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Role and mechanisms of regulation of the basolateral Kir4.1/Kir5.1 K^+ channels in the distal tubules

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Abstract

Epithelial K^+ channels are essential for maintaining electrolyte and fluid homeostasis in the kidney. It is recognized that basolateral inward-rectifying K^+ (K_{ir}) channels play an important role in the control of resting membrane potential and trans-epithelial voltage, thereby modulating water and electrolyte transport in the distal part of nephron and collecting duct. Monomeric $K_{ir}4.1$ (encoded by *Kcnj10* gene) and heteromeric $K_{ir}4.1/K_{ir}5.1$ ($K_{ir}4.1$ together with $K_{ir}5.1$ (*Kcnj16*)) channels are abundantly expressed at the basolateral membranes of the distal convoluted tubule and the cortical collecting duct cells. Loss-of-function mutations in *KCNJ10* cause EAST/SeSAME tubulopathy in humans associated with salt wasting, hypomagnesemia, metabolic alkalosis, and hypokalemia. In contrast, mice lacking $K_{ir}5.1$ have severe renal phenotype that, apart from hypokalemia, is the opposite of the phenotype seen in EAST/SeSAME syndrome. Experimental advances using genetic animal models provided critical insights into the physiological role of these channels in electrolyte homeostasis and the control of kidney function. Here, we discuss current knowledge about K^+ channels at the basolateral membrane of the distal tubules with specific focus on the homomeric $K_{ir}4.1$ and heteromeric $K_{ir}4.1/K_{ir}5.1$ channels. Recently identified molecular mechanisms regulating expression and activity of these channels, such as cell acidification, dopamine, insulin and insulin-like growth factor-1, Src family protein tyrosine kinases etc, as well as the role of these channels in NCC-mediated transport in the distal convoluted tubules, are also described.

Keywords

collecting duct; distal convoluted tubule; Kcnj10; Kcnj16; NCC; resting membrane potential

Introduction

In the kidney, discretionary Na^+ reabsorption and K^+ secretion in the distal part of the nephron and collecting duct (CD) are responsible for the fine tuning of water and electrolyte

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Conflict of Interest

There are no conflicts of interests.

homeostasis (Staruschenko, 2012). Inwardly rectifying K^+ channels (K_{ir}), specifically $K_{ir}4.1$ and $K_{ir}5.1$ (encoded by *Kcnj10* and *Kcnj16* genes, respectively), are essential for the control of basolateral membrane potential and K^+ recycling in the distal convoluted tubules (DCT) and cortical collecting ducts (CCD). This recycling is necessary to maintain a stable source of extracellular K^+ in order to perform trans-cellular Na^+ reabsorption driven by the Na^+/K^+-ATPase (Tanemoto, 2007). Thus, the functional expression of K^+ channels in these tubule segments is critical for electrolyte homeostasis. In humans, loss-of-function mutations in the *KCNJ10* gene have been shown to cause multiple neurological disorders such as epilepsy beginning in infancy, displayed motor impairment with ataxia and sensorineural deafness, and renal salt-losing tubulopathy, all together named SeSAME/EAST syndrome (Bockenhauer *et al.*, 2009, Scholl *et al.*, 2009). Additional analysis revealed that these mutations lead to severe salt wasting, hypomagnesemia, metabolic alkalosis, and hypokalemia, which possibly represent a consequence of defects in the kidney. Moreover, the lack of Kcnj10 resulted in decreased expression of the thiazide-sensitive Na-Cl cotransporter (NCC, encoded by *Slc12a3* gene) in DCT (Zhang *et al.*, 2014) and increased expression of the epithelial Na^+ channel (ENaC; α-, β-, and γ-subunits encoded by *Scnn1a*, *Scnn1b* and *Scnn1g*, respectively) and water channel aquaporin 2 (*Aqp2*) in CCD (Su *et al.*, 2016). Targeted disruption of the *Kcnj16* gene in mice resulted in hypokalemic, hyperchloremic metabolic acidosis with hypercalciuria (Paulais *et al.*, 2011). Despite these important findings, the role of basolateral K^+ channels in the distal parts of nephron and CD has not been extensively studied. The major research focus in this area was devoted to the K^+ secretion mediated by the apical renal outer medullary K^+ channel (ROMK, Kir1.1; encoded by *Kcnj1*) and the Ca^{2+} activated big K^+ channels (big K, BK; pore-forming α-subunit is encoded by *Kcnma1*, there are also several ancillary subunits (Pluznick *et al.*, 2005, Wen *et al.*, 2014, Larsen *et al.*, 2016)). For details please see excellent reviews (Wang and Giebisch, 2009, Welling, 2016) summarizing the role of ROMK and BK channels in the distal renal tubule.

Potassium homeostasis in the kidney and its transport in the distal tubules

K^+ is freely filtered by the glomerulus with final K^+ adjustments occurring in the distal nephron and CD. K^+ secretion begins in the DCT, which can be divided into two functionally distinct portions termed the DCT1 and DCT2 (or the early and late DCTs) (Subramanya and Ellison, 2014, McCormick and Ellison, 2015). The late DCT, connecting tubule (CNT), and CD are often referred to as the aldosterone sensitive distal nephron or ASDN; however, this term is not exactly correct given that the CD morphologically and developmentally is not a part of the nephron. The CD system includes the cortical collecting duct (CCD), the outer medullary CD (OMCD), and the inner medullary CD (IMCD) (Staruschenko, 2012). K^+ is secreted in these segments primarily via apical ROMK channels (Boim *et al.*, 1995, Lee and Hebert, 1995). Multiple mechanisms controlling activity of ROMK channels were previously reported, including high K^+ intake, Angiotensin II, phosphatidylinositides, various kinases, and many others (Wei *et al.*, 2014, Lin *et al.*, 2015, Liu *et al.*, 2015, Dong *et al.*, 2016). BK channels also play a role in K^+ transport under certain conditions, such as shear stress induced increases in intracellular Ca^{2+} shown in isolated CD tubules *ex vivo* (Woda *et al.*, 2001) as well as in BK β1- and β2-subunits

(Pluznick *et al.*, 2005, Larsen *et al.*, 2016) and α-subunit (Rieg *et al.*, 2007) knock out mice *in vivo*. In addition to shear stress, there are some other factors reported that contribute to the activity of BK channels, such as nucleotides (Woda *et al.*, 2002) and vasopressin signaling (Rieg *et al.*, 2007). Therefore, secretion in these nephron segments varies according to physiologic requirements, such as dietary manipulations, and is responsible for most of the urinary K^+ excretion. However, as it will be discussed below, K^+ recycling mediated by $K_{ir}4.1/K_{ir}5.1$ channels significantly contributes to the maintenance of electrolyte homeostasis and control of kidney function.

Shown in Figs. 1 and 2 are the current models of electrolyte transport in the DCT and principal cells of the CCD/CNT, respectively. In both nephron segments discussed here (as well as in many other renal epithelial cells), transport is energized by the Na^+ gradient generated by the Na^+/K^+-ATPase localized in the basolateral membrane (McDonough *et al.*, 1990). In DCT, energy of the electrochemical gradient for Na^+ is harnessed by the NCC cotransporter in the apical membrane and moves Cl^- into the cell against its electrochemical gradient. Cl^- then exits across the basolateral membrane passively via Cl^- channels (Kieferle *et al.*, 1994, Zaika *et al.*, 2016b). Na^+ reabsorption in the principal cells of the CNT and CCDs is mediated by ENaC, which is localized in the apical membranes, and provides a conductive pathway for Na^+ entry into the cell (Canessa *et al.*, 1994, Canessa *et al.*, 1993, Staruschenko, 2012). ENaC is also expressed in the late DCT (not shown in the figure), where it also contributes to Na^+ reabsorption in this segment, in conjunction with NCC (Ciampolillo *et al.*, 1996, Rubera *et al.*, 2003, González-Núñez *et al.*, 2004). The higher permeability of the luminal membrane for Na^+ depolarizes the apical plasma membrane, creating a lumen-negative potential difference (Garcia-Filho *et al.*, 1980), which provides the driving force for secretion of K^+ into the lumen.

Basolateral potassium channels in the distal tubules

The molecular identity of specific channels on the basolateral membrane of the distal tubules is a highly important question investigated by many groups. Initial studies identified several channels with distinct single channel and macroscopic conductance properties (Schlatter *et al.*, 1992, Hirsch and Schlatter, 1993, Wang, 1995, Lu *et al.*, 1997a, Lu *et al.*, 1997b). Most single channel studies identify two types of basolateral K^+ channels. Hirsch et al. identified that the conductance of the smaller channel was approximately 67 and 28 pS in cell-attached and excised patches, respectively. The conductance of the larger K^+ channel was approximately 148 and 85 pS in cell-attached and excised patches, respectively (Hirsch and Schlatter, 1993). Wang et al. also reported two types of native K^+ channels on the basolateral side. Single channel analysis revealed that the conductances of the small-conductance K^+ channel was 28 and 30 pS, in an asymmetrical and symmetrical high KCl solutions, respectively, whereas the conductance of the intermediate-conductance channel was approximately 85 pS in symmetrical high KCl solutions (Wang *et al.*, 1994). Analysis of K^+ channel currents in the basolateral membrane of rabbit DCT (cell attached configuration) also revealed two different conductances of 49 and 61 pS, and both types of channels were completely blocked by Ba^{2+} (Taniguchi *et al.*, 1989). Earlier studies of renal K^+ channels has been comprehensively reviewed by (Wang *et al.*, 1997). Importantly, while different investigators used various solutions, species, methods of preparations etc., the results of

most single channel studies are consistent with two populations of native basolateral channels in both DCT and CD.

New insights into the molecular mechanisms of K^+ transport in the distal nephron and CD have been provided by cloning and identification of numerous K^+ channels. As described by Hamilton and Devor, there are a number of basolateral K^+ channels in the distal tubule segments (Hamilton and Devor, 2012). As an example, Welling described expression, and provided the initial functional analysis of $K_{ir}2.3$ (*Kcnj4*) in an immortalized mouse CCD line, M-1 (Welling, 1997). Millar et al. examined the properties of the K^+ conductance in the principal cells of freshly isolated mouse CCDs, and reported that whole-cell K^+ currents in principal cells show strong inward rectification, high K^+ selectivity, and inhibition by Ba^{2+} in a concentration- and voltage-dependent manner. The authors concluded that the properties of the conductance are consistent with $K_{ir}2.3$ (Millar *et al.*, 2006). Welling and colleagues further studied molecular and signaling mechanisms mediating expression of $K_{ir}2.3$. First, they demonstrated, using an epitope-tag approach, that $K_{ir}2.3$ is exclusively expressed in the basolateral membrane of CCD cells (Le Maout *et al.*, 1997), and that the *C*-terminus coordinates membrane targeting of this channel (Le Maout *et al.*, 2001). Further studies revealed that polarized expression of $K_{ir}2.3$ is influenced by the opposing activities of two different PDZ proteins. Mammalian Lin-7 (mLin-7) interacts with $K_{ir}2.3$, and links the channel with calcium/calmodulin-dependent serine protein kinase (CASK) or related Stardust proteins to coordinate basolateral membrane expression (Olsen *et al.*, 2002, Alewine *et al.*, 2007), whereas the tax interacting protein 1 (TIP-1) competes for interaction with mLin-7 and drives $K_{ir}2.3$ into the endocytic pathway (Alewine *et al.*, 2006). However, there are some inconsistencies within the published studies since Gray et al. reported that the properties of basolateral K^+ conductance in principal cells of rat CCDs are different from previously characterized properties of $K_{ir}2.3$ (Gray *et al.*, 2005).

Another potential candidate for mediating K^+ conductance in the distal tubule is $K_{ir}7.1$ (encoded by *Kcnj13* gene). Using multiple approaches including Western blotting, immunostaining, RNA analysis, and electron microscopic immunocytochemistry, Ookata et al. identified that the $K_{ir}7.1$ channel is located predominantly in the basolateral membrane of the distal tubules (DCT, CNT and CDs). Importantly, staining along the CD was observed only in principal, but not in intercalated cells. Some staining was also reported in the TAL cells. The mRNA levels and immunoreactivity were decreased under low K^+ diet; however it was reversed when diet was supplemented with 4% KCl (Ookata *et al.*, 2000). Interestingly, Derst et al. approximately at the same time reported that tubular fragments of human and guinea pig kidney showed a significant expression of $K_{ir}7.1$ only in the PT and TAL (RT-PCR analysis defined mRNA levels in both segments and immunocytochemical analysis revealed its expression only in PT) (Derst *et al.*, 2001). Additional studies are required to confirm the specific tubule localization, as well as to determine the functional role of the $K_{ir}7.1$ channel in the renal epithelia. This channel may also be of significant interest considering the development of novel small-molecule inhibitors specifically targeting $K_{ir}7.1$ (Bhave *et al.*, 2011, Raphemot *et al.*, 2011).

$K_{ir}4.1$ and $K_{ir}5.1$ channels in the distal tubules

Despite the presence of the aforementioned K^+ channels, it is now viewed that $K_{ir}4.1$ and $K_{ir}5.1$ are the main K_{ir} channels in the basolateral membrane of DCT and CCD responsible for K^+ recycling in these segments. Takumi et al. isolated $K_{ir}4.1$ about 20 years ago (the authors initially designated this new clone as KAB-2, the second type of inward rectifying K^+ channel with an ATP-binding domain). They initially reported that this protein is predominantly expressed in glial cells of the cerebellum and forebrain. In addition to brain, mRNA was also detected in the kidney (Takumi *et al.*, 1995). A subsequent immunohistochemical study revealed that $K_{ir}4.1$ is strongly expressed in the basolateral membrane of renal distal tubular epithelia (Ito *et al.*, 1996). Figure 3 demonstrates the immunohistochemical analysis of $K_{ir}4.1$ (Fig. 3A) and $K_{ir}5.1$ channels (Fig. 3B) in rat kidney cortex as well as colocalization of $K_{ir}5.1$ and AQP2, specific marker of principal cell (Fig. 3C). It is worth noting that $K_{ir}4.1$ is also expressed in the cortical part of TAL (cTAL) where this channel may similarly contribute to the basolateral K^+ conductance (Reichold *et al.*, 2010, Zhang *et al.*, 2015). However, using Kcnj10 knockout ($Kcnj10^{-/-}$) mice, the authors demonstrated that the disruption of $K_{ir}4.1$ has no significant effect on the membrane potential of the cTAL and NKCC2 expression (Zhang *et al.*, 2015). Therefore, additional studies are needed to reveal the physiological role of $K_{ir}4.1$ in cTAL.

$K_{ir}4.1$ forms homomeric channels and co-assembles with $K_{ir}5.1$ to yield $K_{ir}4.1/K_{ir}5.1$ heteromeric channels (Pessia *et al.*, 1996, Pessia *et al.*, 2001, D'Adamo *et al.*, 2011). $K_{ir}4.1/K_{ir}5.1$ has unique properties including greater single channel conductance, and much higher sensitivity to pH within the physiologic range when compared to $K_{ir}4.1$ homomer (Tucker *et al.*, 2000, Pessia *et al.*, 2001, D'Adamo *et al.*, 2011) (see also Fig. 4). We and others reported that the $K_{ir}4.1/K_{ir}5.1$ heteromer is the predominant basolateral K^+ channel in both DCT and CCD (Lourdel *et al.*, 2002, Lachheb *et al.*, 2008, Zaika *et al.*, 2013, Zaika *et al.*, 2016a).

The genetic dissection of renal diseases has identified many components required for normal renal electrolyte homeostasis. Bockenhauer et al. performed linkage analysis in children from two consanguineous families that presenting with epilepsy and abnormal, uncoordinated motor behavior or ataxia, moderate sensorineural hearing loss, and a salt-losing renal tubulopathies. They identified a single significant locus on chromosome 1q23.2, which contained the *KCNJ10* gene. Sequencing of this gene, encoding $K_{ir}4.1$ channel, revealed homozygous missense mutations. Further analysis defined that these mutations cause this autosomal recessive disease, which was called EAST syndrome (epilepsy, ataxia, sensorineural deafness, and tubulopathy) (Bockenhauer *et al.*, 2009). Around the same time, Scholl and colleagues described the same disease, identifying missense or nonsense mutations in $K_{ir}4.1$, in highly conserved amino acids on both alleles in all affected subjects, but named it SeSAME (seizures, sensorineural deafness, ataxia, mental retardation and electrolyte imbalance) (Scholl *et al.*, 2009). Functional analyses revealed that the disease-causing mutations in the channel led to a defect in K^+ conductance. Patients with EAST/SeSAME display hypokalemia, metabolic alkalosis, hypomagnesemia, and elevated levels of renin and aldosterone (Bockenhauer *et al.*, 2009, Scholl *et al.*, 2009). Similarly, $Kcnj10^{-/-}$ mice displayed a phenotype recapitulating the disease including hypokalemia,

hypomagnesemia and metabolic alkalosis, and hypocalciuria, salt-wasting and dehydration (Bockenhauer *et al.*, 2009). Additional disease-causing mutations in *KCNJ10* were further identified by Zdebik and colleagues (Freudenthal *et al.*, 2011, Parrock *et al.*, 2013).

As described by Méndez González et al., there are over 120 coding-region single nucleotide polymorphisms (SNPs) in the *KCNJ10* gene reported in the publicly accessible genome databases (Méndez-González *et al.*, 2016). Electrophysiological analysis revealed that the currents produced by mutant channels carrying disease-associated mutations (R65P, R65P/R199X, G77R, C140R, T164I, and A167V/R297C) were strongly reduced, indicating that the loss of channel function underlies the EAST/SeSAME syndrome (Williams *et al.*, 2010). When mutant $K_{ir}4.1$ subunits were co-expressed together with $K_{ir}5.1$ wild type channels, the reduction in function was similar compared to mutant subunits expressed alone. This data indicates that the studied mutations affect both homomeric and heteromeric channels. Importantly, it was revealed that wild type $K_{ir}4.1$ subunits were unaffected by a reduction of pH_i to 6.8, but currents for mutant subunits were essentially abolished at the lower pH_i. The authors concluded that this effect likely reflects a shift in pH_i sensitivity to more alkaline values, and may contribute to the lower currents observed when these mutants were studied under normal pH_i conditions (Williams *et al.*, 2010). Similarly, Reichold et al. studied several other $K_{ir}4.1$ mutants, specifically the R65P, G77R, R199X, and R175Q. They also observed a decreased current that was mediated by changes in the channel open probabilities (P_o) for both homomeric $K_{ir}4.1$ and heteromeric channels (Reichold *et al.*, 2010). Another group found that a majority of studied $K_{ir}4.1$ mutations (particularly R297C, C140R, R199X, T164I) caused complete loss of channel function, while two mutations (R65P and A167V) resulted only in partial loss of function (Tang *et al.*, 2010). Sala-Rabanal et al. further applied patch clamp analysis together with radiotracer efflux and identified that all of the studied mutations compromised the channel's function, but the underlying mechanisms were different. The authors reported that R65P, T164I, and R297C mutations in $K_{ir}4.1$ caused an alkaline shift in pH sensitivity, indicating that these positions are crucial for pH sensing and pore gating. In R297C, this was due to disruption of the intersubunit salt bridge Glu^{288}-Arg^{297}. C140R breaks the Cys^{108}-Cys^{140} disulfide bond essential for protein folding and function. A167V did not affect channel properties but may contribute to decreased surface expression in A167V/R297C. In G77R, introduction of a positive charge affected channel structure or gating. R199Stop led to a dramatic decrease in surface expression, but channel activity was restored by co-expression with intact subunits, suggesting remarkable tolerance for truncation of the cytoplasmic domain (Sala-Rabanal *et al.*, 2010). Recent studies further identified G83V, L166Q, and Q212R residues as playing a pivotal role in controlling $K_{ir}4.1$ channel function. The G83V rendered the channel to be non-functional. The L166Q variant reduced channel function and the Q212R mutant did not reduce overall conductance, but did demonstrate subtle effects on spermine sensitivity (Méndez-González *et al.*, 2016). Tanemoto et al. further revealed that the A167V mutation in $K_{ir}4.1$ caused compromised trafficking of the mutant channels, and inhibited their expression on the basolateral surface of tubular cells. It was reported that an anchor protein, membrane-associated guanylate kinase with inverted domain structure-1 (MAGI-1), contributes to basolateral K^+ recycling. MAGI-1 directly interacts with $K_{ir}4.1$ and facilitates the basolateral localization of this channel (Tanemoto *et al.*, 2014). All these results provide

important insights for the molecular mechanisms that underlie the EAST/SeSAME syndrome.

Recent studies by Slaats et al. further revealed that $K_{ir}4.1$ along with voltage-gated, kqt-like subfamily, member 1 (*Kcnq1*); voltage-gated, subfamily F, member 1(*Kcnf1*) K^+ channels; and Cl^- channel, voltage-gated 4 (*Clcn4*) regulate renal ciliogenesis in CD through the periciliary diffusion barrier or the ciliary pocket. A siRNA-based reverse genetics screen identified these channels as potential contributors to ciliopathy pathophysiology. Interestingly, the authors reported that damaging mutations in *KCNJ10* as well as in *KCNQ1*, *KCNF1* and *CLCN4* cause defects in cilia structure, but that *KCNJ10* mutations affecting the ion channel function do not affect the cilia (Slaats *et al.*, 2015). This observation requires further investigation, considering the important role of cilia in epithelial cells.

Identification of mutations within the human gene encoding $K_{ir}4.1$ causing the SeSAME/EAST syndrome brought a lot of attention to investigation of this particular channel. However, as discussed above, $K_{ir}4.1/K_{ir}5.1$ heteromeric channels, but not homomeric $K_{ir}4.1$channels, play the main role in the modulation of the basolateral conductance in the distal nephron and CD. Despite this, our knowledge about the role of $K_{ir}5.1$ is very limited. Paulais et al. investigated the role of the $K_{ir}5.1$ subunit in mice with a targeted disruption of the $K_{ir}5.1$ gene (*Kcnj16*) (Paulais *et al.*, 2011). They identified the important role that $K_{ir}5.1$ plays as a pH-sensitive regulator of salt transport in the DCT. It was reported that the $K_{ir}5.1^{-/-}$ mice displayed hypokalemic, hyperchloremic metabolic acidosis with hypercalciuria. To further test the role of $K_{ir}5.1$ for electrolyte transport in the DCT, they tested the effects of hydrochlorothiazide (HCTZ), a NCC inhibitor. The short-term responses to HCTZ were exaggerated, indicating excessive renal Na^+ absorption in this segment. Furthermore, chronic treatment with HCTZ normalized urinary excretion of Na^+ and Ca^{2+}, and abolished acidosis in the $K_{ir}5.1^{-/-}$ mice. Interestingly, increased NCC activity is normally associated with hyperkalemia, not hypokalemia, as observed in $K_{ir}5.1^{-/-}$ mice. However, the mechanism of this hypokalemic renal tubular acidosis is not known. In contrast, inhibition of ENaC in CCD with amiloride was not different in the $K_{ir}5.1^{-/-}$ mice compared to wild type littermates, indicating that ENaC-mediated Na^+ transport is not altered by deletion of $K_{ir}5.1$ (Paulais *et al.*, 2011). To further address the role of $K_{ir}5.1$ in the kidney in the context of a disease state *in vivo*, we recently generated a $Kcnj16^{-/-}$ rat model in Dahl salt-sensitive (SS) background by using ZFN technology. Our preliminary data revealed that knockout of $K_{ir}5.1$ in SS rat induces electrolyte imbalance and blood pressure abnormalities (Palygin *et al.*, 2015). However, this model requires further investigation to precisely determine the role of this channel in the development of salt-sensitive hypertension.

Recent studies also identified mutations in *KCNJ16* (encoding $K_{ir}5.1$) in patients with Brugada syndrome. This cardiac disease is potentially fatal with arrhythmia characterized by abnormal rapid heart rhythms that originate in the heart ventricles (Juang *et al.*, 2014). Furthermore, recent Genome-Wide Association Studies (GWAS) of metabolite quantitative traits also identified *KCNJ16* as having significant loci with 3-hydroxybutyrate, which links this gene to regulation of intermediary metabolites and possibly to ketosis or ketoacidosis,

which results from increased fat metabolism due to a shortage of insulin commonly found in diabetes mellitus (Demirkan *et al.*, 2015). While it is still unclear if these changes play any role in the control of renal function, these observations demonstrate that $K_{ir}5.1$ (despite its lacking ability to form homomeric channel) might be critical for the development of certain pathologies in humans.

Biophysical properties of K_{ir} channels at the basolateral membrane of the distal tubules

The phenomenon of inward or anomalous rectification, as first described by Katz in 1949, means that membrane conductance increases with hyperpolarization and decreases with depolarization (Katz, 1949). K_{ir} channels are primarily responsible for the resting potential and have been found and described in many tissues. In these channels, the increase of current that follows hyperpolarization has been referred to as activation, so that the reduction of channel current at positive potentials has generally been described as deactivation or rectification (Nichols and Lopatin, 1997).

Renal K_{ir} channels, involved in epithelial transport and K^+ homeostasis, include $K_{ir}1.1$ (ROMK), $K_{ir}7.1$, Kir4.1 and Kir5.1, which are all expressed in the distal tubules. The above referenced channels belong to the same subgroup of K^+ transport proteins and have similar biophysical properties. Functional K_{ir} channels are composed of 4 subunits which are either homo- or heterotetramers. Each subunit has a single pore domain and two transmembrane domains. It was predicted that the pore domain and the second transmembrane domain contributes to permeation pore structure, and the other domain is involved in formation of a selectivity filter (Koster *et al.*, 1998, Sepúlveda *et al.*, 2015). To date no K_{ir} channels have been crystallized.

While $K_{ir}4.1$ is able to form a functional homomeric channel, $K_{ir}5.1$ lacks this ability as shown when channel subunits were expressed alone in heterologous expression systems (Pessia *et al.*, 1996). Interestingly it was shown in co-transfected HEK-293 cells that homomeric $K_{ir}5.1$ channel is functional when it forms a complex with PSD-95, a brain anchoring protein (Tanemoto *et al.*, 2002). As it was recently summarized (Sepúlveda *et al.*, 2015), $K_{ir}4.1/K_{ir}5.1$ channels form heteromeric K^+ channel in a specific 4-5-4-5 arrangement that is dependent on external K^+ concentration, which relieves the rectification by K^+-ion binding at external sites by displacing Mg^{2+} from sites deeper inside a multi ion pore. Intriguingly, a 4-4-5-5 tetrameric arrangement (in contrast to 4-5-4-5 position of subunits) produces channels with the properties of homomeric Kir4.1 channels, which provides evidence for the importance of subunit position in the properties of heterotetrameric K_{ir} channels (Pessia *et al.*, 1996, Nichols and Lopatin, 1997). Lourdel et al. performed an elegant analysis of the biophysical properties of K^+ channels on the basolateral membranes of microdissected DCT using a patch clamp approach (Lourdel *et al.*, 2002). It was shown that Mg^{2+} considerably reduced the outward conductance of native K^+ channels, and that the polycation spermine reduced P_o by 50 %. Channel activity was dependent upon the intracellular pH, with acid pH decreasing, and alkaline pH increasing, P_o. Internal ATP and Ca^{2+} had no effect, and channel activity declined irreversibly when the inner side of the

patch was exposed to Mg^{2+} (Lourdel *et al.*, 2002). These channels, similar to other K_{ir} channels, are also inhibited by large cations, such as Cs^{+} and Ba^{2+}. Shown in Fig. 5A is an example of inhibition of native $K_{ir}4.1/K_{ir}5.1$ channels in CCD by Cs^{+} in the presence of extracellular Ba^{2+} (Zaika *et al.*, 2016a).

Several studies in heterologous expression systems reported that tricyclic antidepressants nortriptyline and fluoxetine act as reversible inhibitors of the $K_{ir}4.1$ channel (Furutani *et al.*, 2009, Su *et al.*, 2007, Ohno *et al.*, 2007). We have tested, in isolated CCD, the effect of these drugs on endogenous $K_{ir}4.1/K_{ir}5.1$ channels, and revealed that fluoxetine had only a minor effect (probably through inhibition of homomeric $K_{ir}4.1$ channels), while nortriptyline almost completely abolished macroscopic whole cell currents in CCD principal cells (Fig. 5B) (Zaika *et al.*, 2016a).

As it will be discussed below, these channels are also highly modulated by protein kinase C (PKC) phosphorylation, G protein-coupled receptor (GPCR) activation, as well as some other physiological modulators. Figure 5C demonstrates an example of activation of basolateral K^{+} conductance by insulin (Zaika *et al.*, 2016a).

As described above, basolateral K^{+} channels play a dominant role in determining resting membrane potential and spatial K^{+} buffering in the distal nephron and CD. Shown in Fig. 6 are examples of acute changes in membrane potential followed by modulation of $K_{ir}4.1/K_{ir}5.1$ channels after application of diverse hormones. As shown in Fig. 6, addition of insulin, IGF-1, and dopamine, all of which modulate activity of $K_{ir}4.1/K_{ir}5.1$ channels (Zaika *et al.*, 2013, Zaika *et al.*, 2016a) results in immediate changes in the resting membrane potential level.

Signaling mechanisms controlling properties of potassium channels at the basolateral membrane of the distal tubules

A number of earlier studies revealed several important mechanisms controlling activity of native basolateral K^{+} channels. While these studies do not provide the exact molecular identity of the channels studied, we strongly believe that this information is relevant for understanding signaling pathways controlling basolateral K_{ir} channels. Thus, it was reported that PKC and protein kinase A (PKA) modulate activity of basolateral K^{+} channels. PKC inhibition reduced channel activity by 90% in cell attached patches (Lu and Wang, 1996a). Addition of the catalytic subunit of PKA increased activity of basolateral K^{+} channels (Wang, 1995). Moreover, it was shown that nitric oxide (NO) plays an important role in the regulation of the basolateral K^{+} channels in the CCD (Lu and Wang, 1996b). Inhibition of nitric oxide synthase (NOS) reduced the channels activity. Furthermore, application of a cGMP analogue stimulated basolateral K^{+} channels, and cGMP also restored the channel activity blocked by NOS inhibitors (Lu and Wang, 1996b). It was further proposed that PKC is involved in the stimulation of the small-conductance basolateral K^{+} channels by regulation of NOS (Lu and Wang, 1996a).

Zhang et al. provided evidence that the Src family protein tyrosine kinase is involved in the regulation of K^{+} channels in the basolateral membrane of the mouse DCT1 segment (Zhang

et al., 2013). Western blot analysis showed that $K_{ir}4.1$ was a tyrosine-phosphorylated protein. LC/MS analysis further confirmed that these protein tyrosine kinases phosphorylated $K_{ir}4.1$ at Tyr^8 and Tyr^9. Therefore, the modulation of tyrosine phosphorylation of $K_{ir}4.1$ may play a role in regulating membrane transport function in DCT, similar to its effects on ROMK channels (Lin *et al.*, 2004, Lin *et al.*, 2012). It was further reported that c-Src–induced stimulation of $K_{ir}4.1$ requires coexpression of caveolin-1. Disruption of this scaffolding protein decreased $K_{ir}4.1$ activity and depolarized the membrane potential in the DCT at least partially by suppressing the stimulating effects of c-Src on $K_{ir}4.1$ (Wang *et al.*, 2015).

A potential mechanism involving GPCRs was also reported. It was shown that $K_{ir}4.1$ interacts with the Ca^{2+}-sensing receptor (CaR) in yeast two-hybrid screens, heterologous expression systems, and in DCT (Huang *et al.*, 2007). CaR inactivated Kir4.1 by reducing its cell surface expression. Mutant, activated $G\alpha_q$ reduced cell surface expression and current density of Kir4.1, and these effects were blocked by regulator of G protein signaling 4 (RGS4), a protein that blocks signaling via $G\alpha_i$ and $G\alpha_q$. Similar to the study discussed above (Wang *et al.*, 2015), it was shown that caveolin-1 is involved in this pathway. Knockdown of caveolin-1 blocked the effect of $G\alpha_q$ on $K_{ir}4.1$, whereas knockdown of the clathrin heavy chain had no effect (Cha *et al.*, 2011).

We have reported recently that dopamine, a critical regulator of systemic blood pressure, reversibly decreased the P_o of both homomeric $K_{ir}4.1$ and heteromeric $K_{ir}4.1/K_{ir}5.1$ channels (Zaika *et al.*, 2013). This effect was mediated by D2-like, but not D1-like dopamine receptors, and PKC blockade abolished the inhibition of these channels by dopamine. Importantly, dopamine induced an acute depolarization of basolateral membrane potential, as directly monitored using current-clamp mode in isolated CCDs (Fig. 6A). We also demonstrated that insulin and insulin-like growth factor-1 (IGF-1) acutely activate single channel $K_{ir}4.1/K_{ir}5.1$ P_o, which results in a respective increase in whole cell K^+ currents (Fig. 5C). Inhibition of phosphoinositide 3-kinase eliminates actions of both insulin and IGF-1 on $K_{ir}4.1/K_{ir}5.1$. Importantly, this regulation is capable of controlling basolateral membrane voltage in the CCD (Fig. 6A) (Zaika *et al.*, 2016a). Therefore, insulin and IGF-1 increase the electrochemical driving force for Na^+ reabsorption via its effects on $K_{ir}4.1/K_{ir}5.1$, which further facilitate ENaC-mediated Na^+ absorption as well as Cl^- and K^+ transports in the CCD (Frindt and Palmer, 2012, Pavlov *et al.*, 2013a, Zaika *et al.*, 2015).

Of special interest is the sensitivity of basolateral K^+ channels to inhibition by cell acidification. Even small changes in pH_i, resulting in intracellular acidification, could result in decreased basolateral K^+ conductance, depolarization of the basolateral membrane, and consecutive changes in Na^+ and Cl^- transport. Multiple studies reported that the basolateral K^+ channel, and specifically $K_{ir}4.1$ and $K_{ir}5.1$ are sensitive to changes in cell pH (Lourdel *et al.*, 2002, Paulais *et al.*, 2011). Interestingly, it was shown $K_{ir}5.1$ also serves as an important determinant of neuronal PCO_2/pH sensitivity (D'Adamo *et al.*, 2011, Trapp *et al.*, 2011). Another potential critical regulator of $K_{ir}4.1$, reported in the brain, is DNA methylation (Nwaobi *et al.*, 2014, Nwaobi and Olsen, 2015). However, no data relevant to DNA methylation of either $K_{ir}4.1$ or $K_{ir}5.1$ in the kidney is reported yet.

As it was recently summarized by Ellison et al., there is direct link between the dietary K^+ intake and activity of NCC cotransporter in DCT (Ellison *et al.*, 2016). Sorensen et al. reported that potassium intake followed by a significant rise of plasma aldosterone, and enhanced urinary K^+ and Na^+ excretion, which were accompanied by a rapid and sustained dephosphorylation of NCC and upregulation of proteolytically activated ENaC (Sorensen *et al.*, 2013). Recent data suggest that changes in plasma K^+ concentration signals to NCC by altering intracellular Cl^- concentrations in the DCT (Terker *et al.*, 2015, Bazúa-Valenti *et al.*, 2015). When plasma K^+ concentration is increased, intracellular Cl^- concentration is also high, WNK lysine deficient protein kinases (WNKs) are turned off, and NCC is suppressed (see also Fig. 1). In contrast, when dietary potassium intake, and plasma K^+ concentration, respectively, are decreased, WNKs phosphorylate the downstream kinases Ste20p-related proline alanine-rich kinase (SPAK), and oxidative stress response 1 kinase (OxSR1) to stimulate activity of NCC (Terker *et al.*, 2015). Therefore, activation of NCC results in reduced delivery of Na^+ to the CD, and K^+ secretion, respectively. As it was discussed above, one of the primary functions of basolateral $K_{ir}4.1/K_{ir}5.1$ channels is to recycle K^+ across the basolateral membrane for proper function of the Na^+-K^+-ATPase, among many other functions. Changes in K^+ conductance affect membrane potential, and therefore modulate intracellular Cl^- concentration. Thus, $K_{ir}4.1/K_{ir}5.1$ channels play an important role in the described above pathway. In line with this, Zhang et al. reported that $K_{ir}4.1$ determines the expression of the NCC cotransporter in the DCT. Using the $K_{ir}4.1^{-/-}$ mice, they demonstrated that the disruption of $K_{ir}4.1$ decreased the basolateral Cl^- conductance, and diminished the apical NCC expression in DCT (Zhang *et al.*, 2014). However, it should be taken into account that $Kcnj10^{-/-}$ mice could not survive more than 2 weeks after the birth (Kofuji *et al.*, 2000); therefore, in this manuscript, as well as in some other studies where $Kcnj10^{-/-}$ mice were utilized, experiments were conducted in mice at only a few days postnatal, when the kidney is not fully developed.

Summary and conclusions

In this review, we examined the role of basolateral epithelial K^+ channels in the DCT and CCD. As described above, there are numerous interactions between dietary K^+ intake, and the control of electrolyte homeostasis and blood pressure regulation, respectively. It is clear that basolateral epithelial K^+ channels play vital roles in the kidney electrolyte homeostasis. While there is not much information available, so far, this is an emerging and growing field of research. More molecular and animal studies are clearly needed to increase our knowledge about the function of these channels in the kidney. This is especially important for identification of regulatory mechanism of the basolateral K^+ channels, which is poorly understood. For instance, there is still missing link in the chain of events relating increases in interstitial K^+ concentration to inhibition of NCC activity in DCT although plausible steps include membrane potential to intracellular Cl^- concentration to WNK 1/4 to SPARK and OxSR1 to NCC phosphorylation. Furthermore, additional mechanistic studies are required to precisely uncover molecular mechanisms controlling these channels in CDs. It is expected that novel pharmacological tools modulating basolateral K^+ channels, and especially Kir4.1 and Kir5.1, will be developed. Targeting these channels may be attractive for treatment of

hypertension as well as some renal diseases. Therefore, these channels warrant further studies to uncover their role in cardiorenal physiology.

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References

Alewine C, Kim BY, Hegde V, Welling PA. Lin-7 targets the Kir 2.3 channel on the basolateral membrane via a L27 domain interaction with CASK. Am J Physiol Cell Physiol. 2007; 293:C1733–1741. [PubMed: 17913842]

Alewine C, Olsen O, Wade JB, Welling PA. TIP-1 has PDZ scaffold antagonist activity. Mol Biol Cell. 2006; 17:4200–4211. [PubMed: 16855024]

Bazúa-Valenti S, Chávez-Canales M, Rojas-Vega L, González-Rodríguez X, Vázquez N, Rodríguez-Gama A, Argaiz ER, Melo Z, Plata C, Ellison DH, García-Valdés J, Hadchouel J, Gamba G. The effect of WNK4 on the Na^+–Cl^- cotransporter is modulated by intracellular chloride. J Am Soc Nephrol. 2015; 26:1781–1786. [PubMed: 25542968]

Bhave G, Chauder BA, Liu W, Dawson ES, Kadakia R, Nguyen TT, Lewis LM, Meiler J, Weaver CD, Satlin LM, Lindsley CW, Denton JS. Development of a selective small-molecule inhibitor of Kir1.1, the renal outer medullary potassium channel. Mol Pharmacol. 2011; 79:42–50. [PubMed: 20926757]

Bockenhauer D, Feather S, Stanescu HC, Bandulik S, Zdebik AA, Reichold M, Tobin J, Lieberer E, Sterner C, Landoure G, Arora R, Sirimanna T, Thompson D, Cross JH, van't Hoff W, Al Masri O, et al. Epilepsy, ataxia, sensorineural deafness, tubulopathy, and KCNJ10 mutations. N Engl J Med. 2009; 360:1960–1970. [PubMed: 19420365]

Boim MA, Ho K, Shuck ME, Bienkowski MJ, Block JH, Slightom JL, Yang Y, Brenner BM, Hebert SC. ROMK inwardly rectifying ATP-sensitive K^+ channel. II. Cloning and distribution of alternative forms. Am J Physiol. 1995; 268:F1132–1140. [PubMed: 7611454]

Canessa CM, Horisberger JD, Rossier BC. Epithelial sodium channel related to proteins involved in neurodegeneration. Nature. 1993; 361:467–470. [PubMed: 8381523]

Canessa CM, Schild L, Buell G, Thorens B, Gautschi I, Horisberger JD, Rossier BC. Amiloride-sensitive epithelial Na^+ channel is made of three homologous subunits. Nature. 1994; 367:463–467. [PubMed: 8107805]

Cha SK, Huang C, Ding Y, Qi X, Huang CL, Miller RT. Calcium-sensing receptor decreases cell surface expression of the inwardly rectifying K^+ channel Kir4.1. J Biol Chem. 2011; 286:1828–1835. [PubMed: 21084311]

Ciampolillo F, McCoy DE, Green RB, Karlson KH, Dagenais A, Molday RS, Stanton BA. Cell-specific expression of amiloride-sensitive, Na^+-conducting ion channels in the kidney. Am J Physiol. 1996; 271:C1303–1315. [PubMed: 8897838]

D'Adamo MC, Shang L, Imbrici P, Brown SD, Pessia M, Tucker SJ. Genetic inactivation of Kcnj16 identifies Kir5.1 as an important determinant of neuronal PCO_2/pH sensitivity. J Biol Chem. 2011; 286:192–198. [PubMed: 21047793]

Demirkan A, Henneman P, Verhoeven A, Dharuri H, Amin N, van Klinken JB, Karssen LC, de Vries B, Meissner A, Göraler S, van den Maagdenberg AMJM, Deelder AM, C't Hoen PA, van Duijn CM, van Dijk KW. Insight in genome-wide association of metabolite quantitative traits by exome sequence analyses. PLoS Genet. 2015; 11:e1004835. [PubMed: 25569235]

Derst C, Hirsch JR, Preisig-Muller R, Wischmeyer E, Karschin A, Doring F, Thomzig A, Veh RW, Schlatter E, Kummer W, Daut J. Cellular localization of the potassium channel Kir7.1 in guinea pig and human kidney. Kidney Int. 2001; 59:2197–2205. [PubMed: 11380822]

Dong K, Yan Q, Lu M, Wan L, Hu H, Guo J, Boulpaep E, Wang W, Giebisch G, Hebert SC, Wang T. Romk1 knockout mice do not produce Bartter phenotype but exhibit impaired K excretion. J Biol Chem. 2016; 291:5259–5269. [PubMed: 26728465]

Ellison DH, Terker AS, Gamba G. Potassium and its discontents: new insight, new treatments. J Am Soc Nephrol. 2016; 27:981–989. [PubMed: 26510885]

Freudenthal B, Kulaveerasingam D, Lingappa L, Shah MA, Brueton L, Wassmer E, Ognjanovic M, Dorison N, Reichold M, Bockenhauer D, Kleta R, Zdebik AA. KCNJ10 mutations disrupt function in patients with EAST syndrome. Nephron Physiol. 2011; 119:p40–p48. [PubMed: 21849804]

Frindt G, Palmer LG. Effects of insulin on Na and K transporters in the rat CCD. Am J Physiol Renal Physiol. 2012; 302:F1227–F1233. [PubMed: 22357918]

Furutani K, Ohno Y, Inanobe A, Hibino H, Kurachi Y. Mutational and in silico analyses for antidepressant block of astroglial inward-rectifier Kir4.1 channel. Mol Pharmacol. 2009; 75:1287–1295. [PubMed: 19264848]

Garcia-Filho E, Malnic G, Giebisch G. Effects of changes in electrical potential difference on tubular potassium transport. Am J Physiol. 1980; 238:F235–246. [PubMed: 7369366]

González-Núñez D, Morales-Ruiz M, Leivas A, Hebert SC, Poch E. In vitro characterization of aldosterone and cAMP effects in mouse distal convoluted tubule cells. Am J Physiol Renal Physiol. 2004; 286:F936–F944. [PubMed: 15075189]

Gray DA, Frindt G, Zhang YY, Palmer LG. Basolateral K^+ conductance in principal cells of rat CCD. Am J Physiol Renal Physiol. 2005; 288:F493–F504. [PubMed: 15547117]

Hamilton KL, Devor DC. Basolateral membrane K^+ channels in renal epithelial cells. Am J Physiol Renal Physiol. 2012; 302:F1069–1081. [PubMed: 22338089]

Hirsch J, Schlatter E. K^+ channels in the basolateral membrane of rat cortical collecting duct. Pflugers Arch. 1993; 424:470–477. [PubMed: 7504809]

Huang C, Sindic A, Hill CE, Hujer KM, Chan KW, Sassen M, Wu Z, Kurachi Y, Nielsen S, Romero MF, Miller RT. Interaction of the Ca^{2+}-sensing receptor with the inwardly rectifying potassium channels Kir4.1 and Kir4.2 results in inhibition of channel function. Am J Physiol Renal Physiol. 2007; 292:F1073–F1081. [PubMed: 17122384]

Ito M, Inanobe A, Horio Y, Hibino H, Isomoto S, Ito H, Mori K, Tonosaki A, Tomoike H, Kurachi Y. Immunolocalization of an inwardly rectifying K^+ channel, K(AB)-2 (Kir4.1), in the basolateral membrane of renal distal tubular epithelia. FEBS Lett. 1996; 388:11–15. [PubMed: 8654579]

Juang JM, Lu TP, Lai LC, Ho CC, Liu YB, Tsai CT, Lin LY, Yu CC, Chen WJ, Chiang FT, Yeh SF, Lai LP, Chuang EY, Lin JL. Disease-targeted sequencing of ion channel genes identifies de novo mutations in patients with non-familial Brugada syndrome. Sci Rep. 2014; 4:6733. [PubMed: 25339316]

Katz B. Les constantes electriques de la membrane du muscle. Arch Sci Physiol. 1949; 2:285–299.

Kieferle S, Fong P, Bens M, Vandewalle A, Jentsch TJ. Two highly homologous members of the ClC chloride channel family in both rat and human kidney. Proc Natl Acad Sci U S A. 1994; 91:6943–6947. [PubMed: 8041726]

Kofuji P, Ceelen P, Zahs KR, Surbeck LW, Lester HA, Newman EA. Genetic inactivation of an inwardly rectifying potassium channel (Kir4.1 subunit) in mice: phenotypic impact in retina. J Neurosci. 2000; 20:5733–5740. [PubMed: 10908613]

Koster JC, Bentle KA, Nichols CG, Ho K. Assembly of ROMK1 (Kir 1.1a) inward rectifier K^+ channel subunits involves multiple interaction sites. Biophys J. 1998; 74:1821–1829. [PubMed: 9545044]

Lachheb S, Cluzeaud Fó, Bens M, Genete M, Hibino H, Lourdel Sp, Kurachi Y, Vandewalle A, Teulon J, Paulais M. Kir4.1/Kir5.1 channel forms the major K^+ channel in the basolateral membrane of mouse renal collecting duct principal cells. Am J Physiol Renal Physiol. 2008; 294:F1398–F1407. [PubMed: 18367659]

Larsen CK, Jensen IS, Sorensen MV, de Bruijn PI, Bleich M, Praetorius HA, Leipziger J. Hyperaldosteronism following decreased renal K^+ excretion in KCNMB2 knock-out mice. Am J Physiol Renal Physiol. 2016; doi: 10.1152/ajprenal.00010.2016

Le Maout S, Brejon M, Olsen O, Merot J, Welling PA. Basolateral membrane targeting of a renal-epithelial inwardly rectifying potassium channel from the cortical collecting duct, CCD-IRK3, in MDCK cells. Proc Natl Acad Sci U S A. 1997; 94:13329–13334. [PubMed: 9371845]

Le Maout S, Welling PA, Brejon M, Olsen O, Merot J. Basolateral membrane expression of a K^+ channel, Kir 2.3, is directed by a cytoplasmic COOH-terminal domain. Proc Natl Acad Sci U S A. 2001; 98:10475–10480. [PubMed: 11504929]

Lee WS, Hebert SC. ROMK inwardly rectifying ATP-sensitive K^+ channel. I. Expression in rat distal nephron segments. Am J Physiol. 1995; 268:F1124–1131. [PubMed: 7611453]

Lin DH, Sterling H, Yang B, Hebert SC, Giebisch G, Wang WH. Protein tyrosine kinase is expressed and regulates ROMK1 location in the cortical collecting duct. Am J Physiol Renal Physiol. 2004; 286:F881–F892. [PubMed: 15075184]

Lin DH, Yue P, Rinehart J, Sun P, Wang Z, Lifton R, Wang WH. Protein phosphatase 1 modulates the inhibitory effect of With-no-Lysine kinase 4 on ROMK channels. Am J Physiol Renal Physiol. 2012; 303:F110–F119. [PubMed: 22513846]

Lin DH, Yue P, Yarborough O, Scholl UI, Giebisch G, Lifton RP, Rinehart J, Wang WH. Src-family protein tyrosine kinase phosphorylates WNK4 and modulates its inhibitory effect on KCNJ1 (ROMK). Proc Natl Acad Sci U S A. 2015; 112:4495–4500. [PubMed: 25805816]

Liu BC, Yang LL, Lu XY, Song X, Li XC, Chen G, Li Y, Yao X, Humphrey DR, Eaton DC, Shen BZ, Ma HP. Lovastatin-induced phosphatidylinositol-4-phosphate 5-kinase diffusion from microvilli stimulates ROMK channels. J Am Soc Nephrol. 2015; 26:1576–1587. [PubMed: 25349201]

Lourdel S, Paulais M, Cluzeaud F, Bens M, Tanemoto M, Kurachi Y, Vandewalle A, Teulon J. An inward rectifier K^+ channel at the basolateral membrane of the mouse distal convoluted tubule: similarities with Kir4-Kir5.1 heteromeric channels. J Physiol. 2002; 538:391–404. [PubMed: 11790808]

Lu M, Giebisch G, Wang W. Nitric oxide-induced hyperpolarization stimulates low-conductance Na^+ channel of rat CCD. Am J Physiol Renal Physiol. 1997a; 272:F498–F504.

Lu M, Giebisch G, Wang W. Nitric oxide links the apical Na^+ transport to the basolateral K^+ conductance in the rat cortical collecting duct. J Gen Physiol. 1997b; 110:717–726. [PubMed: 9382898]

Lu M, Wang W. Protein kinase C stimulates the small-conductance K^+ channel in the basolateral membrane of the CCD. Am J Physiol. 1996a; 271:F1045–1051. [PubMed: 8945999]

Lu M, Wang WH. Nitric oxide regulates the low-conductance K^+ channel in basolateral membrane of cortical collecting duct. Am J Physiol. 1996b; 270:C1336–1342. [PubMed: 8967433]

McCormick JA, Ellison DH. Distal convoluted tubule. Compr Physiol. 2015; 5:45–98. [PubMed: 25589264]

McDonough AA, Geering K, Farley RA. The sodium pump needs its beta subunit. FASEB J. 1990; 4:1598–1605. [PubMed: 2156741]

Méndez-González MP, Kucheryavykh YV, Zayas-Santiago A, Vélez-Carrasco W, Maldonado-Martínez G, Cubano LA, Nichols CG, Skatchkov SN, Eaton MJ. Novel KCNJ10 variations compromise function of inwardly rectifying potassium channel 4.1. J Biol Chem. 2016; 291:7716–7726. [PubMed: 26867573]

Millar ID, Taylor HC, Cooper GJ, Kibble JD, Robson L. A $K_{ir}2.3$-like K^+ conductance in mouse cortical collecting duct principal cells. J Membr Biol. 2006; 211:173–184. [PubMed: 17091215]

Nichols CG, Lopatin AN. Inward rectifier potassium channels. Annu Rev Physiol. 1997; 59:171–191. [PubMed: 9074760]

Nwaobi SE, Lin E, Peramsetty SR, Olsen ML. DNA methylation functions as a critical regulator of Kir4.1 expression during CNS development. Glia. 2014; 62:411–427. [PubMed: 24415225]

Nwaobi SE, Olsen ML. Correlating gene-specific DNA methylation changes with expression and transcriptional activity of astrocytic KCNJ10 (Kir4.1). J Vis Exp. 2015; 103:e52406.

Ohno Y, Hibino H, Lossin C, Inanobe A, Kurachi Y. Inhibition of astroglial Kir4.1 channels by selective serotonin reuptake inhibitors. Brain Res. 2007; 1178:44–51. [PubMed: 17920044]

Olsen O, Liu H, Wade JB, Merot J, Welling PA. Basolateral membrane expression of the Kir2.3 channel is coordinated by PDZ interaction with Lin-7/CASK complex. Am J Physiol Cell Physiol. 2002; 282:C183–195. [PubMed: 11742811]

Ookata K, Tojo A, Suzuki Y, Nakamura N, Kimura K, Wilcox CS, Hirose S. Localization of inward rectifier potassium channel Kir7.1 in the basolateral membrane of distal nephron and collecting duct. J Am Soc Nephrol. 2000; 11:1987–1994. [PubMed: 11053473]

Palygin O, Levchenko V, Ilatovskaya DV, Barnett JL, Geurts AM, Jacob HJ, Staruschenko A. Renal phenotype of inwardly rectifying potassium channel Kcnj16 (K_{ir}5.1) knockout in the Dahl salt-sensitive rats. Hypertension. 2015; 66:A113.

Parrock S, Hussain S, Issler N, Differ AM, Lench N, Guarino S, Oosterveld MJS, Keijzer-Veen M, Brilstra E, van Wieringen H, Konijnenberg AY, Amin-Rasip S, Dumitriu S, Klootwijk E, Knoers N, Bockenhauer D, et al. KCNJ10 mutations display differential sensitivity to heteromerisation with KCNJ16. Nephron Physiol. 2013; 123:7–14. [PubMed: 24193250]

Paulais M, Bloch-Faure M, Picard N, Jacques T, Ramakrishnan SK, Keck M, Sohet F, Eladari D, Houillier P, Lourdel Sp, Teulon J, Tucker SJ. Renal phenotype in mice lacking the Kir5.1 (Kcnj16) K^+ channel subunit contrasts with that observed in SeSAME/EAST syndrome. Proc Natl Acad Sci U S A. 2011; 108:10361–10366. [PubMed: 21633011]

Pavlov TS, Ilatovskaya DV, Levchenko V, Li L, Ecelbarger CM, Staruschenko A. Regulation of ENaC in mice lacking renal insulin receptors in the collecting duct. FASEB J. 2013a; 27:2723–2732. [PubMed: 23558339]

Pavlov TS, Levchenko V, O'Connor PM, Ilatovskaya DV, Palygin O, Mori T, Mattson DL, Sorokin A, Lombard JH, Cowley AW Jr, Staruschenko A. Deficiency of renal cortical EGF increases ENaC activity and contributes to salt-sensitive hypertension. J Am Soc Nephrol. 2013b; 24:1053–1062. [PubMed: 23599382]

Persson PB. Good publication practice in physiology 2015. Acta Physiol. 2015; 215:163–164.

Pessia M, Imbrici P, D'Adamo MC, Salvatore L, Tucker SJ. Differential pH sensitivity of Kir4.1 and Kir4.2 potassium channels and their modulation by heteropolymerisation with Kir5.1. J Physiol. 2001; 532:359–367. [PubMed: 11306656]

Pessia M, Tucker SJ, Lee K, Bond CT, Adelman JP. Subunit positional effects revealed by novel heteromeric inwardly rectifying K^+ channels. EMBO J. 1996; 15:2980–2987. [PubMed: 8670799]

Pluznick JL, Wei P, Grimm PR, Sansom SC. BK-β1 subunit: immunolocalization in the mammalian connecting tubule and its role in the kaliuretic response to volume expansion. Am J Physiol Renal Physiol. 2005; 288:F846–F854. [PubMed: 15613616]

Raphemot R, Lonergan DF, Nguyen TT, Utley T, Lewis LM, Kadakia R, Weaver CD, Gogliotti R, Hopkins C, Lindsley CW, Denton JS. Discovery, characterization, and structure-activity relationships of an inhibitor of inward rectifier potassium (Kir) channels with preference for Kir2.3, Kir3.x, and Kir7.1. Front Pharmacol. 2011; 2:75. [PubMed: 22275899]

Reichold M, Zdebik AA, Lieberer E, Rapedius M, Schmidt K, Bandulik S, Sterner C, Tegtmeier I, Penton D, Baukrowitz T, Hulton SA, Witzgall R, Ben-Zeev B, Howie AJ, Kleta R, Bockenhauer D, et al. KCNJ10 gene mutations causing EAST syndrome (epilepsy, ataxia, sensorineural deafness, and tubulopathy) disrupt channel function. Proc Natl Acad Sci U S A. 2010; 107:14490–14495. [PubMed: 20651251]

Rieg T, Vallon V, Sausbier M, Sausbier U, Kaissling B, Ruth P, Osswald H. The role of the BK channel in potassium homeostasis and flow-induced renal potassium excretion. Kidney Int. 2007; 72:566–573. [PubMed: 17579662]

Rubera I, Loffing J, Palmer LG, Frindt G, Fowler-Jaeger N, Sauter D, Carroll T, McMahon A, Hummler E, Rossier BC. Collecting duct-specific gene inactivation of αENaC in the mouse kidney does not impair sodium and potassium balance. J Clin Invest. 2003; 112:554–565. [PubMed: 12925696]

Sala-Rabanal M, Kucheryavykh LY, Skatchkov SN, Eaton MJ, Nichols CG. Molecular mechanisms of EAST/SeSAME syndrome mutations in Kir4.1 (KCNJ10). J Biol Chem. 2010; 285:36040–36048. [PubMed: 20807765]

Schlatter E, Lohrmann E, Greger R. Properties of the potassium conductances of principal cells of rat cortical collecting ducts. Pflugers Arch. 1992; 420:39–45. [PubMed: 1553262]

Scholl UI, Choi M, Liu T, Ramaekers VT, Hausler MG, Grimmer J, Tobe SW, Farhi A, Nelson-Williams C, Lifton RP. Seizures, sensorineural deafness, ataxia, mental retardation, and electrolyte

imbalance (SeSAME syndrome) caused by mutations in KCNJ10. Proc Natl Acad Sci U S A. 2009; 106:5842–5847. [PubMed: 19289823]

Sepúlveda FV, Pablo Cid L, Teulon J, Niemeyer MI. Molecular aspects of structure, gating, and physiology of pH-sensitive background K2P and K_{ir} K^+-transport channels. 2015; 95:179–217.

Slaats GG, Wheway G, Foletto V, Szymanska K, van Balkom BWM, Logister I, Den Ouden K, Keijzer-Veen MG, Lilien MR, Knoers NV, Johnson CA, Giles RH. Screen-based identification and validation of four new ion channels as regulators of renal ciliogenesis. J Cell Sci. 2015; 128:4550–4559. [PubMed: 26546361]

Sorensen MV, Grossmann S, Roesinger M, Gresko N, Todkar AP, Barmettler G, Ziegler U, Odermatt A, Loffing-Cueni D, Loffing J. Rapid dephosphorylation of the renal sodium chloride cotransporter in response to oral potassium intake in mice. Kidney Int. 2013; 83:811–824. [PubMed: 23447069]

Staruschenko A. Regulation of transport in the connecting tubule and cortical collecting duct. Compreh Physiol. 2012; 2:1541–1584.

Su S, Ohno Y, Lossin C, Hibino H, Inanobe A, Kurachi Y. Inhibition of astroglial inwardly rectifying Kir4.1 channels by a tricyclic antidepressant, nortriptyline. J Pharmacol Exp Ther. 2007; 320:573–580. [PubMed: 17071817]

Su X-T, Zhang C, Wang L, Gu R, Lin D-H, Wang W-H. The disruption of KCNJ10 (Kir4.1) stimulates the expression of ENaC in the collecting duct. Am J Physiol Renal Physiol. 2016; doi: 10.1152/ajprenal.00584.2015

Subramanya AR, Ellison DH. Distal convoluted tubule. Clin J Am Soc Nephrol. 2014; 9:2147–2163. [PubMed: 24855283]

Takumi T, Ishii T, Horio Y, Morishige KI, Takahashi N, Yamada M, Yamashita T, Kiyama H, Sohmiya K, Nakanishi S, Kurachi Y. A novel ATP-dependent inward rectifier potassium channel expressed predominantly in glial cells. J Biol Chem. 1995; 270:16339–16346. [PubMed: 7608203]

Tanemoto M. Regulatory mechanism of "K^+-recycling" for Na^+-reabsorption in renal tubules. Clin Exp Nephrol. 2007; 11:1–6. [PubMed: 17384992]

Tanemoto M, Abe T, Uchida S, Kawahara K. Mislocalization of K^+ channels causes the renal salt wasting in EAST/SeSAME syndrome. FEBS Lett. 2014; 588:899–905. [PubMed: 24561201]

Tanemoto M, Fujita A, Higashi K, Kurachi Y. PSD-95 mediates formation of a functional homomeric K_{ir}5.1 channel in the brain. Neuron. 2002; 34:387–397. [PubMed: 11988170]

Tang X, Hang D, Sand A, Kofuji P. Variable loss of Kir4.1 channel function in SeSAME syndrome mutations. Biochem Biophys Res Comm. 2010; 399:537–541. [PubMed: 20678478]

Taniguchi J, Yoshitomi K, Imai M. K^+ channel currents in basolateral membrane of distal convoluted tubule of rabbit kidney. Am J Physiol. 1989; 256:F246–254. [PubMed: 2916658]

Terker AS, Zhang C, McCormick JA, Lazelle RA, Zhang C, Meermeier NP, Siler DA, Park HJ, Fu Y, Cohen DM, Weinstein AM, Wang WH, Yang CL, Ellison DH. Potassium modulates electrolyte balance and blood pressure through effects on distal cell voltage and chloride. Cell Metab. 2015; 21:39–50. [PubMed: 25565204]

Trapp S, Tucker SJ, Gourine AV. Respiratory responses to hypercapnia and hypoxia in mice with genetic ablation of Kir5.1 (Kcnj16). Exp Physiol. 2011; 96:451–459. [PubMed: 21239463]

Tucker SJ, Imbrici P, Salvatore L, D'Adamo MC, Pessia M. pH dependence of the inwardly rectifying potassium channel, Kir5.1, and localization in renal tubular epithelia. J Biol Chem. 2000; 275:16404–16407. [PubMed: 10764726]

Wang L, Zhang C, Su X, Lin DH, Wang W. Caveolin-1 deficiency inhibits the basolateral K^+ channels in the distal convoluted tubule and impairs renal K^+ and Mg^{2+} transport. J Am Soc Nephrol. 2015; 26:2678–2690. [PubMed: 25848073]

Wang W, Hebert SC, Giebisch G. Renal K^+ channels: structure and function. Annu Rev Physiol. 1997; 59:413–436. [PubMed: 9074771]

Wang WH. Regulation of the hyperpolarization-activated K^+ channel in the lateral membrane of the cortical collecting duct. J Gen Physiol. 1995; 106:25–43. [PubMed: 7494137]

Wang WH, Giebisch G. Regulation of potassium (K) handling in the renal collecting duct. Pflugers Arch. 2009; 458:157–168. [PubMed: 18839206]

Wang WH, McNicholas CM, Segal AS, Giebisch G. A novel approach allows identification of K channels in the lateral membrane of rat CCD. Am J Physiol. 1994; 266:F813–822. [PubMed: 8203566]

Wei Y, Liao Y, Zavilowitz B, Ren J, Liu W, Chan P, Rohatgi R, Estilo G, Jackson EK, Wang WH, Satlin LM. Angiotensin II type 2 receptor regulates ROMK-like K^+ channel activity in the renal cortical collecting duct during high dietary K^+ adaptation. Am J Physiol Renal Physiol. 2014; 307:F833–843. [PubMed: 25100281]

Welling PA. Primary structure and functional expression of a cortical collecting duct Kir channel. Am J Physiol. 1997; 273:F825–836. [PubMed: 9374848]

Welling PA. Roles and regulation of renal K channels. Annu Rev Physiol. 2016; 78:415–435. [PubMed: 26654186]

Wen D, Cornelius RJ, Rivero-Hernandez D, Yuan Y, Li H, Weinstein AM, Sansom SC. Relation between BK-α/β4-mediated potassium secretion and ENaC-mediated sodium reabsorption. Kidney Int. 2014; 86:139–145. [PubMed: 24573316]

Williams DM, Lopes CMB, Rosenhouse-Dantsker A, Connelly HL, Matavel A, O-Uchi J, McBeath E, Gray DA. Molecular basis of decreased Kir4.1 function in SeSAME/EAST syndrome. J Am Soc Nephrol. 2010; 21:2117–2129. [PubMed: 21088294]

Woda CB, Bragin A, Kleyman TR, Satlin LM. Flow-dependent K^+ secretion in the cortical collecting duct is mediated by a maxi-K channel. Am J Physiol Renal Physiol. 2001; 280:F786–F793. [PubMed: 11292620]

Woda CB, Leite M Jr, Rohatgi R, Satlin LM. Effects of luminal flow and nucleotides on $[Ca^{2+}]_i$ in rabbit cortical collecting duct. Am J Physiol Renal Physiol. 2002; 283:F437–F446. [PubMed: 12167594]

Zaika O, Mamenko M, Boukelmoune N, Pochynyuk O. IGF-1 and insulin exert opposite actions on ClC-K2 activity in the cortical collecting ducts. Am J Physiol Renal Physiol. 2015; 308:F39–F48. [PubMed: 25339702]

Zaika O, Palygin O, Tomilin V, Mamenko M, Staruschenko A, Pochynyuk O. Insulin and IGF-1 activate Kir4.1/5.1 channels in cortical collecting duct principal cells to control basolateral membrane voltage. Am J Physiol Renal Physiol. 2016a; 310:F311–321. [PubMed: 26632606]

Zaika O, Tomilin V, Mamenko M, Bhalla V, Pochynyuk O. New perspective of ClC-Kb/2 chloride channel physiology in the distal renal tubule. Am J Physiol Renal Physiol. 2016b; doi: 10.1152/ajprenal.00577.2015

Zaika OL, Mamenko M, Palygin O, Boukelmoune N, Staruschenko A, Pochynyuk O. Direct inhibition of basolateral Kir4.1/5.1 and Kir4.1 channels in the cortical collecting duct by dopamine. Am J Physiol Renal Physiol. 2013; 305:F1277–1287. [PubMed: 23986512]

Zhang C, Wang L, Su XT, Lin DH, Wang WH. KCNJ10 (Kir4.1) is expressed in the basolateral membrane of the cortical thick ascending limb. Am J Physiol Renal Physiol. 2015; 308:F1288–F1296. [PubMed: 25834074]

Zhang C, Wang L, Thomas S, Wang K, Lin DH, Rinehart J, Wang WH. Src family protein tyrosine kinase regulates the basolateral K channel in the distal convoluted tubule (DCT) by phosphorylation of KCNJ10 protein. J Biol Chem. 2013; 288:26135–26146. [PubMed: 23873931]

Zhang C, Wang L, Zhang J, Su XT, Lin DH, Scholl UI, Giebisch G, Lifton RP, Wang WH. KCNJ10 determines the expression of the apical Na-Cl cotransporter (NCC) in the early distal convoluted tubule (DCT1). Proc Natl Acad Sci U S A. 2014; 111:11864–11869. [PubMed: 25071208]

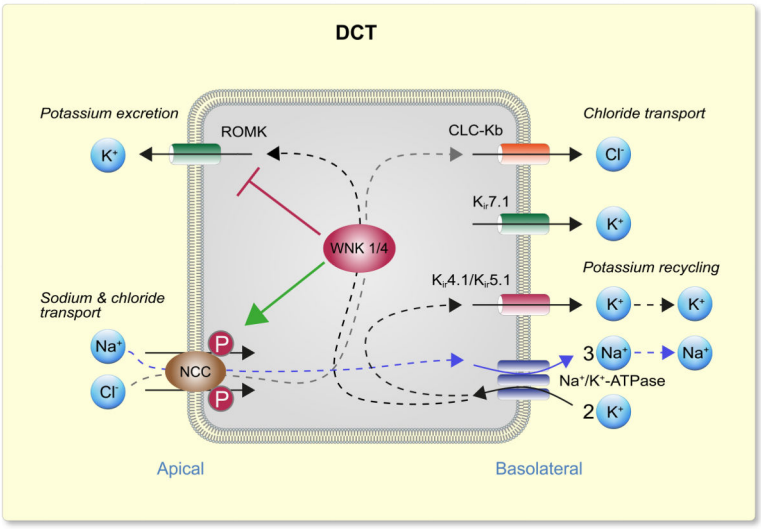

Figure 1.

Model of electrolyte transport in the distal convoluted tubule (DCT). Active electrolyte transport is powered by a Na^+/K^+-ATPase (Na^+/K^+ pump) on the basolateral membrane. In DCT, thiazide-sensitive Na^+-Cl^- cotransporter (NCC) in the apical membrane utilizes the Na^+ electrochemical gradient to drive Cl^- into the epithelial cell against its electrochemical gradient. Cl^- then exits through the basolateral membrane passively via a CLC-K2 (predominantly kidney-specific Cl^- channel). Basolateral $K_{ir}4.1/K_{ir}5.1$ channels recycles K^+ to sustain the Na^+/K^+ pump, and generate driving force for Cl^- and Na^+ transport. In the DCT, WNK (with No Lysine [K] Kinase; discussed below) can stimulate, through activation of downstream kinases, NCC phosphorylation and hence activity. At the same time, WNK kinases can inhibit apical inwardly rectifying channel ROMK ($K_{ir}1.1$) and suppress K^+ excretion. The late distal convoluted tubule (DCT2) expresses both apical Na^+ transport mechanisms: the NCC and amiloride-sensitive Na^+ channel (ENaC, not shown in the figure).

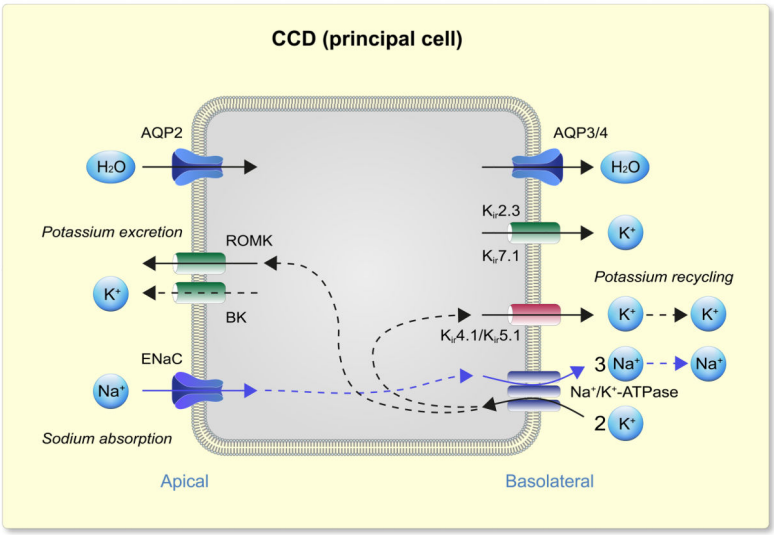

Figure 2.

Model of electrolyte transport in the principal cells of cortical collecting duct (CCD). Basolateral Na^+/K^+-ATPase (Na^+/K^+ pump) create the electrochemical gradient which provides a conductive pathway for Na^+ entry into the cell down through an epithelial Na^+ channel (ENaC) on the apical membrane. The higher permeability of the luminal membrane for Na^+ creates a lumen-negative transepithelial potential difference, and provides an important driving force for the secretion of K^+ into the lumen via apical K^+ channels, apical inwardly rectifying channel ROMK ($K_{ir}1.1$), and the Ca^{2+}-activated K^+ channel (BK). Basolateral membrane K^+ channels (heteromeric $K_{ir}4.1/K_{ir}5.1$ is the main basolateral K_{ir} channel) provide K^+ recycling necessary for Na^+/K^+ pump activity and Na^+ transport in principal cells. Apical aquaporin 2 (AQP2) and basolateral aquaporin 3/4 (AQP3/4) water channels carry H_2O molecules across cell membranes, and are responsible for water transport in the CD.

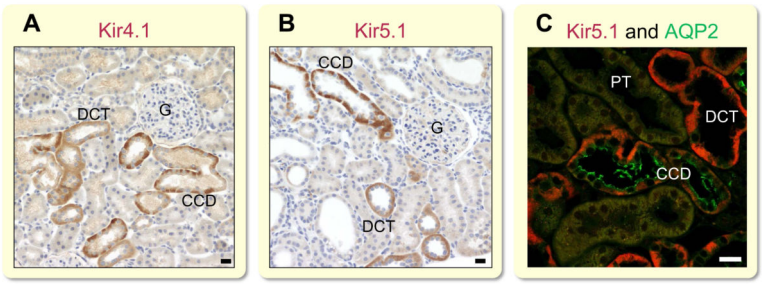

Figure 3.

Immunostaining of $K_{ir}4.1$ and $K_{ir}5.1$ basolateral channels in the renal cortex. Dahl salt-sensitive rats kidney cortex sections stained for $K_{ir}4.1$ (APC-035, Alomone Labs) (**A**) and for $K_{ir}5.1$ (SAB4501636, Sigma) (**B**) shows strong basolateral expression of both proteins. Note absence of protein staining in CD intercalated cells. (**C**) Double staining with $K_{ir}5.1$ (red; SAB4501636, Sigma) and AQP2 (green; sc-28629, Santa Cruz) in Dahl salt-sensitive rats. Immunohistochemical experiments were performed as previously published (Pavlov *et al.*, 2013b) and conform with: Good publication practice in physiology (Persson, 2015). G, glomeruli; PT, proximal tubules; DCT, distal convoluted tubule; CCD, cortical collecting duct. Scale bar is 20 μm.

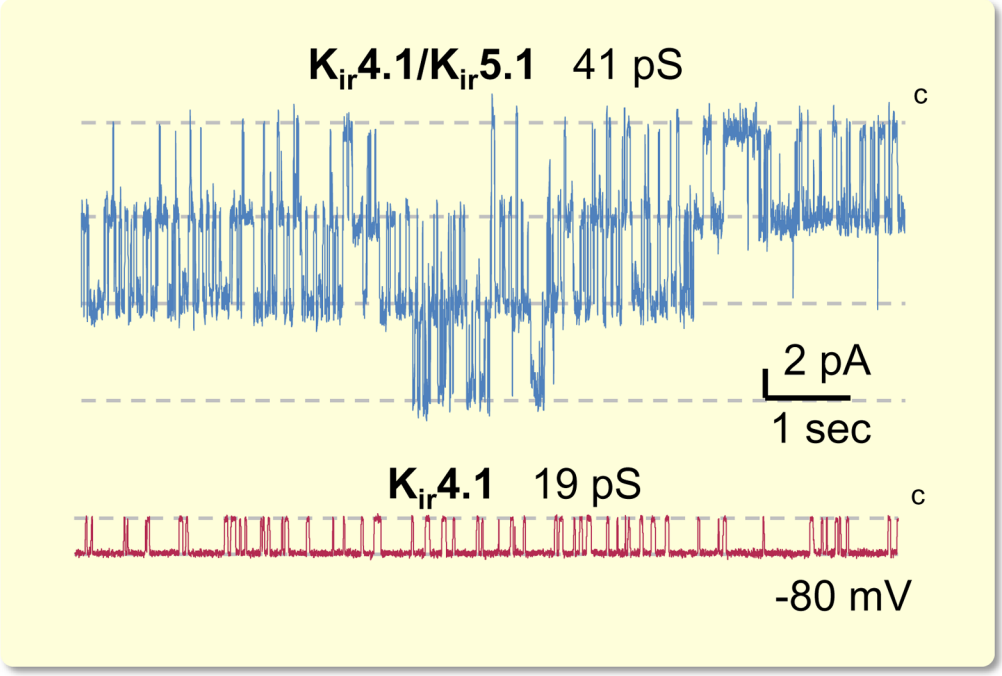

Figure 4.

Single channel recordings of K^+ channels localized on the basolateral membrane of CCD. Shown are representative traces demonstrating activity of highly abundant $K_{ir}4.1/K_{ir}5.1$ heteromeric channel with 41-pS conductance and scarce 19-pS homomeric $K_{ir}4.1$ channel. Cell attached recordings are performed on freshly isolated mice CCDs. Modified from (Zaika *et al.*, 2013) with permission.

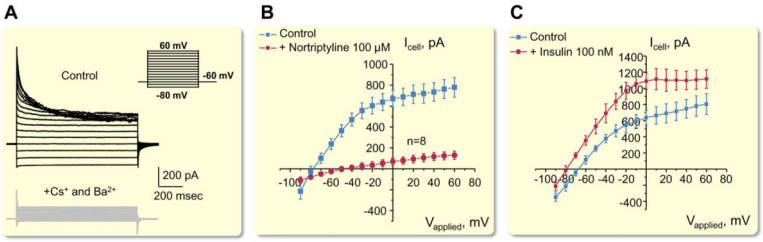

Figure 5.

Biophysical properties of basolateral K_{ir} channels in CCD. (**A**) Macroscopic currents from the basolateral membrane of individual principal cell of CCD isolated from mouse kidney cortex. Equimolar substitution of intracellular K^+ with Cs^+ in the presence of Ba^{2+} in the extracellular medium drastically reduces the amplitude of steady-state current indicating its K_{ir} channel-dependent nature (K_{ir} channels except for $K_{ir}7.1$ are inhibited by Ba^{2+}). (**B**) Inhibition of K^+ macroscopic current with the second-generation tricyclic antidepressant – nortriptyline (100 μM). (**C**) Insulin (and similarly IGF-1) augmented K^+ selective current in principal cells of CCD. Modified from (Zaika *et al.*, 2016a) with permission.

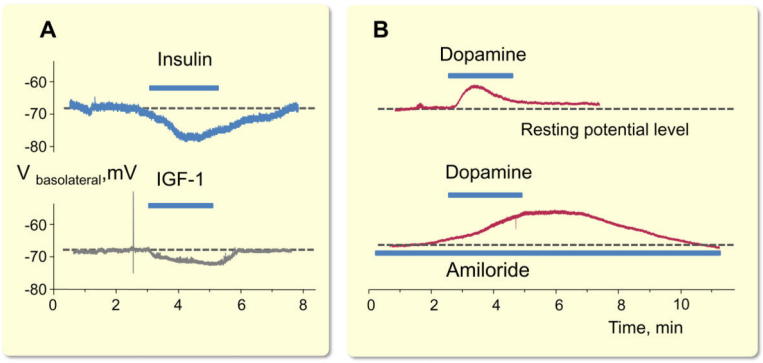

Figure 6.

Pharmacological modulation of basolateral K_{ir} channels activity shifts membrane potential in the cortical collecting duct (CCD). (**A**) Insulin (100 nM) and IGF-1 (500 nM) hyperpolarize basolateral membrane in principal cells by acute increase in open probability of basolateral K^+ channels. (**B**) Dopamine (10 μM) induces acute depolarization of the basolateral membrane via inhibition of $K_{ir}4.1/K_{ir}5.1$ channels. An even greater degree of depolarization is observed when amiloride (2 μM) inhibits ENaC-mediated apical conductance. **A** and **B** modified from (Zaika *et al.*, 2013, Zaika *et al.*, 2016a) with permission.