Genetic Mapping of Soluble Guanylyl Cyclase Genes
Implications for Linkage to Blood Pressure in the Dahl Rat

Mohammad Azam, Gopa Gupta, Wei Chen, Sandra Wellington, Dorothy Warburton, Robert S. Danziger

Abstract—The nitric oxide (NO) signaling system, consisting of NO synthases, soluble guanylyl cyclase, and cGMP, plays a prominent role in salt handling and regulation of blood pressure. Soluble guanylyl cyclases are heme-containing heterodimers (α/β). The α1/β1 isof orm has greater NO sensitivity than the α1/β2. It has recently been shown that expression of the β subunits is altered in the kidney of the Dahl salt-sensitive rat, ie, the β1 subunit is decreased and the β2 subunit increased. However, whether soluble guanylyl cyclase is linked to salt sensitivity is not known. In the present study, we investigated linkage of guanylyl cyclase genes to blood pressure. α1 and β1 gene loci for soluble guanylyl cyclase were mapped to rat chromosome 2, and the β2 gene locus was mapped to rat chromosome 5 using fluorescent in situ metaphase hybridization. By use of a rat radiation hybrid panel, the gene loci were then further mapped with respect to known quantitative trait locus markers of salt-sensitive hypertension in the Dahl rat on chromosomes 2 and 5. Genes for α1 and β1 were closely linked by two-point analysis to Na^+/K^+ -ATPase α1 isof orm (LOD of 15.1 and 14.0, respectively) and calmodulin-dependent protein kinase II-δ loci (LOD of 14.3 and 12.9, respectively), which have been previously shown to flank a quantitative trait locus for blood pressure in the Dahl rat. The α1 and β1 genes were closely linked (LOD of 11.3; 6, 0.4). The β2 gene locus was closely linked to the endothelin-2 (ET-2) locus (LOD of 13.0), which has been shown to cosegregate with blood pressure. We conclude that soluble guanylyl cyclase subunit loci, ie, α1, β1, and β2, are good candidates for genes controlling salt-sensitive hypertension in the Dahl rat. (Hypertension. 1998;32:149-154.)

Key Words: guanylyl cyclase ■ cyclic GMP ■ genetics ■ hypertension, salt-sensitive

The NO signaling system, consisting of NO synthases, SGC, and cGMP, plays a prominent role in salt handling and regulation of blood pressure. In biological systems, SGCs (GTP pyrophosphate-lyase [cyclizing; EC 3.6.1.2]) are pre-eminent receptors for NO. NO and carbon monoxide activate (GTP pyrophosphate-lyase [cyclizing; EC 3.6.1.2]) are pre-

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*Although originally reported as α3 and β3, it is generally accepted that these are human forms of α1 and β1 due to sequence homology.
hypertension. However, the difference between the SS/Jr and SR/Jr kidney at a molecular level remains unclear. We have recently reported that expression of the β2 guanylyl cyclase subunit is increased and that of the β1 subunit is decreased in the kidney of the SS/Jr compared with the SR/Jr. Whether guanylyl cyclase isoforms are genetically linked to blood pressure and salt sensitivity in the Dahl rat is unknown.

QTLs for salt-sensitive blood pressure in the Dahl salt-sensitive rat and gene loci cosegregating with blood pressure have been previously identified using crosses of SS/Jr with SR/Jr, WKY, and MNS rats. Loci segregating with blood pressure were found in chromosome 2, mapping at the GC-A and between the NAK and CAMK, and in chromosome 5 closely linked to the ET-2 locus. In the present study, we tested whether the genes for SGC map to chromosome regions previously identified as containing QTLs for salt-sensitive blood pressure to determine whether they are candidate genes for hypertension in the Dahl rat.

Methods

Chromosomal Localizations

FISH was performed on chromosomal spreads from rat EIC18 cells. Full-length rat cDNAs subcloned into pcDNA-neo were used as probes for the β1 and α1 genes (obtained as a gift from M. Nakane, Abbott, Ill). The probe for the β2 gene was a 22-kb genomic clone obtained by screening a rat genomic library (kidney) in the λ-Dash II vector (Stratagene) using a 320-bp cDNA fragment obtained from an Apol digest of the full-length cDNA subcloned into Bluescript Vector (obtained from P. Yuen, Memphis, Tenn). The β2 probe was random prime-labeled (Random Prime Labeling kit, Amersham), and filters were screened under high stringency. Individual clones were identified after the tertiary screen. λ DNA isolated by

Selected Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>CAMK</td>
<td>calmodulin-dependent protein kinase II-δ</td>
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<tr>
<td>ET-2</td>
<td>endothelin-2</td>
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<tr>
<td>FISH</td>
<td>fluorescent in situ hybridization</td>
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<tr>
<td>GC-A</td>
<td>membrane-bound guanylate cyclase A</td>
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<tr>
<td>LOD</td>
<td>logarithm of odds</td>
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<td>MNS</td>
<td>Milan normotensive strain of rat</td>
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<tr>
<td>NAK</td>
<td>Na⁺,K⁺-ATPase α1 isoform</td>
</tr>
<tr>
<td>NO</td>
<td>nitric oxide</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
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<tr>
<td>QTL</td>
<td>quantitative trait locus</td>
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<tr>
<td>SGC</td>
<td>soluble guanylyl cyclase</td>
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<tr>
<td>SR/Jr</td>
<td>Dahl salt-resistant rat</td>
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<tr>
<td>SS/Jr</td>
<td>Dahl salt-sensitive rat</td>
</tr>
<tr>
<td>WKY</td>
<td>Wistar-Kyoto rats</td>
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the Qiagen Isolation System at a concentration of 10 μg/μL was used for hybridizations. Probes were labeled by nick translation with digoxigenin and hybridized at a concentration of 10 μg/μL overnight. Slides were washed in 1× SSC at 50°C, detected using antidigoxigenin-labeled FITC, counterstained with DAPI, and examined using a Nikon Microphot microscope and Cytovision Image Analysis System (Applied Imaging). Hybridizations were repeated two times. Enhanced DAPI images were karyotyped, and the location of hybridization signals was noted in 20 metaphase spreads.

Radiation Hybrid Mapping

A rat-hamster hybrid panel created by Peter Goodfellow (Cambridge, UK) was obtained from Research Genetics, Inc (Huntsville, Ala). To create the panel, a rat cell line (donor RatFR) was exposed to 3000 rad of x-rays and then fused with nonirradiated thymidine-deficient hamster recipient cells (A23). The panel consists of 106 clones and has an average locus retention rate of 28%.

The presence or absence of each marker was determined using PCR. Each marker was tested separately (none were multiplexed). The PCR primer sets for α1, β1, and β2 subunits of guanylyl cyclase; CAMK; NAK; ET-2; guanylate cyclase-A/atrial natriuretic peptide receptor-A (GC-A); and D2N35 were designed from published sequence data from Genebank (Table 1). PCRs were carried out in a total volume of 50 μL with 0.35 ng of DNA template, 300 nmol/L of each PCR primer, 15 mmol/L MgCl2, 200 μmol/L dNTPs, and 2.6 U of Expand polymerase (Boehringer Mannheim). The PCR profile consisted of 30 cycles of 94°C for 30 seconds (denaturation), 50°C for 60 seconds (annealing), and 72°C for 2 minutes (extension), followed by an additional 10-minute final extension at 72°C.

Optimal annealing temperature was determined for each set of primers on the basis of GC content. The PCR products were resolved on a 2% agarose gel and analyzed using the Bio-Rad gel documentation system.

The radiation hybrid mapping program RHmapper was used to analyze the data. Two-point analysis was performed for gene loci known to cosegregate with blood pressure and SGC genes on appropriate chromosomes. The order of the CAMK, NAK, GC-A, D2N35, and SGC loci was determined by the stepwise ordering strategy with a machine-generated candidate order. Distances were calculated using the “evaluate function” and are reported in cR3000, where 3000 rad indicates the dosage of x-rays used in the irradiation of the hybrids.

Results

Chromosomal Mapping

By FISH, probes for both α1 and β1 were localized on metaphase spreads to rat chromosome 2, band q31. β2 mapped to the most distal band of chromosome 5. Specific signal was identified on at least one chromatid in 20 of 20 pairs examined (Figure).

Radiation Hybrid Mapping

The presence or absence of each of the markers in 92 of 106 radiation hybrid clones was determined by PCR screening using primers as outlined in Table 1. Each of the markers was
detected in 17% to 24% of the hybrids. Retention frequency of the CAMK, NAK, ET-2, GC-A, and D2N35 loci ranged from 17% to 22%. The retention frequency for the α1 loci was 22% and for the β2 loci 24%.

LOD score from two-point analysis (Table 2) showed close linkage of the α1 and β1 gene loci to CAMK, D2N35, NAK, and GC-A with LOD of 9.5 to 15.1 on chromosome 2. The closest linkage determined by two-point analysis for both β1 and α1 was with CAMK and NAK, which are loci previously shown to flank a QTL for blood pressure.27 Linkage between α1 and β1 was also suggested because θ, defined as the probability that two loci are separated by one or more irradiation-induced breaks and an estimate of the physical distance between the markers, was 0.40 with an LOD of 11.3. The loci on chromosome 2 were subjected sequentially to analyses for order. For β1 the most likely order was GC-A, NAK, ET-2, CAMK, D2N35, and for α1 it was GC-A, NAK, α1, CAMK, D2N35, with LOD versus next best of 0.5 and 0.4, respectively. The most likely order for the marker genes, ie, GC-A, NAK, CAMK, D2N35, corresponds to that determined using linkage analysis. Together, the data support a close linkage of α1 and β1 gene loci between NAK and CAMK on rat chromosome 2.

The ET-2 locus was closely linked to the β2 locus on chromosome 5, with an LOD of 13.0. The distance between the ET-2 and β2 loci on chromosome 5 was calculated to be 53.6 cR. Although the resolution of the rat-hamster radiation hybrid panel has not been determined, based on the resolution of human and mouse radiation hybrid panels obtained with 3000 rad, a distance of 4 to 11 Mb is estimated.

Discussion

The present study suggests a link between SGC genes and salt sensitivity in the Dahl rat. We have shown that the genes for SGC α1 and β1 subunits map to chromosome 2 and are closely linked to the GC-A locus, which has been shown to cosegregate with blood pressure,28 and the NAK and CAMK genes, which have previously been shown to flank a QTL for blood pressure in the Dahl salt-sensitive rat in F2 populations of male rats derived from crosses of Dahl salt-sensitive with WKY and MNS rats (LOD score of 5.66 based on the combined population).20,24 Our data also demonstrate that the β2 and ET-2 gene loci on chromosome 5 are closely linked, indicating that the β2 gene for guanylyl cyclase will also cosegregate with blood pressure, since the ET-2 locus has been shown to cosegregate strongly with systolic blood pressure in an F2 population derived from a cross between Dahl salt-sensitive and Lewis rats.25

Pharmacological studies have demonstrated that renal NO signaling and cGMP regulate salt sensitivity and hypertension.30–35 Inhibitors of NO formation, such as Nω-monomethyl-L-arginine, reduce sodium excretion and increase both arterial blood pressure and salt-induced increases in arterial pressure.31,34,35 In addition to influencing sodium handling by regulating renal blood flow, there is growing evidence that NO affects renal sodium transport in the proximal tubule,37 to ATPase,36 Na+-K+ exchange in the proximal tubule,37 and sodium transport in cortical collecting duct cells.38 In the Dahl salt-sensitive rat, administration of L-arginine, a substrate for NO synthases, prevents the development of hypertension,39,40 normalizes pressure natriuresis,40 and increases the glomerular filtration rate.42 Recent studies have demonstrated that as in other tissues, the effect of NO in the kidney is mediated by cGMP generated by SGC. cGMP has been directly linked to inhibition of Na+-H+ exchange in the proximal tubule,37 to regulation of Na+K+-ATPase,43,44 and to reduction in renal vascular resistance, particularly preglomerular arteriolar resistance vessels.45
A decrease in the sensitivity of guanylyl cyclase to NO has been reported in the SS/Jr kidney and postulated to play a central role in the pathogenesis of salt sensitivity.


Further studies have shown that the blood pressure QTL is contained in the region spanning the D2N35 and NEP loci (which includes the NAK and CAMK loci) on chromosome 2 using congenic strains. Further congenic studies with greater resolution of the region will contribute to establishing the physiological link between SGC and salt sensitivity in the Dahl rat.

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References


