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

David M. Harris, Heather I. Cohn, Stéphanie Pesant, Rui-Hai Zhou, and Andrea D. Eckhart

Eugene Feiner Laboratory of Vascular Biology and Thrombosis, Center for Translational Medicine, Thomas Jefferson University, Philadelphia, Pennsylvania

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Vascular smooth muscle \( G_q \) signaling is involved in high blood pressure in both induced renal and genetic vascular smooth muscle-derived models of hypertension. Am J Physiol Heart Circ Physiol 293: H3072–H3079, 2007. First published September 14, 2007; doi:10.1152/ajpheart.00880.2007.— More than 30% of the US population has high blood pressure (BP), and less than a third of people treated for hypertension have it controlled. In addition, the etiology of most high BP is not known. Having a better understanding of the mechanisms underlying hypertension could potentially increase the effectiveness of treatment. Because \( G_q \) signaling mediates vasoconstriction and vascular function can cause BP abnormalities, we were interested in determining the role of vascular smooth muscle (VSM) \( G_q \) signaling in two divergent models of hypertension: a renovascular model of hypertension through renal artery stenosis and a genetic model of hypertension using mice with VSM-derived high BP. Inhibition of VSM \( G_q \) signaling attenuated BP increases induced by renal artery stenosis to a similar extent as losartan, an \( \alpha_1 \)-adrenergic receptor antagonist, only depressed BP by 35%. Inhibition of \( G_q \) signaling also attenuated high BP in our genetic VSM-derived hypertensive model. In contrast, BP remained elevated 25% following treatment with losartan, and prazosin, an \( \alpha_1 \)-adrenergic receptor antagonist, only decreased BP by 35%. Inhibition of \( G_q \) signaling attenuated VSM reactivity to ANG II and resulted in a 2.4-fold rightward shift in \( EC_50 \). We also determined that inhibition of \( G_q \) signaling was able to reverse VSM hypertrophy in the genetic VSM-derived hypertensive model. These results suggest that \( G_q \) signaling is an important signaling pathway in two divergent models of hypertension and, perhaps, optimization of antihypertensive therapy could occur with the identification of particular \( G_q \)-coupled receptors involved.

two-kidney, one-clip renovascular hypertension; \( G \) protein-coupled receptor kinase 2; seven transmembrane-spanning receptor

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 \( G_q \)-coupled heterotrimeric G proteins (29). \( G_q \)-coupled receptors initiate formation of inositol 1,4,5-trisphosphate [Ins(1,4,5)P₃] and diacylglycerol, which respectively increase intracellular \( Ca^{2+} \) and protein kinase C (44) activation, thus causing vasoconstriction. An increase in stimulation of \( G_q \)-coupled receptors would tilt the balance of the blood vessel radius maintenance toward vasoconstriction and increased BP. A peptide inhibitor of \( G_q \) signaling, \( G_q \_I \), has been important in determining which individual seven transmembrane-spanning receptors (7TMRs) are \( G_q \) coupled (29) without affecting \( G_i \) and \( G_s \) signaling (3). It has also been a successful tool used to determine the key role that \( G_q \) 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 \( G_q \) signaling.

Our hypothesis in the current study is that VSM \( G_q \) 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

Two-kidney-one-clip (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 subscapularly, 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 body and Vector VIP peroxidase substrate kit (Cat. No. SK-4600, Vector) was used for immunohistochemistry to reveal endogenous Gq signaling. Normal rabbit IgG was used as the negative control for the primary antibody. A peroxidase-conjugated secondary antibody (1:50) was used as the secondary antibody. Polyclonal anti-Gq(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 orientated 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 (×200) 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 (10⁻⁶ 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 G(12/13) (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 GqI 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 coupling signaled like the 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 β-adrenergic receptor (β-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_q mice with hypertensive mice overexpressing VSM GRK2 (Fig. 2), which we have previously shown to be hypertensive. VSM expression of G_qalone did not change BP (Figs. 1 and 2). Importantly, hybrid mice with VSM overexpression of both GRK2 and G_q have restored normal BP (Fig. 2A). Therefore, inhibition of G_q 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 ~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_q signaling.

Another G_q-coupled 7TMR important to VSM constriction is the α_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 μM prazosin for 7 days before BP determination. Prazosin treatment decreased BP by ~35%, and although this drop was not significant, it does suggest that at least a portion of the high BP is also α_1-adrenergic G_q dependent (Fig. 2B). These data illustrate that at least two different G_q-coupled receptors, AT_1 and α_1-adrenergic receptors, may be involved in the high BP seen in VSM GRK2 mice.

ANG II vasconstriction 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 vasconstriction. Therefore, we examined the impact that our G_q peptide had on ANG II vascular reactivity. ANG II-mediated vasconstriction is attenuated in the presence of G_q (Fig. 3). There was a 2.4-fold rightward shift in the log EC_50 from -8.346 ± 0.1118 (n = 5, NLC) to -7.971 ± 0.0662 (n = 14, G_q; P = 0.0108). This indicates a decreased sensitivity to ANG II. Importantly, there was no change in vasodilation elicited through the G_q-coupled β-adrenergic receptor in response to isoproterenol. Therefore, in our mice, at...
least a portion of the AT1-mediated vasoconstriction is through Gq.

Losartan, but not VSM expression of GqI, 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 Gq 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 Gq 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 Gq 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 GqI normalizes cardiac hypertrophy in VSM-derived hypertension. Although VSM Gq inhibition was unable to prevent cardiac hypertrophy and dysfunction in a renovascular model of hypertension, we were interested in the role of inhibiting Gq 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 GqI 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 Gq signaling also attenuates VSM hypertrophy. Previously, we have shown that overexpression of GRK2 in VSM results in VSM hypertrophy (13). Since Gq signaling is important in regulating cardiac and VSM hypertrophy (2, 11, 44), we also wanted to determine whether inhibiting Gq 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 GqI peptide (Fig. 4). The antibody targets the COOH terminus of Gq, and detects both endogenous Gq and the expression of our GqI specifically in the VSM layer of the aorta (Fig. 4). Importantly, there is no change in VSM thickness in GqI-expressing vessels (Fig. 4). Therefore, in these mice, inhibition of Gq signaling does not affect basal VSM growth. Interestingly, concomitant GqI expression was sufficient to reduce VSM hypertrophy elicited by VSM overexpression of GRK2 (Fig. 4). Therefore, GqI signaling is critical to conveying VSM hypertrophy in our VSM-derived hypertensive model.

DISCUSSION

In the present study, our data suggest that VSM Gq 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 AT1 signaling is involved in both our renal- and VSM-derived hypertensive models and that e1-adrenergic receptors may play at least a partial role in the high BP associated with VSM GRK2 mice. We confirmed in situ AT1 receptors in aorta couple to Gq and mediate vasoconstriction. We also determined that, although decreases in afterload using VSM GqI expression decreased cardiac hypertrophy in our VSM-derived model of hypertension, it was not sufficient to decrease cardiac hypertrophy or dysfunction in the renal-

![Diagram](http://ajpheart.physiology.org/)

**Fig. 3.** ANG II-mediated vasoconstriction is Gq coupled. A: abdominal aortic rings from VSM GqI mice (n = 14) had a decreased sensitivity to ANG II compared with NLC (n = 5). P ≤ 0.05, and two-way ANOVA for both ANG II concentration and presence of GqI was used. B: aortic rings were preconstricted with an E50 dose of 3 × 10−8 M phenylephrine and subjected to increasing concentrations of the β-adrenergic receptor agonist isoproterenol. C: EC50 for ANG II in aorta rings isolated from NLC and GqI (n = 10 for both). *P < 0.05, unpaired two-tailed Student’s t-test.
hypertrophy in VSM-derived hypertension that VSM Gq signaling is critical to the development of two hypertrophy (44), are inhibited. Therefore, this study shows illustrated that inhibition of Gq signaling was sufficient to molecules downstream of Gq important to VSM and cardiac likely that the involvement of protein kinase C, p38, and JNK, decrease not only afterload but also cardiac and VSM hyper-
dysfunction, but whether this phenomenon also occurs in the hypertrophy and dysfunction seems to be dictated by the

dervied 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 2K1C mouse model of hypertension is unclear. Gq-mediated signaling is important to cardiac hypertrophy (2, 3, 16, 17), although this may be mediated, at least in part, through cardiac fibroblasts (43). Our present data suggest that the ANG II-mediated circulating factors/hormones (aldosterone, growth 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 con-

comitant 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 endoge-

nous renin-ANG II system such that it can convert circulating renin into ANG II. Others have shown the necessity of AT1A 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, Gs, and other G proteins (10, 32, 36) and also have G protein-independent signaling (41). Importantly, our data sug-

Table 1. Losartan, but not VSM expression of Gq, reverses cardiac dysfunction and hypertrophy in a renovascular model of hypertension

<table>
<thead>
<tr>
<th></th>
<th>NLC Sham</th>
<th>GqJ Sham</th>
<th>NLC 2K1C</th>
<th>GqJ 2K1C</th>
<th>NLC 2K1C Losartan</th>
<th>GqJ 2K1C Losartan</th>
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<tbody>
<tr>
<td>n</td>
<td>15</td>
<td>20</td>
<td>10</td>
<td>19</td>
<td>10</td>
<td>8</td>
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<td>PWT, mm</td>
<td>0.79±0.02</td>
<td>0.76±0.02</td>
<td>1.00±0.05*</td>
<td>0.93±0.03</td>
<td>0.77±0.03†</td>
<td>0.77±0.02‡</td>
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<td>AWT, mm</td>
<td>0.83±0.04</td>
<td>0.73±0.08</td>
<td>0.92±0.06</td>
<td>0.84±0.06</td>
<td>0.75±0.02</td>
<td>0.77±0.03</td>
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<tr>
<td>EDD, mm</td>
<td>3.52±0.08</td>
<td>3.76±0.05</td>
<td>3.55±0.14</td>
<td>3.66±0.10</td>
<td>3.70±0.10</td>
<td>3.75±0.08</td>
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<td>FS, %</td>
<td>35.7±1.3</td>
<td>34.2±0.8</td>
<td>26.0±1.9*</td>
<td>25.9±1.4*</td>
<td>32.9±0.7†</td>
<td>32.0±0.9†</td>
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<tr>
<td>HR, beats/min</td>
<td>428±14</td>
<td>423±9</td>
<td>412±17</td>
<td>408±15</td>
<td>439±11</td>
<td>430±12</td>
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</table>

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 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 hypertensive

<table>
<thead>
<tr>
<th></th>
<th>NLC</th>
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<th>GRK2/GqJ</th>
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<td>n</td>
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<td>PWT, mm</td>
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<td>0.80±0.02‡</td>
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<td>EDD, mm</td>
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<td>FS, %</td>
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<td>HR, beats/min</td>
<td>458±27</td>
<td>427±15</td>
<td>489±14</td>
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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 coupled 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 Gq 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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