Genetic Isolation of a Region of Chromosome 8 That Exerts Major Effects on Blood Pressure and Cardiac Mass in the Spontaneously Hypertensive Rat

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Abstract

The spontaneously hypertensive rat (SHR) is the most widely studied animal model of essential hypertension. Despite > 30 yr of research, the primary genetic lesions responsible for hypertension in the SHR remain undefined. In this report, we describe the construction and hemodynamic characterization of a congenic strain of SHR (SHR-*Lx*) that carries a defined segment of chromosome 8 from a normotensive strain of Brown-Norway rats (BN-*Lx* strain). Transfer of this segment of chromosome 8 from the BN-*Lx* strain onto the SHR background resulted in substantial reductions in systolic and diastolic blood pressure and cardiac mass. Linkage and comparative mapping studies indicate that the transferred chromosome segment contains a number of candidate genes for hypertension, including genes encoding a brain dopamine receptor and a renal epithelial potassium channel. These findings demonstrate that BP regulatory gene(s) exist within the differential chromosome segment trapped in the SHR-*Lx* congenic strain and that this region of chromosome 8 plays a major role in the hypertension of SHR vs. BN-*Lx* rats. (*J. Clin. Invest.* 1997. 99:577–581.) Key words: hypertension • genetics • blood pressure • rats, inbred spontaneously hypertensive • genetic markers

Introduction

The spontaneously hypertensive rat (SHR)[1] was derived by recurrent selective breeding of Wistar rats in Kyoto, Japan (1). Although this strain has been the subject of extensive physiologic and biochemical investigation, little is known about the primary genetic lesions responsible for the pathogenesis of spontaneous hypertension. Recent linkage studies in recombinant inbred strains and in F2 populations derived from the SHR and normotensive strains have suggested that BP quantitative trait loci (QTLs) may exist on chromosomes 1, 2, 4, 8, 10, 13, 16, 19, and 20 (2–16). However, the individual contributions of each of these chromosomes in the pathogenesis of spontaneous hypertension have not been clearly defined. It is unknown which of these putative QTLs reflect molecular variants necessary for the full expression of hypertension and which represent false positive linkages. By replacing single chromosome regions in the SHR with the corresponding chromosome regions from an appropriate normotensive strain, it should be possible to confirm the existence of putative QTLs, determine their relative contributions to hypertension, and begin fine genetic mapping of specific variants responsible for increased BP. Although consomic strains derived from SHR and normotensive Wistar Kyoto rats have been used to confirm the existence of BP QTLs on the Y chromosome (8), the near total lack of recombination on the Y chromosome precludes the use of these consomic strains for genetic mapping. In a new congenic strain derived from SHR and normotensive Brown-Norway (BN) rats, we have trapped a QTL regulating blood pressure in a region of chromosome 8 that appears to play a major role in the pathogenesis of spontaneous hypertension.

Methods

Strains. The SHR congenic strains were derived from a progenitor strain of SHR (SHR/Ola) that descends 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 × sister mating at the Czech Academy of Sciences (Prague, Czech Republic) for > 15 yr. The rats were in the F48 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 (10, 12, 15).

The SHR-*Lx* congenic strain was derived by a selective breeding protocol in which a segment of chromosome 8 from the normotensive BN-*Lx* strain was transferred onto the genetic background of the progenitor SHR. The BN-*Lx* donor strain (previously referred to as BN.*Lx*, (references 11, 17) was originally derived by introgressing the mutant *Lx* gene of the Polydactylous (PD/Cub) rat onto the BN background (17). The mutant *Lx* gene gives rise to the polydactyly-luxate syndrome in which extra digits develop on the hind feet and sometimes the front feet along with variable luxation of the hind limbs. Because the polydactyly-luxate syndrome maps to rat chromosome 8, the *Lx* mutation was used as a morphogenetic marker for the

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1. *Abbreviations used in this paper:* BN, Brown-Norway; QTL, quantitative trait locus; SHR, spontaneously hypertensive rat.

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inheritance of this region of chromosome 8 during the introgression process. When transferred onto the SHR background, the mutant *Lx* gene is fully recessive and progeny testing is used for carrier detection. The BN-*Lx* strain was first crossed with the SHR progenitor to derive a panel of recombinant inbred strains (11). One of the recombinant inbred strains (BXH11) that inherited a large segment of chromosome 8 including the *Lx* mutation from the BN-*Lx* strain was then used to introgress this region of chromosome 8 onto the SHR background by backcross breeding. After the equivalent of 12 generations of selective backcrossing to the SHR progenitor strain, the differential chromosome segment in the vicinity of *Lx* was fixed and maintained in the homozygous state by brother × sister mating and selective inbreeding of the polydactylous offspring. Animals of the NE12F3 generation were used in the current studies.

The SHR-RT1.N congenic strain (previously referred to as SHR.1N, reference 11) was derived by transferring a piece of chromosome 20 containing the major histocompatibility complex of the BN-*Lx* rat onto the genetic background of the SHR/Ola progenitor strain (11). This was accomplished using a backcross breeding and immunogenetic phenotyping protocol in which the MHC of the SHR (RT1.K haplotype) was replaced with the MHC of the BN-*Lx* rat (RT1.N haplotype). SHR-RT1.N animals of the N14F22 generation were used in the current study. Based on the number of backcross generations and a preliminary assessment of molecular markers within and flanking the MHC (*Tnfa*, *Hspa1*, *D20Arb548*, *D20Arn249*, *Prkacn2*), the size of the differential chromosome segment in the SHR-RT1.N congenic strain is estimated to be ∼ 31 cM. The same markers used to confirm the congenic status of the SHR-*Lx* strain were also used to confirm the congenic status of the SHR-RT1.N strain (described below).

Chromosome 8 mapping. To determine the map position of *Lx* and several candidate genes for hypertension, we genotyped an F2 population derived from the SHR and BN-*Lx* progenitor strains that previously had been used to generate a complete genetic linkage map in the rat (18). For *Lx* genotyping, polydactylous animals were scored as *Lx/Lx* homozygotes; normodactylous animals were scored as +/? because of inability to distinguish between +/+ and +/*Lx* genotypes. PCR techniques were used to determine genotypes for the kidney epithelial potassium channel gene, *Kcnj1*, and the rat brain dopamine receptor gene, *Drd2.* Primers amplifying the 3′ noncoding region of *Kcnj1* were designed from the sequence (Genbank/EMBL/DDBJ); X72341 upstream primer: 5′ -cag atg tag cag tgg ctt ttc c; downstream primer: 5β -atc taa aga gtg gac ttg gc. Restriction fragment length polymorphism that distinguished the three F2 genotype classes was detected by cutting the PCR products with the *Hinf* I enzyme. Single strand conformation polymorphism (SSCP) analysis was used for *Drd2* genotyping. A 729-bp region of *Drd2* was amplified by PCR with the following primers designed from the sequence (Genbank/EMBL/DDBJ); M36831 upstream primer: 5′ ttc ctt gac ctt cct ctt ggg c; downstream primer: 5′ tag tga tgt tac aga gtt gg. After digestion with *Dde* I, SSCP analysis was used to distinguish between the different genotype classes. Linkage analysis was performed using the MAPMAKER program. The initial framework order was set according to the chromosome 8 map determined by Jacob et al. (18) in the identical population of F2 rats used for the current study. The positions of *Lx*, *Kcnj1*, and *Drd2* were determined using the "try" command with loci being placed in their maximum likelihood positions.

Genotype analysis of the SHR-Lx congenic strain. The same markers used to derive the chromosome 8 linkage map were also used to define the size of the differential chromosome segment trapped in the SHR-*Lx* congenic strain. Chromosome 8 genotypes were determined in all of the SHR-*Lx* rats used in the blood pressure studies. The congenic status of SHR-*Lx* and SHR-RT1.N strains was confirmed by PCR analysis of the following markers known to be polymorphic between the SHR and BN-*Lx* strains: *D1Mit9*, *D1Mit14*, *Igf2*, and *Pthr1* (chr.1); *D2Mit4*, *D2Mit16*, *D2N91*, and *Atp1a1* (chr.2); *D3Mit5*, *D3Mit10*, and *D3Mit11* (chr.3); *Eno2*, *Il6*, and *Spr* (chr.4); *D5Mgh8*, *D5Mit1*, and *Slc2a1* (chr.5); *D6Cep8*, *D6Mit8*, *D6Mit9*, and *Ighe* (chr.6); *D7Mgh11*, *D7Mit6*, and *D7Mit8* (chr.7); *D8Mgh4*, *D8Mgh5*, *D8Mgh6*, *D8Mgh7*, *D8Mgh9*, *D8Mit1*, *D8Mit4*, *D8Mit5*, *D8Mit6*, *D8Mit12*, *D8N228*, *Acaa*, *Apoc3*, *Es6*, *Lx*, *Ncam*, *Rbp2*, *Thy1*, and *Tpm1* (chr.8); *D9Mit1*, *D9Mit4*, and *Inha* (chr.9); *D10Mit6*, *Abpa*, *Dcp1*, and *Myh3* (chr.10); *D11Mgh4*, *D11Mgh6*, and *Sst* (chr.11); *D12Mgh4*, *D12Mit8*, and *Mdh2* (chr.12); *D13Mgh3*, *D13Mit2*, and *D13Mit4* (chr.13); *D14Mgh1*, *D14Mit1*, *D14Mit8*, and *Alb* (chr.14); *D15Mgh3*, *D15Mgh5*, and *D15Mit3* (chr.15); *D16Mit2*, *D16Mit3*, and *Mbpa* (chr.16); *D17Mit2*, *D17Mit7*, and *Chrm3* (chr.17); *D18Mit1*, *D18Mit10*, and *Grl* (chr.18); *D19Mit2*, *D19Mit5*, and *D19Mit7* (chr.19); *D20Arb249*, *D20Arb548*, *D20Mgh1*, *D20Mgh2*, *D20Utr1*, *D20Utr2*, *Rt1a*, and *Tnfa* (chr.20); and *DXMgh1*, *DXMit5*, and *Arl* (chr.X). PCR primers were obtained from Research Genetics (Huntsville, AL) or synthesized in the University of California, San Francisco Biomolecular Resource Center according to published sequences.

Cardiovascular phenotyping. Beginning at 8 wk of age, pulsatile arterial pressures and heart rates were measured in unanesthetized, unrestrained male rats with indwelling radiotelemetry transducers

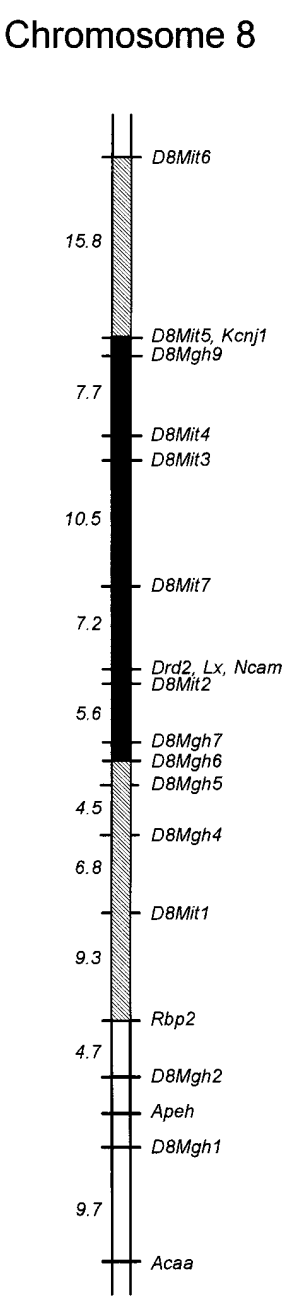

Figure 1. Linkage map showing the transferred segment of chromosome 8 in the SHR-*Lx* congenic strain. The solid bar denotes the chromosome region from the BN-*Lx* strain that has been fixed in the homozygous state on the SHR background. The shaded sections denote regions in which some residual heterozygosity exists within the congenic rats of the NE12F3 generation. The chromosome orientation was determined by fluorescence in situ hybridization mapping of *Acaa* near the telomere (Szpirer, C., and J. Szpirer, unpublished observations).

connected to catheters implanted in the lower abdominal aorta (Data Sciences Intl., St. Paul, MN) (19–22). Pulsatile pressures and heart rates were recorded in 5-s bursts every 5 min during the day (0600–1800) and night (1800–0600) for 3 mo. From these data, single daytime and single nighttime means for systolic and diastolic pressure and heart rate were calculated for each rat for each week of the study. Before and during the BP studies, all rats were given tap water ad libitum and fed a standard pelleted laboratory diet that contained 0.58% NaCl and 1.1% K. Cardiac mass was determined by weighing the heart and correcting for body weight. Statistical analysis was performed using two way repeated measures ANOVA (on weekly averaged BP data) or the Mann Whitney rank sum test (cardiac mass).

Results

Genotype analysis of markers on chromosome 8 verified successful transfer of a large segment of chromosome from the BN-*Lx* strain onto the SHR background. The minimum size of the transferred chromosome segment was delineated by markers for *D8Mit5* and *D8Mgh6*; no recombination was observed within the homozygous portion of the differential chromosome segment (Fig. 1). At the time of these studies, only three generations of inbreeding had been completed after transfer of the BN chromosome segment onto the SHR background. This limited duration of inbreeding accounts for the residual heterozygosity in the proximal and distal portions of the transferred chromosome segment. Genetic mapping studies demonstrated that the transferred chromosome segment includes genes encoding a brain dopamine receptor (*Drd2*) and the renal epithelial potassium channel (*Kcnj1*). Genotype results obtained with 73 widely dispersed polymorphic microsatellite markers confirmed that the congenic strain differs from the SHR progenitor only in the region of chromosome 8 defined in Fig. 1.

Systolic and diastolic BPs determined by radiotelemetry were significantly lower in the SHR-*Lx* congenic strain than in the SHR progenitor strain (Fig. 2, *A* and *B*). Cardiac mass was also significantly lower in the SHR-*Lx* congenic strain than in the SHR progenitor (Fig. 3). The heart rate of the SHR-*Lx* congenic strain tended to be greater than that of the SHR progenitor strain; however, differences in heart rate were not consistently observed throughout the experiment. The strain differences in BP were apparent immediately upon transducer implantation at 8 wk of age and persisted over the entire 3-mo period of the study. Lower BPs were observed in the SHR-*Lx* congenic strain during both the daytime and nighttime light cycles.

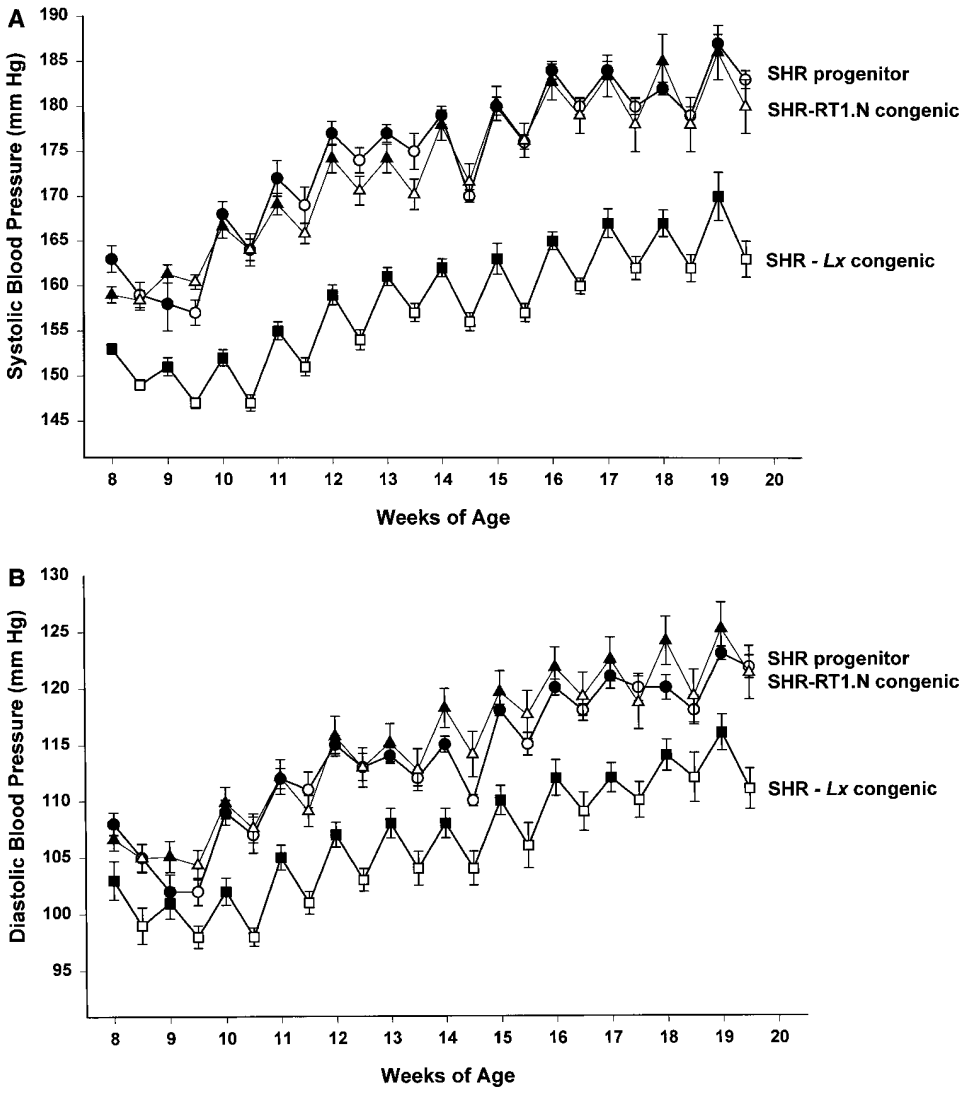

Figure 2. Daytime and nighttime blood pressures determined by radiotelemetry over a period of 3 mo in the SHR progenitor strain, the SHR-*Lx* congenic strain, and SHR-RT1.N congenic strain. Each data point represents the weekly daytime (*open symbols*) or nighttime (*solid symbols*) blood pressure (mean±SEM) in the SHR progenitor strain (*circles*, $n = 8$), the SHR-*Lx* congenic strain (*squares*, $n = 7$), and the SHR-RT1.N congenic strain (*triangles*, $n = 9$) obtained from the weekly averages of ~1,000 daytime or 1,000 nighttime blood pressure measurements in each rat. The sawtooth pattern reflects the circadian variation in blood pressure. (*A*) Systolic blood pressure. The daytime and nighttime systolic pressures of the SHR-*Lx* congenic strain were significantly lower than those of the SHR progenitor strain and the SHR-RT1.N congenic strain ($P < 0.001$). (*B*) Diastolic blood pressure. The daytime and nighttime diastolic pressures of the SHR-*Lx* congenic strain were significantly lower than those of the SHR progenitor strain and the SHR-RT1.N congenic strain ($P < 0.001$).

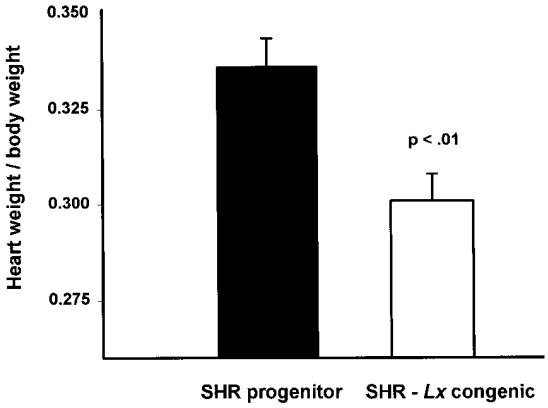

Figure 3. Cardiac mass determined as the ratio of heart/body weight was significantly lower in the SHR-*Lx* congenic strain (*open bar*, $n = 7$) than in the SHR progenitor strain (*solid bar*, $n = 8$).

The differences in BP between the SHR-*Lx* congenic strain and the SHR progenitor strain are not simply a nonspecific consequence of substituting any chromosome region of the SHR with any chromosome region of the BN-*Lx* rat. We also measured pulsatile arterial pressure in the SHR-RT1.N congenic strain that was derived by transferring a segment of chromosome 20 with the MHC of the BN-*Lx* strain onto the SHR background. Genotyping studies of multiple polymorphic markers scattered throughout the genome confirmed that the SHR-RT1.N congenic strain is genetically identical to the SHR progenitor strain except in the vicinity of the MHC on chromosome 20. No consistent differences in systolic or diastolic BP could be detected between the SHR-RT1.N congenic strain and the SHR progenitor strain over a monitoring period of 3 mo (Fig. 2, *A* and *B*). Although previous studies in ether-anesthetized rats revealed modest differences in BP between the SHR-RT1.N congenic strain and the SHR progenitor strain (11), our radiotelemetry measurements in unanesthetized, unrestrained rats indicate that the SHR-RT1.N congenic strain and SHR progenitor strain have identical BPs in the conscious, unrestrained state (similar results were obtained with respect to diastolic or mean arterial pressures and with respect to comparing daytime and nighttime BPs).

Discussion

The SHR has been extensively used to study the pathophysiology of hypertension; however, the genetic mechanisms responsible for increased blood pressure in this model remain to be defined. Although studies in segregating populations derived from the SHR have been useful for the tentative identification of multiple autosomes that may contain genes regulating blood pressure (e.g., chromosomes 1, 2, 4, 8, 10, 13, 16, 19, and 20), considerable uncertainty exists regarding the actual contribution of any of these chromosomes in the pathogenesis of spontaneous hypertension (2–16). In the current studies, we have developed a novel congenic strain of SHR in which we have genetically isolated a region of chromosome 8 that plays a major role in the pathogenesis of spontaneous hypertension in the SHR-BN model. We have found that transfer of a segment of chromosome 8 from the normotensive BN-*Lx* rat onto the SHR background induces significant reductions in both arterial blood pressure and cardiac mass. The reductions in blood pressure were substantial and approached 20 mmHg for systolic pressure and 10 mmHg for diastolic pressure. Given that the differences in BP between the SHR and BN-*Lx* progenitor strains are $\sim$ 80 mmHg systolic and 50 mmHg diastolic (11), these findings demonstrate that the region of chromosome 8 defined by the SHR-*Lx* congenic strain may account for up to 20–25% of the hypertension in SHR vs. BN-*Lx* rats. By trapping a blood pressure QTL within the differential chromosome segment of the SHR-*Lx* congenic strain, the current studies represent a critical step toward the eventual genetic and physical isolation of a QTL regulating blood pressure in the SHR. The difference in cardiac mass between the SHR progenitor strain and SHR-*Lx* congenic strain raises the possibility that the transferred segment of chromosome 8 also contains a QTL(s) that may be relevant to the pathogenesis of cardiac hypertrophy in hypertension. However, further studies will be required to determine whether the effect on cardiac mass is related to, or independent of, the effects on arterial pressure.

The current findings are consistent with the results of linkage studies in SHR and BN rats in which Schork et al. (7) detected a possible BP QTL on chromosome 8 located approximately midway between *D8Mit3* and *D8Mit5*; this region maps within the differential chromosome segment trapped in the SHR-*Lx* congenic strain. In the current study, we have found that a number of interesting candidate genes for hypertension map to this segment of chromosome 8, including *Drd2* (brain dopamine receptor) and *Kcnj1* (renal epithelial potassium channel). Simon et al. (23) have recently demonstrated that mutations in human *KCNJ1* can cause Bartter's syndrome, a condition associated with salt-wasting and low blood pressure. Thus, differences in the expression or structure of *Kcnj1* could conceivably contribute to the differences in blood pressure between the SHR progenitor strain and SHR-*Lx* congenic strain. Although the size of the differential chromosome segment in the SHR-*Lx* congenic strain is large, congenic sublines can now be derived for exclusion mapping and for refining the map position(s) of QTLs with significant effects on BP. If a single gene is responsible for the major changes in blood pressure observed in the current study, the SHR-*Lx* congenic strain could ultimately enable the mapping of a BP QTL on rat chromosome 8 as a simple mendelian locus.

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