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Blood Pressure, Cardiac, and Renal Responses to Salt and Deoxycorticosterone Acetate in Mice: Role of Renin Genes

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Abstract. Several studies have demonstrated that mice are polymorphic for the number of renin genes, with some inbred strains harboring one gene (*Ren-1^c*) and other strains containing two genes (*Ren-1^d* and *Ren-2*). In this study, the effects of 1% salt and deoxycorticosterone acetate (DOCA)/salt were investigated in one- and two-renin gene mice, for elucidation of the role of renin in the modulation of BP, cardiac, and renal responses to salt and DOCA. The results demonstrated that, under baseline conditions, mice with two renin genes exhibited 10-fold higher plasma renin activity, 100-fold higher plasma renin concentrations, elevated BP (which was angiotensin II-dependent), and an increased cardiac weight index, compared with one-renin gene mice (all $P < 0.01$). The presence of two renin genes markedly increased the BP, cardiac, and renal responses to salt. The number of renin genes also modulated the responses to DOCA/salt. In one-renin gene mice, DOCA/salt induced significant renal and cardiac hypertrophy ($P < 0.01$) even in the absence of any increase in BP. Treatment with losartan, an angiotensin II AT_1 receptor antagonist, decreased BP in two-renin gene mice but not in one-renin gene mice. However, losartan prevented the development of cardiac hypertrophy in both groups of mice. In conclusion, these data demonstrate that renin genes are important determinants of BP and of the responses to salt and DOCA in mice. The results confirm that the *Ren-2* gene, which controls renin production mainly in the submaxillary gland, is physiologically active in mice and is not subject to the usual negative feedback control. Finally, these data provide further evidence that mineralocorticoids promote cardiac hypertrophy even in the absence of BP changes. This hypertrophic process is mediated in part by the activation of angiotensin II AT_1 receptors.

The development of genetically engineered animals in which genes are either overexpressed or disrupted has been associated with the increased use of mice in experimental research. Therefore, for investigation of the complex phenotypes of hypertension and cardiovascular diseases, the classic renovascular and mineralocorticoid models of experimental hypertension have been adapted from rats to mice (1–3). However, as recently reported by Johns *et al.* (1), transgenic mouse models relevant to hypertension have sometimes yielded conflicting observations, probably because mouse strains with different genetic backgrounds were used to generate the different models. Previous studies demonstrated that mice are polymorphic for the number of renin genes, with some inbred strains (*e.g.*, C57BL6/J) harboring one gene, *Ren-1^c*, and other strains (*e.g.*, 129 strains) containing two genes, *Ren-1^d* and *Ren-2* (4–6). The *Ren-1* genes express renin in the juxtaglomerular apparatus of the kidney, whereas the *Ren-2* gene controls renin mainly in the submaxillary gland and at very low levels in the kidney. The expression of *Ren-2* in mouse submaxillary glands is, on average, 150-fold greater than that of *Ren-1* in inbred strains (7). Therefore, *Ren-2* is the predominant renin isozyme in mice with two renin genes. Because of the importance of the renin-angiotensin system in the control of BP and electrolyte excretion, characterization of the number of renin genes and analysis of their effects on the experimental model seem to be necessary for the interpretation of results obtained with mice. It was previously demonstrated that the Liddle mutation of the epithelial sodium channel gene differently affects one- and two-renin gene mice (8). Indeed, the BP increase induced by salt was significantly greater in mice with the Liddle mutation and two renin genes, compared with mice with one renin gene (8). Similarly, in 129/SvEvTac mice, which have two renin genes, blockers of the renin-angiotensin system were demonstrated to prevent the development of hypertension and left ventricular hypertrophy induced by deoxycorticosterone acetate (DOCA)/salt; in one-renin gene mice with a different genetic background (C57BL/6J), the same antihypertensive agents prevented cardiac and renal hypertrophy induced by DOCA without affecting the increase in BP (3,9). In this study, we have investigated the effects of 1% salt and DOCA/salt in one- and two-renin gene mice, to investigate the role of renin

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in modulating the BP, cardiac, and renal responses to salt and DOCA.

Materials and Methods

Animals and Experimental Protocol

Wild-type backcross N_{5-6}(129Ola/C57BL/6J) mice that were homozygous for the *Ren-1^c* gene locus (one-renin gene mice) and wild-type backcross N_{5-6}(129Ola/C57BL/6J) mice that were homozygous for the *Ren-1^d*/*Ren-2* gene loci (two-renin gene mice) were used throughout these experiments. These mice were created by crossing C57BL/6J mice, in which only the *Ren-1^c* gene was detected, with 129Ola mice, which possess both the *Ren-1^d* and *Ren-2* genes (10). Because experimental animals could be either heterozygous for the *Ren-1^c* and *Ren-1^d*/*Ren-2* genes or homozygous for these genes, all mice were characterized with respect to their renin gene backgrounds by Southern blot analysis, as demonstrated in Figure 1. Only mice homozygous for the *Ren-1^c* gene and for the *Ren-1^d*/*Ren-2* genes were used for our studies. Six to 10 animals were included in each group. The characteristics of the animals are presented in Table 1. Seven-week-old male animals of each strain (weight, 22 to 26 g) were anesthetized with 1% halothane mixed with oxygen, and the left kidney was removed. A silastic tube (inner diameter, 1.57 mm; outer diameter, 2.4 mm; with sixteen 400-μm micropores) filled with 35 mg of DOCA was subcutaneously placed in the abdomen of each mouse (Figure 2). The DOCA release rate was calculated by serial weighing of the tubes and was estimated to be 21.3 $\pm$ 0.22 μg/h (mean $\pm$ SD, $n = 78$). As demonstrated in Figure 2, the DOCA release rate was constant during the 4 wk of implantation. Mice receiving DOCA (Sigma) were given 1% NaCl to drink. Control mice also underwent uninephrectomy but DOCA was not administered; one control group received 1% saline solution and another group received tap water as the drinking fluid. All mice were investigated after 4 wk of DOCA/salt administration. For evaluation of the changes in mineralocorticoid activity induced by DOCA, the amiloride-sensitive rectal potential difference (PD) was measured with light ketamine anesthesia (11). All mice received the same chow, which contained 3 g Na^+/kg food.

At the end of the experiment, the mice were weighed. BP and heart rate were monitored, and blood was collected for the measurement of serum electrolyte levels, plasma renin activity (PRA), and plasma renin concentration (PRC). After BP measurements, mice were anesthetized and the heart and kidney were rapidly excised, weighed, and fixed as described previously (12).

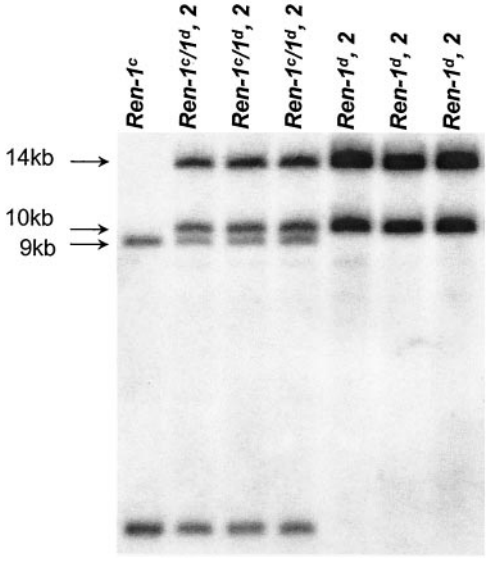

Figure 1. Southern blot analysis of DNA from offspring resulting from the crossbreeding of wild-type C57BL/6J and wild-type 129Ola mice. Southern blot analysis was performed on tail DNA digested with *Pvu*II. A cDNA coding for the mouse renin gene was used as a probe. The *Ren-1^c* gene (from the C57BL6/J strain) was detected as a 9-kb band, and the *Ren-1^d* and *Ren-2* genes (from the 129Ola strain) were detected as 10- and 14-kb bands, respectively. All mice were characterized with respect to their renin gene backgrounds, and only homozygous mice (for *Ren-1^c* or *Ren-1^d*/*Ren-2*) were used in these experiments.

BP Measurements

BP and heart rate were recorded intra-arterially, with a computerized data-acquisition system (13). For placement of the intra-arterial catheter, mice were anesthetized via inhalation of 1 to 2% halothane with oxygen. The right carotid artery was exposed for a length of approximately 4 mm. A PE-10 catheter filled with 0.9% NaCl solution containing heparin (300 IU/ml) was inserted into the artery. After ligation, the catheter was subcutaneously tunneled to exit at the back of the neck. Mice were allowed 3 h to recover from the anesthesia and were placed in Plexiglas tubes for partial restriction of their movements. Thirty minutes later, the arterial line was connected to a pressure transducer; BP and heart rate were then monitored every 20 s for 15 to 30 min.

BP Responses to Blockade of Angiotensin II AT_1 Receptors

For evaluation of the role of the renin-angiotensin system in maintaining BP in the various mice, the angiotensin II receptor antagonist irbesartan was injected intravenously (1.5 mg/kg, in 50 μl) and changes in BP were measured. For injection of the antagonist, a venous catheter was inserted into the left jugular vein.

In a second set of experiments, one- and two-renin DOCA/salt-treated mice were treated with the angiotensin II receptor antagonist losartan. Losartan was added to the drinking water at a dose of 0.2 mg/ml water. The treatment was started on the first day of administration of DOCA and salt. Control groups received the vehicle. To test the ability of the dose of losartan to block the renin-angiotensin system, exogenous angiotensin II was injected intravenously into DOCA/salt-treated mice receiving the vehicle and losartan. The BP response to exogenous angiotensin II (40 ng/kg, administered intravenously) was markedly blunted by losartan (31 mmHg for the control group and 4.5 mmHg for the losartan-treated group, $P < 0.001$).

Biochemical Analyses

For measurement of plasma sodium and potassium levels, 300 μl of blood was obtained from the carotid artery (after BP measurements), in a 0.6-ml Multivette tube containing gel/clot activator (Sarstedt, Numbrecht, Germany). Serum and urinary sodium and potassium levels were measured by flame photometry (model 943; Instrumentation Laboratory). PRA and PRC were measured using methods described previously and adapted for small amounts of blood (13,14). Blood was drawn from the arterial catheter after the BP measurements were completed. Studies performed in our laboratory have demonstrated that blood sampling via an intra-arterial catheter generates less

Table 1. Effects of DOCA and salt in one- and two-renin gene mice with the same genetic background [N_{5-6}(129Ola/C57BL/6J)][a]

	One Renin Gene			Two Renin Genes		
	Tap Water	1% NaCl	DOCA/Salt	Tap Water	1% NaCl	DOCA/Salt
Body weight (g)						
initial	24 ± 1	24 ± 1	23 ± 1	25 ± 1	25 ± 1	25 ± 1
final	27 ±	27 ± 1	26.5 ± 1	29 ± 1	29 ± 1	30 ± 1
Mean BP (mmHg)	120 ± 3	118 ± 3	112 ± 4	141 ± 3[b]	159 ± 4[b,c]	170 ± 4[b,d]
Heart rate (beats/min)	613 ± 18	611 ± 29	572 ± 14	595 ± 23	582 ± 16	578 ± 21
Serum K^+ level (mM)	5.1 ± 1.0	4.9 ± 0.2	2.9 ± 0.2[e]	4.7 ± 1	5.4 ± 0.1[c]	3.4 ± 0.2[e]
Ami. rectal PD (mV)	−11 ± 2	−5 ± 1[f]	−50 ± 1[e]	−19 ± 2[g]	−9 ± 2[c]	−45 ± 3[e]
Cardiac weight (g)	109 ± 3	109 ± 2	127 ± 3[e]	130 ± 5	145 ± 6[f]	160 ± 5
CWI (mg/g)	4.0 ± 0.1	4.1 ± 0.1	4.8 ± 0.1[e]	4.4 ± 0.1[b]	5.0 ± 0.2[b,f]	5.4 ± 0.1[b]
Kidney weight (g)	195 ± 6	213 ± 6[f]	318 ± 10[e]	206 ± 7	243 ± 8[f]	318 ± 12[e]
KWI (mg/g)	7.1 ± 0.2	7.9 ± 0.2[f]	12 ± 0.4[e]	7.0 ± 0.2	8.3 ± 0.2[c]	11 ± 0.4[e]

[a] Ami. rectal PD, amiloride-sensitive rectal potential difference; CWI, cardiac weight index; KWI, kidney weight index; DOCA, deoxycorticosterone acetate; $n = 6$ to 10 mice/group.
[b] $P < 0.01$, two *versus* one-renin gene mice.
[c] $P < 0.01$, NaCl *versus* tap water.
[d] $P < 0.05$ *versus* 1% NaCl.
[e] $P < 0.001$ *versus* 1% NaCl.
[f] $P < 0.05$, NaCl *versus* tap water.
[g] $P < 0.05$, two- *versus* one-renin gene mice.

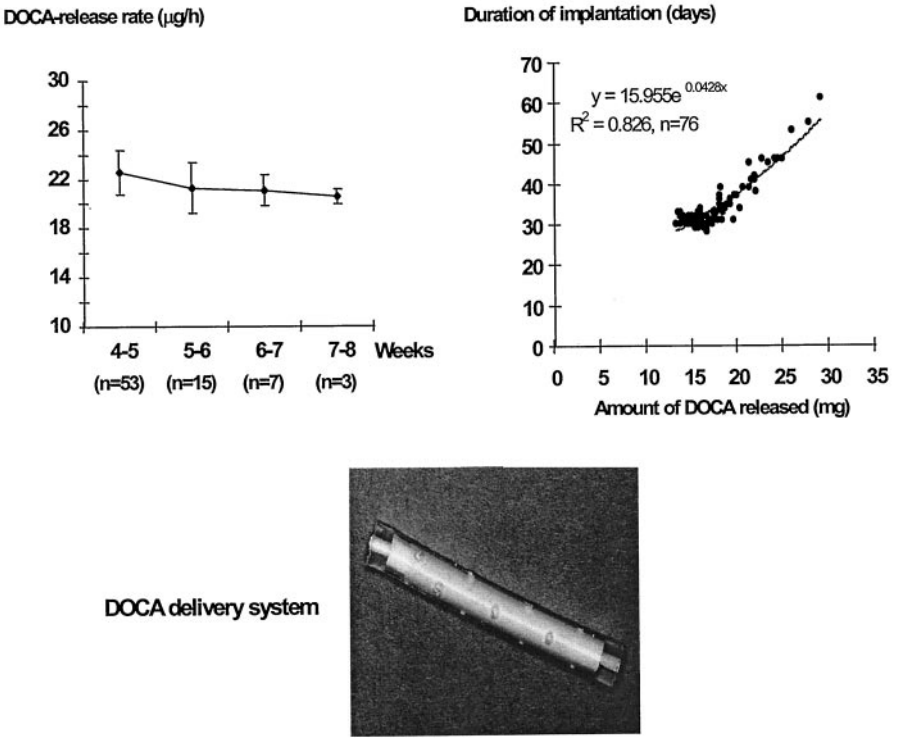

Figure 2. Characteristics of the deoxycorticosterone acetate (DOCA) delivery system. (Upper left) Constant DOCA release rate for several weeks of implantation. (Upper right) Relationship between the amount of DOCA released and the duration of the subcutaneous implantation. Values are means ± SEM. (Lower) Photograph of the homemade delivery system. The system is made of a silastic tube with sixteen 400-μm micropores. It is filled with 35 mg of DOCA and placed subcutaneously in the mouse abdomen.

stress in the mice then does decapitation or retro-orbital blood sampling. Indeed, plasma norepinephrine levels were sixfold higher with decapitation than with the use of a carotid artery catheter and were almost twofold higher with retro-orbital blood sampling (E. Grouzman, C. Cavadas, D. Grand, M. Moratel, J. F. Aubert, H. R. Brunner, and L. Mazzolai, submitted for publication).

Light Microscopy

Heart samples were fixed in 10% neutral buffered formol and embedded in paraffin. Four-micron sections were used for immunochemical analyses with anti-α-skeletal actin 1 (1:10 dilution in Tris-buffered saline) (15). Immunoperoxidase staining was performed essentially as described previously (16). After staining, sections were observed with a Zeiss Axiophot photomicroscope (Zeiss Vision, Oberkochen, Germany). Images were acquired with a high-sensitivity Photonic Coolview color camera (Zeiss), stored, and printed as described above. Images were subsequently analyzed by using KS400 software (Kontron System; Zeiss), as described previously (15).

Statistical Analyses

All results are presented as means ± SEM. Statistical comparisons between groups were performed with ANOVA, followed by *t* test or Dunnett's *t* test when appropriate. $P < 0.05$ was considered to be the minimal level of significance.

Results

Characteristics of the Animals

Table 1 presents the characteristics of the two strains used in these studies and investigated under various experimental conditions. The parameters were measured 4 wk after unilateral nephrectomy, in mice receiving normal mouse chow and either tap water or 1% saline solution as drinking fluid or 1% saline solution and DOCA. Under baseline experimental conditions, two-renin gene mice exhibited higher BP and much greater PRA and PRC values (Figure 3). Amiloride-sensitive rectal PD values were more negative for two-renin gene mice; in the latter mice, intravenous administration of the angiotensin II receptor antagonist irbesartan induced a marked decrease in BP (Figure 4). Cardiac weight indices were also significantly greater at baseline for two-renin gene mice, compared with the one-renin gene mouse strains.

Effects of Salt in One- and Two-Renin Gene Mice

In one-renin gene mice, use of 1% sodium chloride as the drinking fluid had little effect. Indeed, as expected, PRA values decreased slightly and amiloride-sensitive rectal PD values became less negative with salt loading. In addition, a slight but significant increase in the kidney weight index was observed.

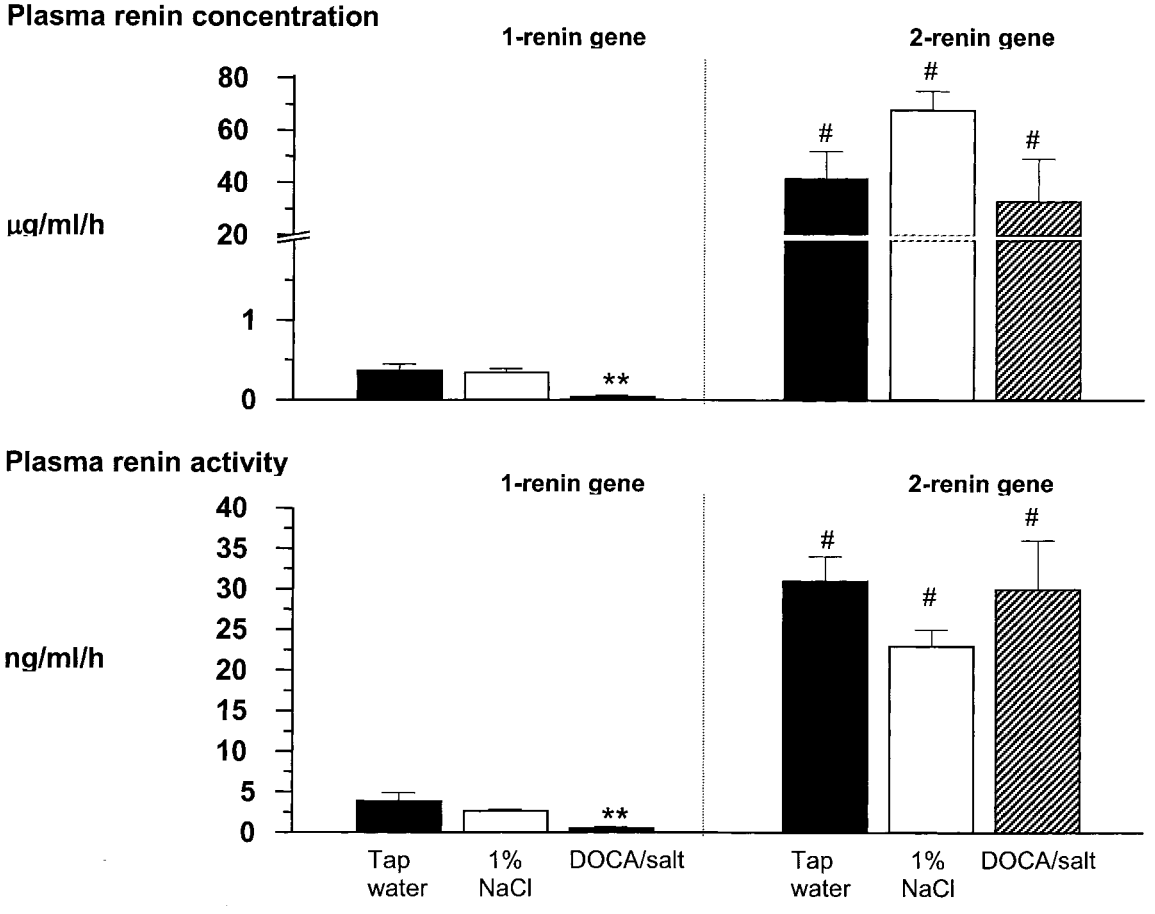

Figure 3. Plasma renin concentrations (upper) and plasma renin activities (lower) in one- and two-renin gene mice receiving tap water, 1% sodium chloride, or DOCA/salt. $^{**}P < 0.01$ *versus* tap water; $\#P < 0.001$ *versus* one-renin gene mice.

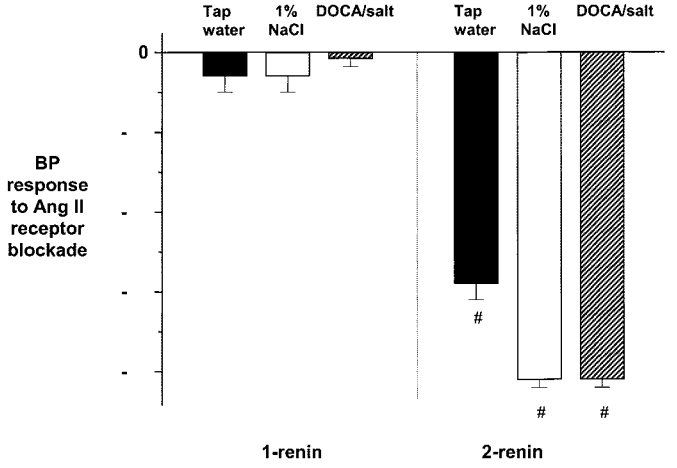

Figure 4. Acute changes in BP induced by intravenous administration of the angiotensin II (Ang II) receptor antagonist irbesartan (1.5 mg/kg, in 50 μl) in one- and two-renin gene mice. #$P < 0.001$ *versus* one-renin gene mice.

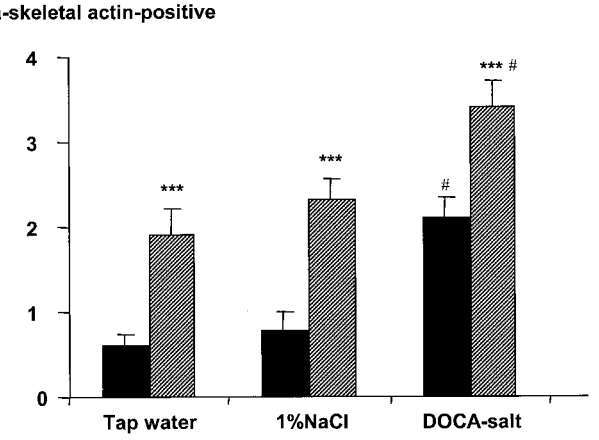

Figure 5. Percentages of α-skeletal actin-positive areas in heart histologic sections from one- and two-renin gene mice that received either tap water, 1% saline, or DOCA/salt. ▨, one-renin gene mice; ■, two-renin gene mice. Bars represent SEM ($n = 4$ or 5 animals). ***$P \leq 0.005$, one-renin gene *versus* two-renin gene mice; #$P \leq 0.01$, DOCA-treated animals *versus* tap water-treated animals with the same renin gene number.

In two-renin gene mice, in contrast, BP was significantly increased with the administration of sodium chloride. PRA and PRC values were not decreased with salt, and both cardiac and kidney indices were significantly increased (Figure 3). During salt-loading, the BP response to angiotensin II receptor blockade was still characterized by a marked decrease in BP in two-renin gene mice (Figure 4).

Effects of DOCA/Salt in One- and Two-Renin Gene Mice

Table 1 also presents the changes induced by the administration of DOCA and salt. As expected, with the administration of DOCA, marked decreases in amiloride-sensitive rectal PD values were observed in association with the development of hypokalemia in both strains. The administration of DOCA was also associated with the development of alkalosis, with blood pH increasing from 7.30 $\pm$ 0.04 to 7.43 $\pm$ 0.02 ($P = 0.01$, $n = 5$ to 7). In one-renin gene mice, PRA and PRC values were almost totally suppressed by the administration of DOCA/salt but BP was not affected (Figure 3). However, both cardiac and renal hypertrophy developed in those mice. In two-renin gene mice, DOCA induced a further increase in BP and the administration of irbesartan still caused a marked decrease in BP. Neither PRA nor PRC values were reduced by the administration of DOCA and salt (Figure 3). In those mice, DOCA produced an additional increase in cardiac and kidney weight indices. Because α-skeletal actin expression increases during cardiac hypertrophy, we evaluated α-skeletal actin expression in mouse myocardium with immunohistochemical staining (Figure 5). The area of positive staining was significantly increased in one-renin gene mice treated with DOCA/salt, compared with one-renin gene mice treated with tap water ($P \leq 0.01$). Moreover, under all experimental conditions, the sizes of α-skeletal actin-positive areas were greater in two-renin gene mice than in the corresponding one-renin gene mice, as demonstrated in Figure 5.

Effects of Losartan in One- and Two-Renin Gene, DOCA/Salt-Treated Mice

Table 2 presents the effects of losartan on BP, serum potassium levels, and cardiac and renal hypertrophy in one- and two-renin gene, DOCA/salt-treated mice. In one-renin gene mice, losartan had no significant effect on BP or serum potassium levels, although the BP response to exogenous angiotensin II was completely blocked. The cardiac weight index was significantly reduced by losartan, however. In two-renin gene mice, losartan induced a significant decrease in BP, which was associated with a marked decrease in the cardiac weight index and little if any change in the kidney weight index.

Discussion

Taken together, the main results of this study are that (*1*) mice with two renin genes exhibit higher, angiotensin II-dependent, baseline BP, in association with an increased cardiac weight index, (*2*) the presence of two renin genes markedly increases the BP, cardiac, and renal responses to salt, (*3*) the renin gene background modulates the response to DOCA/salt, and (*4*) DOCA/salt can induce renal and cardiac hypertrophy in the absence of any change in BP. Cardiac hypertrophy can be prevented by the administration of the angiotensin II AT_1 receptor antagonist losartan in one- and two-renin gene mice. This suggests that activation of AT_1 receptors contributes to the development of the cardiac hypertrophy induced by DOCA and salt.

An intact renin-angiotensin system is fundamental to the maintenance of basal BP in mice. Thus, several gene-targeting experiments in mice noted that mice lacking genes for angiotensinogen (17), angiotensin-converting enzyme (18), or the angiotensin type 1 receptor (19,20) exhibited lower BP, compared with wild-genotype mice. However, it has been more difficult to determine the relative contributions of the *Ren-1*^{d}

Table 2. Effects of losartan in one- and two-renin gene, DOCA/salt-treated mice with the same genetic background [N_{5-6}(129Ola/C57BL/6J)][a]

	One Renin Gene		Two Renin Genes	
	DOCA/Salt	DOCA/Salt + Losartan (0.2 mg/ml)	DOCA/Salt	DOCA/Salt + Losartan (0.2 mg/ml)
n	20	8	9	9
Body weight (g)				
initial	23.2 ± 0.2	22.7 ± 0.4	23.5 ± 0.4	23.7 ± 0.3
final	27.3 ± 0.4	27.3 ± 0.6	28.5 ± 0.6	30.5 ± 0.8
Mean BP (mmHg)	113 ± 3	109 ± 3	165 ± 4	121 ± 6[b]
Heart rate (beats/min)	575 ± 11	574 ± 17	587 ± 16	602 ± 26
Serum K^+ level (mM)	2.8 ± 0.15	2.8 ± 0.15	3.3 ± 0.2	3.4 ± 0.2
Cardiac weight (g)	133 ± 5	116 ± 3[c]	153 ± 3	138 ± 5[b]
CWI (mg/g)	4.96 ± 0.1	4.3 ± 0.07[c]	5.4 ± 0.1	4.5 ± 0.1[b]
Kidney weight (g)	330 ± 8	291 ± 14[d]	342 ± 11	352 ± 17
KWI (mg/g)	12.4 ± 0.3	10.7 ± 0.3[d]	12 ± 0.4	11.5 ± 0.3

[a] CWI, cardiac weight index; KWI, kidney weight index.
[b] $P < 0.001$ *versus* DOCA/salt.
[c] $P < 0.01$ *versus* DOCA/salt.
[d] $P < 0.05$ *versus* DOCA/salt.

and *Ren-2* genes to the maintenance of BP in mice. Bertaux *et al.* (21) observed no change in BP in mice with a disrupted *Ren-1*^{d} gene, suggesting that the product of the *Ren-2* gene is physiologically active and can compensate for the loss of *Ren-1*^{d}, supporting BP. In contrast, Clark *et al.* (22) observed that inactivation of the *Ren-1*^{d} gene decreased BP in female mice but not in male mice. The latter observation suggested that the *Ren-2* protein could not fully compensate for the loss of *Ren-1*^{d} in female mice, whereas the production of renin was sufficient to compensate for the decrease in active renin and the resulting decrease in BP in male mice, which express larger amounts of *Ren-2* in the submaxillary gland. No change in BP was observed in adult mice homozygous for the mutated *Ren-2* gene (10). Whereas most previous comparative studies were conducted with strains with different genetic backgrounds, in this study one-renin gene mice of the C57BL/6J strain were crossbred with 129Ola mice, which have two renin genes, to yield one- and two-renin gene mice with the same genetic background.

Our results clearly demonstrated that mice with two renin genes exhibited higher baseline BP. This finding was mainly attributable to overactivity of the renin-angiotensin-aldosterone cascade. Indeed, we observed that mice with two renin genes exhibited 100-fold higher PRA and PRC values. These differences cannot be attributed to more stressful conditions during sampling for two-renin gene mice, which could release great amounts of salivary gland renin, as suggested previously (23). Indeed, we have observed that catheter blood sampling is much less stressful than decapitation or retro-orbital blood sampling and produces the lowest serum catecholamine levels (E. Grouzmann, personal communication). Furthermore, selective blockade of angiotensin type 1 receptors induced a marked decrease in BP in two- but not one-renin gene mice. The amiloride-sensitive rectal PD, which is an indirect indicator of mineralocorticoid activity (11), was significantly more negative in two-renin gene mice receiving tap water, suggesting that aldosterone activity was also increased in those mice. As a direct consequence of the elevated BP, the cardiac weight index was significantly increased in two-renin gene mice. These observations thus demonstrate that the *Ren-2* gene is physiologically active in two-renin gene mice and participates in the increase in BP under basal conditions, even when the *Ren-1*^{d} gene is active. This finding is consistent with the results of recent studies demonstrating that overexpression of the mouse *Ren-2* gene induces severe hypertension in rats (24). However, the possibility that linked genes may still be producing strain effects and that some differences may not be attributable exclusively to the presence of the *Ren-2* gene cannot be excluded.

The second major finding of these experiments was that the presence of two renin genes markedly increased the sensitivity of the mice to salt. Indeed, in contrast to one-renin gene mice, mice with *Ren-1*^{d} and *Ren-2* genes became hypertensive when 1% NaCl was provided, instead of tap water, as the drinking fluid. The development of salt-sensitive hypertension in two-renin gene mice can most likely be explained by the fact that the renin-angiotensin system was not suppressed by the increased salt intake. Hall *et al.* (25) demonstrated in dogs that BP became salt-sensitive when the activity of the renin-angiotensin system could not be adapted to the sodium intake. Interestingly, two-renin gene mice receiving 1% salt solution to drink became even more dependent on the activity of the renin-angiotensin system, as demonstrated by the greater decrease in BP induced by AT_1 receptor blockade. Whether this is attributable to higher levels of angiotensin II or to upregulation of AT_1 receptors cannot be concluded from these stud-

ies. However, it is also interesting to note that amiloride-sensitive rectal PD values became less negative with a high-salt diet even in two-renin gene mice, suggesting decreased activity of the epithelial sodium channel in the colon. We previously reported that high angiotensin II levels decrease and AT_1 receptor blockade increases amiloride-sensitive rectal PD values in mice (11).

As expected on the basis of the increase in BP, two-renin gene mice receiving salt developed even more cardiac hypertrophy than under normal conditions. However, with respect to renal hypertrophy, increased kidney weight indices were observed for both one- and two-renin gene mice. High dietary salt intake has been demonstrated to induce cardiac hypertrophy and collagen deposition in rats (26,27). With a very high-salt diet (8% NaCl), increased kidney weights have also been documented in rats (28). The effects of salt itself in mice, particularly after uninephrectomy, have not been reported. These results are thus the first to demonstrate that even a modest increase in salt intake induces renal hypertrophy in uninephrectomized mice.

To further investigate the role of renin genes in the development of hypertension in mice, the DOCA/salt model was developed in one- and two-renin gene mice. The deoxycorticosterone/salt hypertension model is a low-renin, salt-dependent model of acquired experimental hypertension that has been extensively used to investigate the epithelial and extra-epithelial effects of mineralocorticoids (29). In contrast to previous studies, we administered DOCA subcutaneously, using a homemade delivery system (Figure 2), and not as a pellet. Our system provides a constant release of DOCA for several weeks. The marked decrease in serum potassium levels and the decrease in amiloride-sensitive rectal PD values clearly demonstrated that a hypermineralocorticoid state was achieved with this system. Despite the development of severe hypokalemia, no increase in BP with the administration of DOCA was observed in one-renin gene mice, whereas an additional increase in BP was measured in two-renin gene animals. PRA and PRC values were totally suppressed in one-renin gene mice but were not affected in two-renin gene mice, again suggesting that renin production in the latter mice was not suppressed by salt or DOCA. Moreover, the BP response to AT_1 receptor blockade remained greater in two-renin gene mice. This finding may be attributable to upregulation of AT_1 receptors or increased production of angiotensin II. Several previous articles suggested that mineralocorticoids could promote upregulation of AT_1 receptors (30,31). Our finding would be in accordance with that hypothesis. The reason why one-renin gene mice remain normotensive with the administration of DOCA/salt, despite the development of hypokalemia, is not yet clear. In this respect, mice differ from rats, which also have one renin gene but still develop low-renin hypertension with DOCA/salt (32). Because BP was measured several hours after anesthesia, small changes in BP soon after surgery might have been masked. The lack of changes in BP with the administration of the angiotensin II receptor antagonist losartan suggests that the mice were not particularly stressed. Another possible explanation involves the lower dose of DOCA used in these experiments, although a hypermineralocorticoid state was clearly obtained in our animals. Of note, most reported DOCA/salt-treated mouse models of hypertension have been created with two-renin gene mice (1–3).

The last finding of our study was that DOCA/salt induced cardiac and renal hypertrophy in both one-renin gene normotensive and two-renin gene hypertensive mice. This DOCA-induced hypertrophic response is in agreement with several other reports demonstrating that hypermineralocorticoid states are associated with cardiac hypertrophy and cardiac fibrosis in rodents (26–30). We did not detect myocardial fibrosis with light microscopy. However, two-renin gene mice were characterized by a significant increase in α-skeletal actin expression, which is an early marker of the development of myocardial muscle hypertrophy (33–35). The DOCA/salt-treated mouse model constitutes an additional example of the induction of α-skeletal actin expression in normotensive animals, as described for mice that overexpressed angiotensinogen exclusively in the heart (36). As mentioned above, there is evidence suggesting that mineralocorticoids could promote the upregulation of AT_1 receptors (30,31). Our observation that losartan prevents the development of cardiac hypertrophy in both normotensive one-renin gene and hypertensive two-renin gene mice supports the hypothesis that DOCA-induced cardiac hypertrophy is mediated by activation of angiotensin II AT_1 receptors.

In summary, this study demonstrates that the number of renin genes is an important determinant of BP and the responses to salt and DOCA in mice. Therefore, careful characterization of the renin gene background should be performed whenever mice are used to investigate the pathophysiologic features of hypertension. Our data also suggest that the *Ren-2* gene, which controls renin production mainly in the submaxillary gland and to a lesser degree in the kidney, is physiologically active in mice and is not subject to the usual negative feedback control. Finally, our data provide further evidence that mineralocorticoids promote cardiac and renal hypertrophy even in the absence of BP changes. Activation of angiotensin II AT_1 receptors seems to contribute to the DOCA/salt-induced cardiac hypertrophy.

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References

1. Johns C, Gavras I, Handy DE, Salomao A, Gavras H: Models of experimental hypertension in mice. *Hypertension* 28: 1064–1069, 1996
2. Gross V, Lippoldt A, Luft FC: Pressure diuresis and natriuresis in DOCA-salt mice. *Kidney Int* 52: 1364–1368, 1997
3. Rhaleb NE, Peng H, Alfie ME, Shesely EG, Carretero OA: Effect of ACE inhibitor on DOCA-salt and aortic coarctation-induced hypertension in mice: Do kinin B_2 receptors play a role? *Hypertension* 33: 329–334, 1999
4. Dickinson DP, Gross KW, Piccini N, Wilson CM: Evolution and variation of renin genes in mice. *Genetics* 108: 651–667, 1984

5. Sigmund CD, Gross KW: Structure, expression, and regulation of the murine renin genes. *Hypertension* 18: 446–457, 1991
6. Field LJ, Gross KW: *Ren-1* and *Ren-2* loci are expressed in mouse kidney. *Proc Natl Acad Sci USA* 82: 6196–6200, 1985
7. Bing J, Poulsen K, Hackenthal E, Rix E, Taugner R: Renin in the submaxillary gland: A review. *J Histochem Cytochem* 28: 874–880, 1980
8. Pradervand S, Wang Q, Burnier M, Beermann F, Horisberger JD, Hummler E, Rossier BC: A mouse model for Liddle's syndrome. *J Am Soc Nephrol* 10: 2527–2533, 1999
9. Peng H, Carretero OA, Alfie ME, Masura JA, Rhaleb NE: Effects of angiotensin-converting enzyme inhibitor and angiotensin type 1 receptor antagonist in deoxycorticosterone acetate salt hypertensive mice lacking *ren-2* gene. *Hypertension* 37: 974–980, 2001
10. Sharp MGF, Fettes D, Brooker G, Clark AF, Peters J, Fleming S, Mullins JJ: Targeted inactivation of the *Ren-2* gene in mice. *Hypertension* 28: 1126–1131, 1996
11. Wang Q, Horisberger JD, Maillard M, Brunner HR, Rossier BC, Burnier M: Salt- and angiotensin II-dependent variations in amiloride-sensitive rectal potential difference in mice. *Clin Exp Pharmacol Physiol* 27: 60–66, 2000
12. Mazzolai L, Pedrazzini T, Nicoud F, Gabbiani G, Brunner HR, Nussberger J: Increased cardiac angiotensin II levels induce right and left ventricular hypertrophy in normotensive mice. *Hypertension* 35: 985–991, 2000
13. Wiesel P, Mazzolai L, Nussberger J, Pedrazzini T: Two-kidney, one clip and one-kidney, one clip hypertension in mice. *Hypertension* 29: 1025–1030, 1997
14. Nussberger J, Fasanella d'Amore T, Porchet M, Waeber B, Brunner DB, Brunner HR, Kler L, Brown AN, Francis RJ: Repeated administration of the converting enzyme inhibitor cilazapril to normal volunteers. *J Cardiovasc Pharmacol* 9: 39–44, 1987
15. Clement S, Chaponnier C, Gabbiani G: A subpopulation of cardiomyocytes expressing α-skeletal actin is identified by a specific polyclonal antibody. *Circ Res* 85: 51e–58e, 1999
16. Schurch W, Skalli O, Seemayer TA, Gabbiani G: Intermediate filament proteins and actin isoforms as markers for soft tissue tumor differentiation and origin. I. Smooth muscle tumors. *Am J Pathol* 128: 91–103, 1987
17. Tanimoto K, Sugiyama F, Goto Y, Ishida J, Takimoto E, Yagami K, Fukamizu A, Murakami K: Angiotensinogen-deficient mice with hypotension. *J Biol Chem* 269: 31334–31337, 1994
18. Krege JH, Jihn SWM, Langenbach LL, Hodgin JB, Hagaman JR, Bachman ES, Jennette JC, O'Brien DA, Smithies O: Male-female differences in fertility and blood pressure in ACE-deficient mice. *Nature (Lond)* 375: 146–148, 1995
19. Sugaya T, Nishimatsu SI, Tanimoto E, Yamagishi T, Imamura K, Goto S, Imaizumi K, Hisada Y, Otsuka A, Uchida H, Sugiura M, Fukuta K, Fukamitsu A, Murakami K: Angiotensin-II type 1a receptor-deficient mice with hypotension and hyperreninemia. *J Biol Chem* 270: 18719–18722, 1995
20. Ito M, Oliveiro ML, Mannon PJ, Best CF, Maeda N, Smithies O, Coffman TM: Regulation of blood pressure by the type 1A angiotensin II receptor gene. *Proc Natl Acad Sci USA* 92: 3521–3525, 1995
21. Bertaux F, Colledge WH, Smith SE, Evans M, Samani NJ, Miller CCJ: Normotensive blood pressure in mice with a disrupted renin *Ren-1^d* gene. *Transgenic Res* 6: 191–196, 1997
22. Clark AF, Sharp MGF, Morley SD, Fleming S, Peters J, Mullins JJ: Renin-1 is essential for normal renal juxtaglomerular cell granulation and macula densa morphology. *J Biol Chem* 272: 18185–18190, 1997
23. Poulsen K, Pedersen EB: Increase in plasma renin in aggressive mice originates from kidneys, submaxillary and other salivary glands, and bites. *Hypertension* 5: 180–184, 1983
24. Mullins JJ, Peters J, Ganten D: Fulminant hypertension in transgenic rats harboring the mouse *Ren-2* gene. *Nature (Lond)* 344: 541–544, 1990
25. Hall JE, Guyton AC, Smith MJ Jr, Coleman TG: Blood pressure and renal function during chronic changes in sodium intake: Role of angiotensin. *Am J Physiol* 239: F271–F280, 1980
26. Frohlich ED, Chien Y, Sesoko S, Pegram BL: Relationship between dietary sodium intake, hemodynamics, and cardiac mass in SHR and WKY rats. *Am J Physiol* 264: R30–R34, 1993
27. Brilla CG, Pick R, Tan LB, Janicki JS, Weber KT: Remodelling of the rat right and left ventricles in experimental hypertension. *Circ Res* 67: 1355–1364, 1990
28. Yu HCM, Burel LM, Black MJ, Wu LL, Dilley RJ, Cooper ME, Johnston CI: Salt induces myocardial and renal fibrosis in normotensive and hypertensive rats. *Circulation* 98: 2621–2628, 1998
29. Young M, Fullerton M, Dilley R, Funder J: Mineralocorticoids, hypertension and cardiac fibrosis. *J Clin Invest* 93: 2578–2583, 1994
30. Robert V, Heymes C, Silvestre JS, Sabri A, Swynghedauw B, Delcayre C: Angiotensin AT_1 receptor subtype as a cardiac target of aldosterone: Role in aldosterone-salt-induced fibrosis. *Hypertension* 33: 981–986, 1999
31. Wang Q, Hummler E, Maillard M, Nussberger J, Rossier BC, Brunner HR, Burnier M: Compensatory upregulation of angiotensin II AT_1 receptors in αENaC knockout heterozygous mice. *Kidney Int* 59: 2216–2221, 2001
32. Gavras H, Brunner HR, Laragh JH, Vaughan ED Jr, Koss M, Cote LJ, Gavras I: Malignant hypertension resulting from deoxycorticosterone acetate and salt excess: Role of renin and sodium in vascular changes. *Circ Res* 36: 300–309, 1975
33. Schwartz K, Boheler KR, de la Bastie D, Lompre AM, Mercadier JJ: Switches in cardiac muscle gene expression as a result of pressure and volume overload. *Am J Physiol* 262: R364–R369, 1992
34. Schwartz K, de la Bastie D, Bouveret P, Oliviero P, Alonso S, Buckingham M: α-Skeletal muscle actin mRNA's accumulate in hypertrophied adult rat hearts. *Circ Res* 59: 551–555, 1986
35. Winegrad S, Wisnewsky C, Schwartz K: Effect of thyroid hormone on the accumulation of mRNA for skeletal and cardiac α-actin in hearts from normal and hypophysectomized rats. *Proc Natl Acad Sci USA* 87: 2456–2460, 1990
36. Clément S, Pellieux C, Chaponnier C, Pedrazzini T, Gabbiani G: Angiotensin II stimulates α-skeletal actin expression in cardiomyocytes *in vitro* and *in vivo* in the absence of hypertension. *Differentiation* 2002, in press