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ION CHANNELS – MEMBRANE TRANSPORT – INTEGRATIVE PHYSIOLOGY

Segment-specific expression of 2P domain potassium channel genes in human nephron

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Segment-specific expression of 2P domain potassium channel genes in human nephron.

Background. The 2P domain potassium (K2P) channels are a recently discovered ion channel superfamily. Structurally, K2P channels are distinguished by the presence of two pore forming loops within one channel subunit. Functionally, they are characterized by their ability to pass potassium across the physiologic voltage range. Thus, K2P channels are also called open rectifier, background, or leak potassium channels. Patch clamp studies of renal tubules have described several open rectifier potassium channels that have as yet eluded molecular identification. We sought to determine the segment-specific expression of transcripts for the 14 known K2P channel genes in human nephron to identify potential correlates of native leak channels.

Methods. Human kidney samples were obtained from surgical cases and specific nephron segments were dissected. RNA was extracted and used as template for the generation of cDNA libraries. Real-time polymerase chain reaction (PCR) (TaqMan®) was used to analyze gene expression.

Results. We found significant ($P < 0.05$) expression of *K2P10* in glomerulus, *K2P5* in proximal tubule and *K2P1* in cortical thick ascending limb of Henle's loop (cTAL) and in distal nephron segments. In addition, we repeatedly detected message for several other K2P channels with less abundance, including *K2P3* and *K2P6* in glomerulus, *K2P10* in proximal tubule, *K2P5* in thick ascending limb of Henle's loop, and *K2P3*, *K2P5*, and *K2P13* in distal nephron segments.

Conclusion. K2P channels are expressed in specific segments of human kidney. These results provide a step toward assigning K2P channels to previously described native renal leaks.

Renal potassium channels contribute to several cellular functions: (*1*) regulation of contractility of mesangial and renal vascular cells in the glomerulus; (*2*) generation and maintenance of the negative cell potential; (*3*) regulation of tubular cell volume; (*4*) recycling of potassium across apical and basolateral membranes; and (*5*) secretion of potassium in distal nephron segments [1, 2]. Therefore, channel activity can affect glomerular filtration rate, facilitate sodium reabsorption and sodium-coupled transport, and maintain potassium homeostasis. Inhibition of proximal potassium channels is expected to decrease sodium reabsorption, resulting in a diuretic effect, while activation of distal potassium channels would increase potassium secretion and thus could help remedy hyperkalemia. Molecular identification of renal potassium channels would establish targets for pharmacologic intervention in these important processes. Insight gained from functional studies of isolated channels—such as their sensitivity to certain blockers and regulators—could be used to understand the individual contribution of each channel to kidney function by applying these agents in patch-clamp experiments of renal cells. Moreover, based on knowledge of the physiology of individual channels, their potential pathologic significance could be tested by assessing the encoding genes for mutations in patients with inherited disorders of kidney function.

2P domain potassium (K2P) channels are a recently discovered superfamily of potassium channels and little is known about their physiologic roles. A defining functional attribute of K2P channels is their ability to pass potassium at all voltages. This characteristic is also called open rectification or potassium leakage [3]. However, potassium leaks are not static, but highly regulated by a diverse array of stimuli, including O_2, pH, and phosphorylation [3]. Patch-clamp studies of renal tubules have described several open rectifier potassium channels that have eluded molecular identification [4–7].

To establish potential K2P channel candidates for these native leak conductances, we have screened human nephron segments for specific expression of the 14 known human K2P channel genes.

Key words: kidney, potassium channels, K2P channels, real-time PCR.

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METHODS

Tissue acquisition

Kidney tissue was obtained from five nephrectomy specimens at Yale-New Haven Hospital under a protocol

approved by the Yale Human Investigation Committee. Nephrectomies were performed because of renal masses. The tissue was obtained immediately after nephrectomy from the macroscopically normal appearing portions of the kidney at the discretion of the surgical pathologist. Segment-specific dissection was performed according to a protocol described by Schafer et al [8]. The dissection succeeded in three specimens (male, age 8; male, age 48; and female, age 51), but failed to yield identifiable segments in two specimens (female, age 72 and female, age 73). Tubule fragments totaling an estimated 30 mm in length or approximately 30 glomeruli were pooled into a single tube. Glomeruli and proximal tubule (S1 and S2), cortical thick ascending limb of Henle's loop (cTAL) and distal nephron (includes portions of distal convoluted tubule, connecting tubule, and cortical collecting duct) were collected and frozen at −80°C until needed.

RNA extraction and cDNA synthesis

Total RNA was extracted from frozen samples (Rnaqueous) (Ambion, Austin, TX, USA) as described [9] and treated with 10 units DNAse (DNAfree) (Ambion). The RNA was reverse transcribed to generate segment-specific cDNA libraries using oligo (dT) primer in a volume of 25 μL with a kit (Superscript II) (Invitrogen, Carlsbad, CA, USA). Separate cDNA libraries were constructed from each nephrectomy specimen.

Primers and fluorogenic probes

Gene expression was analyzed by real-time polymerase chain reaction (PCR) using the TaqMan® technique (Applied Biosystems, Foster City, CA, USA), which utilizes a gene-specific probe labeled with a reporter and a quencher dye, flanked by gene-specific primers. Upon amplification of the gene, exonuclease activity of the polymerase cleaves the probe, thus releasing the reporter dye and the resulting fluorescence is measured in real time. Nonspecific amplification of the primers does not result in fluorescence in this technique, as the dye is only released if the probe binding site is flanked by the primers [10]. Primers and probes for the 14 human K2P channels, the housekeeping enzymes glyceraldehye-3-phosphate dehydrogenase (GAPDH) and β-actin, and the control genes were designed with Primer Express software (Applied Biosystems) and are listed in Table 1. Specificity and sensitivity of all primer pairs was confirmed by agarose gel electrophoresis of PCR reactions performed in the presence or absence of template. Template consisted of genomic DNA for those amplicons not spanning an intron (see below) or of a vector containing the respective cDNA sequence. Probes were dual-labeled with FAM (6-carboxy-fluorescin) as the 5′-prime reporter dye and TAMRA (6-carboxy-tetramethyl-rhodamine) as the 3′-prime quencher dye (Applied Biosystems).

The genomic organization of several K2P channels (*K2P3, K2P7, K2P12, K2P13*, and *K2P15*) includes only one intron, located in the "signature sequence" of potassium channels in the first P loop [11]. Gene-specific amplicons of the length desired for TaqMan® PCR (ideally 60 to 100 bp) could not be developed in this highly homologous region, which encodes the selectivity filter in the pore-forming domain and identifies all potassium channels [12]. Therefore, amplicons for these channels do not span an intron. To assess for any residual contamination of the cDNA libraries with genomic DNA even after DNAse treatment of the extracted RNA, an amplicon located in an intronic sequence of *Troponin I* (*TNNI3)* was used.

Six nephron segment-specific control genes were used to verify the specificity of our libraries, namely the sodium-phosphate exchanger NPT2 (*SLC34A1*, proximal tubule [13, 14]); the bumetanide-sensitive cotransporter NKCC2 (*SLC12A1*, TAL [15, 16]), the thiazide-sensitive cotransporter TSCC (*SLC12A3,* distal tubule [16]); the epithelial sodium channel subunits β- and γ-ENaC (*SCNN1B* and *SCNN1G,* distal tubule [9]); and the inositol polyphosphate 5-phosphatase (*OCRL*, pan-expressed [17]).

TaqMan® PCR

TaqMan® PCR assays were performed in triplicate for each target gene on cDNA samples or DNA standards in 96-well optical plates on a Bio-Rad optical cycler (Bio-Rad, Hercules, CA, USA). Thus, a total of nine reactions was performed for each gene in a given nephron segment (triplicate reactions in three specimens). In addition, three negative controls per gene per specimen were used to assess for contamination of the reagents. For each 25 μL reaction, 1 μL of template (containing either the defined copy number for the standard curves or 0.23 μL of the segment-specific cDNA libraries or H_2O for the negative controls) was mixed with 12.5 μL TaqMan® Universal PCR Master Mix (Applied Biosystems), 9.5 μL H_2O, 0.5 μL each of sense and antisense primer (10 μmol/L), and 1 μL of TaqMan® probe (2.5 μmol/L). PCR parameters were 50°C for 2 minutes, 95°C for 10 minutes, 50 cycles of 95°C for 30 seconds, and 58°C for 1 minute. Negative controls with no added DNA were performed along with the determination of each gene. Standard curves were generated using serial dilutions of known quantities of genomic DNA or cDNA (5000, 500, 50, 5, or 0 copies per reaction) (see Fig. 1) as described by Medhurst et al [18].

Data analysis

All TaqMan® PCR data were captured using iCycler® software (Bio-Rad). For every sample, an amplification plot was generated and the threshold cycle was

Table 1. Primers and probes

Gene	Accession #	Primer	Sequence
KCNK1	NM002245	S	GTGGCCACCCAGTCATCTG
		A	CAACAAATCCTACGCTCAATGG
		P	CTGCGTGGATGGCCCTGCAA
KCNK2	NM014217	S	CAACGTCACAGCCGAATTCA
		A	GGCCCGCTGGAACTTGT
		P	AGAAACCAGGAGGCGACTGAGTGTGGA
KCNK3	NM002246	S	TCGCACTGGAGGTTCAAGCT
		A	GTGTCTGGAAGGCTGAAGTCCTA
		P	ACTGGCCTCCAGCCACATTCTCATAGC
KCNK4	NM016611	S	CGCTGGTGTGGTTCTGGAT
		A	GGACACTACTCGCAGCCAGTT
		P	TGGCTTACTTCGCCTCAGTGCTCACC
KCNK5	NM003740	S	AGCTCTCTGTGCCTTACGAACAG
		A	CCTGCCTCATGTGCCCTT
		P	TGATGAATGAGTACAACAAGGCTAACAGCCC
KCNK6	NM004823	S	GCCTCTGTCTTTCCCTCTTGCT
		A	GCAGTTGACGAGTGGTTAATTGAG
		P	CTGTTTCTCATTCTCTTTCATGTTCCGTCTGTGT
KCNK7	NM033347	S	CCTTCTCCTCCCCAGGTTACTT
		A	GACCTGCGGCAGCTCAGA
		P	TTCTAGGACTCTTGGCCATGCTGCTGG
KCNK9	NM016601	S	CCCATCGCCTATTAGCTCCAT
		A	GACTTCCGGCGTTTCATCA
		P	TCTCCTGGGTTACACAGCTTTACCGACCA
KCNK10	NM021161	S	TTTCACTGTGCCATAAACAACTGA
		A	CGTTTGCTATCTGAAATGAAGTTCTT
		P	AGCTTGCTCTGCCAAAAGGAATCAGAGAA
KCNK12	NM022055	S	CTACTTCTGCTTCGTCACCTTCAG
		A	CCTGCTTGATGAGGATGGAGAT
		P	ACCTGGTGAGCAGCCAGCACGC
KCNK13	NM022054	S	ACGCAGGAACGTGGTGATG
		A	CACCCCGTCTGTCTCTATGGA
		P	CGTCCGGAACCGCTGCAACA
KCNK15	NM022358	S	CTGCAGAGGAAGCTCCCCT
		A	AATGACCGTGAGCCCCAG
		P	CGTGGCCTTCAGCTTCCTCTACATCCT
KCNK16	NM032115	S	CAGGTCAGGTCTTCTGTGTCTTCTAT
		A	CCAGGTGGTTGAGGAAGATCA
		P	CCCTGTTGGGCATCCCGCTTAAC
KCNK17	NM031460	S	TGACATGGGATGTGACTTTCG
		A	CATTGCAGCCCGCTAAAGTAA
		P	CAGCATGCCCTTCTCCCCCACTTC
GAPDH	NM002406	S	CCCACTCCTCCACCTTTGAC
		A	CATACCAGGAAATGAGCTTGACAA
		P	CTGGCATTGCCCTCAACGACCA
β-*Actin*	NM001101	S	TCCACCTTCCAGCAGATGTG
		A	GCATTTGCGGTGGACGAT
		P	CGGACTCGTCATACTCCTGCTTGCTGA
SLC34A1(NPT2)	NM003052	S	CATGACTAGGATAGGCAGGAGTAAGG
		A	GTAACGCAGGCACCAACATCT
		P	CTGGGTATATGACTGTGCAGCTGTTTGTGC
SLC12A1(NKCC2)	NM000338	S	TCCGTGGAAAATTACAGATGCA
		A	TCCTGTAAGAGTTCATTCAGTCGAA
		P	CTGGAAGCAGTCAAGGAAAAGAGTTACCGC
SLC12A3(TSCC)	NM000339	S	ATGATGCAGGCGCACATTAA
		A	CTGGCGCTGGCTTCCTT
		P	CCGTGTTTGACCCAGCGGAGGAC
SCNN1B(β-ENaC)	NM000336	S	CTCCAGGCCAGAGCTTGTGT
		A	GAGCCCTTGGCCAGTAACG
		P	TCAACAGAGAGGCCAGCGGCAAC
SCNN1G(γ-ENaC)	NM001039	S	TCGGGTACTACGCAGCAACA
		A	CCAGTCTCTAGTGACGTGCTAATAGTTC
		P	ACAACTGTCCAGGCTGAGATAAATCCCGG
OCRL	NM000276	S	CCAACCTTATGGCAAGACAGACT
		A	CTTCGCTCCCAAGCAGAAAG
		P	AGTGACCGCCAGCGTGCTATTCAGTTC

Abbreviations are: S, sense; A, antisense; P, probe.

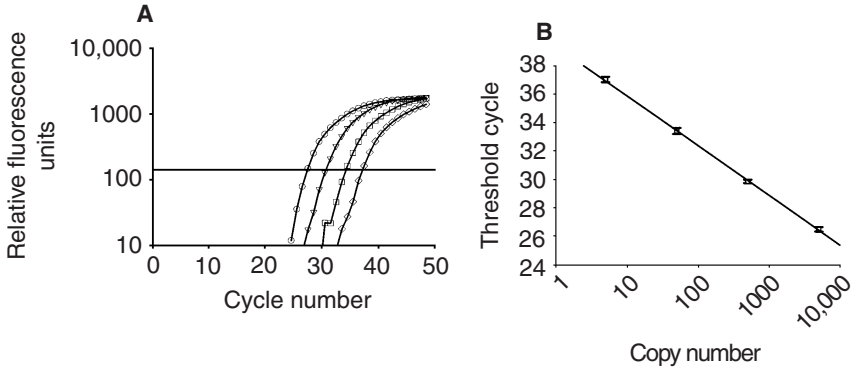

Fig. 1. Establishing standard curves. (*A*) Plotting of measured fluorescence versus cycle number for a target gene, here *K2P1*, with 5000 (○), 500 (△), 50 (□), and 5 (◇) copies of genomic DNA. A negative control with 0 gene copies resulted in no fluorescence. Horizontal line indicates threshold fluorescence, which was arbitrarily defined as ten times the standard deviation of fluorescence during the first 16 cycles. (*B*) Semilogarithmic plot of threshold cycle versus copy number. Squares represent the mean for three measurements of 5, 50, 500, and 5000 gene copies and error bars indicate standard deviation. Line represents linear fit of the data with the formula $y = A - B^*x$, with A representing the threshold cycle for the detection of a single gene copy and calculated here at 38.27. B represents the number of cycles necessary for a tenfold increase in fluorescence and is calculated here at 3.34, which is in good agreement with the theoretical value of 3.32, assuming a doubling of polymerase chain reaction (PCR) product with each cycle.

calculated. Threshold value was arbitrarily defined as ten times the standard deviation of the background fluorescence during the first 16 cycles. Copy numbers of target genes in the sample were calculated from the standard curves, rounded to the closest integer value, expressed in relation to the copy number of *GAPDH* and averaged for each specimen.

Statistics

Significance was calculated using one-tailed one-sample Student *t* test against a hypothetical mean of zero. For calculations of the binomial p-parameter and its 95% exact confidence interval, the quantitative measurements were transformed into binomial data, so that the genes were either detected (calculated copy number ≥ 1 by TaqMan® PCR) or absent. The calculations were made using SAS version 8.02 software (SAS Institute, Inc., Cary, NC, USA).

RESULTS

Libraries are specific

The results of quantitative PCR analysis for individual nephron segments of the three kidneys are shown in Table 2 and Figure 2. The expression profile of the control genes is concordant with their known localization, indicating that the libraries are specific for the respective nephron segments. Thus, *SLC34A1* is exclusively expressed in proximal tubule, *SLC12A1* has high expression in cTAL, and *SLC12A3, SCNN1B*, and *SCNN1G* are in distal nephron. *OCRL* was detected with low copy numbers in all segments, except proximal tubule, probably due to inferior tissue integrity of proximal tubule segments (see below). There was one detection of a single copy of *SCNN1B* in cTAL and three detections of *SLC12A1* in distal nephron with a mean copy number almost 40 times lower than in cTAL, likely reflecting marginal cross-contamination of the cTAL and distal nephron libraries. No copies of *TNNI3* were detected in any of the 36 PCR reactions, indicating the absence of genomic DNA in the libraries.

K2P channels are expressed in specific segments of human kidney

Our results indicate significant ($P < 0.05$) abundance of message for three K2P channels in specific segments of human nephron (Fig. 2): (*1*) glomerulus, in which there is expression of *K2P10*; (*2*) proximal tubule, in which there is expression of *K2P5*; and (*3*) cTAL and distal nephron, which show expression of *K2P1*.

We also repeatedly detected message for other K2P channels, but these failed to reach significance due to higher variability. Because this variability was not in keeping with the assumptions for parametric tests, we transformed the quantitative measurements into binomial data to calculate the likelihood of detecting a particular gene in a given segment (see **Methods** section). The binomial p-parameter describes this likelihood with a value between 0 (absent) and 1 (present). We arbitrarily defined a value for the binomial p-parameter of greater than 0.4 (95% exact CI 0.14 to 0.79) as indicative for the presence of a particular gene in a given segment, which corresponds to detection of the gene in at least four out of the nine determinations. Based on a binomial p-parameter greater than 0.4, the following K2P genes are present: (*1*) there is expression of *K2P10, K2P3*, and *K2P6* in the glomerulus; (*2*) there is expression of *K2P5* and *K2P10* in the proximal tubule; (*3*) there is expression

Table 2. Detection of 2P domain potassium (K2P) and control genes in human nephron

	Glomerulus				Proximal tubule				Cortical thick ascending limb of Henle				Distal nephron			
	Total positive (out of nine)	Copy number Mean (SD) Kidney 1	Copy number Mean (SD) Kidney 2	Copy number Mean (SD) Kidney 3	Total positive (out of nine)	Copy number Mean (SD) Kidney 1	Copy number Mean (SD) Kidney 2	Copy number Mean (SD) Kidney 3	Total positive (out of nine)	Copy number Mean (SD) Kidney 1	Copy number Mean (SD) Kidney 2	Copy number Mean (SD) Kidney 3	Total positive (out of nine)	Copy number Mean (SD) Kidney 1	Copy number Mean (SD) Kidney 2	Copy number Mean (SD) Kidney 3
K2P1	0	0	0	0	0	0	0	0	9	4.0 (3.0)	10.3 (7.2)	1.7 (0.6)	8	4.0 (2.6)	13.7 (13.4)	3.7 (3.2)
K2P2	1	0	0.3 (0.6)	0	2	0.3 (0.6)	0.3 (0.6)	0	2	0.3 (0.6)	0	0.3 (0.6)	0	0	0	0
K2P3	5	5.0 (4.4)	0.3 (0.6)	0.3 (0.6)	0	0	0	0	1	0	0	0.3 (0.6)	4	2.7 (4.6)	2.0 (1.0)	0
K2P4	0	0	0	0	0	0	0	0	1	0.3 (0.6)	0	0	2	0	0.7 (0.6)	0
K2P5	3	0.7 (0.6)	0.7 (1.2)	0	7	3.3 (4.0)	11.3 (4.9)	0.7 (1.2)	4	0	4.3 (1.2)	0.7 (1.2)	4	2.3 (4.0)	29.0 (34.4)	0.3 (0.6)
K2P6	4	6.7 (9.1)	1.7 (1.5)	0	0	0	0	0	0	0	0	0	1	1.3 (2.3)	0	0
K2P7	0	0	0	0	0	0	0	0	0	0	0	0	1	0.3 (0.6)	0	0
K2P9	0	0	0	0	1	0.3 (0.6)	0	0	2	0.3 (0.6)	0	0.3 (0.6)	1	0.3 (0.6)	0	0
K2P10	6	0.7 (0.6)	3.3 (2.1)	0.3 (0.6)	4	4.0 (3.5)	1.0 (1.7)	0	1	0.7 (1.2)	0	0	1	0	0.7 (1.2)	0
K2P12	0	0	0	0	0	0	0	0	1	0.3 (0.6)	0	0	0	0	0	0
K2P13	1	0	0.3 (0.6)	0	2	0.3 (0.6)	0.3 (0.6)	0	2	0	0.3 (0.6)	0.3 (0.6)	4	0.3 (0.6)	11.3 (6.1)	0
K2P15	0	0	0	0	0	0	0	0	0	0	0	0	1	0	3.7 (4.0)	0
K2P16	2	0.3 (0.6)	0.3 (0.6)	0	1	0.3 (0.6)	0	0	1	0	0	0.3 (0.6)	1	0	1.0 (1.7)	0
K2P17	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0.7 (0.6)	0
OCRL	6	0.3 (0.6)	0.7 (0.6)	1.0 (0.0)	1	0	0.3 (0.6)	0	4	1.0 (1.7)	0.7 (0.6)	0.3 (0.6)	4	0	5.7 (5.5)	0.7 (0.6)
SLC34A1	0	0	0	0	9	9.3 (8.5)	10.7 (6.0)	1.0 (0.0)	0	0	0	0	0	0	0	0
SLC12A1	0	0	0	0	0	0	0	0	9	149 (31)	507 (353)	120 (25)	3	0	17.7 (14.0)	0.3 (0.6)
SLC12A3	0	0	0	0	0	0	0	0	0	0	0	0	9	2227 (1451)	37.3 (34.3)	4.7 (7.2)
SCNN1B	0	0	0	0	0	0	0	0	1	0	0	0.3 (0.6)	9	3.3 (0.6)	77.0 (10.8)	15.3 (6.9)
SCNN1G	0	0	0	0	0	0	0	0	0	0	0	0	7	0.3 (0.6)	21.3 (17.6)	7.3 (3.0)
β-*actin*	9	139 (87)	247 (153)	28 (4.0)	6	1.7 (1.2)	7.0 (5.2)	0	9	45 (45)	98 (71)	21 (5.3)	9	48 (38)	474 (225)	163 (78)
GAPDH	9	223 (271)	247 (153)	15.3 (3.2)	9	37 (38.3)	84.3 (64.7)	1.3 (0.6)	9	210 (227)	546 (498)	21 (7.2)	9	339 (234)	1042 (277)	88 (40.1)
TNNI3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

The following are listed for each gene in each nephron segment: the total number of positive assays (copy number ≥1); mean and standard deviation (SD) of copy numbers in each of the three specimens.

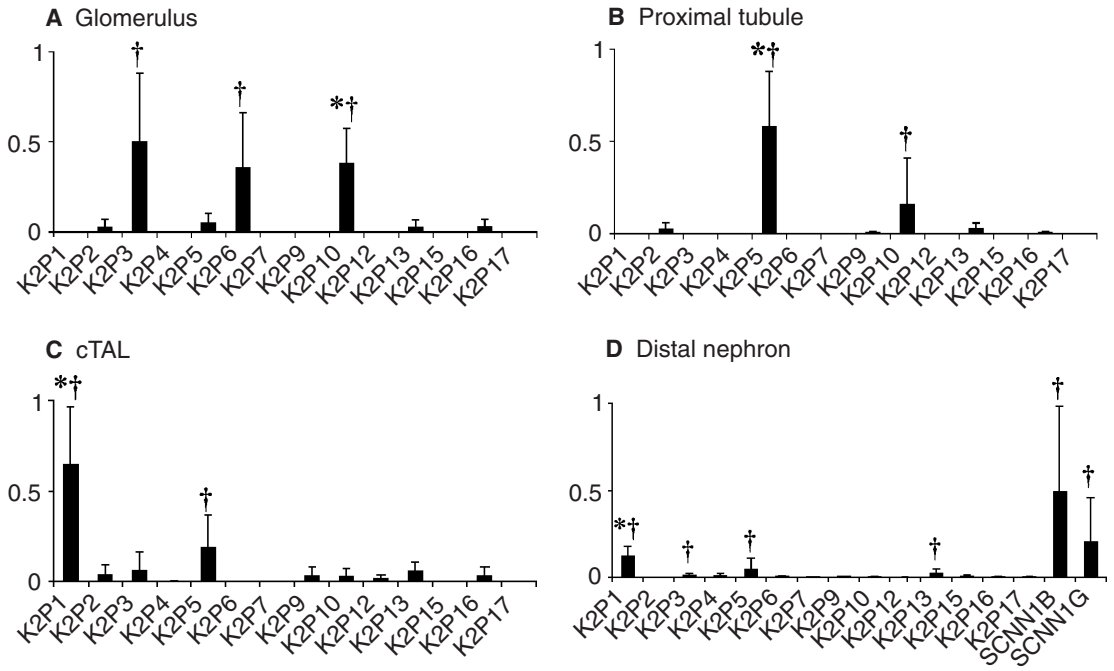

Fig. 2. Expression of K2P channel transcripts in human nephron. (*A*) Glomerulus. (*B*) Proximal tubule. (*C*) Cortical thick ascending limb of Henle. (*D*) Distal nephron. Data are expressed as arbitrary units relative to glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) and are mean ± SD for the indicated segments of the three nephrectomy specimens. *Significant expression ($P < 0.05$); †expression of the gene based on a value for the binomial p-parameter > 0.4.

of *K2P1* and *K2P5* in cTAL; and (*4*) there is expression of *K2P1, K2P3, K2P5*, and *K2P13* in the distal nephron.

Challenges with human kidneys

Compared to our experience with dissection of rabbit kidneys [9, 17, 19], human kidneys were more fibrotic and required a longer collagenase digestion time for segment isolation (60 to 120 minutes). Furthermore, the released segments were short and it was not possible to reliably separate samples from S1, S2, and S3 sections of proximal tubule, nor from distal convoluted tubule, connecting tubule, and cortical collecting duct. Thus, respective fragments were pooled and labeled as proximal tubule or distal nephron. As expected, the degree of fibrosis appeared to correlate with the age of the tissue donor [20]. We were unable to collect sufficient high-quality tubule segments from kidneys from the two oldest donors due to the high degree of fibrosis. Since most nephrectomies are performed on older patients, suitable specimens can be difficult to obtain. The total detected message was low compared to animal kidneys, possibly because of RNA degradation during the processing of the organs by the surgical pathologist and the prolonged collagenase digestion. Signals of both target and control genes were particularly low in the proximal tubule libraries, likely as a result of inferior tissue integrity of proximal tubule during the collagenase treatment. This is not surprising, as proximal tubule is the nephron segment most sensitive to ischemia [21].

DISCUSSION

Renal K2P expression: Comparison with existing literature

Our results establish the expression of several 2P domain potassium channel genes in specific segments of human nephron and are in general agreement with the limited data previously available on renal K2P expression. Segment-specific evaluation of *K2P1* expression in rabbit kidney demonstrated exclusive expression in cTAL and distal nephron [19]. Conversely, analysis of K2P1 protein in rat kidney by immunohistochemistry found a predominant presence in proximal tubule and distal nephron, indicating either species differences or discordance between transcript and protein expression [22]. Here, we find that *K2P1* expression is limited to cTAL and distal segments of human nephron, as seen in rabbit kidney. A tissue distribution analysis of *K2P5* indicated strong expression in human kidney [23] and an immunohistochemical analysis in rat kidney detected the presence of K2P5 throughout the nephron, but at highest levels in proximal tubule [24]. In a genetically modified mouse, with the *K2P5* gene replaced by a gene encoding β-galactosidase, strong blue staining after 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-gal) reaction was

observed in proximal tubule, giving indirect evidence for K2P5 expression in this segment [25]. These data agree well with our finding of significant expression in proximal tubule and presence also in glomerulus, cTAL, and distal segments. *K2P10* has been shown previously to be expressed in human kidney [23] and transcripts were localized exclusively to proximal tubule [26]. Our data agree with the presence in proximal tubule but additionally indicate significant expression in glomerulus. It is unlikely that this finding resulted from contamination of the pooled glomeruli with attached proximal tubule during dissection, since our marker gene for proximal tubule, *SLC34A1*, was detected in 0 of the nine determinations for glomerulus. Presumably, the additional expression in glomerulus can be explained by detection of splice variants. The primers used by Gu et al [26], located at the 5′-end of the gene, detect only a specific isoform of *K2P10* (*K2P10b*, NM138318), while our amplicon is located in exon 7, common to the three known K2P10 isoforms.

We further found expression of three other K2P channels in human nephron: *K2P3* in glomerulus and distal nephron, *K2P6* in glomerulus, and *K2P13* in distal nephron. All three genes were previously shown to be present in human kidney but without localization to specific nephron segments [27–31].

Levels and variability of expression

The amount of message found for K2P channels expressed in the kidney was comparable to that observed for the genes encoding β- and γ-epithelial sodium channel (ENaC) subunits, *SCNN1B* and *SCNN1G* (Fig. 2D) (Table 2). Since the ENaC channel has proven relevance in renal physiology, the low number of channel transcripts detected in this study may reflect the efficiency of ion channels, which can pass more than 10^6 ions per second while transporters move ions 1000- to 10,000-fold less rapidly. The detected number of copies of each channel gene was variable, both between the three measurements for each specimen and between the mean copy numbers of the three specimens. It is unlikely that inconsistencies in the TaqMan® assay itself produced this variability, as repeated determinations of the defined number of gene copies for the establishment of standard curves were remarkably reliable (Fig. 1). Rather, the low number of gene copies may explain the variability. While an equal distribution of the entire DNA in each library can be assumed, this is not necessarily the case for the few copies of individual channel genes. In addition, physiologic differences between the donors could contribute to the observed variability. For example, expression of the ENaC channel subunits is highly regulated, depending on variables such as volume status [32]. Indeed, neither *SCNN1B* nor *SCNN1G* reached statistical significance by Student *t* test despite their prominent expression. It is likely that K2P gene expression is also subject to regulation and therefore the detection of K2P transcripts would not follow a Gaussian distribution. To account for this possibility, we calculated the likelihood of expression (the binomial p-parameter) along with an exact 95% CI [33].

Potential physiologic roles of renal K2P channels

Potassium channels have been observed throughout the nephron. In the glomerulus, they are believed to be the final target in regulation of mesangial cell contraction by setting the resting membrane potential [34]. Because of the inherent difficulties of patch-clamping of glomeruli, most data come from the study of cultured mesangial cells. In that model, the major potassium conductance appears to be a large-conductance, calcium-activated channel [35], but other potassium channels have been observed, including a 65-pS channel sensitive to adenosine triphosphate (ATP) and a 40-pS and a 9-pS channel activated by calcium [36, 37]. Our data suggest K2P10, K2P3, and K2P6 as possible candidates for these native channels. While regulation by ATP and calcium are not reported characteristics of K2P channels, a direct comparison of the biophysical properties of native channels with those of cloned K2P channel is problematic. First, cultured mesangial cells may differ in channel expression from cells in vivo. Second, there can be species differences in gene expression as well as in channel properties, and in vivo studies of ion channels are necessarily restricted to animal models. Third, channels can associate with other subunits, altering the phenotype and thus further complicating their identification [38]. And finally, because of the recent discovery of the K2P channels, limited and sometimes contradictory reports exist about their biophysical properties. For instance, the reported single channel conductance of K2P10 varies between 68 and 170 pS under similar conditions [26, 39, 40]. Clearly, a detailed analysis of channel characteristics and location is necessary before identification can be attempted.

Potassium leak channels have been observed in proximal tubule. Some are sensitive to pH or membrane stretch [6, 7]. Based on our results, K2P5 and K2P10 may constitute these channels, which are involved in establishing membrane potential and thus also in sodium reabsorption. K2P5 is known to be sensitive to pH and K2P10 is responsive to membrane stretch [41, 42]. Indeed, the absence of a swelling-induced, pH-sensitive potassium conductance was reported in cultured proximal tubule cells from K2P5 knockout mice, suggesting a role of K2P5 in this specific function [25].

Impairing apical potassium recycling in TAL with loss-of-function mutations in *KCNJ1* (ROMK) leads to inhibition of salt absorption via NKCC2 and the clinical features of Bartter syndrome [43]. The distribution of K2P1

along the nephron is comparable to ROMK. A 70-pS open rectifier has been described in the apical membrane of TAL, accounting for the majority of the potassium conductance [4, 44]. These studies implicate K2P1 as a potential candidate for this yet unidentified open rectifier. However, studies to compare the biophysical characteristics will need to be deferred until a reliable method for functional expression of K2P1 currents exists [3].

Potassium leak channels have also been observed on the basolateral aspect of principal cells in cortical collecting duct, where they are involved in setting membrane potential and thus enable sodium reuptake via ENaC [5, 45]. These channels are also thought to participate in potassium excretion when kaliuresis is maximally stimulated [46]. Our results suggest that K2P1, K2P3, K2P5, and K2P13 may constitute these yet unidentified potassium leaks.

CONCLUSION

We have defined the segment-specific expression of K2P channel genes in human nephron and identified several candidates for matching potassium channels observed in vivo. Immunohistochemical analysis is now needed to further refine the location, such as vascular or mesangial cell expression for the channels in the glomerulus, and apical versus basolateral expression for those in the tubule. Once localization is clearly defined, attempts can be made to correlate the biophysical characteristics of renal K2P channels with those of native conductances in the respective location. K2P channels with confirmed identity may offer targets for the regulation of glomerular filtration rate, sodium reabsorption, and potassium homeostasis.

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