Proteinuria and glomerulosclerosis in the Sabra genetic rat model of salt susceptibility

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Yagil, Chana, Marina Sapojnikov, Gurion Katni, Zvi Ilan, Sarah Weksler Zangen, Eliezer Rosenmann, and Yoram Yagil. Proteinuria and glomerulosclerosis in the Sabra genetic rat model of salt susceptibility. Physiol Genomics 9: 167–178, 2002. First published April 23, 2002; 10.1152/physiolgenomics.00014.2002.—In search of an experimental model that would simulate the association between proteinuria and salt sensitivity in humans, we studied protein excretion in the Sabra rat model of salt susceptibility. Monthly measurements of urinary protein excretion in animals fed standard rat chow revealed that normotensive salt-sensitive SBH/y developed proteinuria that averaged 65 ± 7 mg/day (n = 10) at 9 mo, whereas proteinuria in normotensive salt-resistant SBN/y was 39 ± 4 mg/day (n = 10) (P < 0.01). Histopathological evaluation revealed focal and segmental glomerulosclerosis (FSGS) lesions grade 2 in SBH/y and normal histology in SBN/y. To amplify the differences between the strains, uninephrectomy was performed. At 9 mo, proteinuria in SBH/y with one kidney (SBH/y-1K) was 195 ± 12 mg/day (n = 10) and in SBN/y was 128 ± 10 mg/day (n = 10) (P < 0.001); histopathology revealed FSGS grade 3 in SBH/y-1K and grade 1–2 in SBN/y-1K. To determine the effect of salt loading, animals were provided with 8% NaCl in chow, causing hypertension in SBH/y but not in SBN/y. Proteinuria markedly increased in both SBH/y with two kidneys (SBH/y-2K) and SBH/y-1K, but not in SBN/y; histopathology revealed FSGS grade 1–2 in SBH/y-2K, grade 2 in SBH/y-1K, no lesions in SBN/y-2K, and grade 0–1 in SBN/y-1K. We concluded that the SBH/y strain is more susceptible to develop proteinuria and glomerulosclerosis than SBN/y. In search for the genetic basis of this phenomenon, we investigated the role of candidate proteinuric gene loci. Consomic strains were constructed by introgressing chromosome 1 (which harbors the rf-1 and rf-2 proteinuric loci) or chromosome 17 (which harbors rf-5) from SBH/y onto the SBN/y genomic background. The resulting consomic strains developed marked proteinuria that was severalfold higher than in SBN/y-1K; histopathological evaluation, however, revealed FSGS lesions grade 1–2, similar to those found in SBN/y-1K and less severe than in SBH/y-1K. These results suggest a functional role of gene systems located on chromosomes 1 and 17 in inducing proteinuria in the salt-susceptible Sabra rat strain. These genetic loci do not appear to harbor major genes for glomerulosclerosis.

Salt sensitivity; SBH/y; SBN/y; consomic strains; quantitative trait loci

Salt sensitivity has been implicated in the development of proteinuria in humans with a variety of clinicopathological conditions (1, 4, 11, 23, 39). The mechanisms of the relationship between salt sensitivity and proteinuria have not been fully elucidated. Evidence from studies in animal models suggests that hypertension that is associated with salt sensitivity is a pathophysiological mediator of proteinuria (13, 34). There have been suggestions, however, that other non-blood-pressure-related mechanisms might be involved (6, 9, 10, 13, 38). The ability to study this blood pressure-independent relationship between proteinuria and salt sensitivity has been hampered by the spontaneous development of hypertension in most experimental models expressing salt sensitivity (13, 34). One notable exception is the Sabra rat model of salt susceptibility, in which salt sensitivity or resistance are inherited genetic traits but in which hypertension develops only during dietary salt loading (3, 41). This model thus provides the unique opportunity to carry the observations from humans back to the experimental animal model and study in depth the relationship between salt sensitivity and proteinuria, without the confounding effect of hypertension.

The Sabra rat model of salt susceptibility consists of two fully inbred contrasting strains: one is salt-sensitive in terms of the development of hypertension (SBH/y), and the other is salt-resistant (SBN/y) (3, 41). Both strains remain normotensive throughout their lifetime unless they are salt-loaded, at which time SBH/y becomes hypertensive while SBN/y remains normotensive. While determining the phenotype of these animals, we found that the “normotensive” salt-sensitive strain that is fed normal chow excretes significantly more protein in the urine than the salt-resistant strain. These findings enable us to differentiate between proteinuria associated with salt susceptibility per se and that which is associated with renal injury caused by hypertension induced by salt loading. In the...
current study, we provide details of these findings as well as of our initial steps in exploring the genetic basis of proteinuria related to salt susceptibility using consomic strains, as a means of investigating the mechanisms involved.

METHODS

Animals

Sabra hypertension-prone (SBH/y) and hypertension-resistant (SBN/y) rats from the colony inbred at the Barzilai Medical Center (Ashkelon, Israel) (41) were used. Animals were housed in the center’s animal facility in strict compliance with institutional regulations and in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication no. 85-23, revised 1985) and the guidelines of the American Physiological Society. Climate-controlled conditions were maintained, and temperature was set at 22–24°C. Regular 12-h diurnal cycles were kept using an automated light-switching device. Tap water and standard rat chow containing 0.65% NaCl (Koffolk, Tel Aviv, Israel) were provided ad libitum, unless stated otherwise. Studies were carried out in male rats, unless stated otherwise.

Study Protocols

The study had two objectives: 1) To investigate the relationship between salt susceptibility and proteinuria in the Sabra model of salt susceptibility, and 2) to initiate the dissection of the genetic basis of proteinuria related to salt susceptibility.

Salt susceptibility and proteinuria. To investigate the relationship between salt susceptibility and proteinuria, we studied urinary protein excretion in three separate sets of experiments.

STUDY 1: NORMOTENSIVE RAT ON REGULAR CHOW. Two groups were studied: SBH/y (n = 10) and SBN/y (n = 10) rats starting immediately after weaning and until 9 mo of age. Animals were fed standard chow and provided tap water ad libitum, under which conditions neither strain normally develops hypertension.

STUDY 2: NORMOTENSIVE RAT AFTER UNINEPHRECTOMY ON REGULAR CHOW. In an attempt to amplify the findings in the intact animals (2K), animals from both strains (n = 10 in each group) were subjected to uninephrectomy (1K) at ∼2 mo. This procedure has been shown not to affect blood pressure in these strains (unpublished results). Animals were studied until 9 mo of age while being fed regular diet.

STUDY 3: HYPERTENSIVE INTACT AND UNINEPHRECTOMIZED RATS DURING SALT LOADING. To differentiate between the effects of salt susceptibility per se and salt-induced hypertension on proteinuria, four additional groups of animals (n = 10 in each group) were studied (SBH/y-2K, SBH/y-1K, SBN/y-2K, and SBN/y-1K), all of which were salt loaded with 8% NaCl in chow and provided tap water ad libitum. Dietary salt loading has been shown to induce hypertension in SBH/y but not in SBN/y. Animals were studied during 4 mo of salt loading until age 6 mo.

For all studies, protein excretion was measured in 24-h urine collections at monthly intervals in standard metabolic cages. Animals were killed upon completion of the studies at 9 mo of age. Serum creatinine as a measure of kidney function was determined, and kidneys were removed for histopathological studies.

All of the above experiments were carried out in male rats. We reasoned that since sex differences have been demonstrated in many of the physiological phenotypes studied so far, this study would not be complete without determining protein excretion in female rats. Therefore, to verify whether the sex of the animal affects proteinuria that is associated with salt susceptibility, we studied intact (SBH/y-2K, n = 9; SBN/y-2K, n = 6) and uninephrectomized (SBH/y-1K, n = 6; SBN/y-1K, n = 6) female rats, as in protocols of studies 1 and 2.

Genetic basis of proteinuria. To study gene systems involved in the pathogenesis of the relationship between proteinuria and salt sensitivity, we adopted a “functional” candidate gene approach which is based on known candidate gene systems and on the use of consomic strains. Specifically, we focused on the rfi-1 and rfi-2 gene loci that are located on chromosome 1 and the rfi-5 gene locus located on chromosome 17. These “candidate gene loci” have been implicated through genetic mapping in mediating proteinuria in another rodent model, the fawn-haired rat, which expresses, among other phenotypes, renal disease with proteinuria (6, 7, 32). We constructed strains that were nearly fully consomic by introgressing >80% of chromosome 1 and >90% of chromosome 17 from the genetic background of one parental strain onto that of the other. We reasoned that if the candidate gene loci had functional significance in terms of proteinuria, then by transferring the respective chromosomes, for example, from SBH/y onto SBN/y, we would affect the phenotype (proteinuria) of the consomic strain so that it would become similar to that of SBH/y rather than of SBN/y.

CONSTRUCTION OF CONSOMIC STRAINS. The consomic lines were constructed by replacing chromosomes 1 or 17 of one strain with the homologous regions of the other. The term “consomic” refers to animal lines in which an entire chromosome is introgressed from one genetic background onto another. In the current study, we were able to achieve transfer of most of the targeted chromosomes. Although the strains that were constructed were thus not fully consomic, they were nearly so and for the purposes of the study were labeled consomic.

The construction of consomic strains was achieved by intercrossing homozygous SBH/y (HH) and SBN/y (NN) rats to form an F1 progeny consisting of heterozygotes (HN) which were backcrossed to the parent strain SBH/y or SBN/y (BC1). The offsprings segregated 1 heterozygote (HN):1 homozygote (HH or NN) at all polymorphic loci. Genotyping of BC1 was determined with chromosome-specific informative microsatellite markers and utilizing standard PCR-PAGE techniques, as previously described (41). Heterozygotes at loci of interest (within the targeted chromosome) were selected, and backcrossed again to SBH/y or SBN/y (BC2). Backcrossing was repeated eight times, always selecting heterozygotes as breeders for the next backcross. During this process, all loci not undergoing selection became homozygous for one strain, whereas the selected allele from the other strain remained heterozygous. After BC8, two heterozygotes were bred. One-fourth of the offsprings were homozygous at the loci of interest. These were bred to each other, thus fixing the allele of interest in the homozygous state on the background of the other strain. This was confirmed by a total genome scan with informative microsatellite markers spread apart at ∼10 cM intervals.

The nomenclature of the resulting consomic strains was based on accepted standards according to which the strains are designated by the order of “RECIPIENT.DONOR-CHROMOSOME NUMBER” (http://pga.mcw.edu/pga/ssp/components/genomics/genomics.jsp).

FUNCTIONAL STUDIES. Once the consomic strains were constructed and the animals were ready for the functional studies, the right kidney was removed and animals were fed regular chow, reproducing thereby the uninephrectomy pro-
toool used in the parental strains. Urinary protein excretion was determined at monthly intervals thereafter and until 9 mo of age, at which time animals were killed and the kidneys were removed for histological studies. A group of uninephrectomized parental SBH/y and SBN/y strains were studied during the same time period and in parallel, to allow between-group comparison.

Measurements of Protein Excretion

Quantitative determination of total protein in the urine, the surrogate end point of this study, was determined in 24-h urine collections by the microprotein-PR method (Sigma Diagnostics). To determine the albumin fraction, we measured in a select number of samples total protein concentration using the pirogallol red molybdate complex and albumin concentration with bromocresol green.

Blood Pressure Measurements

Blood pressure (systolic) was measured in all animals at ambient temperature (27–28°C) in awake animals by the tail-cuff method using a photoelectric oscillatory detection device (ITC Life Science, Woodland Hills, CA), as previously described (41). At least three replicate BP measurements were obtained on three consecutive days (i.e., at least 9 measurements over 3 days) and the average of all measurements was taken as representative of systolic BP.

Histopathology

Animals were killed upon completion of the studies. The kidneys were excised, fixed in 4% formaldehyde, and embedded in paraffin. Sections 6 μm thick were stained with hematoxylin-eosin and periodic acid-Schiff. Slides were examined in a blind fashion. Fifty glomeruli were examined in each kidney and semi-quantitatively graded as follows: grade 0, all glomeruli normal; grade 1, one to two glomeruli were affected; grade 2, more than 2 but less than 17 glomeruli were affected; and grade 3, more than 17 glomeruli were abnormal.

Data Analysis

Data are provided as means ± SE. Groups were compared by paired or unpaired Student’s t-test or ANOVA with the LSD test for post hoc analysis between groups, as applicable. Statistical significance was set at P < 0.05. Histopathological analysis was by counting within each group the number of kidneys in which glomerulosclerosis was found and by averaging the percentage of affected glomeruli (out of 50 examined) as well as the calculated glomerulosclerosis score within each kidney.

RESULTS

Salt Susceptibility and Proteinuria

Study 1: Normotensive rat (2K) without salt loading. PROTEINURIA. The results are shown in Fig. 1A. Upon initiation of the studies at age –2 mo, SBH/y (n = 10) and SBN/y (n = 10) excreted similar amounts of protein, which amounted to less than 20 mg/day, –50% of which was albumin. In the salt-sensitive SBH/y strain, a gradual rise in protein excretion was observed thereafter, rising at 4 mo to levels above 20 mg/day and reaching a protein excretion rate of 65 ± 7 mg/day at 9 mo. In the contrasting salt-resistant SBN/y strain, protein excretion remained below 20 mg/day until age 5 mo, gradually rising thereafter to

Fig. 1. Protein excretion in male SBH/y (A) and SBN/y (B) with 2 kidneys (A, solid lines) or following uninephrectomy (B, “Uni-Nx”, broken lines). Data are means ± SE. * P < 0.05, SBH/y vs. SBN/y by ANOVA.

39 ± 4 mg/day at 9 mo. As of age 4 mo, the differences in protein excretion between SBH/y and SBN/y were significant, yet of relatively small magnitude (~10–25 mg/day), which we considered insufficient for phenotypic differentiation in genetic studies.

KIDNEY FUNCTION. At 9 mo, serum creatinine, a measure of glomerular filtration rate, in SBH/y (0.57 ± 0.02 mg/dl) was slightly higher than in SBN/y (0.50 ± 0.01 mg/dl, P < 0.05).

BLOOD PRESSURE. Systolic blood pressure in SBH/y at baseline was 141 ± 1 mmHg and at 9 mo was 143 ± 1 mmHg; baseline blood pressure in SBN/y was 129 ± 1 mmHg and at 9 mo was 130 ± 1 mmHg. These values are consistent with our previous reports in the aging rat (41) and confirm that blood pressure in these strains does not rise spontaneously with age (45).

Histopathological FINDINGS. The data are shown in Table 1. The histopathology was studied in 8 of 10 SBH/y-2K. Seven of eight kidneys that were examined were found to have changes consistent with glomeru-
Table 1. Histopathological evaluation of kidneys

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<th>Study Group</th>
<th>Strain</th>
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<th>Sex</th>
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<th>Salt Loading</th>
<th>HTN</th>
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<td>1K</td>
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<td>2K</td>
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<td>1K</td>
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<td>3.0 ± 0.4</td>
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Values are means ± SE, where indicated; n, number of kidneys examined/number of animals within the study group that completed the 9-mo study period. M, male; F, female; 2K, 2 kidneys; 1K, 1 kidney; HTN, hypertension. *P < 0.01 and †P < 0.05 SBH/y vs. SBN/y.

Losclerosis. The average percentage of affected glomeruli in each kidney was 6.0 ± 1.5%, and the average glomerulosclerosis score per kidney was 1.1 ± 0.2. The affected glomeruli in SBH/y were located mostly in the inner cortex or the corticomedullary junction. They were of normal size and cellularity. Segmental sclerosis was noted predominantly at the glomerular hilum, involving 25–100% of the glomerular area. Adhesions to Bowman’s capsule were noted, as well as hyalinosis lesions resembling the exudative type of glomerulosclerosis. The blood vessels were unremarkable. Adjacent to globally sclerotic glomeruli, tubular atrophy and interstitial lymphocytic infiltrates were found. In SBN/y-2K, the kidneys were examined in 7 of the 10 animals. Only 0.3 ± 0.2% of the glomeruli were affected, averaging a low glomerulosclerosis score of 0.1 ± 0.1.

Study 2: Uninephrectomized rats without salt loading. Proteinuria. The results are shown in Fig. 1B. In SBH/y subjected to uninephrectomy (SBH/y-1K, n = 10), protein excretion increased over time to a significantly greater extent than in the 2K-SBH/y rats. At age 9 mo, average protein excretion rate was 195 ± 12 mg/day. In SBN/y (SBN/y-1K, n = 10), uninephrectomy had little effect on proteinuria until age 6 mo, when protein excretion began to increase steeply to 128 ± 10 mg/day at 9 mo. The differences in protein excretion between SBH/y and SBN/y became significant as of age 3 mo (1 mo after uninephrectomy) and remained so until the end of the study period.

Kidney function. Seven months after uninephrectomy, serum creatinine in SBH/y (0.68 ± 0.02 mg/dl) was higher than in SBN/y (0.57 ± 0.01 mg/dl, P < 0.05), indicating some loss of glomerular function in the uninephrectomized SBH/y rat. This difference in serum creatinine between the strains could not be accounted for by differences in muscle mass since the body weight of SBH/y 414 ± 9 g was not significantly different from that of SBN/y 393 ± 6 g.

Blood pressure. Systolic BP at baseline was 141 ± 1 in SBH/y and 130 ± 1 mmHg in SBN/y. Blood pressure in the uninephrectomized SBH/y rat at 9 mo was 143 ± 1 and in SBN/y 130 ± 1 mmHg, values which were not different from those at baseline, nor from those measured in the 2K animals.

Histopathological findings. The data are shown in Table 1. The findings were striking in the SBH/y-1K group. All 10 kidneys within this group were examined. All the kidneys showed severe histopathological changes, with the average percentage of glomeruli within each kidney that was affected with glomerulosclerosis being 45.2 ± 7.0% and an average glomerulosclerosis score of 2.9 ± 0.1. The distribution of the affected glomeruli within each kidney was similar as in the 2K animals (study 1), except that it was more diffuse. Most affected glomeruli showed segmental sclerosis, but occasional glomeruli were globally sclerotic (representative pictures of the lesions are shown in Fig. 2). In sharp contrast, the histopathological lesions in the SBN/y-1K group were mild. Five of 10 kidneys were examined in this group. Although four of the five kidneys that were examined showed glomerulosclerosis, only 2.8 ± 0.7% of the glomeruli in each kidney were affected with a glomerulosclerosis score of 0.8 ± 0.1 (range 0–1).

Study 3: Uninephrectomized rats with salt loading. Proteinuria and blood pressure. The results are shown in Fig. 3. During salt loading, urinary protein excretion rate increased very significantly and rapidly in both SBH/y-2K (n = 10) and SBH/y-1K rats (n = 10) (Fig. 3A), concurrent with the development of hypertension in both (Fig. 3B). At age 6 mo, 4 mo after initiation of salt loading, protein excretion was 137 ± 18 mg/day in SBH/y-2K (n = 8) and 142 ± 20 mg/day in SBH/y-1K (n = 10). Compared with non-salt-loaded animals, these values were threefold higher than in age-matched SBH/y-2K and nearly 50% higher than in the age-matched SBH/y-1K rats. In contrast, salt loading in SBN/y-1K (n = 10) and SBN/y-2K (n = 10) caused an only mild increase in protein excretion, without any concurrent rise in blood pressure. As result,
protein excretion was significantly higher in SBH/y than in SBN/y as of 2 mo after initiation of salt loading, at which time SBH/y also became hypertensive.

HISTOPATHOLOGICAL FINDINGS. The data are shown in Table 1. In each subgroup, three kidneys were examined. In the salt-loaded hypertensive SBH/y-2K, all three kidneys examined were affected with glomerulosclerosis, with the average percentage of affected glomeruli being 6.0 ± 0.2% and the average glomerulosclerosis score being 1.3 ± 0.2. In salt-loaded SBH/y-1K, all kidneys examined were also affected; the percentage of affected glomeruli was 12.7 ± 2.4%, which was higher than in SBH/y-1K, but the glomerulosclerosis index was not different at 1.3 ± 0.2. In contrast, the salt-loaded SBN/y exhibited significantly milder lesions; no glomerulosclerotic lesions were detected in SBN/y-2K, and the percentage of affected glomeruli in SBN/y-1K was only 1.3 ± 0.3% with a glomerulosclerosis score of 0.7 ± 0.2.

Effect of Sex Differences: Studies in Female Rats

Proteinuria. The results are shown in Fig. 4. In female SBH/y-2K (n = 9), protein excretion rose to level above 20 mg/day as age 5 mo but tended to be at lower values than in males. Female SBN/y-2K (n = 6) excreted very low levels of protein throughout the study period. In female SBH/y-1K (n = 6), protein excretion increased over time at a similar rate as in males until age 9 mo, at which time it overshot to 292 ± 32 mg/day. In female SBN/y-1K (n = 6), uninephrectomy had no effect on proteinuria throughout the study period, staying at levels below an average of 20 mg/day. The differences in protein excretion between
female SBH/y-1K and SBN/y-1K became highly significant 2 mo after uninephrectomy and remained so until the end of the study.

Kidney function. Seven months after uninephrectomy, serum creatinine (in mg/dl) was 0.48 ± 0.02 in female SBH/y-2K, 0.51 ± 0.01 in SBN/y-2K, 0.50 ± 0.00 in SBH/y-1K, and 0.53 ± 0.02 in SBN/y-1K. There were no statistically significant differences between the groups.

Blood pressure. Systolic blood pressure (in mmHg) was 142 ± 1 in female SBH/y-2K, 129 ± 1 in SBN/y-2K, 142 ± 1 in SBH/y-1K, and 127 ± 1 in SBN/y-1K. As in males, there was ≤15 mmHg difference in systolic blood pressure between SBH/y and SBN/y.

Histopathology. The data are shown in Table 1. In female SBH/y-2K, three of the four kidneys that were examined were affected with glomerulosclerosis, with the average percentage of glomeruli affected in each kidney being 3.5 ± 1.0% and the average glomerulosclerosis score being 1.0 ± 0.3. Although these values tended to be lower than in male SBH/y-2K, the differences between the sexes were not statistically significant. In female SBH/y-1K, all six kidneys examined were affected; the percentage of affected glomeruli was higher at 23.7 ± 5.8% and the glomerulosclerosis index was 2.2 ± 0.2. These values were significantly lower than those found in male SBH/y-1K. In contrast to SBH/y, no lesions consistent with glomerulosclerosis were found in female SBN/y-2K or SBN/y-1K.

The Genetic Basis of Proteinuria

Consomic for chromosome 1. Construction. We were successful in constructing consomic strains with the genetic background of SBH/y, in which most of chromosome 1 was introgressed from SBN/y (SBH.SBN-1), and its reciprocal, in which most of chromosome 1 from SBH/y was introgressed onto the SBN/y background (SBN.SBH-1). The introgressed region of chromosome 1 was nearly identical in the reciprocal strain. The resulting consomic strains incorporated all of the rf-2
and a significant part of the \textit{rf-1} gene loci (7, 32), as well as the salt susceptibility quantitative trait loci (QTLs) \textit{SS1a} and \textit{SS1b} that we previously reported (42–44) (Fig. 5).

\textbf{PROTEIN EXCRETION}. Introggression of chromosome 1 from SBH/y onto the genetic background of SBN/y (SBN.SBH-1) resulted in the uninephrectomized consomic animal (\(n = 6\)) in a pattern of protein excretion which exceeded that of SBN/y-1K and, interestingly enough, as of age 8 mo exceeded also that of the parental SBH/y-1K rat (Fig. 6A). Introggression of chromosome 1 from SBH/y onto the genetic background of SBN/y (SBH.SBN-1) resulted in the uninephrectomized consomic animal (\(n = 6\)) in a protein excretion pattern that was similar to that of the parental SBN/y-1K and significantly lower than that of the parental SBH/y-1K up to age 8 mo, at which time it increased to a level that was intermediate between the two parental strains (Fig. 6B).

\textbf{KIDNEY FUNCTION}. Plasma creatinine levels of SBN.SBH-1 were 0.52 \pm 0.02 mg/dl, which was lower than that found in SBH/y-1K and even lower than that in SBN/y-1K. In contrast, plasma creatinine of SBH.SBN-1 was 0.68 \pm 0.02, which was similar to that of the parental SBH/y-1K and higher than that of the parental SBN/y-1K strains.

\textbf{BLOOD PRESSURE}. Systolic blood pressure of SBN.SBH-1 was 127 \pm 1 mmHg at baseline, a level not different from that found in the parental SBN/y strain.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{rat_chromosome_map.png}
\caption{Linkage map of rat chromosome 1 with representation of the overlapping regions of the consomic strain and of relevant quantitative trait loci (QTLs). The genomic interval that is fully introgressed from SBH/y onto SBN/y or vice versa is indicated by solid black bars. Open white bars indicate the genomic interval spanning the crossover between the two parental strains, as defined by the closest flanking markers. \textit{SS1a} and \textit{SS1b} denote the QTLs for salt susceptibility we previously detected (42). \textit{RF-1} and \textit{RF-2} denote the QTL for proteinuria previously detected by Brown et al. (6) and confirmed by Shiozawa et al. (32).}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{protein_excretion.png}
\caption{Protein excretion in chromosome 1 consomics compared with uninephrectomized (interrupted lines) parental SBH/y (\textit{Œ}) and SBN/y (\textit{’}) strains. A: the consomic strain with chromosome 1 from SBH/y introgressed onto the genetic background of SBN/y (SBN.SBH-1) (\textit{Œ}). \(*P < 0.05\), consomic strain compared with SBN/y. B: the consomic strain with chromosome 1 from SBH/y introgressed onto the genetic background of SBH/y (SBH.SBN-1) (\textit{Œ}). \(*P < 0.05\), consomic strain compared with SBH/y.}
\end{figure}
Interestingly, baseline blood pressure of SBH.SBN-1 was 130 ± 1 mmHg, which was significantly lower than that observed in parental SBH/y and not different from SBN/y. At age 9 mo, blood pressure in SBN.SBH-1 was 129 ± 1 mmHg, indicating that there had been no rise in spontaneous blood pressure during the course of the 7 mo follow-up.

**HISTOPATHOLOGICAL FINDINGS.** The data are shown in Table 1. In the SBN.SBH-1 group, all kidneys were examined. Five of the six kidneys were affected with glomerulosclerosis, with the average percentage of affected glomeruli being 5.3 ± 1.9% and the average glomerulosclerosis score being 1.0 ± 0.2. These values were significantly lower than in male SBH/y-1K and not different from those found in SBN/y-1K. Thus transfer of chromosome 1 from SBH/y onto the SBN/y background did not capture nor transfer the glomerulosclerosis phenotype. In SBH.SBN-1, three of the three kidneys that were examined were affected with glomerulosclerosis, with the average percentage of affected glomeruli being 12.7 ± 2.0% and the average glomerulosclerosis score being 1.7 ± 0.2. These values were intermediate between SBH/y-1K and SBN/y-1K. Thus transfer of chromosome 1 from SBN/y onto SBH/y background appeared to attenuate but not totally remove the glomerulosclerosis phenotype.

**Chromosome 17 consomic.** CONSTRUCTION. We were able to construct a consomic strain in which most of chromosome 17 was introgressed from SBH/y onto genetic background of SBN/y (Fig. 7). We encountered technical difficulties in constructing the reciprocal strain, because of fertility problems. The resulting consomic strain incorporated all of the rf-5 gene locus (32), as well as the salt susceptibility QTLs SS17 detected so far in females only (42, 44).

**PROTEIN EXCRETION.** Introgresion of chromosome 17 from SBH/y onto the genetic background of SBN/y resulted in the uninephrectomized SBN.SBH-17 animal (n = 6) in proteinuria that was not different from the parental SBN/y-1K strain during the first 2 mo, but rose significantly thereafter to levels that were even above those measured in the parental SBH/y-1K at 9 mo (Fig. 8).

**KIDNEY FUNCTION.** Plasma creatinine levels in the consomic strain were 0.53 ± 0.02 mg/dl, which were lower than those found in SBH/y-1K and not different from those in SBN/y-1K.

**BLOOD PRESSURE.** Systolic blood pressure of SBN.SBH-17 was 128 ± 1 mmHg at baseline, a level not different from that found in the parental SBN/y strain. At age 9 mo, blood pressure in SBN.SBH-17 was 129 ± 1 mmHg, indicating that there had been no rise in spontaneous blood pressure during the course of the 7 mo follow-up.

**HISTOPATHOLOGICAL FINDINGS.** The data are shown in Table 1. In the SBN.SBH-17 group, all of the six kidneys that were examined were affected with glomerulosclerosis, with the average percentage of affected glomeruli being 3.0 ± 0.4% and the average glomerulosclerosis score being 1.0 ± 0.0. These values were significantly lower than in male SBH/y-1K and not different from those of SBN/y-1K. Thus transfer of chromosome 17 from SBH/y onto the SBN/y background did not capture nor transfer the glomerulosclerosis phenotype.

**Rat Chromosome 17**

**Linkage Map**

![Fig. 7. Linkage map of rat chromosome 17 with representation of the overlapping regions of the consomic strain and of relevant QTLs. The genomic interval that is introgressed in the consomic strain from SBH/y onto the SBN/y background is indicated by a solid black bar. The open white bar indicates the genomic interval spanning the crossover between SBH/y and SBN/y, as defined by the closest flanking markers. SS17 denotes the QTLs for salt susceptibility we previously detected (42). Rf-5 denotes the QTL for proteinuria previously detected by Brown et al. (6) and confirmed by Shiozawa et al. (32).](http://physiolgenomics.physiology.org/)

**Consomic strain**

- D17Rat1
- D17Wox2
- D17Mgh1
- D17Rat61
- D17Mgh2
- D17Rat136
- D17Mhi1
- D17Mhi8
- D17Mhi12
- D17Mhi2
- D17Mhi4
- D17Rat38
- D17Rat82
- D17Wox8
- D17Mhi8
- D17Mhi11
- D17Rat50
- D17Mgh3
- D17Rat17 D17Rat55 D17Mgh8
- D17Mgh5
- D17Mhi3 D17Wox4 D17Wox5 D17Rat25
- D17Wox12 D17Wox13 D17Mhi12 D17Rat29
- SS17
- Rf-5
Renal injury occurred spontaneously and glomerulosclerosis in the salt-sensitive Sabra strain. **Renal injury occurred spontaneously and glomerulosclerosis in the salt-sensitive Sabra strain.** Proteinuria and glomerulosclerosis are under different genetic and mechanistic control. We demonstrated, using consomic strains, a functional role of chromosomes 1 and 17 in mediating the renal injury, suggesting the presence of candidate gene loci on these chromosomes. Our data also suggest that, contrary to prevailing concepts, proteinuria and FSGS are under different genetic and mechanistic control. The spontaneous development of proteinuria and glomerulosclerosis in the rat is not unique to the Sabra model, as it appears also in other rat strains (2, 5, 12, 17, 22, 27, 33, 35). Even though this phenomenon has been studied in depth over the past two decades, it still remains incompletely understood. In most strains, it is thought to reflect a “natural” age-dependent occurrence rather than a specific disease entity. Proteinuria and glomerulosclerosis in the Sabra model stand out among other strains in their alleged link to salt susceptibility. Our primary aim in the current study was to determine whether the Sabra model of salt susceptibility could be used as a model organism for the study of the relationship between salt sensitivity, which has been implicated in the pathogenesis of renal disease in humans, and proteinuria and glomerulosclerosis.

One problem that we encountered in the study was in the definition of abnormal proteinuria in that rat, which has been well defined in humans. In the rat, the definition is more complex for two main reasons: one is that rats normally excrete small amounts of protein, with the “normal” level not being clearly defined; the other is that with aging, rats tend to develop “age-related” proteinuria and glomerulosclerosis (8, 12, 20, 27). For purposes of this study, we defined normal protein excretion as ≤20 mg/day and increased proteinuria as any value above that. With regard to the effect of aging, we defined the findings in the salt-resistant SBN/y control as “age-related” proteinuria and glomerulosclerosis. We reasoned that any pathology found in SBN/y that surpassed qualitatively and quantitatively that in SBN/y could be attributed to inherent characteristics of that strain that would be associated with salt sensitivity per se.

In the initial studies in the intact two-kidney animals, we found that proteinuria develops earlier and more massively in the normotensive salt-sensitive SBH/y rat than in the salt-resistant SBN/y strain, without the need for salt loading or the development of hypertension. Although protein excretion was normal in both strains immediately after weaning, it began shortly thereafter to increase, preferentially in SBH/y. Of interest is that proteinuria also developed in SBN/y but to a significantly lesser degree. On histological examination, we found kidneys lesions of the FSGS type. A larger percentage of kidneys and more glomeruli were affected in SBH/y than in SBN/y. We attributed the proteinuria and the minimal glomerulosclerotic lesions in SBN/y to aging and attributed the more highly significant differences in protein excretion and in the severity of the histological lesions between SBH/y and SBN/y to another genetically determined factor. Since selective inbreeding had derived these two contrasting strains from a common ancestor, the selection being primarily for salt sensitivity (3, 41), we reasoned that another genetic factor(s) might be causally related to salt susceptibility, with the alternative being fixation of proteinuria and/or glomerulosclerosis-related genes adjacent to the salt susceptibility genetic loci.

In the course of our attempt to simulate the relationship between proteinuria, focal and segmental glomerulosclerosis (FSGS), and salt sensitivity in humans, we demonstrated the preferential development of proteinuria and glomerulosclerosis in the salt-sensitive Sabra (SBH/y) strain. Renal injury occurred spontaneously but was also markedly amplified by uninephrectomy and/or salt loading and the development of hypertension. We were thus able to establish an experimental model in which salt-sensitivity-related proteinuria and glomerulosclerosis are prominent phenotypes. We investigated in depth these phenotypes and demonstrated a time course as well as sex differences in the development and expression of the renal disease. We undertook initial steps in investigating the genetic basis of the association between salt sensitivity and the renal disease as a means of dissecting out pathogenic mechanisms involved. We demonstrated, using consomic strains, a functional role of chromosomes 1 and 17 in mediating the renal injury, suggesting the presence of candidate gene loci on these chromosomes. Our data also suggest that, contrary to prevailing concepts, proteinuria and FSGS are under different genetic and mechanistic control.

To “amplify” the differences in protein excretion between SBH/y and SBN/y and improve thereby our working model, we resorted to uninephrectomy. The drawback in this procedure was that we introduced an additional potentially confounding element in the experimental model, as uninephrectomy per se has been shown in the rat to induce glomerulosclerosis and proteinuria (14, 19, 25–28). To overcome this confounding element, we contrasted in all experiments the salt-sensitive with the salt-resistant strain, with both hav-
ing been subjected to uninephrectomy. As result of uninephrectomy, the magnitude of protein excretion and of the glomerulosclerotic lesions was indeed augmented, and the differences between the salt-sensitive and salt-resistant strains were accentuated. We thus found in uninephrectomized SBH/y severe proteinuria and glomerulosclerosis, whereas in uninephrectomized SBN/y there was significantly milder proteinuria and glomerulosclerosis, which we attributed to the combined effects of aging and uninephrectomy.

The salt-loading experiments yielded two interesting observations. One was that proteinuria increased during salt loading in SBH/y-2K to the same level as in the SBH/y-1K. This finding can be interpreted as signifying either that salt loading per se, or hypertension, or both, constituted a second hit to the kidney (the first hit being related to the genetic predisposition to glomerulosclerosis), which augmented the degree of renal injury in SBH/y-2K to the level of that inflicted in SBH/y-1K. The second observation was that salt loading in SBH/y-1K did not increase the level of proteinuria beyond that found in animals fed regular chow. This finding suggests that the combined effects of genetic predisposition to glomerulosclerosis and uninephrectomy constituted sufficient insult upon the kidney to elicit a degree of injury that could not be augmented further, neither by salt nor by hypertension. Further interpretations of these results would be speculative at this stage.

Also of interest are the sex differences that were found in the severity of proteinuria and glomerulosclerosis in the Sabra model. Similar sex differences have been reported in other strains of rats (29, 35), findings that have not necessarily been confirmed in humans (24). It is noteworthy that sex differences in susceptibility to renal disease have also been observed in experimental animal models simulating other complex diseases such as hypertension (25, 35) and diabetes (40). The significance of these sex differences, their cause, and their relevance to humans are unknown at this time.

The overall results of the current study lead us to attribute the differences in proteinuria and glomerulosclerosis between the salt-sensitive and salt-resistant animals to genetic factors differentiating between the strains, which might causally be related to salt sensitivity but possibly also to other factors. One possibility that needs to be considered is that the 10- to 15-mmHg difference in blood pressure that is normally found between SBH/y and SBN/y might account for the preferential injury in SBH/y. The likelihood that this is the mechanism that mediates the difference in renal injury between the two strains is small, however, as one would have to infer severely impaired autoregulation, considering the relatively low level of blood pressure which is not commonly thought to be associated with the severe level of renal injury such as was observed in SBH/y. Furthermore, the studies using the consomic strains in which SBH/y level proteinuria was transferred to SBN/y by introgressing chromosomes 1 or 17 from SBH/y while maintaining blood pressure as in SBN/y almost rules out the blood pressure difference between the parental strains as a causative factor.

What is currently known about the relationship between salt sensitivity and renal injury? Salt sensitivity is usually defined in experimental animal models in terms of hypertension, i.e., the salt-sensitive strain develops hypertension whereas the salt-resistant strain remains normotensive. And since hypertension per se has been shown to cause renal injury and proteinuria (30), the obvious link between salt sensitivity and renal injury would have been the elevated systemic arterial pressure. The current study demonstrates, however, that the differential renal damage in the Sabra rats, as expressed by increased proteinuria and glomerulosclerosis favoring the salt-sensitive strain, appeared in normotensive animals without any relationship to actual salt loading or an increase in blood pressure. The Sabra model thus enables us to dissect genetic salt susceptibility away from salt-induced hypertension as a mediator of renal injury. What then are the possible mechanisms for renal injury related to salt susceptibility other than hypertension? At present, there appears to be no conclusive explanation. Salt loading, but not salt susceptibility per se, has been claimed to predispose the kidney to injury, for example, by rendering the kidneys more susceptible to angiotensin II-induced injury (16). We have previously shown in the Sabra rat that high salt intake is deleterious to the permselectivity of the glomerular basement membrane and that 1 mo of salt loading was sufficient to reduce the number of anionic sites in the lamina rara externa and interna (15). However, since we observed differential susceptibility to renal injury without salt loading in SBH/y and SBN/y, these mechanisms do not appear to play a role under these circumstances.

As a means by which to dissect the pathogenic mechanisms involved in renal injury related to salt susceptibility, we initiated the detection of genes that would link proteinuria to salt sensitivity in the uninephrectomized salt-sensitive SBH/y rats. We reasoned that identification of these genes might shed light onto pathophysiological mechanisms involved in proteinuria and glomerulosclerosis in the rat. Such findings could then eventually be carried by homology, through comparative genomics, to humans. We used in our initial studies consomic strains for chromosomes 1 and 17. These two chromosomes have been previously shown to harbor genes that mediate proteinuria in another model of renal disease, the fawn-hooded rat (6, 7, 32). By transferring chromosomes 1 and 17 from SBH/y onto the SBN/y genomic background, we were in fact able to significantly augment proteinuria. We were also able to reduce the amount of proteinuria by transferring chromosome 1 from SBN/y onto the SBH/y genomic background. Our data thus indicate that proteinuria in our model is mediated, at least in part, by major gene loci located on chromosomes 1 and 17, which is consistent with the findings in the fawn-hooded rat. Candidate gene loci that have been located on these chromosomes in that model are the rf-1, -2 and...
Further studies with congeneric and subcongeneric strains are needed to confirm or rule out the participation of these particular gene loci in mediating proteinuria in the Sabra model. Other gene loci of relevance on the same or additional chromosomes cannot be ruled out either. In fact, comparative mapping allows us to detect, even at this early stage, additional candidate genes or gene loci will have to be tested in our model.

One is a QTL found for the autosomal dominant form of focal glomerulosclerosis that is located on human chromosome 19q13 and which is homologous to chromosome 1 in the rat (http://rgd.mcw.edu/VCMAP) (21, 37). Another is a locus on human chromosome 1q25–31 (36), which overlaps with a syntenic region of the glomerulosclerosis susceptibility locus Pur1 in the BUF/Mna rat on chromosome 13. A third is the gene encoding α-actinin-4 (ACTN4), an actin-filament crosslinking protein, as the cause of FSGS in several families with the autosomal dominant form of the disease, suggesting altered regulation of the actin cytoskeleton of glomerular podocytes (18). This gene has been detected on chromosome 1q22 in the rat (31).

Finally, with regards to glomerulosclerosis and in contrast to proteinuria, our findings in the studies using the consomic strains do not support a major role for chromosomes 1 or 17 in mediating the renal histological injury, although it does appear that chromosome 1 might contribute to a small degree to glomerulosclerosis. Interestingly, glomerulosclerosis has been mapped in the fawn-hooded rat to a major locus on chromosome 1 (6). On the other hand, proteinuria and glomerulosclerosis in the fawn-hooded rat are not related to salt susceptibility, possibly explaining the divergent findings with the Sabra model. Our data imply that proteinuria and glomerulosclerosis in the Sabra rat are under different genetic control. This presents a truly novel concept in the current understanding of the pathogenesis of FSGS in which proteinuria has been traditionally causally related to sclerosis of the glomerulus. Such dissociation between function and histology, however, is perhaps not truly surprising, as in other renal disease entities, the best example being minimal change glomerulonephritis, proteinuria can be very significant without light microscopy evidence of renal injury.

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REFERENCES


