Abnormal regulation of proximal tubule renin mRNA in the Dahl/Rapp salt-sensitive rat

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Background. The precise pathogenesis of salt-sensitive hypertension in the Dahl rat is unknown. Abnormalities in renal hemodynamics and NaCl handling have been implicated, and may relate to changes in the activity of the intrarenal renin-angiotensin system.

Methods. Circulating, juxtaglomerular and intrarenal (glomerular and proximal tubular) renin were studied in Dahl/Rapp salt-sensitive and salt-resistant rats fed with a normal (0.5%) or high (4%) NaCl diet. Circulating and juxtaglomerular renin were assessed by measurement of plasma renin activity and renin secretory rates. Glomerular and proximal tubular renin mRNA were assessed by microdissection and quantitative competitive RT-PCR.

Results. Circulating and juxtaglomerular renin were suppressed by high dietary NaCl in salt-sensitive rats (plasma renin activity, 0.5%, 10.9 ± 0.7 vs. 4%, 7.9 ± 0.3 ng/ml/hr, P < 0.05; renin secretory rate, 0.5% 220 ± 32 vs. 4%, 58 ± 5 ng/mg/hr, P < 0.05). Glomerular renin mRNA was also suppressed by the higher salt diet in salt-sensitive animals (0.5%, 411 ± 84 vs. 4%, 67 ± 22 × 10³ copies/glomerulus, P < 0.05). In contrast, proximal tubular renin was not suppressed by a high NaCl diet in salt-sensitive animals (0.5%, 13.9 ± 2.7 vs. 4%, 12.1 ± 3.6 × 10³ copies/mm tubule, P = NS), but was suppressed in salt-resistant rats (0.5%, 9.5 ± 2.8 vs. 4%, 3.2 ± 1.2 × 10³ copies/mm, P < 0.05).

Conclusions. Failure to suppress proximal tubular renin in response to high dietary NaCl may result in increased local generation of angiotensin II and enhanced proximal tubular NaCl absorption, and thereby contribute to the generation of salt sensitive hypertension.

Key words: glomerulus, renin-angiotensin system, angiotensin II, hypertension, sodium chloride.

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The pathogenesis of human primary hypertension is unknown. Available evidence from multiple sources suggests that blood pressure is a complex quantitative trait with polygenic and environmental determinants. In many rodent models of genetic hypertension, the kidney plays a pivotal role in the development and/or maintenance of high blood pressure. For example, in the Dahl rat salt-sensitive hypertension is associated with several derangements in renal NaCl handling, including a blunted natriuretic response to an acute NaCl load and a shifted pressure-natriuresis relationship [1–6]. At least part of this aberrant homeostatic mechanism resides primarily in the kidney as suggested by cross transplantation studies, where the hypertensive phenotype can be induced in a normotensive host upon receiving the homograft from a hypertensive donor [7–10], and by the finding of altered sodium handling in isolated perfused kidneys and other models where neuroendocrine influences are eliminated [2, 5, 6]. However, the exact mechanisms that underlie the kidney’s contribution to the generation or maintenance of hypertension remain unclear.

Angiotensin II (Ang II) is an important mediator of renal adaptations in response to alterations in dietary NaCl, affecting both glomerular and tubular function in order to regulate NaCl excretion and restore NaCl balance [11, 12]. Abnormalities in the renin-angiotensin system (RAS) could therefore contribute to the genesis of experimental salt-sensitive hypertension. In support of this hypothesis, a renin gene allele has been found to cosegregate with the salt-sensitive hypertensive phenotype and to impact on blood pressure in a dose-dependent manner in the Dahl rodent model [13]. While many studies have demonstrated suppression of the circulating RAS in salt-sensitive animals, arguing against a central role for the RAS [2, 14–20], the relative activity of the more recently described local, intrarenal RASs in salt-sensitive hypertension has not been examined.

Complete intrarenal RASs have been described in the glomerulus and the proximal tubule, and are distinct from the juxtaglomerular apparatus that is largely responsible for systemic renin responses [21–23]. We have previously demonstrated that in normal Sprague-Dawley rats these intrarenal renin systems are regulated by and may mediate glomerular and proximal tubular adaptations in response to...
changes in dietary NaCl [24]. Because the majority of renal NaCl reabsorption occurs in the proximal tubule, altered activity of this local RAS in particular might contribute to the abnormal NaCl handling characteristic of experimental salt-sensitive hypertension. To examine this hypothesis, we studied the responses of the circulating, juxtaglomerular, glomerular and proximal tubule renin systems to normal and high levels of dietary NaCl in the inbred Dahl/Rapp salt-sensitive rat and its genetic control, the salt-resistant rat.

METHODS

Experimental design

Four-week-old male inbred Dahl/Rapp salt sensitive (SS) and salt resistant (SR) animals (Harlan Sprague Dawley, Indianapolis, IN, USA) were allowed ad libitum access to tap water and normal (0.5%, TD90155) or high (4%, TD90156) NaCl diets (Harlan-Teklad, Madison, WI, USA). Standard rat chow from this vendor contains between 0.4% and 0.8% NaCl. After two weeks, tail cuff blood pressures were obtained and animals were sacrificed for measurement of plasma renin activity (PRA), renin secretory rates (RSR), or microdissection and measurement of glomerular and proximal tubular renin mRNA by quantitative competitive reverse transcription-polymerase chain reaction (RT-PCR).

Blood pressure measurements

Tail cuff blood pressure was measured in awake, unanesthetized SS and SR rats at 37°C with a photoelectric sensor controlled by a programmable pump and coupled to an amplifier (Model 59; IITC, Woodland Hills, CA, USA). Each measurement represents the mean of three to five consecutive measurements in each animal.

Renin studies

Animals were sacrificed by decapitation, and trunk blood and thin renal cortical slices were immediately obtained for measurement of PRA (N = 8 each group) and renin secretory rates (N = 6 each group). Cortical slices (dry wt ≈2 mg) were placed in 15 ml of 37°C KRB (pH 7.4) and preincubilated with 95% O₂-5% CO₂. Following a 15 minute incubation, fresh KRB was added and the slices were further incubated in an oscillating water bath at 37°C for 90 minutes, at which time aliquots were collected for measurement of renin activity. Measurement of renin activity was performed using the antibody trapping method of Poulsen and Jorgensen [25]. Renin activity is expressed as the total amount of angiotensin I generated per hour and in cortical slices is corrected for dry tissue weight.

Microdissection of glomeruli and proximal tubules

Animals were anesthetized with pentobarbital sodium (50 to 60 mg/kg i.p.), and the left kidney perfused with a Heps-buffered solution containing 1% collagenase and 0.5% BSA as previously described [21]. Cortical slices were prepared and incubated in the same solution for 30 minutes at 37°C. Glomeruli and proximal tubular segments were dissected at 4°C. Care was taken to remove all visible attached arteriolar material in the dissection of glomeruli, and dissection time was limited to one hour. Samples were placed in 1 ml of GTC buffer (4 m M GTC, 25 m M sodium acetate, pH 6.0, 0.8% β-mercaptoethanol), snap frozen in liquid nitrogen and stored at −70°C prior to RNA isolation. As a control for background contamination, 30 μl of dissection solution was obtained after dissection and subjected to the subsequent RNA isolation, cDNA synthesis and PCR procedures.

RNA isolation and cDNA synthesis

Tubular and glomerular RNA were isolated as previously described [22]. RNA was retrieved as a 120,000 g pellet (Beckman TL-100 ultracentrifuge; TLS-55 rotor; 55,000 rpm; 16°C; 6 hr) along with 20 μg of E coli rRNA (Boehringer Mannheim, Indianapolis, IN, USA) underneath a discontinuous CsCl gradient (1 ml 97% CsCl bottom, 200 μl 40% CsCl top), resuspended in 0.3 mM sodium acetate and ethanol precipitated.

cDNA was synthesized in a buffer containing: 3 mM Mg²⁺, oligo (15) dt 5 μM, RNasin 40 U, dNTPs 1 mM and DTT 5 mM. The mixture was incubated at 65°C for five minutes and chilled on ice prior to the addition of 200 units of murine Molony leukemia virus reverse transcriptase (RT) (Promega Corp, Madison WI, USA). RT was omitted in RT-controls. The RT profile was as follows: anneal 25°C × five minutes, extension 42°C × 60 minutes, and termination 99°C × five minutes.

Renin quantitative competitive PCR

Polymerase chain reactions (PCR) were performed in a thermal cycler (MJ Research, Watertown, MA, USA) in a total volume of 50 μl containing 0.2 μM primers (forward 5’-TGCCACCTTTGTGTGAGG-3’; reverse 5’-ACCC GATGCGATTGTTATGCGG-3’), 4 mM MgCl₂, 200 μM dNTP, enzyme buffer, and 2.5 U of Taq DNA polymerase (all from Promega Corp.). The primers were previously shown to be in separate exons by the absence of PCR product of predicted size in amplifications of rat genomic DNA (data not shown). The PCR profile was as follows: hot start 94°C × two minutes, 35 cycles 94°C × 30 seconds, 60°C × 60 seconds, 72°C × 75 seconds; final extension 72°C × five minutes. Negative controls included dissection media subjected to RT-PCR, water subjected to PCR, and aliquots from each RNA-containing sample with RT omitted. All negative controls failed to generate PCR products (data not shown).

A mutant renin template (internal 142 bp deletion) was prepared from renin cDNA by composite primers as an internal standard as described previously [21]. Aliquots of
sample cDNA from $\frac{1}{2}$ to 1 glomerulus or 3 mm of proximal tubule were competitively amplified against a fivefold step dilution series of mutant template, and consistently produced two bands of the predicted size (renin 374 bp, mutant 232 bp). PCR products were size-fractionated by agarose gel electrophoresis, transferred to nylon membranes, and probed with a $^{32}$P end-labeled internal oligonucleotide common to both templates ($5'$-GCTTGCATGATCAACTGCAGG-3'). Hybridizations were performed as previously described. PCR products were quantified by autoradiography densitometry, and the ratio of renin and mutant product plotted against the starting amount of mutant template. Linear regression lines were calculated, and the number of molecules of renin cDNA in each sample was determined by a renin/mutant ratio of one. In pilot studies, the ability of the quantitative RT-PCR assay to reliably detect known differences in starting amounts of cDNA was verified, and the assay was shown to be accurate in detecting differences as small as 50% (data not shown).

**Statistical analysis**

Data are expressed as mean ± SEM. Comparisons between the various groups were performed using one way analysis of variance with Student-Newman-Keuls analysis. Statistical significance was defined as a $P$ value < 0.05.

**RESULTS**

Mean arterial pressures in salt-sensitive (SS) and salt-resistant (SR) animals after two or three weeks on a normal (0.5%) or high (4%) NaCl diet. Values are means ± SEM. *$P < 0.05$ versus SR on same diet; †$P < 0.05$ versus 0.5% NaCl. Symbols are: (▼) SS 4%; (▲) SS 0.5%; (●) SR 4%; (■) SR 0.5%.

![Graph showing mean arterial pressures in SS and SR animals after two or three weeks on normal or high NaCl diet.](image)

Table 1. Effect of dietary NaCl on circulating and juxtaglomerular renin

<table>
<thead>
<tr>
<th>Rat strain</th>
<th>Plasma renin activity (ng/mg/hr)</th>
<th>Renin secretory rate (ng/mg/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SR 0.5%</td>
<td>18.7 ± 1.3</td>
<td>911 ± 83</td>
</tr>
<tr>
<td>SR 4%</td>
<td>10.0 ± 0.3*</td>
<td>426 ± 42*</td>
</tr>
<tr>
<td>SS 0.5%</td>
<td>10.9 ± 0.7</td>
<td>220 ± 32</td>
</tr>
<tr>
<td>SS 4%</td>
<td>7.9 ± 0.3*</td>
<td>58 ± 5*</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Abbreviations are SR, salt resistant; SS, salt sensitive. *$P < 0.05$ vs. 0.5% diet; †$P < 0.05$ vs. SR on same diet.

Mean arterial pressures in SS animals were still evolving at the two-week time point, when systemic and local renin responses were assessed, these studies reflect the "generation" rather than the "maintenance" phase of salt-induced hypertension.

Renin secretory rate (RSR; Table 1), reflecting the activity of the juxtaglomerular renin system, was suppressed by excess dietary NaCl in both SS and SR animals. On a high salt diet, RSR decreased by 53% in SS animals, and by 74% in SS animals. RSR was also markedly reduced in SS compared to SR animals on both diets (0.5%, 4-fold reduction; 4%, 7-fold reduction). A similar pattern was seen with plasma renin activity (PRA; Table 1). High dietary NaCl resulted in suppression of PRA in both SR and SS rats, and PRA levels were lower in SS compared to SR rats on both diets. Thus, SS rats have lower baseline circulating and juxtaglomerular renin compared to SR rats, and high dietary sodium suppressed the circulating and juxtaglomerular renin systems in both strains.

In view of the fact that the endocrine RAS is suppressed in SS animals on a high salt diet, it is not likely to be pathogenic for the hypertensive phenotype. However, a disparate activation of the local renal RASs may nevertheless contribute to hypertension. To investigate the possibility that the intrarenal RAS behaved differently than the endocrine RAS in response to high dietary NaCl, renin transcript levels were measured in microdissected glomeruli and PT using quantitative RT-PCR. Southern blots and linear quantitation of competitive PCR from representative experiments in microdissected glomeruli are shown in Figure 2. Figure 3 is a summary of the data from all the animals studied ($N = 7$ to 11, each group). High dietary NaCl resulted in a sixfold suppression of glomerular renin mRNA in SS animals (in $10^3$ copies/glomerulus, 0.5% 411 ± 84 vs. 4% 67 ± 22, $P < 0.05$). In contrast, glomerular
renin transcript abundance was unaffected by dietary sodium in SR animals (in \(10^3\) copies/glomerulus, 0.5\% 909 ± 140 vs. 4\% 871 ± 151, \(P = \text{NS}\)). On both the normal and high salt diets glomerular renin gene expression was significantly reduced in SS compared to SR animals (0.5\%, 2.2-fold suppression; 4\%, 13-fold suppression).

A markedly different pattern was seen in the proximal tubule, where dietary NaCl excess resulted in suppression of tubular renin mRNA in SR but not SS animals. One set of representative Southern blots and linear quantitations is shown in Figure 4, while Figure 5 summarizes the data from all animals studied (\(N = 7\) to 11, each group). A high NaCl diet decreased proximal tubular renin mRNA by 66\% in SR animals (in \(10^3\) copies/mm tubule, 0.5\% 9.5 ± 2.8 vs. 4\% 3.2 ± 1.2, \(P < 0.05\)). In contrast, tubular renin gene expression was not down-regulated by dietary sodium in SS animals (in \(10^3\) copies/mm tubule, 0.5\% 13.9 ± 2.7 vs. 4\% 12.1 ± 3.6, \(P = \text{NS}\)). Furthermore, on the high sodium diet tubular renin transcript levels were nearly fourfold higher in SS compared to SR animals.

**DISCUSSION**

The precise mechanisms underlying salt-induced hypertension in the Dahl rat remain unclear despite decades of study. Cross transplantation studies indicate that the kidney plays an integral role [7–10], and abnormalities in renal hemodynamics [1, 28–32] and NaCl handling [1–6] have been implicated in the pathogenesis of salt-sensitive hypertension. As angiotensin II plays a critical role in the modulation of renal hemodynamics and NaCl excretion, abnormalities in the RAS could conceivably contribute to these functional derangements as well as salt-induced hypertension. Inbred SS Dahl rats manifest differences in the renin and angiotensin converting enzyme (ACE) genomic sequences that cosegregate with blood pressure [13, 33], but it is unclear whether these genetic differences result in changes in the activity of the RAS that actually contribute to hypertension.

Our findings of suppression of circulating and juxtaglomerular renin in SS animals compared to SR are consistent with previous studies showing reductions in PRA as well as...
total renal renin activity, renin release and renin mRNA in SS animals [2, 14–20]. Since juxtaglomerular cells are responsible for the vast majority of total renal renin synthesis, studies of whole kidney or cortical renin, like renin secretory rates, primarily reflect the activity of the juxtaglomerular renin system. Suppression of circulating and juxtaglomerular renin likely reflect activation of renal baroreceptors (via increases in perfusion pressure) or macula densa mechanisms (via increased loop chloride reabsorption [5, 6, 34]), both of which down-regulate juxtaglomerular renin secretion. While arguing against a central role for the endocrine RAS in the pathogenesis of abnormal renal hemodynamics or salt sensitive hypertension, these findings do not exclude the potential for altered activity of the paracrine/autocrine intrarenal RAS’s.

Recent evidence suggests that all the components of the RAS exist locally in both the glomerulus and the proximal tubule, with the potential for local generation of Ang II and regulation of function in an autocrine or paracrine fashion [21, 23]. The existence of an intrarenal RAS is further
supported by the finding that angiotensin concentrations in glomerular filtrate and in the proximal tubular lumen are 100- to 1,000-fold higher than those found in plasma [35–37]. While the functional significance of these local RASs has not been fully defined, in vivo microperfusion studies strongly support a functional role of the proximal tubule RAS in transport regulation [38]. From a quantitative standpoint the amount of renin made in the proximal tubule is very small, and the physiological significance of locally produced renin in local Ang II generation has not yet been clearly demonstrated. Nevertheless, local glomerular and proximal tubule renin gene expression have been shown to be regulated by chronic changes in dietary NaCl in normal Sprague-Dawley rats [24], and to be regulated independent of the endocrine RAS in the uninephrectomized rat [22]. The present studies are the first to uncouple the regulation of juxtaglomerular and local intrarenal renin in the setting of systemic hypertension.

In the proximal tubule Ang II is a potent stimulator of NaCl and HCO₃ absorption, and locally produced Ang II has been shown to modulate rat proximal tubular transport [38]. In the present studies, proximal tubular renin mRNA was suppressed by dietary sodium in SR animals, consistent with previous studies in normal Sprague-Dawley rats [24]. In contrast, tubular renin transcript levels were not suppressed by a high salt diet in SS animals, and were significantly higher than those seen in SR animals on that diet. In the present study, we make the tacit presumption that changes in renin transcript result in changes in renin protein. This failure to suppress tubular renin in response to dietary NaCl could conceivably result in enhanced local generation of Ang II, increased proximal tubular NaCl and water reabsorption, and generation of hypertension in these animals. The absence of suppression of brush border Na-H antiporter activity in SS animals fed a high salt diet are consistent with such an increased Ang II effect [39].

The abnormal renal NaCl handling seen in SS Dahl rats includes blunted pressure-natriuresis relationships and, in some but not all studies, impaired handling of an acute NaCl load [1–6]. The tubular segments and mechanisms responsible for these differences have not been fully defined. Reductions in glomerular filtration rate, blunted renal vasodilatory responses, and enhanced water and chloride reabsorption in the proximal tubule and loop of

![Fig. 4. Southern blots and linear regression from representative competitive quantitative RT-PCR experiments in proximal tubule (PT) after two weeks of 0.5% and 4% NaCl diets.](image_url)
Henle have all been implicated. While micropuncture studies addressing segmental renal NaCl handling in SS rats have primarily found increased chloride uptake by the loop of Henle [5, 6, 34], studies of Na-H antiporter activity in brush border membranes do support a possible role for the proximal tubule [39]. In addition, fractional urinary lithium excretion is reduced in volume expanded pre-hypertensive SS animals, consistent with a component of increased proximal tubule reabsorption [40].

Local glomerular renin mRNA is suppressed by dietary sodium in SS animals and is therefore unlikely to play a pathogenetic role in glomerular injury or the development of hypertension. Other studies of SS animals have shown that glomerular capillary pressure, proteinuria and glomerular structural damage are not affected by angiotensin converting enzyme inhibition [41], also arguing against a significant role for Ang II in mediating glomerular changes.

The absence of suppression of glomerular renin by high dietary NaCl in SR animals is of unclear significance, and differs from previous findings in normal Sprague-Dawley rats [24]. It is possible that this reflects an intrinsic difference between the Sprague-Dawley and the Dahl rat. Suppression of glomerular renin by dietary NaCl in the Dahl rat may depend on the presence of systemic hypertension seen in the Dahl SS strain, perhaps as a result of related changes in glomerular hemodynamics. Along similar lines,
hypertension-related increases in delivery of filtrate to the proximal tubule could conceivably contribute to changes in tubular renin mRNA in SS animals. Further studies of local renin gene expression in SS animals in whom blood pressure elevations are controlled would be of interest in addressing these issues.

Our data suggest that tubular but not glomerular renin may be important in the generation of salt retention and hypertension in the Dahl rat, despite the fact that from a quantitative standpoint renin is much more abundant in the glomerulus than in the proximal tubule. One explanation may relate to the relative availability of other components of the RAS in the microenvironments of the glomerulus and proximal tubule. For example, in situ hybridization studies demonstrate that intrarenal angiotensinogen mRNA is primarily localized to the proximal tubule, with much smaller amounts in the glomerular tuft [42]; ACE activity also is low in the glomerulus compared to the proximal tubule [43]. Given this relative abundance of ACE and angiotensinogen, even small amounts of local generated renin may be important in influencing Ang II production by the proximal tubule. In addition, the seemingly low levels of renin transcript in the proximal tubule may not be low given the fact that it is acting as an autocrine/paracrine hormone.

In summary, early in the evolution of salt-induced hypertension when circulating, juxtaglomerular and glomerular renin were suppressed, proximal tubular renin was not down-regulated by excess dietary NaCl. This failure to suppress proximal tubular renin could result in increased local generation of Ang II and enhanced proximal tubule NaCl absorption, thereby contributing to renal salt retention and exacerbating the hypertensive response to exogenous dietary NaCl. Additional mechanisms are undoubtedly operative in the development and maintenance of hypertension, and may include abnormalities in adrenal steroidogenesis, the central nervous system, urinary kallikreins, renal responsiveness to atrial natriuretic factor (ANF) and dopamine, nitric oxide, renal prostaglandins and yet unidentified humoral factors [44]. The present studies suggest that a primary defect exists in the intrarenal RAS of the proximal tubule. Further studies will be necessary to define the precise contribution of this abnormality to the generation of salt-sensitive hypertension.

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APPENDIX

Abbreviations used in this article are: ACE, angiotensin converting enzyme; ANF, atrial natriuretic factor; Ang II, angiotensin II; Pgc, glomerular capillary pressure; PRA, plasma renin activity; RAS, renin-angiotensin system; RSR, renin secretory rate; RT-PCR, reverse transcription-polymerase chain reaction; SR, salt-resistant rat strain; SS, salt-sensitive rat strain.

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