Renin–Angiotensin System and Y Chromosome

Origin of the Y Chromosome Influences Intrarenal Vascular Responsiveness to Angiotensin I and Angiotensin (1-7) in Stroke-Prone Spontaneously Hypertensive Rats


Abstract—The lineage of the Y chromosome accounts for up to 15 to 20 mm Hg in arterial pressure. Genes located on the Y chromosome from the spontaneously hypertensive rat (SHR) are associated with the renin–angiotensin system. Given the important role of the renin–angiotensin system in the renal regulation of fluid homeostasis and arterial pressure, we hypothesized that the origin of the Y chromosome influences arterial pressure via interaction between the intrarenal vasculature and the renin–angiotensin system. Sixteen-week-old normotensive rats (Wistar Kyoto [WKY]), spontaneously hypertensive stroke-prone rat (SHRSP), and 2 reciprocal Y consomic rat strains, 1 comprising the WKY autosomes and X chromosome with the Y chromosome from the hypertensive rat strain (WKY.SP$_{60}$Y) and vice versa (SP.WKY$_{60}$Y), were examined. SP.WKY$_{60}$Y had lower systolic blood pressure than SHRSP (195±5 versus 227±8 mm Hg; *P*<0.03), whereas WKY.SP$_{60}$Y had higher systolic blood pressure compared with WKY (157±3 versus 148±3 mm Hg; *P*<0.05), measured by radiotelemetry. Compared with WKY rats, SHRSP had higher plasma angiotensin(I-7) (Ang (1-7)):Ang II ratio (WKY: 0.13±0.01 versus SHRSP: 1.33±0.4; *P*<0.005), greater angiotensin II receptor type 2 and Mas receptor mRNA expression, and a blunted renin constrictor response to intrarenal Ang I and Ang(1-7) infusions. Introgenesis of the normotensive Y chromosome into the SHRSP background (SP.WKY$_{60}$Y) restored responses in the SHRSP to WKY levels, evidenced by a reduction in plasma Ang(I-7):Ang II ratio (SP.WKY$_{60}$Y: 0.24±0.02; *P*<0.01), angiotensin II receptor type 2, and Mas receptor mRNA expression and an increased vasoconstrictor response to intrarenal Ang I and Ang(I-7) infusion. This study demonstrates that the origin of the Y chromosome significantly impacts the renal vascular responsiveness and therefore may influence the long-term renal regulation of blood pressure. (Hypertension. 2014;64:1376-1383.)

Key Words: gene expression ■ hypertension ■ rats ■ rats, inbred SHR ■ renal circulation ■ renin-angiotensin system

Sex-specific differences in blood pressure regulation and renal function are well documented, with men reported to have higher arterial pressure compared with women.1,2 The mechanisms regulating arterial pressure in men are multifactorial.3,4 In fact, the genetic lineage from which the Y chromosome originates, whether it is inherited from a hypertensive or normotensive father, plays an integral role.5,6 The lineage of the Y chromosome accounts for up to 15 to 20 mm Hg in arterial pressure. Genes located on the Y chromosome from the spontaneously hypertensive rat (SHR) are associated with the renin–angiotensin system. Given the important role of the renin–angiotensin system in the renal regulation of fluid homeostasis and arterial pressure, we hypothesized that the origin of the Y chromosome influences arterial pressure via interaction between the intrarenal vasculature and the renin–angiotensin system. Sixteen-week-old normotensive rats (Wistar Kyoto [WKY]), spontaneously hypertensive stroke-prone rat (SHRSP), and 2 reciprocal Y consomic rat strains, 1 comprising the WKY autosomes and X chromosome with the Y chromosome from the hypertensive rat strain (WKY.SP$_{60}$Y) and vice versa (SP.WKY$_{60}$Y), were examined. SP.WKY$_{60}$Y had lower systolic blood pressure than SHRSP (195±5 versus 227±8 mm Hg; *P*<0.03), whereas WKY.SP$_{60}$Y had higher systolic blood pressure compared with WKY (157±3 versus 148±3 mm Hg; *P*<0.05), measured by radiotelemetry. Compared with WKY rats, SHRSP had higher plasma angiotensin(I-7) (Ang (1-7)):Ang II ratio (WKY: 0.13±0.01 versus SHRSP: 1.33±0.4; *P*<0.005), greater angiotensin II receptor type 2 and Mas receptor mRNA expression, and a blunted renin constrictor response to intrarenal Ang I and Ang(1-7) infusions. Introgenesis of the normotensive Y chromosome into the SHRSP background (SP.WKY$_{60}$Y) restored responses in the SHRSP to WKY levels, evidenced by a reduction in plasma Ang(I-7):Ang II ratio (SP.WKY$_{60}$Y: 0.24±0.02; *P*<0.01), angiotensin II receptor type 2, and Mas receptor mRNA expression and an increased vasoconstrictor response to intrarenal Ang I and Ang(I-7) infusion. This study demonstrates that the origin of the Y chromosome significantly impacts the renal vascular responsiveness and therefore may influence the long-term renal regulation of blood pressure. (Hypertension. 2014;64:1376-1383.)

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The stroke-prone spontaneously hypertensive rat (SHRSP) is a model of essential hypertension displaying similar features to the human condition. To investigate the role of the Y chromosome, we have developed 2 consomic strains of rat, 1 with the normotensive Wistar Kyoto (WKY) autosomes and X chromosome in which the SHRSP Y chromosome has been introgressed (WKYSYSPm_Y), and vice versa (SP.WKYGlaY). Given the important role of the RAS in renal regulation of blood pressure and vascular tone together with in vitro evidence that the Y chromosome interacts with the RAS, we hypothesize that the origin of the Y chromosome influences blood pressure regulation via the intrarenal vascular responsiveness to RAS stimulation.

Methods

All studies were conducted in accordance with the United Kingdom Home Office regulations and the National Health and Medical Research Institute Animal Welfare Committee guidelines and were approved by the Alfred Medical Research and Education Precinct ethics committee (Approval No: E/1090/2011/B). From weaning, rats were maintained on normal rat chow (standard rat diet, 0.8% NaCl, Special Diet Services, United Kingdom, and Specialty Feeds, Australia) and water ad libitum.

Consomic Cross

The reciprocal consomic strains of rat studied were derived as described previously. One strain comprised the WKY autosomes and X chromosome with the SHRSP Y chromosome introgressed (WKYSYSPm_Y), and the other strain comprised the SHRSP autosomes and X chromosome with the WKY Y chromosome introgressed (SP.WKYGlaY).

Experimental Protocol

At 16 weeks of age, rats were anesthetized with isoflurane (4% in O2) and a radiotelemetry probe (C40, Data Sciences International) was chronically implanted into the abdominal aorta for measurements of arterial pressure variables into WKY, WKYSYSPm_Y, SP.WKYGlaY, and SHRSP. After 1 week recovery, 2 weeks of recordings were made for 10 seconds every 10 minutes which were analyzed as weekly 12-hour averages.

In Vivo Renal Function

In vivo renal function tests were performed in a separate cohort of 16-week-old rats. Briefly, rats were anesthetized with thiobutabarbital (150 mg/kg IP, Inactin, Sigma-Aldrich, Australia), and the left jugular vein and left carotid artery were catheterized for intravenous infusions and arterial pressure measurements via a Biopac MP100 data-acquisition system, respectively. Animals received a continuous infusion containing BSA (2%, Sigma), inulin (7.5%, Inuest, Fresenius Kabi, Germany), and para-aminohippuric acid (1.5%, Sigma). The bladder was cannulated for urine collections. After a 45-minute equilibration period, a timed 30-minute urine collection began with a blood sample taken at the 15-minute mark via the carotid artery. Urine volume was measured gravimetrically.

Renal Function Calculations

Blood samples and urine samples were analyzed for inulin and para-aminohippuric acid which were determined by spectrophotometry. Glomerular filtration rate and effective renal plasma flow (eRPF) were calculated using the following equations: glomerular filtration rate=urinary concentration of inulin×urine flow rate/plasma inulin concentration, eRPF=urinary concentration of para-aminohippuric acid×urine flow rate/plasma para-aminohippuric acid concentration.

Intrarenal Infusions of Ang I, Ang II, and Ang (1-7)

The carotid artery and jugular vein were catheterized for blood pressure measurements and infusion of BSA (2%, Sigma), respectively. For direct drug infusion into the left renal artery, a catheter was inserted into the left femoral artery and advanced ≈3 mm into the left renal artery. The left renal artery was cleared of perivascular fat, and a 0.5 V Transonic renal flow probe (Transonic Systems, Ithaca, NY) was placed around the renal artery distal to the catheter for measurement of RBF in real time. Recordings were made using a TS420 peripheral flowmeter (Transonic Systems, Ithaca, NY). After 30-minute equilibration, intrarenal graded bolus doses of Ang I (1–100 ng/kg), Ang II (1–100 ng/kg), or Ang (1–7) (1–100 ng/kg) were administered in increasing concentrations. The peptides were administered in random order with a 10-minute recovery period between doses and a 20-minute washout period between peptides. Responses are presented as the maximum reduction in RBF (percentage change) from baseline.

mRNA Gene Expression

Total RNA was extracted from whole kidney and renal artery using RNeasy kits (Qiagen) as described previously. Normalization was confirmed by performing real-time polymerase chain reaction (Taqman, Applied Biosystems) of Actb (β-actin; Promega) with comparable threshold cycles. Taqman predesigned assays (Applied Biosystems) for ACE (Rn00561094_m1), ACE2 (Rn01416293_m1), angiotensin II receptor type 1a (Rn02750772_s1), and angiotensin II receptor type 2 (AT2R, Rn00560677_s1), and Mas receptor (MasR, Rn00562673_s1) were multiplexed with Actb (4352340E-labeled VIC). Gene expression for each gene of interest relative to Actb in each sample was derived using the comparative (∆∆CT) threshold change method. Briefly, an average change in cycle threshold (∆CT) value was obtained for each sample run in triplicate. The average ∆CT for the calibrator group (in this case the WKY group) was subtracted from each sample to generate a ∆∆CT which was then substituted into the equation 2−<sup>ΔΔCT</sup>. This transforms the raw data to a relative gene expression format, where each sample is expressed relative to the calibrator group.

Plasma Ang II and Ang (1-7) Concentration and ACE and ACE2 Activity

Plasma samples were collected from centrifuged heparinized blood samples (40 U/mL) and treated with an endopeptidase inhibitor cocktail containing 0.2 mol N-ethylmaleimide, 50 mmol Na3EDTA, and 2 trypsin inhibitor units/mL aprotinin. Plasma Ang II and Ang (1–7) were measured using a commercial radioimmunoassay (ProSearch International, Malvern, Australia). Plasma ACE and ACE2 activity in samples collected from centrifuged heparinized blood samples (40 U/mL) were determined as previously described. Briefly, ACE activity was determined after incubation with the intramolecularly quenched synthetic ACE-specific substrate o-aminobezoic acid-FRK(Dnp)-P (Peptides International, Inc, Kentucky). Plasma ACE2 activity was determined after incubation with the intramolecularly quenched synthetic ACE2 substrate o-aminobezoic acid-Ser-Pro-Tyr(NO)<sub>2</sub>-OH (Peptides International, Inc, Kentucky). Five microliters of plasma was incubated at 37°C for 1 to 18 hours with 95 μL of Tris (100 mmol/L; pH. 6.5), NaCl (1.5 mmol/L), phenylmethanesulfonyl fluoride (1 mmol/L); N-ethylmaleimide (1 mmol/L), 2 μg BSA, and 33 μmol/L of either ACE or ACE2 substrate. A blank well was run for each sample which comprised all components as the testing wells with the addition of 1 mmol/L EDTA to inhibit any ACE or ACE2 activity. Fluorescence was measured using an F-Max plate fluorescence reader with an excitation of 355 nm and emission of 450 nm. Blank well fluorescence was subtracted from each sample.

Statistical Analysis

Data are presented as mean±SEM. Systolic arterial pressure and RBF responses to intrarenal infusions were analyzed using a repeated measures 1-way ANOVA with the factor strain (P<0.05). Body weights, kidney weights, gene expression, eRPF, and glomerular filtration rate were analyzed using a 1-way ANOVA (using the factor strain; P<0.05) with Tukey post hoc test to account for multiple comparisons. Statistical significance was accepted at P≤0.05.

Results

As expected, systolic and diastolic blood pressure were higher in the SHRSP compared with the WKY (P<0.05; Figure 1).
Hypertension

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Introgression of the SHRSP Y chromosome into the WKY rats (WKY .SPGlaY) resulted in higher systolic and diastolic blood pressure compared with WKY, whereas introgression of the WKY Y chromosome into SHRSP rats (SP.WKYGlaY) resulted in significantly lower blood pressure compared with SHRSP (P <0.05; Figure 1). Heart rate was similar in all strains (Figure 1).

Body weights were higher and kidney weights not different in the WKY and WKY.SPGlaY compared with both SHRSP and SP.WKYGlaY (Table). Kidney:body weight ratio was therefore significantly lower in the WKY and WKY.SPGlaY strains than the SHRSP and SP.WKYGlaY animals. Renal function was not influenced by the origin of the Y chromosome. Glomerular filtration rate and eRPF were higher in WKY and WKY.SPGlaY compared with SHRSP and SP.WKYGlaY (Table).

To determine whether the Y chromosome–dependent upregulation of RAS gene promoter activity reported in vitro has functional consequences in vivo, we quantified (1) renal RAS gene expression (Figure 2), (2) plasma Ang II and Ang (1-7) levels (Figure 3), and (3) plasma ACE and ACE2 activity (Figure 3). Total kidney ACE mRNA gene expression was greater in the SHRSP compared with the WKY with no differences in expression between the consomics and their parental strain, demonstrating that renal ACE mRNA expression was not Y chromosome dependent. Renal gene expression of ACE2 and nephrilysin was similar in all strains (Figure 2). In contrast, expression of angiotensin II receptor type 1a, AT2R, and MasR were all significantly higher in whole kidney homogenate from SHRSP compared with WKY, and introgression of the WKY Y chromosome into SHRSP rats reduced expression by >50% (Figure 2). Expression levels of angiotensin II receptor type 1a, AT2R, and MasR were similar in the WKY and WKY.SPGlaY animals (Figure 2).

Expression of ACE mRNA in the renal artery was similar in all strains (Figure 2). In contrast to the in vitro findings, ACE2 expression in the renal artery of SHRSP was greater than WKY (11.4±4 versus 1.1±0.3; P<0.01; Figure 2). Introgression of the WKY Y chromosome into SHRSP background (SP.WKYGlaY) reduced ACE2 expression to levels similar to the WKY strain, suggesting that the higher ACE2 expression in the SHRSP is Y chromosome dependent. Nephrilysin gene expression in the renal artery was similar in all strains (Figure 2). Although we observed no difference in angiotensin II receptor type 1a or MasR expression in the renal artery between strains, AT2R expression in the renal artery was significantly greater in the SHRSP compared with the WKY (Figure 2) and influenced by the origin of the Y chromosome as evidenced by a 60% reduction in AT2R expression in the SP.WKYGlaY compared with SHRSP.

We observed a greater ratio of Ang (1-7):Ang II in the plasma of SHRSP compared with WKY (1.33±0.4 versus 0.13±0.01, respectively; Figure 3; P<0.01), attributable to both a lower Ang II and higher Ang (1-7) concentration in the SHRSP. Introgression of the Y chromosome from the WKY into the SHRSP significantly reduced the Ang (1-7):Ang II ratio to WKY levels (SP.WKYGlaY: 0.24±0.02; Figure 3), suggesting that the elevated ratio in the SHRSP is Y chromosome dependent. There was no

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**Table.** Body Weights, Kidney Weights, Glomerular Filtration Rate, and Effective Renal Plasma Flow (n≥6 Per Group)

<table>
<thead>
<tr>
<th>Strain</th>
<th>BW, g</th>
<th>KW, g</th>
<th>KW/BW, g/kg</th>
<th>GFR, mL·min⁻¹·g⁻¹·KW</th>
<th>eRPF, mL·min⁻¹·g⁻¹·KW</th>
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<tr>
<td>WKY</td>
<td>350.6±11.7</td>
<td>2.02±0.06</td>
<td>5.79±0.2</td>
<td>1.03±0.12</td>
<td>4.26±0.6</td>
</tr>
<tr>
<td>WKY.SPGlaY</td>
<td>332.4±3.6</td>
<td>1.88±0.08</td>
<td>5.66±0.25</td>
<td>1.08±0.08</td>
<td>3.94±0.5</td>
</tr>
<tr>
<td>SP.WKYGlaY</td>
<td>263.3±9.3</td>
<td>2.33±0.13</td>
<td>8.47±0.46</td>
<td>0.77±0.09</td>
<td>2±0.3</td>
</tr>
<tr>
<td>SHRSP</td>
<td>280.4±7.7</td>
<td>2.39±0.08</td>
<td>8.53±0.13</td>
<td>0.66±0.05</td>
<td>1.63±0.3</td>
</tr>
</tbody>
</table>

All data are presented as mean±SEM. BW indicates body weight; GFR, glomerular filtration rate; KW, kidney weight; SHRSP, spontaneously hypertensive stroke-prone rats; and WKY, Wistar Kyoto.

*P<0.05, †P<0.01, ‡P<0.001 compared with WKY.
Activity of ACE and ACE2 in the WKY was higher than all other strains, with no difference in activity levels among WKY SP_{GlaY}, SP.WKYGlaY, or SHRSP (Figure 3). There was no difference in the RBF response to Ang I between WKY and WKY SP_{GlaY} at 1, 3, 10, or 30 ng/kg. However, the vasoconstrictor response to the highest dose (100 ng/kg) was greater in the WKY SP_{GlaY} group compared with the WKY group (RBF reduction of 34.1±11.9% versus 16.6±2.8%; P=0.05; Figure 4). SHRSP were markedly less sensitive to the intrarenal bolus dose of Ang I, we saw the greatest reduction in RBF at the lowest dose (1 ng/kg) with each higher dose resulting in a smaller reduction in RBF in all strains (Figure 4). Given the important role of the renal RAS in the regulation of blood pressure and the observed Y chromosome–dependent RAS gene expression in the kidney, we hypothesized that the vascular responsiveness to intrarenal RAS stimulation in the SHRSP would be Y chromosome dependent. In response to...
the vasoconstrictor effects of Ang I compared with WKY, with significantly less vasoconstriction at 1 (50.8±8.5% versus 88.4±5.9%; \(P=0.014\)), 3 (27.6±8.7% versus 80.5±5.9%; \(P=0.005\)), 10 (17.5±6.4% versus 58.2±6.4%; \(P=0.040\)) and 30 ng/kg (9.4±2.2% versus 32.2±5.3%; \(P=0.003\); Figure 4). We observed a significant increase in Ang I–induced vasoconstriction in the SP.WKYGlaY compared with the SHRSP at all doses with responses in the SP.WKYGlaY rats similar to both WKY and WKY.SPGlaY at 1, 3, 10, or 30 ng/kg (Figure 4). Next, we examined whether the attenuated vasoconstrictor response to Ang I in SHRSP was attributable to altered sensitivity to the effector peptides Ang II or Ang (1-7). We observed a dose-dependent decrease in RBF in response to Ang II which was similar in all strains demonstrating that the blunted constrictor response to Ang I in the SHRSP is not attributable to altered sensitivity to the constrictor effects of Ang II (Figure 4). Interestingly, vasoconstrictor responses to intrarenal Ang (1-7) closely resembled Ang I responses with the greatest constrictor response at the lowest dose with blunted constriction observed in response to increasing Ang (1-7) concentrations (Figure 4). Similar to Ang I responses, we observed little to no change in RBF in the SHRSP compared with all other strains. Notably, introgression of the WKY Y chromosome into the SHRSP background (SP.WKY\textsubscript{GlaY}) improved vasoconstrictor responses to WKY levels (Figure 4). Taken together, these data demonstrate that the blunted vasoconstrictor response to Ang I in the SHRSP is Y chromosome dependent and is likely mediated by a blunted constrictor response to Ang (1-7).

**Discussion**

The contribution of the sex chromosomes to the regulation of arterial pressure was highlighted by Ji et al\textsuperscript{25} in studies which dissected the contribution of the sex chromosome complement (XX or XY) from the contribution of the sex hormones. Ji et al\textsuperscript{25} concluded that there are indeed sex chromosome–dependent effects in arterial pressure regulation, in particular the arterial response to chronic systemic Ang II infusion. It is now well recognized that the Y chromosome is significantly associated with blood pressure regulation in humans\textsuperscript{4,5} and animal models of essential hypertension such as the SHR.\textsuperscript{6,26} Our study presents the novel finding that the origin of the Y chromosome influences circulating Ang II and Ang (1-7) levels, renal RAS receptor expression, and renal responsiveness to RAS stimulation.

We observed a greater Ang (1-7):Ang II ratio in SHRSP compared with WKY, consistent with previous reports of greater plasma Ang (1-7) concentration in hypertensive compared with normotensive rats.\textsuperscript{27,28} These data suggest that the SHRSP have a paradoxical upregulation of the vasodilator arm of the RAS. To confirm this upregulation of the vasodilator arm of the RAS, we hypothesized that the SHRSP would have a blunted intrarenal vasoconstrictor response to Ang I. Indeed, the response to intrarenal Ang I was significantly attenuated in SHRSP compared with WKY. To determine whether the blunted responsiveness was mediated by decreased vascular sensitivity to Ang II and increased vascular sensitivity to Ang

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**Figure 3.** Plasma angiotensin II (Ang II) and Ang (1-7) concentration, angiotensin-converting enzyme (ACE), and ACE2 activity in Wistar Kyoto (WKY), WKY.SPGlaY, spontaneously hypertensive stroke-prone rats (SHRSP), and SP.WKY\textsubscript{GlaY} (\(n\geq5/strain\)). Data are presented as mean±SEM with *\(P<0.05\), **\(P<0.01\) compared with WKY; #\(P<0.05\), ##\(P<0.01\) compared with SHRSP.

**Figure 4.** Renal blood flow (RBF) responses to intrarenal angiotensin I (Ang I), Ang II and Ang (1-7) in (A) Wistar Kyoto (WKY; ●) and WKY.SPGlaY (○) and (B) spontaneously hypertensive stroke-prone rats (SHRSP; ▲) and SP.WKY\textsubscript{GlaY} (Δ), \(n\geq6/strain\). Data are presented as mean±SEM. *\(P<0.05\), **\(P<0.01\) compared with WKY; #\(P<0.05\), ##\(P<0.01\), ###\(P<0.005\) compared with SHRSP.
(1-7), we quantified reductions in RBF in response to graded intrarenal doses of Ang II and Ang (1-7). The reductions in RBF in response to Ang II in the WKY rats were consistent with previous reports. Although we observed no difference in response to Ang II in any strain, the RBF responses to Ang (1-7) were strikingly similar to responses observed to Ang I, suggesting that the RBF responses to intrarenal Ang I in all strains are largely mediated by the conversion of Ang I to Ang (1-7). Indeed, our findings are in agreement with the growing body of work reporting that renal metabolism of Ang I to Ang (1-7) is greater than Ang I to Ang II. Consistent with previous work, we observed an increase in renal AT2R and MasR expression in SHRSP compared with WKY, which may suggest that the blunted vasoconstrictor response to Ang (1-7) in the SHRSP may be explained, in part, by the increased AT2R and MasR gene expression in the SHRSP compared with WKY.

Importantly, this study provides novel evidence that introgression of the WKY Y chromosome into the SHRSP background (SP.WKY<sub>Gla</sub>Y) is sufficient to restore the responsiveness of the renal circulation to the vasoconstrictive effects of intrarenal Ang I and Ang (1-7) infusion to WKY levels. Similarly, expression of the AT2R and MasR was reduced in the SP.WKY<sub>Gla</sub>Y compared with the SHRSP. Although we observed an increase in systolic blood pressure in the WKY.S<sub>Gla</sub>Y consistent with previous reports, we did not observe any striking differences in renal function or RBF when compared with the WKY strain. There was also no difference in plasma Ang II levels between WKY.S<sub>Gla</sub>Y and WKY, in contrast to the finding that transient overexpression of Sry3 in the kidney of WKY increased renal Ang II content. This highlights 2 important points. First, that RAS responses to transient tissue-specific upregulation of the Sry3 are not indicative of long-term upregulation, and second, that circulating levels of Ang II do not always reflect renal levels, as has been previously shown. “It is possible that the normotensive WKY background strain has a greater ability to compensate for any changes that may occur because of the introgression of the Y chromosome from the hypertensive strain.” In any case, these data suggest that the increase in arterial pressure in the WKY.S<sub>Gla</sub>Y compared with WKY is not mediated by the renal RAS.

There are 3 possible explanations for the Y chromosome–dependent blunted constrictor response to Ang I in the SHRSP. First, the blunted constriction may be a consequence of increased basal vascular tone in the SHRSP, evidenced by the lower eRPF in SHRSP compared with the WKY. The greater renal vascular tone in SHRSP is not surprising given the well-described endothelial dysfunction and reduced RBF reported previously in this model. However, this explanation seems unlikely for 2 reasons: (1) we observed no difference in the vasoconstrictor response to Ang II in the SHRSP despite increased basal vascular tone and (2) renal vasoconstrictor responses to Ang (1-7) were greater in the SP.WKY<sub>Gla</sub>Y compared with the SHRSP despite a similar level of basal vascular tone in both strains. Second, it is possible that the conversion of Ang I to Ang (1-7) in the SHRSP is greater than in all other strains resulting in a higher intrarenal Ang (1-7) and lower Ang II concentration. Although the data do not definitively discount this hypothesis, the renal ACE and ACE2 mRNA expression and circulating ACE activity and ACE2 activity profiles do not support an upregulation of the enzymes critical for this conversion. In addition, the similarity between the intrarenal constrictor responses to Ang I and Ang (1-7) suggest that the responses to Ang I were mediated by the production of Ang (1-7) in all strains, not only the SHRSP. The third and most likely explanation is that the blunted response in the SHRSP is mediated by increased vasodilatory receptor activation, namely activation of the AT2R and MasR. Indeed, we observed a greater expression of the AT2R and MasR in the kidney and AT2R in the renal artery compared with the WKY. In addition, the restored vasoconstrictor response in the SP.WKY<sub>Gla</sub>Y was associated with a lower expression of the AT2R and MasR receptor compared with SHRSP, to levels similar to the WKY, further supporting this hypothesis.

Although we did observe an increase in ACE mRNA expression in the SHRSP in the current study, this was not Y chromosome dependent as has been reported in vitro. Given that the ACE promoter contains ≥2 shear stress responsive elements as well as 2 cAMP response elements which also regulate gene promoter activity, it is not surprising that the activity of the ACE promoter in vivo is different to the in vitro activity. In the in vivo setting, multiple genetic and environmental factors contribute to ACE promoter activity, particularly in the SHRSP where shear stress is likely to be a main contributor. ACE2 gene and protein expression has been previously shown to be similar in SHRSP to WKY consistent with the current study but in contrast to the increase in ACE2 gene promoter activity reported by Milsted et al. Therefore, our in vivo evidence demonstrates that ACE and ACE2 gene expression and activity are not dependent on the lineage of the Y chromosome.

Identification of candidate genes located on the Y chromosome from the SHRSP that directly or indirectly mediate the renal RAS is essential for the translation of these findings into humans. We therefore await the completion of the rat Y chromosome sequencing with great anticipation. Although genes located on the Y chromosome, such as the Sry genes, have been shown to upregulate RAS genes in vitro, the functions of genes in vitro do not always reflect the in vivo situation. Future studies that examine gene expression profiles in a whole-genome, tissue-specific manner will provide the best insights into candidate genes and pathways involved in the Y chromosome–dependent regulation of the renal RAS observed in the present study.

**Perspectives**

We demonstrate that the origin of the Y chromosome mediates intrarenal vascular responsiveness to RAS stimulation in the SHRSP. The blunted constrictor response to intrarenal Ang I in the SHRSP was mediated by a blunted constrictor response to Ang (1-7) which we suggest is attributable to upregulation of the vasodilatory AT2R and MasR. Introgresion of the WKY Y chromosome into the SHRSP (SP.WKY<sub>Gla</sub>Y) restored intrarenal constrictor responses to Ang I and Ang (1-7) and reduced AT2R and MasR expression to WKY levels. Renal vascular responsiveness to changes in the hormonal
milieu are critical for long-term renal regulation of body fluid homeostasis and blood pressure control. Therefore, our data provide functional evidence that the origin of the Y chromosome significantly impacts the renal vascular responsiveness and therefore may influence the long-term renal regulation of blood pressure.

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Disclosures
None.

References


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**Novelty and Significance**

**What Is New?**

- The origin of the Y chromosome, whether it is inherited from a hypertensive or normotensive father, influences circulating angiotensin II (Ang II) and Ang(1-7) levels, renal renin–angiotensin system (RAS) receptor expression, and renal responsiveness to RAS stimulation.
- The blunted constrictor response to intrarenal Ang I in the spontaneously hypertensive stroke-prone rats was mediated by a blunted constrictor response to Ang(1-7) which we suggest is attributable to the upregulation of the vasodilatory angiotensin II receptor type 2, and Mas receptor expression.
- Introggression of the WKY Y chromosome into the spontaneously hypertensive stroke-prone rats restored intrarenal constrictor responses to Ang I and Ang(1-7) and reduced angiotensin II receptor type 2 and Mas receptor expression to Wistar Kyoto levels.

**What Is Relevant?**

- The origin of the Y chromosome is significantly associated with blood pressure regulation in humans. Our study provides evidence that the origin of the Y chromosome influences circulating and renal RAS, suggesting that the involvement of the RAS in blood pressure regulation in men may be Y chromosome dependent. Therefore, the efficacy of antihypertensive therapeutics that target the RAS may also be dependent on the Y chromosome lineage.

**Summary**

Renal vascular responsiveness to changes in the hormonal milieu are critical for long-term renal regulation of body fluid homeostasis and blood pressure control. Our data provide functional evidence that the origin of the Y chromosome significantly impacts the renal vascular responsiveness and therefore may influence the long-term renal regulation of blood pressure.
Origin of the Y Chromosome Influences Intrarenal Vascular Responsiveness to Angiotensin I and Angiotensin (1-7) in Stroke-Prone Spontaneously Hypertensive Rats

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