Genetically defined risk of salt sensitivity in an intercross of Brown Norway and Dahl S rats

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Genetically defined risk of salt sensitivity in an intercross of Brown Norway and Dahl S rats. Physiol Genomics 2: 107–115, 2000.—A genetic segregation analysis was performed to identify genes that cosegregate with arterial blood pressure traits reflective of salt sensitivity. A population of 113 F2 male rats was derived from an intercross of inbred SS/JrHsd/Mcw (Dahl salt-sensitive) and BN/SS/N/Mcw (Brown Norway) rats. Rats were maintained on an 8% salt diet from the age of 9 to 13 wk, and arterial pressure was measured for 3 h daily during the 4th wk of high salt intake in unanesthetized rats using implanted arterial catheters. At the end of the 3rd day of high-salt pressure recordings, the arterial pressure response to salt depletion was determined 1.5 days following treatment with Lasix and a low-sodium (0.4%) diet. A genome-wide scan using 265 polymorphic simple sequence length polymorphism (SSLP) markers found that seven arterial pressure phenotypes determined at different times and circumstances, and representing two distinct indexes of salt sensitivity, mapped to the same region of rat chromosome 18. The trait of salt sensitivity was strongly influenced by the presence of SS alleles in this region of chromosome 18, and those rats which were homozygote SS/SS exhibited a significantly greater reduction of mean arterial pressure following sodium depletion (29 ± 2 mmHg) than homozygote BN/BN (17 ± 3 mmHg) or heterozygotic (22 ± 2 mmHg) rats. This region of rat chromosome 18 corresponds to the long arm of human chromosome 5 and a region of human chromosome 18 that has been linked to hypertension in humans. Given the unlikely chance of these different blood pressure traits mapping to the same region, we believe these data provide evidence that this region of rat chromosome 18 plays an important role in salt-induced hypertension.

hypothesis; salt; blood pressure; rat chromosome 18

Based upon a large number of observational and interventional trials, relatively consistent but only modest effects of sodium intake upon arterial pressure have been found (16, 23). Yet, it is evident that arterial pressure in certain individuals can be markedly influenced by changes in sodium intake or by use of diuretics as antihypertensive agents. This is especially evident in the African-American population, where as many as 70% of hypertensive subjects have been reported to be salt sensitive (34, 39).

Despite efforts to stratify salt sensitivity in the general population based on age, race, and gender, these approaches have shown little utility as predictors of an individual’s risk to salt intake. With rapid progress in the mapping of multifactorial traits in the mouse, rat, and human genomes and eventual sequencing of all three organisms, it is now possible to consider new approaches to this problem that may enable future genetic epidemiologists to identify individuals with gene(s) that confer an increased risk to develop hypertension with a high-salt diet. To this end, it will first be necessary to define the gene(s) and their allelic variants that contribute to salt sensitivity. The present study represents a step in this direction by utilizing techniques of quantitative genetics to identify genes responsible for salt sensitivity in rats and illustrates the differences between gene effects within population vs. the gene effects within the individual.

With the advent of a dense rat genetic map (2, 11, 32), inbred hypertensive rat strains provide particularly robust models for the genetic dissection of various pathways that contribute to hypertension and related phenotypes. Genetic analysis of large rat crosses overcomes many of the problems faced in human studies of complex genetic traits in which multiple genes, moderate gene penetrance, environmental interactions, and genetic heterogeneity (allelic differences within the same gene, or different gene combinations causing the same phenotype) are present in the population. Hereditary hypertension in rats is a result of naturally occurring allelic variants captured during the selection and inbreeding process. Use of inbred rats removes the problem of heterogeneity. Furthermore, since the coding region of the rat genome is more than 90% similar to the human genome (24), there is a strong possibility that disease genes identified in the rat can be studied in...
regions of conserved gene order between humans and rats.

The data reported in this manuscript represent the initial analysis carried out as part of a more extensive complex genetic linkage analysis to map nearly 247 traits which were considered to be likely determinants (intermediate phenotypes) of arterial blood pressure. Toward this end, a multiple cosegregation analysis of these many traits was carried out using an F2 intercross. The analysis was carried out using an inbred line of SS/J HsdDMcw (Dahl Salt-sensitive rats) and BN/SSN/Mcw (Brown Norway) rats. A collection of 265 PCR-based genetic markers (2, 11, 32) were used to scan the entire rat genome for regions that harbor genes, so-called “quantitative trait loci” (QTLs), responsible for these 247 traits.

The present report focuses upon arterial pressure salt sensitivity, a trait that was characterized both by the level of arterial pressure achieved following 4 wk of a high-salt diet (8%) starting at 9 wk of age and also by the reduction of pressure achieved following 36 h of sodium depletion at the end of the 4-wk salt-feeding period. The results of these studies indicate that rat chromosome 18 contains a gene region that plays an important role in determining arterial pressure salt sensitivity.

METHODS

Generation of the F2 rat population. Experiments were performed in an F2 rat population from an intercross between inbred normotensive SS/J HsdDMcw (Dahl salt-sensitive rats) and BN/SSN/Mcw (Brown Norway) rats. These parental strains originated from colonies of rats purchased from Harlan Sprague Dawley (Indianapolis, IN). The BN/SSN/Mcw colony was inbred from a single pair of SSN line rats obtained from the Alabama colony (Harlan Sprague Dawley). The SS/J HsdDMcw strain was inbred from a congenic control group of Dahl S rats (SS/Ren) obtained from Dr. Theodore Kurtz (UCSF, CA) which were originally derived from the Harlan SS/J r colony (13). The derived inbred MCW strains will be referred to herein as the BN and SS strains of rats.

The SS rats have been maintained in our Medical College of Wisconsin vivarium since June 1991, and the BN have been maintained since June 1995. Both of these strains required considerable marker-assisted breeding to eliminate residual heterozygosity and genetic contamination that plagues nearly all commercially available rat strains sold worldwide. To further assure homozygosity, a panel of 200 microsatellite markers (Research Genetics, Huntsville, AL) that provided even coverage of the 21 chromosomes (20-cM intervals) were used to genotype the rats used for generation of the F2 population. The parental rats selected as the progenitors for the present study rats were homozygous for all regions tested. In the F2 progeny, we did not observe any unexpected alleles in the 265 markers used, further suggesting the rats are inbred. Each of these parental strains have since undergone periodic total genome scans to assure allelic homogeneity. F1 generation rats (n = 19) obtained from the intercross of the inbred male SS and female BN rats were brother-sister mated to produce the 113 F2 generation rats used as the F2 study population. A total of 35 male Dahl SS, 42 male BN, and 113 F2 generation male rats that were phenotyped are reported in this report.

Sodium dietary regimen and surgical protocol. Breeding animals were maintained on a 0.4% NaCl rat chow diet (Dyets, Allentown, PA), since a 0.1% salt diet impairs fertility. Weaned rats were placed on a 0.4% salt diet until 5 wk of age to allow for normal development and then placed on a lower salt diet (0.1% NaCl) until 9 wk of age. At this time, when the postnatal development of the kidney is complete, the NaCl content in the rat chow was increased to 8%. After 3 wk on the high-salt diet, rats were anesthetized with ketamine (30 mg/kg ip) and xylazine (2 mg/kg ip), and a chronic arterial catheter was implanted in the left femoral artery, exteriorized at the shoulders, and run up a spring which was attached to the rat with a small jacket. Surgery was followed by an injection of penicillin (300,000 U/kg im) to prevent infection, and buprenorphine (0.1 mg/kg sc) was administered the first day to minimize pain. One week was allowed for full recovery from surgery prior to initiating the measurement of arterial pressure.

Phenotyping protocol. All rats were housed individually in their home cage, which can double as a metabolic cage (Suburban Surgical, Wheeling, IL), within a specially designed chronic rodent hemodynamic monitoring facility. At 13 wk of age (1 wk following catheter surgery), the experimental protocol began. Coat quality, grooming patterns, activity, and food and water consumption were assessed daily as indexes of animal health. Any animal that did not meet strict criteria of health were eliminated from the study. Arterial pressures (systolic, diastolic, and mean) were recorded using solid-state pressure transducers (Argon Medical Technologies, Athens, TX). The output of the analog pressure signals was amplified (StemTech, GPA-4; Quintron, Menominee Falls, WI), low-pass filtered (30 Hz, 4 pole), and sampled at 100 s⁻¹ (Significat, Newton, MA). The digitized signals were processed (Apollo DN3500, Hewlett-Packard) and converted to 1-s (for time series analysis) or 1-min averages. The frequency response of the entire analog and digital system (catheter, transducer, amplifier, analog-to-digital, computer) was evaluated and found to be of second order with a damping ratio of 0.4, a roll off frequency of 16 s⁻¹, and an average amplitude ratio of 0.993 over the range of 0 to 35 s⁻¹. These frequency response characteristics therefore enabled determination of systolic and diastolic arterial pressures in the rat. Daily measurements of pressure were collected at the same time each day for 3 h during the “inactive” light cycle during week 4 of high salt intake while the rats rested unrestrained in their home cages. During the second day of pressure recordings, an additional 4-h recording of pressure was also made during the “active” dark cycle. A 24-h urine collection was also obtained during this 2nd day of pressure recording.

After pressure was recorded for 3 days, the rats were then given an intraperitoneal injection of furosemide (10 mg/kg Lasix) to induce salt depletion and consequently switched to a 0.4% sodium chloride rat chow to maintain a salt-depleted state. After 36 h on the low-salt diet, blood pressure was measured again for 3 h during the light cycle, and a 24-h urine was again collected as described above.

DNA extraction and genome scan. At the end of the experimental protocols, the rats were anesthetized with ketamine (20 mg/kg ip) and Inactin (25 mg/kg ip). The liver was excised, snap frozen in liquid N₂, and stored at –80°C for later DNA extraction. Upon thawing, this tissue was minced and incubated overnight in lysis buffer containing 100 μg/ml proteinase K. This solution was precipitated with 100% isopropanol and centrifuged at 1,200 g. The pellet was washed with 75% ethanol, air dried, resuspended in Tris-HCl/EDTA (TE) buffer, pH 7.5, and DNA was quantified by spectrophotometry (model DU640; Beckman Instruments,

http://physiolgenomics.physiology.org
A genome-wide scan was performed using 265 simple sequence length polymorphism (SSLP) genetic markers with an average spacing of 10 cM, as described previously (31), and analyzed using the MAPMAKER/QTL computer package as described by Lander and Kruglyak (22).

Parametric linkage analysis. Normality of all phenotypes was examined by a panel analysis that included examination of quantiles, cumulative distribution, and application of Kolmogorov-Smirnov test (38). Phenotypes that did not pass the normality test were then transformed (either square root or log) and retested. Phenotypes that passed the normality test (transformed or not) were then mapped using a parametric linkage analysis (20). Those that did not pass the normality test were mapped using a nonparametric analysis (18). Unless otherwise noted, LOD scores reported resulted from parametric analysis. For the parametric tests, an LOD threshold of 4.3 was used to establish significant linkage for our cross structure, whereas an LOD threshold of 2.8 was considered to be suggestive of linkage as described by Lander and Kruglyak (22). A permutation test, by randomly assigning the phenotypes relative to the genotypes in 1,000 replicated tests was used to determine the threshold of significance after multiple comparisons. For this cross with 113 animals and 265 genetic markers, the Lander-Kruglyak thresholds are appropriate surrogates for multiple comparison. Different genetic models (additive, dominant/recessive, and free) were evaluated for each of the traits reported here.

Nonparametric linkage analysis. The statistic $(X_w)$ testing both additive and dominant components present in the genetic model was used for the nonparametric analysis. The threshold, $T$, was selected such that under the assumption of no linkage, the probability that the nonparametric statistic exceeds $T$ at any given point on the genome was equal to the targeted false-positive rate ($0.05$). The threshold estimate for this study was calculated to be 4.45, based on assumptions related to map marker density, map length, number of markers, number of chromosomes, and estimated crossovers per Morgan (18).

Allele sharing. To illustrate the effect of a single gene region on salt sensitivity in both the F2 population and the individual rats, we selected genetic markers around the peak of the LOD score for the reductions of mean arterial pressure (MAP) that occurred with salt depletion. This interval spans 28 cM and contains five genetic markers: D18Mit8, D18Rat57, D18Rat18, D18Mit5, and D18Mgh9. Genotypes of all F2 rats at these genetic markers were counted and frequencies of common genotypes for each marker were calculated.

Statistics for phenotypes. Physiological data are presented as mean ± 1 SE or, when noted, as ± SD. The significance of differences in mean values within and between groups was determined with an analysis of variance followed by a Tukey multiple comparison test. A probability level of $P < 0.05$ (two-tailed test) was considered significant.

RESULTS

Blood pressures in parental and F2 populations on high salt intake. As summarized in Fig. 1, MAP determined during the “inactive” light cycle of male BN rats ($n = 42$) maintained for nearly 4 wk on 8% NaCl rat chow averaged 109 ± 2 mmHg and ranged from 89 to 145 mmHg; SS rats ($n = 35$) were significantly higher, averaging 185 ± 5 mmHg and ranged from 127 to 230 mmHg. Systolic arterial pressure (SAP) of the parental BN rats averaged 127 ± 3 mmHg, and diastolic arterial pressure (DAP) averaged 94 ± 2 mmHg. SAP of SS rats were significantly higher averaging 206 ± 6 mmHg, and DAP averaged 167 ± 4 mmHg. The levels of MAP were significantly greater than those achieved in another group of SS rats ($n = 28$) maintained on a low-salt diet (0.4%) throughout their life, in which MAP (measured in the same manner) at 12 wk of age averaged 125 ± 3 mmHg. F2 rats in the present study (Fig. 1) were significantly different from both parental strains with an average MAP of 133 ± 2, SAP of 159 ± 2, and DAP of 112 ± 2 mmHg.

Significant parental strain differences in arterial pressure were also observed when recorded during the “active” dark cycle of the day. “Active” MAP of BN rats on day 2 of recording averaged 114 ± 4 mmHg, SS rats 172 ± 7 mmHg, and F2 rats 135 ± 2 mmHg. “Inactive” MAP of BN rats on this day averaged 109 ± 3 mmHg, SS rats 173 ± 7 mmHg, and F2 rats 131 ± 2 mmHg. There was thus a significant difference in the change of MAP that occurred from the light to dark cycle with BN rats increasing on average 4.5 ± 2 mmHg, whereas SS rats decreased 7.0 ± 7 mmHg.

Frequency histograms of the change of MAP in response to salt depletion. The two overlapping histograms presented in Fig. 2 represent the distribution of MAP in the F2 rats as determined while receiving either a high (8%) daily sodium intake (top right distribution) or following sodium depletion with maintenance of a low 0.4% sodium intake (bottom left distribution). It is seen that MAP of F2 male rats on the high sodium intake is skewed moderately toward higher pressures. However, it is evident that there was a downward shift in the MAP following sodium depletion ($P < 0.05$), and low-salt MAP of the F2 population appears more normally distributed.

Arterial pressure responses of parental strains to sodium depletion. The reduction of MAP (Fig. 3, left) that occurred during the 36 h following sodium depletion was significantly greater in parental SS rats, averaging 45 ± 4 mmHg compared with BN rats, which averaged 14 ± 2 mmHg. Following sodium depletion, the average MAP remained significantly different be-
between the parental strains, averaging 96 ± 3 mmHg in BN (n = 21) and 141 ± 5 mmHg in SS (n = 24). The slope of the relationship between daily sodium excretion and MAP is used in this study as an index of salt sensitivity. The slope of the linear regression line of this relationship was significantly greater in the SS rats (SS, MAP = 3.6 × UNaV + 142; r² = 0.5; where UNaV is the urinary sodium excretion rate) compared with the value seen BN rats (BN, MAP = 1 × UNaV + 94; r² = 0.3), which was not significantly different than the average of the F2 male population shown in Fig. 4.

Figure 3, right, represents the same relationship of F2 rats whose reduction of pressure following sodium depletion was greater than 1 standard deviation from the mean representing the 10% tails, which contain over 80% of the genetic variance (20). When the slopes of the relationships between sodium excretion and MAP of the individual rats at the tails (± 1 SD) of the F2 distribution were compared with the parental BN and SS, it is evident these F2 rats in the tails appear phenotypically similar to their respective parental strains (Fig. 3, left). The slope of the sodium excretion/MAP relationship of F2 rats in the lower tail in which ΔMAP was less than 11 mmHg (1 SD from the mean) with salt depletion (MAP = 0.3 × UNaV + 112; r² = 0.05) was not significantly different from the parental BN/Mcw rats. Conversely, the slope of those F2 rats in the upper tail in which MAP fell greater than 34 mmHg with salt depletion (MAP = 2.1 × UNaV + 115; r² = 0.5) was not significantly different from the parental SS rats. As expected, the animals in the two extremes are much more representative of the parental strains, suggesting that they are carrying more SS or more BN blood pressure alleles than the average F2 animal.

Arterial pressure responses of F2 rats to sodium depletion. The individual responses of the male F2 rats...
to sodium depletion are illustrated in Fig. 4, in which the relationship between the 24-h sodium excretion and associated MAP is represented. It is apparent from this graph that some of the individual F2 rats exhibited a substantial reduction of MAP with sodium depletion while others did not. The calculated regression equation (MAP = 1 × UNaV + 112; r² = 0.3) indicates that on average the reduction of MAP in the F2 population as a whole (23 ± 1 mmHg) was significantly less than that seen in the parental SS rats (45 mmHg) but greater than that seen in the BN (14 mmHg) rats illustrated in Fig. 3, left. MAP of F2 rats following sodium depletion fell to an average 111 ± 1 mmHg. For these three traits, the F2 averages were intermediate between the parental strains. This is consistent with a multifactorial trait.

Genetic linkage analysis. Figure 5 illustrates the results of the QTL linkage analysis, in which it is apparent that seven blood pressure-related phenotypes mapped to a broad region of chromosome 18 with the 95% confidence intervals of these QTLs falling within a 36-cM region between D18Mit8 and D18Mit1. These QTLs fell into two broad categories defining salt sensitivity. One category represents the level of hypertension reached after 4 wk of a high salt intake. Specifically, the average MAP during the inactive period recorded over 2–3 days following 4 wk of high salt intake mapped with a LOD score of 3.9 (nonparametric). Also, the “active” dark cycle MAP of the day 2 mapped with a LOD score of 4.4 (nonparametric), and the “inactive” light cycle MAP recorded on day 1 of the 3-day high-salt recording period mapped with a LOD score of 3.5 (nonparametric). MAP and DAP recorded during the “inactive” light cycle of day 2 also mapped to this same region with LOD scores of 3.3 and 2.8 (parametric), respectively.

Phenotypes in the second category of salt sensitivity represent the reduction of pressure that occurred following sodium depletion. Specifically, the reduction of MAP and SAP mapped with respective LOD scores of 4.6 (nonparametric) and 3.6 (parametric). The fraction of the variance in salt sensitivity that could be accounted for by the QTLs for ΔMAP and ΔSAP was 16.5 and 17%, respectively. Although both the average arterial pressures recorded over the 2- to 3-day period and the pressure reduction with sodium depletion are both generally considered indexes of salt sensitivity, these two entities were not statistically correlated.

Taken together, the mapping of these seven phenotypes representing salt sensitivity within the same broad locus strongly suggests that a QTL for blood pressure exists on chromosome 18.

Allele sharing of salt-sensitive and salt-insensitive F2 rats. Figure 6 presents the degree of reduction of MAP following sodium depletion with each rat ranked by the degree of pressure reduction. The center horizontal line represents the average 23 ± 1 (SE) mmHg MAP reduction of the F2 male population. The top and bottom horizontal lines in Fig. 6 represent ± 1 SD of the mean, referred to as the “left tail” and “right tail” of this distribution.

Fig. 5. Results of the genetic linkage analysis found that several indexes of blood pressure salt sensitivity phenotypes mapped to chromosome 18. Mapped polymorphic markers are represented with centimorgan (cM) distances indicated. Short bar (left) represents the 36-cM region containing all of the 95% confidence intervals of the parametric analysis and the peak LOD scores of each quantitative trait loci (QTL) (horizontal hatch marks). Indexes of salt sensitivity fell into two broad categories. Category 1 phenotypes represent the level of hypertension reached after 4 wk of a high salt intake. QTLs under this category were as follows: “inactive” average day 2–3 MAP which mapped with a LOD score of 3.9 (NP; [D18Mit8 to D18Rat18]); day 2 “active” dark cycle MAP with a LOD score of 4.4 (NP; [D18Mit8 to D18Mit5]); day 1 “inactive” light cycle MAP with a LOD score of 3.5 (NP; [D18Mit8 to D18Rat18]); day 2 “inactive” MAP and DAP mapped with LOD scores of 3.3 (P; [D18Mit8 to D18Mit1]) and 2.8 (P; [D18Mit8 to D18Mit7]), respectively. Category 2 phenotypes represent the reduction of pressure which occurred following sodium depletion. Specifically, the reduction of MAP and SAP mapped with respective LOD scores of 4.6 (NP; [D18Mit8 to D18Mit8]) and 3.6 (P; [D18Mit8 to D18Mit1]). SAP, systolic arterial pressure; DAP, diastolic arterial pressure; D2–3, two- to three-day high-salt average; D1, day 1; D2, day 2; P, parametric analysis; NP, nonparametric analysis; 95% confidence intervals are indicated by square brackets, above.
F2 distribution. The rats in the right tail (n = 14) exhibited an average MAP reduction of 43 ± 2 mmHg, whereas rats in the left tail averaged 5 ± 2 mmHg (n = 9). It is the slopes of these relationships between salt depletion and MAP of the rats in these respective “tails” (the salt sensitivity index) that were compared with the parental rats in Fig. 3.

Figure 6 contains a second important feature in that the genotype of each of these rats is defined by color coding. Specifically, rats are represented in orange were homozygous SS using the marker at the peak of the QTL for this salt sensitivity phenotype; those that were homozygous BN are shown in blue; and heterozygotes are in lavender. An allele-sharing analysis using 5 genetic markers that covered the 95% confidence limits of the salt-sensitive QTL region on chromosome 18 also demonstrated that F2 rats (n = 14) within the right tail of the distribution (>1 SD) were either SS homozygote (64%) or heterozygote (36%). Those rats (n = 9) within the left tail of the F2 distribution (<1 SD) were either BN homozygote (56%) or heterozygote (42%). These results show that the trait of salt sensitivity is strongly influenced by the presence of SS alleles in this QTL region of chromosome 18.

Figure 7 utilizes the genotype data shown in Fig. 6 to illustrate the relationships between the reduction of MAP with salt depletion and the genotypes of F2 male rats. Using the single marker at the peak of the QTL, we can see that the trait of salt sensitivity was strongly influenced by the presence of SS alleles in this region of chromosome 18, and those rats which were homozygote SS/SS (seen in orange in Fig. 6) exhibited a significantly greater reduction of MAP following sodium depletion (29 ± 2 mmHg) than homozygote BN/BN (17 ± 3 mmHg; shown in blue in Fig. 6) or heterozygotic (22 ± 2 mmHg) rats.

DISCUSSION

In the present study, a cosegregation analysis using total genome scans was carried out to determine QTLs for salt sensitivity of blood pressure in an F2 cross of BN and DS rats. The data summarized in this report are part of a more extensive linkage analysis utilizing more than 247 measured or derived phenotypes that reflect many likely determinants of blood pressure. The primary focus of the present analysis was to define chromosomal regions that segregated with the trait of blood pressure salt sensitivity. Although there is no single accepted definition of salt sensitivity, the results show that two different indexes of this trait mapped to the same region of chromosome 18. The reduction of blood pressure (ΔMAP and ΔSAP) that occurred with salt depletion following treatment with Lasix and a low-salt diet was used as one index of salt sensitivity. This index was chosen because it is analogous to that

![Fig. 6. F2 male rat population ranked by the degree of reduction of MAP following sodium depletion. Middle horizontal line represents the mean reduction of MAP in the F2 rats. Top and bottom horizontal lines (right tail and left tail) delineate those rats which exhibited reductions of MAP with sodium depletion that exceeded ±1 SE of the mean. The genotype of each of these rats is defined by color coding: homozygous SS are represented in orange, homozygous BN are in blue, and heterozygotes are in lavender. Rats that showed the greatest reduction of MAP were either homozygote SS (64%) or heterozygote (36%) based on an allele-sharing analysis using 5 genetic markers that covered the 95% confidence limits of the salt-sensitive QTL region of chromosome 18.](http://physiolgenomics.physiology.org)
commonly used to define human salt sensitivity as summarized by Weinberger et al. (39). Since we have shown previously that rats rapidly achieve salt balance within a day following a change of sodium intake (29), the 36 h of low salt intake (0.4%) following Lasix administration would have enabled achievement of a new state of sodium balance. The other equally important index of salt sensitivity used in the present study was the level of arterial pressure achieved following 4 wk of a high (8%) salt intake. Specifically, a number of different arterial pressure phenotypes obtained during the final 4th week of the high-salt diet and representing both active and inactive periods of the day, all mapped to the same region of chromosome 18.

Finally, it should be noted that Tonellato et al. (37) carried out a time series analysis using the pressure data obtained from these same F2 rats. This robust stochastic mathematical model was capable of reproducing distinct features of the time plots, power spectra, and autocorrelation functions of the recordings. It was found that several of the parameters of this model that represented arterial pressure fluctuations over time mapped to this same locus of chromosome 18 (15).

Taken together, the results of the present analysis indicate that no matter whether salt sensitivity is defined as the response to sodium depletion or the level of arterial pressure achieved over a prolonged period of a daily high-salt diet, these traits map to the same region of chromosome 18. Furthermore, these indexes represent independent traits, because the levels of MAP or DAP achieved following 4 wk of high salt intake were not significantly correlated with the reduction of arterial pressure achieved with salt depletion. Given the unlikely chance of all of these blood pressure traits mapping to the same region, we believe that these data provide evidence that this region of rat chromosome 18 plays an important role in arterial pressure regulation and salt sensitivity.

Prediction of salt sensitivity from genotype and allele sharing of F2 rats. In contrast to the linkage analysis and LOD scores alone the extent to which the allelic characterization of rats in the F2 population could predict the trait of salt sensitivity. As was shown in Fig. 6, the F2 male rats that shared more of the SS alleles in this QTL region of chromosome 18 were salt sensitive, whereas those that shared more of the BN alleles were more likely to be salt insensitive. Furthermore, within the F2 population of male rats, the arterial pressure of those rats that genotyped as homozygote SS fell to a significantly greater extent than that which occurred in the homozygote BN rats (Fig. 7). The results show that the trait of salt sensitivity is strongly influenced by the presence of SS alleles in this region of chromosome 18.

The traditional manner of representing salt sensitivity in human population studies has been to relate the amount of daily excreted sodium to the measured level of arterial pressure, since it is not generally feasible to collect a 24-h urine sample first, with the patient on a controlled low-sodium diet and then later on a high-sodium diet. When studies are carried out in this manner and a regression analysis is performed to define the relationship between sodium excretion and blood pressure within the general population, usually the relationship is rather unimpressive (23). Such a relationship is represented for the F2 general population in Fig. 4 by the regression line obtained from all data points, and it is evident that when salt sensitivity is expressed in this manner, the relationship between sodium excretion and arterial pressure in our rat study population is unimpressive (e.g., value of the slope is low). However, when arterial pressure of each rat was evaluated before and following sodium depletion, it is clear that some of the F2 rats resembled parental BN rats and are quite salt insensitive, whereas others resemble parental SS rats and are salt sensitive (Figs. 3 and 6). These data illustrate why it is so difficult to characterize salt sensitivity in human population studies even though many individuals can be quite salt sensitive.

Homologous regions in humans and potential candidate genes. The broad region of rat chromosome 18 related to salt sensitivity corresponds to that reported by Jacob et al. (12) to be linked with blood pressure after a salt load in an F2 cross of spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats. Studies by others have found associations between blood pressure and sodium intake or salt depletion such as that found in the Milan hypertensive strain (MHS) rat model of hypertension, which linked the alpha-adducin gene with hypertension and sodium metabolism (1). A random genome screen in the Sabra rat model of hypertension found two QTLs on chromosome 1 that contributed to the salt sensitivity in these rats (40). Many regions of the DS genome may contribute to hypertension as recently reviewed by Rapp (25). Indeed, suggestive or significant blood pressure-related QTLs have been found on rat chromosomes 1 (9), 2 (5, 6, 7), 3 (9), 5 (9), 7 (3), 9 (27), 10 (7, 8), 12 (14), and 13 (26). A suggestive linkage of blood pressure on chromosome 18 was reported by Garrett et al. (9) in a DS × Lewis cross, and a strong QTL on this chromosome was found in an F2 cross of SHR and BB/OK (spontaneously diabetic rat) by Kovacs et al. (17).

Failure to find common areas of linkage between different crosses is to be anticipated, given the differences in the genetic backgrounds of the parental strains used to generate the different crosses. This genetic diversity provides the opportunity for different gene interactions in these different crosses whereby salt-sensitive alleles may result in hypertension when expressed on one of the genetic backgrounds but not the other. Such heterogeneity would indicate that there should exist different forms of salt-sensitive hypertension depending on the expression of salt-sensitive genes on a different genetic background. This would indeed conform with our physiological understanding of the many complex pathways and neuroendocrine controllers that can influence sodium homeostasis.

Comparative maps indicate that the region of rat chromosome 18 found in the present study corresponds to the long arm of human chromosome 5 between
5q22–5q32 and a region of human chromosome 18 between 18q12-q21. A number of interesting candidate genes, which could potentially influence salt sensitivity, are contained within the 95% confidence limits of the QTL on chromosome 18. For example, the β2-adrenergic receptor gene is located in this region. Interestingly, evidence of linkage at the β2-adrenergic receptor locus of human chromosome 5q31–32 to salt sensitivity in African Americans has been reported (35). The role of this receptor in the regulation of heart rate and contractility, vascular tone, lipid mobilization through lipolysis in fat cells, and in the control of renin secretion from the juxtaglomerular cells of the kidney is well established. The dopamine receptor gene is also located within this QTL region, as determined by comparative mapping from the human genome map (4). Both of these candidate genes have been linked to hypertension in humans (19). Also contained within the chromosome 18 QTL region is PDGFRB (platelet-derived growth factor, receptor beta), which has been implicated in atherogenesis and belongs to the subfamily of receptor tyrosine kinases (41), and EGR1 (early growth response transcription factor) and FGFR1 (fibroblast growth factor 1), which are growth factors and could influence vascular tone (see the “Online Mendelian Inheritance in Man” site, at http://www.ncbi.nlm.nih.gov/omim).

Clinical relevance of the rat model system: comparative genomics strategy. Linkage studies using inbred strains of rats offer a number of advantages over studies in human populations. Genetic heterogeneity and the multifactorial inheritance of hypertension makes the identification of genes of susceptibility to hypertension difficult in human studies, since the contribution of individual genes to hypertension may be small and the expression of hypertension in any individual is likely to result from the inheritance of a different combination of genes (25). Genetic and physical mapping can localize naturally occurring mutations using inbred rat models that can also be generated in large numbers for extensive phenotyping and mechanistic studies. Because of the high degree of conservation of gene order and expressed proteins between organisms, comparative alignment of genes controlling traits across species can then be used to generate candidate regions for comparative human studies.

The extent to which model systems can be used to understand the basic pathophysiology of disease has long been debated. Evidence is mounting that the natural variant models of hypertension found in rat strains can predict QTLs that contain a gene(s) that contributes to human hypertension. Fifty-seven QTLs for 33 blood-pressure traits have been identified in the progenies of seven F2 rat intercrosses for genetic hypertension, and this translates into 26 homologous regions in the human genome using a comparative mapping strategy (33). To date, five of the six known QTLs for human hypertension have been correctly predicted from the genetic studies of hypertension in rat models (33). As seen from the present study, the suggested QTL of chromosome 18 corresponds to the homologous region related to systolic pressure variation found in a human linkage and association study by Krushkal et al. (19).

In summary, the present study indicates that chromosome 18 contains a loci that plays an important role in determining the influence of sodium intake upon arterial pressure. The utility of this finding is that by comparative mapping, these data may point to a potential genomic region of importance for salt sensitivity in human populations. As the technologies of genotyping improve in efficiency, accuracy, and cost, it is hoped that these genetic approaches may allow for better risk assessment and point toward the more effective ways to predict the best therapeutic approach for subjects with essential hypertension.

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REFERENCES


