Effect of Chromosome 19 Transfer on Blood Pressure in the Spontaneously Hypertensive Rat

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Abstract—Linkage studies in the spontaneously hypertensive rat (SHR) have suggested that a gene or genes regulating blood pressure may exist on rat chromosome 19 in the vicinity of the angiotensinogen gene. To test this hypothesis, we measured blood pressure in SHR progenitor and congenic strains that are genetically identical except for a segment of chromosome 19 containing the angiotensinogen gene transferred from the normotensive Brown Norway (BN) strain. Transfer of this segment of chromosome 19 from the BN strain onto the genetic background of the SHR induced significant decreases in systolic and diastolic blood pressures in the recipient SHR chromosome 19 congenic strain. To test for differences in angiotensinogen gene expression between the congenic and progenitor strains, we measured angiotensinogen mRNA levels in a variety of tissues, including aorta, brain, kidney, and liver. We found no differences between the progenitor and congenic strains in the angiotensinogen coding sequence or in angiotensinogen expression that would account for the blood pressure differences between the strains. In addition, no significant differences in plasma levels of angiotensinogen or plasma renin activity were detected between the 2 strains. Thus, transfer of a segment of chromosome 19 containing angiotensinogen from the BN rat into the SHR induces a decrease in blood pressure without inducing any major changes in plasma angiotensinogen levels or plasma renin activity. These results indicate that the differential chromosome segment trapped in the SHR chromosome 19 congenic strain contains a quantitative trait locus that influences blood pressure in the SHR but that this blood pressure effect is not explained by differences in plasma angiotensinogen levels or angiotensinogen expression. (Hypertension. 1999;33[part II]:256-260.)

Key Words: hypertension, experimental ■ angiotensinogen ■ genetics ■ blood pressure ■ rats

In humans, linkage and association studies have suggested that a blood pressure (BP) regulatory gene exists in or near the angiotensinogen (Agt) gene on chromosome 1.1-3 Linkage studies in the spontaneously hypertensive rat (SHR) also have suggested that at least 1 quantitative trait locus influencing BP exists on rat chromosome 19, which is homologous to human chromosome 1, in the vicinity of the Agt gene.4,5 However, in a segregating population derived from stroke-prone SHRs and normotensive Wistar-Kyoto (WKY) rats, no relationship was found between a polymorphism in the Agt gene and BP.6

To investigate whether chromosome 19 plays a role in the greater BP of SHRs versus normotensive Brown Norway (BN) rats, we measured BP in an SHR progenitor strain and an SHR congenic strain that are genetically identical except for a segment of chromosome 19 that contains the Agt gene. To derive the SHR chromosome 19 congenic strain, we used backcross breeding combined with molecular selection to transfer the Agt gene and an associated segment of chromosome from the BN strain onto the genetic background of the SHR. We found that transfer of this segment of chromosome 19 induced decreases in systolic and diastolic BPs in congenic SHRs fed both normal and high salt diets. However, the effects of this chromosome region on BP did not appear to be related to effects on plasma levels of Agt or on tissue-specific Agt gene expression.

Methods

Strains

The SHR congenic strain was derived from a progenitor strain of SHR (SHR/Ola) descended from inbred SHRs originally obtained from the National Institutes of Health. This progenitor strain of SHR is commercially available in Europe and has been maintained by brother×sister mating at the Czech Academy of Sciences in Prague.
for >15 years. The results of DNA fingerprint and polymerase chain reaction (PCR) microsatellite tests have confirmed that the SHR progenitor strain is highly inbred.4

The SHR congenic strain was derived by a selective breeding protocol, as described previously for other congenic strains,7,8 in which a segment of chromosome 19 from the normotensive BN/Cr strain was transferred onto the genetic background of the progenitor SHR. A restriction-fragment-length polymorphism in the Agt gene was used for selection of heterozygous carriers in each backcross generation. After 8 generations of selective backcrossing to the SHR progenitor strain, the transferred segment of chromosome 19, including Agt, was fixed by intercrossing heterozygotes and then maintained in the homozygous state by brother×sister mating. Rats of the N8F5 generation were used in the present studies. The resulting congenic strain was designated SHR.BN-Agt.

Genotype Analysis of the SHR.BN-Agt Congenic Strain

Agt genotyping was performed using PCR primers amplifying a 320-bp fragment of exon 2 in the Agt gene containing a Pvu II restriction site present in SHRs but not BN rats. The upstream primer was 5′-CGC ATG TAC TAC AAG ATG CTG AGT-3′; the downstream primer was 5′-AAA TGG CTG CTG TTT TAG GCC CAA-3′. To determine the length of the differential chromosome 19 segment transferred and fixed on the SHR genetic background, we used PCR to genotype the congenic strain using the following microsatellite markers polymorphic between the SHR and BN progenitor strains: D19Rat57, D19Rat5, D19Rat2, D19Rat3, D19Rat7, D19Rat49, D19Mgh3, D19Mit7, D19Mgh2. Primers were synthesized by the University of California at San Francisco (UCSF) Biomolecular Resource Center according to sequences obtained from the Whitehead Institute for Biomedical Research/MAssachusetts Institute of Technology (WIBR/MIT) rat genome map or Jacob et al.9 We found that the size of the homozygous BN segment transferred was at minimum 8.2 cm and at maximum 12.2 cm on the basis of the map distances of the WIBR/MIT map and the published maps of Jacob et al10 and Pravenec et al11 (Figure 1). As previously described,7,8 we used PCR analysis of >50 widely dispersed polymorphic microsatellite markers on other chromosomes to confirm that the congenic strain differs from the SHR progenitor only in the region of chromosome 19 defined in Figure 1. The map position of the Agt gene was determined by genotyping an F2 population derived from the SHR and BN progenitor strains: D19Rat57, D19Rat5, D19Rat2, D19Rat3, D19Rat7, D19Rat49, D19Mgh3, D19Mit7, D19Mgh2. Primers were synthesized by the University of California at San Francisco (UCSF) Biomolecular Resource Center according to sequences obtained from the Whitehead Institute for Biomedical Research/MAssachusetts Institute of Technology (WIBR/MIT) rat genome map,9 Jacob et al,10 and Pravenec et al.11 Map position of Agt was determined as described.

Cardiovascular Phenotyping

Pulsatile arterial pressures and heart rates were measured continuously in 6 male progenitor SHRs and 7 male congenic SHR.BN-Agt rats for 8 weeks beginning at 11 weeks of age. Indwelling aortic radiotelemetry transducers were implanted under ketamine/xylazine anesthesia as described previously.13,14 Systolic and diastolic BPs and heart rates were recorded in anesthetized, unrestrained rats in 5-second bursts every 5 minutes throughout the day and night. From these data, separate daytime and nighttime 12-hour averages for systolic and diastolic BPs and heart rate were calculated for each rat for each day from 11 to 18 weeks of age.

From weaning through 13 weeks of age, all rats were given tap water ad libitum and fed a standard pelleted laboratory diet that contained 0.58% NaCl and 1.1% K. To test for interactions between dietary salt and the effect of the differential chromosome 19 segment on BP, 1% NaCl was added to the drinking water at age 14 weeks for 1 week. Rats were then switched back to tap water for the remainder of the study (age, 15 to 18 weeks). A baseline nighttime and daytime BP for each rat was determined by averaging the daily BP measurements obtained during the 2-week period before salt administration (age, 11 to 13 weeks). The BPs of older rats (age, 15 to 18 weeks) were determined by averaging the daily BP measurements beginning 1 week after the supplementary dietary salt was stopped. Average BPs obtained at baseline (age, 11 to 13 weeks), during salt admin-

Sequence Analysis of Agt cDNA

The Agt coding sequences in BN and SHR.BN-Agt congenic rats were obtained by reverse transcription of kidney messenger RNA, PCR amplification of the cDNA, and direct sequencing of the PCR amplicon. Sequencing was performed by the Genome Analysis Core Facility at the UCSF Cancer Center with ABI377 automated sequencers.

Agt Expression

Total RNA was extracted from aorta, brain, kidney, heart, spleen, and liver tissue collected from 5 month-old SHRs (n=6) and SHR.BN-Agt rats (n=6) fed standard laboratory chow and tap water ad libitum. To test for age-dependent variation in Agt expression, we also measured aortic Agt mRNA levels in 1-month-old progenitor (n=2) and congenic (n=2) SHRs. Tissue-specific Agt expression was analyzed by RNase protection assay with an RNA probe designed to simultaneously detect Agt and β-actin as an internal standard. To construct the probe, a 790-base segment of Agt cDNA (ATCC 87105, American Type Culture Collection) was inserted into a commercially available antisense actin template that protects a
the purposes of data presentation, the results are all expressed as mean±SEM.

Plasma Agt and Plasma Renin Activity Measurements

PRA and plasma Agt levels were determined in progenitor SHRs (n=6) and SHR.BN-Agt congenic rats (n=7) as previously described. In brief, to measure plasma renin activity (PRA), phosphate-buffered plasma (pH 6.25) was incubated at 37°C in the presence of inhibitors of converting enzyme and angiotensinases, and the angiotensin I generated during the incubation was measured by radioimmunoassay. PRA was expressed in nanograms per milliliter per hour (nanograms of angiotensin I per 1 mL of original plasma per hour of incubation at 37°C). To measure renin substrate concentration (Agt), excess hog renin (sufficient to convert all substrate to angiotensin I) was added to phosphate-buffered plasma (pH 6.25), and the mixture was incubated at 37°C in the presence of inhibitors of converting enzyme and angiotensinases. The angiotensin I generated during the incubation was measured by radioimmunoassay, and the results were expressed in nanograms per milliliter (nanogram of angiotensin I generated per 1 mL of original plasma). PRA and plasma renin substrate (Agt) levels were analyzed by ANOVA.

Results

Genotype analysis of >50 widely dispersed polymorphic microsatellite markers verified that the SHR.BN-Agt congenic strain differs from the SHR progenitor strain only in the vicinity of the Agt gene. The minimum size of the transferred chromosome 19 segment was delineated by markers for D19Rat57 (at the end of 19p) and D19Rat49; the maximum size of the segment in the centromeric direction was defined by the marker D19MIt7. Thus, the size of the transferred chromosome segment is between 8.2 and 12.2 cM (Figure 1). Agt, which was used as the selection marker in the derivation of the congenic strain, is within the transferred chromosome segment.

Twelve-hour average daytime and nighttime systolic BPs determined by radiotelemetry were significantly lower in the SHR.BN-Agt congenic rats than in the progenitor SHRs at 14 weeks of age during 1% NaCl–water administration and at 15 to 18 weeks of age on a normal salt diet (Figure 2a and 2b; all P≤0.015). At baseline (11 to 13 weeks of age), systolic BPs tended to be lower in the SHR.BN-Agt congenic rats (daytime and nighttime systolic BPs, P≤0.03), but these differences did not meet strict criteria for statistical significance when corrected for multiple comparisons. Average daytime and nighttime diastolic BPs of the SHR.BN-Agt congenic strain were significantly lower than those of the SHR progenitor strain throughout the study period (all P<0.015), except for nighttime diastolic BP at 11 to 13 weeks of age (P=0.02). Both the SHR progenitor and congenic strains showed increases in BP during 1% NaCl–water administration at 14 weeks of age. However, the magnitude of the NaCl-induced increase in systolic BP in the congenic rats (5.7±1.2 mm Hg) was lower than that in the SHR progenitor rats (10.7±0.7 mm Hg in SHR rats, P<0.01). The magnitude of the NaCl-induced increase in diastolic BP was also significantly lower in the congenic than in the progenitor rats (data not shown). Daytime (but not nighttime) 12-hour average heart rates also were significantly lower in the SHR.BN-Agt congenic rats compared with SHR.
progenitor rats (P<0.01) (data not shown). There were no differences in pulse pressures between the progenitor and congenic strains. In addition, cardiac mass (corrected for body weight) was not significantly different between the progenitor and congenic strains (data not shown).

To test for tissue-specific differences in Agt expression, we compared Agt mRNA levels (corrected for β-actin expression) in aorta, kidney, brain, heart, spleen, and liver between the SHR progenitor and congenic strains. Figure 3 shows the results of quantitative analysis of aorta, brain, kidney, and liver Agt mRNA levels in SHRs and SHR.BN-Agt congenic rats. There were no significant differences between the progenitor and congenic SHRs with respect to liver and brain Agt expression. There was no detectable Agt mRNA in heart or spleen in either strain (not shown). However, SHR.BN-Agt congenic rats had significantly higher levels of Agt mRNA levels in the aorta and kidney than did progenitor SHRs (P<0.005). To investigate whether increased aortic Agt gene expression was age dependent, we also measured aortic Agt mRNA expression in 1-month-old progenitor and congenic rats (data not shown).

The sequences of the BN and SHR.BN-Agt congenic strain Agt coding regions were compared with the published Agt sequences of SHR and WKY rats.5,10 We found no nucleotide differences that would change the predicted amino acid sequence of the protein in BN or SHR.BN-Agt congenic rats.

We also found no significant differences between progenitor and congenic rats in either Agt levels (1238.8±57.3 versus 1357±47.8 ng/mL for progenitor versus congenic, P=0.15) or PRA (2.17±0.15 versus 2.04±0.18 ng·mL⁻¹·h⁻¹ for progenitor versus congenic, P=0.59).

**Discussion**

The renin-angiotensin system plays a well-known physiological role regulating salt and water homeostasis and arterial BP. In humans, sequence variants in the Agt gene have been associated with increased serum Agt levels and increased BP in some ethnic groups.1,2,17,18 Studies in gene-targeted animals have shown that artificially induced changes in the number of copies of the Agt gene can influence plasma Agt levels and BP.19 These studies suggest the possibility that transfer of naturally occurring Agt gene variants between inbred rat strains, such as the SHR and BN strain, might affect BP.

In the present study, we constructed a new congenic strain of SHR that carries an 8- to 12-cM segment of chromosome 19 that includes the Agt gene transferred from the normotensive BN rat. The SHR.BN-Agt congenic strain is genetically identical to the progenitor SHR strain, except for the defined segment of chromosome 19. We found that transfer of this chromosome region was sufficient to induce a significant reduction in systolic and diastolic BPs and heart rate. The present findings are consistent with the results of previous studies in which a similar region of chromosome 19 was linked to effects on mean arterial pressure or pulse pressure in the SHR.4,5 On a normal salt diet, the reductions in BP in the congenic strain were 12 to 14 mm Hg for systolic BP and 11 to 13 mm Hg for diastolic BP. On the high salt diet, the difference in systolic BP between the SHR progenitor and congenic strains was even greater (17 mm Hg). Given that the difference in systolic BP between the progenitor SHR and BN strains is ≈80 mm Hg, the region of chromosome 19 isolated in the SHR.BN-Agt congenic strain could account for up to 15% to 20% of the hypertension of SHRs versus BN rats. These differences in systolic and diastolic BPs are similar in magnitude to BP effects that we found previously in SHR congenic strains carrying segments of chromosomes 1 and 8 transferred from the BN rat.7,8 The differences in systolic and diastolic BPs in the SHR.BN-Agt congenic strain are not simply a random effect of substituting chromosome segments in the SHR with corresponding chromosome segments in the BN rat. For example, we found no major differences in systolic or diastolic BP between the SHR strain and SHR congenic strains carrying segments of chromosomes 13 or 20 transferred from the BN rat.7,20

To investigate whether replacing the Agt gene in the SHR with the Agt gene from the normotensive BN rat would affect Agt expression, plasma Agt levels as well as BP, we measured plasma levels of Agt and tissue-specific Agt expression in SHR and SHR.BN-Agt congenic rats. We found no differences in plasma Agt levels or PRA that could account for the BP differences observed between the progenitor and congenic SHRs. In addition, we found no differences between progenitor and congenic SHRs in liver Agt expression, which is the main source of circulating plasma Agt,21 or in brain Agt mRNA levels. We did observe greater expression of the Agt gene in the aorta and kidney of adult congenic versus progenitor SHRs. However, greater Agt expression in the aorta and kidney would not explain the lower BP of the congenic strain. The difference in Agt expression was not present in 1-month-old rats and may actually be a secondary effect of the difference in BP. Recently, Lodwick et al19 also
found greater Agt expression in the aorta and kidney of normotensive WKY rats versus SHRs. Although our Agt expression results do not explain the lower BP in the SHR congenic strain, the mechanism of the increased Agt mRNA levels in the congeneric strain merits further investigation. For example, it might be interesting to investigate whether the increased aortic and/or renal Agt expression in the SHR.BN-Agt congenic strain enhances the susceptibility to hypertension-induced vascular damage in the aortas or kidneys of the congeneric rats.

Sequence analysis of the coding regions of the Agt gene of SHR and SHR.BN-Agt congenic rats revealed no differences between the progenitor and congenic strains. It should be noted that although we did not find any sequence variation in the coding regions of the Agt gene between the SHR progenitor and congenic strains, this does not exclude the possibility of functionally significant variants in the noncoding or upstream promoter regions of the Agt gene that could affect Agt expression.

The present findings indicate that the differential chromosome segment trapped in the SHR chromosome 19 congenic strain exerts directionally opposite effects on BP and Agt mRNA levels in the aorta and kidney. These findings, together with the lack of any detectable changes in circulating levels of Agt or PRA, suggest that sequence variation in the Agt gene itself does not contribute to the effect of this chromosome region on BP. The new SHR.BN-Agt congenic strain should provide a useful model for the investigation of other genes on rat chromosome 19 that might contribute to hypertension in the SHR. Specifically, congenic sublines can now be derived for exclusion mapping and further genetic dissection of quantitative trait loci in the target region of chromosome 19 that influence BP in the SHR.

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References

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