Molecular Physiology in Renal Water, Sodium and Acid-base Metabolism

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Water is the most abundant component of all living organisms, however cells and tissues are remarkably different in their ability to absorb or release water. Water selective channels (aquaporins) had long been suspected to provide rapid water permeation of certain tissues including kidney, however the molecular identity of these membrane proteins remained unknown until the serendipitous discovery of aquaporin-1 by Peter Agre and his colleagues. The subsequent identification of several renal aquaporins provided insight into the fundamental physiology of water balance and the pathophysiology of water balance disorders.

Key Words : Aquaporin, Urine concentration, Membrane protein

Renal Water Balance

A major function of the kidney is to regulate body water and sodium balance. This function is achieved and finely regulated by a number of cellular and molecular processes, including tubular reabsorption of water and sodium through renal water channels (aquaporins) and sodium (co) transporters under the control of hormones and nerves along with intracellular signaling pathways[1-5]. Since water can slowly diffuse through lipid bilayers, all biological membranes exhibit some degree of water-permeability [6]. Nevertheless, specialized membrane water transport molecules must exist in renal tubules which have distinctively high water permeability, hence this is crucial for maintaining the fluid reabsorption and the urinary concentration mechanism. In this context, the discovery of aquaporin membrane water channels by Agre and co-workers[7-9] therefore answered a long-standing biophysical question of how water crosses biological membranes in kidney specifically, and provided insight into the fundamental physiology of water balance and the pathophysiology of water balance disorders.

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In kidney, the aquaporins are therefore present at the 1) proximal tubules, 2) descending thin limbs and vasa recta, and 3) collecting ducts. In the proximal tubule which has an extraordinarily high water permeability[10-12], aquaporin-1 (AQP1) is abundantly present at the apical plasma membranes as well as basolateral plasma membranes[13]. The AQP1 mediates near-isosmotic fluid reabsorption that is driven by the active sodium transport via a variety of renal sodium transporters which will be described below. In the descending thin limbs and descending vasa recta, the presence of AQP1 is involved in the countercurrent exchange process of the renal medulla[14]. In kidney collecting ducts, at least four aquaporins (AQP2, AQP3, AQP4, and AQP6) are known to be expressed[5]. AQP2 is the apical water channel of collecting duct principal cells and is highly abundant in the apical plasma membrane and subapical vesicles [15, 16]. Water reabsorption in the collecting duct is regulated by both short-term regulation and long-term adaptational mechanisms, both of which have been shown to depend critically on AQP2 expression[16-20]. Because the collecting duct is the final site for regulation of renal water excretion, the changes in both AQP2 trafficking and abundance hence the

changes in the water permeability of the collecting duct have been known to play a significant role in the a variety of conditions demonstrating altered capacity to concentrate urine[5, 21]. Vasopressin is a peptide hormone that controls systemic osmolality through regulation of renal water excretion/reabsorption. Its main site of action is the renal collecting duct, where it regulates water, urea, and sodium transport[22]. In collecting duct principal cells, vasopressin binds to the V_2 receptor, which stimulates an increase in intracellular cyclic AMP content via adenylyl cyclase. Subsequently cAMP binds to the regulatory subunit of protein kinase A, resulting in dissociation of the regulatory subunit from the catalytic subunit. This activates the catalytic subunit, which phosphorylates various proteins including AQP2[23]. AQP2 is then translocated from intracellular vesicles to the plasma membrane thereby increasing the water permeability of the apical plasma membrane[16]. Binding of vasopressin to the V_2 receptor is also associated with intracellular calcium mobilization mediated by calcium release from ryanodine-sensitive intracellular stores via the type I ryanodine receptor, which triggers calmodulin-dependent regulatory processes within the cell[24]. Moreover, multiple studies have demonstrated changes in AQP2 abundance in several inherited and acquired water balance disorders [1, 5, 25]. This includes inherited forms of nephrogenic diabetes insipidus (NDI), acquired states of NDI (e.g., lithium treatment, hypercalcemia, hypokalemia), and other diseases associated with urinary concentrating defects (e.g., acute and chronic renal failure, ureteral obstruction) where AQP2 abundance (and/or targeting) is affected. Conversely, AQP2 abundance and targeting appears to be increased in some conditions with water retention such as pregnancy and congestive heart failure. These long-term actions are thought to be associated with regulatory processes at a transcriptional level, involving either the transporter genes themselves or regulatory molecules that indirectly alter water channel protein abundance. Transcriptional regulation of AQP2 is thought to be a result of vasopressin-induced increase in intracellular cAMP levels with concomitant increases in activation of PKA. This view is supported by the results of molecular cloning and sequencing of the 5′-flanking region of the AQP2 gene, which has demonstrated the presence of a putative cAMP-response element (CRE) motif[26]. Moreover activation of CRE can increase AQP2 gene transcription in the response to increased intracellular cAMP levels[27, 28]. Water transport across the basolateral plasma membrane of collecting duct principal cells is thought to be mediated by AQP3 and AQP4[29, 30]. Consistent with this, rats with lithium-induced NDI have a dramatically reduced AQP2 and AQP3 expression levels along with a marked polyuria and urinary concentrating defect[31, 32]. Moreover, transgenic mice lacking AQP3 are severely polyuric[33] and inner medullary collecting ducts from AQP4 deficient mice have a significant reduction in vasopressin-stimulated water permeability[34]. Therefore, these findings demonstrate that basolateral membrane water transport can also become a rate limiting factor for water reabsorption.

In contrast to the AQP2, AQP3, and AQP4 which are present in the collecting duct principal cells, AQP6 (previously referred to as rat WCH3 and human hKID) is present in the collecting duct intercalated cells[35]. It is therefore possible that AQP6 is involved in the acid/base homeostasis, or regulation of water balance and indeed altered expression of AQP6 in the intercalated cells was observed in the corresponding physiological or pathophysiological conditions[36]. AQ7 mRNA has been identified in the kidney and immunocytochemistry revealed that AQP7 is expressed in the apical plasma membrane of the proximal tubule[37]. In addition, AQP8 is expressed at low levels in rat kidney proximal tubules and collecting ducts[38]. Continued analysis of the aquaporins is providing detailed molecular insight into the fundamental physiology and pathophysiology of water balance and water balance disorders and renal AQPs are discussed in detail in the another chapter in this issue (Dr. S-W Kim).

Renal Na (co)transporters

The renal tubular water reabsorption is driven by

the active sodium transport via a variety of sodium transporters. Proximal tubule, the site of reabsorption of approximately two thirds of the sodium that enters the tubular fluid by glomerular filtration[39], carry out active sodium reabsorption mainly through apically expressed Na/H exchanger (type 3 Na/H exchanger: NHE3) and basolaterally expressed Na,K-ATPase and Na-HCO3 cotransporter (kNBC1). The type II Na/Pi cotransporters (NaPi-2) and sodium-glucose cotransporters (SGLT-2) are also expressed apically in the proximal tubules and contribute the sodium reabsorption in this segment[40, 41]. Concentration of the urine requires establishment and maintenance of a hypertonic medullary interstitium[3]. The loop of Henle generates a high osmolality in renal medulla by driving the countercurrent multiplication[42], which is dependent on the active NaCl absorption by the thick ascending limb (TAL). The apically expressed Na-K-2Cl cotransporter (rat type 1 bumetanide-sensitive cotransporter: BSC-1 or NKCC2) and NHE3, as well as basolaterally expressed Na,K-ATPase are key components responsible for sodium reabsorption by the TAL[43-47]. Furthermore, urinary dilution is also mediated by NaCl absorption in the TAL and distal convoluted tubule (DCT)[48-51]. The collecting duct is the site for the fine regulation of sodium excretion and α-, β- and γ-subunits of the epithelial sodium channel (ENaC) are chiefly involved in this [52-56].

The regulation of renal sodium transporters is importantly involved in the renal sodium handling. The NHE3, which is expressed apically in the proximal tubules[57], is believed to be the protein that mediates a major fraction of the transcellular sodium and bicarbonate reabsorption[39]. Consistent with the proposed roles of the NHE3 in kidney tubules, the proximal convoluted tubules from NHE3 gene knockout mice have a marked decrease in the fluid and bicarbonate absorption by 69% and 61%, respectively[58]. These findings therefore indicate that NHE3 is critically involved in the proximal tubule sodium, fluid, and bicarbonate reabsorption. BSC-1 (NKCC2) expression has been demonstrated to be regulated and this appears to play a significant role in the urinary concentration mechanism[43, 59]. An increase in the delivery of NaCl to the loop of Henle by chronic oral saline loading[60] or vasopressin treatment (shown by use of dDAVP : a V2-selective agonist) [59] is known to upregulate BSC-1 levels, whereas hypercalcemia- or hypokalemia-induced NDI is associated with reduced BSC-1 expression[61, 62]. Indeed remnant kidney induced by 5/6 nephrectomy[63, 64], where plasma vasopressin levels and the sodium delivery to the loop of Henle are increased, is associated with upregulation of BSC-1 expression (density per nephron). Since V_2-receptor is coupled to activation of adenylyl cyclase, it is possible that the upregulation of BSC-1 by vasopressin is a result of elevated levels of cAMP levels. Consistent with this, a cAMP-regulatory element (CRE) was identified in the 5′-flanking region of the mouse NKCC2 gene[65]. Thus, expression of BSC-1 in the TAL is significantly involved in the long-term regulation of the countercurrent multiplication system. Moreover an apical potassium channel in the TAL (Kir 1.1 or ROMK) is now known as another critical component of the countercurrent multiplication mechanism and the characteristic lumen-positive voltage in thick ascending limb arises from the secretion and recycling of potassium through the apical ROMK channel[66]. This ROMK channel is upregulated by vasopressin administration as well[67, 68] and downregulated in hypercalcemia-induced NDI[62]. The calcium sensing receptor expressed at the thick ascending limb may be linked to the regulation of thick ascending limb sodium transporters. Epithelial sodium channel ENaC is regulated by the adrenal mineralocorticoid hormone, vasopressin, and insulin which markedly increase the apical permeability of the collecting duct to sodium[56, 69, 70]. The importance of the ENaC in the extracellular fluid volume regulation has been demonstrated in recent studies that have identified mutations in ENaC as the basis of the pathogenesis of Liddle's syndrome, a disorder characterized by volume expansion and hypertension[71, 72] as well as type I pseudohypoaldosteronism, a disorder characterized by volume depletion and hypotension[73].

The renin-angiotensin-aldosterone system has been

demonstrated to play a critical role in the regulation of renal sodium and water metabolism through a variety of physiological pathways. Aldosterone increases the sodium reabsorption in part by increasing the abundance of the thiazide-sensitive Na-Cl cotransporter (TSC) in the DCT cells[49] and the α-subunit of epithelial sodium channel in the collecting duct principal cells[70]. In contrast, oral administration of spironolactone, a mineralocorticoid receptor antagonist, decreases substantially the abundances of the thiazide-sensitive Na-Cl cotransporter (TSC or NCC) and the α-subunit of the amiloride-sensitive epithelial Na channel (ENaC)[74]. A dependency of TSC expression on aldosterone was confirmed by showing increased TSC expression in response to aldosterone infusion in adrenalectomized rats[74]. Moreover, angiotensin II (AngII) has known effects on the regulation of renal hemodynamics, glomerular filtration rate, aldosterone secretion, as well as more direct effects on renal tubule transport in the proximal tubule[75, 76]. Increased NHE3 abundance in medullary and cortical TAL cells as well as in the proximal tubule brush border was observed in response to Ang II treatment and this may contribute to the previously observed enhanced renal Na and HCO3 reabsorption by AngII treatment[77]. Thus renal expression of sodium transporters plays a critical role in the renal sodium handling and altered expression could be expected in conditions with urinary concentration defect or deranged renal sodium metabolism and this is discussed in more detail in the another chapter in this issue (Dr. G-H Kim).

Na-dependent bicarbonate cotransporters

In addition to the water and sodium balance, the kidney maintains acid-base balance by secretion or reabsorption of H^+ and HCO_3^-[78]. Recently several members of the Na-dependent HCO_3 cotransporter (NBC) family have been identified and functionally characterized in vitro, and they could potentially play an important role in mediating acid/base transport in kidneys or in maintaining intracellular pH levels[79-81]. The proximal tubule cells reabsorb HCO_3^- by the apically expressed NHE3 in conjunction with the basolaterally expressed Na, HCO3 cotransporter (kNBC1) that plays an important role in mediating electrogenic bicarbonate efflux[82]. Consistent with this, immunocytochemistry demonstrated that electrogenic Na,HCO3 cotransporter (kNBC1) is associated with the basolateral plasma membrane domains of predominantly S1 and S2 segment in proximal tubules of rat kidney[83, 84].

Moreover, the electroneutral NBCn1 was cloned from rat smooth muscle cells[80], and it has been demonstrated that NBCn1 immunolabeling in normal rat kidney is associated with the basolateral plasma membrane domains of TAL cells in the outer medulla [85]. Consistent with this, studies of intracellular pH measurement confirmed that the sodium dependent recovery from acidosis in the presence of $HCO3^-$ and amiloride is present in the thick ascending limbs[85, 86] (Fig. 1). In addition, collecting duct intercalated cells in the inner stripe of the outer medulla (ISOM) and in the inner medulla (IM) also exhibit NBCn1 immunolabeling[85]. Electroneutral NBC3[81], which was isolated from the human skeletal muscle cells and is 89-92% identical to NBCn1, is exclusively associated with intercalated cells in connecting tubules and in cortical, outer medullary and initial inner medullary collecting ducts of rat kidney[87]. In particular, the electroneutral NBC3 labeling in connecting tubule and cortical collecting duct is associated with both type-A and type-B intercalated cells. NBC3 colocalizes with the H^+-ATPase in the apical domains in the type-A intercalated cells or in the basolateral do-

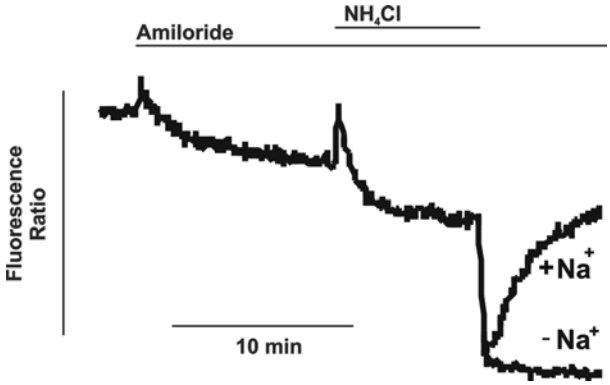

Fig 1. *Bicarbonate transport in mTAL. The trace marked +Na^+ shows the pH recovery in the presence of Na^+ and HCO_3^-.*

mains in the type-B intercalated cells[87, 88]. Therefore, NBC3 may participate in the H^+/base transport in the collecting duct. The role of sodium-dependent HCO_3 transport outside the proximal tubules is still uncertain, however chronic metabolic acidosis (induced by chronic NH_4Cl loading in rats) was associated with 1) significantly increased abundance of the electroneutral NBCn1 as well as enhanced bicarbonate transport in mTAL and 2) significantly increased abundance of the electroneutral NBC3 in the intercalated cells of the collecting ducts.

The protein abundance of NBCn1 is dramatically enhanced in response to CMA induced by oral NH_4Cl loading (Fig 2). This supports a previous study demonstrating a DIDS sensitive and sodium and bicarbonate dependent recovery from acidosis in kidney slices from the ISOM[85]. Nevertheless, the electroneutral Na,HCO_3 cotransport activity in rat mTAL is not well understood. Moreover the dramatic increase in expression of basolateral NBCn1 and uptake of HCO3 in CMA seems unexpected. One possible explanation may be though that NBCn1 plays a role in supporting NH_4^+ reabsorption in the mTAL, which is significantly enhanced in response to CMA as an adaptive change (Fig. 3)[89]. A major fraction of the ammonium (NH_4^+) is reabsorbed at the TALs mainly through the Na-K-2Cl cotransporter presumably by substituting for K^+ ion[90]. Then, in TAL cells lipid soluble ammonia (NH3) from ammonium (NH_4^+) can passively diffuse into the interstitium through basolateral plasma membrane, whereas hydrogen ion from ammonium (NH_4^+) combines intracellulary with bicarbonate to form H_2CO_3. This is converted to CO_2 and H_2O, and CO_2 diffuse into the interstitium where it combines with H_2O to yield hydrogen ion and bicarbonate. In the medullary interstitium, hydrogen ion combines with ammonia (NH_3) to form ammo-

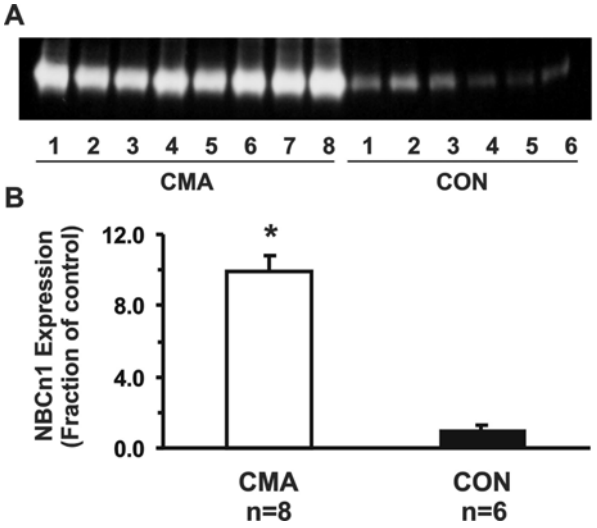

Fig. 2. Semiquantitative immunoblotting of membrane fractions of whole kidneys. A) The immunoblots were reacted with anti-NBCn1 and revealed a broad band centered approximately at 180 kDa. B) Densitometric analyses revealed that the abundance of whole kidney NBCn1 in rats treated with 0.28M NH4Cl in drinking water for 2 weeks (CMA) was dramatically increased to 995±87% of control levels (100±27%, $^*p<0.05$).

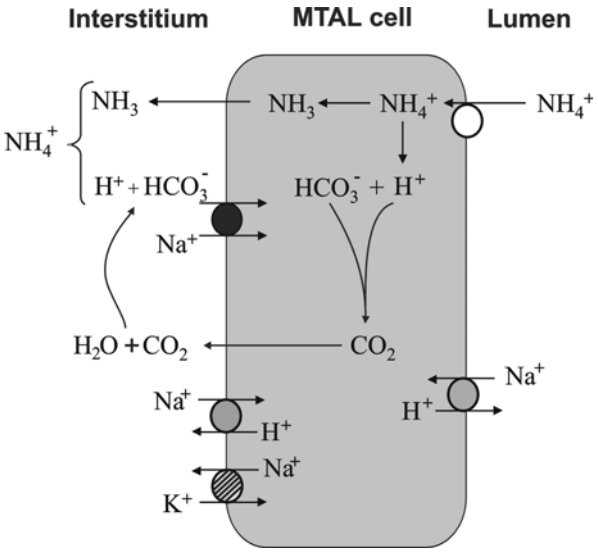

Fig. 3. The possible role of the NBCn1 in the ammonium ($NH4^+$) reabsorption in the medullary thick ascending limb. $NH4^+$ is mainly reabsorbed at the medullary thick ascending limbs (MTAL) through Na-K-2Cl cotransporter (open circle) presumably by substituting for K^+ ion. Then, in MTAL cells lipid soluble ammonia (NH3) from ammonium ($NH4^+$) can passively diffuse into the interstitium through basolateral plasma membrane. Hydrogen ion from ammonium ($NH4^+$) combines intracellulary with bicarbonate to form H2CO3 (carbonic acid), which is converted to CO2 and H2O. CO2 diffuse into the interstitium where it combines with H2O to yield hydrogen ion and bicarbonate. In the medullary interstitium, hydrogen ion combines with ammonia (NH3) to form ammonium ($NH4^+$), whereas bicarbonate is transported into the cells though NBCn1 (closed circle) which is located in the basolateral plasma membrane with sodium ion. Thus, NBCn1 may play an important role in 1) the ammonium ($NH4^+$) reabsorption, medullary accumulation, and urinary excretion of ammonium; and 2) the basolateral bicarbonate transport into the TAL cells. MTAL cells also express apical and basolateral Na-H exchanger as well as basolateral Na,K-ATPase.

nium (NH_4^+), whereas bicarbonate is transported into the cells though basolaterally expressed NBCn1. Consistent with both increase in the abundance and functional activity of NBCn1, CMA has been known to be associated with an increased urinary excretion of total ammonia (NH_4^+/NH_3). Upregulation of NH4Cl reabsorption in CMA[89] would in this case therefore be expected to be associated with an increase of NBCn1 activity in the basolateral membrane. It can also be noted that in this model the basolateral influx of HCO_3^- does not lead to a net transport of HCO_3^- towards the lumen. Thus, NBCn1 is possible to play an important role in 1) the ammonium ($NH4^+$) reabsorption, medullary accumulation, and urinary excretion of ammonium and 2) the basolateral bicarbonate transport into the TAL cells.

Conversely, it may be possible to speculate that increased basolateral NBCn1 activity in response to CMA may contribute to the increase intracellular Na^+ which in turn decreases the inward gradient for apical Na,K-2Cl cotransporter, hence it may reduce ammonium reabsorption in the TAL. However, CMA is known to inhibit NaCl and fluid reabsorption in the proximal tubule and increase NaCl delivery to the loop of Henle[91]. Moreover a chronic increase in sodium delivery mediates an adaptive increase in bicarbonate and ammonium reabsorption in the mTAL independent of changes in acid-base balance presumably by increasing both apical Na-K-2Cl cotransporter[60] as well as basolateral membrane Na,K-ATPase activity[89, 92, 93]. Therefore the increased ability of the mTAL to absorb $NH4^+$ during CMA is associated with fine regulation of mTAL $NH4^+$ transport via coordinated effects on various apical and basolateral transporters.

The inhibition with DIDS (about 70% in ref no. 86) was much larger than in the cloning studies (about 25%). However it should be pointed out that in smooth muscle cells[94] and cardiac myocytes[95] where electroneutral NBC was first demonstrated the inhibition induced by DIDS was at least 70%. The smaller effect of DIDS on the NBCn1-B expressed in oocytes may have several explanations. The observation in the present study of a significant effect of DIDS supports the view that an electroneutral NBC is expressed. However, it cannot be excluded that another DIDS sensitive electroneutral NBC isoform, distinct from NBCn1, may also be expressed and may play a major role in the basolateral plasma membrane of the thick ascending limb cells. Further studies are therefore warrented to define this.

A novel Cl/HCO_3 exchanger pendrin

Recently it has been demonstrated that pendrin is expressed in the apical domain of all type B intercalated cells as well as in nonA-nonB intercalated cells in the connecting tubule (CNT) and cortical collecting duct (CCD) of both mouse and rat kidney[96]. The demonstration that pendrin is expressed in type B intercalated cells is in agreement with observations by Royaux and co-workers[97] and provide further support for pendrin representing the apical anion exchanger of type B intercalated cells as indicated by their elegant transport studies in isolated CCD segments from pendrin-deficient mice[97]. The results of immunoelectron microscopic studies revealed strong labeling for pendrin in the apical plasma membrane as well as in apical intracellular vesicles of both type B and nonA-nonB intercalated cells[96]. As reported previosly[97], there was no expression of pendrin in the AE1-positive type A intercalated cells. Therefore these observations indicate that both type B and nonA-nonB intercalated cells are capable of pendrin-mediated bicarbonate secretion in the CCD and CNT and suggest that bicarbonate secretion may be regulated by trafficking of pendrin between intracellular vesicles and the apical plasma membrane.

It is generally accepted that type B intercalated cells are involved in bicarbonate secretion which is mediated by an apical Cl^-/HCO_3^- exchanger that is distinct from AE1. To establish whether pendrin might represent the apical anion exchanger in the B cells, Royaux, Wall and colleagues[97] examined bicarbonate transport in isolated perfused CCD segments from pendrin-deficient mice (Pds-knockout mice). In contrast to tubules from wild type mice, CCD seg-

ments from pendrin-deficient mice did not secrete bicarbonate in response to an alkali load. Those findings indicated that pendrin is responsible, at least in part, for bicarbonate secretion in the CCD and thus may correspond to the apical Cl^-/HCO_3^- exchanger in the type B intercalated cells.

The nonA-nonB intercalated cell was first described in the rat kidney by Kim et al.[98] and Madsen et al.[99] who reported that a few intercalated cells in the CNT exhibited ultrastructural characteristics that were distinct from those of type A and type B intercalated cells. The nonA-nonB intercalated cells are larger than type A and type B intercalated cells; they are rich in mitochondria and have numerous apical microprojections similar to those described in type A intercalated cells. It was suggested that they might correspond to the AE1-negative intercalated cells with apical H^+-ATPase which, according to a previous study by Alper et al.[100], constituted approximately 1% of the intercalated cells in the renal cortex of the rat. Subsequent studies revealed that nonA-nonB cells were present also in the mouse and confirmed that these cells exhibit strong labeling for H^+-ATPase in the apical plasma membrane, but do not express AE1[101, 102]. Moreover, the prevalence of the nonA-nonB intercalated cells in the CNT and CCD was found to be higher than previously anteciated, and it was demonstrated that the majority of intercalated cells in the CNT of the mouse belongs to the nonA-nonB subtype[101].

The function of the nonA-nonB intercalated cells remains to be established. It is not known whether they represent a distinct subtype of intercalated cell or a modified form of either the type A or type B intercalated cell. Moreover, the response of the nonA-nonB intercalated cells to changes in acid-base balance has not been investigated in detail, and there are no studies of acid-base transport in the CNT of the mouse, where nonA-nonB cells constitute a major proportion of the cells. However, the demonstration that these cells express both H^+-ATPase and the anion exchanger, pendrin, in the apical plasma membrane suggests that they are capable of both proton and bicarbonate secretion. Thus, non A-non B intercalated cells appear to represent a unique cell type which may be involved in the systemic acid-base regulation by either apical proton secretion or apical bicarbonate secretion.

Perspectives

Reabsorption of water and sodium or acid-base regulation by the kidney was initially characterized by a combination of micropuncture studies, microdissection and isolated perfused tubule studies, and vesicle studies. The evidence from these studies and subsequent studies was instrumental in the eventual cloning of cDNAs for these channels and transporters. The cDNA sequences have now allowed us to produce an ensemble of affinity-purified, peptide-directed polyclonal antibodies to these water channel proteins, sodium transporters and sodium-dependent bicarbonate cotransporters. This has allowed us to use comprehensive sets of antibodies against proteins relevant to a given physiological process and to investigate entire pathways through simultaneous assessment of the regulatory state of all members of the pathway. The approaches in the experiments are 1) to characterize the antibodies, immunolocalize the channels and transporters in kidney; 2) to employ the entire ensemble of antibodies to screen kidney membranes enriched membrane fractions as well as intracellular vesicle fractions to determine whether changes of expression of the AQPs, sodium transporters and sodium-dependent bicarbonate cotransporters play a role in the corresponding water and sodium balance disorders as well as in the acid-base disturbance.

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