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Topical Review

Use of Knock-out Mouse Models for the Study of Renal Ion Channels

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Introduction

The development of transgenic mice bearing null mutations of different genes encoding channel proteins has opened a large field of investigation, which allows a better understanding of the physiological mechanisms controlled by these proteins. For instance, a number of ion channels have been identified in the mammalian kidney, but their physiological role remains largely unknown. Therefore, it was interesting to use knock-out (KO) mice for these channels to provide insights into their function under in vitro and in vivo conditions, as well as on the potential compensating mechanisms induced by the KO. Thus, *cftr* –/– mice provided important information on the functional expression of the cystic fibrosis transmembrane conductance regulator (CFTR) as a Cl^- channel as well as a regulatory protein. The data obtained from this model compared with those obtained from wild-type mice have shown that CFTR is clearly a cAMP-activated Cl^- channel in the apical membrane of both the distal convoluted tubule (DCT) (Barriere et al., 2003b) and cortical collecting tubule (CCT) (Barriere et al., 2003b), but not in the proximal convoluted tubule (PCT) (Barriere et al., 2003b) and cortical thick ascending limb (Marvao et al., 1998). In PCT, DCT and CCT cells, CFTR participates in the control of the regulatory volume decrease (RVD) process during hypotonicity by increasing the swelling-activated currents (Barriere et al., 2003c; Belfodil et al., 2003).

In the same way, KO mice showed that mouse kidney contains at least two types of K^+ channels involved in the RVD process: TASK2 and BK channels (Belfodil et al., 2003). This has been confirmed by the study of the swelling-activated K^+ channels in proximal tubule cells from *task*2 –/– mice (Barriere et al., 2003a). In this case, using this mouse, the role of TASK2 both in the RVD process and in bicarbonate reabsorption has been demonstrated (Barriere et al., 2003a; Warth et al., 2002).

Using *kcne1* –/– mice has revealed a role for the KCNE1 subunit in the control of swelling-activated Cl^- and K^+ currents (Barriere et al., 2003c). Unfortunately, the mechanism by which KCNE1 modulates these currents remains unknown. The lack of these currents might explain the origin of the enhanced fractional urinary excretion of Na^+, Cl^-, and glucose in null mice, by preventing electrogenic Na^+-coupled transport of glucose (Vallon et al., 2001).

The generation of transgenic mice is thus an efficient tool to probe the physiological role of some channels expressed in the kidney, but it is also an essential tool for understanding the pathophysiology of channels in the kidney. This is particularly true for ClC and ROMK channels, which participate in some essential renal functions, such as endocytosis, NaCl reabsorption, or urinary concentration in specific nephron segments. Many similarities exist between human renal channel diseases and kidney mouse phenotypes induced by the knock-out of the genes encoding these channels. For instance, the mice lacking ClC5 exhibit an alteration of endocytosis in the proximal tubule that is consistent with the phenotype of patients with Dent's disease (Piwon et al., 2000; Wang et al., 2000). Furthermore, the nephrogenic diabetes insipidus observed in *Clcnk1* –/– mice suggests that mutations in *Clcnka* could be responsible for nephrogenic diabetes insipidus in humans lacking mutations in V2R and AQP2 (Uchida, 2000). Concerning mice lacking ROMK, they exhibit a type-II Bartter's syndrome. This observation supports the hypothesis that ROMK plays a crucial role in K^+ recycling in the tAL, which is required for maximum Na^+ reabsorption via NKCC2, and also shows that a significant amount of Na^+ reabsorption can take place in its absence (Lorenz et al., 2002).

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Finally, mouse models are powerful tools to investigate disease-modifying genes, as well as to give insights into complex pathways involving ion channels in the kidney.

CFTR KO Mice

Cystic fibrosis is a human disease mainly characterized by defective Cl^- secretion in secretory epithelia. Since the discovery of the gene encoding CFTR, an impressive number of studies has been performed to elucidate the role of this protein in the organs affected by the disease. All these studies have converged toward the conclusion that CFTR is a small linear Cl^- channel regulated by cAMP and that the ΔF508 mutation (present in 70% of the CF patients) is associated with a loss of this cAMP sensitivity. However, although the vital role of CFTR is now widely accepted in secretory epithelia, its role in reabsorbing epithelia remains unclear. For example, the kidney of CF patients does not display major changes in renal function. This absence of an obvious renal phenotype is *a priori* paradoxical, because the gene encoding CFTR is abundantly expressed in human kidney. Therefore, the kidney has become an interesting model, since it can be surmised that the function of CFTR is compensated by other Cl^- channels or/and that CFTR is not expressed as a cAMP-dependent Cl^- channel in the renal cell membrane.

In Vitro Studies

Using antibodies and immunostaining, CFTR was first detected in the apical membrane of both proximal and distal tubules of human kidney (Crawford et al., 1991). Later, segmental analysis by RT-PCR showed that CFTR mRNAs were expressed in proximal tubule (PT) (Morales et al., 1996; Rubera et al., 1998), Henle's loop (Devuyst et al., 1996), distal tubule (DT) (Rubera et al., 1998) and collecting ducts (CD) (Bens et al., 2001). Moreover, whole-cell clamp analysis has revealed the existence of Cl^- currents with properties similar to CFTR in cultured rabbit DCT cells (Distal Convoluted Cells) (Rubera et al., 1998), in rat mouse inner medullary CD cells (Husted et al., 1995; Vandorpe et al., 1995) and in mouse cortical cells (Letz & Korbmacher, 1997). These currents were activated by cAMP. In rabbit DCT, Cl^- currents exhibited striking similarities to the cAMP-dependent small conductance CFTR Cl^- channels recorded in secretory epithelia (Poncet et al., 1994).

However, the presence of CFTR mRNA along the nephron, together with the existence of a cAMP-activated Cl^- conductance in the terminal segments, do not prove that CFTR controls renal channels in the normal epithelium. Therefore, *cftr* knockout mouse models were developed as a tool to elucidate the renal role of this protein. Different knockout mouse models have been engineered (Dorin et al., 1992; Snouwaert et al., 1992; O'Neal et al., 1993; Ratcliff et al., 1993; Hasty et al., 1995; Rozmahel et al., 1996). One of them, the $cftr^{tm1Unc}$ mouse was obtained by introducing a stop codon in exon 10 (Snouwaert et al., 1992). These transgenic mice were used to study the effect of CFTR deletion on the ion conductances along the nephron. For this purpose, primary cultures of proximal convoluted tubule (PCT), distal convoluted tubule (DCT) and cortical collecting tubule (CCT) microdissected from the kidney of wild type mice (*cftr* +/+) and KO mice (*cftr* –/–) were established (Barriere et al., 2003b).

A whole-cell clamp study carried out on these cell cultures has provided important information on the localization and role of CFTR in the control of different Cl^- and K^+ conductances. In *cftr* +/+ mice, external application of forskolin activated a linear Cl^- current in DCT and CCT. These currents shared characteristics with the CFTR Cl^- currents. In contrast, in DCT and CCT cells from *cftr* –/– mice, addition of forskolin had no effect on Cl^- conductance. Taken together, these results clearly confirmed that in DCT and CCT cells, the activity of forskolin-activated Cl^- channels is consistent with CFTR (Barriere et al., 2003b). Interestingly, the application of forskolin did not stimulate any Cl^- current in primary cultures of PCT cells from *cftr* +/+ mice. A similar observation was made in primary culture of rabbit PCT in which no CFTR Cl^- currents were detected despite the presence of CFTR mRNA (Rubera et al., 1998). This finding is consistent with that of Kibble et al. (2001) who could find no evidence for a cAMP-dependent whole-cell Cl^- conductance in PCT cells from wild-type mouse.

In secretory epithelia, the cAMP-sensitive Cl^- conductance on the apical membrane is related to a basolateral K^+ conductance, which provides the driving force for electrogenic Cl^- secretion. In crypt cells of small and large intestine this conductance is supported by the KCNQ1 channel (Warm et al., 1996). In murine renal tissue, mRNA encoding KCNQ1 has been detected in PCT (Barriere et al., 2003c), DCT and CCT (Demolombe et al., 2001), whereas cAMP-sensitive K^+ channels were recorded in DCT and CCT only. These channels were blocked by chromanol derivatives, indicating that they belong to the KCNQ family. In epithelia, KCNQ1 associates with the small regulatory β-subunit KCNE1, or KCNE3, to form a K^+ channel that could be involved in cAMP-stimulated Cl^- secretion (Bleich & Warth, 2000). Using RT-PCR and Southern-blot experiments, transcripts encoding the KCNQ1 and KCNE1 sequence were found in cultured PCT cells from wild-type mice (1). In contrast, DCT cells also expressed KCNQ1, but not KCNE1, excluding a role

for this subunit in the cAMP- sensitive K^+ channel in this nephron segment. Whether KCNQ1 expression in DCT and CCT cells requires an additional subunit, such as KCNE3, remains to be elucidated.

Interestingly, the cAMP-activated K^+ currents disappeared in *cftr* –/– mice (Belfodil et al., 2003). As in other tissues, CFTR controls these channels, but the mechanism of the interaction between CFTR and K^+ channel protein is far from being completely understood. This mechanism probably depends on the nature of the K^+ channels, because in addition to KCNQ1, other types of channels, such as the ROMK family, could interact directly with CFTR (McNicholas et al., 1997; Ho, 1998; Boucherot, Schreiber & Kunzelmann, 2001). Finally, in the distal tubule, KCNQ1 K^+ currents are clearly dependent on CFTR expression. Nevertheless, such an interaction remains controversial since some studies led to the conclusion that KCNQ1 currents were activated by cAMP but independent of the presence of CFTR (Boucherot et al., 2001; Mall et al., 2000; Warth et al., 1996); whereas other studies demonstrate that CFTR directly activates KCNQ1 conductances (Cunningham et al., 1992; Mall et al., 1996). This K^+ conductance was also not detected in the epithelial cell line CFPAC expressing ΔF508-CFTR (Loussouarn et al., 1996).

It is now evident that CFTR is not only a cAMP-regulated Cl^- channel, but it can also serve as a regulator of other ion channels and transporters. For instance, it has been shown that intestinal crypts isolated from *cftr* –/– mice have lost their ability to regulate their volume following osmotic stress (Valverde et al., 1995). A similar observation was made in PCT, DCT and CCT cells from *cftr* –/– mice, which also showed an impairment of the RVD process following a hypotonic shock (Barriere et al., 2003b). In DCT and CCT this impairment was due to the absence of swelling-activated Cl^- and K^+ channels, while in PCT it was due to the absence of the swelling-activated Cl^- channel (the swelling-activated K^+ conductance still being functional) (Belfodil et al., 2003) (Fig. 1*A* and 1*B*). This highlights the existence of two different swelling-activated K^+ channels involved in cell volume regulation. In DCT and CCT cells they could belong to the BK K^+ channel family, in PCT cells the pharmacological profile corresponds to that of TASK2 channels (Belfodil et al., 2003). This will be discussed further in the section on TASK2.

Besides these data, the *cftr* –/– mouse model has clarified the mechanism of activation of swelling-activated channels during cell volume regulation (Barriere et al., 2003b). According to this mechanism: a hypotonic shock stimulates ATP release from PCT and DCT cells; A1 receptors are then activated by adenosine generated by the degradation of ATP by membrane ecto-enzymes and stimulation of A1

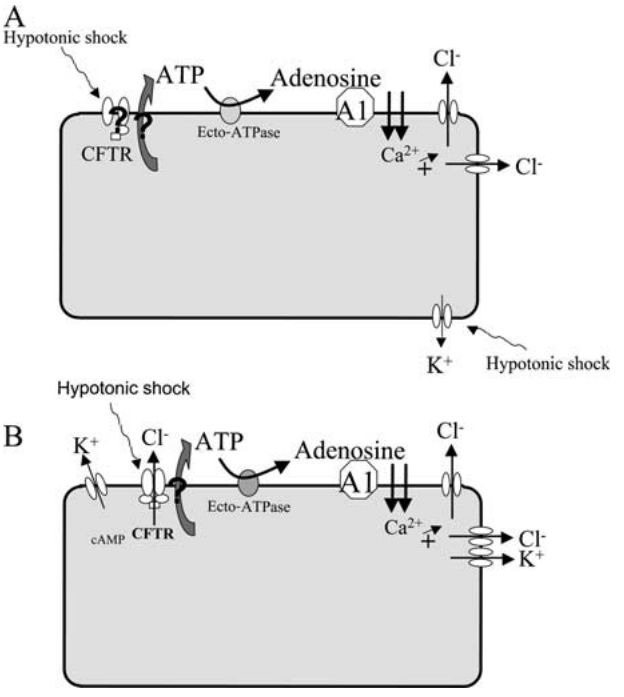

Fig. 1. Model of CFTR and purinergic signalling in normal proximal (*A*) and distal (*B*) cells. A hypotonic shock in PCT cells stimulates ATP release controlled by CFTR. A1 receptors are then activated by adenosine generated by the degradation of ATP by membrane ectoenzymes; stimulation of A1 receptors induces an influx of extracellular Ca^{2+} just beneath the membrane. Finally, this Ca^{2+} influx activates the swelling-activated Cl^- channels in PCT cells and both swelling-activated Cl^- and K^+ channels in DCT cells. Since for the moment no data are available on the distribution of Cl^- and K^+ channels between the apical and basolateral membranes, these channels have been drawn in their putative positions.

receptors in turn induces an influx of extracellular Ca^{2+}; finally, this Ca^{2+} influx activates Cl^- and K^+ channels in DCT cells and only a Cl^- channel in PCT cells (Fig. 1). The observation that adenosine restores both swelling-activated Cl^- conductance and RVD in *cftr* –/– cells confirms that adenosine is a mediator of RVD, at least in renal epithelium. Therefore, in *cftr* –/– cells there is no volume-sensitive ATP release and the cascade of events that triggers the final increase in Cl^- conductance does not occur.

PCT, DCT and CCT are not the only segments of the nephron that exhibit CFTR mRNA. Such transcripts were also found in the *cftr* +/+ mouse cortical thick ascending limb (CTAL) (Marvao et al., 1998), together with a 9 pS Cl^- channel activated by cAMP at the basolateral membrane (Guinamard, Chraibi & Teulon, 1995), suggesting a role for CFTR in this segment. However, in *cftr* –/– mice, although CFTR mRNA was undetectable, the cAMP-sensitive Cl^- channels were still present (Marvao et al., 1998), indicating that CFTR protein has no influence on Cl^- reabsorption in this part of the nephron.

In Vivo Studies

The demonstration in vitro that CFTR is able to modulate different ion channels associated with the existence of a possible tubular Cl^- secretion in several nephron segments (Barriere et al., 2003b) supported a role for this protein in whole kidney function. This is strengthened by the observation that cystic fibrosis patients exhibit a defect in renal diluting capacity, indicating increased NaCl reabsorption. *cftr* –/– mice would offer a good model in which to study the role of CFTR in the kidney in vivo. Unfortunately, these mice exhibit significant growth and intestinal dysfunction and generally die within 6 weeks of birth. To extend their life, the animals are fed a special liquid diet. In spite of this, animals remain very weak and their renal function has not so far been investigated. Therefore, kidney function could be impaired, but the very poor physiological state of these animals could mask this.

Recently, *cftr*^{tm2Cam} mice were used to investigate proximal tubule function (Kibble et al., 2001). These mice were obtained by homologous recombination and exhibit the ΔF508 mutation. Clearance experiments showed that under basal conditions there was no difference between wild type and *cftr* ΔF508 mice in respect to NaCl excretion. Both mice have comparable glomerular filtration rate (GFR) and fractional NaCl excretion rates. However, during volume expansion, *cftr* ΔF508 mice did not increase their GFR to the same extent as *cftr* +/+, indicating a relative decrease of proximal Na^+ reabsorption. Finally, *cftr* ΔF508 mice do not exhibit a real functional deficit in proximal tubule. This absence of a clear phenotype may be due to the constitutive lack of a whole-cell cAMP-dependent Cl^- conductance in mouse proximal tubule cells.

TASK2 KO Mice

TASK2 (twik-related acid-sensitive K^+ channel) is a K^+ channel belonging to the two-pore-domain K^+ channel family. According to its properties in heterologous expression systems, it is suggested that TASK2 serves as a background K^+ channel sensitive to external pH variations in the physiological range (Duprat et al., 1997).

In Vitro Studies

TASK2 mRNA is abundant in renal tissue, (Reyes et al., 1998). In primary cultures of PCT cells Belfodil et al. described that hypotonicity activated a K^+ current, which exhibits properties and a pharmacological profile similar to TASK2 (Belfodil et al., 2003). Barriere et al. investigated the role of TASK2 in volume regulation in primary cultures of mouse proximal tubules from *task2* +/+ and *task2* –/– mice (Barriere et al., 2003a). The knock-out mice for *task2* were generated by a gene-trap strategy (Skarnes, 2000; Leighton et al., 2001; Mitchell et al., 2001). Replacing the TASK2 gene by the gene encoding β-galactosidase allowed an indirect localization of TASK2 protein. This protein is expressed in cortical PCT in situ and in cultured proximal cells in vitro. Whole-cell clamp analysis on primary culture of PCT demonstrated the existence of K^+ currents sensitive to external pH in *task2* +/+ and not in *task2* –/– mice, and provided evidence that this channel was involved in the RVD process during hypotonic shock. The swelling-activated K^+ channels disappeared in PCT cells from *task2* –/– mice, leading to the conclusion that the channel involved in this process is TASK2. The null mutation of *task2* has no effect on swelling-activated Cl^- currents (Barriere et al., 2003a).

Results from further experiments permit to propose the following mechanism: In hypotonic conditions, the activation of swelling-activated Cl^- channels is coupled to the activation of Cl^-/HCO_3^- exchange. In the extracellular space, the exit of HCO_3^- induces an increase in external pH, which then activates basolateral TASK2 K^+ channels (Fig. 2*A*) (Poujeol et al., 2003). With this mechanism, Cl^- efflux occurs before K^+ efflux and therefore the exit of K^+ can maintain the membrane potential during the RVD process.

Besides its role in cell volume regulation TASK2 could well be involved in the mechanism of bicarbonate reabsorption in isotonic conditions. It is now well established that proximal bicarbonate reabsorption occurs via apical proton secretion, carbonic acid dehydration with CO_2 diffusion to the cell, new HCO_3^- formation, and finally exit of HCO_3^- across the basolateral membrane. In the S1, S2 segments of the proximal tubule, the major mechanism of this exit is the electrogenic $Na^+/3HCO_3^-$ cotransporter, in addition to the Cl^-/HCO_3^- exchanger. A series of experiments performed on PCT cells, using the whole-cell clamp technique, indicated that TASK2 could be activated by the increase in external pH that results from a Na^+-dependent efflux of HCO_3^- (Fig. 2*B*). In contrast, this could not be demonstrated in PCT cells from *task2* –/– mice (Poujeol et al., 2003). These data confirmed the observations made by Warth et al., using clearance techniques in these knock-out mice (Warth et al., 2002). Therefore, it is possible that the efflux of K^+ via basolateral TASK2 channels participates in the repolarization of the cell membrane potential, which is depolarized by the electrogenic exit of HCO_3^-.

Finally, in vitro studies on *task2* –/– mice demonstrated that RVD following hypotonic shock was mediated via TASK2 K^+ channels in PCT and via BK channels in DCT cells. Moreover, under isotonic conditions, TASK2 also participated in bicarbonate reabsorption by PCT cells.

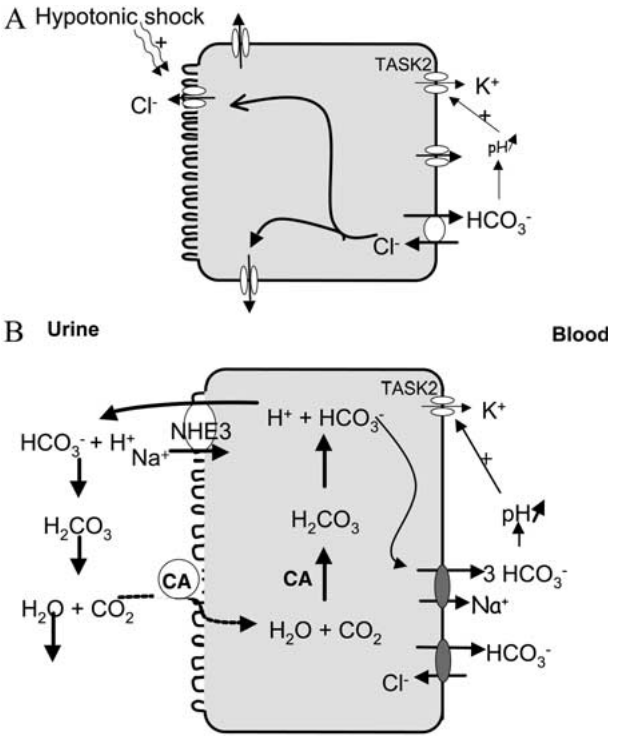

Fig. 2. Model of putative TASK2 functions in proximal tubule cells. (*A*) Under isotonic conditions, during HCO_3^- reabsorption, 3 HCO_3^- leave the cell together with 1 Na^+, not shown here, by depolarizing the basolateral membrane. In the extracellular space, a rise in HCO_3^- concentration causes an increase in pH, which then activates basolateral TASK2 K^+ channels. TASK2 activity will repolarize the membrane, thus restoring the driving force for ongoing $NaHCO_3$ efflux. (*B*) In hypotonic conditions, the activation of swelling-activated Cl^- channels is coupled to the activation of Cl^-/HCO_3^- exchange. In the extracellular space, the exit of HCO_3^- induces an increase in external pH, which then activates basolateral TASK2 K^+ channels. Finally, the efflux of Cl^- and K^+ will permit an efficient RVD process in these cells.

In Vivo Studies

Concerning the physiological implications, only one study has investigated the effect of knocking out *task2* on renal function in vivo in the mouse (Warth et al., 2002). In order to unmask the role of the alkalosis-activated TASK2 channel in salt reabsorption in the kidney, $NaHCO_3$ was administered to increase blood pH and Na^+ load. Under these conditions, the increase in blood pH was identical in *task2* +/+ and *task2* –/– mice but urinary flow rate and salt loss were enhanced in *task2* –/– mice. From these findings, it was concluded that during $NaHCO_3$ reabsorption, an increase in extracellular pH stimulates TASK2 K^+ channels, leading to a hyperpolarization. Since $Na^+/3HCO_3^-$ cotransport is electrogenic, this hyperpolarization facilitates Na^+ and HCO_3^- reabsorption in PCT of *task2* +/+ mice.

In summary, TASK2 in mouse kidney is involved in two important process. TASK2 is the swelling-activated K^+ channel in PCT and is probably activated by an increase in external pH due to the activation of a Cl^-/HCO_3^- exchanger (Barriere et al., 2003a). The second process involving TASK2 is its role in the repolarization of the proximal cell membrane during $NaHCO_3$ reabsorption mediated by the $Na^+/3HCO_3^-$ cotransporter (Warth et al., 2002).

KCNE1 KO Mice

KCNE1, also known as mink or IsK, belongs to a family of small transmembrane proteins. The *kcne1* gene was cloned from rat kidney. It encodes the subunit of a K^+ channel. This subunit is associated with KCNQ1 to form a slowly activating K^+ channel (Barhanin et al., 1996). In order to analyze the function of the KCNE1-KCNQ1 association, mice carrying a null mutation of the *kcne1* gene were engineered by replacing it by either the neomycin resistance gene (Vetter et al., 1996) or the *lacZ* gene (Kupershmidt et al., 1999). Homozygous null mutant mice are deaf and exhibit classic shaker/waltzer behavior (Vetter et al., 1996). So far, a functional role for KCNE1 has been proposed in the heart and in the inner ear (Vetter et al., 1996). In kidney, *kcne1* mRNA was found in rat PCT (Sugimoto et al., 1990) and in mouse PCT (Barriere et al., 2003c), DCT and CCT (Demolombe et al., 2001). The *kcnq1* mRNA is found in mouse PCT. Using immunostaining, KCNE1 protein was found in rat PCT (Sugimoto et al., 1990) and in mouse PCT (Vallon et al., 2001). The KCNQ1 channel was also found in mouse PCT cells (Vallon et al., 2001).

In Vitro Studies

In primary culture of PCT cells, whole-cell clamp experiments revealed that the *kcne1* null mutation impaired the RVD process following hypotonic shock. Similar observations were also reported by Lock & Valverde in ciliated epithelial cells from murine tracheal *kcne1* –/– mice (Lock & Valverde, 2000). The analysis of the swelling-activated Cl^- and K^+ currents during RVD indicated that they were completely abolished in PCT cells from *kcne1*–/– mice. However, the K^+ conductance involved in RVD was not due to the KCNQ1 channel, because it has been clearly demonstrated that the currents remain insensitive to chromanol derivatives. Furthermore, as stated above, the only swelling-activated K^+ conductances found in PCT are the TASK2 channels. The fact that this channel has never been recorded in PCT cells from *kcne1* –/– mice, indicates that the null mutation of KCNE1 affects this TASK channel. However, this conclusion is at variance with that of Lock and Valverde (2000), who proposed that the KCNQ1/KCNE1 complex is implicated in the chan-

nel that is activated during the K^+ secretion linked to RVD in murine airways epithelial cells.

That the PCT swelling-activated Cl^- conductance is also affected by the null mutation of KCNE1 remains an enigma. Although it was demonstrated that KCNE1 may act as an activator of both K^+ and Cl^- channels in heterologous expression systems, nothing is known about the nature of these Cl^- channels. Moreover, DCT cells from *kcne1* –/– cells exhibited functional RVD associated with the activation of both K^+ and Cl^- currents, indicating that 1) KCNE1 protein is not always necessary for controlling the swelling-activated Cl^- conductance and/or 2) the nature of the swelling-activated currents is different in PCT and DCT cells, and 3) the KCNE subunit is different in these two epithelia.

In Vivo Studies

Concerning in vivo studies (Vallon et al., 2001), micropuncture experiments in *kcne1* –/– and *kcne1* +/+ mice indicated that the lack of KCNE1 in the proximal tubule decreased luminal K^+ concentration in the late proximal and early distal tubule. The KCNE 1-dependent K^+ movement from the cell to the lumen contributes to the maintenance of the electrical driving force for Na^+-coupled transport in the proximal tubule. Clearance experiments revealed an enhanced fractional urinary excretion of fluid, Na^+, Cl^- and glucose in *kcne1* –/– compared with *kcne1* +/+ mice.

In summary, in the proximal convoluted tubule, KCNE1 modulates the swelling-activated Cl^- and K^+ currents during hypotonic shock (Barriere et al., 2003c) and offsets the membrane depolarization due to electrogenic Na^+-coupled transport of glucose and amino acids (Vallon et al., 2001).

ROMK KO Mice

In Vitro Studies

The ROMK channel (Kir 1.1), an adenosine triphosphate (ATP)-sensitive K^+ channel, was cloned from rat kidney outer medulla (Ho et al., 1993). This channel is a member of the inward rectifier family of K^+ channels (Nichols & Lopatin, 1997). The ROMK channel is expressed at the apical membrane of the TAL and CCD segments in cortical medullary rays in mouse kidney (Lu et al., 2002) and in the distal tubule of rat kidney (Lee & Hebert, 1995; Xu et al., 1997).

This channel plays an important role in the movement of K^+ in the Henle loop. On the apical membrane there is a co-transporter, NKCC2 (Na-K-2Cl), that permits the entry of Cl^- and K^+ according to the Na^+ gradient. Na^+ and Cl^- will be extruded from the cell by the basolateral Na^+ -K^+ -ATPase and a chloride channel, ClC-K (Hebert, 1998; Estevez et al., 2001). ROMK "recycles" reabsorbed K^+ back into the tubule lumen. The activity of ROMK is essential, because the K^+ concentration in the luminal fluid is much lower than that of Na^+ and Cl^- . Finally, K^+ secretion into the lumen via ROMK and the basolateral Cl^- efflux via CLC-Kb/barttin (Birkenhager et al., 2001; Estevez et al., 2001) contribute to the epithelial electrical potential difference, producing a driving force for Na^+ reabsorption across the paracellular pathway (Hebert & Andreoli, 1984). Bartter's syndrome is due to null mutation of genes encoding NKCC2 (Simon et al., 1996a), ROMK (Simon et al., 1996b), ClC-Kb (Simon et al., 1997), and barttin (Birkenhager et al., 2001). The development of knockout mouse models has facilitated our understanding of kidney disorder. For instance, two knockout mice related to this syndrome exist. The first model is the mouse model for Bartter's syndrome type I, involving NKCC2, and the second is the *Romk* –/– mouse model, which develops Bartter's syndrome type II.

Patch-clamp analysis shows that the null mutation eliminates the activity of the apical small conductance (SK) K^+ channel in tAL and CCD (Lu et al., 2002). In other words, the *Romk* gene encodes the SK channel in the apical membranes of both tAL and principal cells of the CCD.

In Vivo Studies

In vivo studies performed by Lorenz and colleagues revealed that the ROMK-deficient mice develop hydronephrosis, polydipsia, polyuria, extracellular fluid volume depletion, and a urinary concentrating defect (Lorenz et al., 2002). Micropuncture analysis of single nephron function showed that tubuloglomerular feedback is severely impaired and Na^+ reabsorption is significantly reduced, but not eliminated, in the TAL of *Romk* –/– mice. This study underlined the important role of ROMK in K^+ recycling in this segment (Fig. 3). The phenotype of this mouse can be likened to Bartter's syndrome type II.

ClC-5 KO Mice

This channel has now been well studied, because it was a positional candidate for Dent's disease. ClC-5 in kidney is highly expressed in the three segments (S1–S3) of the proximal tubule and in both types of intercalated cells of the collecting duct (Gunther et al., 1998; Luyckx et al., 1998; Devuyst et al., 1999; Sakamoto et al., 1999). In the proximal tubule, this protein has been colocalized with the proton pump beneath and apically in the brush border membrane (Gunther et al., 1998; Sakamoto et al., 1999). There is

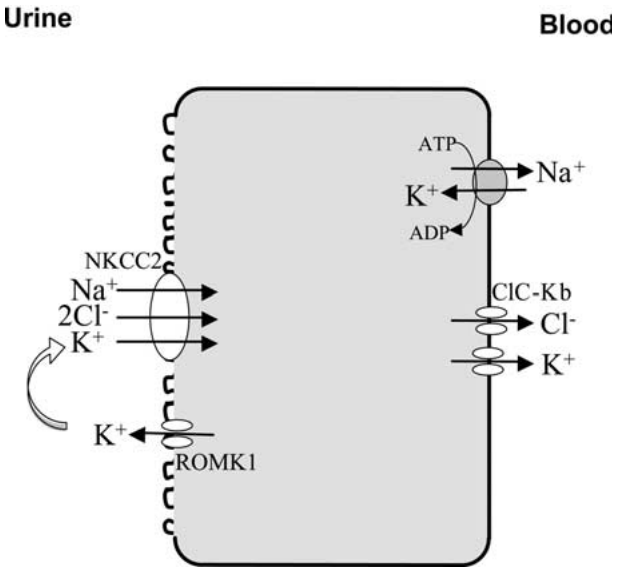

Fig. 3. As fluid flows along the thick ascending limb, ions are reabsorbed across the cells that line the tubule. Using energy supplied by hydrolysis of ATP, the Na, K-ATPase imports K^+ ions from the interstitium and exports Na^+ ions. The resulting concentration gradients allow another ion transporter, NKCC2 (*left*), to import Na^+, Cl^- and K^+ ions from the tubule fluid. K^+ and Cl^- ions can then leave the cell by diffusing through selective channels (including ROMK1 and ClC-Kb channels). K^+ ions recycle across the apical and basolateral membranes. The net result is that Na^+ and Cl^- ions are reabsorbed from lumen to blood.

no in vitro study of mouse kidney cells, probably due to the fact that this channel is largely confined to mostly intracellular compartments.

Three mouse models have been produced to elucidate the physiological function of ClC-5 and the pathophysiology of Dent's disease (Luyckx et al., 1999; Piwon et al., 2000; Wang et al, 2000).

In Vivo Studies

Clcn5 gene mutations cause Dent's disease. This disease is characterized by a renal tubular disorder that includes low-molecular-weight proteinuria, generalized proximal tubule dysfunction, hypercalciuria, nephrocalcinosis, kidney stones and renal failure (Fisher et al., 1994) and is a form of Fanconi syndrome. Expression of ClC-5 in the proximal tubule and its co-localization with H^+-ATPase to endosomes suggests that this channel could have a role in endosomal acidification. It has been hypothesized that impaired acidification of the endosomal apparatus due to the loss of function of ClC-5 could affect endocytosis, resulting in the low-molecular-weight proteinuria of Dent's disease (Lloyd et al., 1996; Gunther et al., 1998; Devuyst et al., 1999). Three distinct mouse models of disrupted ClC-5 expression have been used to investigate the potential role of ClC-5 in endocytosis and Ca^{2+} homeostasis. The mouse model created by a ribozyme knock-down approach did not present an obvious phenotype except for hypercalciuria. This mouse did not exhibit proximal tubule dysfunction, nor low-molecular-weight proteinuria (Luyckx et al., 1999). Two other groups have also generated and characterized knock-out mouse models for Dent's disease by targeted disruption of part of the exon 5 and/or exon 6 of *Clcn5*, using homologous recombination (Piwon et al., 2000; Wang et al., 2000). The *Clcn5* mice reported by Wang et al. exhibited low-molecular-weight proteinuria, generalized amino-aciduria, glycosuria, phosphaturia and hypercalciuria (Wang et al., 2000). In the *Clcn5* KO mice reported by Piwon et al., the loss of ClC-5 also caused low-molecular-weight proteinuria by reducing proximal tubular apical endocytosis and phosphaturia (Piwon et al., 2000). However, these mice were not hypercalciuric (Piwon et al., 2000).

Finally, ClC-5 is the first intracellular chloride channel for which a role is now well established in vesicle trafficking. Therefore, these mice are a good model for investigating Dent's disease.

ClC-K1 KO Mice

The ClC-K channels are the only group of ClC channels that is almost exclusively expressed in the kidney and urinary tract. For this reason, the cloning of ClC-K1 (Uchida et al., 1993) and ClC-K2 (Adachi et al., 1994) channels from rat, and the similar human channels, ClC-Ka and ClC-Kb (Kieferle et al., 1994), helped in understanding their role in renal physiology and pathophysiology. Immunohistochemistry in rat kidney revealed that ClC-K1 is restricted in the thin ascending limb of Henle's loop (tAL) and that it is present in both the apical and basolateral plasma membranes (Uchida et al., 1995). In contrast to the restricted localization of ClC-K1, ClC-K2 showed a relatively broader expression. In rat kidney, in situ hybridization revealed that ClC-K2 is mainly expressed in distal tubules, connecting tubules and cortical collecting ducts (Yoshikawa et al., 1999). The KO of *clc-K2* is not available at present, but mutations in the gene encoding for ClC-K2 are responsible for Bartter's syndrome type III with a phenotype that includes salt wasting, hypokalemic alkalosis and hypotension (Simon et al., 1997). In contrast, concerning *clcnk1* gene, a mouse *clcn-k1* knockout was engineered (Matsumura et al., 1999).

In Vitro Studies

For the moment, there is no study comparing the ClC-K1 currents between *clcnk1* –/– and *clcnk1* +/+ mice. For instance, the data on the characteristics of these currents have been obtained in *Xenopus* oocytes. The study of Waldegger & Jentsch showed

Table 1. Results from knock-out experiments

Channel	Mouse	Background	Molecular technique	Effects	Reference
CFTR	*cftr*^{tm1Unc}	C57BL/6 B6DZ	Inframe stop (exon 10)	Disappearance of whole-cell cAMP-activated K^+ currents in DCT and CCT cells	Barriere, 2003 Belfodil, 2003
		BALB/C		Disappearance of whole-cell swelling-activated Cl^- currents in PCT, DCT and CCT cells Disappearance of whole-cell swelling-activated K^+ currents in DCT and CCT cells	Snouwaert, 1992
				CFTR is not the 9 pS Cl-channel in mouse TAL	Marvao, 1998
	cftr^{tm2Cam}	C57BL/6	Homologous recombination	Increased amiloride- sensitive Na^+ reabsorption during chronic salt restriction	Kibble, 2000 Colledge, 1995
			(ΔF508)	Lack of whole-cell cAMP-activated Cl^- currents in PCT	
TASK-2 (KCNK5)	*kcnk5*	129/Svj C57BL/6	Gene-trap insertion	Disappearance of whole-cell swelling-activated K^+ currents in PCT cells Renal salt and water loss	Barriere, 2003 Warth, 2002 Skarnes, 2000
KCNE1 co-assembly with KCNQ1	*isk*	129/Sv C57BL/6	Gene-trap insertion?	Disappearance of whole-cell swelling-activated Cl^- and K^+ currents in PCT cells Urinary salt, water, and glucose loss, hypokalemia, hemoconcentration	Barriere, 2003 Vallon, 2001
		C57BL/6	Homologous recombination		Kupershmidt, 1999
ROMK	*kcnj1*	129/Svj		Disappearance of small-conductance (SK) K^+ channel in tAL and CCD	Lu, 2002
				Reduction of Na^+ reabsorption, hydronephrosis, polydipsia, polyuria, extracellular fluid volume depletion, and urinary concentrating defect	Lorenz, 2002
ClC-5	*clcn-5*	C57BL/6	Ribozyme approach	Urine exhibits low-molecular-weight proteinuria, generalized amino-aciduria, glycosuria, phosphaturia and hypercalciuria	Luyckx, 1999
					Wang, 2000
			Homologous recombination	Reduction of the apical proximal tubular endocytosis	Piwon, 2000
ClC-K1	*Clcnk1*	C57BL/6	Homologous recombination	Disappearance of Cl^- transport in tAL cells Increased urine volume and polyuria	Matsumura, 1999

that the injection of cRNA encoding ClC-K1 produced currents that exhibit outward rectification, activation by external calcium, and sensitivity to external pH (that is stimulation by alkaline pH) (Uchida et al., 1995; Waldegger & Jentsch, 2000). More recently, another study reported the existence of a Cl^- channel in the basolateral membrane of the mouse distal convoluted tubule, which exhibits similar properties to that described in *Xenopus* oocytes after injection of cDNA encoding ClC-K1 (Lourdel et al., 2003). However, for the moment it is difficult to know with certainty which ClC-K channel has been characterized in this study, because it is unknown whether this current is still present in *clcnk1* –/– mice. Also, the in vitro microperfusion of renal tubules from *clcnk1* +/+ and *clcnk1* –/– mice demonstrated the presence of a Cl^- transport pathway in tAL that is sensitive to acidification and NPPB; this Cl^- transport is abolished in *clcnk1* –/– (Matsumura et al., 1999).

In Vivo Studies

The *Clcn-k1* mouse model showed that ClC-K1 is a major Cl^- channel mediating transepithelial Cl^- transport in tAL, thereby participating in urinary concentration (Matsumura et al., 1999). Clearances in *Clcn-k1* –/– and *Clcn-k1* +/+ mice revealed that the urine volume of the *Clcn-k1* –/– mice is increased by five-fold. This increase is coupled to a decrease in urine osmolality. Moreover, when the mice are deprived of water, the *Clcn-k1* –/– mice exhibited a three-fold greater weight loss compared with *Clcn-k1* +/+ mice. The fractional excretion of Na^+, Cl^- and urea and creatinine clearance were not altered in *Clcn-k1* –/– mice, indicating that the polyuria observed in these mice was due to a water diuresis (Akizuki et al., 2001). This loss of water induced a higher plasma osmolality in the *Clcn-k1* –/– than *Clcn-k1* +/+ mice. Correspondingly, urinary AVP excretion was increased in *Clcn-k1* –/– mice.

In conclusion, the data obtained in *Clcn-k1* –/– mice showed that the absence of this channel in the tAL affects the countercurrent mechanism in vivo. Also, the hypertonicity observed in the inner medulla is probably a result of multiple interactions between NaCl and urea permeability in the tAL (Akizuki et al., 2001). Finally, ClC-K1 mediates Cl^- transport sensitive to nitro-phenyl-parabenzoic acid (NPPB) or pH in this segment and the paracellular shunt pathway presents a selective Na^+ permeability (Liu et al., 2002).

Conclusion

Generation of KO animals is an important strategy for studying the physiological role of epithelial ion channels. Despite the fact that conventional gene disruption is a powerful method to explore gene function in vivo, this approach may lead to embryonic lethality or premature death if the gene is crucial for development or survival. It is likely that many research teams have made attempts to knock out genes encoding other channel proteins, but we do not know the results generated by these efforts because the KO animals were not viable. For instance, in *cftr* –/– mice many deaths occur during the first 5 days of post-natal development and after 30 days less than 10% survive (Snouwaert et al., 1992). To circumvent this limitation, a conditional gene-targeting strategy, using a site-specific Cre-loxP recombination system, is being developed to control gene inactivation in specific tissues. Therefore, it would be possible in the future to delete the gene of interest in restricted parts of the nephron. One essential part of the technique is to engineer a mouse strain that expresses Cre recombinase controlled by a specific promoter. Actually, two strains express Cre recombinase specifically in podocytes of the renal glomerulus, using either the promoter Nphs1 (Eremina et al., 2002) or the promoter Nphs2 (Moeller et al., 2003). Another mouse strain expresses a tagged form of Cre in the thick ascending limb under the control of the Tamm-Horsfall promoter (Stricklett et al., 2003). Shao et al. (Shao, Somlo & Igarashi, 2002) have developed a transgenic strain that expresses Cre recombinase in collecting ducts and thick ascending limbs of Henle's loops by using the Ksp-cadherin promoter. Nelson et al. have developed another one that expresses a Cre-tagged recombinase under the AQP2 promoter in collecting duct principal cells (Nelson et al., 1998). Yu et al have engineered a mouse strain that drives Cre recombinase under the control of the HoxB7 promoter/enhancer specifically within the mesonephric duct and its derivatives (Yu, Carroll & McMahon, 2002). Recently, Rubera et al. have constructed a transgenic mouse line that expresses Cre recombinase under the control of the sglt2 5′ region specifically in the renal proximal tubule (Rubera et al., 2004). Up to now these different lines were never used for specific gene recombination in the kidney, but in the near future it is likely that different strains of mice exhibiting a null mutation of a given channel in a specific nephron segment will soon be available. This will improve considerably our knowledge of channel function in the kidney.

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