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The voltage-gated potassium channel subunit, Kv1.3, is expressed in epithelia

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Abstract

The Shaker-type voltage-gated potassium channel, Kv1.3, is believed to be restricted in distribution to lymphocytes and neurons. In lymphocytes, this channel has gained intense attention since it has been proven that inhibition of Kv1.3 channels compromise T lymphocyte activation. To investigate possible expression of Kv1.3 channels in other types of tissue, such as epithelia, binding experiments, immunoprecipitation studies and immunohistochemical studies were performed. The double-mutated, radiolabeled peptidyl ligand, ^{125}I-HgTX$_1$-A19Y/Y37F, which selectively binds Kv1.1, Kv1.2, Kv1.3 and Kv1.6 channels, was used to perform binding studies in epithelia isolated from rabbit kidney and colon. The equilibrium dissociation constant for this ligand was found to be in the sub-picomolar range and the maximal receptor concentration (in fmol/mg protein) 1.68 for colon and 0.61–0.75 for kidney epithelium. To determine the subtype of Kv1 channels, immunoprecipitation studies with ^{125}I-HgTX$_1$-A19Y/Y37F labeled epithelial membranes were performed with specific antibodies against Kv1.1, Kv1.2, Kv1.3, Kv1.4 or Kv1.6 subunits. These studies demonstrated that Kv1.3 subunits constituted more than 50% of the entire Kv1 subunit population. The precise localization of Kv1.3 subunits in epithelia was determined by immunohistochemical studies.

Keywords: Potassium channel; Epithelia; Kv1.3 subunit; Radiolabeled hongotoxin-1; Binding studies; Immunoprecipitation; Immunohistochemistry

1. Introduction

The Kv family of voltage-gated channels constitutes the largest family of potassium channels. At least nine different subfamilies of Kv channels named Kv1 to Kv9 exist, and a number of the genes within the single subfamilies have been described (for reviews, see Refs. [1,2]). The Kv subfamilies are able to form heterotetrameric channel complexes both in vitro [3–5] and in vivo [6–8]. In addition, a number of modulatory Kv β-subunits have been identified and taken together the potential complexity of Kv potassium channels is enormous [9–12]. The group of Kv1 or *Shaker* channels has been subject to the most intense investigation among Kv channels. These channels have predominantly been found and characterised in the CNS where they participate in the control of neuronal excitability and the large number of different combinations of Kv channel complexes give optimal opportunities for strict control of the firing pattern in excitable tissues [1,13]. An exception is Kv1.3 channels, which were described as early as 1984 in lymphocytes [14]. The Kv1.3 channels were originally cloned from brain tissue [9–11,15–17] but shortly after also isolated from lymphocytes [18]. The presence of Kv1.3 channels in lymphocytes has attained intense attention since this channel type is responsible for setting the membrane potential in these cells. Inhibition of the Kv1.3 channel can prevent the activation response of lymphocytes [19–21], although Ca^{2+}-activated intermediary conductance K^+ channel (IK channels) may also be important for lymphocyte proliferation [22,23].

In addition to their importance for regulation of the membrane potential, ion channels play an important role for transport in epithelia. For example in kidney and colon, the overall transport of salt and water is dependent on the activity of several types of K^+ channels (see, e.g. Refs. [24,25]). It is therefore of interest that Kv channels have also been identified in epithelia [26,27]. However, only sparse information is available concerning the abundance of Kv channels in epithelial cells, and in particular little in known concerning the subtype distribution.

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In the present study, Kv channels were identified and quantified in kidney and colon epithelia by use of hongotoxin-1 ($HgTX_1$), a peptidyl toxin from the scorpion *Centruroides limbatus*. An iodinated double-mutated form of this toxin, ^{125}I-$HgTX_1$-A19Y/Y37F, binds with equally high affinity to Kv1.1, Kv1.2 and Kv1.3 and also recognize Kv1.6, although with lower affinity [27]. Epithelial membranes prelabeled with ^{125}I-$HgTX_1$-A19Y/Y37F were subsequently immunoprecipitated with specific antibodies raised against the different Kv1 channel subunits. This gave not only the possibility to quantify Kv channels in the epithelial membranes, but also provided information about the presence of various Kv1 channel subtypes. Such experiments revealed that Kv1.3 channel subunits are the most abundant among the epithelial Kv subtypes, and the localization of channels containing Kv1.3 subunits in kidney and colon was subsequently determined in immunohistochemical experiments.

2. Materials and methods

2.1. Tissue preparation

New Zealand white rabbits (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 distal colon was immediately dissected, cut into pieces of approx. 3 cm, flushed with 0.9% NaCl to remove fecal contents and placed on ice. The segments were then opened along their length, and placed with the luminal side upward on a 150 mm cooled Petri dish. After cleaning the colon thoroughly with gauze to remove mucus, the colon mucosa was harvested by gentle scraping with two glass slides [28]. The colon mucosa was then placed in liquid nitrogen and stored at − 80 °C.

The kidneys were immediately removed and placed on a 150 mm ice-cooled Petri dish and cortex, outer medulla and inner medulla were separated with a scalpel. For a single kidney, this resulted in approximately 2.50 g of cortex, 0.90 g of outer medulla and 0.50 g of inner medulla. Kidney tissue was then placed in liquid nitrogen and stored at − 80 °C.

2.2. Vesicle preparation

2.2.1. Colon

Two grams of tissue scrapings were homogenized with 30 ml 250 mM sucrose, 1 mM EGTA, 10 mM MOPS–Tris, pH 7.0 Tris with 10 strokes at 1000 rpm in a glass/teflon homogenizer (Braun-Melsungen) at 0 °C. 1 mM DTT and 0.2 mM PMSF was added just before homogenization. The homogenate was subjected to low-speed differential sedimentation 500 × *g* for 10 min at 4 °C in a Sorvall SS 34 rotor. The supernatant containing soluble proteins and crude membrane fractions was decanted and subjected to a high-speed centrifugation at 207 000 × *g* for 30 min at 4 °C in a Beckman Ti 70.1 rotor. The supernatant was discarded and mucus from the inner side of the centrifuge glasses was removed before the pellet was resuspended in 0.5 ml 20 mM Tris–HCl pH 7.2 and homogenized in the glass/teflon homogenizer with 10 strokes at 1000 rpm. These membrane preparations (''crude membranes''), which contain plasma membranes as well as intracellular membranes from surface and crypt cells were stored at − 20 °C until further use.

2.2.2. Kidney

Approximately 1 g of kidney cortex, outer medulla or kidney inner medulla were homogenized with 10 ml 250 mM sucrose, 50 mM NaCl, 10 mM MOPS, 1 mM EGTA Tris–HCl pH 7.2 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* × 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. The resuspended pellet was subjected to another low-speed centrifugation (Sorvall SS-34) 6300 × *g* × 15 min at 4 °C. The two supernatants were mixed and subjected to a high-speed centrifugation (Sorvall SS-34) 41 700 × *g* × 35 min at 4 °C. The supernatant was discarded and the pellet resuspended in 20 mM Tris pH 7.2 and homogenized in the glass/teflon homogenizer (10 strokes) at 0 °C. Membrane preparations were stored at − 20 °C until further use.

2.3. Binding assay

The double-mutated, radiolabeled analog of hongotoxin named ^{125}I-$HgTX_1$-A19Y/Y37F was used as ligand for binding to kidney and colon epithelia membranes. Incubation was carried out in 2–4 ml medium consisting of 2.5–100 mM KCl, 10 mM NaCl, 20 mM Tris–HCl pH 7.4, 0.1% BSA in polystyrene tubes. Nonspecific binding was defined in the presence of 1 nM non-labeled $HgTX_1$-A19Y/Y37F and incubation was carried out at room temperature for 2–4 h except saturation experiments where incubation was allowed to proceed for 24 h. All serial toxin dilutions were performed in 150 mM NaCl, 0.1% BSA, 20 mM Tris–HCl pH 7.4 and always added directly to the incubation medium to avoid adsorption phenomena. Digitonin was allowed to incubate with the membranes for 20 min at room temperature before the ligand was added. The protein concentration of the membrane vesicles was 100 μg/ml for colon, kidney cortex and outer medulla and 50 μg/ml for kidney inner medulla except for initial experiments where different protein concentrations ranging from 12.5 to 500 μg/ml were tested. At the end of incubation, the samples were rapidly filtered through Toyo Advantec GC 50 glass fiber filters (presoaked for at least 60 min in 0.3% (w/v) polyethylenimine) on a Milipore 1002530 filter apparatus, followed by two washes with ice-cold buffer consisting of 150 mM NaCl, 20 mM Tris–HCl pH 7.4 (3 ml per wash). In each experiment, measurements in duplicate were routinely performed and the data averaged.

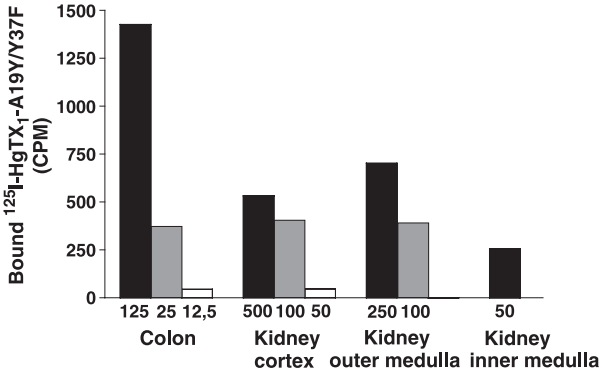

Fig. 1. Binding of ^{125}I-HgTX$_1$-A19Y/Y37F to epithelial membranes. Different amounts of membranes from colon or kidney were incubated with 6.8 pM ^{125}I-HgTX$_1$-A19Y/Y37F in the absence (total binding) or presence (unspecific binding) of 10 nM unlabeled HgTX$_1$-A19Y/Y37F. Specific binding was determined as the difference between total and unspecific binding. The membrane concentration used in the experiments are given in μg/ml on the abscissa. The figure shows the specific binding as the mean of determinations in duplicate.

2.4. Immunoprecipitation assay

Kidney or colon epithelia membranes (10 mg) were incubated with 50–100 pM ^{125}I-HgTX$_1$-A19Y/Y37F for 1–2 h at room temperature in 10 ml incubation buffer consisting of 5 mM KCl, 150 mM NaCl, 20 mM Tris–HCl pH 7.4. The suspension was subjected to high-speed centrifugation at 195 000 × g × 20 min. The supernatant was discarded and the pellet resuspended in 1 ml ice-cold incubation buffer and subjected to another high-speed centrifugation at 195 000 × g × 20 min. This washing step was repeated twice to remove unbound ^{125}I-HgTX$_1$-A19Y/Y37F from the pellet. After the last wash, the pellet was resuspended in 5 ml ice-cold incubation buffer and 100 mg Triton X-100 to a final Triton concentration of 2%. Membranes were allowed to solubilize for 30–45 min at 0 °C. Solubilized membranes were subjected to high-speed centrifugation at 272 000 × g × 20 min. The supernatant containing solubilized membranes were stored at 0 °C and solubilization efficiency determined.

Immunoprecipitation was carried out in Eppendorf tubes initially prepared by addition of 75 μl 1:2 diluted Protein A Sepharose (PAS), 200 μl incubation buffer and various amounts of Kv1.1, Kv1.2, Kv1.3, Kv1.4 or Kv1.6 antibodies. The specificity of these antibodies has earlier been proven [6]. The recognition sequence of the different antibodies within the primary amino acid sequence of the different Kv1 channels was as follows: Kv1.1 aa 458–475; Kv1.2 aa 461–

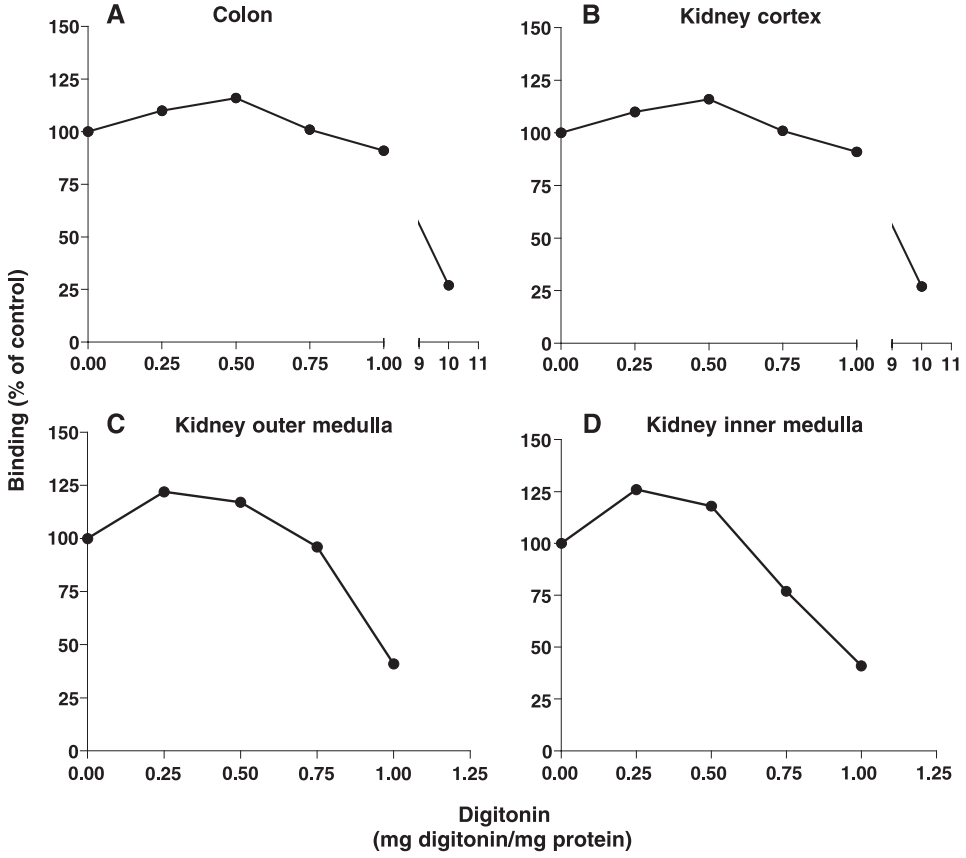

Fig. 2. Demasking of ^{125}I-HgTX$_1$-A19Y/Y37F binding by digitonin: 100 μg/ml of colon vesicles (A), kidney cortex vesicles (B), kidney outer medulla vesicles (C) or kidney inner medulla vesicles (D) were incubated for 20 min at room temperature with increasing concentrations of digitonin before binding experiments with constant concentrations of ^{125}I-HgTX$_1$-A19Y/Y37F (4.0 pM) were performed. Specific binding in percentage of control (no digitonin added) is shown as a function of the digitonin to membrane protein ratio. Data points are mean of determinations in duplicate.

480; Kv1.3 aa 456–474 or aa 485–502; Kv1.4 aa 605–623 and Kv1.6 aa 652–669. These suspensions were allowed to incubate at least 15 min at 4 °C with slow rotation before two washes with 500 μl ice-cold incubation buffer were performed. To save the pellet, suspensions were pulse-spun at 18 500 × *g* × 30 s between each wash. The washed suspensions were kept at 4 °C until addition of solubilized membranes.

Antibody binding was performed by adding 250 μl solubilized membranes and 250 μl ice-cold incubation buffer to the washed PAS/antibody complex. Incubation was carried out for 4 h at 4 °C with slow rotation. After incubation, eppendorf tubes were pulse-spun for 30 s before removal of approximately 400 μl supernatant. Four washes with 1 ml ice-cold incubation buffer were then performed before pellets were resuspended in 3 × 250 μl incubation buffer and transferred to polystyrene tubes for counting in a 1470 Wizard™ gamma counter from Wallac.

2.5. Immunohistochemical studies

2.5.1. Kidney

Wistar male rats were anaesthetised with Midizolam/Hypnorm (i.p.) and perfusion fixed through the left ventricle. As fixative was used phosphate buffered saline (PBS) plus 3000 i.e. heparin per liter PBS followed by 0.1 M phosphate buffer with 4% paraformaldehyde (PFA) for 10 min. The kidneys were removed, and post-fixed for an additional 24 h in 0.1 M phosphate buffer plus 4% PFA. Kidney fixed in 4% PFA was embedded in paraffin and 5 μm serial sections were cut and mounted on Super Frost Plus object slides. Sections were dehydrated in xylene–ethanol series, endogenous peroxidase activity was blocked in 1% H_2O_2 in methanol, washed three times for 5 min in PBS plus 0.1% Triton X-100, followed by incubation in PBS plus 1% human serum albumin (hSA) for 1 h. Primary rabbit Kv1.3 antibody was diluted 1:1500 in PBS plus 1% hSA and sections were incubated overnight at 4 °C, then washed three times for 5 min in PBS and incubated in donkey–anti-rabbit (Fab_2) biotinylated antibody 1:200 in PBS plus 1% hSA for 1 h at room temperature. Washed three times in PBS followed by incubation with StreptAvidin-Biotin-Complex 1:500 Vector Elite (Vector, Burlingame, USA) for 1 h, washed three times in PBS and then incubated with 0.05% di-amino-benzidine (DAB) in 0.01% H_2O_2 in PBS + 0.1% Triton X-100 for 10 min and finally washed three times in distilled water. One of the two sections on each slide was counterstained with hematoxylin, before mounting in DEPEX.

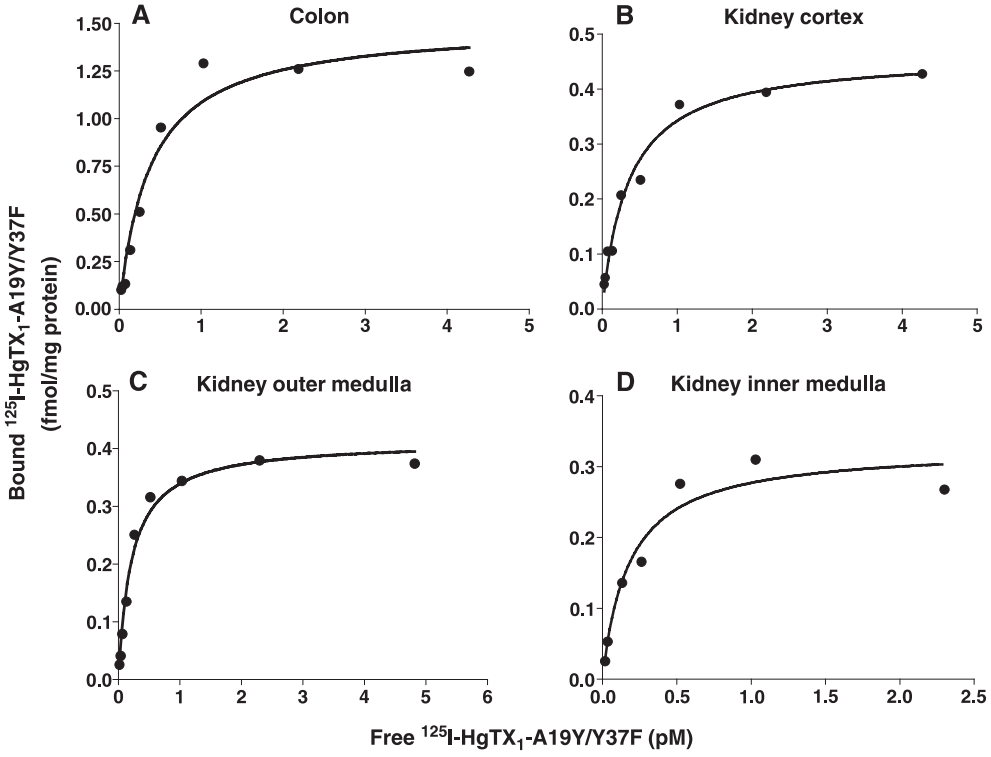

Fig. 3. Quantification of ^{125}I-HgTX$_1$-A19Y/Y37F binding sites. Saturation binding experiments with increasing concentrations of ^{125}I-HgTX$_1$-A19Y/Y37F were performed to colon membranes (A), kidney cortex membranes (B), kidney outer medulla membranes (C) and kidney inner medulla membranes (D). Protein concentration was 100 μg/ml for colon, kidney cortex and kidney outer medulla and 60 μg/ml for kidney inner medulla. Samples were pre-incubated for 20 min with 0.50 mg digitonin/mg protein (colon membranes) or 0.25 mg digitonin/mg protein (kidney membranes) before addition of the indicated amounts of ^{125}I-HgTX$_1$-A19Y/Y37F. Receptor-ligand binding was allowed to proceed for 24 h before specific binding was determined. Each data point shows the average result of measurements in duplicate on pooled membranes from six animals.

2.5.2. Colon

Wistar male rats were anaesthetised and perfusion-fixed in 4% PFA in PBS as described above. The colon was removed, post-fixed for 24 h in 4% PFA, embedded in paraffin and 5 μm serial sections were cut. The sections were dehydrated in tolouen-ethanol series, blocked in blocking buffer (PBS containing 1% BSA, 0.3% Triton X-100 and 5% swine serum) and incubated overnight at 4 °C with the anti-Kv1.3 antibody in blocking buffer. After rinses, sections were incubated with Alexa 568-goat anti-rabbit IgG (1:800) for 2 h at room temperature, rinsed again and mounted with Prolong Antifade kit before being examined by laser scanning confocal microscopy using the LEICA TCS SP2 system.

2.6. Protein determination

The concentration of membrane protein was determined according to Grunnet et al. [29], using BSA as a standard.

2.7. Analysis of data

The results from saturation binding experiments were subject to a Michaelis–Menten analysis, where the equilibrium dissociation constant (K_d) and the maximal receptor concentration (B_{max}) were determined using the one-site binding equation $Y = B_{max} \times X/(K_d + X)$ where Y = receptor concentration and X = radioligand concentration. The correlation coefficient for these plots was >0.94. The data from saturation experiments were transformed into Scatchard plots and the B_{max} and K_d values determined from this linear regression were not significantly different from the values determined from the one-site binding equation.

2.7.1. Materials

Antibodies against Kv1 channels and recombinant, double-mutated $HgTX_1$-A19Y/Y37F (expressed in *E. coli*, purified and iodinated as described earlier [27]) were kind gifts from Dr. Hans Guenther Knaus; donkey-anti-rabbit (Fab_2) biotinylated antibody was from Jackson Immunoresearch Laboratories (Pennsylvania, USA); swine serum was from DAKO A/S (Denmark); Alexa 568-goat anti-rabbit IgG and Prolong Antifade kit were from Molecular Probes (Leiden, The Netherlands); Digitonin was from Serva (Germany), polystyrene tubes were from InterMed (Denmark) and all other chemicals were from Sigma.

3. Results

3.1. Binding of ^{125}I-$HgTX_1$-A19Y/Y37F to epithelial membranes

For binding studies the radiolabeled, double-mutated, hongotoxin analog ^{125}I-$HgTX_1$-A19Y/Y37F was employed. This labeled peptide, which specifically recognizes Kv1.1, Kv1.2, Kv1.3 and Kv1.6, shows no difference in affinity as

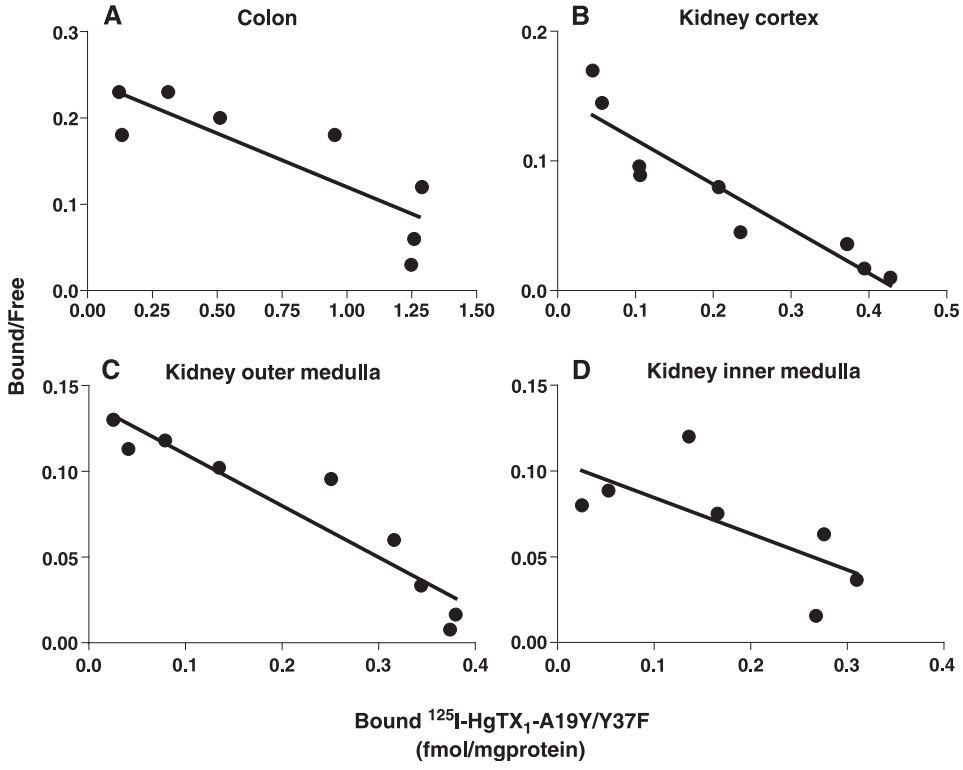

Fig. 4. Scatchard analysis of saturation binding experiments. Data obtained from the non-linear saturation experiments in Fig. 3 were subject to a Scatchard analysis after linear transformation. The results are shown for colon membranes (A), kidney cortex membranes (B), kidney outer medulla membranes (C) and kidney inner medulla membranes (D). Calculated maximal receptor concentrations (B_{max}) and equilibrium dissociation constants (K_d) are summarized in Table 1.

compared to native hongotoxin and has been a valuable tool for the investigation of Kv1 channel distribution in brain [27]. Fig. 1 shows an initial experiment, where ^{125}I-HgTX$_1$-A19Y/Y37F binding to different amounts of partially purified membranes from colon and kidney epithelia was measured. The figure shows that the specific binding is dependent on the amount of membranes although the specific binding as a function of the membrane concentration in these experiments does not show a linear relationship. Membrane concentrations of 12.5 μg/ml (colon) and 50 μg/ml (kidney cortex) are practically below the detection level and in the case of a membrane concentration of 500 μg/ml (kidney cortex) the binding assay is most likely saturated. In all further experiments, membrane concentrations of 60–100 μg/ml were used as indicated in the figure legends.

As ^{125}I-HgTX$_1$-A19Y/Y37F recognizes and binds to the outer vestibule of channels containing Kv1.1, Kv1.2, Kv1.3 and Kv1.6 subunits, binding is only possible if the membrane vesicles are orientated right-side-out. Since membrane vesicle preparations may consists of a heterogenous population of right-side-out, inside-out and leaky vesicles, it may be necessary to demask the binding sites to obtain reliable quantification of channels by ligand binding. Digitonin has been shown to be a suitable detergent for demasking of epithelial vesicles [30]. In Fig. 2, the vesicles from colon and kidney were pre-incubated with increasing concentrations of digitonin, before ^{125}I-HgTX$_1$-A19Y/Y37F binding was measured with constant ligand and receptor concentrations. As can be seen, maximal binding to colon- and kidney vesicles was obtained in the presence of a detergent/receptor ratio between 0.25 and 0.50 mg digitonin/mg protein. For all further quantification studies, these digitonin concentrations were therefore applied to secure full access to all ^{125}I-HgTX$_1$-A19Y/Y37F binding sites. At detergent concentrations exceeding approximately 1.0 mg digitonin/mg protein, the specific binding of ^{125}I-HgTX$_1$-A19Y/Y37F was severely attenuated. This is most likely due to the fact that high detergent concentrations compromise filter retention.

For a number of peptide toxins, specific binding to potassium channels is reduced in the presence of high concentrations of K^+, since K^+ competes with the toxin for binding to the channel pore. However, to obtain ^{125}I-HgTX$_1$-A19Y/Y37F binding to Kv1.3 channels, a certain amount of K^+ is necessary, most likely to prevent "pore-collapse" [31]. To optimize binding conditions with respect to the K^+ concentration, ^{125}I-HgTX$_1$-A19Y/Y37F binding to colon and kidney vesicles was performed at constant ^{125}I-HgTX$_1$-A19Y/Y37F and protein concentrations in the presence of increasing K^+ concentrations. In these experiments, K^+ between 2.5 and 10 mM increased specific binding to both membrane preparation by approx. 40% (data not shown). All further binding experiments were therefore done in the presence of 5 mM K^+.

Table 1
Quantification of Kv channels in membranes from kidney and colon epithelia

Tissue	B_{max} (fmol/mg protein)	K_d (pM)
Colon	1.68 ± 0.21	0.38 ± 0.09
Kidney cortex	0.75 ± 0.26	0.35 ± 0.06
Kidney outer medulla	0.68 ± 0.28	0.22 ± 0.03
Kidney inner medulla	0.61 ± 0.32	0.18 ± 0.06

The values for B_{max} (maximum receptor concentration) and K_d (equilibrium dissociation constant) were determined from 15 to 25 independent experiments (colon, $n=25$; kidney cortex, $n=25$, kidney outer medulla, $n=15$ and kidney inner medulla, $n=15$), where specific binding was determined in duplicate. The membranes originated from six different animals. Results are given as means ± S.E.

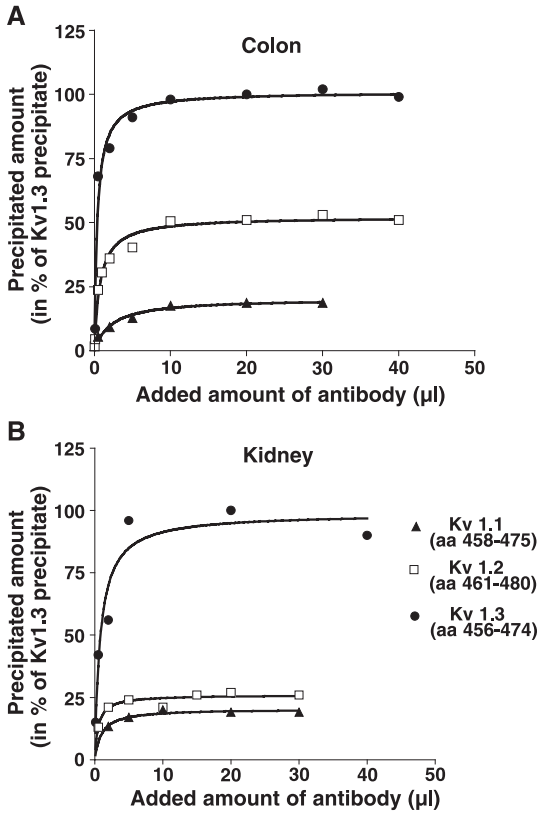

Fig. 5. Kv1 subtype classification by immunoprecipitation. Triton X-100 solubilized colon membranes (A) or kidney membranes (B) pre-labeled with ^{125}I-HgTX$_1$-A19Y/Y37F were subject to precipitation with increasing amounts of Kv1.1, Kv1.2 or Kv1.3 antibodies. The amounts immunoprecipitated ^{125}I-HgTX$_1$-A19Y/Y37F receptor are shown relative to the maximal immunoprecipitation by the Kv1.3 antibody as a function of the amount of added antibody. Numbers in brackets indicate the epitope sequence for the antibodies. Each data point shows the average result of measurements in duplicate on pooled membranes from six animals.

3.2. Quantification of Kv1 channels in epithelia

Having optimized assay conditions for ^{125}I-HgTX$_1$-A19Y/Y37F binding, studies were performed to obtain information about the absolute number of Kv1 channels in colon- and kidney epithelia. When membrane vesicles were incubated with increasing concentrations of ^{125}I-HgTX$_1$-A19Y/Y37F ranging from approximately 10 times below

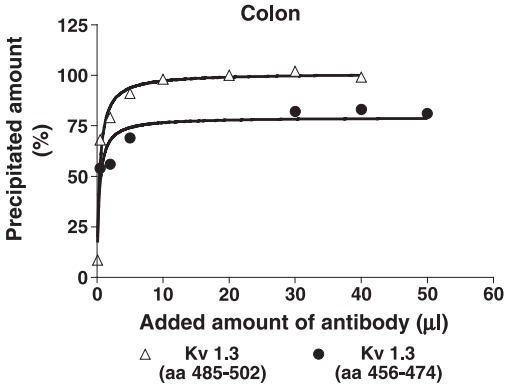

Fig. 6. Kv1.3 immunoprecipitation with alternative Kv1.3 antibodies. Triton X-100 solubilized colon membranes pre-labeled with ^{125}I-HgTX$_1$-A19Y/Y37F were subject to immunoprecipitation as in Fig. 5 with two different Kv1.3 antibodies raised against unique epitopes. Numbers in brackets indicate the epitope sequence for the antibodies. Each data point shows the results of measurements in duplicate as in Fig. 5.

to 30 times above the expected K_d value, the toxin associated in a concentration-dependent manner (Fig. 3). For all membrane fractions, specific binding was found to be a saturable function of the ^{125}I-HgTX$_1$-A19Y/Y37F concentration. Non-linear binding data obtained from ^{125}I-HgTX$_1$-A19Y/Y37F saturation binding studies were transformed into Scatchard plots. As can be seen in Fig. 4, the transformations resulted in straight lines, indicating that ^{125}I-HgTX$_1$-A19Y/Y37F interacts with a single class of receptor sites in both kidney and colon membranes. The kinetic parameters for ^{125}I-HgTX$_1$-A19Y/Y37F binding to the epithelial membranes are given in Table 1.

According to earlier studies [27], ^{125}I-HgTX$_1$-A19Y/Y37F binds to homotetrameric Kv1.1, Kv1.2 and Kv1.3 channels with approximately identical affinity (K_d = 0.1–0.25 pM) and to Kv1.6 channels with a considerable lower affinity (K_d = 9.6 pM). The K_d values obtained in the present studies (0.18–0.38 pM, Table 1), therefore, are consistent

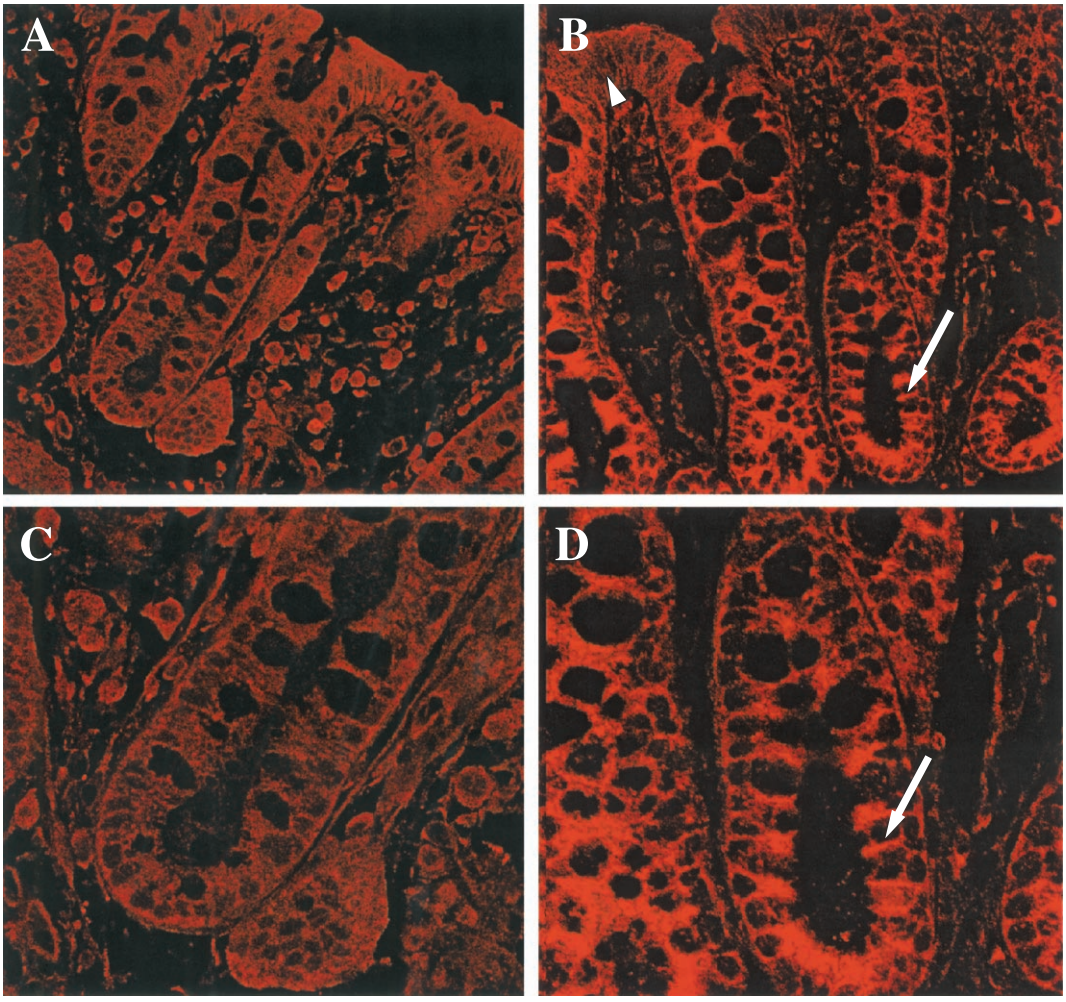

Fig. 7. Immunohistochemical identification of Kv1.3 channels in colon. Confocal scanning images of paraffin tissue sections of rat distal colon stained as described in Materials and methods without (A,C) and with (B,D) addition of primary antibodies against Kv1.3. Panel B shows a section of colonic epithelium, and surface cells (arrowhead) and crypt cells (arrow) can be identified. Specific staining is mainly located to the crypt cells. Panel D shows an enlargement of the crypts and the staining indicate that Kv1.3 is located in the basolateral membrane of the crypt cells (arrow).

with the presence of homotetrameric or heterotetrameric Kv channels consisting of Kv1.1, 1.2 or 1.3 subunits.

3.3. Subtype characterization of Kv1 channels present in epithelia

To determine the relative abundance of the Kv1 channel subunits in the epithelia, colon or kidney membranes were initially labeled with ^{125}I-HgTX$_1$-A19Y/Y37F, before immunoprecipitations were performed with specific antibodies against Kv1.1 (Kv1.1-aa 458–475), Kv1.2 (Kv1.2-aa 461–480), Kv1.3 (Kv1.3-abI-aa 456–474), Kv1.3 (Kv1.3-abII-aa 485–502). Kv1.4 (Kv1.4-aa 605–623) or Kv1.6 (Kv1.6-aa 652–669). All precipitation experiments were carried out with increasing amounts of antibody until a saturable level of precipitation was achieved (Fig. 5). As can be seen, it was possible to obtain saturable precipitation levels for the three antibodies raised against Kv1.1, Kv1.2 and Kv1.3 in both colon- and kidney epithelia vesicles. As expected, no specific precipitation of ^{125}I-HgTX$_1$-A19Y/Y37F labeled membranes could be demonstrated with antibodies raised against Kv1.4 or Kv1.6 (data not shown). Fig. 5 shows that the most abundant Kv1 subtype in both colon and kidney epithelia was Kv1.3. For colon membranes 59.5 ± 2.8% and for kidney membranes 68.9 ± 8.9% of the ^{125}I-HgTX$_1$-A19Y/Y37F receptors immunoprecipitable by all Kv1 antibodies could be chelated with antibodies against Kv1.3. In contrast, much smaller fractions of labeled receptor could be precipitated with antibodies against Kv1.2 (30.4% for colon membranes and 17.5% for kidney membranes) and against Kv1.1 (10.8% for colon membranes and 13.7% for kidney membranes). The finding that Kv1.3 seems to be a predominant Kv channel subunit in epithelia was somewhat surprising, since Kv1.3 channels have been believed to be located mainly in lymphocytes and neuronal tissue. Therefore, the immunoprecipitation experiments were repeated using another antibody directed against Kv1.3, namely Kv1.3-abII-aa 485–502. These experiments confirmed the results obtained with Kv1.3-abI-aa 456–474 antibody for colon membranes (Fig. 6) as well as for kidney membranes (data not shown). Taken together, these results strongly indicate that the Kv1.3 is a predominant subunit among epithelial Kv1 channels.

3.4. Immunohistochemical localization of Kv1.3 channels in epithelia

The presence of Kv1.3 subunits in epithelia demonstrated by the binding and immunoprecipitation studies does not give information concerning the localization of Kv channels containing the 1.3 subunit, and it could a priori not be excluded that the membrane preparations could be contam-

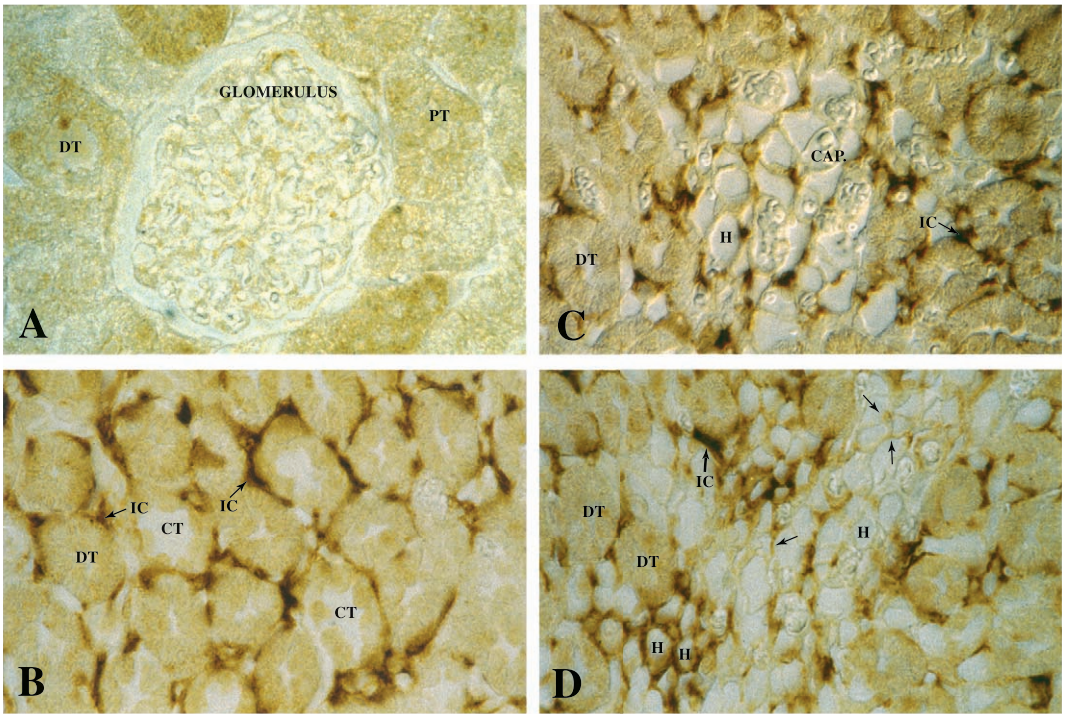

Fig. 8. Immunohistochemical identification of Kv1.3 subunits in kidney. Sections of rat kidney were immunostained with specific antibodies against Kv1.3. Panel A shows a section of the renal cortex. The glomerulus, the proximal tubule (PT) and the distal tubule (DT) can be identified, and none of these structures show staining. Panel B shows a section of the outer medulla. The interstitial cells (IC) show profound staining, whereas no staining is detected in the DT and the collecting ducts (CT). Panels (C and D). Sections of inner medulla showing DT without Kv1.3-staining and Henle's loop (H) with Kv1.3-staining. Position of capillaries (CAP) and DT is indicated. Arrows without any notion show the position of the true thin part of the loop of Henle.

inated with lymphocytes or neuronal tissue. To determine the localization of Kv1.3 subunit-containing channels in epithelia, sections from kidney and colon were stained with the Kv1.3 antibody (Kv1.3-abI-aa 456–474). In the colon, staining was exclusively identified in the basolateral membrane of the crypt epithelium, whereas no staining could be detected either in the apical membranes of these cells or in the surface epithelium (Fig. 7). In kidney, specific staining was observed mainly in the interstitial cells of the medulla (Fig. 8A–D). In addition, some staining was observed in the loop of Henle, in particular at the transition zone to the distal tubule (Fig. 8D), whereas the actual thin loop showed only sparse staining (Fig. 8D, arrows). Staining was not observed in the glomerulus, pars convuluta of proximal and distal tubule and collecting ducts (Fig. 8A–C).

4. Discussion

In the present paper, we determine the abundance of certain Shaker-type Kv1 channels in kidney and colon by means of binding studies using radiolabeled hongotoxin-1 (^{125}I-HgTX$_1$-A19Y/Y37F). This radioligand specifically binds homotetrameric Kv channels of the Kv1.1, Kv1.2, Kv1.3 and Kv1.6 types and heterotetrameric Kv channels consisting of these subtypes [27]. The B_{max} values for the ^{125}I-HgTX$_1$-A19Y/Y37F binding showed a density for these Kv channels 1.68 ± 0.21 fmol/mg protein in colon and from 0.61 ± 0.32 to 0.75 ± 0.26 fmol/mg protein in kidney. In addition, the K_d values of 0.18–0.32 pM suggested the presence of Kv1.1, Kv1.2 and Kv1.3 subunits. The immunoprecipitation studies showed that the Kv1.3 subunit is the most abundant of the Kvs in these organs; the relative distributions of Kv1.1:Kv1.2:Kv1.3 subunits are 1:3:6 in colon and 1:1:5 in kidney. Immunohistochemistry showed that in kidney, the Kv1.3 subunits are mainly expressed in medullary interstitial cells and in the distal part of the loop of Henle, whereas in colon our results indicated that Kv1.3 subunits are expressed exclusively in the basolateral membrane of the crypt cells.

From the total amount of ^{125}I-HgTX$_1$-A19Y/Y37F receptors and from immunoprecipitation studies, it can be estimated that the density of Kv1.3. channel subunits in kidney is in the range of 0.5 fmol/mg protein. This is a rather low number compared to, e.g. rat brain membranes, where the overall number of Kv1.3 subunits using the same methods has been determined to approx. 100 fmol/mg protein [27]. It could a priori be expected that the number of Kv channels would be considerably higher in the CNS considering the massive amount of Kv1 channels needed for conducting action potentials along neurons, but also in comparison with other renal channels, the abundance of the Kv1.3 channels in kidney is low; e.g. we have recently determined that the abundance of high-conductance Ca^{2+}-activated K^+ channels (BK channels) in kidney is approx. 10 fmol/mg protein. From the immunohistochemical experiments, it can be seen that the reason for the low overall abundance of Kv1.3 subunits in kidney is that the subunits are only expressed in rather specific parts of the kidney, namely the epithelial cells in the distal part of the loop of Henle and in the medullary interstitial cells, and it must be expected that the local density in these particular cells is considerably higher.

In the membranes from colon the abundance of Kv1.3 subunits can be calculated to be approx. 1 fmol/mg protein from the binding experiments and the immunoprecipitation experiments. This is also a very sparse distribution in comparison with other K^+ channels in this epithelium, since we have shown in a similar membrane preparation that the abundance of BK channels is approx. 40 fmol/mg protein [32]. In contrast to the kidney, the Kv1.3 subunits in the colon seem to be expressed exclusively in epithelial cells and more precisely in the basolateral membrane of the secretory crypt cells and not in the absorptive surface cells (cf. Fig. 7).

Functional Kv channels may consist of heterotetrameric complexes of up to four different subunits. It has been demonstrated that Kv channels can form heterotetrameric complexes of subunits within the family of Kv1 channels but not from more remote families such as Kv2 to Kv9 channels. In the brain, it has been possible to determine the precise composition and relative abundance of such complexes by additive immunoprecipitation studies [6]. This approach was also attempted in the present study but unfortunately the distribution of epithelial Kv1 channels was too sparse to obtain such information. Even though such analysis could not be performed, it can be concluded that heterotetrameric complexes containing Kv1.4 and Kv1.6 subunits are unlikely to be present in epithelia since no immunoprecipitation could be demonstrated using specific antibodies for these channels in experiments with ^{125}I-HgTX$_1$-A19Y/Y37F labeled membranes. The presence of homotetrameric Kv1.6 channels can also be ruled out as very improbable since ^{125}I-HgTX$_1$-A19Y/Y37F recognizes Kv1.6 channels although with lower affinity than for Kv1.1, 1.2 and 1.3 channels. In contrast, ^{125}I-HgTX$_1$-A19Y/Y37F does not recognize Kv1.4 homotetrameric channels and the presence of such channel complexes cannot be ruled out based on the binding and immunoprecipitation studies performed in this study. Since our data shows that Kv1.3 is the most abundant of the Kv subtypes examined, the present study is in agreement with papers identifying Kv1.3 or Kv1.3-like channels in a renal library [26] and prostate epithelium [33]. However, the significance of the presence of voltage-gated potassium channels such as, e.g. Kv1.3 in epithelia is rather obscure, since the basolateral membrane potential of these cells in most cases under normal physiological conditions is believed to be negative to −60 mV. Kv1.3 channels are not activated before depolarization to approximately −45 mV and would therefore not readily be expected to play a role in epithelia cells. However, it has been suggested that under

certain circumstances, extracellular potassium concentrations in kidney interstitium can be as high as 40 mM, meaning that the membrane potential will be sufficiently depolarized to activate Kv1.3 channels [26,34]. Indeed a model considering a possible function of Kv1.3 channels in renal epithelial cells has been proposed [26]. However, it should be kept in mind that the present study shows that in kidney, Kv1.3 channels are found in the highest concentration in interstitial cells, where a possible function for the channels remains to be established. Also in the colonic epithelial cells further studies are clearly needed to establish a possible function of Kv1.3 channels.

Given the existence of Kv1 channels in epithelia it does, however, make sense that the subtypes identified, namely Kv1.1 Kv1.2 and Kv1.3, all belong to the group of relatively slowly inactivating Kv channels, which could be activated in epithelial cells, provided the membrane potential exceeds their threshold for activation, e.g. due to a rise in extracellular K^+. This is in contrast to the very fast inactivating Kv channels, e.g. Kv1.4, for which it would be difficult to assign a role in non-excitable tissues, such as epithelia.

To date Kv1.3 channels have in particular caused attention because of their involvement in lymphocyte proliferation [21] and they have been proposed as a target for immunosuppressive drugs [20]. However, even though the amount of Kv1.3 channels in epithelia was found to be modest, these findings raise the question as to whether Kv1.3 channels will constitute an appropriate target for immunosuppressive drugs before detailed investigations of the role of these channels in other cell types have been performed.

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