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# Quantification and distribution of big conductance $Ca^{2+}$-activated $K^+$ channels in kidney epithelia

Morten Grunnet[a,c,*], Anders Hay-Schmidt[b], Dan A. Klaerke[a]

[a]Department of Medical Physiology, The Panum Institute, University of Copenhagen, Blegdamsvej 3, DK-2200 Copenhagen N, Denmark
[b]Department of Anatomy, The Panum Institute, University of Copenhagen, Blegdamsvej 3, DK-2200 Copenhagen N, Denmark
[c]NeuroSearch A/S, Pederstupvej 93, 2750 Ballerup, Denmark

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**Abstract**

Big conductance $Ca^{2+}$ activated $K^+$ channels (BK channels) is an abundant channel present in almost all kind of tissue. The accurate quantity and especially the precise distribution of this channel in kidney epithelia are, however, still debated. The aim of the present study has therefore been to examine the presence of BK channels in kidney epithelia and determine the actual number and distribution of these channels. For this purpose, a selective peptidyl ligand for BK channels called iberiotoxin or the radiolabeled double mutant analog $^{125}$I-IbTX-D19Y/Y36F has been employed. The presence of BK channels were determined by a isotope flux assay where up to 44% of the total $K^+$ channel activity could be inhibited by iberiotoxin indicating that BK channels are widely present in kidney epithelia. Consistent with these functional studies, $^{125}$I-IbTX-D19Y/Y36F binds to membrane vesicles from outer cortex, outer medulla and inner medulla with $B_{max}$ values (in fmol/mg protein) of 6.8, 2.6 and 21.4, respectively. These studies were performed applying rabbit kidney epithelia tissue. The distinct distribution of BK channels in both rabbit and rat kidney epithelia was confirmed by autoradiography and immunohistochemical studies. In cortical collecting ducts, BK channels were exclusively located in principal cells while no channels could be found in intercalated cells. The abundant and distinct distribution in kidney epithelia talks in favor for BK channels being important contributors in maintaining salt and water homeostasis.
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## 1. Introduction

In mammalians, salt concentrations are maintained within a very narrow range despite large variations in daily dietary intake. The kidneys play an essential role in obtaining this homeostasis by paracellular and transcellular re-absorption of NaCl across specialized epithelia cells [1,2]. The transport is mediated by the coordinated function of $Na^+/K^+$-ATPases, $Na^+/2Cl^-/K^+$ co-transporters and various kind of ion channels [3–5]. Different studies have revealed that $K^+$ channels are essential in this transport, and the existence of a wide range of channels has been reported so far [6–13] but it is still debated which type of $K^+$ channels plays the most important role in the trans-epithelial transport. Firm evidence has been reported for the ROMK (Kir1.1 or KCNJ1) channel in both humans and mice. ROMK knock-out mice have an nearly complete lethal phenotype, with only 5% of littermates surviving more than 3 weeks. Adult knock out mice suffer from a severe phenotype partly as a consequence of reduced NaCl absorption [14]. Also in humans, mutations in the ROMK channel display a serious

* Corresponding author. Department of Medical Physiology, The Panum Institute, University of Copenhagen, Blegdamsvej 3, DK-2200 Copenhagen N, Denmark. Tel.: +45 35327569; fax: +45 35327526.
*E-mail address:* mgrunnet@mfi.ku.dk (M. Grunnet).

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phenotype, resulting in Type II Bartter's syndrome manifested by reduced kidney function [15].

Another candidate for an essential role in trans-epithelial transport could be the big conductance $Ca^{2+}$-activated $K^+$ channel (BK channel or maxi K channel) which has the highest single channel conductance among the $K^+$ channels characterized until now, and which is known to be strictly regulated by physiological concentrations of intracellular $Ca^{2+}$, cAMP, phosphorylation/dephosphorylation and sheer stress [16–22]. There is good evidence that this channel is involved in flow dependent potassium secretion in the kidney [23,24]. A functional role for BK channels in kidney is further emphasized by experiments performed knock out mice where the BK-β1 subunit to the BK channel has been deleted. These animals failed to increase potassium secretion in the distal nephrons as a response to volume expansion and increased flow rate [25]. Despite these indications of a functional role for BK channels in kidney epithelium, there has been some disagreement about the presence, distribution and amount of BK channels in the kidney. Some experiments has reported the existence of BK channels in kidney cortex (proximal tubule and cortical collecting duct), and kidney outer medulla (Thick Ascending Limb) while others have not observed BK channels at all in the kidney [4,13,18,26–31].

Different peptidyl and non-peptidyl agents such as $Ba^{2+}$, TEA, charybdotoxin (ChTX) and iberiotoxin (IbTX) have been valuable in the characterization of BK channels [32–35]. All of these agents are able to prevent flux through the channel when applied to the extracellular face. This blocking event is obtained by a physical occlusion of the channel pore. IbTX is the only agent considered specific for BK channels.

Attempts to perform IbTX binding were originally hampered by the fact that native IbTX loses its biological activity upon iodination [36]. However, a double mutant of IbTX called IbTX-D19Y/Y36F has been constructed and iodinated with full preservation of the affinity and specificity for BK channels [36]. This recombinant toxin has a low dissociation rate and has turned out to be a useful ligand in quantification of BK channels in both excitatory tissue and colon epithelium [36–39].

In the present study, we have performed flux assays and binding experiments with IbTX and the double mutant IbTX-D19Y/Y36F, respectively, and for the first time, it has been possible to obtain reliable information about the exact number and localization of BK channels in kidney epithelium. The distribution of the channels was confirmed by autoradiography and immunohistochemical studies. Here, it was demonstrated that BK channels were exclusively located in principal cells known to be involved in sodium and water re-absorption. In contrast, no channels could be found in neighboring intercalated cells, which are believed to participate in secretion of protons and bicarbonate.

## 2. Experimental procedures

### 2.1. Materials

Trizma (Tris base), MOPS, EGTA, BSA, SUCROSE and polyethylenimine were obtained from Sigma; KCl, NaCl, $BaCl_2$ and $MgCl_2$ were from Merck; Digitonin was from Serva; Tubes from InterMed; Filters were from Advantec; Ouabain and Dowex beads (50 W × 8) were from Fluka. The Dowex beads were converted to the Tris form by 16–24 incubation with Tris base (suspension pH > 10) and subsequent repetitive washing with distilled water until the suspension pH dropped to 7.0–7.4. $^{86}$RbCl (0.5–12 mci/mg $Rb^+$) was purchased from Amersham, Purification and iodination of $^{125}$I-IbTX-D19Y/Y36F was done as previously described [36].

### 2.2. Tissue preparation

New Zealand white rabbits (female, approximately 2.5 kg) maintained on a standard commercial diet (0.13% $Na^+$ and 0.8% $K^+$) were killed by cervical dislocation and bled. The kidneys were immediately removed and placed on a 150-mm ice cooled Petri dish and outer cortex, inner cortex, outer medulla and inner medulla were separated with a scalpel. For a single kidney, this resulted in approximately 1.25 g of outer cortex, 1.40 g of inner cortex, 0.90 g of outer medulla and 0.50 g of inner medulla. The four different kinds of kidney tissue were placed in liquid nitrogen and subsequent stored at −80 °C.

### 2.3. Vesicle preparation

Approximately 1 g of kidney outer cortex, inner cortex, outer medulla or kidney inner medulla were homogenized either with 10 ml 250 mM sucrose, 50 mM NaCl, 10 mM MOPS, 1 mM EGTA Tris–HCl pH 7.2 (for binding experiments) or with 250 mM sucrose, 50 mM KCl, 10 mM MOPS, 1 mM EGTA Tris–HCl pH 7.2 (for flux assays) with 5 strokes at 1000 rpm in a glass/teflon homogenizer (Braun-Melsungen) at 0 °C. The homogenate was subjected to low-speed centrifugation (Sorvall SS-34) 6300×$g$ for 15 min at 4 °C. The supernatant containing membrane fractions was decanted and saved at 0 °C while the pellet was resuspended in 10 ml 250 mM sucrose, 50 mM NaCl, 10 mM MOPS, 1 mM EGTA Tris–HCl pH 7.2 (for binding experiments) or in 250 mM sucrose, 50 mM KCl, 10 mM MOPS, 1 mM EGTA Tris–HCl pH 7.2 (for flux assays).

The resuspended pellet was subjected to another low-speed centrifugation (Sorvall SS-34) 6300×$g$ for 15 min at 4 °C. The two supernatants were mixed and subjected to a high-speed centrifugation (Sorvall SS-34) 41700×$g$ for 35 min at 4 °C. The supernatant was discarded and the pellet resuspended in 20 mM Tris pH 7.2 (for binding experiments) or in 250 mM sucrose, 50 mM KCl, 10 mM MOPS, 1 mM EGTA Tris–HCl pH 7.2 (for flux assays) and

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