Chromosomal mapping of the genetic basis of hypertension and renal disease in FHH rats

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Submitted 5 January 2007; accepted in final form 21 September 2007

The Fawn Hooded Hypertensive (FHH) rat is a genetic model of systolic hypertension and renal disease (17–19, 38). The development of elevated arterial blood pressure and focal glomerulosclerosis results in end-stage renal disease that is associated with a reduced life span (19) and is preceded by hypertension and proteinuria (4). The FHH rat was transferred onto the normal Brown Norway (BN) genetic background. A standardized phenotyping protocol was performed in each sex of each strain in addition to the FHH and BN parental rats to quantify phenotypes related to hypertension [conscious mean arterial blood pressure (MAP) and heart rate (HR)] and renal disease (plasma creatinine, albumin excretion, and protein excretion). The time course for the development of these disease phenotypes was accelerated by feeding the animals a diet containing high salt (8.0% NaCl) and adding the nitric oxide synthase (NOS) inhibitor Nω-nitro-L-arginine methyl ester (L-NAME; 12.5 mg/l) to the drinking water for 2 wk before the experiment and throughout the experimental protocol (23).

METHODS

Experimental animals. Experiments were performed on inbred lines of FHH/EurMcwi (FHH) rats, BN/SSNHSdMcwi (BN) rats, and the panel of consomic rats. The consomic rat panel consisted of 22 strains in which each of the 20 autosomes as well as the X and Y chromosomes of the normal Brown Norway (BN) rat was transferred onto the FHH genetic background. A standardized phenotyping protocol was performed in each sex of each strain in addition to the FHH and BN parental rats to quantify phenotypes related to hypertension (conscious mean arterial blood pressure (MAP) and heart rate (HR)) and renal disease (plasma creatinine, albumin excretion, and protein excretion). The time course for the development of these disease phenotypes was accelerated by feeding the animals a diet containing high salt (8.0% NaCl) and adding the nitric oxide synthase (NOS) inhibitor Nω-nitro-L-arginine methyl ester (L-NAME; 12.5 mg/l) to the drinking water for 2 wk before the experiment and throughout the experimental protocol (23).

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typing with a set of 182 microsatellite markers that provided even coverage across all chromosomes. Both the progenitor strains and the consomic rats used for the present study were homozygous for FHH alleles at all markers tested outside of the substituted chromosome (http://pga.mcw.edu/). The rats were maintained as inbred colonies at Medical College of Wisconsin. A total of 221 male and 194 female rats were studied (average group size for each phenotype was 7.8 ± 0.3 rats). The Medical College of Wisconsin Institutional Animal Care and Use Committee approved all experimental protocols.

The breeders 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 purifiedAIN-76A rodent diet containing 0.4% NaCl (Dyets, Bethlehem, PA) which we previously found accelerates the progression of renal disease in hypertensive strains of rats (22). 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). Measurement of drinking water consumed indicated that this route of administration provided the rats with an average of 2.6 mg kg⁻¹ day⁻¹ of l-NAME. The rats were maintained on this regimen for the 17 days leading up to the experimental study and throughout the experimental protocol. As we previously described (23), untreated FHH rats at this age exhibit only a moderate elevation of arterial pressure with minimal development of renal disease compared with BN rats. The present protocol was therefore adopted following a number of pilot studies and was designed to accelerate the development of hypertension and renal disease in the FHH rats while having a minimal effect in BN rats. The degree of proteinuria and renal histologic changes observed using this protocol fully resemble those that develop naturally in 20-wk-old FHH (20).

Surgical preparation. The surgical preparation was performed following 17 days on the high-salt diet with l-NAME in the drinking water. Rats were deeply anesthetized with ketamine (35 mg/kg ip), xylazine (10 mg/kg ip), and acepromazine (0.5 mg/kg ip); supplemental anesthesia was administered when needed. With the use of aseptic technique, 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 UI/kg penicillin G im) and analgesic (0.1 mg/kg buprenex sc) were administered post-surgically. Following recovery from surgery, the rats were placed in individual stainless steel cages that permit daily measurement of arterial blood pressure and overnight urine collection.

Experimental protocol. The rats recovered 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 postoperative days 3–5 to quantify MAP and HR. After the second day of blood pressure measurement, a timed overnight urine collection was obtained for measurement of 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).

Histological analysis. Kidneys were obtained for histological analysis from four male rats of each parental strain and representative consomic strains with minimal proteinuria (FHH-15BN, FHH-16BN, FHH-18BN). All rats were fed the high-salt (8% 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% formalin solution in phosphate buffer. The kidneys were paraffin-embedded using an automatic tissue processor (Microm HMP 300), cut in 3-μm sections (Microm HM355S), mounted on silanized/charged slides, and stained with Gomori’s One-Step Trichrome (sections from all rats were simultaneously stained). Tissue sections were photographed using a Nikon E-400 fitted with a Spot Insight camera; digital micrographs were obtained to visualize differences in renal injury among the strains. Glomerular damage (40–50 per rat) was scored using the semiquantitative index method of Raij et al. (32); glomeruli were scored from 0 (best) to 4 (worst) based on the percentage of the glomerular capillary area filled with matrix material (22, 23). The fraction of renal outer medullary tissue containing dilated tubules was quantified by determining the proportion of red-stained structures in this region using Metamorph Image Analysis software (version 4.6, Universal Imaging Systems) (22, 23).

Statistical analysis. Data are presented as means ± 1 SE. A two-way ANOVA with a Holm-Sidak post hoc test was used to assess sex differences for each phenotype within the different consomic and parental rats. A one-way ANOVA with a Fisher least significant differences post hoc test was used to evaluate differences between the parental and the different consomic strains within each sex. Due to the large number of groups and variance in this analysis, this comparison was only useful to identify differences on the extreme ends of the distribution. In some cases, changes in phenotypic values which were indicative of significant effects were determined with a second one-way ANOVA between the parental FHH and those consomic strains in which the means ± 1 SE did not overlap with the FHH. Any differences detected in this secondary analysis are not indicated on the figures but are instead designated in the text as changes which are “suggestive.” The 95% confidence interval was considered significant.

RESULTS

MAP values in the male and female rats that were chronically treated with l-NAME and fed a high-salt (8.0% NaCl) diet are illustrated in Fig. 1. Average MAP was significantly greater in the male and female FHH (181 ± 3 and 166 ± 8 mmHg, respectively) than in the male and female BN rats (121 ± 3 and 126 ± 9 mmHg, respectively). As a group, MAP was significantly greater in the male than the female rats. There was also a significant influence of chromosomal substitution on MAP in both male and female consomic rats. Substitution of chromosome 20 in the male rats and chromosome 15 in the female rats from the disease-resistant BN onto the FHH genetic background led to a significant decrease in MAP compared with that observed in the parental FHH. While all of the male consomic strains had MAP levels significantly different than the BN male, MAP was not significantly different from the BN in the female FHH-5BN, FHH-8BN, FHH-15BN, FHH-19BN, and FHH-20BN. Differences in HR in the consomic rats are illustrated in Fig. 2. HR was significantly lower in the male and female FHH rats compared with the FHH of the same sex. Substitution of chromosomes 3, 15, and 20 led to a significant reduction in HR in the male rats of these consomic strains compared with the FHH rats. No significant differences in HR were detected between the female rats of the consomic strains and the parental FHH strain.

The albumin excretion rates in the l-NAME-treated male and female FHH rats were significantly higher than that observed in the BN (Fig. 3). Moreover, albumin excretion was significantly greater in the male than the female rats of the FHH and the consomic strains. In male rats, substitution of chromosomes 1, 15, 16, and 18 from the BN onto the FHH genetic background significantly reduced albumin excretion compared with the FHH parental strain. Urinary albumin excretion was significantly elevated in the female FHH-9BN and FHH-10BN rats compared with the female FHH. Similar to the differences observed for albumin excretion, the male and female FHH rats excreted significantly more protein than the
BN rats of the same sex (Fig. 4). Protein excretion rate was significantly lower than observed in the FHH in the male consomic FHH-1BN, FHH-14BN, FHH-15BN, and FHH-16BN rats. Substitution of chromosome 10 led to a significant elevation in protein excretion in female rats of this consomic strain compared with the female FHH.

Photomicrographs of trichrome-stained sections of the glomeruli and outer medullary regions of male BN, FHH, and selected consomic strains (FHH-15BN, FHH-16BN, and FHH-18BN) are presented in Fig. 5. Marked histological damage was observed in the kidneys of the male FHH compared with the BN rats; extensive glomerular injury (blue fibrotic tissue and collapsed capillary structure) and a large number of dilated tubules were observed in the outer medulla (red protein deposition casts) of the FHH. The glomerular damage and percentage of outer medullary area comprised of dilated tubules in the parental and selected consomic strains are quantified in Fig. 6. Substitution of chromosomes 15, 16, and 18 led to a significant improvement in the glomerular injury score while substitution of chromosomes 15 and 16 decreased the number of blocked tubules observed in the renal outer medulla.

Plasma creatinine levels in the consomic panel are presented in Fig. 7. Plasma creatinine concentration was significantly elevated in the male FHH compared with the BN rats. Substitution of chromosome 20 led to a significant decrease while substitution of chromosome 3, 17, or X led to a significant elevation of plasma creatinine concentration in male rats of these consomic strains compared with the male FHH. No significant differences in plasma creatinine concentration were detected between the female BN and FHH. PRA was significantly elevated in the male and female FHH compared with the BN (Fig. 8). The PRA was significantly lower in the male FHH-7BN, FHH-17BN, and FHH-20BN rats and in the female FHH-2BN compared with levels observed in the FHH rats of the same sex. The PRA in the female rats of the FHH-4BN and FHH-18BN strains was significantly greater than was observed in the female rats of the FHH parental strain.

To evaluate the overall influence of each chromosome to hypertension/renal disease in the FHH rat, the beneficial effect of each chromosomal substitution was ranked for each phenotype relative to the values measured from the FHH and BN parental rats of the same sex. To facilitate comparisons between the sexes and phenotypes, the relative rankings for each phenotype were compared with the ranked values for the FHH (arbitrarily set to 100) and the BN (arbitrarily set to 0) of that sex. The ranking for each phenotype was generated by dividing the absolute difference in the mean values between each strain and the BN by the absolute difference between the FHH and BN {for blood pressure: \( [(\text{MAP}_{\text{FHH}} - \text{MAP}_{\text{BN}})/(\text{MAP}_{\text{FHH}} - \text{MAP}_{\text{BN}})] \times 100 \) \}. The relative ranks for each disease-related...
phenotype which were significantly different between the FHH and BN rats (MAP, albumin excretion, and protein excretion in both sexes; plasma creatinine in male rats) were then averaged to yield an “Overall Phenotype Rank” for each chromosome. This ranking method indicated that substitution of chromosomes 1, 15, and 20 had the most beneficial overall effect on phenotypes related to hypertension and kidney disease in the present study (Fig. 9). A further analysis indicated that the changes observed in the FHH-2BN, FHH-8BN, and FHH-18BN suggested beneficial effects of substitution of these chromosomes on hypertension and kidney disease. In contrast to the beneficial effects of chromosome substitution, exchange of chromosome 10 from the BN to the FHH rat significantly increased the degree of disease compared with FHH rats.

**DISCUSSION**

Chromosome substitution from the disease-resistant BN to the disease-prone FHH had a significant impact on the development of hypertension and several phenotypes related to the development of renal disease. In general, the FHH rats of both sexes had greater levels of arterial blood pressure, higher heart rates, greater albumin and protein excretion rate, elevated PRA, and increased plasma creatinine compared with BN rats of the same sex. The exchange of chromosomes 1, 15, and 20 from the normal BN rat onto the FHH genetic background reduced the severity of disease phenotypes compared with the FHH rat. Among those strains, the male FHH-20BN rats had significantly lower values of MAP, HR, plasma creatinine, and PRA than the male FHH rats. The substitution of chromosomes 2 and 8 also had a beneficial effect on the development of hypertension and kidney disease-related phenotypes. These results indicate that there are genes that contribute to the development of L-NAME-induced hypertension and renal disease on chromosomes 1, 2, 8, 15, and 20 of the FHH rat.

**Hypertension.** The present consomic rat study demonstrated significant effects of substitution of chromosome 20 on MAP in the male rats while the exchange of chromosome 15 significantly decreased MAP in female rats. Correspondingly, HR was decreased in the male FHH-20BN rats, indicating that mechanisms altering HR may mediate the decrease in MAP. Blood pressure-related genes in the FHH have not been previously identified on these chromosomes. As reviewed by Rapp (35), a number of studies demonstrated a role for rat chromosome 20 on blood pressure in other genetic models of hypertension. QTLs for blood pressure have been localized to chromosome 20 in female rats from a cross of SS and BN rats (24), in Milan hypertensive rats (MHS) (50), and in a backcross of a BN and ACI rat F1 generation (28). Moreover, QTLs for blood pressure were identified on chromosome 15 in female SS rats (24) and SHR stroke-prone rats (14), and a QTL cluster containing genes of hypertension in human studies was local-
ized to rat chromosome 15 by comparative mapping techniques (49). The consomic strategy has therefore identified chromosomes harboring hypertension-related loci which had previously not been recognized in the FHH. These results demonstrate the mapping power of the consomics, as well as the likely different genetic basis for the phenotypes between FHH and BN, compared with the previous FHH and ACI cross.

Kidney disease. The development of hypertension-induced renal disease in the male rats, as assessed by urinary albumin and protein excretion and/or plasma creatinine levels, was significantly attenuated by substitution of chromosomes 1, 14, 15, 16, 18, and 20 from the BN onto the FHH genetic background. Moreover, a histological examination of kidneys from these strains indicated a reduction in glomerular damage.
in the male rats with chromosomes 15, 16, and 18 substituted from the BN onto the FHH background. Since these strains, aside from the SS-20BN, had equivalent levels of arterial blood pressure, genes important in the resistance to hypertension-induced renal disease appear to be present on these chromosomes. Of interest, L-NAME-induced hypertension was attenuated in the male FHH-20BN and the female FHH-15BN groups. Surprisingly, albuminuria and proteinuria were not significantly reduced in either of these strains, indicating that the decrease in blood pressure did not prevent the development of proteinuria in these consomic strains. These data indicate that renal disease is at least partially independent of arterial blood pressure in these strains; the mechanism for this observation is not known.

QTL associated with renal disease and hypertension have been identified in previous linkage analysis studies between the FHH and the ACI strains. Distinct genetic loci on chromosomes 1, 3, 14, and 17 of FHH rats have been found to be associated with hypertension and/or renal disease (2, 40). The results of the present experiments confirm the mapping data in regard to the existence of a major gene on chromosome 1 that influences proteinuria. Since this chromosome appeared to possess loci with the greatest influence on blood pressure and kidney disease in genetic crosses with the FHH (2, 40), and congenic strains have confirmed the presence of renal disease loci on chromosome 1 of the FHH (31, 33), this finding is not unexpected. In contrast to previous mapping studies, however, a significant influence of chromosomes 3 and 17 was not observed for any of the measured phenotypes in the present consomic panel. Instead, the substitution of chromosomes 15, 16, 18, and 20 tended to normalize the disease phenotypes in the present study. It is not unexpected that there could be significant differences between the results of linkage analysis studies that utilized an F1 backcross or an F2 intercross approach of FHH with ACI rats compared with the present results obtained by chromosome substitution between FHH and BN rats. Genetic mapping studies can only identify regions that are different between the strains compared. Another likely reason for the disparity in the results are the differing genetic strategies. In a cross design, each animal is unique and has the potential to reveal potential gene-gene interactions (epistasis) between the renal failure loci on chromosome 1 and QTLs on chromosomes 3, 14, and 17. In contrast, the consomics provide for replication, but will not reveal epistasis, at least as studied here, which likely also contributes to the variation in loci mapped. In either case, the loci in each study have been replicated either via congenics for the cross design, and replicate animals with the consomics.

The present study demonstrated that genes contributing to albuminuria and proteinuria also reside on chromosomes 15, 16, and 18 of the FHH rat. Loci related to kidney disease have not been previously identified on these chromosomes of the FHH. Renal function-related loci, however, have been identified on chromosomes 15 and 18 in the SS rat (24, 48), and the substitution of chromosomes 16 or 18 from the BN onto the SS genetic background reduces proteinuria and albuminuria (22).
Despite these previous studies which have implicated loci on these chromosomes in renal disease in other rat strains, there is little known about the potential genes on these chromosomes which may lead to disease in the FHH rat.

**Potential mechanisms of hypertension and kidney disease.** The mechanisms that led to the increased degree of hypertension and renal disease in the FHH rat compared with the BN are not known. Chronic administration of NOS inhibitors has been reported to increase arterial blood pressure (1, 8, 37) and renal disease (1, 7, 8, 37) in rats, but the relative insensitivity of the BN rat compared with the FHH is somewhat surprising. Chronic oral or intravenous administration of L-NAME in doses ranging from 5 to 8.6 mg·kg<sup>-1</sup>·day<sup>-1</sup> to Munich Wistar or Sprague-Dawley rats led to an elevation of MAP which ranged from 20 to 40 mmHg (1, 7, 21, 26). The severe hypertension which developed in the male and female FHH with a relatively small dose of L-NAME (2.6 mg·kg<sup>-1</sup>·day<sup>-1</sup>) in the present study indicates that the FHH is more sensitive to NOS inhibition than other rat strains. Since the substitution of chromosomes 1, 2, 8, 15, 18, and 20 tended to reverse the L-NAME-mediated disease process, genes harbored on these chromosomes may be important in the differential sensitivity to NOS inhibition. A number of genes related to NO-mediated effects on blood pressure and renal disease are present on these chromosomes. Isoforms of dimethylarginine dimethylaminohydrolase, an enzyme that degrades an endogenous competitive inhibitor of NOS, are found on chromosomes 2 and 20; phosphodiesterases, including phosphodiesterases specific for cGMP, are located on chromosomes 2, 8, 18, and 20; the gene for arginase 1, which degrades NOS substrate L-arginine into L-ornithine and urea, is found on chromosome 1; and genes encoding proteins which transport L-arginine into cells are found on chromosomes 1, 2, and 15 (34). Potential functional differences or alterations in expression in these gene products may mediate the differential sensitivity to NOS inhibition in FHH and BN rats. While these are attractive candidate genes, we cannot exclude other genes in the absence of formally defining the underlying mechanisms.

**Sex effects.** Sex affected the development of hypertension and renal disease in the FHH and the consomic rats. Significant differences between male and female FHH rats were observed for MAP, albumin excretion rate, protein excretion rate, and PRA. The only sex-related difference noted between the male and female BN rats was for HR, but there were no differences in the FHH male and female rats for this phenotype. Substitution of some chromosomes prevented the sex-related differences in MAP, albuminuria, and proteinuria. In strains in which chromosomes 1, 2, 3, 6, 7, 9, 10, 11, 14, 16, 17, 20, or X were substituted, there were no differences in MAP between male and female rats. Consomic rats with BN chromosomes 3, 4, 11, 12, 14, 15, 16, or 18 had no sex-related differences in albumin excretion rate. Finally, there were no sex-related differences in protein excretion rate between male and female FHH-1BN, FHH-3BN, FHH-14BN, FHH-16BN, or FHH-20BN.

It is not clear what mechanism decreases the vulnerability of the female FHH and consomics to hypertension and renal disease. The sex differences in blood pressure and kidney disease observed in the present study are consistent with earlier reports in FHH rats (38) as well as reports regarding sex differences in blood pressure (36) and kidney disease (13, 27) in humans and experimental animals. Explanations for these differences, including the role of sex hormones (27, 36), remain to be explored. Interestingly, substitution of the Y chromosome from the BN to the FHH background did not have a beneficial effect on any of the disease phenotypes, indicating that genes specific to the Y chromosome of the FHH were

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**Fig. 8.** Plasma renin activity in male (top) and female (bottom) FHH, BN, and consomic rats (indicated by the substituted chromosome) fed a high-sodium diet (8.0% NaCl) with L-NAME added to the drinking water (12.5 mg/l). *P < 0.05 vs. FHH of the same sex. †P < 0.05 vs. the BN rat of the same sex. ‡P < 0.05 vs. the male rat of the same strain.

**Fig. 9.** Influence of chromosomal substitution on an aggregate of hypertension/renal disease-associated phenotypes in FHH, BN, and consomic rats (indicated by the substituted chromosome) fed a high-sodium diet (8.0% NaCl) with L-NAME added to the drinking water (12.5 mg/l). *P < 0.05 vs. FHH of the same sex. †P < 0.05 vs. the BN rat of the same sex.
likely not a causative factor in the sex-related differences which were observed in the present study.

**PRA.** PRA was significantly greater in the FHH than the BN parental rats and was influenced by both sex and chromosome substitution. Unlike the other phenotypes, PRA, a component of a prohypertensive pathway, was greater in the FHH female than the male rats; moreover, the PRA value in a number of the consomic strains was elevated in the females compared with the male rats. While substitution of chromosome 2 in the female rats and chromosomes 7, 17, and 20 in the males led to a significant reduction in PRA compared with the FHH, the only strain with a reduction in both PRA and MAP compared with the FHH was the FHH-20BN male. It should be noted that the renin gene itself on rat chromosome 13 was not linked to PRA. The influence of PRA on the hypertensive phenotype in the present study is therefore unclear. Of interest, a number of studies examined renin as a candidate gene in hypertension. In an F1 backcross study between the hypertensive SHR and the normotensive diabetic BB/OK rat, the renin allele of the SHR strain promoted a lowering of blood pressure (16). Moreover, transfer of the renin gene from the Dahl SS to the SR (Dahl salt resistant) rat resulted in a reduction of blood pressure and decreased plasma renin (45). Results from the present study indicate that the absolute PRA level is likely not important in the disease process which occurs in FHH rats.

**Quantification of effects of chromosome substitution.** This unique data set provides an opportunity to assess the genetic contribution of individual chromosomes to the final disease or disease-related phenotypes in FHH rats. Data previously obtained from cosegregation studies and from experiments with congenic rat strains indicated that the genetic effects of some individual QTL on hypertension and other disease phenotypes may be additive in SHR and SS rats (6, 29, 39). To estimate whether the genetic effects of the full chromosomal complement of the FHH are additive for the phenotypes examined in this study, we compared the absolute differences observed between the parental FHH and BN for each phenotype to the sum of the differences between the FHH parental and each individual consomic strain. For the sake of comparison between different phenotypes and sexes, these data are normalized and presented in Fig. 10. If we had captured all the genetic components and there was no epistasis, we would expect that the observed difference between the parental strains would approximate the sum of the differences between the FHH and the consomic strains. For each phenotype that was significantly different between the FHH and BN, the sum of the differences between the FHH and the consomic strains was at least 60% different than the difference between the FHH and BN rats. In fact, the absolute value of the mean calculated difference between the FHH and the consomics for each of the phenotypes was significantly different than that observed between the parental rats, and averaged 209 ± 39% of the observed FHH-BN difference. These data indicate that the effects of the different loci harbored on the different chromosomes are not additive for the hypertension and renal disease-related phenotypes measured in this panel of consomic rats.

Based on our observations in these chromosome-substituted animals, we propose that there are multiple genes on a number of different chromosomes which by themselves can influence any of the phenotypes examined in this study. As described above, however, the predicted sum of the effects of the individual genes is significantly greater than the observed phenotypic differences between the two parental strains. This is likely due to the mechanisms inherent in physiological control systems which serve to buffer large alterations to the system. As such, the effects of any individual QTL or groups of QTLs are buffered, and the effects of multiple genes would not be predicted to be additive; instead, the inherent control systems serve to maintain homeostasis. This conclusion is not surprising when the multiple positive and negative feedback control systems responsible for homeostasis are considered (10, 11). Potential feedback and interactions may occur between the gene products of each chromosome at the level of transcription and translation, between proteins which are part of a single pathway, between pathways, or at the level of the effector mechanisms. As such, the effects of any individual gene may be modified by the actions of other genes which exist in the same or different molecular pathways. Indeed, our data support this type of epistasis. Finally, feedback from the effector mechanism back to the pathway can also have a profound impact on function.

**Conclusions.** Chromosome substitution from the disease-resistant BN to the disease-prone FHH rat had a significant impact on a number of hypertension and renal disease-related phenotypes. In general, the FHH rats of both sexes had greater levels of arterial blood pressure, higher HRs, greater albumin and protein excretion rate, elevated PRA, and decreased creatinine clearance compared with BN rats of the same sex. Substitution of chromosome 20 from the BN onto the FHH genetic background reversed L-NAME hypertension, normalized PRA, and decreased protein excretion. Urinary excretion of albumin in males was decreased by substitution of chromosomes 1, 15, 16, and 18 from the FHH to the BN, indicating a prevention of renal disease. These data indicate that genes contributing to L-NAME-induced hypertension and renal disease are found on chromosomes 1, 15, 16, 18, and 20. These studies also demonstrated that hypertension alone induced by L-NAME is not sufficient to induce renal disease in this model.
ACKNOWLEDGMENTS

The authors thank J. Jursinic, M. P. Kunert, H. Lund, L. James, M. Bregantini, J. Powlas, A. Piro, E. Liss, S. Kaplan, G. Slocum, and C. Bobrowitz for assistance with different portions of these studies.

GRANTS

The work in this manuscript was partially supported by National Institutes of Health Grants HL-66579, HL-54998, and DK-62803.

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