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Review Article

REGULATION OF ACID-BASE HOMEOSTASIS BY THE KIDNEY

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INTRODUCTION

To maintain normal acid-base homeostasis, the kidneys must not only reabsorb all of the filtered bicarbonate (HCO_3^- reclamation), but they also have to regenerate the bicarbonate which has been lost as a result of buffering the acid produced in the body from catabolism of dietary proteins. The body produces acids (organic and inorganic) from sources other than CO_2 which are called nonvolatile or fixed acids. These include phosphoric and sulfuric acid generated during catabolism of proteins and other organic molecules containing sulfur and phosphorus, and lactic acid, ketone bodies (acetoacetic acid and β-OH butyric acid) and other acids (more than 30 have been identified). Those consuming a high protein diet (Western diet) normally have a net daily production of 75-100 mmoles (1-1.5 mmol/kg body weight) of nonvolatile acids. In contrast, those who are on a strict vegetarian diet have a net metabolic production of bicarbonate (net loss of H^+). To regenerate the bicarbonate which is lost in buffering the nonvolatile acids, the kidneys secrete H^+ (proton) in the distal nephron. For each H^+ which is secreted in the distal nephron, a new bicarbonate is generated inside the cell which is then transported into the plasma. This process results in net acid (H^+) excretion (or bicarbonate regeneration) and is the final step in the regulation of acid-base balance.

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Proximal tubular acidification (bicarbonate reclamation)

Plasma bicarbonate is completely filterable at the glomerulus. The daily filtered load of bicarbonate is about 4500 mmoles. The majority (85-90%) of the filtered HCO_3^- is reabsorbed in the proximal tubule. The remainder is reabsorbed in the thick ascending limb of the loop of Henle (about 5-10%) and the collecting duct (5-10%). Bicarbonate reabsorption in the proximal tubule requires active H^+ secretion to buffer the filtered bicarbonate, and depends on a variety of transporters and enzymes (Fig. 1, for general reviews see references 1-3).

Active H^+ secretion in the proximal tubule is carried out by two different H^+ secretory pumps. About two-thirds of the H^+ secretion in the proximal tubule is via Na^+: H^+ antiporter (Na^+:H^+ exchanger). This is an electroneutral, secondary active pump which exchanges one Na^+ for one H^+. This pump does not directly utilize ATP as a source of energy but is energized by the concentration gradient of Na^+ across the luminal membrane of the proximal tubular cells. The tenfold higher sodium concentration in the lumen (sodium concentration in lumen =150 mmol/L versus sodium concentration inside the cell=15 mmol/L) drives Na^+ into the proximal tubular cells in exchange for H^+ which is secreted into the lumen. In order to keep this pump functioning, sodium concentration inside the proximal tubular cells must be kept low. This is achieved by the Na^+, K^+-ATPase, a primary active pump, which is located in the basolateral membrane of these cells. This pump extrudes three Na^+ ions in exchange for two K^+ ions. The result is a lowered intracellular sodium concentration as well as a net negative membrane potential of -70 mv inside the cells. The latter is

due to more positive charges being extruded than brought into the cell by the Na^+, K^+-ATPase pump. However, in steady state the total amount of ions entering the proximal tubule cells from the lumen equals the total amount of ions that exit the cells through their basolateral membrane into the plasma.

The Na^+: H^+ exchanger can function in either direction based on the concentration gradients of Na^+ and H^+. It can also exchange other ions such as NH_4^+ instead of H^+, and Li^+ instead of Na^+. This pump is the major mechanism for NH_4^+ secretion in the proximal tubule which is of great importance for net acid excretion (discussed later). The other mechanism for H^+ secretion in the proximal tubule is a "vacuolar" type H^+-ATPase pump, a primary active pump which utilizes ATP as a source of energy. The nomenclature "vacuolar" is because this type of H^+-ATPase pump is widely distributed among mammalian intracellular organelles (clathrin-coated vesicles, endosomes, lysosomes, Golgi membrane and endoplasmic reticulum) involved in endocytic and exocytic pathways, collectively referred to as the vacuolar system. This pump is also present in the plasma membrane of H^+-transporting cells in the kidney (proximal tubular cells, and intercalated cells in the collecting duct).[1,2,4-7]

Although the Na^+: H^+ antiporter is the major mechanism for H^+ secretion in this segment,[3] the vacuolar H^+-ATPase present in the brush border membrane[8-11] has been shown to contribute to 30-40% of the overall proximal H^+ secretion in the rat kidney.[12-14] A recent report has suggested a differential regulation of the Na^+:H^+ antiporter and H^+-ATPase by pH and bicarbonate in the proximal tubule.[15] It demonstrated that luminal Na^+: H^+ exchanger is predominantly regulated by pH whereas H^+-ATPase is mainly regulated by bicarbonate and/or PCO_2. It also showed that H^+-ATPase adaptation was likely mediated via endocytic/exocytic pathways, whereas adaptation in Na^+: H^+ exchanger was via non-endocytic/exocytic, but kinetic regulatory pathways.[15]

Secreted H^+ instantly combines with the filtered HCO_3^- to generate carbonic acid (H_2CO_3) which in the presence of membrane bound carbonic anhydrase 4 (present on the surface of brush borders of the proximal tubular cells) is rapidly converted to $CO_2 + H_2O$. This latter reaction would have been very slow in the absence of carbonic anhydrase (CA).

$$HCO_3^- + H^+ \rightarrow H_2CO_3 \text{ -- CA-}\rightarrow CO_2 + H_2O$$

The CO_2 which is generated from dehydration of the carbonic acid diffuses freely into the proximal tubular cells where in the presence of cytosolic carbonic anhydrase 2 it combines with H_2O and yields carbonic acid. The latter molecule dissociates into H^+ and HCO_3^-.

$$CO_2 + H_2O \text{ -- CA} \rightarrow H_2CO_3 \rightarrow HCO_3^- + H^+$$

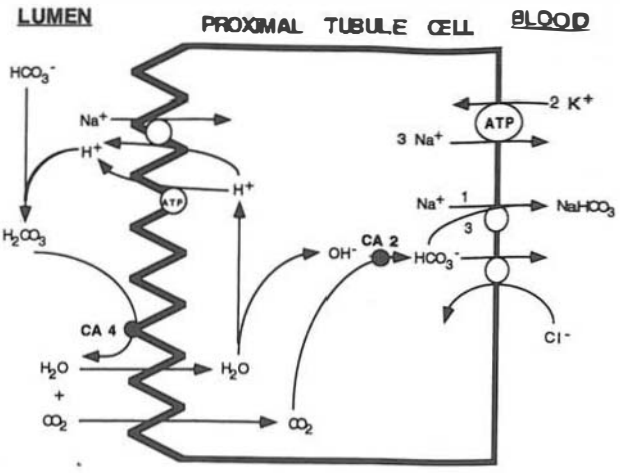

Fig. 1. The proximal renal tubules reabsorb about 85% of the filtered bicarbonate (HCO_3^-), without pronounced reliance on electrical or chemical gradients, through its buffering by the protons which are secreted into the lumen. Proton secretion by the proximal tubular cell is predominantly via an electroneutral Na^+: H^+ exchanger and to a lesser degree via an electrogenic ATP utilizing proton pump (the H^+-ATPase pump). The resulting titration of intraluminal HCO_3^- creates H_2CO_3, the substrate for production of CO_2 by mem bound carbonic anhydrase 4 (CA4). The CO_2 freely into the cell, where in the presence of cytoplasmic CA2 it combines with OH^- and reconstitutes bicarbonate which is transported across the basolateral membrane into the blood, predominantly via the Na^+, 3 HCO_3^- cotransporter, and to a lesser extent by the Cl^-: HCO_3^- anion exchanger.

The above mentioned reactions (traditional view) may be an oversimplification and the more recently held view is that the H_2O inside the cell dissociates into H^+ and OH^-. The OH^- molecule in the presence of carbonic anhydrase combines with CO_2 to yield a bicarbonate.

$$H_2O \rightarrow H^+ + OH^-$$
$$OH^- + CO_2 \text{ - - CA} \rightarrow HCO_3^-$$

The H^+ is secreted into the lumen via the afore-mentioned H^+ secretory pumps. The HCO_3^- generated in the cell leaves the cell through the basolateral membrane into the peritubular interstitial space and finally into the peritubular capillaries and the systemic circulation. Thus, it is clear that filtered HCO_3^- is not simply reabsorbed via the proximal tubular cells and is rather regenerated. However, for each HCO_3^- which is regenerated a filtered HCO_3^- is consumed by a secreted H^+. Thus, reabsorption of filtered HCO_3^- involves consumption of the luminal HCO_3^- and generation of new cellular HCO_3^- which is subsequently transported into the blood. Importantly, this process is not associated with a net change in the plasma HCO_3^-.

Extrusion of HCO_3^- through the basolateral membrane is mainly via a cotransporter which extrudes one Na^+ with 3

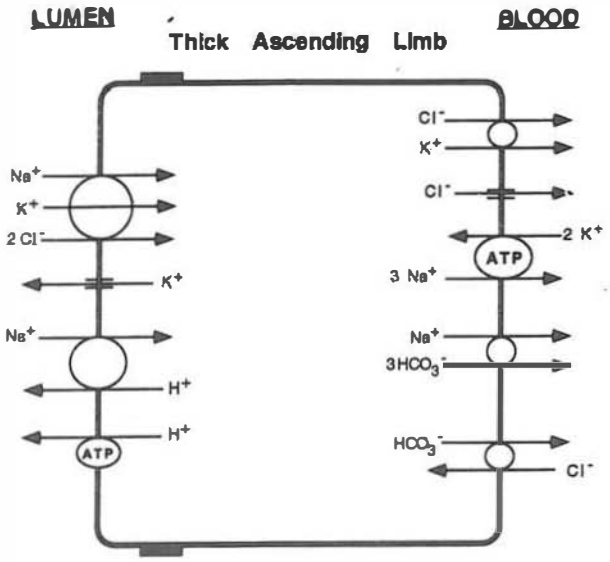

Fig. 2. The thick ascending lunb of the loop of Henle reabsorbs 5-10% of the filtered bicarbonate. Proton secretion in this segment is predominantly (80%) via the Na^+: H^+ exchanger, and to a lesser extent (20%) via the H^+-ATPase pump. The bicarbonate generated inside the cell, as a result of proton secretion, is transferred to plasma through the basolateral membrane predominantly by Na^+, 3 HCO_3^- co-transporter, and to a lesser extent by Cl^-: HCO_3^- anion exchanger. Additionally, cells in this segment also carry luminal Na^+, K^+, $2Cl^-$ co-transporter (this pump is blocked by loop diuretics) in which NH_4^+ can substitute for K^+ resulting in NH_4^+ reabsorption (recycling) in this segment. The other channels and transporters shown in this figure are: K^+ channel in the luminal membrane, Cl^- channel, Cl^-, K^+ co-transporter and Na^+, K^+-ATPase in the basolateral membrane.

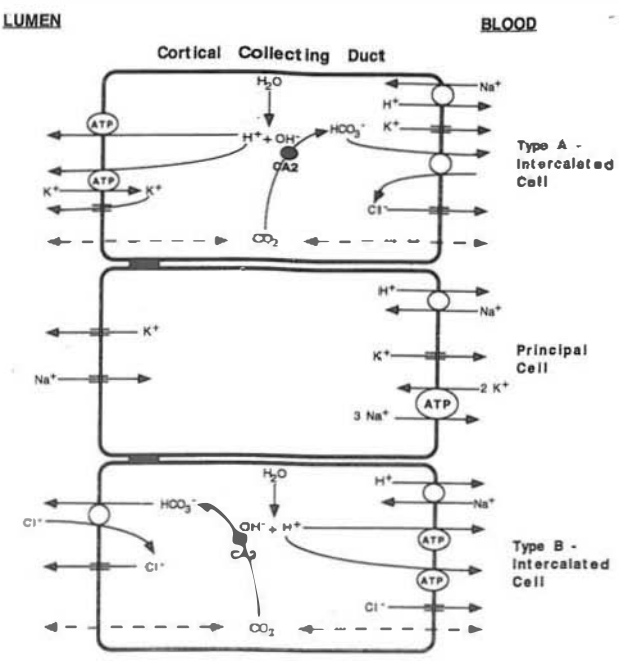

Fig. 3. The cortical collecting duct is comprised of principal cells and intercalated cells. Principal cells carry Na^+ and K^+ channels in their luminal membrane, and K^+ channels, Na^+, K^+-ATPase and Na^+: H^+ exchanger in their basolateral membrane. The type A intercalated cells (acid secreting) secrete H^+ into urine via H^+-ATPase and H^+, K^+-ATPase pumps located in the luminal membrane. The latter pump is predominantly responsible for K^+ reabsorption. The bicarbonate generated inside the cell exits the cell via Cl^-: HCO_3^- exchanger located in the basolateral membrane. These cells also carry K^+ and Cl^- channels, and Na^+: H^+ exchanger in the basolateral membrane. In the states of K^+ depletion the K^+ that enters the cell is transported to plasma through the K^+ channels in the basolateral membrane; however, in the states of K^+ repletion (adequate K^+ supply) K^+ that enters the cell is returned back to urine via luminally located K^+ channels. The type B intercalated cells secrete bicarbonate into the lumen via Cl^-: HCO_3^- anion exchanger located in the luminal membrane. The protons which are generated inside these cells are transferred to plasma via the H^+-ATPase and H^+, K^+-ATPase pumps located in the basolateral membrane. The Na^+: H^+ exchanger located in the basolateral membranes of type A and B intercalated cells and principal cells are not involved in acid-base regulat on. Their primary function is regulation of intracellular pH. In the intercalated cells bicarbonate is generated inside the cell from interaction of CO_2 and hydroxyl ions (OH^-) in the presence of cytoplasmic carbonic anhydrase 2 (CA2).

HCO_3^- molecules [or one Na^+ with one carbonate (CO_3^{--}) and one bicarbonate (HCO_3^-)] with a net result of two negative charges (sum of one positive and three negative charges) being extruded from the cell. This cotransporter is energized by the negative membrane potential (-70 mv) inside the cell. HCO_3^- can also exit the basolateral membrane via a Cl^-: HCO_3^- anion exchanger. This pump exchanges one Cl^- for one HCO_3^- and appears to play a less important role.

The remaining (10-15%) of the filtered HCO_3^- is reabsorbed in the more distal nephron segments. About half of it is reabsorbed in the thick ascending limb via Na^+: H^+ exchanger (80%) and vacuolar H^+-ATPase pump (20%) with a mechanism similar to that which occurs in the proximal tubular epithelia (Fig. 2)[16,17] and the rest is reabsorbed in the cortical and medullary collecting ducts.

Distal nephron acidification (net acid excretion)

In the cortical and medullary collecting ducts, not only the remaining filtered HCO_3^- is reabsorbed, but additional H^+ is also secreted into the lumen. The latter leads to the generation of new HCO_3^- inside the cell which is then transported basolaterally into the circulation, and net acid excretion in the urine. Thus, the distal nephron is responsible for the excretion of the daily nonvolatile acid load which is produced in the body. Net acid excretion in the distal

nephron can only happen after all of the filtered bicarbonate has been reabsorbed.

Since the free H^+ concentration in urine is extremely low, the daily acid load can not be excreted as free H^+ ions. Distally secreted H^+ ions are excreted by binding to either a filtered buffer (e.g., $HPO_4^{--} + H^+ \rightarrow H_2PO_4^-$) or, to NH_3 ($NH_3 + H^+ \rightarrow NH_4^+$). In the steady state, the net amount of H^+ excreted in 24 hour urine is roughly equal to the dietary acid load.

The net acid excretion can markedly increase (>300 mmol/day) if acid production is increased (e.g., acidosis). This response is predominantly mediated by increase in NH_4^+ excretion. Since urinary concentration of free H^+ is negligible, the net quantity of H^+ excreted in the urine is equal to the amount of H^+ excreted as titratable acidity (predominantly $H_2PO_4^-$) and NH_4^+, minus any H^+ added to the body because of urinary bicarbonate loss:

Net acid excretion rate = titratable acidity (mmol/24 hr urine) + NH_4^+ (mmol/24 hr urine) - urinary HCO_3^- (mmol/24 hr urine)

Because of its favorable pKa of 6.8 and its relatively high urinary excretion rate, HPO_4^{--} is the major urinary buffer ($HPO_4^{--} + H^+ \rightarrow H_2PO_4^-$) contributing to the titratable acid excretion, with lesser contributions from other weak acids, such as creatinine and uric acid. Titratable acidity is measured by the amount of NaOH that must be added to the 24 hour urine to titrate the urine pH back to 7.4 (plasma pH). Although the amount of H^+ buffered by HPO_4^{--} increases as the urine pH is reduced, however, once urine pH is <5.5, virtually all of the urinary phosphate exists as $H_2PO_4^-$ and no more H^+ can be buffered by this system unless more phosphate becomes available in urine. Acid loading decreases proximal phosphate reabsorption and so increases urinary phosphate concentration. The latter leads to augmented H^+ buffering capacity in the urine by increasing titratable acidity. Nevertheless, the ability to enhance net acid excretion by this phosphaturic response is limited, and it is the increased NH_4^+ excretion that generally constitutes the major adaptation to an acid load (see NH_4^+ excretion).

Cellular anatomy of the distal nephron

In the cortical collecting duct, two different cell types exist (Fig. 3).[1,2,9,11,18-22] Principal cells comprise 60% of the cells in this nephron segment and are mainly involved in Na^+ and water reabsorption, and K^+ secretion. They have basolateral Na^+, K^+-ATPase pumps which extrude $3Na^+$ in exchange for $2K^+$. Activity of this pump is increased by the presence of high levels of aldosterone in the plasma. Principal cells also have Na^+ and K^+ channels in their luminal membrane which allows Na^+ to be reabsorbed and K^+ to be secreted into the lumen. These channels are also under the influence of plasma aldosterone. Reabsorption of cationic Na^+ in the cortical collecting duct generates an electronegative charge

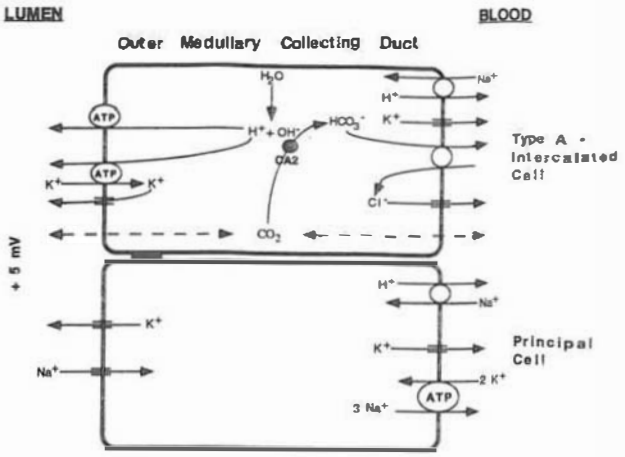

Fig. 4. The outer medullary collecting duct is comprised of only two cell types, the principal cells and the type A intercalated cells (acid secreting). In the early parts of this segment, the principal cells carry channels and transporters similar to their counterparts in the cortical collecting duct and are responsible for Na^+ and water reabsorption and K^+ secretion. However, in the latter part of this segment the principal cells are no more involved in electrolyte transport but continue to be responsible for water reabsorption, a critical function of principal cells throughout the collecting duct. The type A intercalated cells are very similar to their counterparts in the cortical collecting duct.

(-5 to -30 mv) in the lumen which facilitates secretion of both H^+ and K^+, indirectly influencing distal acid and K^+ secretion.

The other cell population present in the cortical collecting duct is referred to as intercalated cells (Fig. 3). These cells are mainly involved in acid-base regulation, and ultrastructurally are distinguished from the principal cells by a darker cytoplasm which contains numerous mitochondria and is very rich in carbonic anhydrase. Intercalated cells do not transport Na^+ and do not possess basolateral Na^+, K^+-ATPase. They constitute about 40% of the cells in the cortical collecting duct and are further divided into two subtypes. The majority (60-70%) of the intercalated cells in the cortical collecting duct secrete H^+ and reabsorb HCO_3^-; they are called type A (acid secreting) intercalated cells. H^+ secretion by type A intercalated cells is carried out predominantly by a vacuolar H^+-ATPase pump which is present in its luminal plasma membrane. This is very similar to the H^+-ATPase pump already described in the proximal tubule. For each H^+ which is secreted into the urine, one HCO_3^- is generated inside the cell which leaves the cell through the basolateral membrane in exchange for a Cl^-. This process is via a Cl^-: HCO_3^- anion exchanger (a truncated form of AE1 anion exchanger present in the red blood cells, also called band-3 protein) located in the basolateral membrane and is crucial in the regulation of net acid excretion and HCO_3^- regeneration. The other, less

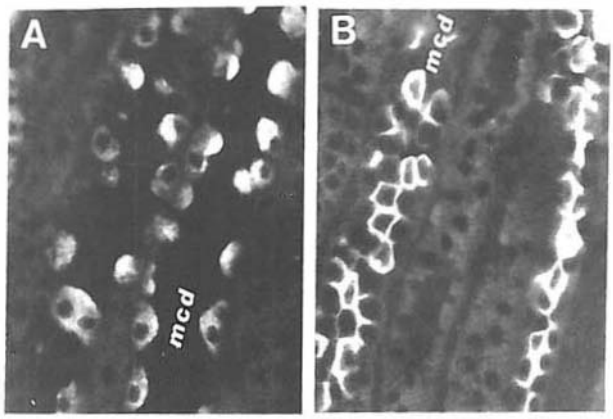

Fig. 5. Panel A, the medullary collecting duct (mcd) of a normal rat. The type A intercalated cells show significant H^+-ATPase staining in the apical cytoplasmic vesicles. Panel B, chronic acid-loaded rat. After two weeks of oral acid-loading with NH_4Cl in drinking water, intercalated cells in the medullary collecting duct show very prominent H^+-ATPase staining in the luminal plasma membrane (rim pattern) with almost total disappearance of the H^+-ATPase staining in the cytoplasmic vesicles. This represents redistribution of the H^+-ATPase pumps from cytoplasmic vesicles into the luminal plasma membrane leading to significant expansion of the luminal membrane and appearance of the "rim cells". We have shown similar adaptive changes in the models of remnant kidney, acute or chronic respiratory acidosis, chronic desoxycorticosterone administration, and chronic K^+ depletion diet (see references 22,26,29,30,31). For immunocytochemical staining a monoclonal antibody against the 31kD subunit of H^+-ATPase was used. Antibody binding was identified with FITC-(fluorescein isothiocyanate) labeled appropriate secondary antibody. The slide was viewed with an epifluorescent microscope (Magnification, 400×).

frequent (30-40%) sub-type is the type B (HCO_3^- secreting) intercalated cell. This cell is very similar to a type A intercalated cell that has reversed its polarity. That is to say, type B intercalated cell secretes HCO_3^- in exchange for Cl^- in the apical membrane, and reabsorbs H^+ through the basolateral membrane. Type B cell plays an important role in HCO_3^- excretion in response to an alkali load (decreased proximal HCO_3^- reabsorption can also contribute to its urinary excretion).

It has long been shown that cortical collecting ducts simultaneously secrete both H^+ and HCO_3^- in the urine, and can implement net H^+ or HCO_3^- excretion based on the acid-base status of the animal.[23-25] We have recently shown that chronic acid-loading, with NH_4Cl in drinking water, results in increased polarization of the H^+-ATPase pumps to the apical pole of type A intercalated cells, resulting in a higher percentage of intercalated cells showing a well polarized apical staining for H^+-ATPase.[22] This was accompanied with a concomitant proportional reduction in the percentage of intercalated cells showing poorly polarized apical, diffuse, or bipolar staining for H^+-ATPase.[22] We have also shown that respiratory acidosis can result in similar changes, albeit to a much lower extent.[26] Our findings confirm the previous ultrastructural studies suggesting that metabolic or respiratory acidosis result in fusion of cytoplasmic tubulovesicular structures carrying 10 nm "studs" (presumably representing the H^+-ATPase pumps) to the luminal plasma membrane of type A intercalated cells.[27,28] We have also shown that in models of 7/8 nephrectomy (removing one kidney and infarcting 3/4 of the other kidney, a model of remnant kidney),[29] chronic low potassium diet (our unpublished preliminary observation), and chronic desoxycorticosterone (DOCA) administration,[30] there is a significant increase in the percentage of intercalated cells that show well polarized H^+-ATPase distribution to both apical and basolateral poles, suggesting activation of both type A and type B intercalated cells.

In the medullary collecting duct (Fig. 4), about two-thirds of the cells are principal cells which are involved in water and to some extent (only in the early outer medulla) Na^+ reabsorption. The remaining one-third of the cells are type A intercalated cells which are involved in H^+ secretion and HCO_3^- reabsorption as described before. We have recently shown that the main adaptation of the intercalated cells in the medullary collecting ducts to chronic acid-loads is by redistribution of the H^+-ATPase pumps from apical cytoplasmic vesicles to the luminal cell membrane. This results in almost total disappearance of the H^+-ATPase pumps in the cytoplasm, and a concomitant expansion of the luminal plasma membrane that shows bright staining for H^+-ATPase, an appearance that we have named "rim cell" pattern of staining.[22,31] Our finding confirms an earlier ultrastructural study that showed chronic acid loading results in fusion of cytoplasmic tubulovesicular structures that carried 10 nm "studs" (H^+-ATPase pumps) to the luminal plasma membrane, resulting in marked expansion of the latter (Fig. 5).[32] We have seen similar adaptive changes in response to respiratory acidosis,[26] remnant kidney model,[29] chronic desoxycorticosterone (DOCA) administration,[30] and chronic low potassium diet (our unpublished preliminary results).

Recently a pump similar to the one present in the gastric mucosa (which acidifies stomach contents) has also been found in the distal nephron segments. This is an H^+, K^+-ATPase pump, a primary active pump, which utilizes ATP to secrete H^+ in exchange for reabsorbing K^+. In the kidney, this pump is predominantly responsible for potassium reabsorption, and its expression is tightly regulated by changes in dietary potassium. Thus, a low K^+ diet increases and a high potassium diet suppresses its expression in the distal nephron. This pump has been immunocytochemically localized in the intercalated cells of the cortical and medullary collecting ducts, and has been shown to have the same polar distribution as the H^+-ATPase pump.[33] The relative

importance of this pump, compared to the vacuolar H^+-ATPase pump, in the daily acid-base regulation has not been well defined yet.[34-36]

Ammonium production and excretion

Ammonium (NH_4^+) is produced primarily in the proximal tubular cells from the metabolism of aminoacids, particularly glutamine:

$$1 \text{ Glutamine} \rightarrow 2\, NH_4^+ + 2\, HCO_3^-$$

The HCO_3^- generated in the above reaction is transported into the systemic circulation via the basolaterally located Na^+, $3HCO_3^-$ cotransporter. The NH_4^+ produced in the proximal tubular cells is secreted into the lumen via the Na^+: H^+ exchanger (antiporter), which can also function as a Na^+: NH_4^+ exchanger.

Ammonium (NH_4^+) and ammonia (NH_3) are interconvertable based on the pH of the media and their respective concentrations ($NH_3 + H^+ <---> NH_4^+$). With a pKa of 9.3, at a urine pH of 6.0 the ratio of NH_4^+ to NH_3 is 1000 to one. NH_3, being electroneutral, can easily diffuse across the cell membranes and the tubular cells. However, inside the lumen it is exposed to a high H^+ concentration (e.g., in the medullary collecting duct where the urine pH is at its lowest ~5.5) and converts to NH_4^+ which is lipid-insoluble and therefore is "trapped" in the lumen.

More than 75% of the ammonium in the proximal tubular fluid that enters the loop of Henle is recycled in the medulla so that very little enters the distal convoluted tubule and the cortical collecting duct. The primary step in the medullary recycling is NH_4^+ reabsorption in the thick ascending limb (impermeable to NH_3) by substitution of NH_4^+ for K^+ on the Na^+, K^+, $2Cl^-$ cotransporter (Figure 2). In the less acidic medullary interstitium some of the NH_4^+ converts to NH_3. Some of the latter diffuses into the straight segment (pars recta, S3 segment) of the proximal tubule located in the outer medulla where it combines with H^+ in the lumen and converts to NH_4^+ which is then trapped in the lumen and further recycled in the thick ascending limb. The net effect is maintenance of a high medullary interstitial NH_3 concentration. A large amount of medullary interstitial NH_3 diffuses into the medullary collecting duct. This is due to a relatively low NH_3 concentration in the lumen (due to NH_4^+ removal in the thick ascending limb, and the low urine pH in this segment which favors conversion of NH_3 to NH_4^+) that generates a concentration gradient favoring this diffusion. The NH_3 is then converted to NH_4^+ which is trapped in the lumen and excreted in urine. Acid loading (acidosis) increases the urinary excretion rate of NH_4^+ from a baseline of 30-40 mmol/day to >250 mmol/day. This is done by both increased trapping of NH_4^+ in a more acidic urine (acute adaptation), and an increase in proximal NH_4^+ production from glutamine (chronic adaptation to acidemia).

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