Congenic strains confirm the presence of salt-sensitivity QTLs on chromosome 1 in the Sabra rat model of hypertension

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Submitted 23 August 2002; accepted in final form 14 November 2002

Yagil, Chana, Norbert Hubner, Reinhold Kreutz, Detlev Ganten, and Yoram Yagil. Congenic strains confirm the presence of salt-sensitivity QTLs on chromosome 1 in the Sabra rat model of hypertension. Physiol Genomics 12: 85–95, 2003. First published November 19, 2002; 10.1152/physiolgenomics.00111.2002.—We previously detected by linkage analysis in segregating populations derived from crosses between the Sabra hypertension-prone rat (SBH/y) and the hypertension-resistant strain (SBN/y) two QTLs for salt susceptibility on chromosome 1, with sex specificity: in males SS1a and SS1b, and in females SS1b only. To provide support for a functional role of these QTLs in relation to hypertension, we constructed congenic strains by replacing most or selected segments from chromosome 1 from SBN/y with the homologous chromosomal regions of SBH/y, or reciprocally from SBH/y with segments of SBN/y, leaving the other chromosomes unperturbed. Genetic screening with over 150 microsatellite markers confirmed the homozygosity of the targeted genomic inserts and of the remainder of the genomic background. The phenotype of the congenic strains was tested by salt loading with DOCA-salt over a 4-wk period and measuring blood pressure by tail-cuff (in all animals) or radiotelemetry (in select groups) at baseline and during salt loading. In the congenic strains in which a chromosomal segment incorporating QTL SS1a from SBN/y was introgressed onto the genomic background of SBH/y, the blood pressure response to salt loading, as measured by tail-cuff, was decreased by 16 mmHg in both males and females compared with the parental SBH/y; replacing the QTL SS1a reduced the blood pressure response by 30 and 21 mmHg, respectively. In the congenic strains in which both SS1a and SS1b were introgressed from SBN/y onto the genomic background of SBH/y, the reduction in blood pressure was 34 mmHg in males and 38 mmHg in females; these latter results were confirmed by radiotelemetry. When either one or both QTLs were introgressed from SBH/y onto the SBN/y genomic background, tail-cuff measurements failed to detect an increase in blood pressure above baseline; telemetric measurements in the congenic strains introgressing both QTLs together, however, detected a significant rise in blood pressure after 3 and 4 wk of salt loading. Neither the origin of the Y chromosome nor the sex of the parental strain had any significant impact on the magnitude of the blood pressure response to salt loading. We conclude that the congenic rat strains that we constructed for the chromosome 1 QTLs provide functional evidence for the role of gene systems within QTLs SS1a and SS1b in the blood pressure response to salt loading. The unexpected finding was that QTL SS1a contributes to the hypertensive response also in females. The data indicate the lack of a Y chromosomal effect or of parental imprinting.

salt susceptibility; congenic strains; microsatellites; gene loci

WE PREVIOUSLY CARRIED OUT a total genome scan in the Sabra rat model of hypertension to detect blood pressure-related salt-susceptibility genes (22, 23). We reported on chromosome 1 the presence of two quantitative trait loci (QTLs) (SS1a and SS1b) in male rats and of one QTL (SS1b) in female rats. In the current study, we constructed multiple congenic strains to provide functional evidence for the role of these QTLs in mediating salt-sensitive hypertension. The resulting congenic strains introgressed either nearly all of chromosome 1 or the individual QTLs SS1a and SS1b from SBN/y onto the genomic background of SBH/y, or from SBH/y onto the background of SBN/y. The functional significance of these QTLs was assessed by measuring the blood pressure response to salt loading.

METHODS

Animals

Sabra hypertension-prone (SBH/y) and hypertension-resistant (SBN/y) rats from colony at the Barzilai Medical Center Campus in Ashkelon, Israel (21), were used for generating the congenic strains. Animals were housed in the center’s animal facility in strict compliance with institutional regulations and with the guidelines set forth by the American Physiological Society. Climate-controlled conditions were maintained, and temperature was set at 22°C. Regular 12-h diurnal cycles were kept using an automated light/dark switching device. Animals were provided ad libitum tap wa-
ter and standard rat chow containing 0.65% NaCl (Koffolk, Tel-Aviv, Israel), unless stated otherwise.

Construction of Congenic Strains

We initially constructed congenic strains which replaced most of chromosome 1 and consequently both QTLs SS1a and SS1b from one strain with the homologous regions of the contrasting strain. These near-genomic strains, not quite meeting the criteria of "conomics," are referred to hitherto as "large congenics." From these lines we subsequently derived multiple congenic lines that incorporated one QTL each. In these lines, we accounted for sex effects by studying male and female progeny in separate studies. In addition, we accounted for the sex of the parental strain and the origin of the Y chromosome by constructing separate congenic lines in which the initiation of the construction was by crossing female SBHy/w with male SBNy/y and, reciprocally, male SBHy/w with female SBNy/y. This complex design, which led to our attempts to construct a total of 12 congenic lines, was necessitated by the need to account for a Y chromosomal effect, which has been claimed by some (6, 12, 13, 15) to be of significance, and by our previous observations that in the two sexes, different QTLs might account for salt-sensitive hypertension (22).

The construction of the congeneric strains was initiated by crossing homozygous SBHy/w with SBNy/y in a reciprocal design to form F1 progenies consisting of heterozygotes. In congenic lines 1, 3, and 5, female SBHy/w were crossed with male SBNy/y, thus fixing the Y chromosome to SBNy/y; in lines 2, 4, and 6, male SBHy/w were crossed with female SBNy/y, fixing thereby the source of the Y chromosome to SBHy/w. In lines 7 and 9, female SBNy/y were crossed with male SBHy/w, the source of the Y chromosome thus being SBHy/w; in lines 8 and 10, male SBNy/y were crossed with female SBHy/w, the source of the Y chromosome being SBNy/y. By definition, lines 1, 3, 5, 7, and 9 can also be considered as "double congenics," as in the process of construction, in addition to introgressing the targeted genomic inserts (QTLs SS1a and/or SS1b), the Y chromosome was transferred from one strain into the contrasting strain. The resulting congeneric lines are shown in Table 1.

The F1 progenies were subsequently backcrossed as a rule either to the parental female SBHy/y or SBNy/y strain (BC1). The genotype of the BC1 progenies was determined by genome screening with chromosome-specific informative microsatellite markers. Heterozygotes at loci of interest (within the targeted chromosome) were selected, and backcrossed again to female SBHy/y or SBNy/y (BC2) in a design similar to that described for BC1. Backcrossing was repeated 8–10 times, always selecting heterozygotes as breeders for the next backcross. During this process, >99.9% of loci not undergoing selection became homozygous for one strain, while the selected allele from the other strain remained heterozygous. After BC8–10, two heterozygotes were crossedbred. The resulting offspring were that were homozygous at loci of interest were bred to each other, thus fixing the alleles of interest in the homozygous state on the background of the other strain. A total genome scan was then carried out with informative microsatellite markers at ~10 cM intervals. Lines showing homozygosity for the targeted introgressed chromosome (chromosome 1) or for a chromosomal segment incorporating either QTL SS1a or SS1b and for the native genetic background were used for generating the targeted congeneric lines.

Determination of Genotype

Genomic DNA was prepared from tail clipping of each animal by salt precipitation, followed by phenol-chloroform cleaning, as previously described (21). Purity and quantity of extracted DNA were assessed spectrophotometrically (GeneQuant II, Pharmacia Biotech, Cambridge, UK). Microsatellite markers were obtained from Research Genetics (Huntsville, AL). Genotyping was carried out by PCR amplification, as previously described (21). In brief, genomic DNA (50 ng) was amplified by PCR. The forward primer was labeled with [32P]ATP (DuPont, NEN) using T4 polynucleotide kinase (Promega). The PCR reactions were processed on a thermal cycler (model PTC 100; MJ Research, Water- town, MA). The product of each reaction (3 μl) was loaded onto a 7% polyacrylamide gel and run using a Base Ace apparatus (Stratagene, La Jolla, CA) at 60 W (Feathervolt 3000, Stratagene) for 4 h and exposed to XAR-5 film (Kodak, Rochester, NY) for autoradiography.

Determination of Phenotype

The phenotype of interest in the current study was the blood pressure response to salt loading. Blood pressure was measured in all strains by the tail-cuff method in the parental and large congeneric strains also by radiotelemetry. The protocol for measuring blood pressure consisted of basal blood pressure measurements shortly after weaning, while the animals were fed standard chow and provided tap water. Salt loading was then initiated by implanting subcutaneously in the back of the neck a 25 mg deoxycorticosterone acetate (DOCA) pellet (Innovative Research, Tampa, FL) and providing 1% NaCl as drinking water (here forth salt loading) for a total of 4 wk, as previously described (21). Blood pressure was measured again after 4 wk of salt loading (by the tail-cuff method) or at weekly intervals during salt loading (radiotelemetry). Blood pressure was measured by tail-cuff in

### Table 1. Congenic lines

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<th>Line</th>
<th>Genetic Background</th>
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<th>Donor</th>
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M, male; F, female; QTL, quantitative trait locus.

Physiol Genomics • VOL 12 • www.physigeneomics.org
all study groups. Because of technical constraints related to the high cost and resulting limitation in availability of telemetry equipment, blood pressure was measured by telemetry only in the parental SBH/y and SBN/y strains as well as in the large congenic strains.

Tail-cuff measurements. Blood pressure (systolic) was measured 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 (21). Multiple blood pressure measurements were made by the same operator at timed intervals on at least two consecutive days. The average of all measurements at each experimental time point was taken as representative of systolic blood pressure.

Telemetry measurements. Intra-arterial systolic and diastolic blood pressure and heart rate were measured by telemetry, using the Dataquest IV system (Data Sciences, Minneapolis, MN). Animals were prepared for telemetry as previously described (21). In brief, the abdominal cavity was opened under ether anesthesia, a pressure probe attached to a transmitter-transducer was inserted into the abdominal aorta below the renal arteries, the transducer-transmitter was sutured ventrally to the peritoneum, and the abdominal cavity was closed with 3-0 sutures. Animals were allowed to recover from surgery for 1 wk, which was considered adequate time for hemodynamic stabilization. Blood pressure was then recorded in individual cages over 24-h periods at 2-min intervals, 10 s per interval, at baseline and at 2, 3, and 4 wk after initiation of salt loading. Data are provided as hourly averages over the 24-h period.

Statistical Analysis

Blood pressure data are provided as means ± SE. Between groups analysis was by paired t-test, one-way analysis of variance or repeated measures analysis, as applicable. Statistical significance was set at the P < 0.05 level.

RESULTS

Blood Pressure in the Parental Strains

Tail-cuff measurements. Basal systolic blood pressure and blood pressure after 4 wk of salt loading, as measured by the tail-cuff method, are shown in Table 2. Basal systolic blood pressure was significantly higher in SBH/y than in SBN/y, both in male and female rats. Salt loading with DOCA-salt over 4 wk increased systolic blood pressure in male and female SBH/y significantly by 55 mmHg, whereas it had no effect on blood pressure in SBN/y. These results are entirely consistent with those we previously reported in these strains (21).

Radiotelemetry measurements. Baseline blood pressure (systolic, diastolic, and mean arterial pressures) and the blood pressures after 2, 3, and 4 wk of salt loading in SBH/y and SBN/y rats as measured by telemetry are shown in Fig. 1 (males) and 2 (females). The data in both sexes demonstrate the gradual evolution of hypertension (systolic and diastolic) during salt loading in SBH/y and the only minimal rise in blood pressure in SBN/y. The peak hypertensive effect was achieved in SBH/y in both sexes after 4 wk of salt loading. The animals were, however, clearly hypertensive already at 2 and 3 wk.

A diurnal variation in blood pressure was noted in both SBH/y and SBN/y, with lower arterial pressures during daytime and a rise in arterial pressure during the night, at which time the animals usually consume chow and are active. It is also noteworthy that blood pressure measurements in females tended to be lower than in males of the same strain, both at baseline and during salt loading. This observation held true for systolic, diastolic, and mean arterial pressures.

Compared to tail-cuff measurements which were carried out, as a rule, between 10:00–14:00, the telemetry systolic blood pressure measurements were lower in males by 10–20 mmHg and in females by 15–25 mmHg. During night time activity, the telemetry measurements generally approached those obtained by tail-cuff during daytime. These differences in blood pressure between the tail-cuff and telemetry methods were very consistent throughout the studies and were therefore attributed largely to inherent features of the different methodologies.

Construction of Congenic Lines

The chromosomal span of the congenic strains is shown in Fig. 3. We were able to construct on the SBH/y background congenic strains which incorporated one QTL, either SS1a (lines 1, 2) or SS1b (lines 3, 4) from SBN/y. On the SBN/y background, we attempted to construct a congenic line for QTL-SS1a from SBH/y but encountered severe fertility problems that did not allow us to perpetuate this line; we were successful, however, in constructing single congenics for QTL-SS1b (lines 7, 8). We also constructed large congenic strains with the genetic background of SBH/y in which most of chromosome 1 was introgressed from SBN/y (lines 5, 6), and its reciprocal where most of chromosome 1 from SBH/y was introgressed onto the SBN/y background (lines 9, 10). These latter lines, which came as close as we could to constructing con- somic lines for chromosome 1, our original intent, incorporated both QTLs SS1a and SS1b.

Blood Pressure in the Congenic Strains

Tail-cuff systolic blood pressure data are shown in Table 3 at baseline and after 4 wk of salt loading for all lines, for
both sexes and in the reciprocal strains. Blood pressure measurements by telemetry are shown in Figs. 4–7.

Background SBH/y, Insert SBN/y

Congenics for SS1a. In animals that originated from backcrossing to female SBH/y, the source of the Y chromosome was SBN/y. Within this subgroup, male congenic SBH/y.SBN/y-(D1Mgh2-D1Rat101),YSBN/y rats had a baseline systolic blood pressure that was 12 mmHg lower than in the parental SBH/y (P < 0.01) and not significantly different from that in the parental SBN/y. Blood pressure after salt loading was 16 mmHg...
lower in the congenic than in the parental SBH/y strain (P < 0.01) and 53 mmHg above SBN/y (P < 0.01). In females, baseline systolic blood pressure was 11 mmHg lower than in the parental SBH/y (P < 0.01) and not significantly different from that in the parental SBN/y. Blood pressure after salt loading was 16 mmHg lower in the congenic than in the parental SBH/y strain (P < 0.01) and 52 mmHg above SBN/y (P < 0.01).

In animals that originated from backcrossing to female SBN/y, the source of the Y chromosome was SBH/y. Within this subgroup, male SBH/y.SBN/y-(D1Wox11-D1Rat137),YSBH/y congenic line had a baseline systolic blood pressure that was 12 mmHg lower than in the parental SBH/y (P < 0.01) and not significantly different from that in the parental SBN/y. Blood pressure after salt loading was 25 mmHg lower in the congenic than in the parental SBH/y strain (P < 0.01) and 44 mmHg above SBN/y (P < 0.01). In female rats, baseline systolic blood pressure in males was 8 mmHg lower than in the parental SBH/y (P < 0.01) and 7 mmHg higher than in the parental SBN/y strain (P < 0.01). Blood pressure after salt loading was 32
mmHg lower in the congenic than in SBH/y (P < 0.01) and 36 mmHg above SBN/y (P < 0.01).

**Congenics for SS1b.** In the subgroup in which the source of the Y chromosome was SBN/y, male SBH/y.SBN/y-(D1Rat137-D1Rat83), Y<sup>SBH/y</sup> rats had a baseline systolic blood pressure that tended only to be lower than in SBH/y and that was not significantly different from that in the parental SBN/y. Blood pressure after salt loading was 30 mmHg lower in the congenic than in SBH/y (P < 0.01) and 39 mmHg above that of SBN/y (P < 0.01). In female rats, baseline systolic blood pressure was 11 mmHg lower than in the parental SBH/y (P < 0.01) and not significantly different from that in SBN/y. Blood pressure after salt loading was 40 mmHg lower in the congenic than in SBH/y (P < 0.01) but still 35 mmHg higher than in the parental SBN/y strain (P < 0.01). In female rats, baseline systolic blood pressure in the congenic strain was 9 mmHg lower than in SBH/y (P < 0.01), but 5 mmHg above that of the parental SBN/y (P < 0.01). Blood pressure after salt loading was 38 mmHg lower in the congenic strain than in SBH/y (P < 0.01) but 30 mmHg above that of SBN/y (P < 0.01).

In the reciprocal SBH/y.SBN/y-(D1Mgh2-D1Rat74), Y<sup>SBH/y</sup> line, baseline systolic blood pressure in males was 11 mmHg lower than in the parental SBH/y (P < 0.01) and 4 mmHg above that in SBN/y (P < 0.01). Blood pressure after salt loading was 21 mmHg lower in the congenic than in the SBH/y (P < 0.01) and 47 mmHg above that of SBN/y (P < 0.01).

In the subgroup in which the source of the Y chromosome was SBH/y, male congenic SBH/y.SBN/y-(D1Rat137-D1Rat123), Y<sup>SBH/y</sup> rats had a baseline systolic blood pressure that was 12 mmHg lower than in SBH/y (P < 0.01) and not significantly different from that in the parental SBN/y. Blood pressure after salt loading was 28 mmHg lower in the congenic than in SBH/y (P < 0.01) and 41 mmHg above that in SBN/y (P < 0.01). In female rats, baseline systolic blood pressure in male rats was 10 mmHg lower than in the parental SBH/y (P < 0.01) and 4 mmHg lower that in the parental SBN/y (P < 0.01). Blood pressure after salt loading was 32 mmHg lower in the congenic than in the parental SBH/y strain (P < 0.01) and 36 mmHg above that in SBN/y (P < 0.01).

**Large congenics**

**Tail-cuff measurements.** In male SBH/y.SBN/y-(D1Mgh2-D1Mgh11), Y<sup>SBH/y</sup> rats, baseline systolic blood pressure was 11 mmHg lower than in the parental SBH/y (P < 0.01) and not significantly different from that in SBN/y. Blood pressure after salt loading was 34 mmHg lower in the congenic strain than in SBH/y (P < 0.01) but still 35 mmHg higher than in the parental SBN/y strain (P < 0.01). Blood pressure after salt loading was 38 mmHg lower in the congenic strain than in SBH/y (P < 0.01) but 30 mmHg above that of SBN/y (P < 0.01).

Blood pressure after salt loading was 34 mmHg lower in the congenic strain than in SBH/y (P < 0.01) but still 35 mmHg higher than in the parental SBN/y strain (P < 0.01). Blood pressure after salt loading was 38 mmHg lower in the congenic strain than in SBH/y (P < 0.01) but 30 mmHg above that of SBN/y (P < 0.01).

Blood pressure after salt loading was 41 mmHg lower in the congenic strain than in SBH/y (P < 0.01) and 30 mmHg higher than in the parental SBN/y strain (P < 0.01). Blood pressure after salt loading was 38 mmHg lower in the congenic strain than in SBH/y (P < 0.01) but 30 mmHg above that of SBN/y (P < 0.01).

**Radiotelemetry.** We found no difference in the blood pressure data that was measured by radiotelemetry when the Y chromosome originated from SBN/y [SBH/y.SBN/y-(D1Mgh2-D1Mgh11), Y<sup>SBN/y</sup>] or from SBH/y [SBH/y.SBN/y-(D1Mgh2-D1Rat74), Y<sup>SBH/y</sup>]. The blood pressure data are shown therefore combined for both groups. In males (Fig. 4), baseline blood pressure (systolic, diastolic and mean arterial pressure) was lower by an average of 5–10 mmHg in the congenic strain compared with SBH/y. The data clearly demonstrate that over the subsequent 4 wk, the rise in blood pressure in the con-
genic strain was significantly attenuated compared with the that observed in the parental SBH/y strain, with an approximate ~30 mmHg reduction in systolic blood pressure, a ~20–25 mmHg reduction in diastolic blood pressure, and a 15–20 mmHg reduction in mean arterial pressure. In females (Fig. 5) interestingly enough, baseline blood pressure in the congenic strain was similar to that of SBH/y. The rise in blood pressure during salt loading was also attenuated as in males, but to a lesser degree, with an average reduction of ~10–20 mmHg in
systolic, diastolic, and mean arterial pressure compared with female SBH/y.

**Background SBN/y, Insert SBH/y**

Congenics for QTL-SS1b. In the SBN/y.SBH/y-(D1Rat27-D1Mit7),YSBH/y and SBN/y.SBH/y-(D1Rat101-D1Rat74),YSBN/y congenic lines, systolic blood pressure at baseline and after salt loading was not different from SBN/y in male and female rats.

**Large Congenics**

Tail-cuff measurements. In the congenic SBN/y.SBH/y-(D1Mgh17-D1Mgh14),YSBH/y and SBN/y.SBH/y-(D1Rat148-D1Rat89),YSBN/y lines, basal systolic blood pressure as well as blood pressure after salt loading was not different from that in the parental SBN/y strain in both male and female rats.

Radiotelemetry. Since no differences in blood pressures were detected by the source of the Y chromosome, the data are shown combined, irrespective of the source of chromosome Y. In males (Fig. 6), baseline systolic but not diastolic or mean arterial pressure tended to be higher in the congenic strain compared with the parental SBN/y strain. During the subsequent weeks of salt loading, there was a significant increment in blood pressure in the congenic strain of an average of ~10–20 mmHg which had not been observed during the tail-cuff measurements. Compared with the parental SBN/y strain, systolic, diastolic, and mean arterial pressures of the congenic strain were significantly higher as of 2 wk into salt loading. In females (Fig. 7), there was no difference in blood pressure at baseline, nor at 2 wk; at 3 and 4 wk, a significant rise in blood pressure (systolic, diastolic, and mean arterial pressure) occurred in the congenic strain, of an order of magnitude similar to that observed in the male congenic rats.

**DISCUSSION**

The use of congenic strains constitutes an integral part of the research paradigm that is nowadays applied for investigating the genetic basis of complex diseases (2, 3, 8, 11, 14, 18–20). It usually follows detection of quantitative trait loci and has two purposes: the first and foremost is to confirm the functional significance of the QTL, and the second is to narrow down as much as possible the chromosomal span of the QTL to allow identification of likely candidate genes within. In the current study, we set out to construct congenic strains that incorporate the chromosome 1 QTLs SS1a and SS1b, together or in separate, based on data we previously generated in our search for salt-susceptibility QTLs in the Sabra rat model of salt-sensitive hypertension (22). Our aims were to test the hypothesis that QTLs SS1a and SS1b incorporate each genes or gene systems that mediate the blood pressure susceptibility to salt and to functionally evaluate their respective contributions to the blood pressure response to salt loading. Our findings unequivocally confirm that chromosome 1 incorporates gene loci that are involved in blood pressure salt susceptibility in the Sabra rat model, that at least two separate salt-sensitivity QTLs can be detected on chromosome 1, and that each in separate functionally contributes to the blood pressure response to salt loading. Additional findings are that...

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**Fig. 6. Telemetric blood pressure measurements in male large congenics lines 9 [SBN/y.SBH/y-(D1Mgh17-D1Mgh14),YSBH/y] and 10 [SBN/y.SBH/y-(D1Rat148-D1Rat89),YSBN/y] combined (n = 11), at baseline and after 2, 3, and 4 wk of salt loading. The blood pressure of the large congenic strain is shown as open symbols and of the SBN/y parental strain as solid symbols. Data are provided as hourly averages over a period of 24 h; ▲ and ▼, systolic blood pressure; v and v, diastolic blood pressure; • and ○, mean arterial pressure.**
neither the source of the Y chromosome nor the sex of the parental strain appears to affect the phenotype expression of either QTL and that contrary to what has been suggested by linkage analysis, both QTLs contribute similarly in males and in females to the genetic variance of the blood pressure response to dietary salt intake. In addition, chromosome 1 appears to harbor a gene(s) that accounts for the ~10–15 mmHg difference in basal blood pressure between SBH/y and SBN/y.

The “large” congenic strain in which most of chromosome 1 was introgressed from SBN/y onto the background of SBH/y brought about the anticipated reduction in the blood pressure response to salt loading with an order of magnitude that was within the range predicted during linkage analysis (22). It is of interest that introgression of chromosome 1 from SBH/y onto the background of SBN/y elicited only a small increase in blood pressure, significantly less than that predicted by linkage analysis and detectable only by radiotelemetry and not by tail-cuff measurements. It has been speculated that this small magnitude of rise in blood pressure may be due to yet unidentified elements that confer “hypertension resistance” to the animal and that are not affected by recombinant manipulations of the kind we performed.

From the data obtained upon introgression of QTLs SS1a and SS1b in separate from SBN/y upon the genetic background of SBH/y, it is apparent that in male rats neither congenic strain elicited alone the full response (reduction in the magnitude of salt sensitivity) that we observed in the large congenics incorporating both QTLs together. It appears that the blood pressure effect of the two QTLs was additive or at least synergistic, as the sum of the reduction by the two QTLs added up to the total effect or beyond that observed in the large congenic strain. An interesting and unexpected finding was that similar results were also obtained in female rats, suggesting a functional role in females of not only QTL SS1b but also of SS1a. We thus failed to confirm the existence of sexual dimorphism in the genetic basis of salt susceptibility that we previously detected by linkage analysis in the Sabra model of salt susceptibility with respect to the QTLs on chromosomes 1 (22). In our previous study, we found the LOD score for QTL SS1a in males to be 4.52 and in females to be nonsignificant (22). Only QTL SS1b had a significant or suggestive LOD score for both males and females. We would have then expected the congenic lines 1 and 2 not to impact the blood pressure response in female rats. And yet our current findings clearly indicate that these two congenic lines significantly reduce blood pressure in females. This observation suggests that gene loci that are not detected by linkage analysis can be detected with the use of congenic strains. Similar findings have been reported by other groups. Saad et al. (17) by using congenic sublines were able to define three separate QTLs on chromosome 1, whereas linkage analysis had detected only one large QTL. Thus there seems to surface a lack of full correlation between results derived from linkage analysis and from studies in congenic strains. This important conclusion leads us to suggest that linkage analysis may prove not to be sensitive enough to detect individual QTLs, perhaps with small effects, and that the construction of congenic/consomic strains for all of
the autosomes and sex chromosomes may be more informative and productive than hitherto perceived.

The current study suggests that the source of the Y chromosome carries no weight in determining the blood pressure response to salt loading in the Sabra rats. This is contrary to what has been suggested in the SHR (6, 12, 13, 15). We also found no evidence that the sex identity of the parental strain influences the expression of salt sensitivity in the congenic lines, suggesting a lack of parental imprinting. We did observe, however, a difference in blood pressure between male and female rats in the course of the study. Even though tail-cuff measurements did not reveal differences in blood pressure at baseline or after salt loading in the parental SBH/y and SBN/y strains, blood pressure by radiotelemetric measurements was found to be lower in females. In the congenic strains, a similar trend was observed: blood pressure at baseline and after salt loading was not different between males and females when measured by tail-cuff but tended to be lower in females when monitored by radiotelemetry. The lower blood pressure in females was irrespective of the identity of the maternal strain (SBH/y or SBN/y). The significance of the difference in blood pressure between the sexes is yet unclear.

The detection of blood pressure differences between the sexes by radiotelemetry but not by the tail-cuff methodology deserves comment and emphasis, as it raises the question of the sensitivity and validity of the blood pressure measurements by tail-cuff. It could be argued that the sensitivity of tail-cuff is lower than telemetry. Furthermore, tail-cuff measurements allow only sporadic and intermittent systolic blood pressure measurements. In favor of tail-cuff measurements is that they are, in qualified hands, highly reproducible and therefore reliable. In fact, it is striking that the tail-cuff measurements in the current as well as in our previous studies are and have been highly consistent within sex, allowing us to detect group differences in the blood pressure response to salt loading. Telemetry yields, in contrast, abundant information that is recorded continuously over prolonged time intervals (1, 18). The amount of information yielded by telemetry, in particular the 24 h pattern as opposed to a single set of morning measurements by tail-cuff, carries a definite advantage. This is exemplified by the detection of a rise in blood pressure when SBH/y was introgressed onto the SBN/y background, which could not be detected by tail-cuff. Because of similar observations by other researchers, there is an ongoing controversy as to the need to apply telemetry to all blood pressure measurements in genetic studies of this kind. But this is clearly not feasible, as the major drawbacks of telemetry are its high cost and its low throughput. In contrast, tail-cuff measurements constitute a high-throughput and low-cost methodology. In fact, most studies of the kind presented here have to resort to tail-cuff measurements to allow a large number of animals to be studied within a reasonable period of time and with a limited budget. It is thus apparent that both methods are valuable and can and should be applied together but judiciously to answer issues of relevance.

Finally, in the male and female large congenic strains in which most of chromosome 1 from SBN was introgressed onto the SBH/y background, baseline systolic blood pressure was significantly lower than in the parental SBH/y and not significantly different from that in the parental SBN/y. These findings suggest that the SS1a and SS1b QTL segments in SBH/y incorporate a gene or a set of genes that contribute to the difference in basal blood pressure between SBH/y and SBN/y and that the effects of SS1a and SS1b are not additive for this variable. The data in the single congenic strains showed similarly that introgression of QTL SS1a from SBN/y onto the SBH/y background brought about a significant 11 mmHg reduction in baseline systolic blood pressure, rendering the blood pressure closer to the parental SBN/y and suggesting that SS1a incorporates a gene or a set of genes that contribute to the difference in basal blood pressure between SBH/y and SBN/y. In congenics for SS1b, baseline systolic blood pressure was 7 mmHg lower than in the parental SBH/y and closer to that in the parental SBN/y, although it appears to exert a lesser effect than SS1a. Linkage analysis had not previously provided us with any clue as to the loci of the genes that account for the 10–15 mmHg difference observed all along between the two parental strains, stressing once again the added value of congenic strains in detecting QTLs per se.

Now that we have positively confirmed the functional validity of the previously detected QTLs for salt susceptibility on chromosome 1 and have identified that SS1a also contributes to the blood pressure response to salt in females and that both SS1a and SS1b contribute to the difference in basal unstimulated blood pressure between SBH/y and SBN/y, the stage is set to proceed into the second phase of this research paradigm. Further research is needed to initiate the construction of multiple congenic sublines that should allow us to narrow down the span of the QTLs to a desired 1–2 cM range and attempt to detect within the significantly narrowed chromosomal segments genes for salt susceptibility.

We acknowledge the contributions of Marina Sapojnikov, Gurion Katni, Marina Grinyok, Svetlana Rosenblum, and Natalya Kornfeld for technical support.

This study was supported by grants from the German-Israeli Foundation for Scientific Research and Development, the Israel Science Foundation, and the EUROMYPGEN II Concerted Action of the Biomedical Program of the European Community.

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