Renal Actions of RGS2 Control Blood Pressure

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ABSTRACT

G protein-coupled receptors (GPCRs) have key roles in cardiovascular regulation and are important targets for the treatment of hypertension. GTPase-activating proteins, such as RGS2, modulate downstream signaling by GPCRs. RGS2 displays regulatory selectivity for the Gαq subclass of G proteins, and mice lacking RGS2 develop hypertension through incompletely understood mechanisms. Using total body RGS2-deficient mice, we used a kidney crosstransplantation strategy to examine separately the contributions of RGS2 actions in the kidney from those in extrarenal tissues with regard to BP regulation. Loss of renal RGS2 was sufficient to cause hypertension, whereas the absence of RGS2 from all extrarenal tissues including the peripheral vasculature did not significantly alter BP. Accordingly, these results suggest that RGS2 acts within the kidney to modulate BP and prevent hypertension. These data support a critical role for the renal epithelium and/or vasculature as the final determinants of the intra-arterial pressure in hypertension.


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The role of G protein-coupled receptors (GPCRs) in hypertension and cardiovascular diseases is well established.1 Moreover, pharmacologic antagonists of GPCRs, such as β-adrenergic and angiotensin receptors, are cornerstones of therapy in the treatment of hypertension and its complications.2 Signaling by GPCRs is triggered by ligand-induced conformational changes in the receptor that promote exchange of guanosine 5’-diphosphate for guanosine 5’-triphosphate on the Gα subunit of the G protein complex,3 followed by dissociation of Gα from the Gβγ dimer. The dissociated subunits can then interact with effector molecules to propagate the signal. The duration and intensity of signaling are further regulated by GTPase-activating proteins.4 The regulators of G protein signaling (RGSs) are a family of proteins with GTPase-activating protein activity.4 Among these, RGS2 displays regulatory selectivity for the Gαq subclass of G proteins.5 Many key cardiovascular hormones such as angiotensin II, endothelin-1, thromboxane A2, and norepinephrine activate receptors that couple to Gαq. A specific role for RGS2 in maintaining normal vascular tone and BP was established using genetically modified mice.6,7 RGS2-deficient mice have hypertension6,7 along with abnormal vascular contraction and relaxation responses.7 In addition to its actions to influence the contractile state of vascular smooth muscle, regulated expression of RGS2 has been described in other tissues that are important for BP regulation including the central nervous system8 and the kidney.9 Here, we use a kidney crosstransplantation strategy to distinguish contributions of RGS2 actions in the kidney from extrarenal tissues to the regulation of BP and the development of hypertension. Our studies indicate that RGS2 effects within the kidney are critical for regulation of BP, suggesting that altered renal epithelial and/or vascular functions are responsible for hypertension in this genetic model.

To determine the relative contributions of RGS2 in renal versus extrarenal tissues to the pathogenesis of hypertension, we used a kidney crosstransplantation strategy. By varying the genotype of the transplant donor and recipient, we generated four groups of animals in which renal function was provided entirely by the single transplanted kidney. The wild-type group consisted of wild-type mice transplanted with kidneys from wild-type donors, having normal expression of RGS2 in the kidney transplant and in all systemic tissues. For the systemic knockout (KO) group, RGS2-deficient recipients were transplanted with kidneys from wild-type donors; these animals lack RGS2 in all tissues except the kidney. Kidney KO animals are wild-type recipients of RGS2-deficient kidneys lacking expression of RGS2 only in renal parenchyma and vasculature but with normal expression of receptors in the peripheral vasculature. Using total body RGS2-deficient mice, we used a kidney crosstransplantation strategy to examine separately the contributions of RGS2 actions in the kidney from those in extrarenal tissues with regard to BP regulation. Loss of renal RGS2 was sufficient to cause hypertension, whereas the absence of RGS2 from all extrarenal tissues including the peripheral vasculature did not significantly alter BP. Accordingly, these results suggest that RGS2 acts within the kidney to modulate BP and prevent hypertension. These data support a critical role for the renal epithelium and/or vasculature as the final determinants of the intra-arterial pressure in hypertension.


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all systemic, nonrenal tissues including peripheral vessels. Finally, the total KO group consists of RGS2-deficient recipients of RGS2-deficient kidneys and therefore completely lacks RGS2 in all tissues.

The absence of RGS2 did not significantly affect the normal diurnal variation in BP in any of the groups (Figure 1). Among the transplanted animals, mean systolic BP levels for the period of baseline recording in the wild-type group (123 ± 2 mmHg; n = 7) were in a range similar to previous measurements in nontransplanted, wild-type C57BL/6 mice, supporting our previous observations that the surgical procedure and the presence of only a single transplanted kidney do not significantly alter baseline levels of BP. By contrast (Figure 2), BP levels were significantly increased in the total KO animals completely lacking RGS2 (129 ± 2 mmHg; n = 6) compared with the wild-type controls (P = 0.04). Thus, elimination of RGS2 in all tissues in the total KO group recapitulates the original phenotype of elevated BP described in Rgs2−/− mice.6–11

BP levels in the systemic KO group (120 ± 3 mmHg; n = 6) were not different from wild-type controls. Thus, deletion of RGS2 from all extrarenal tissues including the central nervous system and peripheral vasculature is not sufficient to cause hypertension. On the other hand, BP levels in the kidney KO group (131 ± 3.0 mmHg; n = 7) were significantly increased compared with the wild-type controls (P = 0.046) and comparable with those of the total KO group. This finding is consistent with the view that the kidney is a major determinant of the chronic level of BP and indicates that the absence of signaling pathways linked to RGS2 in the kidney and its vasculature is sufficient to increase BP. The patterns of BP differences between the groups were similar when daytime and nighttime BPs were examined separately (not shown). Furthermore, feeding a high-salt (6% NaCl) diet for 7 days did not significantly affect BP in any of the groups except the total KO group, in which an increase in BP from 131 ± 3 mmHg on the regular (0.4% NaCl) diet to 137 ± 11 mmHg on the high-salt diet was observed, which approached statistical significance (P = 0.0503).

At the end of the studies, kidneys and hearts were harvested, and organ weights were determined. As shown in Table 1, whereas the heart-to-body weight ratio was numerically highest in the kidney KO group, there were no significant differences across the four groups. Thus, the relatively modest differences in BP between the groups did not generate appreciable differences in heart weight. Likewise, there was no systematic evidence of cardiac hypertrophy in the Rgs2−/− recipients, which had experienced life-long RGS2 deficiency before transplantation.

Because the renin-angiotensin system (RAS) is a key regulator of BP homeostasis and Gq-linked GPCRs may influence responsiveness to angiotensin II, we measured mRNA expression of renin, a key rate-limiting enzyme regulating the activity of the RAS. As shown in Figure 3, renin mRNA levels in the transplanted kidneys were not significantly different between the groups. In particular, there was no evidence for enhanced renin expression in the kidneys of the kidney KO and total KO groups with the highest BP, indicating that activation of the systemic RAS was not a mechanism driving the elevated BP in these groups.

Table 1. Organ weights in transplant groups

<table>
<thead>
<tr>
<th>Transplant Group</th>
<th>Body Weight (g)</th>
<th>Kidney Weight (mg)</th>
<th>Heart Weight (mg)</th>
<th>Kidney Weight/BODY Weight (mg/g)</th>
<th>Heart Weight/BODY Weight (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>26.7 ± 0.6</td>
<td>201.4 ± 8</td>
<td>126.0 ± 5</td>
<td>7.6 ± 0.3</td>
<td>4.7 ± 0.1</td>
</tr>
<tr>
<td>Systemic KO</td>
<td>27.7 ± 1.6</td>
<td>262.9 ± 11</td>
<td>137.1 ± 9</td>
<td>9.1 ± 0.6*</td>
<td>4.9 ± 0.1</td>
</tr>
<tr>
<td>Kidney KO</td>
<td>30.5 ± 0.8</td>
<td>263.8 ± 29</td>
<td>196.1 ± 34</td>
<td>8.7 ± 1.1</td>
<td>6.5 ± 1.3</td>
</tr>
<tr>
<td>Total KO</td>
<td>27.3 ± 0.9</td>
<td>215.2 ± 11</td>
<td>139.2 ± 6</td>
<td>7.9 ± 0.3</td>
<td>5.1 ± 0.1</td>
</tr>
</tbody>
</table>

*P = 0.03 compared with wild-type.
RGS2 in BP control, kidney transplantation was carried out between genetically matched C57BL/6 wild-type and RGS2-deficient mice homozygous for a targeted disruption of the Rgs2 gene locus.23 Except for the presence or absence of RGS2, the donors and recipients are genetically identical, so there is no rejection and no need for immunosuppressive therapy. The major finding in our study is that the pool of RGS2 in the kidney is required for maintenance of normal systemic BP. This is clearly illustrated in the kidney KO group animals, in which the lack of RGS2 only in the kidney and its vasculature is sufficient to recapitulate the phenotype of hypertension seen in Rgs2+/− mice with global deficiency of RGS2. Conversely, the systemic KO group has normal BP despite the absence of RGS2 from extrarenal tissues, including key areas that potentially affect BP homeostasis including the brain, the heart, the peripheral vasculature, and the adrenal gland. In this case, providing normal levels of RGS2 expression at key sites within the kidney rescues the hypertensive phenotype.

Although these studies strongly support the importance of renal RGS2 in maintaining normal levels of BP, they are inadequate to distinguish the precise functions of RGS2 responsible for producing this hypertensive phenotype. Specifically, these experiments cannot distinguish between actions of RGS2 to modulate renal epithelial functions versus regulation of vasomotor tone in the renal vasculature. RGS2 is highly expressed within the kidney in a number of cell types including epithelium and vascular smooth muscle cells. The actions of GPCRs coupled to Gαq such as type 1 angiotensin receptors affect fluid and solute reabsorption by epithelial cells along the nephron, thereby modulating BP.22 RGS2 may act to attenuate these effects and promote natriuresis. In addition, GPCRs expressed along the renal vasculature regulate renal blood flow and thereby have secondary effects to influence renal sodium handling.24 For example, renal vasconstriction caused by angiotensin II reduces medullary blood flow, thus blunting the kidney’s excretory capacity for sodium.25 RGS2 would attenuate these actions, and these effects may be exaggerated in RGS2-deficient mice, potentially promoting hypertension. Therefore, RGS2 in the kidney may affect BP through direct actions on epithelial function and/or renal vascular resistance, and abrogation of these actions causes hypertension. However, with our current data, we cannot dissect which of these precise compartments plays the dominant role or the exact mechanism(s) involved. Future studies will address these questions.

**CONCISE METHODS**

**Experimental Animals**

A null mutation in the Rgs2 gene23 was backcrossed onto the C57BL/6 genetic background, and inbred C57BL/6-Rgs2+/− and −/− male mice were used as kidney transplant donors and recipients. The experimental procedures described below were approved by the respective IACUCs of the Durham Veterans Affairs and Duke University Medical Centers.

**Renal Crosstransplantation in RGS2-deficient Mice**

Transplantation of a single mouse kidney with bilateral native nephrectomy was performed as we have described previously.10 Overall surgical mortality was approximately 20%.

**Measurement of BP in Conscious Mice**

We used a radiotelemetry system (Data Sciences International/Transoma Medical, St. Paul, MN) to monitor BP in conscious mice as described previously.10 The pressure-sensing catheter was implanted via the left carotid artery as described,10 6 to 8 days after transplantation. The mice were allowed to recover for 7 days after surgery to regain their normal circadian rhythms before BP measurements were initiated; the mice were housed in a separate light cycle-controlled “monitoring” room in the animal facility where quiet is maintained and no other activities are permitted. The BP data were collected continuously with sampling every 5 minutes at 10-second intervals26 during the prescribed time periods.

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**Figure 3.** There are no significant differences in renin mRNA expression in transplanted kidneys among the experimental groups.
using Dataquest A.R.T. software (Data Sciences International/Transoma Medical, St. Paul, MN).

Experimental Protocol
Baseline BPs were measured on 10 consecutive days while the animals ingested a conventional diet containing 0.4% sodium chloride. BP response to a high-sodium diet (6% sodium chloride; Harlan Teklad, Madison, WI) was also assessed over a 1-week period. The animals were sacrificed 6 weeks after the initial surgery, and the hearts and kidneys were removed, weighed, and snap frozen in liquid nitrogen.

RNA Isolation and Analysis
Total RNA was extracted from the transplanted kidneys of mice from each group (RNEasy; Qiagen) using standard techniques. Renin mRNA levels were measured using real-time quantitative PCR, which was performed using the fluorogenic 5′-exonuclease assay with primers and dual-labeled probe (5′-FAM and 3′-TAMRA) on the basis of previously published sequences as described previously. Gene expression was quantified using the ΔΔCt method for relative quantitation.

Statistical Analysis
The values for each parameter within a group are expressed as the means ± the SEM. For comparisons between groups, statistical significance was assessed using a t test or ANOVA followed by Tukey’s test for multiple comparisons. A paired t test was used for comparisons within groups.

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DISCLOSURES
None.