Effect of Renin Gene Transfer on Blood Pressure in the Spontaneously Hypertensive Rat

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Abstract—To investigate whether molecular variation in the renin gene contributes to the greater blood pressure of spontaneously hypertensive rats (SHR) versus normotensive Brown Norway (BN) rats, we measured blood pressure in an SHR progenitor strain and an SHR congenic strain that are genetically identical except at the renin gene and an associated segment of chromosome 13 transferred from the BN strain. Backcross breeding and molecular selection at the renin locus were used to create the SHR congenic strain (designated SHR BN-Ren) that carries the renin gene transferred from the normotensive BN strain. We found that transfer of the renin gene from the BN strain onto the genetic background of the SHR did not decrease blood pressure in rats fed either a normal or high-salt diet. In fact, the systolic blood pressures of the SHR congenic rats tended to be slightly greater than the systolic blood pressures of the SHR progenitor rats. However, the congenic strain exhibited lower serum high-density lipoprotein cholesterol, and greater levels of total cholesterol, very-low-density lipoprotein, and intermediate-density lipoprotein cholesterol during administration of a high-fat, high-cholesterol diet. These findings demonstrate that (1) under the environmental circumstances of the current study, the greater blood pressure of SHR versus BN rats cannot be explained by strain differences in the renin gene and (2) a quantitative trait locus affecting lipid metabolism exists on chromosome 13 within the transferred chromosome segment. The SHR BN-Ren congenic strain may provide a useful new animal model for studying the interaction between high blood pressure and dyslipidemia in cardiovascular disease (Hypertension. 1998;31[part 2]:373-377.)

Key Words: hypertension • cholesterol • genetics • congenic • quantitative trait locus • renin • rat

Linkage studies in the SHR have suggested that QTLs influencing blood pressure and lipid phenotypes might be linked on several chromosomes. For example, previous studies in RI and congenic strains derived from SHR and BN rats have demonstrated the presence of QTLs regulating blood pressure and lipid phenotypes on chromosomes 8, 19, and 20. In the SHR x BN RI strains, we have also observed cosegregation between blood pressure and the renin gene on chromosome 13, as well as a suggestive association (P < 0.04) between the D13Ceb9s3 marker (which maps close to Ren) and a serum subfraction of HDL cholesterol (M. P., 1997, unpublished observation). Other linkage studies in the SHR, as well as the Dahl salt-sensitive rat, have suggested that a QTL influencing blood pressure might exist on rat chromosome 13 in or near the renin gene.

To investigate whether molecular variation in the renin gene contributes to the greater blood pressure of SHR versus normotensive BN rats, we measured blood pressure in an SHR progenitor strain and an SHR congenic strain that are genetically identical except at the renin gene and an associated segment of chromosome 13 transferred from the BN strain. A secondary objective was to determine whether a QTL or QTLs affecting lipid phenotypes might exist in or near the renin gene on chromosome 13. To accomplish these objectives, we replaced the SHR chromosome 13 segment that contains the renin gene with the corresponding chromosome region from the normotensive BN rat. We found that transfer of the renin gene from the BN strain onto the genetic background of the SHR did not decrease blood pressure in rats fed either a normal or a high-salt diet. In fact, the systolic blood pressures of the SHR congenic strain carrying the BN renin allele tended to be slightly greater than the systolic blood pressures of the SHR progenitor strain. During administration of a high-fat, high-cholesterol diet, the SHR congenic strain carrying the chromosome 13 segment transferred from the BN rat exhibited significantly lower levels of serum HDL cholesterol, and higher levels of total cholesterol, VLDL, and IDL cholesterol when compared with the progenitor SHR strain. These findings indicate that a QTL affecting serum lipoprotein levels in response to high dietary fat intake exists on chromosome 13 in the rat. The current findings also indicate that strain differences in the renin gene are not sufficient to explain the greater blood pressure of SHR versus BN rats.
Methods

Strains

The SHR congenic strain was derived from a progenitor strain of SHR (SHR/Ola) descended from inbred SHR originally obtained from the National Institutes of Health. This progenitor strain of SHR is commercially available in Europe and has been maintained by brother x sister mating at the Czech Academy of Sciences in Prague for more than 15 years. The rats were in the F46 generation when the SHR colony was established in Prague. The results of DNA fingerprint and PCR microsatellite tests have confirmed that the SHR progenitor strain is highly inbred.

The SHR congenic strain was derived by a selective breeding protocol in which a segment of chromosome 13 from the normotensive BN (Crl) strain was transferred onto the genetic background of the progenitor SHR. A microsatellite marker within the renn gene was used for selection of heterozygous carriers in each backcross generation. After 10 generations of selective backcrossing to the SHR progenitor strain, the renn gene was fixed and maintained in the homozygous state by brother x sister mating and selective inbreeding of the offspring. This strain was designated SHR BN-Ren.

Chromosome 13 Mapping

Renn genotyping was performed either by using PCR primers amplifying a polymorphic HindIII site in the fifth intron of the renn gene or by amplifying the CT microsatellite marker D13UW1 (Rena2) within the gene. To determine the length of the differential chromosome 13 segment transferred onto the SHR genetic background, we typed the congenic strain using the following markers polymorphic between the SHR and BN progenitor strains D13Mgh1, D13Mgh2, D13Mgh3, D13Mgh4, D13Mgh5, D13Mgh7, D13Mgh8, D13Mtr1, D13Mtr2, D13Mtr3, D13Mtr4, D13Mtr5, D13N1, and Syt2. Unless otherwise specified, primers were obtained from Research Genetics with sequences as published by Jacob et al.

We found that the size of the homozygous BN chromosome fragment transferred was 2.5 cm, estimated from the map distances of Jacob et al. and Remmers et al. (see Fig 1)

Genotype Analysis of the SHR.BN-Ren Congenic Strain

The congenic status of the SHR BN-Ren strain was confirmed by PCR analysis of the following markers polymorphic between the SHR and BN strains: D1Mtr9, D1Mgh22, and D1Mtr14 (chromosome 1), D2Mgh11, D2Mgh12, and D2Mtr16 (chromosome 2), D3Mgh3, D3Mtr10, and D3Mtr11 (chromosome 3), D4Mgh17, Eno2, and Il6 (chromosome 4), D5Mgh2, D5Mgh8, and D5Mtr1 (chromosome 5), D6Mgh8, D6Mtr9, and Hmgcr3a (chromosome 6), D7Mgh1, D7Mtr6, and D7Mtr8 (chromosome 7), Aca8, D8Mgh6, and D8Mtr6 (chromosome 8), D9Mtr4, and D9Mtr4 (chromosome 9), D10Mgh8, D10Mtr1, and D10Mtr6 (chromosome 10), D11Mgh4, D11Mgh6, and D11Mtr1 (chromosome 11), D12Mgh2, D12Mtr4, and D12Mtr8 (chromosome 12), D14Mgh1, D14Mtr1, D14Mtr7, and D14Mtr8 (chromosome 14), D15Mgh3, D15Mtr5, and D15Mtr13 (chromosome 15), D16Mgh2, D16Mtr2, and D16Mtr3 (chromosome 16), D17Mtr2, D17Mtr4, and D17Mtr7 (chromosome 17), D18Mgh1, D18Mtr1, and D18Mtr10 (chromosome 18), D19Mtr2, D19Mtr5, and D19Mtr7 (chromosome 19), D20Mgh5 and D20Mtr1 (chromosome 20), and Anh, DXMgh1, and DXMtr5 (chromosome X). PCR primers were obtained from Research Genetics or synthesized in the UCSF Biomedical Resources Center according to published sequences.

Cardiovascular Phenotyping

Pulsatile arterial pressures and heart rates were measured continuously in 15 male progenitor SHR and 12 male congenic SHR BN-Ren rats for 10 to 11 weeks beginning at 10 weeks of age. Indwelling radiotelemetry transducers were implanted under ketamine/xylazine anesthesia and connected to catheters implanted in the lower abdominal aorta (Data Sciences). Systolic and diastolic blood pressures and heart rates were recorded in unanesthetized, unrestrained rats in 5-second bursts every 5 minutes during the day (6 AM to 6 PM) and night (6 PM to 6 AM). From these data, single 24-hour averages for systolic and diastolic blood pressure and heart rate were calculated for each rat at 10 to 14 weeks of age and 16 to 19 weeks of age.

From weaning through 14 weeks of age, all rats were given tap water ad libitum and fed a standard pelleted laboratory diet that contained 0.5% NaCl and 1% K. In a subset of six SHR progenitor and six SHR congenic rats, 1% NaCl was added to the drinking water for 1 week beginning at 14 weeks of age. These rats were then switched back to tap water for 3 weeks (ages 16 to 19 weeks). Blood pressures were measured again at age 20 weeks after a 2nd week of 1% NaCl water administration. The remaining nine SHR progenitor rats and six SHR congenic rats remained on tap water throughout the study. Twenty-four-hour average blood pressures were combined and analyzed by ANOVA for rats in both groups while on the normal salt diet at weeks 10 to 14 and 16 to 19 of age. Blood pressures of rats during 1% NaCl administration at 15 and 20 weeks of age were analyzed by ANOVA separately.

All procedures performed involving animals were in accordance with institutional guidelines on animal welfare.

Serum Lipid Phenotyping

From weaning until 7 weeks of age, a separate series of six SHR progenitor and six SHR BN-Ren congenic rats were fed a commercial pelleted diet. After this baseline period, the rats were fed the commercial diet supplemented with 5% (wt/wt) olive oil and 2% (wt/wt) cholesterol for 4 weeks as previously described. Lipid analysis was performed as described previously. Total cholesterol and triglyceride levels were measured before and after the high-fat diet. Lipoprotein fractions were also measured after administration of the high-fat diet. Briefly, blood samples were obtained either from tail veins with the rats under light anesthesia or from the aorta at killing. Sera were cooled and kept at 4°C until ultracentrifugation. Lipoprotein fractions (VLDL, IDL, LDL, IDL, and HDL) were isolated by density gradient ultracentrifugation. Serum total cholesterol, triglyceride, and lipoprotein cholesterol concentrations in each fraction were determined using enzymatic test kits (Boehringer-Mannheim GmbH). Lipoprotein cholesterol concentrations were corrected for recovery. Between-strain differences in cholesterol, triglyceride, and lipoprotein cholesterol concentrations were analyzed using the Student’s t test and the Bonferroni correction. Statistical significance was defined as an adjusted value of P < 0.05.

Results

Genotype analysis of 59 widely dispersed polymorphic microsatellite markers verified that the SHR BN-Ren congenic strain differs from the SHR progenitor strain only in the vicinity of the renn gene. Analysis of markers on chromosome 13 revealed that the size of the segment transferred from the BN rat was 2.5 cm with an additional 16 cm region of heterozygosity (Fig 1). The 24-hour average systolic and diastolic blood pressures of the SHR congenic rats carrying the renn gene transferred from the BN rat were not lower than the blood pressures of the SHR progenitor rats (Fig 2A and 2B). In fact, the 24-hour
Comparison of the SHR Progenitor with the SHR.BN-Ren Congenic Strain

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>SHR</th>
<th>SHR.BN-Ren</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal cholesterol</td>
<td>1.68±0.07</td>
<td>1.98±0.14</td>
</tr>
<tr>
<td>Final cholesterol</td>
<td>1.86±0.17</td>
<td>2.37±0.19†</td>
</tr>
<tr>
<td>Basal triglycerides</td>
<td>0.38±0.06</td>
<td>0.43±0.14</td>
</tr>
<tr>
<td>Final triglycerides</td>
<td>0.44±0.11</td>
<td>0.63±0.14∗</td>
</tr>
<tr>
<td>Final VLDL cholesterol</td>
<td>0.81±0.10</td>
<td>1.47±0.14‡</td>
</tr>
<tr>
<td>Final LDL cholesterol</td>
<td>0.07±0.02</td>
<td>0.17±0.03‡</td>
</tr>
<tr>
<td>Final IDL cholesterol</td>
<td>0.18±0.04</td>
<td>0.18±0.03</td>
</tr>
<tr>
<td>Final HDL1 cholesterol</td>
<td>0.61±0.11</td>
<td>0.43±0.04∗</td>
</tr>
<tr>
<td>Final HDL2 cholesterol</td>
<td>0.12±0.01</td>
<td>0.08±0.01†</td>
</tr>
</tbody>
</table>

Basal values were obtained from rats on commercial rat chow before feeding the high-fat, high-cholesterol diet; final values were obtained after feeding the high-fat, high-cholesterol diet. All values (in mmol/L) are means±SD.

∗P<.05; †P<.005; ‡P<.0001, statistical significance by Student’s t test comparing SHR vs SHR.BN-Ren congenic strain.

average systolic blood pressures of 10- to 14-week-old SHR.BN-Ren congenic rats tended to be slightly higher than the systolic blood pressures of the SHR progenitor rats (mean SBP±SEM, 171±0.9 mm Hg versus 167±1.4 mm Hg, P=.01). However, there were no significant differences in diastolic blood pressure or heart rate between the SHR progenitor rats and the SHR.BN-Ren congenic rats at 10 to 14 or 16 to 19 weeks of age. In addition, we found no difference in systolic or diastolic blood pressure between the SHR progenitor and SHR.BN-Ren congenic rats fed the high-salt diet, either at 15 or 20 weeks of age (data not shown). Addition of 1% NaCl to the drinking water increased systolic and diastolic blood pressures to the same extent in the progenitor and the congenic strains. There were no significant differences in cardiac mass between the progenitor and congenic strains (data not shown).

Before administration of the high-fat, high-cholesterol diet, the SHR progenitor and the SHR.BN-Ren congenic strains showed similar total serum cholesterol and triglyceride levels (Table). The Table shows that after rats were fed the high-fat, high-cholesterol diet, the SHR.BN-Ren congenic strain showed significantly greater levels of serum total cholesterol (P<.005), VLDL (P<.0001), and IDL (P<.0001). The SHR.BN-Ren congenic strain also showed significantly lower levels of HDL1 cholesterol (P<.0001) and a tendency for lower levels of HDL2 cholesterol (P<.05).

Discussion

In genetic linkage studies using F2 and backcross populations derived from SHR and normotensive Lewis rats or SHR and Wistar-Kyoto rats, the renin allele inherited from the SHR progenitor strain was found to cosegregate with increased blood pressure. In R1 strains derived from SHR and normotensive BN rats, the renin allele of the SHR also cosegregated with increased blood pressure. In R1 strains fed a high-fat, high-cholesterol diet, we detected a suggestive association (P<.004) between a serum fraction of HDL cholesterol and the D13Ceh953 marker, which maps close to renin on chromosome 13. Taken together, these linkage studies suggest the possibility that QTLs affecting blood pressure and/or lipid metabolism might exist on chromosome 13 in the vicinity of the renin gene.

In the present study, we constructed a new congenic strain of SHR that carries the renin gene transferred from the BN rat. The SHR congenic strain is genetically identical to the progenitor SHR strain except for the renin gene and an associated segment of chromosome 13. We found that transfer of the renin allele from the normotensive BN rat onto the SHR background was not sufficient to decrease blood pressure in rats fed either a normal or high-salt diet. In fact, the blood pressure of the SHR.BN-Ren congenic strain tended to be higher than that of the SHR progenitor strain. However, transfer of this segment of chromosome 13 from the BN rat onto the SHR genetic background did induce a significant decrease in serum HDL cholesterol and an increase in serum total cholesterol, VLDL, and IDL cholesterol levels in rats fed a high-fat, high-cholesterol diet.

The current SHR.BN-Ren congenic strain was constructed to test the hypothesis that molecular differences in the renin gene contribute to the greater blood pressure of SHR versus...
BN rats Given that the SD of our radiotelemetry blood pressure measurement is approximately 5 mm Hg, the current study had a 99% power of detecting a blood pressure difference of 10 mm Hg between the congenic and progenitor strains, and a 70% power of detecting a 5 mm Hg strain difference in blood pressure (assuming a two-tailed significance threshold of 0.05). Therefore, our blood pressure measurements in the congenic and progenitor strains indicate with reasonable certainty that molecular differences in the renn gene do not explain much, if any, of the greater blood pressure in the SHR versus BN strain. It is possible, however, that a chromosome 13 blood pressure QTL detected in previous linkage studies exists outside of the chromosome segment transferred in the SHR BN-Ren congenic strain.

It should be noted that negative results obtained by comparing congenic and progenitor strains should be interpreted with caution. The possibility exists that a QTL on the differential chromosome segment may exhibit an effect on blood pressure only when in the presence of certain gene variants located in other regions of the genome. For example, although transfer of the BN renn gene itself did not affect blood pressure in the recipient congenic strain, transfer of the BN renn gene together with the BN angiotensinogen gene might reduce blood pressure. This possibility could be investigated by measuring blood pressure in an SHR double congenic strain in which both the SHR renn and angiotensinogen genes have been replaced by the corresponding BN genes.

In the current study, we demonstrate the existence of a QTL that affects lipid metabolism in the vicinity of the renn gene on chromosome 13. These results confirm our previous finding of a suggestive linkage between HDL levels and markers on chromosome 13 in the rat. Of interest, linkage studies in the mouse have suggested that a QTL influencing lipid metabolism exists on chromosome 1 in a region homologous to rat chromosome 13. In an F2 population derived from NZB/B1NJ and SM/J mice, Purcell-Huynh et al. found linkage between markers on mouse chromosome 1 and levels of total cholesterol, triglycerides, and HDL cholesterol. Analysis of the corresponding region of mouse chromosome 1 may thus reveal...
important candidate genes that map to the region of rat chromosome 13 isolated in the SHR BN-Ren congenic strain.

In the SHR BN-Ren congenic strain, the chromosome segment transferred from the BN rat was associated with decreased HDL levels and increased cholesterol levels in response to a high-fat diet. Thus, the SHR BN-Ren congenic strain represents a hypertensive model that is highly susceptible to dietary-induced changes in serum lipids. Accordingly, this strain may provide a useful new animal model for studying the combined effects of high-blood pressure and dyslipidemia on susceptibility to stroke and other forms of target organ damage in hypertension.

Acknowledgments

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References


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