Vascular Smooth Muscle Gq Signaling is Involved in High Blood Pressure in Both
Induced Renal and Genetic Vascular Smooth Muscle-Derived Models of Hypertension

David M Harris PhD, Heather I Cohn BS, Stéphanie Pesant MSc,
Rui-Hai Zhou MD, Andrea D Eckhart* PhD

Eugene Feiner Laboratory of Vascular Biology and Thrombosis, Center for Translational
Medicine, Thomas Jefferson University, Philadelphia PA 19107-5001

Running Title: Gq signaling contributes to high BP

*Address for Correspondence:
Andrea D Eckhart, PhD
Associate Professor
Thomas Jefferson University
Rm 309 College Bldg
1025 Walnut St
Philadelphia PA 19107
215.955.9992 (phone)
215.503.5731 (fax)
Andrea.Eckhart@jefferson.edu

Non-standard abbreviations: GqI: inhibitor of Gq signaling; VSM: vascular smooth
muscle; 2K1C: 2 kidney, 1 clip renovascular hypertension; GRK2: G protein-coupled
receptor kinase 2; BP: blood pressure; 7TMR: 7 transmembrane-spanning receptor;
Abstract

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 effectiveness of treatment. Because Gq signaling mediates vasoconstriction and vascular function can cause BP abnormalities, we were interested in determining the role of vascular smooth muscle (VSM) Gq 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 Gq signaling attenuated BP increases induced by renal artery stenosis to a similar extent as losartan, an angiotensin II receptor blocker and current antihypertensive therapy. Inhibition of Gq 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 Gq signaling attenuated VSM reactivity to angiotensin II and resulted in a 2.4-fold right-ward shift in EC\textsubscript{50}. We also determined that inhibition of Gq signaling was able to reverse VSM hypertrophy in our genetic VSM-derived hypertensive model. These results suggest that Gq signaling is an important signaling pathway in two divergent models of hypertension and perhaps, optimization of antihypertensive therapy could occur with identification of particular Gq-coupled receptors involved.
Hypertension is a considerable health problem in the United States. Importantly, of the population with hypertension, less than 35% have their BP controlled (1). 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 2 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 which 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 angiotensin II (AngII) 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 IP$_3$ and diacylglycerol, which respectively increase intracellular Ca$^{2+}$ and protein kinase C (44) activation, thus causing vasoconstriction. An increase in stimulation of Gq-coupled receptors would tilt the balance of blood vessel radius maintenance towards vasoconstriction and increased BP. A peptide inhibitor of Gq signaling, GqI, has been important in determining which individual 7 transmembrane-spanning receptors (7TMRs) are Gq-coupled (29) without affecting Gi and Gs signaling (3). It has also been
a successful tool used to determine the key role 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-AngII system leading to increased levels of renin, AngII, aldosterone and other circulating factors (34). Renal artery stenosis results in an increase in BP and cardiac remodeling (34). More recently, VSM-derived hypertensive models have been developed through genetic manipulation. We have created 2 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-derived 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**

**Two Kidney, 1 Clip Surgery (2K1C)**

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 prior to closing the incision. 2K1C success was verified by left-to-right kidney ratio. A group of mice were treated with 30mg/kg/d losartan for 28d in the drinking water (gift from Merck).

**Hybrid Mice**

VSM-GqI mice in a C57Bl/6 background(29) were bred with VSM-GRK2 (13) (same background) in accordance with IACUC guidelines at Thomas Jefferson University. All male progeny were studied (non-transgenic littermate control (NLC), single transgenic (VSM-GRK2) or hybrid mice(VSM-GRK2/GqI)) between 2 and 5 months of age. In addition, in a group of NLC and VSM-GRK2 mice, Alzet osmotic mini-pumps containing 10 µM prazosin(14) were implanted subscapularly and the mice were allowed to recover for 7d prior to BP determination.

**Blood Pressure 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, freely-moving BP was measured 4 days post insertion.

**Echocardiography Measurements**
Mice were anesthetized with 1-2% 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 \((\text{End-Diastolic diameter-End systolic diameter}) / \text{End diastolic diameter} \times 100\%\).

**Histochemistry and Immunohistochemistry**
Animals were sacrificed and blood vessels were perfused at 100mmHg in vivo with 4% PBS-buffered paraformaldehyde, excised and fixed further in 4% PBS-buffered paraformaldehyde for 10 hours. After fixation, the aorta were orientated in tissue processing/embedding cassettes, subjected to dehydration with serial concentrations of ethanol, clarification with xylene, and paraffinization and then embedded in paraffin. Sections of the parallel arteries were obtained at several planes 1mm apart for immunohistological assay. Polyclonal anti-Gqα (1:50) was used as the primary antibody. Normal rabbit IgG was used as negative control for the primary antibody. Peroxidase-conjugated secondary antibody and Vector VIP peroxidase substrate kit (Vector, Cat# SK-4600, CA) 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 hours. 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 above. The tissue was embedded in paraffin with both 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 1mm apart for Gomori staining. 200x images were analyzed using ImageTool. 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 aorta 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 gently scraping the lumen with a steel wire. Verification of endothelial cell removal was confirmed by administration of acetylcholine (10^{-5} M) and lack of response indicated endothelial cell removal. Rings were then treated to cumulative increasing doses of AngII or isoproterenol at 3 minute intervals. Data was obtained and analyzed offline by Chart 5.0. For isoproterenol, phenylephrine was used to generate pre-constriction to detect relaxation. Importantly, responses were normalized to an EC_{50} dose of PE.

**Statistical Analysis**

All data are presented as mean±SEM. For simple comparisons between 2 groups, an unpaired, 2-tailed Student’s t test was used. One-way ANOVA with post hoc Bonferroni multiple comparisons 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 non-transgenic littermate control (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 (Figure 1). BP increased in NLC mice following 4 weeks of renal artery stenosis using the 2K1C Goldblatt model of hypertension (Figure 1). Importantly, the presence of VSM-GqI was able to attenuate elevated BP in this renovascular model of hypertension (Figure 1).

The 2K1C model is a model in which hypertension is derived from an increased renin-AngII-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 AngII in hypertension (7). AngII is a 7TMR capable of coupling to Gq, Gi and G12/13 (32, 36). We were interested in determining the contribution of VSM AngII-Gq signaling to the development of hypertension in the 2K1C model of high BP. Therefore, we compared the results of VSM Gq signaling inhibition to global AngII AT1 receptor inhibition with losartan, which would inhibit all downstream signaling of the AT1R, and not just Gq-coupled signaling like the GqI. Losartan decreased BP to a similar extent in both NLC and VSM-GqI mice (Figure 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 Gq signaling can attenuate increased BP in the 2K1C model and that losartan is likely acting primarily via Gq-coupled AT1 receptors to inhibit BP. In addition, it suggests that at least in this model, VSM AT1 receptors play an important role in hypertension.

**Inhibition of VSM Gq signaling attenuates high BP in a VSM model of hypertension**

To understand the role of VSM Gq 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 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 βAR-mediated dilation contributes to the high BP but the role of constriction and in particular VSM Gq-coupled signaling is not appreciated.

We mated our VSM-GqI mice with hypertensive mice overexpressing VSM-GRK2 (Figure 2) in which we have previously shown to be hypertensive. VSM expression of GqI alone did not change BP (Figure 1,2). Importantly, hybrid mice with VSM overexpression of both GRK2 and GqI have restored normal BP (Figure 2A). Therefore, inhibition of Gq signaling was sufficient to ameliorate high BP in VSM-GRK2 mice.
We next tested the efficacy of losartan in our 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 approximately 75% (Figure 2B). Therefore, it is likely that AngII 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-AT1 mediated yet dependent on Gq signaling.

Another Gq-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 mini-pump with $10\mu$M prazosin for 7 days prior to BP determination. Prazosin treatment decreased BP by approximately 35%, although this drop was not significant, it does suggest that at least a portion of the high BP is also $\alpha_1$-adrenergic-Gq dependent (Figure 2B). These data illustrate that at least 2 different Gq-coupled receptors, AT1 and $\alpha_1$-adrenergic receptors, may be involved in the high BP seen in VSM-GRK2 mice.

**AngII vasoconstriction is mediated by Gq signaling**

An important 7TMR involved in the 2K1C model is the AT1 receptor as is shown by the losartan data above (Figure 1,2B). We wanted to verify that in our mice, AT1 receptors are coupled to Gq and mediate vasoconstriction. Therefore, we examined the impact our GqI peptide had on AngII vascular reactivity. AngII mediated vasoconstriction is attenuated in the presence of GqI (Figure 3). There was a 2.4-fold right-ward shift in the logEC$_{50}$ from $-8.346\pm0.1118$ (n=5, NLC) to $-7.971\pm0.0662$ (n=14,
This indicates a decreased sensitivity to AngII. Importantly, there was no change in vasodilation elicited through the Gs-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. We (29) and others (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 (Figure 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 AngII, which are inhibited by systemic losartan but not VSM 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-AngII system which convey high BP (Figure 1) and cardiac hypertrophy (Table 1), hypertension in our VSM-GRK2 model is due to the desensitization of VSM 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 if 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 (Figure
4). The antibody targets the C-terminus of $G_\alpha q$ and detects both endogenous Gq and the expression of our GqI specifically in the VSM layer of the aorta (Figure 4). Importantly, there is no change in VSM thickness in GqI expressing vessels (Figure 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 (Figure 4). Therefore, Gq 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 \( \alpha_1 \)-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-derived model of hypertension. This is likely due to non-VSM related effects of the activated renin-AngII-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 likely 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. We and others (7, 29) 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 AngII-mediated circulating factors/hormones (aldosterone, growth factors) are more likely responsible for cardiac hypertrophy in the renal hypertensive model than absolute BP. Alternatively, non-Gq coupled AngII signaling mediated through $G_{12/13}$ and/or transactivation of growth factor receptors such as EGF receptor may play a more important role in VSM and 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 AngII levels are increased at 7d but restored to normal at 25d (34). In contrast, renin levels remain elevated for at least 4 weeks (46). Importantly, VSM (19, 42) and heart (19) contain an endogenous renin-AngII system such that it can convert circulating renin into AngII. Others have shown the necessity of AT1A receptors in the development of high BP in rats with 2K1C (6, 20). Our data confirms that vasoconstriction mediated by AngII 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 7TMRs, including AngII 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 7-member family (GRK1-7) of serine/threonine kinases (27, 37). Therefore, 7TMRs and regulation of their signaling by GRKs, is critical to normal VSM homeostasis. Studies from animal models provide support for the important role of 7TMRs and GRK2 in hypertension. There is a downregulation of the aorta βAR-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 βARs to K_ATP channels via the heterotrimeric G protein, G_s, 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 SHR and Dahl salt-sensitive hypertensive rats as well as in lymphocytes of hypertensive patients (23-25). Importantly, not only is βAR 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, 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 $\alpha$-adrenergic receptor subtypes, $\alpha_{1A}$- and $\alpha_{1D}$-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 able to couple to Gq and be desensitized by GRK2 whereas 5-HT$_{2A}$ 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. Therefore, 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).
Acknowledgments

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**Figure Legends**

**Figure 1.** Inhibition of VSM Gq signaling attenuates hypertension in a renal-derived model of hypertension to a similar extent as inhibition of AT1 receptors. Both NLC (n=9) and VSM-GqI mice (n=8) had similar basal BP in the sham operated group. BP is increased in NLC mice following 2K1C (n=8). Increased BP is attenuated in VSM-GqI mice (n=7). Losartan was administered for 28 d (30mg/kg/d) in the drinking water in sham mice or immediately following surgery in the 2K1C group. Losartan did not affect resting BP in sham NLC or VSM-GqI mice (data not shown) but attenuated high BP in both (n=10,6 respectively). *P<0.05 vs. respective sham, †P<0.05 vs. NLC 2K1C, Two-way ANOVA, Bonferroni post test.

**Figure 2.** Inhibition of VSM Gq signaling attenuates hypertension in a genetic VSM-derived model of hypertension whereas inhibition of AT1 receptors and α₁-adrenergic receptors was less effective. A, Concomitant VSM expression of GqI attenuated VSM-GRK2 induced increase in BP. Hybrid VSM GqI/GRK2 mice are normotensive compared to GRK2 overexpressing mice. n=9,8,7,5. *P<0.05 vs. NLC, †P<0.05 vs. GRK2, one-way ANOVA, Bonferroni post-test. B, AngII receptor inhibition using losartan attenuates hypertension in VSM-GRK2 hypertensive mice although BP is still significantly elevated. Treatment of mice with prazosin to block α₁-adrenergic receptors did not significantly affect BP in our VSM-derived hypertensive model. n=10,6,6,5,3,4 respectively. One-way ANOVA, Bonferroni multiple comparison post-test. *P<0.05 versus NLC.
Figure 3. AngII-mediated vasoconstriction is Gq-coupled. A. Abdominal aortic rings from VSM Gql mice (n=14) had a decreased sensitivity to AngII compared to NLC (n=5). *P<0.05 two-way ANOVA for both AngII concentration and presence of Gql. *P<0.05 for the EC50 dose of AngII in Gql versus NLC in an unpaired 2-tailed t-test. B. Aortic rings were preconstricted with an EC50 dose of 3 x 10^{-7}M phenylephrine and subjected to increasing concentrations of the β-adrenergic receptor agonist isoproterenol. C. EC50 for AngII in aorta rings isolated from NLC and Gql (n=10,10). *P<0.05 unpaired two-tailed t-test.

Figure 4. Gql is expressed specifically in VSM and Gql mediated inhibition of Gq signaling attenuates VSM hypertrophy concomitant with VSM overexpression of GRK2. Top panels Left and Right, Aortas were fixed and immunohistochemistry was performed in NLC and VSM Gql mice. Dark purple staining confirms expression endogenous expression of Gq and transgenic expression of Gql in VSM Gql mice. Middle panels, Left and Right and bottom panel left, Carotid arteries were removed from NLC, GRK2, and hybrid VSM Gql/GRK2 mice and stained with Gomori’s trichrome. GRK2 mice have increased VSM hypertrophy which is reversed in hybrid VSM Gql/GRK2 mice. As shown in the bottom right-hand corner histogram, there was no change in thickness between NLC (n=3) and VSM Gql (n=3) carotid arteries. VSM GRK2 overexpressing mice (n=3) have increased carotid artery thickness that is normalized with concomitant VSM Gql expression (n=6). *P<0.05 vs. NLC, †P<.05 vs. GRK2, both using one-way ANOVA with Bonferroni multiple comparison post-test.
Table 1 Losartan, but not VSM expression of GqI, reverses cardiac dysfunction and hypertrophy in a renovascular model of hypertension

Four weeks following 2K1C surgery, mice were anesthetized with 1.0-1.5% Isoflurane and m-mode echocardiography was performed. Losartan (30 mg/kg/day) was administered via drinking water immediately following surgery.

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n=number of mice, PWT=Posterior Wall Thickness, AWT=Anterior Wall Thickness, EDD=End Diastolic Diameter, FS=Fractional shortening, HR=heart rate (beats per minute)*P<.05 versus respective sham, **P<.05 versus NLC 2K1C, †P<.05 versus GqI 2K1C
Table 2 VSM expression of GqI normalizes cardiac hypertrophy in VSM-derived hypertension. At 2 months of age, NLC, GRK2 overexpressing mice and hybrid GRK2/GqI mice were anesthetized with 1.5-2.0% isofluane and echocardiography was performed.

<table>
<thead>
<tr>
<th></th>
<th>NLC</th>
<th>GRK2</th>
<th>GRK2/GqI</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>6</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>PWT, mm</td>
<td>0.74±0.02</td>
<td>0.92±0.03*</td>
<td>0.80±0.02†</td>
</tr>
<tr>
<td>AWT, mm</td>
<td>0.73±0.02</td>
<td>0.89±0.06*</td>
<td>0.74±0.02†</td>
</tr>
<tr>
<td>EDD, mm</td>
<td>3.72±0.14</td>
<td>3.66±0.16</td>
<td>3.80±0.19</td>
</tr>
<tr>
<td>FS, %</td>
<td>34.9±1.3</td>
<td>32.2±2.1</td>
<td>34.9±1.0</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>458±27</td>
<td>427±15</td>
<td>489±14</td>
</tr>
</tbody>
</table>

n=number of mice, PWT=Posterior Wall Thickness, AWT=Anterior Wall Thickness, EDD=End Diastolic Diameter, FS=Fractional shortening, HR=heart rat (beats per minute) one-way ANOVA, Bonferroni post-test, *P<.05 versus respective NLC, †P<.05 versus GRK2
Figure 1
Figure 2
Figure 3

A

AngII Response (% of Maximum)

AngII (logM)

-11 -10 -9 -8 -7 -6 -5

25 50 75 100

* NLC • GqI

B

Tension (% of Phenylephrine EC50)

Isoproterenol (logM)

-10 -9 -8 -7 -6 -5 -4

60 80 100

* NLC • GqI

C

EC50 (M)

5e-009 1e-008

NLC • GqI

*