# RENAL COLLECTING DUCT PHYSIOLOGY AND PATHOPHYSIOLOGY

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RENAL COLLECTING DUCT PHYSIOLOGY AND PATHOPHYSIOLOGY

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Abstract

In the kidney, the collecting duct (CD) is composed of at least four cell types: principal, type-A intercalated cells (IC), type-B IC and non-A, non-B IC. Although this heterogeneous composition has been recognized since the end of the 19th century, the physiological role of the various cell types in the CD continues to be deciphered as of today. Principal and IC are essential in ion/water balance and acid-base homeostasis, respectively. However, recent research has revealed a striking interplay and overlap between the specific functions of these cell types. This review summarizes the recent findings on CD cells and their role in multiple pathophysiologies.

Keywords: Kidney, collecting duct, intercalated cells, principal cells, acid-base balance, pH homeostasis
1. The Functional Unit of the Kidney: the Nephron

Our everyday life and diet generates wastes that our bodies need to excrete. The human body relies on two main organs to detoxify and excrete unwanted toxins, chemicals and metabolites: the liver and the kidneys. The kidneys play an essential role in (i) ion, pH and water homeostasis, also contributing to hormonal regulation of these processes, (ii) excretion of acids generated by our metabolism and (iii) conservation of key molecules (amino acids, glucose, etc). With its complex structure encompassing the functional unit called nephron, it provides a sophisticated machinery to specifically filter, excrete, secrete and reabsorb molecules to/from the blood. Blood from the afferent arteriole that enters each renal corpuscle is filtered through fenestrated glomerular endothelial cells, the basement membrane and podocytes foot processes prior to entering the capsular space where it flows into the proximal tubule. Within 24 hours, an average of 180 liters of plasma is filtered by the kidneys.

Each kidney contains about 1 million nephrons. Each nephron is composed of a sequence of tubular segments that are defined by transitions in the epithelial cells underlying each segment (Kriz and Kaissling 2013, Chen et al. 2017, Park et al. 2018b). The various series of epithelial segments include the proximal tubule (PT), the loop of Henle (the thin descending limb, thin ascending limb, thick ascending limb), the distal convoluted tubule (DCT), the connecting tubule (CNT) and finally the collecting duct (CD). The type of cells, their tight junction properties as well as the solute carriers (SLC) and channels expressed in these cells define the function of each segment. Interestingly, in some sections of the nephron, the transition from one segment to the next occurs in a gradual way, with for example overlapping expression of some key proteins such as the epithelial sodium channel (ENaC) and the sodium/chloride cotransporter (NCC) in the DCT/CD transition.

The composition of the filtrate in the early part of the proximal tubule is similar to the plasma except that it is devoid of blood cells and contains less proteins (Koeppen and Stanton 2007). As the filtrate flows through the nephron, its composition is modified through reabsorption and excretion. The bulk reabsorption of water, ions and solutes occurs in the PT. Unlike the thin descending limb, the thin ascending limb, the thick ascending limb and the DCT all together contribute to sodium, chloride, calcium and magnesium reabsorption. Lastly, the CNT and CD are the segments responsible for the fine tuning of urine composition and urine acidification. The CD tightly regulates the movement of water, sodium, chloride, potassium, bicarbonate and protons using a combination of both transcellular and paracellular pathways involving at least 3 different cell
types: PCs, type-A IC (also called α-IC) and type-B IC (also called β-IC). This review focuses on the structure and function of CD cells, specifically on principal and intercalated cells.

2. CD cell types and key genes

A. Principal Cells

Various cell types of heterogeneous structural composition have been identified by electron microscopy in the embryonic CD epithelium (Kloth et al. 1993). "Light PCs" and "dark IC" were distinguished based on these morphological observations. In cortical rat (Hansen et al. 1980, Kim et al. 1999), mouse (Teng-umnuay et al. 1996, Kim et al. 1999) and rabbit (LeFurgey and Tisher 1979) CD, two third of the cells were found to be PCs. These cells are simple cuboidal epithelial cells that carry fewer organelles and mitochondria than the dark IC. The mitochondria also appear to be smaller in size and randomly distributed in the cytoplasm. Lysosomes, autophagic vacuoles, multivesicular bodies, both rough and smooth endoplasmic reticulum are also evident by transmission electron microscopy (Madsen and Tisher 1986).

The major role of the PCs in the CD is to reabsorb sodium and water from primary urine and to excrete potassium to the urine. They achieve this by the concomitant action of specific transporters including the epithelial sodium channel ENaC, aquaporin 2 (AQP2), the renal outer medullary potassium channel (ROMK) (Figure 1) and to a lesser extent the Ca\(^{2+}\) activated K\(^{+}\) channel (BK) (Madsen et al. 1988). In the PCs, aldosterone and arginine vasopressin play a key role for regulation of ENaC, ROMK and AQP2 to facilitate sodium, potassium and water transport, respectively (Pearce et al. 2015).

The amiloride-sensitive sodium channel ENaC is expressed at the apical membrane of the PC. Via the action of aldosterone, arginine vasopressin and other hormones (Duc et al. 1994, Hager et al. 2001), high and low sodium diets result in low and high apical expression of ENaC, respectively (Loffing et al. 2000). Its apical abundance is tightly regulated via ubiquitination as detailed below.

Potassium secretion through the apical membrane of the PC is mediated by ROMK and is regulated by mineralocorticoids. In rabbit isolated perfused CCD, mineralocorticoids stimulate potassium secretion and sodium reabsorption (O’neil and Helman 1977, Schwartz and Burg 1978). The Na\(^+\)/K\(^+\)-ATPase activity is also enhanced by mineralocorticoid stimulation in the CCD of rat (Mujais et al. 1984) and rabbit (Garg et al. 1981).

Apical expression of ROMK in the DCT, CNT or CD can be increased in a high potassium diet (Wade et al. 2011).
Water molecules in the PC follow the lumen-to-interstitium osmotic gradient and their reabsorption is mediated by the synchronized expression and activity of apical AQP2 and basolateral AQP3 and AQP4 (Ishibashi et al. 1997, Kim et al. 2005, Pearce et al. 2015).

**B. Intercalated Cells**

The other cell types found in the CD are IC or “dark” cells. In comparison with the PCs, IC have a high density of mitochondria, a dark cytoplasm, microprojections at the apical membrane, and they do not have a central cilium (Schuster 1993). All IC are positive for carbonic anhydrase II (CA II) and V-H\(^+-\)ATPase proteins expression.

IC can be subdivided into 3 subtypes: type-A, type-B, and non-A, non-B IC. The location of the V-H\(^+-\)-ATPase expression in addition to the expression of other key proteins define the IC subtype (Teng-umnuay et al. 1996, Roy et al. 2015).

Type-A IC express V-H\(^+-\)-ATPase at the apical membrane and the kidney anion exchanger 1 (kAE1) at the basolateral membrane. These cells significantly contribute to acid/base balance by secreting protons via the apical V-H\(^+-\)-ATPase, and reclaiming bicarbonate via basolateral kAE1, both ions being generated from hydrolysis of CO\(_2\) and water by the CA II (see section 3. B). On the other hand, type-B IC express V-H\(^+-\)-ATPase at the basolateral membrane and pendrin at the apical membrane. In addition to contributing to acid/base balance by secreting bicarbonate and reclaiming protons in case of alkalosis, type-B IC also contribute to electrolyte homeostasis as they are involved in chloride reabsorption (see section 3. A) (Teng-umnuay et al. 1996, Roy et al. 2015).

Although IC can be morphologically, structurally and functionally distinguished from the PCs, experimental evidence support that both cell types originate from the same precursor (Trepiccione et al. 2017). Immortalized type-B IC plated at a high density were able to convert to type-A IC and secrete acid instead of alkali (van Adelsberg et al. 1994). This ability to convert from one cell type to the other was due to the secretion of the extracellular matrix hensin protein by the type-B IC (Gao et al. 2010). In support of these findings, a hensin knock-out mouse model displayed a predominant abundance of type-B IC, the absence of type-A IC in the CD, and development of metabolic acidosis (Gao et al. 2010).
Lastly, non-A, non-B IC express both V-H\(^+\)-ATPase and pendrin at the apical membrane. The function of non-A, non-B IC is still unclear. However, the fact that both V-H\(^+\)-ATPase and pendrin are at the apical membrane suggests that these cells are not involved in acid/base balance but instead may be involved in electrolyte homeostasis. It is also thought that these cells may represent a transition state between the other two types depending on diet and plasma pH (Roy et al. 2015). A very recent publication showed that the mouse CD contains a third type of cells in addition to PC and IC. These cells have features characteristics of PC and IC as they were positive for both AQP2 and V-H\(^+\)-ATPase, indicating that they may represent a previously un-identified transitional state between the two main cell types (Park et al. 2018b).

The distribution of the different IC types in the distal nephron varies among species. In mouse, type-A IC make 40%, 60% and 100% of the IC in CNT, CCD and OMCD/IMCD, respectively. On the other hand, type-B and non-A, non-B IC make 10% and 50%, 20% and 20%, and 0% of the IC in CNT, CCD and OMCD/IMCD, respectively (Kim et al. 1999).

Recent years of research have demonstrated a clear interplay between PC and IC. Indeed, in a similar finding to what was observed in RTA patients (Sebastian et al. 1976), mice knockouts on the B1 subunit of the V-H\(^+\)-ATPase displayed a defective conservation of sodium and chloride due to altered function of ENaC and decreased abundance of pendrin (Gueutin et al. 2013). Thus, a knockout in IC results in functional defects of not only IC but also PC. These animals displayed elevated levels of urinary ATP and prostaglandin E2 (PGE2) originating from type B-IC but acting on PCs in a paracrine process. Thus, these findings highlight that the function of one cell type is linked to that of its neighbor cells in the CD.

C. Tight Junctions

Tight junctions are separating epithelial cells thereby controlling the nature and amount of molecules that are transported between rather than through the cells. Their role in ion and water homeostasis is less well-defined than the transcellular pathways. Within these junctions, claudins are dynamically participating in the renal epithelial function, and specifically in the so-called "chloride shunt" (Hou 2016a). In mouse CD cells that express claudin 3, 4, 6, 7, 8 and 10 (Kiuchi-Saishin et al. 2002, Li et al. 2004), claudin-4 and -8 form heterodimers that are expressed in both principal and IC. Claudin-4 abundance is regulated by dietary NaCl and aldosterone (Moellic et al. 2005). PC-specific claudin-4 or claudin-8 knockout mice developed
hypotension, hypochloremia and metabolic alkalosis (Gong et al. 2014, 2015), thereby demonstrating that in CD cells, the tight junction proteins claudin-4 and claudin-8 are involved in paracellular chloride reabsorption and in ion homeostasis.

3. Functions of the CD cells

A. Ion homeostasis and blood pressure

The extent of renal excretion and reabsorption of ions varies to maintain an appropriate extracellular fluid volume and acid-base balance (Pearce et al. 2015). In the CD, IC and PC regulate the homeostasis of acid-base, fluid and electrolytes, respectively (Gueutin et al. 2013, Kriz and Kaissling 2013). Interestingly, in this segment, reabsorption of sodium is partially separated from that of chloride. In PC, sodium reabsorption takes place via ENaC (Reeves and Andreoli 2008), which results in the generation of a lumen-negative transepithelial potential (Figure 2). This potential generates the driving force for potassium secretion via the apical channel ROMK and favors proton secretion from the IC (see next paragraph) (Pearce et al. 2015).

Variations in blood pressure or an increase in plasma pH trigger the release of various hormones including aldosterone, insulin and angiotensin II, which in turn regulate the function of ENaC (Pao 2016, Wall 2017). Upon aldosterone stimulation, the serine-threonine kinase (SGK1) phosphorylates and thereby inhibits Nedd4-2, an ubiquitin ligase that normally ubiquitinates ENaC to initiate its termination and ultimately degradation (Kabra et al. 2008, Soundararajan et al. 2010). Thus, SGK1 activation results in increased abundance of ENaC at the apical membrane and further sodium reabsorption (Bhalla et al. 2006). SGK-1 has a small regulatory effect on ROMK as well (Lang et al. 2010). Aldosterone also triggers the activity of basolateral Na⁺/K⁺-ATPase in PC (Verrey et al. 1987)

Chloride reabsorption takes place partially in a paracellular way through the tight junctions and trancellularly via type-B IC (Gueutin et al. 2013, Hou 2016a). The transepithelial potential generated by ENaC-mediated electrogenic sodium reabsorption is enough to promote lumen-to-blood flux of chloride ions, despite the unfavorable chloride concentration gradient (Hou 2016b). Claudin-4 and claudin-8 form the paracellular pathway for chloride permeation, which is regulated by a number of proteins including Cap1 and Kelch-like 3 (KLHL3) (See section 4. C) (Gong et al. 2014, 2015).
The transcellular chloride reabsorption pathway takes place through type-B IC and is coupled with the electroneutral reabsorption of sodium in the mouse model (Figure 2). This process requires the concomitant action of apical pendrin and SLC4A8, and the basolateral SLC4A9 (although the stoichiometry of this transporter remains a matter of debate) and ClC-Kb in a process energized by the basolateral V-H\(^+\)-ATPase (Leviel et al. 2010, Gueutin et al. 2013). Bicarbonate apically excreted in exchange for chloride uptake through pendrin is recycled back into the cells by SLC4A8 together with a sodium ion and in exchange for a chloride ion. Basolaterally, the sodium and chloride ions are transported to the interstitium via SLC4A9 (together with bicarbonate ions) and ClC-Kb, respectively. This functional coupling of apical and basolateral transporters/channels results in the net and electroneutral absorption of both sodium and chloride ions. This process is amiloride-resistant, thiazide-sensitive, and regulated by the Nedd4-2 ubiquitin ligase (Nanami et al. 2018). Of note, although SLC4A8 has been represented at the apical membrane of type-B IC in figure 2, its precise location remains to be confirmed and further in vitro studies confirming the location and contribution of each transporter need to be performed.

In parallel to ion reabsorption, water is also passively reabsorbed in PC, in a process regulated by the arginine vasopressin (Olesen and Fenton 2017). In the normal kidney, an increase in plasma arginine vasopressin concentration results in the arginine vasopressin receptor 2 gene (AVPR2) activation, which triggers a cAMP-dependent cytosolic signal to relocate intracellular vesicles containing AQP2 to the apical membrane. The apical membrane then becomes more water permeable.

**B. pH homeostasis**

With our acid-generating Western diet, type-A IC is the predominant cell type that dictates the final urinary pH. In these cells, CO\(_2\) diffuses into the type A-IC and is hydrolyzed via CA II in the cytosol producing H\(_2\)CO\(_3\), which in turn dissociates to proton and bicarbonate.

The bicarbonate ions are transported to the interstitial fluid in exchange for chloride via kAE1 at the basolateral membrane. On the other hand, the protons are secreted to the lumen via the apical proton pump V-H\(^+\)-ATPase and the H\(^+\)-K\(^+\)-ATPase. Both the pH difference across the apical membrane and the potential difference across the epithelium affect the function of the V-H\(^+\)-ATPase (Andersen et al. 1985). In the lumen, the secreted protons bind either to ammonia (NH\(_3\)\(^+\)) and generate ammonium (NH\(_4\)\(^+\)) or bind to phosphate ions (HPO\(_4^{2-}\)) to generate titratable acids that are excreted in the urine. The extent of urinary acidification is
regulated by the ratio of type-A versus other CD cell types and by aldosterone (Wagner 2014). Any malfunction of the 3 main contributors of pH acidification (CA II, kAE1 or V-H^+ATPase) results in a disease characterized by a low blood pH called distal renal tubular acidosis (dRTA) (See section 4.B).

Various molecules are sensitive to intracellular and extracellular pH variations and affect IC's function and plasma pH. The soluble adenylate cyclase senses bicarbonate concentration and indirectly acts on the apical V-H^+ATPase (Gong et al. 2010). Another example is the G protein-coupled receptor 4 (GPR4) whose deletion results in decreased renal acid excretion and a metabolic acidosis in mice (Sun et al. 2015). Additional pH sensors identified in the kidney are reviewed elsewhere (Brown and Wagner 2012).

C. Innate immunity

IC also play a previously un-recognized role in defending renal epithelia against bacterial infections. Uropathogenic Escherichia coli (UPEC) preferentially bind to CD cells and specifically to type-A IC. Therefore, in addition to being involved in urine acidification, these cells also play a significant protective role in keeping the urine sterile. They achieve this goal in two major ways. First, these cells secrete protons to the urine, thereby creating an acidic environment unfavorable for bacterial growth (Paragas et al. 2014). Second, upon infection by UPEC, these cells specifically secrete a bacteriostatic protein called neutrophil gelatinase-associated lipocalin (NGAL) or lipocalin-2 (LCN2). This protein specifically interacts with enterochelin, a protein secreted by gram negative bacteria, and thereby prevents iron’s transfer to the bacteria. Additionally, LCN2 is necessary for Toll-like receptor 4 (TLR4) activation in IC, via the microRNA Let-7i (Sadio et al. 2018), and initiation of innate immune response by IC. This process is also hormonally regulated through AVPR2 activation (Chassin et al. 2007). Therefore, the function of type-A IC is not restricted to acid secretion to the urine but also encompasses immune defense against bacterial invasions.

4. Diseases associated with mutated proteins in the CD & phenotype of mouse models

A. Liddle Syndrome

Liddle syndrome, an autosomal dominant inherited disease, was first reported by Grant Liddle and his co-workers in a kindred from Alabama, US presenting some characteristics of hypertensive symptoms with negligible aldosterone secretion (Liddle 1963). The clinical presentation of patients with this syndrome includes hypertension, hypokalemia, metabolic alkalosis associated with low plasma renin and aldosterone
(Warnock and Bubien 1994). A successful treatment approach by administering spironolactone and triamterene (two potassium-sparing diuretic) provided the first hint that the cause of the disease could be somewhere between the mineralocorticoid receptor and ENaC. In fact, an over-activity of the hetero-trimeric ENaC due to germline mutations in the α, β or γ-subunits can be a cause of Liddle syndrome (Hansson et al. 1995, Schild et al. 1996). The cytosolic domain of the 3 ENaC subunits contains a conserved PY (proline tyrosine) motif, which serves as the Nedd4-binding domain (Staub et al. 1996). This interaction results in the channel's ubiquitination and proteosomal degradation. Mutations that cause Liddle syndrome were found to disrupt this binding motif, thereby resulting in prolonged and uncontrolled sodium reabsorption (Rotin et al. 1994, Schild et al. 1996, Staub et al. 1996).

One mouse model of Liddle’s syndrome was developed by knocking out the β-subunit of ENaC. Under a high salt diet, these mice displayed a high BP, metabolic alkalosis and hypokalemia, thereby recapitulating the symptoms observed in patients (Pradervand et al. 1999). Mice lacking Nedd4-2 developed a similar phenotype accompanied by increased ENaC expression (Huysse et al. 2012), supporting the important role of the channel and this ubiquitin ligase in development of the disease.

B. Distal renal tubular acidosis

Type 1 or distal renal tubular acidosis (dRTA) results from a renal defect in acid secretion (Mohebbi and Wagner 2018) and consequently in bicarbonate reabsorption in tubules of the distal nephron (Cordat and Casey 2009). Mutations in the genes encoding either kAE1 (Bruce et al. 1997, Karet et al. 1998, Jarolim et al. 1998), the V-H+ATPase (Karet et al. 1999) or CA II (Lewis et al. 1988) can result in dRTA. dRTA patients carrying dominantly or recessively-inherited mutations develop renal stones, hypokalemia, hyperchloremia, nephrocalcinosis, metabolic acidosis and a defective urine acidification in addition to facing difficulties to thrive (Trepiccione et al. 2017). AE1 is a 14 transmembrane domains Cl-/HCO3- exchanger that is expressed in erythrocytes (eAE1) and in the kidney (kAE1) (Arakawa et al. 2015). The kAE1 protein is a truncated version of the eAE1 protein, as it lacks the first 65 amino-terminal residues of the erythroid protein. At this time, point or frameshift mutations in the SLC4A1 gene encoding kAE1 protein are reported to cause dRTA, in a homozygous, heterozygous or compound heterozygous state (Zhang et al. 2012, Fry et al. 2012, Cordat and Reithmeier 2014, Park et al. 2018a). Investigations in Madin-Darby canine kidney (MDCK) cells showed that
DRTA-causing kAE1 mutants were either non-functional or mis-trafficked to the apical membrane, the Golgi or the endoplasmic reticulum (Devonald et al. 2003, Toye et al. 2004, Cordat et al. 2006) (Figure 3). Co-expression of dominant dRTA mutants with the wild-type (WT) kAE1 protein, thereby mimicking the situation found in patients with a dominant form of the disease, showed that the mutant affected the trafficking of the WT protein in these cells (Cordat et al. 2006). In contrast, co-expression of recessive mutants with kAE1 WT, as found in parents of the patients with recessive dRTA, showed that the WT protein rescued the mutant's trafficking. These findings provided a molecular mechanisms for development of dRTA.

However, recent in vivo findings challenged our understanding of dRTA pathophysiological mechanisms. Indeed, when expressed in mouse inner medullary collecting duct (mIMCD3) or mouse cortical collecting duct M1 cells, the dominant mutant kAE1 R607H (corresponding to the human dominant kAE1 R589H dRTA mutation) showed a normal function and proper targeting of the protein to the basolateral membrane (Mumtaz et al. 2017). This mutant was previously reported to be retained in the endoplasmic reticulum in MDCK cells (Toye et al. 2004, Cordat et al. 2006). Moreover, in a kAE1 R607H knock-in mouse model that developed dRTA upon acid challenge, the protein was properly targeted to the basolateral membrane, although its expression level was lower compared with wild type mice (Mumtaz et al. 2017). In fact, these mice had a lower amount of V-H^+^-ATPase and were unable to relocate this protein to the apical membrane upon acid challenge. In fact, the number of type-A IC in these mice was significantly lower in comparison with the WT mice. A recent study investigating the interactome of the V-H^+^-ATPase identified the nuclear receptor coactivator 7 (Ncoa7) as an interactor (Merkulova et al. 2015). A targeted deletion of this protein in mice resulted in incomplete dRTA (Merkulova et al. 2018). These recent findings may be the first step towards deciphering the functional link between basolateral kAE1 and apical targeting of the V-H^+^-ATPase. Therefore, our understanding of the pathophysiology associated with dRTA remains obscure and further studies will be necessary to fully understand the molecular mechanisms of this complex disease.

The CA II enzyme is found in the cytosol of the PT cells, loop of Henle and in the IC of the CD (Laing et al. 2005). CA II converts CO\(_2\) and water into bicarbonate and protons in PT cells and IC. Accordingly, the lack of or dysfunction of CA II results in an impaired bicarbonate reabsorption and acid secretion (Ring et al. 2005), defined as Type 3 renal tubular acidosis. Patients with type 3 RTA have acidemia, alkaline urine, osteopetrosis,
cerebral calcification and mental retardation. Beside the kidney, the tissues and organs affected in type 3 RTA correlate with tissue-expression of the CA II. A recent study of CA II deficient mice showed that CA II also plays a significant role in urine concentration (Krishnan et al. 2017). In addition to type 3 RTA, these mice had polyuria and polydipsia without altered sodium or calcium reabsorption/excretion indicating that they had a specific defect in water reabsorption.

C. Pseudohypoaldosteronism type II

Another disease that manifests in acid/base dysregulation is pseudohypoaldosteronism type II (PHAII). This condition originates from defects in the DCT, CNT and CD but for the purpose of this review, we will focus on the role of the CD. Patients with PHA II present symptoms of increased blood pressure, hyperkalemia and hyperchloremic acidosis (Wilson et al. 2001). PHA II is either caused by a deletion in the first intron or missense mutations in the genes encoding with-no-lysine-kinase 1 or 4 (WNK1 or WNK4), respectively. Both deletion or mutations lead to an increase in WNK activity. WNK1/WNK4 are both expressed in IMCD cells (Uawithya et al. 2008), with specific expression of the long WNK1 in IC (Webb et al. 2016). While WNK1 is mainly cytoplasmic, WNK4 is localized to the tight junction in the distal nephron (Wilson et al. 2001). Both proteins are sensitive to intracellular chloride concentration \([\text{Cl}^-]\) changes (Terker et al. 2016). An increase in \([\text{Cl}^-]\) inhibits WNK1/WNK4 activities. However, WNK4 is inhibited at a lower concentration of \([\text{Cl}^-]\) than WNK1. Elevated \([\text{Cl}^-]\) inhibits WNK1 activity by inhibiting its auto-phosphorylation (Piala et al. 2014). Overexpression of WNK1 and disease-causing WNK4 mutants in MDCK II cells increased chloride permeability through phosphorylation of the tight junction protein claudin-4 (Ohta et al. 2006). WNK4 also inhibits the activity of the sodium channel ENaC, therefore PHAII-causing WNK4 mutations result in increased ENaC conductivity and un-regulated sodium reabsorption (Ring et al. 2007).

KLHL3 and culin 3 (Cul3) are two additional proteins whose malfunction causes PHAII (Boyden et al. 2012, Louis-Dit-Picard et al. 2012). These two proteins form the “culin-ring E3 ligase” (CRL) complex where KLHL3 serves as a substrate adaptor for the Cullin-3-mediated ubiquitylation of several proteins, including WNK1, WNK4 and claudin-8 (Gong et al. 2015, Sohara and Uchida 2016). KLHL3 mutations are either dominant or recessive and impair KLHL3 interaction with the target protein, while Cul3 mutations are all dominant and alter the structure and stability of the CRL complex. These mutations result in inappropriate
sodium and chloride reabsorption in the CD by at least two pathways. One pathway involves the ubiquitylation of claudin-8 by KLHL3 (Hou et al. 2010, Gong et al. 2015). Knocking down claudin-8 in immortalized mouse IMCD cells resulted in the loss of claudin-4 localization to tight junctions and as a result a decrease in paracellular chloride conductance. PHAII-causing mutations in KLHL3 disrupt its interaction with claudin-8, thereby preventing claudin-8 ubiquitination and degradation, and causing an increase in paracellular chloride flux. The second pathway involves the ubiquitylation of WNK proteins by KLHL3. In a mouse model knocked-in with the PHAII-causing KLHL3 R528H dominant mutation, WNK1 and WNK4 expression level were increased compared to control littermates due to a loss of interaction with the KLHL3 mutant and impaired WNK1 and 4 ubiquitylation (Susa et al. 2014). This disease illustrates the complex interplay between tight junction properties and transcellular ion fluxes.

D. Pendred syndrome

Pendred syndrome is an autosomal recessive disease characterized by sensorineural deafness, non-endemic goitre and in some cases iodide organification (Kopp and Bizhanova 2011). This disease was first described by British practitioner Vaughan Pendred from his observation of the association of goitre and deafness (Pendred 1896, Kopp and Bizhanova 2011). Pendred syndrome is the underlying cause for 10% of all syndromic deafness with an estimated incidence rate of 7.5-10 in 100,000 population (Reardon et al. 1997).

Encoded by the SLC26A4 gene, pendrin is a transmembrane electroneutral exchanger for various anions including bicarbonate and chloride (Wall 2016) and is responsible for the Pendred syndrome (Everett and Green 1999). The gene is highly expressed in various organs including inner ear, thyroid gland (symptoms of this disease include deafness and goitre) and in the kidney. In this organ, pendrin can increase the urinary bicarbonate concentration in alkalosis, a process synchronized with reabsorption of NaCl and thus in maintenance of blood pressure as detailed in section 3.A.

Pendrin is expressed at the apical surface of the type-B and in non-A-non-B IC in the CD (Royaux et al. 2001, Soleimani et al. 2001). During metabolic alkalosis, pendrin expression increases significantly and the protein localizes predominantly at the apical membrane of mice CD cells (Wagner et al. 2002). In contrast, the mice demonstrated a dwindled expression and cytosolic pendrin upon metabolic acidosis. Compared with their WT counterparts, pendrin knockout mice fed an alkaline diet were not able to secrete bicarbonate into the urine (Royaux et al. 2001). When fed a low NaCl diet, pendrin knockout mice demonstrated a lower blood
pressure and higher plasma pH than WT mice, most likely due to a reduced chloride/bicarbonate exchange 
activity (Verlander et al. 2003). The knockout mice also displayed a significantly reduced ENaC expression, a 
finding that could explain the hypotension observed at steady-state (Kim et al. 2007). Interestingly however, 
patients with Pendred syndrome as well as the pendrin knockout mice neither have abnormal renal function, 
acid-base homeostasis nor fluid and electrolyte homeostasis at steady state, which suggests that other renal 
chloride/bicarbonate exchangers are able to compensate the loss of pendrin (Royaux et al. 2001, Verlander et 

E. Nephrogenic Diabetes Insipidus

First introduced in 1947, the term ‘nephrogenic diabetes insipidus’ (NDI) emerged in a study of 7 
cases in one family where the condition was transmitted by mothers to their male progeny (Williams and 
Henry 1947). This disease can be defined as the nephrogenic incompetence to concentrate urine in response to 
the antidiuretic hormone arginine vasopressin. This results in defective permeability to water in the DCT or 
CD, or an increase of the corticopapillary interstitial osmotic gradient or a combination of both (Valtin and 
Schafer 1995). Therefore, NDI patients excrete a large volume of water through urine (water diuresis) 
(Morello and Bichet 2001). Diuresis is also associated with increased fluid/water intake (polydipsia), and 
could also lead to secondary issues like hypernatremia and dehydration.

About 90 % of patients with congenital NDI carry a mutation in the gene encoding AVPR2, while the 
remaining 10 % have a mutation in the gene encoding the water channel AQP2 (Bech et al. 2018). AQP3 
knockout mice also developed NDI (Ma et al. 2000), but there is no NDI patient with a mutation in this gene 
described at this time. The most common non-hereditary cause of NDI is chronic lithium therapy 
(administered for psychiatric disorders including depressive or bipolar disorder) due to an inhibition of the 
vasopressin-triggered cAMP signaling cascade (Bech et al. 2018).

Although no viable AQP2 knockout mouse model has been described, knocking-in an NDI-causing 
AQP2 mutation (T126M) in mice confirmed the essential role of this channel in NDI (Yang et al. 2001). The 
mutant pups developed normally until the first 2-3 days but then started failing to thrive and did not survive 
past day 6 unless given supplemental fluids. When heterologously expressed in *Xenopus* oocytes or Chinese 
hamster ovarian CHO cell line, the human ortholog of this mutant was functional but retained in the 
endoplasmic reticulum unless chemical chaperones were provided. In the CD epithelial cells, AQP3 is
expressed at the basolateral membrane. Deletion of this gene resulted in normally developed mice that presented polyuria and a dilute urine (Ma et al. 2000). In these mice, expression of AQP1 and AQP4 was not affected, however, AQP2 abundance was reduced significantly. Arginine vasopressin administration to these animals revealed a mild urine-concentrating defect.

A mouse model carrying an NDI-causing AVPR2 mutation also displayed an inability to concentrate its urine and a dilute basal urine. Similarly to the AQP2 knocked-in mice described above, these pups failed to thrive and died within 2 weeks from birth from dehydration (Yun et al. 2000).

Over the past decade, considerable research efforts have aimed at identifying therapeutic strategies to overcome the molecular defects causing NDI. Strategies have focused on bypassing or potentiating AVPR2 signaling and on increasing the abundance of apical AQP2 channel in principal cells as reviewed elsewhere (Sands and Klein 2016).

5. Conclusions

The renal collecting duct is the site where the final urine composition is set. This occurs through fine hormonal regulations, the removal or relocation of specific proteins from/to the apical membrane and the dynamic regulation of the abundance of various cell types. As illustrated above, this segment of the kidney plays essential roles in ion, water and acid-base homeostasis. Many pathologies are associated with dysfunctional CD. However, despite our current understanding of the CD function, we are still uncovering unsuspected features of these cells. The recent report of the role of type-B IC in blood pressure regulation illustrates that there remains many gaps in our knowledge of the CD. There is more to discover, including the respective role of electrogenic versus electroneutral ion reabsorption in PC and type-B IC, respectively. What regulates one pathway versus the other in maintenance of blood pressure? What is the role of tight junctions in this process? Are mutated tight junction proteins also causing abnormal blood pressure? Similarly, the role of non-A, non-B IC remains obscure. The regulation of the CD cells interconversion is not well-understood either. Further, the interplay between the various CD cell types needs further investigation, but a robust cell model recapitulating all the features of CD cells is still lacking. Thus, further research is needed to decipher the role of this kidney segment in health and diseases.
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References


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Figure legends

Figure 1: Types of transepithelial transport. As the plasma membrane is poorly permeable to ions and water, two pathways are possible for transepithelial transport of molecules: the transcellular (green and blue arrows and molecules) and paracellular (burgundy arrow) pathways. If molecules X or Y cross the epithelium through the transcellular pathway, they need to cross a series of two membranes, the apical and the basolateral membrane in addition to diffusing through the cytosol to reach the opposite membrane. Specific channels and primary or secondary active transporters are expressed in these membranes to facilitate and regulate the nature, direction and number of molecules crossing the plasma membrane. The localization of these transporters is essential to drive molecules’ movement in one direction versus the other across the epithelium (absorption versus secretion).

The second pathway facilitates the movement of a molecule Z in a paracellular way through tight junctions (burgundy arrow). For this type of transport, molecules are passively moving between epithelial cells down their electrochemical gradient, and only have to cross one “barrier”, the tight junctions (as opposed to crossing apical and basolateral membranes in the transcellular pathway). Tight junctions are composed of occludins, claudins, junction adherens molecules and tricellulins. The highly dynamic nature of tight junctions allows a fast and specific regulation of the amount and the nature of molecules moving through this pathway. Note that primary active transporters can be apically or basolaterally located and can carry one or more substrates (in a co-transport or antiport mode of action).

Figure 2: Schematic diagram illustrating the two NaCl reabsorption pathways identified in CD cells. The electrogenic pathway (shown on the left) includes the activity of PC and type-A IC. The activation and opening of ENaC (1) results in a lumen-negative transepithelial potential. Apically reabsorbed sodium is actively exported outside of the cells via the basolateral Na⁺/K⁺-ATPase (represented as “NKATPase”) (2). This transepithelial potential generates a driving force for the apical secretion of potassium through KCC and ROMK in PC and of protons through the V-H⁺-ATPase (represented as “HATPase”) and H⁺/K⁺-ATPase (represented as...
“HKATPase”) in type-A IC (3). This potential also favors paracellular fluxes of chloride ions through the tight junctions, specifically via claudin-4 and 8 (not shown) (4). Water passively follows ion reabsorption through apical AQP2 and basolateral AQP3/4.

The electroneutral pathway (shown on the right) includes the reabsorption of sodium and bicarbonate ions in exchange for a chloride ion through SLC4A8. The bicarbonate is provided by the activity of apical pendrin which exports bicarbonate in exchange of chloride reabsorption. At the basolateral membrane, chloride ions are transported to the blood via CIC-Kb and sodium and bicarbonate via the cotransporter SLC4A9.

Figure 3: Schematic diagram illustrating the previous and the new model for SLC4A1-mediated dRTA.

Left, dRTA-causing SLC4A1 mutant proteins were described as either non-functional (1), intracellularly retained (2) or apically mis-trafficked (3), based on MDCK cell studies. Right, recent in vivo findings showed that a dominant dRTA-causing mutation did not cause mis-trafficking of the protein, but rather a lack of relocation of the V-H+-ATPase to the apical membrane upon plasma acidification, possibly resulting in a dramatic loss of type-A IC in the CD.
Transcellular secretion

Transcellular absorption

Paracellular absorption

Primary active transporters

Channel

Cotransporter

Ion exchangers

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Figure 1 Lashhab & Ullah
Principal cell-mediated electrogenic NaCl reabsorption

B-IC cell-mediated, electroneutral NaCl reabsorption

Figure 2 Lashhab & Ullah
Previous model for SLC4A1-mediated distal RTA

New model for SLC4A1-mediated distal RTA

Figure 4 Lashhab & Ullah