Expression of the Amiloride-Sensitive Sodium Channel β Subunit Gene in Human B Lymphocytes

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Abstract. The amiloride-sensitive, epithelial sodium channel (ENaC) is composed of at least three subunits (α, β, and γ). This study demonstrates that the ENaC β subunit gene is expressed in human B cell lines, peripheral blood lymphocytes, and lymph node at the mRNA level. Further, this study shows that both wild-type and mutated alleles of the ENaC β subunit gene are transcribed in human B lymphocytes derived from an individual affected with Liddle’s syndrome, an autosomal dominant form of human hypertension. (J Am Soc Nephrol 8: 126–129, 1997)

The epithelial sodium channel (ENaC), present in many sodium-absorptive epithelia, provides an apical entry pathway for sodium ions (1). ENaC activity has also been found in nonepithelial cells, including B lymphocytes (2), thyroid cells (3), and vascular smooth muscle cells (4). The physiologic role of ENaC in these nonepithelial cells is unknown. Human ENaCs have been cloned and are composed of at least three homologous subunits: α, β, and γ (5). Recent genetic linkage analysis has shown that Liddle’s syndrome, an autosomal dominant form of human hypertension, can be caused by mutations in the carboxy terminus of the ENaC β (6) or γ (7) subunit genes. These mutated ENaC subunits, when expressed in oocytes, produce constitutively activated sodium channels (7,8).

Although patch clamp analysis has clearly demonstrated that human B lymphocytes express sodium channels that are functionally and pharmacologically similar to ENaCs (2), the molecular and biochemical properties of human lymphocyte sodium channels are poorly understood. In this study, we demonstrate that human B cells from an individual with Liddle’s syndrome contain mRNAs transcribed from both wild-type and mutated alleles of the ENaC β subunit gene.

Materials and Methods

Cell Preparation

Human B lymphocytes were cultured in suspension with RPMI 1640 supplemented with 10% fetal bovine serum (FBS). The following human B cells were used: LS12 and LS13, provided by Dr. R. Lifton; and Daudi, PA-1, and RPMI 8226, provided by Dr. J. Bubien. Human blood lymphocytes were prepared as previously reported (2).

Upon receipt, human blood samples were diluted 1:1 with RPMI 1640 and centrifuged over Ficoll-Hypaque (Pharmacia, NJ). The blood lymphocytes were washed twice with RPMI 1640 and frozen at -70°C for later analysis.

RT-PCR

Total RNA were extracted from cultured human B lymphocytes and blood lymphocytes with a single-step guanidinium thiocyanate/phenol/chloroform method (9). mRNAs were prepared using the Micro mRNA Purification Kit (Pharmacia). cDNA was synthesized by the oligo(dT)-priming method using Avian myeloblastosis virus (AMV) reverse transcriptase (RT), and polymerase chain reaction (PCR) was performed as previously described (9). Human lymph node cDNA was obtained from the Clontech (CA).

We designed the PCR primers to amplify the second transmembrane and/or the carboxy-terminal domain of the human ENaC β subunit. Among several primer sets tested, the following PCR primers gave the best results (Figure 1): the forward primer corresponds to nucleotide position 1334–1353 (numbered according to GenBank, L36592), and the reverse primer corresponds to 1591–1573. This amplified region is known to contain at least one intron (6). Negative controls for RT-PCR, such as omission of RT, showed no DNA amplification.

On 2% agarose gel, 10 µl of the PCR products were analyzed by electrophoresis. The PCR products were validated by restriction map analysis and/or nucleotide sequence determination. Nucleotide sequences were determined by using an ABI 373 automated DNA sequencer at the UAB CFAR DNA Sequencing Core (Birmingham, AL).

Analysis of the ENaC β Subunit Gene Transcription

As an initial approach, we amplified the carboxy-terminal of the human ENaC β subunit, which contains a mutation in the original Liddle’s kindred, the K100 pedigree (6), using the forward primer corresponding to nucleotides 1609–1631 with one nucleotide substitution (A to C at 1612) to create a Xho I site, and the reverse primer corresponding to nucleotides 1790–1814 with two nucleotide substitutions (GG to CA at 1807–8) to create an Acc I site. The PCR products were restricted with Xho I and Acc I, and ligated into prβENaC-O1 that was digested with Xho I and Acc I. The
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Figure 1. (A) Polymerase chain reaction (PCR) amplification of the ENaC β subunit from cultured human B lymphocytes, LS12 (lane 2), human lymph node (lane 3), and normal human peripheral blood lymphocytes, S100 (lane 4). Lane 1 contains 100 bp marker. (B) Restriction map analysis of the amplified β subunits from the S100. Lane 1 contains 100 bp marker, and lane 2 is the PCR products without enzyme treatment. PCR products were treated with either Sty I (lane 3) or Hph I (lane 4). (C) A partial restriction map in the amplified region. The same restriction map patterns were obtained when the PCR products from the LS12 or the human lymph node were used. The results demonstrate that the ENaC β subunit gene is transcribed in cultured human B lymphocytes, lymph node, and peripheral blood lymphocytes.

Figure 2. Allelic expression of the ENaC β subunit gene in human B lymphocytes (LS13) derived from the K100. (A) Restriction analysis of the polymerase chain reaction (PCR) products from eight different single bacterial colonies (lanes 1-8). Lane 9 contains a positive control, a PCR product from a wild-type human ENaC β subunit cDNA. M represents 100 bp marker. The PCR products from wild-type ENaC β subunit allele are expected to contain two Hph I sites, while those from the mutated K100 allele are expected to contain three Hph I sites in the amplified region. (B) A partial restriction map in the amplified region including Hph I. The results show that both the wild-type allele (Lanes 1,2,3,4,8) and the mutated allele (Lanes 5,6,7) of the ENaC β subunit gene are transcribed in cultured LS13 human B lymphocytes.

Results

We found the ENaC β subunit mRNAs in Epstein-Barr virus (EBV)-transformed human B lymphocytes, LS12 (Figure 1A) and LS13 (Figure 2), and peripheral human blood lymphocytes (Figure 1A and Figure 3). The ENaC β subunit was also amplified from human lymph node cDNA (Figure 1A) and from three other human B cell lines, Daudi, PA-1, and RPMI 8226 (data not shown).

Interestingly, both wild-type and mutated alleles of the ENaC β subunit gene were equally well transcribed in blood lymphocytes (Figure 3) and cultured B lymphocytes (Figure 2) derived from the K100 pedigree. Two slightly different procedures were used to confirm this observation. First, the PCR products from the K100 blood lymphocytes were digested with restriction enzymes to create cohesive ends at both 5' and 3' ends of the PCR products, which were then cloned into a vector also digested with the same enzymes (Figure 3). Alternatively,
the PCR products were directly cloned into the linearized pCR II vector (Figure 2). Applying the former method, out of the six analyzed bacterial colonies derived from the K100 blood lymphocytes, three contained normal β subunit sequences, and three contained mutated β subunit sequences (Figure 3B). With the direct cloning method, out of eight bacterial colonies derived from the K100 blood lymphocytes, three contained normal β subunit sequences, and five contained mutated β subunit sequences (data not shown). Similarly, in the cultured K100 B lymphocytes, out of eight bacterial colonies, five contained normal β subunit sequences, and three contained mutated β subunit sequences (Figure 2A).

**Discussion**

The current observation is important in several aspects. First, this study is the first to show the expression of the ENaC gene in human non-epithelial cells and tissue, namely B lymphocytes and lymph node, suggesting that human ENaC gene expression is not restricted to epithelial cells or tissue. Second, it is important to demonstrate whether a candidate gene for a certain disease, identified through genetic linkage, is transcribed at the mRNA level in an appropriate cell. This study shows the presence of the mutated ENaC β subunit mRNAs in human B lymphocytes derived from an individual affected with Liddle’s syndrome. Third, the presence of both normal and mutated ENaC β subunit mRNAs in blood lymphocytes from an individual with Liddle’s syndrome provides further evidence that the underlying cause of the abnormally regulated sodium channel activities observed in these patients (2) is the expression of the mutated ENaC β subunit. Consistent with this speculation, coexpression of an equal amount of normal and mutated ENaC β subunits together with wild-type ENaC α and γ subunits in *Xenopus* oocytes produces highly activated amiloride-sensitive currents (Oh et al., submitted for publication). Fourth, although it might be argued that the mRNAs detected in B cells by RT-PCR may simply reflect “illegitimate” transcription, the fact that constitutively activated amiloride-sensitive sodium channel activity has been demonstrated in these same cells (2) supports the functional expression of an entire sodium channel complex in human B lymphocytes. In the future, examining whether other ENaC subunits are expressed in human lymphocytes, as well as whether both normal and truncated ENaC β subunits are equally represented in forming functional sodium channels in the membrane, will be important.

**Acknowledgments**

We thank Dr. J.K. Bubien for his help in preparing human peripheral blood lymphocytes, and Dr. M.J. Welsh for providing us the human ENaC clones. We also thank Ms. Lily Shen for her technical support. This work was supported in part by grants from the National Institute of Health, NS-34877 (YO), DK-19407 (DGW), and the Research Service of the Department of Veterans Affairs (DGW).

**References**


