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Immunolocalization of the TASK2 Potassium Channel in Frog Kidney*

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TASK2 ($K_{2P}5.1$, KCNK5) is a two-pore domain K^+ channel belonging to the TALK subgroup of the K_{2P} family of proteins. TASK2 expression has been reported in a variety of cells and tissues ranging from kidney to immune cells and including specific neurons, its proposed functions spanning from involvement in the regulation of cell volume to control of excitability. The purpose of this study was to determine the tubule location of the TASK2 K^+ channel protein in frog kidney applying polyclonal antibody against the carboxyl terminus of human TASK2 (KCNK5) protein. Immunohistochemical analysis revealed that TASK2 is expressed on distal tubules and proximal epithelial cells. TASK2 is strongly expressed predominantly on the luminal part of the proximal epithelial cells and slightly cytoplasmatic staining is expressed. Distal tubules showed diffuse cytoplasmatic staining as well as slight staining on the apical parts of the cells. These findings suggest that the TASK2 K^+ channel has cell-specific roles in renal potassium ion transport.

Key words: TASK2 potassium channel, frog, kidney, immunohistochemistry.

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K^+ channels are located in both apical and/or basolateral membranes of epithelial cells and occur in a wide range of structures of various organs including kidney tubules, respiratory airways, small and large intestine, gall bladder, sweat duct, pancreatic duct, and epididymis of the male and fallopian tubes of the female reproductive tracts (HAMILTON & DEVOR 2012). TASK2 ($K_{2P}5.1$, KCNK5) is a two-pore domain K^+ channel that belongs to the TALK subgroup of the K_{2P} family of proteins (CID *et al.* 2013). K_{2P} channels are generally classified as background or "leak" K channels and exist as dimers, each subunit comprising four transmembrane domains (TMD_s) and two pore-forming domains (LESAGE & LAZDUNSKI 2000). TASK2 expression has been reported in a variety of cells and tissues ranging from kidney to immune cells and including specific neurons, its proposed functions spanning from an involvement in the regulation of cell volume to control of excitability (CID *et al.* 2013). TASK2 channels have been shown to be involved in regulatory volume decrease (RVD) of Ehrlich cells (NIEMEYER *et al.* 2001), a classical model used in cell volume regulation field (HOFFMANN & PEDERSEN 2011). In these cells, a K^+ channel insensitive to Ca^{2+} but activated by osmotic cell swelling and termed $I_{K,vol}$ is a functional correlate of TASK2 (NIEMEYER *et al.* 2000). TASK2 expressed in HEK-293 cells responds to changes in cell volume and enhances the RVD response (NIEMEYER *et al.* 2001). The involvement of native TASK2 in RVD has also been shown in mouse renal proximal tubule cells using TASK2 KO mice (BARRIERE *et al.* 2003) and TASK2 channels have also been implicated in apoptotic volume decrease (AVD) that precedes cell death (L'HOSTE *et al.* 2007). Other cells where TASK2 has been proposed to play a role in RVD

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are spermatozoa (BARFIELD *et al.* 2005), retinal Müller glial cells (SKATCHKOV *et al.* 2006) and T-lymphocytes (BOBAK *et al.* 2011). Experiments using TASK2 K^+ channel null mice have shown a central role for TASK2 in the proximal tubule bicarbonate reclaim process (WARTH *et al.* 2004), as well as in the signaling in central CO_2 and O_2 sensitive neurons in control of breathing (GESTREAU *et al.* 2010).

The purpose of this study was to determine the tubule location of the TASK2 K^+ channel protein in frog kidney applying polyclonal antibody against the carboxyl terminus of human TASK2 (KCNK5) protein.

Material and Methods

The research protocol was approved by the local ethics committee of the Medical School of the University in Belgrade and the Ministry of Education and Science of Republic of Serbia (353-01-2508/2010-03) and fulfils the standards of good laboratory practice, recommended by the Helsinki Convention.

Rana esculenta (<50g) of both sexes (adult frogs) were kept in aquariums at 4°C. After anaesthesia with tricaine methanesulfonate (1.5g/l adjusted to pH 7.0), the kidneys from five frogs were carefully removed from animals and placed in 10 ml Ringer solution. Amphibian Ringer solution of the following composition (in mmol/l) was used: NaCl 90, KCl 2.5, $NaHCO_3$ 10, NaH_2PO_4 0.5, $CaCl_2$ 1.8, $MgCl_2$ 1, glucose 2.2, dextran (mol. wt. 80000) 15 g/l and heprin 2000 U/l. The Ringer solution was equilibrated with 1% CO_2 - 99% O_2 to pH 7.6. The kidneys were doubly perfused with aortic and portal vein perfusions, maintained using hydrostatic pressure (50 cm H_2O for aortic perfusion, 30 cm H_2O for portal vein perfusion). After that, the doubly perfused kidneys were dissected. The kidneys were harvested and kept in 4% paraformaldehyde solution at room temperature, at which point they were embedded in paraffin, using the Leica ASP3000 automated paraffin wax tissue processor. Five-micrometer sections were cut and blocked with 0.05 M Tris-EDTA, pH 9.0 for 20 min in a microwave oven. Then, sections were incubated with TASK2 (KCNK5) antibody (catalogue no. sc - 28632; Santa Cruz Biotechnology) at 1:50 dilution dissolved in PBS (Phosphate Buffered Saline, pH 7.4, 50 mM NaH_2PO_4, 150 mM NaCl, 0.1% Tween 20 and 3% BSA) for 60 minutes. TASK2 (H-170) is a rabbit polyclonal antibody raised agains amino-acids 330-499 mapping at the C terminus of the TASK2 of human origin. We used this antibody on frog renal tissues since, according to the NCBI database (http://www.ncbi.nlm.nih.gov/gene/), the TASK2 protein sequence in Homo sapiens (Gene ID 8645) and TASK2 protein sequence in Xenopus laevis (Gene ID 496362) have equally conserved domains, and they match in 73.4 % .

Localization of TASK2 was accomplished using immunoperoxidase procedures as we have done previously (MŰLLER *et al.* 2002). Universal Dako ChemMete™ EnVision kit (catalogue no. K5007, Dako Corporation, CA93013 USA) and 3-amino-9-ethylcarbazole (AEC, catalogue no. K 3469, Ready-to-use, Dako Corporation, CA93013 USA), were used for detection and visualization of TASK2 expression. Afterwards sections were analyzed on a Nikon Coolscope microscope. Controls in immunostaining procedure were obtained by replacement of the primary antibody with PBS or by blocking antibodies (sc-10646 P) Santa Cruz.

Results

In the present study we analyzed TASK2 potassium channel expression in frog kidney by immunohistochemical analysis. The pattern of immunoreactivity was consistent on all slides (Fig. 1). Observation by light microscopy under low magnification (Fig. 1A) revealed the expression of the TASK2 K^+ channel on distal tubules and proximal epithelial cells. At higher magnification it was clear that TASK2 is strongly expressed predominantly on the luminal part of the proximal epithelial cells and slightly cytoplasmatic staining is also expressed (Fig. 1B). In addition, distal tubular cells, recognized by their typical morphology including flattened epithelium and basically localized nuclei, expressed diffuse cytoplasmatic staining as well as slight staining on the apical parts of the cells (Fig. 1C).

Labelling was not observed in sections from frog kidney when the primary antibody was omitted or blocking antibody was applied (Fig. 1D).

Discussion

In this study we clearly show that the TASK2 K^+ channel is expressed on distal tubules and proximal epithelial cells. However, the pattern of expression of the TASK2 K^+ channel differs between distal and proximal tubules. TASK2 is strongly expressed predominantly on the luminal part of the proximal epithelial cells; slightly cytoplasmatic staining is also expressed. Distal tubules expressed diffuse cytoplasmatic staining as well as slight staining on the apical parts of the cells. These findings suggest that the TASK2 K^+ channel has cell-specific roles in renal potassium ion transport.

Theoretically, any K^+ channel that retains activity around the resting membrane potential can con-

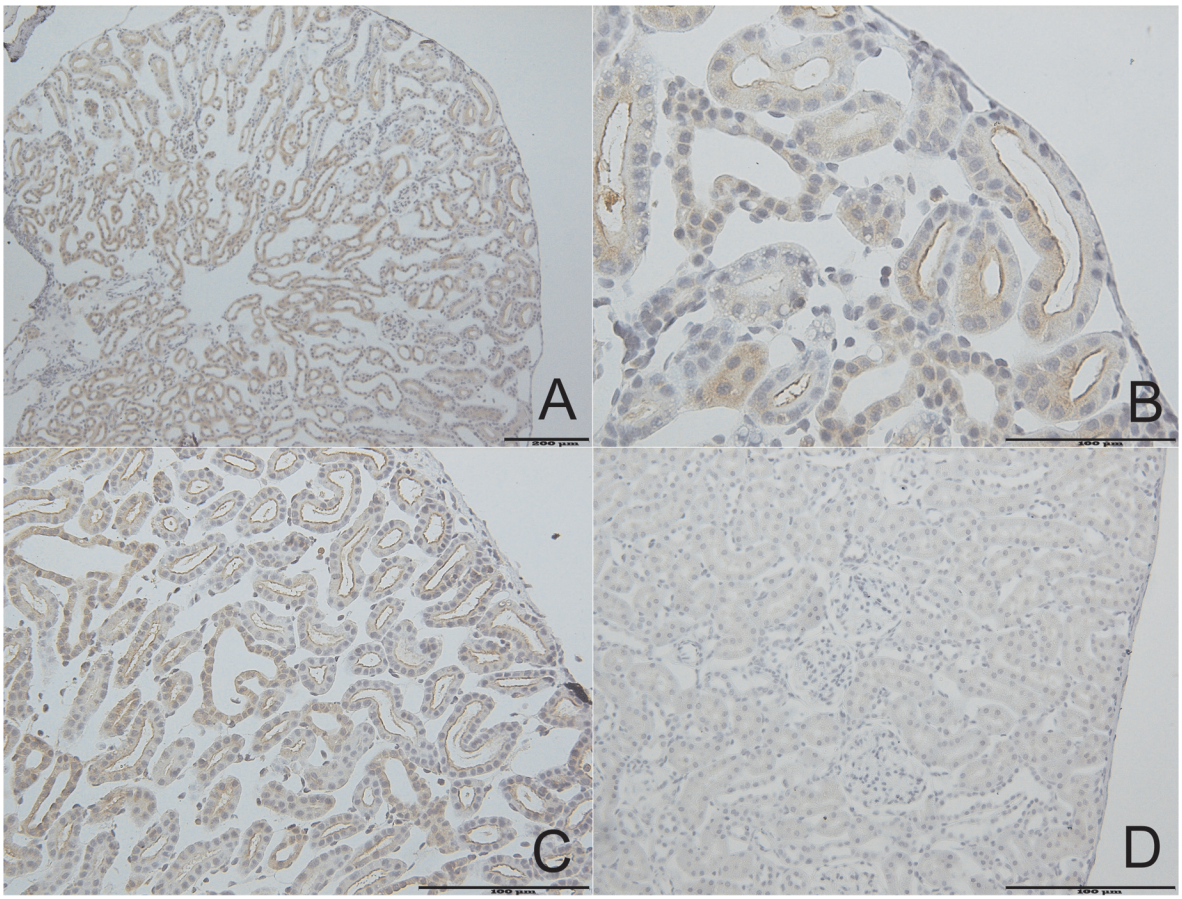

Fig. 1. TASK2 potassium channel expression in frog kidneys. A – TASK2 is expressed on distal tubules and proximal epithelial cells (Immunoperoxidase staining, bar = 200 μm); B – TASK2 is strongly expressed predominantly on the luminal part of the proximal epithelial cells and slight cytoplasmatic staining is also visible (Immunoperoxidase staining, bar = 100 μm); C – Distal tubules expressed diffuse cytoplasmatic staining; slight expression is also visible on the apical part of the cells (Immunoperoxidase staining, bar = 100 μm); D – Control slide, labelling was not observed.

tribute to background K^+ conductance, and persistent activity of both voltage-dependent K^+ (Kv) and inwardly rectifying (Kir) channels probably plays a role in some cell contexts (BAYLISS & BARRET 2008). However, the K_{2P} channels (TASK2 is one of them), which are structurally distinct from the Kv or Kir channels, seem to be uniquely positioned to provide a leak K^+ conductance. These channels display little voltage dependence and, thus, they carry K^+ currents over a wide range of membrane potentials. Despite their contribution to leak K^+ conductance, the activity of K_{2P} channels is not variant; they are stongly modulated by physicochemical factors, endogenous neurochemicals and clinically relevant drugs. Background K^+ channels have a key role in regulating membrane potential and cellular activity and K_{2P} channels are major contributors to these background currents (BAYLISS & BARRET 2008). Different K^+ channels are expressed in different ways from species to species. For example, in mouse the KCNQ1 K^+ (KvLQT1, Kv7.1) channel is expressed in renal proximal tubules (JESPERSEN *et al*. 2004; 2005), on the luminal membrane, and the TASK2 K^+ channel is present on the basolateral membrane of the proximal tubule and papillary collecting ducts (WARTH *et al*. 2004). We did not prove KCNQ1 expression in the proximal cell tubule of the frog kidney, but we found evidence that this channel exists in the distal convoluted and collecting duct (CEMERIKIC *et al.* 2007). The function of the KCNQ1 K^+ channel in the kidney has not been fully established, but KCNQ1 knockout mice were found to suffer from lower potassium sera level, urinary and faecal salt wasting and volume depletion, thereby indicating an important role for the IsK (KCNQ1) channel and renal function (VALLON *et al*. 2005). In this study, we show that the TASK2 K^+ channel is strongly expressed predominantly on the luminal part of the proximal epithelial cells. One of the possibilities is that the TASK2 K^+ channel in the proximal tubule of frog kidney functions instead of the KCNQ1 K^+ channel.

Likewise, extracellular pH increases brought about by HCO_3^- efflux from proximal tubule epithelial cells have been proposed to couple to

TASK2 activation to maintain electrochemical gradients favourable for HCO_3^- reabsorption (LOPEZ-CAYUQUEO *et al.* 2015). In that study TASK2 is expressed at the basolateral membrane of the same proximal tubule cells that express apical membrane Na^+-H^+-exchanger NHE-3 and basolateral membrane Na^+-HCO_3^- cotransporter NBCe1-A, the main components of the HCO_3^- transport machinery. Warth and coworkers have shown a central role for TASK2 in the proximal tubule bicarbonate reclaim process in experiments using TASK2 null mice (WARTH *et al.* 2004). Most of the bicarbonate of the glomerular filtrate is salvaged in the proximal tubule by mechanisms that have been largely elucidated (SKELETON *et al.* 2010). Under the action of carbonic anhydrase (CA) IV, HCO_3^- together with H^+ secreted into the lumen produce CO_2 and H_2O. CO_2 finds its way into the cell where CA II catalyzes the hydration of CO_2 to produce HCO_3^-. Intracellular HCO_3^- is then transported into the peritubular plasma by the electrogenic Na^+/HCO_3^- cotransporter (NBCe1-A) with a 3 to 1 HCO_3^- to Na^+ stoichiometry. Proximal tubule cells have to respond to changing demands in acid base regulation of the organism to regulate HCO_3^- reabsorption accordingly (BROWN & WAGNER 2012). Dysregulation will lead to disease as in proximal tubular acidosis (pRTA) and hypotension secondary to renal loss of HCO_3^- (WARTH *et al.* 2004). Reabsorption of HCO_3^- requires the activity of a basolateral K^+ channel to recycle K^+ taken up in the pumping cycle of the Na^+-K^+ pump and to maintain a hyperpolarized membrane potential compatible with NBCe1-A activity through extracellular basolateral alkalinization brought about by the HCO_3^- efflux. We demonstrated that TASK2 is strongly expressed predominantly on the luminal part of the proximal epithelial cells and slight cytoplasmatic staining was also noted. This result may be explained by species difference in TASK2 expression or functional difference of TASK2 in frog kidney. However, our results correspond to the findings that TASK2 is involved in the process of HCO_3^- reabsorption. In proximal tubules, Na^+-H^+-exchanger, carbonic anhydrases, Na^+-HCO_3^- cotransporter and TASK2 K^+ channels appear to act in concert during $NaHCO_3$ reabsorption (WARTH *et al.* 2004). Sodium bicarbonate cotransporters, important in bicarbonate reabsorption, are members of an emerging superfamily of Na^+-coupled HCO_3^- transporters (SCHMITT *et al.* 1999) and are widespread throughout the animal kingdom, including invertebrates (DEITMER & SCHLUE 1989), birds (KIM *et al.* 1997), amphibia (BORON & BOULPAEP 1983) and mammals (JENTSCH *et al.* 1984). On the other hand, TASK2 is activated by extracellular alkalinization, pH_o (REYES *et al.* 1998), but are also sensitive to changes in intracellular pH-pH_i (NIEMEYER *et al.* 2010). TASK2 located on the luminal part of proximal tubule cell can be activated by intracellular pH changes that appear because of increased bicarbonate absorption through the basolateral tubular membrane. TASK2 K^+ current that arises on the luminal membrane of the proximal tubule can be transferred onto the basolateral membrane through the intercellular conductive pathway, and serves to maintain an electrochemical gradient favorable for HCO_3^- reabsorption. Of course, this assumption can be the aim of further investigation.

One possibility is that the TASK2 K^+ channel in the proximal tubule cell participates in a process termed regulatory volume decrease (RVD). Most cells are capable of adjusting their volume on the face of acute changes in intra- or extracellular osmotic pressure followed by rapid fluxes of water across their plasma membrane (HOFFMANN & PEDERSEN 2011). Osmotically swollen cells release KCl and organic osmolytes such as taurin, with a concomitant osmotically-forced loss of cell water leading to a reduction in volume and recovery toward pre-swelling values. This regulatory volume decrease is often achieved by parallel activation of osmosensitive K^+ and Cl^- channels. It has been shown that the TASK2 K^+ channel is involved in RVD in Ehrlich cells (NIEMEYER *et al.* 2001). In these cells, a K^+ channel insensitive to Ca^{2+} but activated by osmotic cell swelling and termed $I_{k,vol}$ is a functional correlate of TASK2 (NIEMEYER *et al.* 2000). More evidence that TASK2 is involved in RVD of proximal tubule cells was put forth in Beldofil's study (BELDOFIL *et al.* 2003). They studied the role of CFTR (cystic fibrosis transmembrane conductance regulator) Cl^- channel in the control of K^+ currents in mouse kidney. In proximal (PCT) cell from wild-type (WT) and CFTR knock-out (KO) mice, the hypotonicity-induced K^+ currents were activated at extracellular pH 8.0 and inhibited at pH 6.0, suggesting that the corresponding channel was TASK2.

Our experiments were carried out on frog kidneys. The question can be why we used this animal species in our study? More common and successful comparisons of transport mechanisms in kidneys have concerned amphibians and mammals. The basis for this common comparison are, first, the greater ease with which amphibian renal anatomy lends itself to experimentation at the cellular level, and, second, man's interest in his own vertebrate class. Fewer data obtained for a given system in mammals often accords with a more extensive description in amphibians, so that the overall similarity of the system in the two classes is stressed at this level of analysis. Furthermore, experimentally induced adaptation often reveals an underlying

similarity of cellular transport mechanisms, even when initial description has stressed the differences between amphibians and mammals (LONG & GIEBISCH 1979). Also, the effects of the amino acid phenylalanine (Na^+– dependent amino acid cotransports), on electrical properties of proximal tubule cells, for the first time were described in frog kidney (MESSNER *et al.* 1985). Since we showed that TASK2 potassium channel was expressed on proximal and distal epithelial tubule cells, frog kidney might be used as experimental model to determine detailed function of this channel in epithelial tubule cell transport.

We showed in a previous study that all epithelial cells of distal convoluted tubules revealed basolateral expression of the KCNQ1 potassium channel, while only single cells of the collecting duct, probably intercalated cells, show diffuse cell surface staining (CIROVIC *et al.* 2010). In this study, the presence of TASK2 was demonstrated in proximal, but also in distal tubules. Distal tubules expressed diffuse cytoplasmatic staining as well as slight expression on the apical part of the cells. One possible role for the TASK2 potassium channel in frog distal tubules is maintaining the resting membrane potential. Beldofil and coworkers showed that CFTR is implicated in the control of KCNQ1 and Ca^{2+}-sensitive swelling-activated K^+ conductances in distal convoluted tubule cells (DCT) and cortical collecting tubule cells (CCT), but not in proximal convoluted tubule cells (BELDOFIL *et al.* 2003). In CFTR knockout mice, impairment of the regulatory volume decrease process in DCT and CCT could be due to loss of an autocrine mechanism, implicating ATP and adenosine, which controls swelling-activated Cl^- and K^+ channels (BELDOFIL *et al.* 2003). We also showed that all epithelial cells in the distal tubule of frog kidney revealed basolateral expression of the KCNQ1 K^+ channel (CIROVIC *et al*. 2010). Moreover, in this study, we showed that the TASK2 K^+ channel was slightly expressed on the apical part of the cells. Possibly the KCNQ 1 and TASK2 K^+ channels act in concert during swelling-activated processes in distal tubule cells.

Although the kidneys of amphibians possess a diluting segment (early distal tubule) having virtually the same transport properties as the thick ascending limb of Henle of mammalian kidneys, amphibian kidneys cannot form hyperosmotic urine because of their lack of architectural structure (NISHIMURA & FAN 2003). Wang and coworkers performed experiments concerning transport mechanisms in the diluting segment of frog kidney (WANG *et al*. 1987). They concluded that a Na^+ dependent rheogenic HCO_3^--transport system exists across the peritubular cell membrane in this part of the amphibian nephron. This rheogenic peritubular Na^+-HCO_3^- contransporter cooperates with a Na^+/H^+ exchanger in the luminal membrane, thus driving HCO_3^- reabsorption. Reabsorption of HCO_3^- and secretion of H^+ depend upon the presence of carbonic anhydrase (WANG *et al*. 1987). The TASK2 K^+ channel can be involved in maintaining a proper value of membrane potential during this transport process, but the detailed function of this channel in epithelial transport in this part of the nephron remains to be determined.

We clearly showed TASK2 K^+ expression in proximal and distal tubules in frog kidney, but there is still much to be learned about how cell signals tune the activity of TASK2 to a specific function.

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