Cloning and localization of a double-pore K channel, KCNK1: exclusive expression in distal nephron segments

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Orias, Marcelo, Heino Velázquez, Freeman Tung, George Lee, and Gary V. Desir. Cloning and localization of a double-pore K channel, KCNK1: exclusive expression in distal nephron segments. Am. J. Physiol. 273 (Renal Physiol. 42): F663–F666, 1997.—The K-selective channel, TOK1, recently identified in yeast, displays the unusual structural feature of having two putative pore regions, in contrast to all previously cloned K channels. Using the TOK1 pore regions as probes, we identified a human kidney cDNA encoding a 337-amino acid protein (hKCNK1) with four transmembrane segments and two pore regions containing the signature sequence of K channels. Amino acid identity to TOK1 is only 15% overall but 40% at the pores. Northern analysis indicates high expression of a 1.9-kb message in brain > kidney > heart. Nephron segment localization, carried out in rabbit by reverse transcription-polymerase chain reaction, reveals that KCNK1 is expressed in cortical thick ascending limb, connecting tubule, and cortical collecting duct. It was not detected in the proximal tubule, medullary thick ascending limb, distal convoluted tubule, and glomerulus. We conclude that KCNK1 is a unique, double-pore, mammalian K channel, distantly related to the yeast channel TOK1, that is expressed in distal tubule and is a candidate to participate in renal K homeostasis.

In contrast to the 2-TM and 6-TM classes, which have been well characterized in many species including mammals, virtually nothing is known about the 4-TM and 8-TM classes of K channels. Data from the Caenorhabditis elegans genome sequencing project suggest that the 4-TM class of K channels contains at least 23 different genes (9). These channels display the unusual structural feature of having two putative pore regions in contrast to all previously cloned K channels (2-TM and 6-TM), which only have one pore. The first member (TOK1) of the 8-TM class, recently identified in yeast, has two pore domains and encodes an outward-rectifying K channel that has an activation potential coupled to the reversal potential of K (3). A 4-TM K channel (ORK1) cloned from Drosophila also has two pores and mediates K currents with outward rectification dependent on external K concentration (1).

Here, we describe the cloning and nephron segment localization of a mammalian, 4-TM, double-pore K channel (KCNK1) and show that expression of KCNK1 in rabbit kidney is limited to the distal nephron.

MATERIALS AND METHODS

Cloning of hKCNK1. The amino acid sequences of each pore region of TOK1 (GenBank accession no. X77087) (pore 1, NALYFCTVSLTTLVGLDIL; pore 2, NCIYFCFLCLLLT1GYG-DYAP) were aligned using the BLAST algorithm to the sequences in GenBank translated into all six frames. One clone was identified, amplified by polymerase chain reaction (PCR), and used to screen a λZap human kidney cDNA library (Stratagene). A total of $1 \times 10^6$ clones were screened in duplicate at 42°C in buffer containing 0.5–10$^6$ cpm/ml of labeled probe, 50% formamide, 0.5 M Na$_2$PO$_4$, 1 mM EDTA, 7% sodium dodecyl sulfate (SDS), and 1% bovine serum albumin (BSA), pH 7.2. Filters were washed in 2× SSC (300 mM NaCl, 30 mM sodium citrate, pH 7) and 0.5% SDS at room temperature for 1 h and then in 0.2× SSC and 0.1% SDS at 42°C for 45 min. Twelve clones were isolated and characterized. Overlapping sequences of both strands were obtained by either subcloning the appropriate restriction fragments or by using sequence-specific oligonucleotides. Nucleotide and protein sequence analysis was carried out using the program MacVector (Oxford Molecular Group). The GenBank accession number for hKCNK1 is U90065.

Tissue distribution of hKCNK1 gene expression. Human multiple tissue Northern blots (MTN blot and MTN blot II, Clonetech) were tested using a $^{32}$P-labeled cDNA probe (bp
556–955) and following the manufacturer’s recommended protocol. The final wash was carried for 30 min at 60°C in a solution containing 0.1 × SSC and 0.1% SDS.

Nephron segment localization of rabKCNK1. A partial-length (780-bp excluding primer sequences) rabbit clone, rabKCNK1, was amplified from rabbit kidney cortex cDNA by PCR (denature initially at 94°C for 3 min, then denature 94°C for 1 min, anneal/extend at 65°C for 1 min, 40 cycles) using hKCNK1-specific primers. The PCR product was cloned into the EcoRV site of pBluescript and sequenced in both directions. The GenBank accession number for rabKCNK1 is AF004695.

The primers used for amplification of cDNA synthesized from nephron segments (sense, 5′ CGGGCCTCTGCTCTACCTGGTTATTCGGTG 3′; antisense, 5′ AGTTCCAGTATCCTCCAGGACAGAGA 3′) were first tested in preliminary experiments to confirm that they amplified rabKCNK1 cDNA template but not genomic DNA. Tubules segments (glomerulus, proximal tubule (S2 and S3), medullary thick ascending limb (MTAL), cortical thick ascending limb (CTAL), distal convoluted tubule (DCT), connecting tubule (CNT), cortical collecting duct (CCD), and outer medullary collecting duct (OMCD)] were hand dissected from adult New Zealand White rabbits using sharpened forceps. Tubes, all glassware, and instruments were rinsed with diethyl pyrocarbonate (DEPC)-treated water and autoclaved to reduce potential ribonuclease contamination. Each sample consisted of 2 mm of tubule adsorbed to 0.5-mm diameter glass beads and placed in 10 µl of lysis solution (2% Triton, 1 mM dithiothreitol, 2.2 U/µl RNasin, and DEPC-treated water). Each sample was snap frozen and stored at −70°C until needed.

Reverse transcription was carried out in the sample tube using an oligo(dT) primer and the reverse transcriptase enzyme Superscript II (BRL) in a final volume of 20 µl. The manufacturer’s recommended protocol was followed except for the addition of BSA at a final concentration of 0.5 µg/µl and the use of one-half of the recommended amount of enzyme. Amplification of rabKCNK1 cDNA was carried out using rabbit-specific primers and 5 µl of the cDNA. Samples were denatured at 94°C for 3 min followed by denaturation for 1 min at 94°C, annealing and extension for 1 min at 68°C (40–45 cycles). Every PCR experiment included a negative control that contained all the necessary components for PCR except template cDNA and a positive control that contained cDNA made from 17.5 ng of rabbit kidney cortex total RNA. The reaction products were separated by agarose gel electrophoresis, visualized by ethidium bromide staining, and then blotted onto GeneScreen (DuPont/New England Nuclear).

**Fig. 1.** Hydropathy analysis of hKCNK1 and amino acid comparison with the 4-TM K channel cloned from Drosophila (ORK1). A: potential TMs (TM1–TM4) were identified by analyzing (MacVector, IBI, Kodak) the deduced amino acid sequence using the Kyte-Doolittle algorithm with a window size of 15. P1 and P2, pore regions.

B: the computer program Align (DNAStar) was used to compare the amino acid sequence of hKCNK1 (337 amino acids) to the first 320 amino acids (from a total of 618) of ORK1 (GenBank accession no. U55321). Areas of perfect alignment lie within the boxed regions. Putative transmembrane segments (TM1– TM4) and pore regions (P1 and P2) are indicated above the alignment. Single letter amino acid code is as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; Y, Tyr.
Southern blotting was carried out using a rabKCNK1 probe to confirm the specificity of the amplification reaction and to also identify nephron segments that may contain amplified products that fall below the level of detection afforded by ethidium bromide staining.

RESULTS AND DISCUSSION

The amino acid sequences of each pore region of TOK1 were aligned using the BLAST algorithm to the sequences in GenBank translated into all six frames. A 471-bp human cDNA clone (HSC3AH031) containing an open-reading frame (ORF) encoding a protein with significant homology to the pore 1 of TOK1 was identified and used to probe a human kidney λZap cDNA.

Twelve clones were isolated and characterized. The longest one was 1,882 nucleotides long and contained an ORF of 1,187 bp (3–1190) and a 3'-untranslated region of 692 bp (1191–1882). It is likely to represent a full-length cDNA, since the message detected by Northern blot is 1.9-kb long (see Fig. 2). In addition, the first methionine (bp 183–185) encountered in the longest ORF is in a context (GAGAAGATGC) that is sufficient to support the initiation of translation (4). Hydropathy analysis of the deduced amino acid sequence indicates the presence of two pore regions, P1 and P2, each flanked by transmembrane segments (TM1–TM4) (Fig. 1A). P1 contains the signature sequence of K channels (glycine/tyrosine/glycine, GYG), whereas P2 has a glycine/leucine/glycine (GLG) motif. After consultation with the Human Gene Nomenclature Committee, the clone was named hKCNK1. Although amino acid identity between hKCNK1 and TOK1 is only 15% overall, it is 38 and 45% at P1 and P2, respectively. Human KCNK1 is more closely related to ORK1, a 4-TM, double-pore K channel recently cloned from Drosophila, which is thought to mediate leak current in neurons (1). Indeed, compared with TOK1, amino identity between hKCNK1 and ORK1 is higher overall (28%), at P1 (65%), and at P2 (52%) (Fig. 1B). The hKCNK1 sequence is identical to that of TWIK-1 (6), which when expressed in Xenopus oocytes, mediates the expression of a barium-sensitive, K-selective current. On the basis of the kinetic properties of the expressed current, and since TWIK-1 is expressed in a wide variety of tissues, it has been hypothesized that it mediates background K currents and maintains baseline membrane potential.

Although Northern analysis of human tissue using a hKCNK1-specific probe indicated wide expression of a 1.9-kb message, the highest levels were detected in brain, kidney, and heart (Fig. 2). To determine whether KCNK1 expression was limited to specific nephron segments, more precise localization was carried out...
using reverse transcription-PCR of dissected nephron segments as described in MATERIALS AND METHODS. A partial-length (780 bp) rabbit clone was amplified by PCR using hKCNK1-specific primers. Amino acid identity between rabbit and human was 96%. Rabbit-specific primers were then used to amplify rabKCNK1 message in dissected nephron segments. Specific message could only be detected in distal tubular segments including CTAL (4/6), CNT (6/6), CCD (6/6), and OMCD (2/2). Figure 3A depicts a representative experiment in which KCNK1 message was strongly amplified in CTAL, CNT, and CCD. We were unable to detect rabKCNK1 message in S2 (0/6), S3 (0/6), MTAL (0/6), and DCT (0/2). Although message was amplified in 1 of 6 glomeruli, we feel that this result should be interpreted cautiously, since it is possible that glomerular samples also contain other tubular segments such as macula densa, CTAL, and CNT. The preceding results, depicted in Fig. 3B, indicate that KCNK1 gene expression is limited to the distal nephron. We do not know whether the KCNK1 protein is located in the luminal or basolateral membrane because there are no specific antibodies available at the present time. However, since the channel appears to have a very high open probability, independent of voltage and ATP, it is possible that it may control baseline membrane potential and provide additional exit pathways for K recycling and/or K secretion.

In conclusion, KCNK1 is a 4-TM, double-pore, mammalian K channel that is distantly related to yeast TOK1 and to Drosophila ORK1. It is highly expressed in the rabbit distal nephron and may participate in renal K homeostasis.

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