Substitution of chromosome 1 ameliorates L-NAME hypertension and renal disease in the fawn-hooded hypertensive rat

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THE FAWN-HOODED HYPERTENSIVE (FHH) rat is a genetic model of systolic hypertension and renal disease (17–19, 29). The development of elevated arterial blood pressure and focal glomerulosclerosis proceeds slowly and progressively over the lifetime of the FHH (17, 19) and is preceded by glomerular hypertension and proteinuria (7, 31, 32). The glomerular lesions in the FHH rats eventually progress to end-stage renal disease, which is associated with a reduced life span (19).

A number of quantitative trait loci (QTLs) associated with renal disease and hypertension have been identified in F1 backcross and F2 intercross studies of the FHH and the August Copenhagen Irish (ACI) rat. Several distinct genetic loci associated with proteinuria and hypertension have been identified on chromosome 1 of FHH rats (3, 30). In addition to the renal disease-associated loci, a locus associated with systolic blood pressure in the FHH (Bpfr1) has also been identified on chromosome 1 (3). The renal disease-associated loci, termed Rf-1 and Rf-2, were estimated to explain a significant portion of the genetic variance in proteinuria and glomerulosclerosis in the FHH. Genes important in hypertension and renal disease are likely to be present on chromosome 1 of the FHH rat.

Genetic loci related to renal disease and hypertension have also been identified on chromosome 1 in other rat genetic models. Several studies with congenic rat strains have demonstrated that regions of chromosome 1 in the Brown Norway (BN) rat render the kidney more susceptible to hypertension-induced damage compared with the spontaneously hypertensive rat (SHR) (4, 33, 34). In addition, QTLs for urinary albumin and protein excretion have been identified on chromosome 1 of the Dahl salt-sensitive rat in an F1 backcross with the SHR (10). Regions of chromosome 1 also cosegregated with elevated arterial blood pressure in crosses which used the SHR (14, 26), the Dahl salt-sensitive rat (12, 26), and the Sabra hypertensive rat (26, 42). These data demonstrate that genes important in hypertension and renal disease are present on chromosome 1 of other inbred rat strains as well as the FHH. Finally, QTLs for renal disease on rat chromosome 1 are concordant with loci linked to end-stage renal disease in humans (13, 16), indicating that the study of kidney disease loci on rat chromosome 1 may have important implications for human disease.

A recent study performed in congenic rats demonstrated that transfer of the region of chromosome 1 containing the Rf-1 locus from the disease-prone FHH ratonto the ACI genetic background increased the susceptibility for renal disease (24). Despite the positive results in this study, the degree of proteinuria, albuminuria, and focal glomerulosclerosis in these congenic rats was blunted relative to that observed in the FHH at the same age (24). The relative insensitivity of this congenic rat strain to renal disease indicates that other genes in the ACI background may have obscured the effects of the chromosomal transfer from the FHH or that multiple genes within the FHH are required for full expression of the disease phenotypes. As the ACI is normotensive, the congenic study also did not address the importance of the hypertension-related loci. The present studies were therefore performed to determine the importance of chromosome 1 in the development of hypertension and renal disease in the FHH rat by substituting chromosome 1 from the normal BN rat to the disease-sensitive FHH genetic background (FHH-1BN). We hypothesized that this chromosomal substitution strategy would prevent or attenuate the development of hypertension and renal disease in N1-nitro-L-arginine methyl ester (l-NAME)-treated male and female rats.

METHODS

Experimental animals. Experiments were performed on inbred lines of FHH/EurMcwi rats, BN/SSNHsdMcwi rats, and consomic...
FHH-1B/N(Mcw), rats maintained as inbred colonies at the Medical College of Wisconsin (MCW). The MCW Institutional Animal Care and Use Committee approved all experimental protocols. The FHH-1B/N consomic rat line was derived from inbred BN and FHH rats using at least six generations of backcross breeding as previously described (5, 23). The degree of residual heterozygosity and genetic contamination was determined by using a set of 182 microsatellite markers for genotyping that provided even coverage across all 21 chromosomes. Both the progenitor strains and the FHH-1B/N rats used for the present study were homozygous for all markers tested.

The FHH, FHH-1BN, and BN breeding stock of each strain were maintained on chow containing 0.4% NaCl obtained from Harlan Teklad (3075S, Madison, WI) with tap water. At weaning, the rats to be studied were placed on a purified AIN-76A rodent diet containing 0.4% NaCl (Dyets, Bethlehem, PA). At ~9.5 wk of age, the rats were placed on the AIN-76A chow containing 8.0% NaCl; and the tap water was replaced with water containing the nitric oxide synthase inhibitor l-NAME (12.5 mg/l). The rats were maintained on this regimen for the 17 days leading up to the experimental study and throughout the experimental protocol.

The above-described protocol, which includes high-salt chow and l-NAME in the drinking water, was adopted following a number of pilot studies designed to maximize the hypertensive and renal disease-associated phenotypes in the FHH rats. Preliminary experiments demonstrated that 12-wk-old male FHH rats maintained on a 4.0% NaCl diet for 3 wk had minimal hypertension; mean arterial pressure (MAP) averaged 124 ± 1 in FHH (n = 10) compared with 101 ± 2 mmHg (n = 4) in male BN rats on the same dietary regimen. In addition, protein and albumin excretion in the FHH (37 ± 5 and 12 ± 2 mg/day, respectively) were only slightly greater than that observed in the BN rats (17 ± 3 and 1 ± 0.3 mg/day, respectively). Both hypertension and renal disease have been previously reported in 12-mo-old FHH rats that have been uninephrectomized and treated with l-NAME in the drinking water (75–250 mg/l) for up to 3 mo (24, 36, 37). To study the rats in this protocol at a relatively young age (12 wk), the disease process was accelerated by administering an 8.0% NaCl diet and adding l-NAME to the drinking water (12.5 mg/l) for approximately 3 wk.

Surgical preparation. After 17 days on the high-salt diet with l-NAME in the drinking water, the surgical procedures were performed. The rats were deeply anesthetized with an intraperitoneal injection of ketamine (35 mg/kg), xylazine (10 mg/kg), and acepromazine (0.5 mg/kg); supplemental anesthesia was administered when needed. Using aseptic technique, polyvinyl catheters were implanted in the femoral artery, tunneled subcutaneously, and exteriorized at the back of the neck in a lightweight tethering spring. Both antibiotic (100,000 U/kg im penicillin G) and analgesic (0.1 mg/kg sc buprenex) were administered postsurgically, and the rats were allowed to fully awaken from anesthesia on a temperature-controlled pad. Following recovery from anesthesia, all rats were placed in individual stainless steel cages that permit daily measurement of arterial blood pressure and overnight urine collection.

Experimental protocol. The rats were permitted to recover for 2 days following catheter implantation before daily blood pressure measurements began. During this time, they were maintained on the high-salt (8.0% NaCl) diet with l-NAME (12.5 mg/l) in the drinking water. Daily arterial blood pressure measurements were made from 9 AM to 12 PM on postsurgical days 3-5 to quantify MAP and heart rate (HR). After the second day of blood pressure measurement, a timed overnight urine collection was obtained for measurement of high-salt urinary sodium, potassium, and creatinine excretion. Following the daily blood pressure recording obtained the following morning, arterial plasma samples were obtained for measurement of plasma creatinine and plasma renin activity (PRA) while the rats were maintained on the high-sodium diet.

Histological analysis. Kidneys were obtained for histological analysis from FHH, BN, and FHH-1BN rats maintained on the high-NaCl diet with l-NAME in the drinking water as described above. The rats were deeply anesthetized with pentobarbital sodium (50 mg/kg ip); the kidneys were then removed, bisected along the midsagittal plane, and placed in a 10% formaldehyde solution in phosphate buffer. The tissue was paraffin embedded using an automatic tissue processor (Microm HM300), cut in 3-μm sections (Microm HM355S), mounted on silanized/charged slides, and stained with Gomori’s One-Step Trichrome. Tissue sections were photographed using a Nikon E-400 fitted with a Spot Insight camera; digital micrographs were taken at different magnifications. Individual glomeruli (25–35 per rat) were graded from 0 (best) to 4 (worst) on the basis of glomerulosclerosis and mesangial expansion as described previously (5, 21, 25). The outer medulla was also assessed for tubular necrosis. The number of blocked tubules in the outer medulla was determined using Metamorph Image Analysis software (version 4.6, Universal Imaging Systems). The quantification of renal damage was performed by a blinded observer.

Statistical analysis. All data are presented as the mean ± 1 SE. A two-way ANOVA was used to determine the differences in parameters between the male and female FHH, FHH-1BN, and BN rats in this study. The differences in individual values between the groups were evaluated using a Student-Newman-Keuls post hoc test. The differences in parameters between genders of an individual strain were assessed with a t-test. The 95% confidence interval was considered significant.

RESULTS

Differences in MAP and HR in the males and females of the different groups of rats are depicted in Fig. 1. Arterial pressure...
averaged 188 ± 3 mmHg in the male FHH rats treated with \( l-\text{NAME} \); a value significantly higher than MAP in the male BN rats. MAP in the male FHH-1\(^{BN}\) rats was midway between the values of the two parental strains. HR was not significantly different in the male FHH-1\(^{BN}\) rats than that of the male FHH (435 ± 9 beats/min), although HR in the male BN rats (350 ± 8 beats/min) was significantly different than either of the other strains.

Qualitatively similar differences in MAP and HR were observed between the female rats of these strains. MAP was greatest in the female FHH, averaging 166 ± 7 mmHg, a value significantly greater than MAP in the female BN (137 ± 6 mmHg); MAP in the female FHH-1\(^{BN}\) was not different from the females of either parental strain. HR was not significantly different between the female FHH and FHH-1\(^{BN}\), although HR in the BN female was significantly less than observed in either the FHH or FHH-1\(^{BN}\). Within-strain gender differences in MAP were observed in the FHH, where MAP was significantly greater in the male than the female rats, and in the BN, where MAP was significantly greater in the female than in the male rats.

The albumin and protein excretion rates in the male and female FHH rats were significantly elevated relative to the BN rats (Fig. 2). Substitution of chromosome 1 in the male rats led to a significant decrease in both albumin and protein excretion compared with the parental FHH rats. A similar pattern was observed for the female rats. Finally, when comparing the male and female rats within strains, both albumin and protein excretion were significantly lower in the female FHH than the female, and albumin excretion was significantly lower in the female FHH-1\(^{BN}\) rat compared with the male FHH-1\(^{BN}\).

Creatinine clearance, normalized to kidney weight, was significantly higher in the male BN and FHH-1\(^{BN}\) rats compared with the FHH (Fig. 3). Consistent with the clearance data, plasma creatinine concentration was highest in the male FHH, lowest in the male BN, and midway between the two values in the FHH-1\(^{BN}\) (Table 1). Creatinine clearance (Fig. 3) and plasma creatinine concentration (Table 1) in the female rats were not significantly different between the FHH, FHH-1\(^{BN}\), and BN rats. Within-strain comparisons indicated that creatinine clearance was significantly greater in the female than the male FHH and significantly lower in the female FHH-1\(^{BN}\) compared with the male rats.

PRA was remarkably high in both male and female FHH (Fig. 3). The PRA was not different between male FHH and FHH-1\(^{BN}\) rats, although PRA was significantly lower in the BN than in the FHH. In the female rats, PRA was highest in the FHH and significantly lower in both the FHH-1\(^{BN}\) and the BN. PRA was significantly elevated in the female FHH compared with the male FHH.

Additional experimental parameters in the \( l-\text{NAME} \)-treated rats are presented in Table 1. Both body weight and kidney weight were significantly greater in the male than the female rats, with the BN rats of either gender tending to be significantly smaller than either the FHH or FHH-1\(^{BN}\). Plasma sodium and potassium concentrations were not different between the different groups of rats. Hematocrit was not different...
between the strains, although the female FHH and FHH-1BN rats had lower hematocrits than the male rats of these strains. Sodium and potassium excretion were not different within a gender between the different groups, although the female rats tended to have lower excretion rates of both electrolytes than the male rats, indicating lower food intake.

Table 1. Comparison of experimental parameters in male and female FHH, FHH-1BN, and BN rats maintained on an 8% NaCl diet with l-NAME (12.5 mg/l) added to the drinking water

<table>
<thead>
<tr>
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<th>FHH</th>
<th>FHH-1BN</th>
<th>BN</th>
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<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>293±6 (13)</td>
<td>200±4 (16)†</td>
<td>288±6 (10)</td>
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<tr>
<td>Kidney weight, g</td>
<td>2.9±0.1 (13)</td>
<td>2.1±0.2 (14)†</td>
<td>3.0±0.1 (10)</td>
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<tr>
<td>Plasma creatinine, mg/dl</td>
<td>0.50±0.04 (11)</td>
<td>0.39±0.03 (13)</td>
<td>0.35±0.03 (7)*</td>
</tr>
<tr>
<td>Plasma sodium, meq/l</td>
<td>143±1 (11)</td>
<td>141±2 (12)</td>
<td>140±1 (7)</td>
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<tr>
<td>Plasma potassium, meq/l</td>
<td>4.6±0.2 (11)</td>
<td>4.4±0.1 (12)</td>
<td>4.9±0.1 (7)</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>43±2 (11)</td>
<td>35±2 (13)†</td>
<td>45±1 (6)</td>
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<tr>
<td>Sodium excretion, meq/day</td>
<td>19.2±1.3 (13)</td>
<td>14.1±1.8 (16)†</td>
<td>19.3±1.1 (10)</td>
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<tr>
<td>Potassium excretion, meq/day</td>
<td>1.1±0.1 (13)</td>
<td>0.9±0.1 (12)†</td>
<td>1.3±0.1 (10)</td>
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Values are means ± SE. The number of animals per group is indicated in parenthesis. *P < 0.05 vs. fawn-hooded hypertensive rat (FHH) of the same gender. †P < 0.05 vs. male rat of the same strain. BN, Brown Norway rat; FHH-1BN, transfer of chromosome 1 from BN to FHH genetic background.

Fig. 4. Light microscopy images of whole kidneys (1× original magnification, A, C, and E) and outer medulla (10× original magnification, B, D, and F) of kidneys obtained from male BN rats (A and B), male FHH-1BN rats (C and D), and male FHH rats (E and F) maintained on a high-salt diet (HS; 8.0% NaCl) and administered l-NAME (12.5 mg/l) in drinking water for 3 wk. Severe interstitial fibrosis (blue staining in interstitium) and tubular necrosis (red protein deposition casts) are evident in the kidneys of the FHH and FHH-1BN rats. Visibly less renal injury is evident in the kidney of the BN rat.
5. Severe glomerular damage (blue fibrotic tissue with collapsed capillaries) and blocked tubules in the outer medulla (red protein deposition casts) are evident in the kidney of the male FHH rat. Visibly less glomerular and tubular injury is evident in the kidney of the male BN rats. The extent of glomerular injury and the number of blocked tubules per unit area of the renal outer medulla were quantified in male and female rats of each strain and are presented in Fig. 6. The glomerular injury index was significantly greater in the male FHH rats than in the FHH-1BN or BN rats. There was no significant difference in the number of blocked tubules in the outer medulla of the male FHH and FHH-1BN rats, as assessed by protein casts counted in the outer medullary region, although there were significantly fewer in the outer medulla of the male BN. The index of glomerular injury was similar in the female FHH, FHH-1BN, and BN rats compared with the male rats of each strain. The number of protein casts in the outer medulla, however, was significantly reduced in the female FHH and FHH-1BN rats compared with the males.

**DISCUSSION**

The present experiments indicate that chromosome 1 contains alleles that increase the susceptibility for the development of hypertension and renal disease in FHH rats. Substitution of chromosome 1 from the normal BN rat into the FHH genetic background attenuated L-NAME hypertension and renal disease in male rats as assessed by a decreased level of arterial blood pressure, increased creatinine clearance, reduced albuminuria and proteinuria, and a reduction in glomerular damage in male rats. Similarly, the substitution of chromosome 1 in female rats led to a reduction in albumin and protein excretion and a decrease in glomerular damage.

QTLs associated with renal disease and hypertension have been identified in a linkage analysis between the FHH and the ACI rat. Distinct genetic loci on chromosome 1 of FHH rats have been found to be associated with hypertension and renal disease (3). Results of the present experiments therefore serve to confirm the mapping data. Further fine mapping studies are needed to identify the specific genomic regions responsible for these phenotypes.
necessary to determine the causative genes of disease in these rats, although chromosome 1 harbors a number of potential candidates. A search of the Rat Genome Database (http://rgd.mcw.edu/) identified 823 genes on rat chromosome 1. Any of these or other unidentified genes could be responsible for the kidney disease and hypertension in the FHH. Among the genes on rat chromosome 1 are: alpha 2 and beta 1 adrenergic receptors, nephrin, kallikrein, superoxide dismutase 2, members of the cytochrome P-450 family, several fibroblast growth factors, and many different ion channels.

The mechanisms that led to the increased degree of hypertension and renal disease in the FHH rat are not clear. Chronic administration of L-NAME to rats is well known to lead to an elevation of arterial blood pressure (2, 9, 28) and renal disease (2, 8, 9, 28), but the greater sensitivity of blood pressure in the male FHH relative to the BN rat is somewhat surprising. It is presently unclear whether the enhanced arterial pressure sensitivity to L-NAME in the FHH reflects differences in NO synthetic capacity between the FHH and BN rat or whether NO plays a greater role to buffer the prohypertensive effects of neural or other circulating factors in the FHH. The FHH rats have a significantly greater PRA than the BN rats, indicating that the renin-angiotensin system may be activated to a greater degree in the FHH rats. Consistent with this observation is the finding that chronic administration of an angiotensin-converting enzyme inhibitor leads to a profound decrease in blood pressure in the FHH rat (31, 40, 41). Although alterations in the level of the renin-angiotensin system may be important in the

difference in blood pressure levels attained in the present study, the PRA levels are suppressed in L-NAME-treated FHH rats compared with rats maintained on a 0.4% NaCl or 4.0% NaCl diet (23). These data indicate that stimulation of the renin-angiotensin system is not the mechanism of L-NAME hypertension in these rats, although the mechanism for the enhanced hypertension in the FHH remains to be determined.

It is similarly unclear by what mechanism L-NAME leads to an increase in renal disease-related phenotypes in the FHH. In the present experiments, the interstitial fibrosis, as assessed by blocked tubules, was not significantly attenuated by substitution of chromosome 1; although the glomerular injury score, the urinary excretion of albumin and protein, and the reduction in creatinine clearance in the FHH were attenuated in the FHH-1BN. The glomerular disease was therefore the most affected by chromosomal substitution. It has been previously proposed that glomerular hypertension contributes to the development of renal damage in the FHH (31, 32). Moreover, several studies have demonstrated that the FHH rat does not autoregulate renal blood flow or glomerular capillary pressure as efficiently as control rats (6, 35, 38, 39). Interestingly, chronic administration of L-NAME to normal rats leads to glomerular hypertension (2) with accompanying glomerular damage (2, 8, 9, 28). It is therefore possible that the enhanced elevation of arterial pressure in the FHH rat leads to an abnormally large increase in glomerular capillary pressure in the FHH, which enhances the development of the glomerular disease in the male rats. Consistent with this idea is the present observation that arterial blood pressure was greatest in the FHH, least in the BN, and midway between the two in the FHH-1BN males. The glomerular injury may therefore be a result of the elevation in systemic arterial pressure in the male rats in the present study. Of note, the glomerular injury index and albumin excretion rate were elevated in the female FHH compared with the female FHH-1BN despite a lack of significant difference in systemic arterial blood pressure. Because the present experimental design was not designed to directly address the influence of arterial blood pressure on kidney disease, further study will be required to elucidate the mechanisms of renal disease in this model.

Gender also affected the development of hypertension and renal disease in the FHH and FHH-1BN rats. The glomerular injury scores were similar between the male and female rats of both strains, but the degree of hypertension, the albumin and protein excretion rates, and the number of protein casts in the outer medulla are dramatically reduced in the female rats of each strain. The female FHH and FHH-1BN rats appear to be less vulnerable than male rats to hypertension and renal disease even though the degree of glomerular damage observed was similar between the genders of each strain. In contrast to the elevated MAP in the male compared with the female FHH and FHH-1BN rats, the female BN had elevated MAP compared with the male BN. It is unclear what mechanism leads to the greater blood pressure in the female BN rats. The sodium excretion data (Table 1), which reflect the steady-state sodium intake in this experiment, indicate that the female BN consumed as much sodium as the male BN rats while having ~60% of the body mass of the male rats. Because L-NAME hypertension is sodium sensitive (8, 20), the elevated blood pressure in the female BN may reflect the elevated sodium intake in these rats. The gender differences in blood pressure

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Fig. 6. Glomerular injury score (top) and number of protein casts per mm² in the renal outer medulla (bottom) in kidneys of male and female BN, FHH-1BN, and FHH rats maintained on a high-salt diet (8.0% NaCl) and administered L-NAME (12.5 mg/l) in drinking water for 3 wk. The number of animals per group is indicated in parenthesis. *P < 0.05 vs. FHH of the same gender. †P < 0.05 vs male rat of same strain.
and kidney disease observed in the present study are consistent with earlier reports in FHH rats (11, 29) as well as reports regarding gender differences in blood pressure (27) and kidney disease (1, 15, 22) in humans and other experimental animals. Explanations for these differences including the role of androgens and/or estrogens (1, 22, 27) or interactions with genes on the Y chromosome remain to be explored in the FHH rat.

In conclusion, the present experiments indicate that a gene or genes found on chromosome 1 of the FHH are important in the development of L-NAME-induced hypertension and/or renal disease in both male and female rats. Substitution of chromosome 1 from the disease-resistant BN rat into the genetic background of the FHH rat led to a significant decrease in MAP, albuminuria, proteinuria, and glomerular damage in male rats administered L-NAME and high salt.

**REFERENCES**


