Cardiovascular and Renal Phenotype in Mice With One or Two Renin Genes

Craig Lum, Edward G. Shesely, D’Anna L. Potter, and William H. Beierwaltes

Abstract—We compared the phenotype of two common mouse models, C-57BL/6J (C57), which carries only the Ren-1c gene, and 129/SvJ (Sv-129), with both Ren 1c and Ren-2. We hypothesized two renin gene Sv-129 would have increased blood pressure and the renin-angiotensin system would be more influential in regulating renal function compared with one renin gene mice. Sv-129 consistently had higher blood pressure than C-57, whether conscious (128 versus 108 mm Hg, P<0.001) or anesthetized (102 versus 88 mm Hg, P<0.001). Plasma renin concentration in both conscious and anesthetized C-57 mice was 3- to 4-fold higher than in Sv-129 (P<0.05), whereas renal cortical renin content was 2.5-fold higher (P<0.005). Renal blood flow and renal vascular resistance were the same in C-57 and Sv-129. Exogenous angiotensinogen produced identical pressor and renal vasoconstrictor responses in both strains. Blocking AT1 receptors with losartan reduced blood pressure by 19 mm Hg in both strains. Nitric oxide synthase inhibition by l-NAME increased blood pressure by 29 mm Hg in C-57 and 35 mm Hg in Sv-129 mice, but the decrease in renal blood flow was 30% less in C-57 (P<0.025). We conclude that Sv-129 mice with two renin genes have higher blood pressure but lower plasma and renal renin than C-57 mice with one renin gene. Renin substrate may limit angiotensin II production in the mouse. In Sv-129, the influence of nitric oxide on renal but not systemic resistance may be exaggerated. Renin from Ren-2 may act independently of normal renin control mechanisms. (Hypertension. 2004; 43:79-86.)

Key Words: angiotensin ■ angiotensinogen ■ endothelium ■ mouse ■ nitric oxide ■ nitric oxide synthase ■ renin

The potent vasoconstrictor hormone angiotensin II (AngII) is the active product of the renin-angiotensin system. AngII is typically produced through the rate-limiting cleavage of the substrate angiotensinogen by the enzyme renin.1 In man and in most experimental animals such as dogs, rats, and rabbits, the inactive renin precursor, preprorenin, is synthesized by a single highly-regulated gene designated Ren-1c, predominantly in the kidney, where it is located in the juxtaglomerular (JG) cells of the afferent arteriole. Pathways for the regulation of renin secretion include the renal baroreceptor, macula densa, and renal sympathetic nerves.2 Renin synthesis and secretion are enhanced by dietary sodium restriction, reduced renal perfusion or renal ischemia (as in renovascular hypertension), and conversely, they can be suppressed by excess sodium intake or elevated renal perfusion pressure.2 Abnormal regulation or production of AngII has been implicated as a significant pathogenic factor in many forms of hypertension, as well as heart and renal failure.3

In contrast to most experimental models, the renin genes in mice are polymorphic.4 Some strains have only the typical Ren-1c gene, whereas others have two alternative genotypes at the renin locus, and thus two renin genes, designated Ren-1d and Ren-2.5 In these strains, the genes are tightly linked in tandem within chromosome 1, spanning over 40 kb. Both genes have the same overall genomic organization, encoding proteins that are different, yet exhibit 97% homology; however, they differ with respect to their glycosylation sites.6 mRNA derived from the two renin genes accumulates in the kidney of two-gene strains in approximately equal levels,7 and homozygous deletion of Ren-1d reduces total renin mRNA by 50% within the kidney.6 However, in mice with two renin genes, Ren-1d gene expression is reported to be higher (70% to 80% of total), and is thought to be the source of the renin activated in JG cell granules and released constitutively in response to classic renin-stimulating pathways.8 Using chronically uninephrectomized inbred mice, similar with the exception that they had either one or two renin genes, Wang et al9 reported that mice with two renin genes had much higher plasma renin concentration (PRC) than mice with only one renin gene. Blood pressure was also greater in mice with two renin genes. They concluded that the second renin gene was not subject to the normal renin control mechanisms, and that the higher renin they observed in the mice with two renin genes suggests that extrarenal renin in this model may become a more important regulator of blood pressure.
Although the function of the second renin gene is not understood, it represents a unique, redundant renin-producing gene in addition to the established Ren-1-derived renin pathway. Both Ren-1 and Ren-2 are found in the kidney, though Ren-2 also exists at some nonrenal sites. Mice lacking a functional Ren-1 gene have no JG-cell granulation and exhibit atypical macula densa morphology, whereas blood pressure in females is lower (though it is similar in males). In the absence of Ren-1, plasma active renin is reduced, whereas inactive prorenin is increased, suggesting that Ren-1 is a prerequisite for the formation of renin storage granules despite the presence of Ren-2-generated renin, and further that renin from Ren-1 and Ren-2 genes is secreted through different pathways. Alternatively, Sharp et al found that if they deleted the Ren-2 gene from Sv-129 mice by gene targeting, leaving only Ren-1, no morphological abnormalities or changes in blood pressure resulted, though active renin was increased and inactive prorenin was decreased. They suggested that Ren-1 is fully capable of regulating blood pressure in the absence of the second renin gene in this mutant strain.

In addition to the presence of a second renin gene, the renin angiotensin system in the mouse may be different from other species because of reported high levels of renin, low levels of angiotensin system in the mouse may be different from other strains, we would uncover inherent differences in basal cardio-renal dynamics and the renin-angiotensin system. We hypothesized that SV-129 mice with two renin genes would have enhanced renin secretion compared with one renin gene C-57.

**Methods**

Male C-57 and Sv-129 mice weighing 28 to 30 g from Jackson Laboratories (Bar Harbor, Me) were used in protocols approved by the Institutional Animal Care and Use Committee and conformed to federal guidelines.

**Instrumented Conscious Mice**

Mice were catheterized under barbiturate anesthesia (Nembutal, 125 mg/kg bw, i.p., Abbott, Chicago, Ill) using antiseptic techniques. The femoral artery was cannulated using PE-10 tubing welded into PE-50 tubing to see if it would affect either systemic blood pressure or RBF. Inactin-anesthetized mice were instrumented as described above and then given semipurified sheep angiotensinogen sufficient to produce 0-, 2-, 5-, 10-, 25-, and 50-ng Ang I in 50-µL aliquots boluses of saline IV, followed by an additional 50-µL saline to clear the dead space. The next dose was not administered until 10 to 15 minutes after blood pressure and RBF had returned to baseline. We used sheep angiotensinogen because it is a universal renin substrate and is routinely used in our laboratory for PRC.

**Surgical Protocols in Anesthetized Mice**

Mice fasted overnight but had water ad libitum. Anesthetized mice (125-mg/kg IP thiobutabarbitual; Inactin, 14) were prepared for acute determination of blood pressure and renal blood flow (RBF) using a 0.5SVB Transonic ultrasonic Doppler flow probe and Transonic flowmeter (Ithaca, NY), calculating renal vascular resistance (RVR) in mm Hg/mL/min/100 mg kidney weight (kw), or resistance units (RU), as previously described. 50 µL samples of renal venous blood were collected directly from the renal vein using a Hamilton microsyringe and transferred to a siliconized capillary tube for centrifugation to measure hematocrit and renin concentration, replacing the sample volume with 6% bovine serum albumin. Urine was removed directly from the bladder using a 23-g needle on a 1-cc syringe. Mice were terminated by pneumothorax and aortic transection, kidneys removed, decapsulated, weighed, and hemodynamic values normalized by 100 mg (wet) kw.

**Determination of Renin in Mice**

We evaluated circulating renin using both plasma renin activity (PRA) and PRC assays. PRA was determined by incubating the plasma sample solely with endogenous substrate. Alternatively, PRC samples were incubated with an excess of exogenous partially purified sheep angiotensinogen and incubated at 37°C for 30 minutes. The supernatant was frozen for later analysis.

Tissue renin content (renal cortex, brain, submandibular gland) was determined similarly to PRC using an excess of partially purified sheep angiotensinogen with supernatant from homogenized tissue. Tissue was blotted, weighed, and placed in cold bicarbonate buffer (10% wet tissue weight; 90% buffer) pre-equilibrated with a mixture of 95% O2/5% CO2 to a pH of 7.4 at 37°C, and incubated with an inhibitor cocktail of 3% PMSF and 3.8% disodium EDTA. PRA, PRC, and tissue content were then analyzed by radioimmunoassay (RIA) for generation of AngI using a Gamma Coat kit (DiaSorin, Stillwater, Minn). Values were corrected by tissue weight and designated as ng AngI/hr/mg/30 minutes.

**Response to Exogenous Angiotensinogen**

We found that PRA was below the assay detection range of PRC in both strains of mice. Although the lower limit of the assay is 0.2 ng AngI/mL/h, the dilutions necessary to bring PRC into the standard curve raised the lower limit to 20. To confirm the (apparently) low-circulating levels of angiotensinogen, we added exogenous renin substrate to see if it would affect either systemic blood pressure or RBF. Inactin-anesthetized mice were instrumented as described above and then given semipurified sheep angiotensinogen sufficient to produce 0-, 2-, 5-, 10-, 25-, and 50-ng Ang I in 50-µL aliquots boluses of saline IV, followed by an additional 50-µL saline to clear the dead space. The next dose was not administered until 10 to 15 minutes after blood pressure and RBF had returned to baseline. We used sheep angiotensinogen because it is a universal renin substrate and is routinely used in our laboratory for PRC.

**Angiotensin AT1-Receptor Blockade With Losartan**

To assess the contribution of AngII to blood pressure control, mice were anesthetized with Inactin and prepared to monitor blood pressure and RBF as described above. A 10-mg/kg bw bolus of the AT1 antagonist losartan was given, and depressor and renal vasodilator responses were observed over a 30-minute period. The role of endogenous nitric oxide (NO) was tested using a 10-mg/kg

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Because rat plasma contains excess angiotensinogen, as a control we ran 6 Inactin-anesthetized adult Sprague-Dawley rats (Charles River Labs, Wilmington, Mass; 350 to 450 g bw), using basically the same protocol, adjusting the amount of sheep substrate to the greater body weight of the rats. Boluses of semipurified sheep angiotensinogen sufficient to produce 0-, 2-, 5-, 10-, 25-, and 50-ng Ang I in 50-µL saline to clear the dead space. The next dose was not administered until 10 to 15 minutes after blood pressure and RBF had returned to baseline. We used sheep angiotensinogen because it is a universal renin substrate and is routinely used in our laboratory for PRC.

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TABLE 1. Comparison of Hemodynamic Parameters in Inactin-Anesthetized C-57 and SV-129 Mice

<table>
<thead>
<tr>
<th>Variable</th>
<th>C-57</th>
<th>SV-129</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP (mm Hg)</td>
<td>88±1 (20)</td>
<td>102±1 (25)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Heart Rate (bpm)</td>
<td>343±13 (25)</td>
<td>366±14 (20)</td>
<td>ns</td>
</tr>
<tr>
<td>RBF (mL/min/100 mg kw)</td>
<td>1.66±0.09 (13)</td>
<td>2.00±0.13 (12)</td>
<td>ns</td>
</tr>
<tr>
<td>RVR (RU)</td>
<td>56.7±3.0 (13)</td>
<td>56.9±6.2 (12)</td>
<td>ns</td>
</tr>
</tbody>
</table>

P-value is comparing C-57 to SV-129.

bw of the nitric oxide synthase (NOS) inhibitor L-nitroarginine methyl ester (L-NAME, Sigma, St. Louis, Mo). Blood pressure and RBF responses to L-NAME in the presence of losartan were monitored over 15 minutes.

Response to Reduced Renal Perfusion Pressure and NOS Inhibition

We compared two different manipulations of renal perfusion involving NOS inhibition. First, we compared the pressor and renal vasoconstrictor response to a 10-mg/kg bw blocking dose of L-NAME in the two strains. Additionally, changes in RBF in response to sequential decreases in renal perfusion pressure were monitored using an adjustable aortic constrictor as previously described. Renal perfusion pressure was reduced in 10-mm Hg decrements by constricting the aortic loop.

Analytical Methods

Strains were compared using Student t test. Changes were analyzed using a paired t test. Dose-response protocols were evaluated by ANOVA for repeated measures, followed by a Bonferroni post-hoc test. Renal pressure/flow relationships were evaluated by regression analysis. For all tests, a probability value (or adjusted probability value) of 0.05 was considered to be significant.

Results

Conscious Instrumented Mice

Blood pressure measured directly in 13 conscious instrumented C-57 mice was 108±2 mm Hg, compared with 128±3 in 13 conscious Sv-129 mice (P<0.001). Heart rate was 656±21 bpm in C-57 and 608±30 in Sv-129. Hematocrit was 44±2% in C-57 and 48±2% in Sv-129.

Inactin-Anesthetized Mice

Inactin anesthesia lowered basal blood pressure in both strains, but the difference between strains remained significant (Table 1) as Sv-129 results were 14 mm Hg higher than in C-57 (P<0.001). Anesthesia decreased basal heart rate by 40% to 45% in both strains. We did not observe any significant differences in RBF or RVR between strains.

Renin

Contrary to our expectations, PRC in conscious C-57 mice was 4-fold greater than in Sv-129 (P<0.05, Table 2). Under Inactin anesthesia, PRC tended to increase in both strains, but the difference between strains was maintained in all study groups. In contrast to PRC, PRA was always below the limit of our standard curves (<20 ng AngI/mL/h, see Methods section). We did not repeat the PRA assay with greater sensitivity and longer incubation times because if PRA does not approximate renin concentration, it is governed by two unknown variables (angiotensinogen and renin).

Consistent with PRC, renal renin content was increased 2.5-fold in C-57 mice compared with Sv-129 (P<0.005, Table 2). However, contrary to our expectations, submandibular gland renin content was similar in both strains, though Sv-129 glands were 20% heavier than C-57 (P<0.05). In the brain, where we expected to find no difference, renin content in C-57 mice was half that of Sv-129 (P<0.05).

Response to Renin Substrate

Basal blood pressure in 7 C-57 mice was 85±2 mm Hg, significantly lower than Sv-129 (102±2 mm Hg, n=13; P<0.05). Basal RBF was the same in C-57 and Sv-129 (1.47±0.22 versus 1.40±0.10 mL/min/100 mg kw, respectively), as was RVR (66.2±9.1 versus 77.6±5.3 RU). Graded doses of exogenous renin substrate produced identical pressor and renal vasoconstrictor responses in the two strains (Figure 1). Notably, responses were graded between 2 and 10 ng, reaching a maximum pressor response between 10 and 15 mm Hg and a 60% decrease in RBF, which remained consistent even at 5-fold greater doses.

When we ran similar experiments in rats using equivalent doses of angiotensinogen (adjusted for increased body weight), we found neither a pressor response nor a decrease in RBF at any dose of substrate. Thus, even at the highest dose, addition of exogenous substrate had no hemodynamic effects in rats, contrary to our results in mice.

AT-1 Blockade

Basal blood pressure in C-57 mice (n=13) was 86±2 mm Hg, significantly lower than in Sv-129

TABLE 2. Comparison of Renin Values in C-57 and SV-129 Mice

<table>
<thead>
<tr>
<th>Variable</th>
<th>C-57</th>
<th>SV-129</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRC (conscious), µg AngI/mL/h</td>
<td>8.18±0.35</td>
<td>1.92±2.27</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>PRC (anesthetized), µg AngI/mL/h</td>
<td>11.90±0.20</td>
<td>3.80±0.53 (8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Renal Cortical RC, ng/mL/hr/mg tissue wt</td>
<td>77.0±12.3</td>
<td>30.1±3.7</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Submandibular RC, ng/mL/hr/mg tissue wt</td>
<td>7.1±2.1</td>
<td>5.5±0.6</td>
<td></td>
</tr>
<tr>
<td>Brain RC, ng/mL/hr/mg tissue wt</td>
<td>0.064±0.023</td>
<td>0.115±0.004</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>24.6±0.5</td>
<td>23.3±0.5</td>
<td></td>
</tr>
<tr>
<td>Kidney wt, mg</td>
<td>115±5</td>
<td>105±4</td>
<td></td>
</tr>
<tr>
<td>Submandibular gland wt, mg</td>
<td>4.48±0.28</td>
<td>5.45±0.38</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Brain wt, mg</td>
<td>420±7</td>
<td>366±7</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

P-value compares C-57 to SV-129. Unless otherwise noted, n=13 in all data sets. PRC indicates plasma renin content; RC (tissue) renin content. Kidney Weight is a mean of both kidneys.
Basal heart rate was 38 ± 16 bpm in C-57, significantly greater than in Sv-129 (31 ± 20 bpm; P < 0.025). There was no difference between C-57 and Sv-129 with regard to either RBF (1.39 ± 0.13 mL/min/100 mg kw) or RVR (62.7 ± 3.4 versus 75.2 ± 7.0 RU). Similarly, losartan reduced blood pressure by 19 ± 2 mm Hg in C-57 and by 19 ± 4 mm Hg in Sv-129, and also increased RBF and decreased RVR in the two strains (-24.1 ± 3.3 RU in C-57 versus -25.6 ± 5.9 RU in Sv-129, Figure 2). Thus, despite different basal blood pressures, the depressor and renal hemodynamic responses to AT1 blockade were identical.

L-NAME reversed the hemodynamic changes evoked by losartan in both strains (Figure 2), increasing blood pressure to 91 ± 4 mm Hg in C-57 (+26 ± 2 mm Hg) and to 104 ± 3 mm Hg in Sv-129 (+25 ± 4 mm Hg). Likewise, L-NAME reversed the renal vasodilatation induced by losartan (Figure 2), causing RVR to fall by 24.1 ± 3.3 RU in C-57, and by 25.6 ± 5.9 RU in Sv-129. Thus, neither the hemodynamic responses to AT1 blockade nor subsequent reversal with NOS inhibition differed between strains.

Response to NOS Inhibition
The acute pressor response to NOS inhibition using L-NAME was similar in Inactin-anesthetized C-57 (n = 13) and Sv-129 (n = 12), increasing by 29 ± 3 versus 35 ± 2 mm Hg (Figure 3). However, the absolute decrease in RBF after acute L-NAME administration in C-57 mice was 30% less compared with Sv-129 (-0.63 ± 0.05 versus -0.92 ± 0.09 mL/min/100 mg kw; P < 0.025). The changes in RVR evoked by L-NAME tended to be greater in Sv-129 than in C-57, but the difference was not significant (+65.6 ± 6.6 RU in C-57 versus +78.0 ± 4.9 RU in Sv-129).

Separate groups of 9 C-57 mice and 8 Sv-129 mice were fitted with a suprarenal constricting loop, and the relationship between renal perfusion pressure and RBF was studied, first under control conditions and then after a 10 mg/kg bw dose of L-NAME. Basal blood pressure in the C-57 mice was 83 ± 3 mm Hg, and RBF was 1.69 ± 0.11 mL/min/100 mg kw. Decreasing renal perfusion pressure lowered RBF at all pressures below 80 mm Hg (Figure 4). In Sv-129, blood pressure was 97 ± 2 mm Hg, significantly higher than C-57 (P < 0.05), whereas RBF was 2.01 ± 0.11 mL/min/100 mg kw. Decreasing renal perfusion pressure lowered RBF at all levels below 90 mm Hg (Figure 3). Although basal pressure was greater in Sv-129, there was no difference in the slopes of the pressure-flow curves.
After L-NAME, blood pressure in C-57 rose to 108/110 mm Hg (P < 0.001) and RBF was decreased by 41% to 0.99/0.10 mL/min/100 mg kw (P < 0.001). In Sv-129 mice, L-NAME increased blood pressure to 127/133 mm Hg and decreased RBF by 54% to 0.93/0.03 mL/min/100 mg kw (P < 0.001). Although the pressor response to L-NAME was the same in both strains, the change in RBF after L-NAME was significantly greater in Sv-129 than in C-57 (P < 0.025). In each strain, the reduction in renal perfusion pressure after L-NAME caused a gradual decrease in RBF, whereas the slopes of the responses were the same in both strains despite their different starting points (Figure 4).

Discussion

We found that Sv-129 mice with two renin genes consistently had higher blood pressure than C-57 mice with one renin gene, whether they were conscious or anesthetized. This is similar to Wang’s finding comparing inbred, backcrossed, uninephrectomized mice with two renin genes to backcrossed homozygous mice with one renin gene. Although none of the blood pressures we measured would be considered hypertensive, the differences between strains persisted throughout all of our protocols. Contrary to our expectations, PRC in both conscious and anesthetized C-57 mice was 3- to 4-fold higher than in Sv-129, and renal cortical renin content was also significantly greater in C-57, in contrast to nonrenal rennin, which was similar or lower in C-57.

We have compared commonly used strains of mice that naturally have either one or two renin genes. Previous reports used genetic inbreeding to look at the influence of different configurations of renin genes. Renin mRNA from both renin genes has been shown to be essentially proportional in the renal cortex. Pentz et al9 reported that mutant Ren-1d-deficient mice (with Ren-2 intact) had suppressed circulating PRC as well as lower blood pressure compared with mice with intact Ren-1d. Clark et al5 found that Sv-129 mice with Ren-1d deleted had reduced active renin and increased inactive renin, combined with total absence of JG cell secretory granules and abnormal JG cell morphology. They indicated that deletion of the Ren-1d gene from Sv-129 mice only lowered blood pressure in females. However, Bertaux et al4 found that mice with disruption of Ren-1d had similar blood pressure to Sv-129 mice with Ren-1d intact.

Mullins et al11 reported that mice lacking Ren-1d had normal blood pressure, despite lacking JG cell granulation and atypical JG cell morphology. This suggests that the normal JG cell activation and renin secretion depends on expression of a viable Ren-1 gene. Homozygous Ren-1d deletions lead to a 50% reduction in total renin mRNA. In Sv-129 mice, when the Ren-2 gene was deleted by gene targeting, there was no abnormal morphology and no change in blood pressure. In the absence of Ren-2, circulating active renin increased whereas prorenin decreased, suggesting Ren-2 is a major source of inactive prorenin. Thus Ren-1 alone can likely assume full regulatory control of renin secretion, so that Ren-2 expression is probably not important in controlling blood pressure. Moreover, the pathways of expression of the two renin genes appear completely different.5

Wang et al9 also compared the effects of deoxycorticosterone acetate (DOCA) treatment in 4-week uninephrectomized...
inbred mice with either one or two renin genes. They found that, after breeding two renin gene 129Ola mice with one renin gene C57BL/6J, the wild type backcross 129Ola/C57BL/6J mice that were homozygous for two renin genes had PRCs 100-fold greater than wild-type backcrossed 129Ola/C57BL/6J mice with only one renin gene 4 weeks after uninephrectomy. Although the results in 2 renin gene mice contrasts with our data, the influence of chronic uninephrectomy on renin from mice with two renin genes was not addressed. DOCA/salt treatment for 4 weeks reduced renin in the mice with one renin gene but not in those with two. Blood pressure was 21 mm Hg greater in mice with two renin genes (similar to our results) after 4 weeks of uninephrectomy, and 4 weeks of DOCA treatment reduced blood pressure in mice with one renin gene but increased it by another 30 mm Hg in mice with two renin genes. Consistent with the effect of DOCA/salt on renin, blocking the AT1 receptors with losartan decreased blood pressure in mice with two renin genes but not in those with only one renin gene. These authors concluded that the second renin gene was not subject to the normal renin control mechanisms, and that the higher renin they observed in mice with two renin genes suggests that extrarenal renin may become a more important regulator of blood pressure in this model. They also suggested that the Ren-2 gene is physiologically active in mice and not subject to what they called the “usual negative feedback controls.”

Although the differences between mice with one and two renin genes in these studies is striking, the data are obscured by the difficulty in interpreting the effects of 4 weeks of uninephrectomy, renal hypertrophy, DOCA/salt treatment, and hypertension on renin from mice with one renin gene. All of these manipulations could suppress renal (Ren-1)-derived renin, thereby artificially reducing any contribution by this source of renin and allowing both renal and extrarenal Ren-2 renin to predominate, or perhaps be upregulated. Although this is of considerable interest, it may have little to do with understanding the role of Ren-1 versus Ren-2-derived renin in the control of normal renal function or even blood pressure.

In a preliminary abstract, Hansen et al compared PRC in three strains of mice containing only the Ren-1 gene (C57BL6, Balb C, AIAR) with three strains containing two renin genes (J129, Swiss Webster, AIAR). They reported that PRC in the mice with one renin gene was similar (289 to 426 ng AngI/mL/h), but PRC in the mice with two renin genes varied greatly and was either lower or higher than PRC in one renin gene (224 to 2407 ng AngI/mL/h). They suggested that in pure inbred strains of mice, renin is dependent on the number of renin genes. However, their data also suggest that the result of having two renin genes may vary considerably without any relationship between the Ren-2 gene and PRC.

Both of these reports present values for PRA as well as PRC, and as in our study, PRA was only a very small percentage of PRC (1/1000th). However, the utility of reporting PRA is debatable, as the assay for PRA is an approximation of PRC, which assumes an excess of renin substrate, maintaining the enzymatic reaction forming angiotensin I in first-order kinetics, a prerequisite for an accurate determination. Thus, if PRA is only a fraction of paired values for PRC, it is invalid as a meaningful measurement of renin because both substrate and renin are unknown variables. Although you can still determine values for PRA in mice, it is not a quantitative representation of anything, but only a gross index of the lack of sufficient renin substrate, as we have shown. The pressor responses to exogenous substrate we found in mice directly address this issue. Therefore, in our results we did not try to adjust the sensitivity or prolong incubation times of our assay to get an absolute value for PRA, but only report it as being below the range of sensitivity of the PRC assay.

In our studies, we only used male mice, and though both strains were normotensive, we still found that the Sv-129 had higher blood pressure. However, it is unclear from these experiments whether that difference has anything to do with the number of renin genes or simply a trait common to this strain. Wang’s experiments in mice with two renin genes also showed increased blood pressure, though PRC was different from ours. Our observation that mice with higher blood pressure also had lower PRC and renal renin content may merely be due to renin suppression through the renal baroreceptor rather than some inherent trait of the renin genes. Because basal blood pressure was different, the difference cannot be explained within the constraints of our observations. However, other parameters of renal function were similar and both strains responded similarly to AT1 blockade and NOS inhibition, suggesting that these differences in renin may not be critical to overall regulation of renal function or blood pressure.

Inactin anesthesia in mice has been reported to increase PRC as much as 10-fold. However, we found that although anesthesia did increase PRC slightly, it was generally less than double, similar to the rat. Previous reports of large increases may be because of the concurrent use of ketamine, which we found induced transient hypotension and stimulated renin (unpublished observations, Beierwaltes, 1999). Although Inactin by itself takes much longer to take effect in mice compared with rats, this is more than compensated for by the sustained stable blood pressure it provides.

We did not measure inactive prorenin in these studies. Previous reports suggest that both prorenin and the ratio of inactive to active renin are higher in mice with a second Ren-2 gene. Clark et al reported that PRC (active renin) was similar in Sv-129 mice with two renin genes and in mice with the Ren-1 gene knocked out. Inactive prorenin in Sv-129 was twice the level of active renin, and deletion of Ren-1 increased prorenin 3-fold compared with intact Sv-129.

We found that the addition of exogenous renin substrate evoked significant hemodynamic changes, including a pronounced pressor response and renal vasoconstriction. These data suggest that there is little circulating angiotensinogen in the mouse compared with other species. Angiotensinogen levels in mice are reportedly only one-tenth of that found in humans. Our values for PRA, which depend on endogenous substrate for generation of AngI, were a small fraction of PRC. This contrasts somewhat with reports indicating that PRA, although only a fraction of PRC, still gives measurable results, and may in fact be similar to PRA in the rat. Meneton et al suggested that angiotensinogen levels are higher in females than in males, and also higher in mice with...
one rather than two renin genes. Hansen et al reported PRA values in different strains that tended to correlate with PRC but were only a fraction of their activity, and like PRC, were quite variable in mice with two renin genes. Our data suggest that high renin levels may consume all available substrate as it is produced. Our finding of a significant dose-dependent pressor response in mice, compared with the lack of a response to similar doses of renin substrate in rats, suggests that substrate availability may be a critical factor in the mouse. Contrary to renin acting as the rate-limiting factor in angiotensin formation, the small amount of circulating substrate and the hemodynamic responses to exogenous substrate suggests that angiotensinogen availability might be a limiting step in angiotensin formation in the mouse.

One might question whether the remarkably different levels of PRC in our two strains reflect a difference in circulating angiotensin II levels, or in the influence of renin on blood pressure or vascular resistance. Although we did not measure AngII, we observed equipotent hemodynamic responses to AT1 receptor blockade, suggesting that the influence of angiotensin in these strains was similar. Thus, although PRC was different, it is possible that decreased substrate does not allow AngII levels that reflect PRC.

There is growing evidence that elements of the renin-angiotensin system occur in a diverse array of tissues, including the kidney, submandibular gland, brain, heart, adrenals, and sex organs. We found that nonrenal renin content was different from renal renin. Submandibular renin was similar in the two strains, whereas brain renin, which was much lower overall, was almost twice as high in SV-129 as in C-57. It is possible that nonrenal sources of renin may be influenced more by the Ren-2 gene, which is not constrained by the classical regulatory pathways of Ren-1 found in the kidney.

When we compared the RBF response to reduced renal perfusion pressure, the pressure-dependent decrease in flow was similar in both strains. We then inhibited NOS because we have found that, in anesthetized rats and mice, the decrease in RBF after L-NAME was dependent on the renin-angiotensin system. We did observe that the renal vasoconstrictor response in Sv-129 was significantly greater than in C-57, despite their lower PRC. These data might be interpreted as suggesting a greater influence of endogenous renal renin in Sv-129, or, alternatively, that the buffering effect of endothelial NO in Sv-129 is more profound. This remains to be resolved.

In summary, our observations comparing mice with one renin gene (C-57) and two renin genes (Sv-129) suggest that mice with two renin genes have higher blood pressure, but lower PRC and renal renin content. Varied reports of diverse values for PRC in mice with two renin genes but not one renin gene suggest that the classical renal regulation of renin release by Ren-1 is absent with Ren-2, and that this alternative source of renin may be quite different and independent of these regulatory pathways. In both strains, the systemic and renal pressor responses to exogenous angiotensinogen suggest that renin substrate may be an important regulator of angiotensin formation in the mouse. It appears that NO may exert an exaggerated influence on renal but not systemic resistance in mice with two renin genes.

**Perspectives**

Although there have been several reports of higher blood pressure in mice with two renin genes, and even another report of different renin levels, we believe our work represents the first rigorous attempt to describe the cardiovascular and renal phenotype of two commonly used wild-type control strains of mice. This is extremely important because of the numerous mutant and knockout strains currently used to investigate hypertension and cardiorenal physiology. Although both our strains seem to be characterized by normal and similar physiology, we and others have found that mice with two renin genes have higher blood pressure. Unlike man and other experimental models, another novel feature of mice is the apparent depletion of circulating renin substrate in the face of renin concentrations much greater than in other species, suggesting that in the mouse, renin may not be the rate-limiting step in angiotensin formation. Although the renin derived from the Ren-1 gene in mice seems to be regulated similarly to that in other species, understanding the expression of renin derived from the Ren-2 gene is confounded by the apparent lack of normal regulating control mechanisms, so that the variety, fluctuations, and seemingly unpredictable results for renin in our study and others may be attributable to this apparently renegade gene. Overall, the utility of strains of mice possessing two renin genes for cardiovascular or renal studies of the renin-angiotensin system is highly suspect due to these inconsistencies, as well as our lack of understanding of how the second renin gene may be regulated or contribute to cardiorenal homeostasis.

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**References**


19. Pentz ES, Lopez ML, Kim HS, Carretero O, Smithies O, Gomez RZ. Ren 1\(^{\text{a}}\) and Ren2 cooperate to preserve homeostasis: evidence from mice expressing GFP in place of Ren 1\(^{\text{a}}\). Physiologic Genomics. 2002;1:45–55.


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Craig Lum, Edward G. Shesely, D’Anna L. Potter and William H. Beierwaltes

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