Vascular smooth muscle Gq signaling is involved in high blood pressure in both induced renal and genetic vascular smooth muscle-derived models of hypertension

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Hypertension is a considerable health problem in the United States. Importantly, of the population with hypertension, less than 35% have their blood pressure (BP) controlled (35a). Additionally, race/ethnicity contributes to the complexity of this disease, likely due to differences in etiology. For example, African American individuals have a higher prevalence of hypertension, and they also have an increased incidence of two or more risk factors. Therefore, although there are already current antihypertensive therapies that are adequate, they are not successful or appropriate for all, and it is essential that we better understand mechanisms underlying hypertension to develop novel therapeutic strategies that will improve the efficacy and success of treatment to prevent further cardiovascular complications.

Although to date the origin of hypertension has principally been attributed to the kidney, more recently it is appreciated that increases in BP can also arise from primary abnormalities in vascular cell function (33). Elevated levels of catecholamines and peptide hormones such as ANG II are often associated with hypertension. Within the vasculature, we have shown that these ligands can signal through Gq-coupled heterotrimeric G proteins (29). Gq-coupled receptors initiate formation of inositol 1,4,5-trisphosphate [Ins(1,4,5)P3] and diacylglycerol, which respectively increase intracellular Ca2+ and protein kinase C (44) activation, thus causing vasoconstriction. An increase in stimulation of Gq-coupled receptors would tilt the balance of the blood vessel radius maintenance toward vasoconstriction and increased BP. A peptide inhibitor of Gq signaling, GI, has been important in determining which individual seven transmembrane-spanning receptors (7TMRs) are Gq coupled (29) without affecting Gq and Gs signaling (3). It has also been a successful tool used to determine the key role that Gq signaling plays in cardiac hypertrophy in the setting of pressure overload (3, 15).

A typical method of studying the etiology of hypertension in animal models has been to use a surgical manipulation creating renal artery stenosis (28, 46). Reduced blood flow to one kidney activates the renin-ANG II system, leading to increased levels of renin, ANG II, aldosterone, and other circulating factors (34). Renal artery stenosis results in an increase in BP and cardiac remodeling (34). More recently, vascular smooth muscle (VSM)-derived hypertensive models have been developed through genetic manipulation. We have created two different VSM models of hypertension (13, 30), which take advantage of the fact that classical 7TMR signaling is tightly controlled by a class of proteins: the G protein-coupled receptor kinases (GRKs). Elevated levels of GRK2 in both the lymphocytes and VSM are associated with human hypertension and animal models of the disease (18, 23–25), and we have shown that transgenic VSM overexpression of GRK2 is sufficient to increase BP (13). Therefore, we used both a renal- and VSM-derived model of hypertension to investigate the role of Gq signaling.

Our hypothesis in the current study is that VSM Gq signaling is critical to the development of hypertension. To understand...
the role VSM Gq signaling plays in hypertension, we have investigated the use of the GqI peptide in two divergent models of hypertension: renal and VSM based. Interestingly, we found class-specific inhibition of Gq signaling was successful at attenuating high BP in both the renal- and VSM-derived hypertensive mouse models. In contrast, losartan, a current antihypertensive therapy, was only successful in the renal-derived hypertensive model. Our data suggest that VSM Gq signaling is critical to hypertension. Comparing the etiology of two different models of hypertension will allow us to better understand the mechanisms underlying hypertension and allow us to identify more optimized antihypertensive therapeutic strategies.

METHODS

2K1C surgery. Mice were anesthetized with ketamine (50 mg/kg) and xylazine (2.5 mg/kg). The left kidney, renal artery, and vein were exposed via an incision on the lower left flank. Carefully, the renal artery and vein were separated, and a stainless steel U-shaped clip (0.12 mm inner diameter) was placed around the renal artery. Care was taken not to harm the exposed kidney. The incision was then sutured closed with 5-0 vicryl. Sham operations were identical except that the clip was removed before closing the incision. Two-kidney, one-clip renovascular hypertension (2K1C) success was verified by left-to-right kidney ratio. A group of mice were treated with 30 mg·kg⁻¹·day⁻¹ losartan in drinking water (a gift from Merck) for 28 days.

Hybrid mice. VSM GqI mice in a C57Bl/6 background (29) were bred with VSM-GRK2 (13) (with the same background). Procedures were approved by the Institutional Animal Care and Use Committee at Thomas Jefferson University and conducted in accordance with their guidelines. All male progeny were studied [nontransgenic littermate control (NLC), single transgenic (VSM GRK2), or hybrid mice (VSM GRK2/GqI)] between 2–5 mo of age. In addition, in a group of NLC and VSM GRK2 mice, Alzet osmotic minipumps containing 10 μM of prazosin (14) were implanted subcutaneously, and the mice were allowed to recover for 7 days before BP determination.

BP measurements. Mice were anesthetized with ketamine (50 mg/kg) and xylazine (2.5 mg/kg). A gel-filled catheter (PC-10, Data Sciences International) using radiotelemetry was inserted into the left carotid artery and the battery stored in the subcutaneous subscapular layer. Mice were allowed to recover, and conscious, free-moving BP was measured 4 days postinsertion.

Echocardiography measurements. Mice were anesthetized with 1% to 2% isoflurane. Echo was performed using a Vevo 770 (Visual Sonics) and images taken in M-mode in the short axis. Data was obtained and analyzed using Visual Sonics software. Fractional shortening was determined by (end-diastolic diameter – end-systolic diameter)/end-diastolic diameter × 100%.

Histochemistry and immunohistochemistry. Animals were euthanized, and blood vessels were perfused at 100 mmHg in vivo with 4% PBS-buffered paraformaldehyde, excised, and fixed further in 4% PBS-buffered paraformaldehyde for 10 h. After fixation, the heart was sectioned in longitudinal and transverse sections and then embedded in paraffin. Sections of the parallel arteries were obtained at several planes 1 mm apart for immunohistochemical assay. Polyclonal anti-Gqα (1:50) was used as the primary antibody. Normal rabbit IgG was used as the negative control for the primary antibody. A peroxidase-conjugated secondary antibody and Vector VIP peroxidase substrate kit (Cat. No. SK-4600, Vector) was used for immunohistochemistry to reveal endogenous Gq and GqI expression. To examine VSM thickness and area, carotid arteries of both sides were perfused in situ with 4% PBS-buffered paraformaldehyde and were excised with the heart and the tissues surrounding the carotid arteries intact for further fixation in 4% PBS-buffered paraformaldehyde for 10 h. After fixation, both the left and right carotid arteries, with the aortic arch intact, were further dissected under a microscope and oriented in tissue processing/embedding cassettes. The tissues were then subjected to dehydration as described in Histochemistry and immunohistochemistry. The tissue was embedded in paraffin with both the left and right carotid artery oriented in parallel and at a similar position longitudinally relative to the branching of the internal and external carotid arteries. Sections of the parallel arteries were obtained at several planes 1 mm apart for Gomori staining. Images (x200) were analyzed using ImageTool software. Thickness was determined at 10 individual sites for each section and averaged. VSM area was determined as the area between the external and internal elastic lamina.

Aortic rings. Abdominal aortas were dissected and 2.5-mm segments hung on a force pressure transducer as described previously (13, 30). Rings were denuded of endothelial cells by the gentle scraping of the lumen with a steel wire. Verification of endothelial cell removal was confirmed by the administration of acetylcholine (10⁻⁵ M), and a lack of response indicated endothelial cell removal. Rings were then treated to cumulative increasing doses of ANG II or isoproterenol at 3-min intervals. Data were obtained and analyzed offline by Chart 5.0. For isoproterenol, phenylephrine was used to generate preconstriction to detect relaxation. Importantly, responses were normalized to an EC₅₀ dose of phenylephrine.

Statistical analysis. All data are presented as means ± SE. For simple comparisons between two groups, an unpaired two-tailed Student’s t-test was used. One-way ANOVA with a post hoc Bonferroni multiple-comparison test was used to compare multiple groups. Two-way ANOVA was used when a dose response was analyzed.

RESULTS

Inhibition of VSM Gq signaling attenuates high BP in a renovascular model of hypertension. The etiology of more than 90% of human high BP is unknown (35). VSM Gq signaling causes vasoconstriction; therefore, it is possible that Gq signaling is a major contributor to increased BP. To test this, we first examined the efficacy of inhibiting Gq signaling in VSM in a renovascular model of hypertension. We performed 2K1C on NLC, and mice with VSM expression of the inhibitor of Gq (GqI) signaling using a portion of the SM22α promoter (29). Basal BP was not altered by the presence of VSM GqI (Fig. 1). BP increased in NLC mice following 4 wk of renal artery stenosis using the 2K1C Goldblatt model of hypertension (Fig. 1). Importantly, the presence of VSM GqI was able to attenuate elevated BP in this renovascular model of hypertension (Fig. 1).

The 2K1C model is a model in which hypertension is derived from an increased renin-ANG II-aldosterone system (34), which can have multiple effects on multiple organs and cell types although it is believed that the kidney is primarily responsible for the actions of ANG II in hypertension (7). ANG II is a 7TMR capable of coupling to Gq, Gi, and G12/13 (32, 36). We were interested in determining the contribution of VSM ANG II Gq signaling to the development of hypertension in the 2K1C model of high BP. Therefore, we compared the results of VSM Gq signaling inhibition with global ANG II type 1 (AT₁) receptor inhibition with losartan, which would inhibit all downstream signaling of the AT₁ receptor and not just GqI. Losartan decreased BP to a similar extent in both NLC and VSM GqI mice (Fig. 1). In addition, there was no further decline in BP in the VSM GqI mice treated with losartan. These data suggest that inhibition of
VSM $G_q$ signaling can attenuate increased BP in the 2K1C model and that losartan is likely acting primarily via $G_q$-coupled AT$_1$ receptors to inhibit BP. In addition, it suggests that, at least in this model, VSM AT$_1$ receptors play an important role in hypertension.

Inhibition of VSM $G_q$ signaling attenuates high BP in a VSM model of hypertension. To understand the role of VSM $G_q$ signaling in another divergent model of hypertension, we took advantage of our genetic hypertensive mouse model that is VSM derived. GRK2 mRNA expression levels are increased in the lymphocytes of young human hypertensive subjects (23, 24) as well as in lymphocytes and VSM of spontaneously hypertensive rats (24). Previously, we generated transgenic mice with VSM overexpression of GRK2 in which BP was increased and VSM and hearts were hypertrophied (13). We have previously determined that desensitization of $\beta$-adrenergic receptor ( $\beta$-AR)-mediated dilation contributes to the high BP, but the role of constriction and, in particular, VSM $G_q$-coupled signaling is not appreciated.

We mated our VSM G$_{qI}$ mice with hypertensive mice overexpressing VSM GRK2 (Fig. 2), which we have previously shown to be hypertensive. VSM expression of G$_{qI}$ alone did not change BP (Figs. 1 and 2). Importantly, hybrid mice with VSM overexpression of both GRK2 and G$_{qI}$ have restored normal BP (Fig. 2A). Therefore, inhibition of G$_{qI}$ signaling was sufficient to ameliorate high BP in VSM GRK2 mice.

We next tested the efficacy of losartan in the VSM-derived model of high BP. Unlike in the 2K1C model, the underlying mechanisms of high BP in the VSM GRK2 model are not fully appreciated. Losartan did not affect basal resting BP in NLC mice, but it did decrease BP in the VSM GRK2 mice by $\sim$75% (Fig. 2B). Therefore, it is likely that ANG II receptors are somehow involved in the high BP in VSM GRK2 mice. In addition, based on our data, at least 25% of the high BP in VSM GRK2 mice is non-AT$_1$ mediated yet dependent on G$_{qI}$ signaling.

Another G$_q$-coupled 7TMR important to VSM constriction is the $\alpha_1$-adrenergic receptor. We were interested in determining the role this receptor plays in the development of high BP in the VSM GRK2 mice. The mice were treated by osmotic minipump with 10 $\mu$M prazosin for 7 days before BP determination. Prazosin treatment decreased BP by $\sim$35%, and although this drop was not significant, it does suggest that at least a portion of the high BP is also $\alpha_1$-adrenergic G$_q$ dependent (Fig. 2B). These data illustrate that at least two different G$_q$-coupled receptors, AT$_1$ and $\alpha_1$-adrenergic receptors, may be involved in the high BP seen in VSM GRK2 mice.

ANG II vasoconstriction is mediated by G$_q$ signaling. An important 7TMR involved in the 2K1C model is the AT$_1$ receptor, as is shown by the losartan data (Figs. 1 and 2B). We wanted to verify that in our mice AT$_1$ receptors are coupled to G$_q$ and mediate vasoconstriction. Therefore, we examined the impact that our G$_{qI}$ peptide had on ANG II vascular reactivity. ANG II-mediated vasoconstriction is attenuated in the presence of G$_{qI}$ (Fig. 3). There was a 2.4-fold rightward shift in the log $EC_{50}$ from $-8.346 \pm 0.1118$ (n = 5, NLC) to $-7.971 \pm 0.0662$ (n = 14, G$_{qI}; P = 0.0108$). This indicates a decreased sensitivity to ANG II. Importantly, there was no change in vasodilation elicited through the G$_q$-coupled $\beta$-adrenergic receptor in response to isoproterenol. Therefore, in our mice, at
least a portion of the AT$_1$-mediated vasoconstriction is through G$_q$.

Losartan, but not VSM expression of G$_q$I, reverses cardiac dysfunction and hypertrophy in a renovascular model of hypertension. Increases in cardiac hypertrophy accompany chronic increases in high BP. It is believed that this compensatory hypertrophy ultimately progresses to cardiac dysfunction and failure. Our laboratory (29) and Crowley et al. (7) have found that for the most part, hypertension per se conveys the increase in cardiac size. However, we wanted to further test this concept in our current renovascular- and VSM-derived models of hypertension. Despite the ability of VSM G$_q$ inhibition at lowering BP, there was a persistent increase in cardiac size and dysfunction with 2K1C hypertension (Table 1). Therefore, it does not appear that afterload alone is conferring the increased cardiac size in this renovascular model of hypertension.

We further examined the ability of losartan, which lowered BP to a similar extent as VSM G$_q$ inhibition (Fig. 1), to restore cardiac size and dysfunction (Table 1). Treatment with losartan normalized the cardiac hypertrophy and function (Table 1). Therefore, it is likely that paracrine effects due to the increased circulating renin and/or ANG II, which are inhibited by systemic losartan, but not VSMm G$_q$ inhibition, mediate cardiac hypertrophy in this model, and cardiac hypertrophy is not due solely to an increase in afterload in this model of hypertension.

VSM expression of G$_q$I normalizes cardiac hypertrophy in VSM-derived hypertension. Although VSM G$_q$ inhibition was unable to prevent cardiac hypertrophy and dysfunction in a renovascular model of hypertension, we were interested in the role of inhibiting G$_q$ signaling in VSM in a VSM-derived model of hypertension. Unlike renal artery stenosis where there is an increase in circulating levels of the renin-ANG II system which convey high BP (Fig. 1) and cardiac hypertrophy (Table 1), hypertension in our VSM GRK2 model is due to the desensitization of VSMm 7TMRs by GRK2 and a disruption of the tightly controlled balance between constriction and dilation. Although we did not expect any differences, we verified that there were in fact no changes in circulating catecholamines in the VSM GRK2 mice (data not shown). We found that concomitant expression of VSM G$_q$I with VSM GRK2 was able to decrease cardiac hypertrophy associated with VSM-derived hypertension (Table 2). Therefore, in this model, it is likely that the increase in cardiac hypertrophy is due to a direct increase in afterload.

Inhibition of G$_q$ signaling also attenuates VSM hypertrophy. Previously, we have shown that overexpression of GRK2 in VSM results in VSM hypertrophy (13). Since G$_q$ signaling is important in regulating cardiac and VSM hypertrophy (2, 11, 44), we also wanted to determine whether inhibiting G$_q$ signaling in VSM could decrease the VSM hypertrophy seen in our GRK2 mice. We first verified, using immunohistochemistry, that we had VSM-specific expression of our G$_q$I peptide (Fig. 4). The antibody targets the COOH terminus of G$_q$q and detects both endogenous G$_q$q and the expression of our G$_q$I specifically in the VSM layer of the aorta (Fig. 4). Importantly, there is no change in VSM thickness in G$_q$I-expressing vessels (Fig. 4). Therefore, in these mice, inhibition of G$_q$ signaling does not affect basal VSM growth. Interestingly, concomitant G$_q$I expression was sufficient to reduce VSM hypertrophy elicited by VSM overexpression of GRK2 (Fig. 4). Therefore, G$_q$ signaling is critical to conveying VSM hypertrophy in our VSM-derived hypertensive model.

DISCUSSION

In the present study, our data suggest that VSM G$_q$ signaling is involved in high BP of two divergent models of hypertension: one that is renal derived and one that is VSM derived. We also determined that AT$_1$ signaling is involved in both our renal- and VSM-derived hypertensive models and that AT$_1$-adrenergic receptors may play at least a partial role in the high BP associated with VSM GRK2 mice. We confirmed in situ AT$_1$ receptors in aorta couple to G$_q$q and mediate vasoconstriction. We also determined that, although decreases in afterload using VSM G$_q$I expression decreased cardiac hypertrophy in our VSM-derived model of hypertension, it was not sufficient to decrease cardiac hypertrophy or dysfunction in the renal-
derived model of hypertension. This is likely due to non-VSM-related effects of the activated renin-ANG II-aldosterone system or AT1 G protein-independent signaling (41). Finally, we illustrated that inhibition of Gq signaling was sufficient to decrease not only afterload but also cardiac and VSM hypertrophy in our VSM-derived hypertensive model since it is likely that the involvement of protein kinase C, p38, and JNK, molecules downstream of Gq important to VSM and cardiac hypertrophy (44), are inhibited. Therefore, this study shows that VSM Gq signaling is critical to the development of two divergent models of hypertension and associated cardiovascular risk factors including cardiac and VSM hypertrophy.

Given our data in the current study, the role of BP on cardiac hypertrophy and dysfunction seems to be dictated by the model. Our laboratory (29) and Crowley et al. (7) have previously shown that BP determines cardiac hypertrophy and dysfunction, but whether this phenomenon also occurs in the renal hypertensive model than absolute BP. Alternatively, factors) are more likely responsible for cardiac hypertrophy in this model. The role of these non-Gq-coupled signaling pathways and cardiac myocytes versus fibroblasts/myofibroblasts (4) in cardiac hypertrophy (44) in this model. The role of these non-Gq-coupled signaling pathways and cardiac myocytes versus fibroblasts/myofibroblasts (4) in cardiac hypertrophy concomitant with renovascular hypertension remains to be determined.

The 2K1C model of hypertension is a high renin, normal volume model of hypertension (46). Plasma ANG II levels are increased at day 7 but restored to normal at day 25 (34). In contrast, renin levels remain elevated for at least 4 wk (46). Importantly, VSM (19, 42) and heart (19) contain an endogenous renin-ANG II system such that it can convert circulating renin into ANG II. Others have shown the necessity of AT1 receptors in the development of high BP in rats with 2K1C (6, 20). Our data confirm that vasoconstriction mediated by ANG II is Gq coupled, at least in the mouse. This is an important distinction since AT1 receptors have been shown to couple to both Gq, Gi, and other G proteins (10, 32, 36) and also have G protein-independent signaling (41). Importantly, our data suggest that, although the 2K1C model is a renovascular model of hypertension, VSM Gq signaling is also important in the development of hypertension and therapies tailored to targeting mechanisms both at the level of the kidney and VSM will improve efficacy of treatments.

The predominant regulation of 7TMs, including ANG II receptors, occurs with the targeted phosphorylation of activated receptors leading to G protein uncoupling, a process termed desensitization. This process is initiated by phosphorylation of the agonist-occupied receptor by GRKs, a seven-member family (GRK1–7) of serine/threonine kinases (27, 37). Therefore, 7TMs and the regulation of their signaling by GRKs are critical to normal VSM homeostasis. Studies from animal models provide support for the important role of 7TMs and GRK2 in hypertension. There is a downregulation of the aorta α-AR-adenylate cyclase system due to humoral and hemodynamic factors in vivo in spontaneously hypertensive (SHR) and Dahl salt-sensitive rats (45). In addition, the coupling of β-ARs to ATP-sensitive K+ channels via the heterotrimeric G protein, Go, is compromised, thereby preventing K+ influx and subsequent vasodilation (21). We have found that VSM overexpression of GRK2 is sufficient to result in hypertension (13). Others have shown that increased GRK2 protein expression has been correlated with increased BP in lymphocytes and VSM of SHRs and Dahl salt-sensitive hypertensive rats as well as in lymphocytes of hypertensive patients (23–25). Importantly, not only is β-AR signaling compromised in this VSM-derived hypertensive model (13) but, in this current study, we provide evidence that there must also be an exacerbation of Gq signaling such that when it is inhibited,

| Table 1. Losartan, but not VSM expression of Gq, reverses cardiac dysfunction and hypertrophy in a renovascular model of hypertension |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | NLC Sham       | GqJ 2K1C        | NLC 2K1C        | GqJ 2K1C         | NLC 2K1C Losartan |
| n               | 15             | 20              | 10              | 19              | 19              |
| FWT, mm         | 0.79±0.02      | 0.76±0.02       | 1.00±0.05*      | 0.93±0.03*       | 0.77±0.03†      |
| AWT, mm         | 0.83±0.04      | 0.73±0.06       | 0.92±0.06       | 0.84±0.06        | 0.75±0.02       |
| EDD, mm         | 3.52±0.08      | 3.76±0.05       | 3.55±0.14       | 3.66±0.10        | 3.70±0.10       |
| FS, %           | 35.7±1.3       | 34.2±0.8        | 26.0±1.9*       | 25.9±1.4*        | 25.9±1.4*       |
| HR, beats/min   | 428±14         | 423±9           | 412±17          | 408±15           | 439±11          |

Values are means ± SE; n, number of mice. Four weeks following two-kidney, one-clip renovascular hypertension (2K1C) surgery, mice were anesthetized with 1.0% to 1.5% isoflurane, and M-mode echocardiography was performed. Losartan (30 mg·kg⁻¹·day⁻¹) was administered via drinking water immediately following surgery. NLC, nontransgenic littermate control; PWT, posterior wall thickness; AWT, anterior wall thickness; EDD, end diastolic diameter; FS, fractional shortening; HR, heart rate. *P < 0.05 vs. respective sham; †P < 0.05 vs. NLC-2K1C; ‡P < 0.05 vs. GqJ-2K1C.

| Table 2. VSM expression of GqJ normalizes cardiac hypertrophy in VSM-derived hypertension |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | NLC             | GRK2            | GRK2/GqJ         |
| n               | 6               | 5               | 5               |
| FWT, mm         | 0.74±0.02       | 0.92±0.03*      | 0.80±0.02†      |
| AWT, mm         | 0.73±0.02       | 0.89±0.06*      | 0.74±0.02†      |
| EDD, mm         | 3.72±0.14       | 3.66±0.16       | 3.80±0.19       |
| FS, %           | 34.9±1.3        | 32.2±2.1        | 34.90±1.0       |
| HR, beats/min   | 458±27          | 427±15          | 489±14          |

Values are means ± SE; n, number of mice. At 2 mo, NLC, G protein-coupled receptor kinase 2 (GRK2)-overexpressing mice, and hybrid GRK2/GqJ mice were anesthetized with 1.5% to 2.0% isoflurane, and echocardiography was performed. One-way ANOVA and Bonferroni posttest were used. *P < 0.05 vs. respective NLC; †P < 0.05 vs. GRK2.
normal BP is restored. It was unexpected that losartan was able to reduce BP in this VSM-derived model of hypertension because the literature suggests that, at least in heart, GRK2 phosphorylates and desensitizes AT1 receptors (26). The role of GRK2 on VSM AT1 and AT2 receptors obviously needs further investigation. Interestingly, prazosin also decreased BP 35% in VSM GRK2 mice. Although our previous data suggest that GRK3 phosphorylates and desensitizes α1B-adrenergic receptors in the heart (12), the role of GRK2 on the two important VSM α-adrenergic receptor subtypes, α1A- and α1D-adrenergic receptors, remains to be determined. Importantly, neither losartan or prazosin completely attenuated VSM GRK2 increased BP, and, therefore, it remains to be determined which specific Gq-coupled 7TMRs are involved. Importantly, other VSM receptors such as PDGF (38) and endothelin (38) receptors have also been shown to be capable to Gq and be desensitized by GRK2, whereas 5-HT2A serotonin receptors, also Gq coupled, are not substrates for GRK2 (22); therefore, investigation into these and other Gq receptors and their role in BP regulation will be essential to better understand hypertension and develop more appropriate candidate therapies.

We have shown that VSM Gq signaling is important in two diverse models of hypertension. Our GqI peptide, which was only expressed in VSM, was as effective as losartan, which was administered globally at decreasing hypertension in the renal-based model of hypertension, suggesting that VSM AT1 Gq-coupled receptors are critical to the development of high BP in this renal-derived model. To our knowledge, this study is the first to show the importance of both Gq and the VSM component of AT1 signaling in this 2K1C model. These data suggest that numerous VSM Gq receptors are affected by GRK2. Therefore, in human hypertension, when GRK2 levels are increased, it is apparent that multiple therapeutics are required to successfully manage the disease. Understanding the correct receptors to target is critical to improve therapy. There-
fore, this study illustrates the importance of VSM $G_q$ signaling in different etiologies of high BP and suggests that, perhaps, development of better antihypertensive therapeutic strategies will improve as mechanisms underlying hypertension are more fully understood (44).

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