Sequence Analysis of the α1 Na⁺,K⁺-ATPase Gene in the Dahl Salt-Sensitive Rat

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In the inbred Dahl salt-sensitive rat (SS/Jr strain), it has been proposed that a T for A transversion in the DNA sequence encoding amino acid 276 in the α1 subunit isoform of Na⁺,K⁺-ATPase may impair ion transport and contribute to the pathogenesis of hypertension. This hypothesis is of major scientific interest because it represents the first attempt to explain the pathogenesis of salt-sensitive hypertension on the basis of a specifically defined mutation at the DNA level. We devised a polymerase chain reaction technique to screen the genomic DNA of multiple SS/Jr rats for the T for A transversion reported in the complementary DNA (cDNA) encoding the α1 subunit of Na⁺,K⁺-ATPase. When eight Dahl SS/Jr rats from Harlan Sprague Dawley Inc. were tested with the polymerase chain reaction technique, we found no evidence of this mutation in the Na⁺,K⁺-ATPase gene. Direct sequence analysis of the gene in three SS/Jr rats also did not show the T for A transversion. These results 1) strongly suggest that commercially available Dahl SS/Jr rats do not carry a T for A transversion in the genomic DNA sequence encoding amino acid 276 in the α1 subunit isoform of Na⁺,K⁺-ATPase and 2) raise the possibility that the previous finding of a mutation in the cDNA of the SS/Jr rat may have been due to a reverse transcriptase error during cDNA synthesis. (Hypertension 1991;18:689-693)
ing mean arterial pressures in unanesthetized, unrestrained rats 3 days after implantation of indwelling femoral artery catheters.3,4

To further insure that the SS/Jr rats had not been switched with the SR/Jr rats during the shipping process or after arrival in our laboratory, each rat was tested for a Bgl II restriction fragment length polymorphism (RFLP) in the first intron of the renin gene; the SS/Jr strain is known to exhibit a 2.7 kb fragment, the SR/Jr strain a 1.5-1.7 kb fragment.4,5 Renin genotyping was performed as previously described.4,6

**Polymerase Chain Reaction Analysis**

According to the sequence data published by Herrera and Ruiz-Opazo,2 the T for A transversion in the α1 Na⁺,K⁺-ATPase gene of the SS/Jr rat should abolish an Eae I restriction site. Eae I recognizes the sequence pyGGCCpu where py represents a pyrimidine (C or T) and pu represents a purine (A or G). In the SS/Jr rat, a substitution of T for A at nucleotide position 1,079 corresponding to amino acid number 276 in the α1 Na⁺,K⁺-ATPase gene (position numbering according to Shull et al7) would convert the sequence CGGCCA to the sequence CGGCCT and give rise to an Eae I RFLP.

We designed PCR primers to amplify a 129-base pair (bp) fragment that includes this restriction site and thereby enable us to distinguish the SS/Jr allele from the SR/Jr allele by gel electrophoresis of the Eae I digested PCR products. The upstream primer was CTT GCT TCT GGG CTG GAA GGC; the downstream primer was GTA CTC AAG GAT CAG AGA GAG. PCR was performed as described for amplification of the Eae I site. The residual PCR primers were removed from the amplified product by centrifugation in a Centricon-100 column (Amicon, Danvers, Mass.). Automated sequencing of the purified PCR product was performed using fluorescent dye terminator chemistry on a 373A analyzer (Applied Biosystems, Foster City, Calif.) in the Biomedical Resource Center at UCSF. The same primers used to amplify the target sequence were used to prime the sequencing reactions. One sample was sequenced in both the forward and reverse directions, the other samples were sequenced in the reverse direction.

**Results**

**Polymerase Chain Reaction Analysis**

Figure 1 depicts the results of PCR analysis of the putative T for A transversion in the α1 Na⁺,K⁺-ATPase gene of two SS/Jr rats and two SR/Jr rats. The size of the undigested PCR target sequence in the SS/Jr strain was similar to that in the SR/Jr strain. In the SR/Jr strain, Eae I digestion of this 129 bp target should yield a small 21-bp fragment and two larger fragments (56 bp and 52 bp) because the target sequence contains two Eae I sites. If the T for A transversion reported by Herrera and Ruiz-Opazo2 exists in the genomic DNA of the SS/Jr rat, it should abolish one of the Eae I restriction sites in this 129-bp PCR product. Accordingly, Eae I digestion of the PCR product from the SS/Jr strain should yield a 77-bp fragment (21 + 56) and a 52-bp fragment. Thus, if the reported mutation truly exists in the genomic DNA of the SS/Jr strain, one should be able to distinguish the SS/Jr rat from the SR/Jr rat by observing the presence of a unique 77-bp Eae I restriction fragment in the SS/Jr strain and the absence of the smaller 21-bp fragment. However, upon Eae I digestion of the PCR target sequence, the SS/Jr and SR/Jr strains exhibited the same pattern of restriction fragments (i.e., the 21-bp, 56-bp, and 52-bp fragments expected only for the SR/Jr strain).

To confirm the results of the Eae I digest in the SR/Jr strain, a separate shipment of six SS/Jr rats was obtained for testing. Figure 2 shows the results of the PCR analysis in these additional SS/Jr rats. All of the SS/Jr rats exhibited the Eae I restriction pattern...
FIGURE 1. Polymerase chain reaction (PCR) analysis of the putative T for A transversion site at nucleotide position 1,079 in the \( \alpha_1 \) Na\(^{+}\),K\(^{+}\)-ATPase gene of the inbred strain of Dahl salt-sensitive (SS/Jr) rats. In the SS/Jr rat, the T for A transversion should have abolished one of the two \( \text{Eae}\_I \) restriction sites in the segment of the Na\(^{+}\),K\(^{+}\)-ATPase gene that we amplified by PCR. Lanes 1 and 2 contain undigested PCR products from two Dahl SS/Jr rats. Lanes 3 and 4 contain \( \text{Eae}\_I \)-digested PCR products from two SS/Jr rats. Lanes 5 and 6 contain \( \text{Eae}\_I \)-digested PCR products from two inbred Dahl salt-resistant (SR/Jr) rats. Lanes 7 and 8 contain undigested PCR products from two SR/Jr rats. Lane 9 contains \( \phi X 174 \) DNA/Hae III size markers (the last band is the 72-bp size marker). SS/Jr rats exhibit the same restriction fragments as the SR/Jr strain. Smallest fragment corresponds to the single-stranded PCR primers.

FIGURE 2. Polymerase chain reaction (PCR) analysis of the putative T for A transversion site in the \( \alpha_1 \) Na\(^{+}\),K\(^{+}\)-ATPase genes of six of the inbred strain of Dahl salt-sensitive (SS/Jr) rats. Lane 1 contains undigested PCR product from an SS/Jr rat. Lanes 2–7 contain \( \text{Eae}\_I \)-digested PCR products from six SS/Jr rats.

Sequence Analysis

None of the three SS/Jr rats tested showed a T for A transversion at nucleotide position 1,079. The DNA sequence around the putative T for A mutation site was: GAA GGC GGC CAG ACC CCC ATT (the underlined base is the site where the SS/Jr rat was reported to have a T instead of an A). This sequence around nucleotide position 1,079 is identical to that reported by Shull et al\(^7\) in the \( \alpha_1 \) Na\(^{+}\),K\(^{+}\)-ATPase cDNA of a Sprague-Dawley rat. The mean arterial pressures in these rats after 4 weeks of supplemental dietary NaCl were markedly increased: 172 mm Hg, 175 mm Hg, and 192 mm Hg.

Discussion

The inbred SS/Jr strain was derived by John Rapp from a colony of Brookhaven Dahl salt-sensitive rats.\(^8\) In 1986, Rapp provided the inbred SS/Jr strain (F\(_w\) generation) to Harlan Sprague Dawley, the only commercial source of these rats in the United States (J. Rapp, personal communication). The current results would seem to indicate that SS/Jr rats from Harlan Sprague Dawley do not carry a mutation in the genomic DNA encoding amino acid 276 in the \( \alpha_1 \) subunit of the Na\(^{+}\),K\(^{+}\)-ATPase. These findings appear to conflict with the results of the study by Herrera and Ruiz-Opazo\(^2\) in which a T for A transversion was observed at this site in the cloned \( \alpha_1 \) Na\(^{+}\),K\(^{+}\)-ATPase cDNA of a single SS/Jr rat. We also found no evidence of the T for C substitution observed by these investigators in the sequence encoding amino acid position 890 in the \( \alpha_1 \) Na\(^{+}\),K\(^{+}\)-ATPase of the SS/Jr rat.\(^2\)
Several possible explanations for the discrepancies between our study and that of Herrera and Ruiz-Opazo require consideration: 1) The single SS/Jr rat studied by Herrera and Ruiz-Opazo may have been genetically different from the SS/Jr rats currently sold by Harlan Sprague Dawley. This explanation seems unlikely because the SS/Jr strain was not distributed until after it was highly inbred. However, given that a certain degree of genetic heterogeneity can exist within inbred strains, this explanation cannot be excluded. 2) The mutations detected by Herrera and Ruiz-Opazo may have been generated by a unique form of messenger RNA (mRNA) editing and therefore, the mutations can only be detected in cDNA, not genomic DNA. Although such RNA editing is theoretically possible, we are unaware of any precedent for single base substitutions in mammalian mRNA other than the C to U modification observed in the intestinal form of apolipoprotein B mRNA.9 3) The mutations detected by Herrera and Ruiz-Opazo may have been generated by reverse transcriptase errors or cloning errors and are not present in the native DNA of SS/Jr rats. Reverse transcriptases lack 3'→5' exonuclease activity and cannot proofread errors made during DNA synthesis. The relatively low fidelity of reverse transcriptases has been well-documented,10 and we believe that reverse transcriptase errors may well account for the nucleotide substitutions observed by Herrera and Ruiz-Opazo in their single SS/Jr rat. Although the Taq polymerase used for the PCR analysis in our study is also error-prone, it is unlikely that the enzyme would commit identical errors in the multiple DNA samples we tested. Another possibility is that the unexpected PCR products resulted from the amplification of pseudogenes in the SS/Jr strain that have sequence homology to the α1 subunit gene of the SR/Jr strain. This explanation would require not only that pseudogenes exist in the SS/Jr rat that carry the α1 subunit sequence of the SR/Jr strain but also that such pseudogenes are preferentially amplified compared with the specific α1 subunit gene from which the current PCR primers were designed.

Herrera and Ruiz-Opazo2 have proposed that the T-A transversion they observed in the SS/Jr rat might impair ion transport and contribute to the pathogenesis of salt-sensitive hypertension. The current findings appear to demonstrate that in the Dahl SS/Jr rat, a T for A transversion in the genomic sequence encoding the α1 Na⁺,K⁺-ATPase is not required for the expression of severe hypertension. It should also be noted that in an F₂ population derived from SS/Jr and SR/Jr rats, Rapp and Dene 11 found the blood pressure of rats inheriting an RFLP marking the α1 Na⁺,K⁺-ATPase allele of the SS/Jr strain to be similar to that of rats inheriting an RFLP marking the Na⁺,K⁺-ATPase allele of the SR/Jr strain. Although we have not been able to detect either the T for A transversion or the T for C transition reported by Herrera and Ruiz-Opazo2 in their SS/Jr rat, we do not wish to imply that the entire sequence of the α1 Na⁺,K⁺-ATPase gene in the SS/Jr strain is identical to that in the SR/Jr strain. In fact, it would appear that comparisons of the α1 Na⁺,K⁺-ATPase gene sequences from the SS/Jr rat and SR/Jr rat have never been reported. Although Herrera and Ruiz-Opazo2 published sequencing gels comparing the cDNA sequence of a single “S” rat to that of a single “R” rat, it should
be recognized that these gels actually portray the cDNA sequences of an SS/Jr rat (designated “S”) and a Sprague-Dawley rat (designated “R”), not an SR/Jr rat. Thus, even if most of the published cDNA sequence of the Na⁺,K⁺-ATPase gene in the SS/Jr rat is correct, it is unknown whether it differs from that in the SR/Jr rat. It should also be noted that in Figure 4A in the report of Herrera and Ruiz-Opazo, the 5’ end of the sequence for the α1 Na⁺,K⁺-ATPase gene is incorrectly labeled as the 3’ end.

In summary, we have found that in the Dahl SS/Jr rat, increased blood pressure may not be attributed to a mutation in the genomic sequence encoding amino acid 276 in the α1 subunit of Na⁺,K⁺-ATPase. The previous finding of a T for A transversion in the SS/Jr cDNA corresponding to this region of the Na⁺,K⁺-ATPase gene could have been due to an error in nucleotide processing by the reverse transcriptase used to generate the cDNA from RNA. Less likely explanations for the discrepant results between the present study and that of Herrera and Ruiz-Opazo include: 1) genetic variability in the SS/Jr strain, 2) the existence of some unique form of mRNA editing, or 3) SS/Jr rats carry pseudogenes with sequence homology for the α1 subunit gene of the SR/Jr strain and such pseudogenes have been preferentially amplified in our PCR experiments.

References

KEY WORDS • genetics • sodium-dependent hypertension • blood pressure • polymorphism
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