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REVIEW

Roles of renal ammonia metabolism other than in acid–base homeostasis

I. David Weiner[1,2]

Abstract The importance of renal ammonia metabolism in acid–base homeostasis is well known. However, the effects of renal ammonia metabolism other than in acid–base homeostasis are not as widely recognized. First, ammonia differs from almost all other solutes in the urine in that it does not result from arterial delivery. Instead, ammonia is produced by the kidney, and only a portion of the ammonia produced is excreted in the urine, with the remainder returned to the systemic circulation through the renal veins. In normal individuals, systemic ammonia addition is metabolized efficiently by the liver, but in patients with either acute or chronic liver disease, conditions that increase the addition of ammonia of renal origin to the systemic circulation can result in precipitation and/or worsening of hyperammonemia. Second, ammonia appears to serve as an intrarenal paracrine signaling molecule. Hypokalemia increases proximal tubule ammonia production and secretion as well as reabsorption in the thick ascending limb of the loop of Henle, thereby increasing delivery to the renal interstitium and the collecting duct. In the collecting duct, ammonia decreases potassium secretion and stimulates potassium reabsorption, thereby decreasing urinary potassium excretion and enabling feedback correction of the initiating hypokalemia. Finally, the stimulation of renal ammonia metabolism by hypokalemia may contribute to the development of metabolic alkalosis, which in turn can stimulate NaCl reabsorption and contribute to the intravascular volume expansion, increased blood pressure and diuretic resistance that can develop with hypokalemia. The evidence supporting these novel non-acid–base roles of renal ammonia metabolism is discussed in this review.

Keywords Ammonia · Hyperammonemia · Hypokalemia · Signaling · Collecting duct · ENaC · H-K-ATPase

Introduction

Renal ammonia[1] metabolism plays a critical role in mammalian biology. Ammonia's role as a critical component of both basal net acid excretion and the response to acid–base disorders, such as metabolic acidosis, is well known. Less well recognized is that ammonia produced in the kidney can be added to the systemic circulation and increase systemic ammonia levels. In patients with impaired hepatic ammonia metabolism, this may lead to either precipitation or worsening of hyperammonemic (hepatic) encephalopathy. Also less well recognized is that intrarenal ammonia may function as a paracrine signaling molecule. Intrarenal ammonia is produced in the proximal tubule through mechanisms regulated by extracellular potassium and then acts in the collecting duct to alter potassium secretion by the collecting duct in a manner that allows feedback regulation of the initiating potassium disorder. This review discusses these less well-known effects of ammonia, which are independent of the traditional view of the role of ammonia in systemic acid–base homeostasis.

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[1] Ammonia can exist in two molecular forms, NH_3 (free ammonia) and NH_4^+ (ammonium cation). Throughout this review, "ammonia" refers to the combination of both molecules; "NH_3" refers specifically to the molecular form of NH_3; "NH_4^+" refers specifically to the molecular form NH_4^+.

Brief review of ammonia's role in acid–base homeostasis

Overview of acid–base homeostasis

Acid–base homeostasis is necessary for the normal maintenance of health. Disordered acid–base homeostasis leads to a wide variety of complications, including failure to thrive, skeletal osteopenia and/or osteoporosis, recurrent nephrolithiasis, impaired thyroid hormone action and impaired glucose tolerance [1, 2]. The kidney's contribution to acid–base homeostasis involves two fundamental processes: (1) reabsorption of filtered bicarbonate and (2) generation of new bicarbonate [3, 4]. Filtered bicarbonate reabsorption is necessary for acid–base homeostasis; however, on its own biocarbonate reabsorption is not sufficient to maintain a state of homeostasis because systemic bicarbonate is used to buffer endogenous acid production, resulting in depletion of bicarbonate stores. Thus, even with 100 % reabsorption of filtered bicarbonate, the kidneys need to generate "new" bicarbonate to maintain acid–base homeostasis. New bicarbonate generation is equivalent to net acid excretion and involves the separate processes of titratable acid excretion and ammonia excretion. Under basal conditions, ammonia excretion is the quantitatively larger component of new bicarbonate generation, and in response to acid–base disorders, almost all of the change in new bicarbonate generation is attributable to variations in ammonia metabolism [5, 6]. Organic anion excretion can also contribute to acid–base homeostasis, but in humans it is quantitatively a substantially lesser component [3, 7, 8].

Renal ammonia metabolism

The renal handling of ammonia differs fundamentally from that of almost every other urinary solute. Other solutes are delivered to the kidney via the renal artery and undergo variable components of glomerular filtration and renal epithelial cell transport that result in urinary excretion. This results in a renal vein content that is less than the renal arterial content. Moreover, urinary excretion is only a minor component of the filtered load (Table 1). The paradigm for renal ammonia metabolism is different. Total filtered ammonia, assuming an arterial ammonia level of 25 μmol/L [9] and a normal adult glomerular filtration rate (GFR) of 120 mL/min, is 4.32 mmol/24 h; this contrasts with the normal urinary ammonia excretion of ~30 mmol/24 h [10]. Thus, the majority of urinary ammonia is not excreted via glomerular filtration.

Kidneys actually generate ammonia from the metabolism of specific amino acids, predominantly glutamine, with a lesser component from alanine metabolism [4, 6, 11]. The proximal tubule is the primary site of ammonia generation and is the site of changes in ammonia generation in response to physiologic stimuli [12–16]. Ammonia generation from glutamine, when carried to its completion, results in generation of two NH_4^+ and two bicarbonate molecules [6]. Several recent reviews of the mechanisms of renal ammonia generation are available for the interested reader [3, 4, 6, 11]. The bicarbonate generated is added to the systemic circulation through the renal vein, thereby serving as a source of "new bicarbonate." Ammonia generated in the kidney is either excreted into the urine or enters peritubular capillaries and is added to the systemic circulation via the renal vein [3, 6, 17, 18]. As a result, the kidneys, even though they excrete ammonia, also act as a source of systemic ammonia generation (Fig. 1).

Differential handling of the ammonia generated in the kidneys, urinary excretion versus systemic addition, has a critical effect on the acid–base aspects of ammonia metabolism. Ammonia added to the systemic circulation undergoes, in the individual with normal hepatic function, rapid hepatic metabolism; this is necessary to maintain low arterial ammonia levels and prevent hyperammonemic encephalopathy. There are two pathways of hepatic ammonia metabolism—one produces urea and the other glutamine [20–24]. The ureagenic pathway uses equal amounts of bicarbonate and NH_4^+. As a result, the bicarbonate produced during renal ammonia generation is used in the hepatic metabolism of ammonia to urea. Ammonia can also be metabolized by glutamine synthetase in an enzymatic process that regenerates glutamine. However, this reaction generates one H^+ for each NH_4^+ metabolized [25], and the

Table 1 Common urine solutes

Solute	Units	Amount filtered per day	Amount excreted in the urine per day	Percentage of filtered load excreted in urine
Water	L	180	~1	~0.6
Na^+	mmol	25,200	100–200	0.4–0.8
K^+	mmol	720	60	8.3
HCO_3^-	mmol	4320	0	0
Ammonia	mmol	4.3	30	~700

Calculations are based upon normal adult glomerular filtration rate (GFR) of 120 mL/min, serum Na^+ level of 140 mmol/l, serum K^+ level of 4.0 mmol/L and serum bicarbonate level of 24 mmol/L. Urinary solute excretion is variable, and typical norms are shown
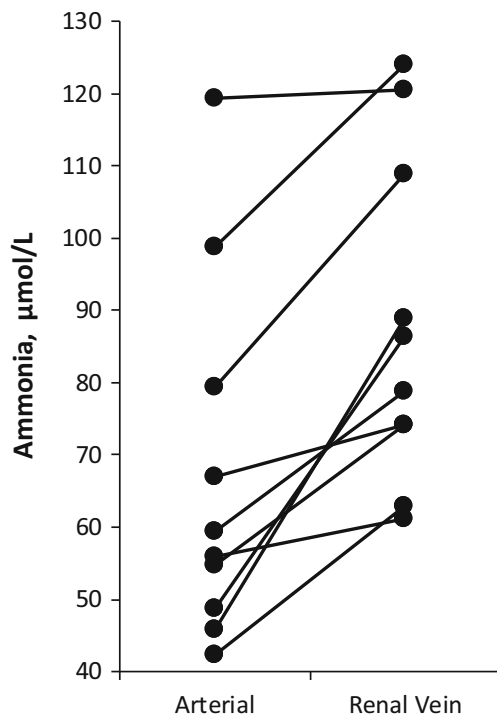
Fig. 1 Renal vein ammonia concentration is higher than the concentration of ammonia in the renal artery, indicating systemic ammonia addition of renal origin. Graph was constructed using measurements of arterial and renal vein ammonia concentrations in ten patients. Data were obtained from [19]. All patients had impaired liver function, likely contributing to mild elevation in arterial ammonia levels

H^+ is then buffered by bicarbonate. Consequently, hepatic ammonia metabolism, whether resulting in urea or glutamine production, consumes an equal amount of bicarbonate as was made during the renal generation of ammonia. Thus, only the ammonia generated in the kidneys and excreted in the urine is associated with a net addition of bicarbonate to the extracellular body fluids.

Clinical implications of renal ammonia addition to the systemic circulation

The addition of renal ammonia to the systemic circulation has important clinical implications. The acid–base effect has been described in the preceding sections. The second effect is related to changes in arterial ammonia concentrations. In individuals with intact hepatic function, rapid hepatic metabolism prevents significant changes in arterial ammonia levels [26–28]. However, in patients with acute or chronic liver disease, who have an impaired ability to metabolize ammonia, increased renal ammonia addition may lead to hyperammonemia. The renal contribution to hyperammonemia probably occurs under a wide variety of conditions, including hypokalemia,

gastrointestinal (GI) bleeding, high protein diet and valproic acid-induced hyperammonemia.

Hypokalemia

Hypokalemia is one of the more common electrolyte disorders observed in clinical medicine. It occurs in 3–8 % of hospitalized patients, with an increased incidence in patients treated with diuretics, whether for hypertension, heart failure or other volume overload conditions [29–31]. Not as well-recognized, however, is that hypokalemia also can increase arterial ammonia levels in patients with liver disease (Fig. 2). In one study, K^+ depletion increased arterial ammonia levels from ~45 to ~80 μmol/L, with subsequent K^+ supplementation reversing the effect [34]. Changes in renal ammonia metabolism appear to account for this effect of hypokalemia. Specifically, hypokalemia increases renal ammonia generation, urinary ammonia excretion and, importantly, systemic ammonia addition from the kidneys [32, 33]. Studies in animal models show that hypokalemia stimulates all major components of ammonia metabolism, renal glutamine uptake, ammonia generation and multiple components of ammonia transport by renal epithelial cells [35–38]. The mechanism through which hypokalemia stimulates these components has not been definitively determined. Some [39, 40], but not other [41], studies have shown that hypokalemia causes intracellular acidosis in renal cortical cells, presumably those of the proximal tubule, and this process might be the proximate signal initiating increased renal ammoniagenesis.
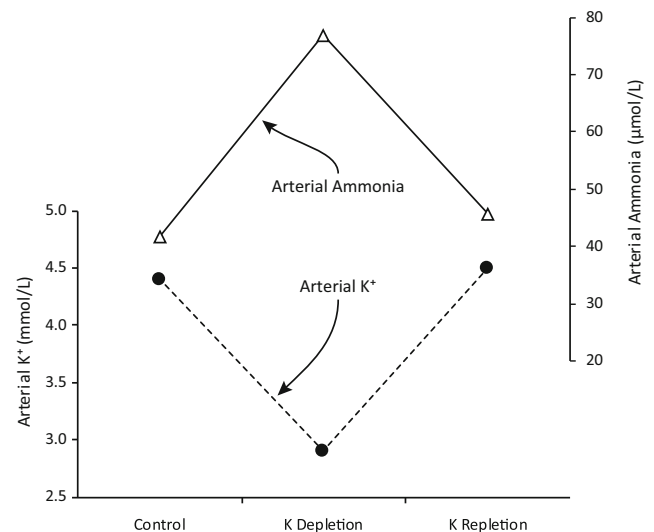

Fig. 2 Hypokalemia increases arterial ammonia levels in patients with cirrhosis. Data in graph show the effects of diuresis-induced potassium (K^+) depletion and then correction (K Repletion) by either diet or diet with supplemental K^+ salts. All changes were statistically significant. Data obtained from [32, 33]. All patients had impaired liver function, likely contributing to the observed mild elevation in arterial ammonia levels

This stimulation of renal ammonia metabolism increases the addition of ammonia of renal origin to the systemic circulation. In one set of studies, patients on a normal potassium diet and following induction of a potassium restricted diet were studied [32]. Arterial ammonia levels and renal venous ammonia levels were measured to assess the addition of renal ammonia to the systemic circulation (Fig. 3). When the patients were on a normal potassium diet, the renal vein ammonia concentration was an average of 23 μmol/L higher than the renal arterial ammonia concentration due to the generation of renal ammonia and its addition to the systemic circulation. When hypokalemia was induced by a potassium restricted diet, the addition of ammonia of renal origin to the systemic circulation, measured as the increase in the concentration of ammonia in the renal venous fluid, increased by ~2.5 fold. Although renal plasma flow was not determined in this study, it is highly unlikely that hypokalemia decreased renal plasma flow by more than 70 %, which would be necessary to account for this change in renal vein ammonia levels. These human studies have been extended by animal studies showing that dialysis-induced hypokalemia substantially increases the addition of ammonia of renal origin to the systemic circulation [32].

Another experimental model tested whether the effects of diuresis-induced hypokalemia and the addition of ammonia of renal origin to the systemic circulation were reversible with acute intravenous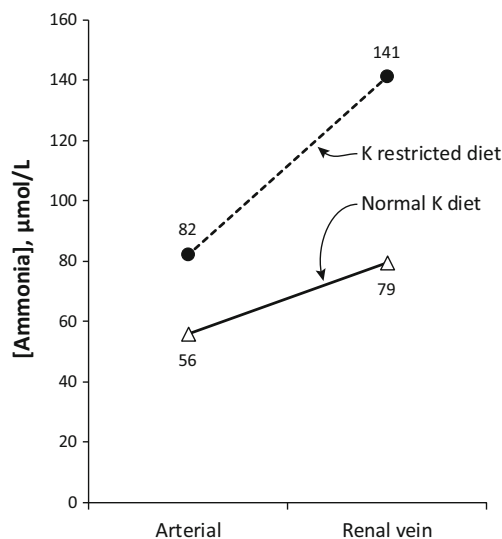 potassium replacement [32]. During the diuresis-induced hypokalemia, ammonia concentration in the renal vein exceeded that in the renal artery by an average of 100 μmol/L (Fig. 4). Following intravenous potassium replacement, the increment decreased substantially, to an amount similar to that observed in the patients on a normal potassium diet in the previous study, i.e. 22 μmol/L. These results suggest that hypokalemia's stimulation of renal-to-systemic ammonia addition appears to be rapidly reversible with correction of the hypokalemia.

Gastrointestinal bleeding

Another clinical condition associated with precipitating and/or worsening hepatic (hyperammonemic) encephalopathy is GI bleeding. Traditionally, the increase in arterial ammonia levels during GI bleed is thought to result from increased colonic ammonia production. However, increased addition of ammonia of renal origin to the systemic circulation is also likely to contribute substantially to the observed increase.

In one study, intestinal and renal ammonia balance was measured in patients with cirrhosis and active variceal bleeding [42]. Intestinal tract net ammonia consumption averaged 121 nmol/kg/min. When extrapolated to a 70-kg individual, this is an average rate of ammonia consumption of 12 mmol/24 h. Thus, worsening hyperammonemia could not be attributed in this circumstance to net intestinal ammonia production. Unfortunately, the measurements of "net intestinal ammonia production" in this study resulted
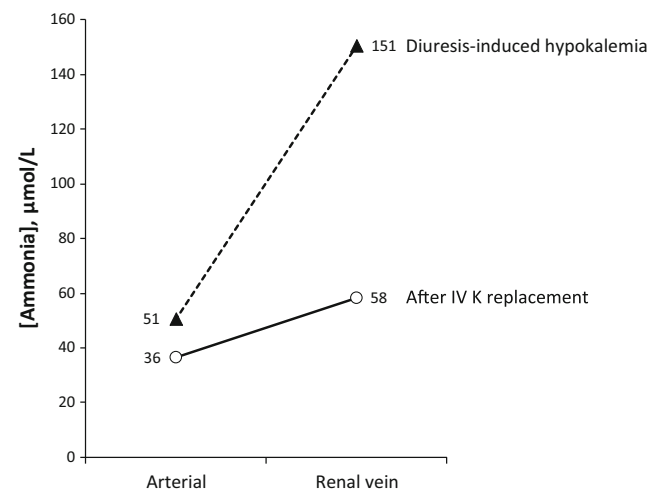

Fig. 3 Hypokalemia increases the addition of ammonia of renal origin to the systemic circulation. This graph is a representative example of the effect of spontaneous hypokalemia induced by dietary potassium restriction on the addition of ammonia of renal origin to the systemic circulation. Results were obtained from a patient with chronic liver disease on a normal potassium diet (65 mEq per day) an then on a low potassium diet (30 mEq per day). Serum potassium decreased from 3.5 to 2.7 mEq per liter. There was no significant change in arterial pH. Data were obtained from [32]. In this study, all patients had impaired liver function, likely contributing to mild elevation in arterial ammonia levels

Fig. 4 Effect of intravenous (*IV*) K$^+$ replacement on the addition of ammonia of renal origin to the systemic circulation. This graph is a representative example of the effect of correcting hypokalemia with IV K$^+$ supplementation on the addition of ammonia of renal origin to the systemic circulation. Acute IV potassium replacement decreases arterial ammonia concentration and decreases the addition of ammonia of renal origin to the systemic circulation, as indicated by the decrease in the gradient between arterial and renal vein ammonia concentration. Data were obtained from [32]. In this study, all patients had impaired liver function, likely contributing to mild elevation in arterial ammonia levels

from the measurement of arterial ammonia, hepatic vein ammonia and hepatic venous blood flow rates.

However, the authors of the study did assess renal ammonia production by measuring arterial ammonia, renal vein ammonia and renal plasma flow [42], documenting a net ammonia production of 572 nmol/kg/min. When extrapolated over 24 h in a 70-kg individual, this is equivalent to an increase in the addition of ammonia of renal origin to the systemic circulation of 58 mmol/24 h. Because similar measurements were not made in similarly ill patients without active bleeding, it is not possible to conclude definitively that the addition of renal ammonia to the systemic circulation was increased. However, this rate of renal ammonia addition, i.e. ~60 mmol/day, is approximately twofold higher than the average of 30 mmol/day typically seen.

In the same study, a more detailed analysis of the site of ammonia generation during a GI bleed was assessed during more controlled circumstances, i.e. not during an acute GI bleed [42]. The authors studied patients with biopsy-proven cirrhosis undergoing assessment of transjugular intrahepatic portosystemic shunt (TIPSS) patency. They measured arterial ammonia, intestinal tract-specific (i.e. not involving the liver) ammonia production and renal ammonia production, both before and after a simulated GI bleed. The GI bleed was mimicked by providing an intestinal load of 46 g of an amino acid solution designed to mimic the amino acid composition of hemoglobin, administered via a nasogastric tube. Intestinal tract ammonia production increased over the 4-h time course of the experiment, from ~600 to ~1000 nmol/kg/min, i.e. an increase of 400 nmol/kg/min. In addition, the addition of ammonia of renal origin to the systemic circulation increased from ~100 to ~600 nmol/kg/min. These findings indicate that nasogastric administration of an amino acid load, designed to mimic hemoglobin, stimulates renal ammonia generation and addition to the systemic circulation that is at least as much, if not greater, than the increase in intestinal ammonia production.

Whether these changes in ammonia addition also occur in response to changes in dietary protein has been addressed indirectly in studies assessing the renal response to dietary protein changes. High dietary protein increases multiple components of renal ammonia production and metabolism, and low-protein diets decrease renal ammonia metabolism [43–45]. These effects on renal ammonia metabolism are paralleled by changes in urinary ammonia excretion, and they appear to reflect a renal response designed to maintain acid–base homeostasis in the face of varying levels of endogenous acid production that result from changes in dietary protein metabolism. The finding, discussed above, that nasogastric amino acid administration increases the addition of ammonia of renal origin to the systemic circulation suggests that changes in dietary protein intake will cause parallel changes, but this has not been determined specifically.

Valproic acid encephalopathy

Valproic acid is an effective anticonvulsant used in many types of epilepsy; it is usually well tolerated, but occasionally causes hyperammonemic encephalopathy in the absence of overt liver disease. As many as 10–15 % of pediatric patients treated with valproic acid develop hyperammonemia [46], and this, at least in part appears to be due to increased renal ammonia generation. Valproic acid and several of its metabolites increase renal glutamine uptake and renal ammonia production quite dramatically [47–49]. The increased ammonia production results in both an increase in urinary ammonia excretion and a simultaneous quantitatively similar increase in the addition of ammonia of renal original to the systemic circulation [50–55]. Another study demonstrated the critical role of the addition of renal ammonia to the systemic circulation by showing that valproic acid-induced hyperammonemia did not occur in animals with a previous bilateral nephrectomy [55]. There is also likely to be a hepatic contribution, resulting from impaired hepatic ammonia metabolism, that contributes to the hyperammonemia that develops.

Intrarenal roles of ammonia other than acid–base homeostasis

A variety of studies over many years have investigated the observation of a correlation between potassium disorders and abnormal ammonia metabolism. As discussed previously, hypokalemia stimulates renal ammonia metabolism and increases urinary ammonia excretion. Simultaneous with the changes in urinary ammonia excretion are changes in potassium excretion [56, 57].

Tannen and colleagues investigated whether these changes were causally related by stimulating renal ammonia metabolism independent of changes in systemic acid–base status or serum potassium concentration [58]. They took advantage of the finding that glutamine is a limiting factor for renal ammonia metabolism, under both basal and acidotic conditions, and stimulated renal ammonia generation by administering glutamine. The results showed that glutamine administration increased urinary ammonia excretion rapidly and simultaneously decreased urinary potassium excretion (Fig. 5). The potassium conservation could not be accounted for by changes in urinary sodium excretion or in plasma potassium or acid–base parameters. Quantitatively, the change in urinary ammonia excretion, an increase of ~32 μmol/min, was significantly greater than the change in urinary potassium excretion, a decrease of ~14 μmol/min. Thus, the change in K^+ excretion could not be simply attributed to a renal K^+ for NH_4^+ exchange process. These results suggest that there might be a correlation between the effects of renal ammonia metabolism and renal potassium handling.
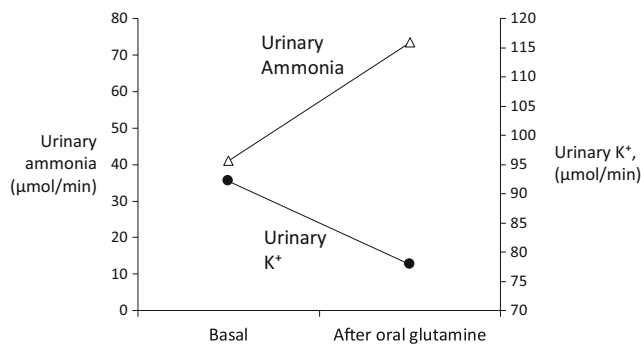
Fig. 5 Effect of stimulating renal ammonia metabolism with glutamine on urinary ammonia and K^+ excretion. Normal healthy men first ingested a constant formula diet of normal electrolyte content for 3 days, followed by the ingestion of glutamine at 4.3 mmol/kg body weight. Glutamine ingestion resulted in an increase in urinary ammonia excretion and a concomitant decrease in potassium excretion. Data were obtained from [58]

Fig. 6 Effects of ammonia on cortical collecting duct (CCD) K^+ secretion. Net K^+ secretion was determined in isolated perfused rabbit CCD segments. Ammonia addition to extracellular solutions significantly inhibited net K^+ secretion. Data are from [60]

Further studies in animal models sought to determine where in the kidney changes in ammonia metabolism altered potassium transport. Jaeger and colleagues observed that the administration of intravenous glutamine to rats had the exact same effect on urinary ammonia and potassium excretion as observed in humans [59]. In their study, free-flow micropuncture was used to assess the site along the nephron/collecting duct where potassium transport was altered. Glutamine administration did not significantly alter the fractional delivery of potassium to the micropuncturable distal tubule, indicating no change in K^+ reabsorption by the combined proximal tubule and loop of Henle [59]. Instead, all of the regulation of potassium handling occurred distal to the "micropuncturable distal tubule," a region which includes portions of the distal convoluted tubule (DCT) and connecting segment and the entire collecting duct. In the absence of glutamine stimulation, there was net potassium secretion; however, following glutamine treatment, there was net potassium reabsorption. Thus, stimulating ammonia metabolism with glutamine decreased renal potassium excretion, with all of the effects on potassium transport occurring in the "distal nephron," i.e. somewhere in the DCT, connecting segment and collecting duct.

The next set of studies examining this issue originated from the laboratory of Hamm, et al, and involved the use of in vitro microperfusion of cortical collecting duct segments [60]. This technique allows the effects of specific stimuli on transport to be examined without having to worry about systemic and/or nonspecific effects. These authors showed that ammonia rapidly and significantly decreased potassium secretion by the cortical collecting duct by ~40 % (Fig. 6). Thus, the effects of glutamine administration increase renal ammonia metabolism and alter "distal tubule" potassium handling, and are attributable, at least in part, to the ability of ammonia to significantly and substantially inhibit net potassium secretion in the cortical collecting duct.
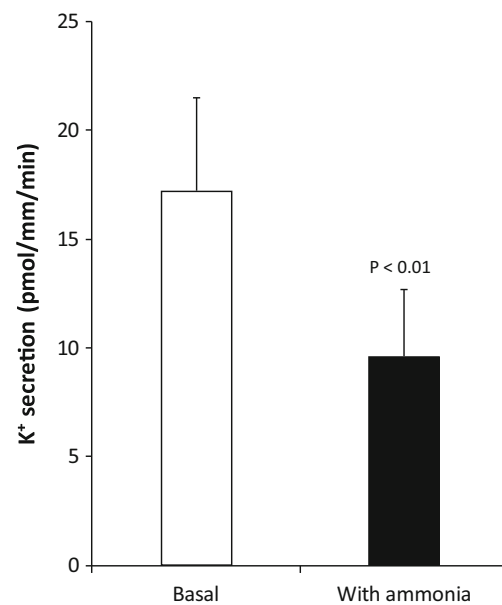
Potassium handling by the collecting duct involves separate components of potassium secretion and potassium reabsorption [29, 61, 62]. Potassium secretion in the principal cell is primarily linked to a mechanism of sodium reabsorption occurring through the epithelial sodium channel (ENaC), coupled to potassium secretion. Hamm et al., using the isolated-perfused collecting duct approach, also showed that ammonia significantly and rapidly impaired sodium reabsorption with a similar time course and magnitude as its effects on potassium secretion [60]. Whether this effect was due to changes in intracellular pH was not examined specifically. However, chronic ammonia exposure has been found to decrease intracellular pH in collecting duct intercalated cells [63]. If similar intracellular pH changes occur in Na^+-transporting principal cells, this could explain, at least in part, the inhibition of Na^+ reabsorption because decreased intracellular pH is known to inhibit ENaC [64, 65].

In a study involving the heterologous expression of ENaC in the Xenopus oocyte and detailed electrophysiologic studies, Nakhoul et al. showed that ammonia directly affected the inhibition of ENaC-mediated sodium transport (Fig. 7) [66]. This effect was independent of the effects of ammonia on intracellular pH. Thus, at least a component of the effects of ammonia on collecting duct potassium secretion can be linked to ammonia acting as a "potassium-sparing diuretic" that blunts the secretion of potassium by the collecting duct.

Potassium handling by the collecting duct also involves potassium reabsorption, which occurs primarily via H-K-ATPase [29, 67, 68]. Two sets of studies have addressed the effects of ammonia on this component of potassium handling.
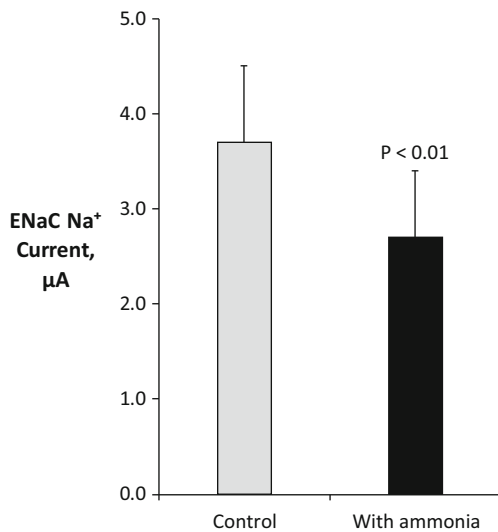
Fig. 7 Effect of ammonia on epithelial sodium (Na^+) channel (*ENaC*)-mediated Na^+ transport. ENaC was expressed in Xenopus oocytes, and the effects of ammonia on ENaC-mediated Na^+ transport, measured as current, were measured. Data are from [66]

intracellular pH through alternative mechanisms not involving changes in either extracellular or intracellular pH did not alter H^+ secretion [63]. These observations indicate that ammonia stimulates H-K-ATPase, directly contributing to the observed increased H^+ secretion and likely stimulating unidirectional K^+ reabsorption.

This effect of ammonia and H-K-ATPase appears to be a specific defect involving specific intracellular signaling pathways. Further studies have shown that ammonia activates H-K-ATPase through mechanisms involving intracellular calcium, microtubules, SNARE proteins and activation of mitogen-activated protein kinases (MAPKs) ([69] and unpublished observations). Although ammonia altered intracellular pH, equivalent intracellular pH changes induced by an alternative, nonspecific mechanism did not alter H-K-ATPase activity [63].

Overall, these observations of a correlation between systemic potassium, renal ammonia metabolism and renal potassium transport suggest that ammonia can be considered to be an intrarenal, paracrine signaling molecule. A paracrine signaling molecule is produced by one set of cells in a tissue in response to a stimuli, and it acts on other cells in the same tissue to enable correction of the initiating stimuli. Hypokalemia directly stimulates proximal tubule ammonia generation and luminal secretion [38]. Because ammonia is reabsorbed in the thick ascending limb by the transport of NH_4^+ at the K^+-binding site of NKCC2 [70], hypokalemia increases ammonia reabsorption, leading to increased interstitial ammonia concentrations, particularly in the cortex and outer medulla [71], the major sites of K^+ transport in the collecting duct. This ammonia can then act in the collecting duct to regulate net K^+ transport, inhibiting unidirectional potassium secretion and stimulating unidirectional potassium reabsorption [60, 63, 66, 69, 72]. Thus, ammonia meets all of the criteria necessary to be identified as a renal paracrine signaling molecule. However, the

The work of Hamm and colleagues on the isolated, perfused collecting duct showed that ammonia stimulated the reabsorption of the potassium analog, rubidium [60], and that by Frank and colleagues on the isolated, perfused cortical collecting duct showed that ammonia stimulated proton secretion [63]. There are two mechanisms of apical proton secretion in the cortical collecting duct, namely, H-ATPase and H-K-ATPase. The inhibition of apical H-K-ATPase completely blocked the effects of ammonia on proton secretion, whereas the inhibition of H-ATPase did not [63]. Although continuous ammonia exposure caused intracellular acidification in H^+-transporting intercalated cells [63], this process was considered by the authors as unlikely to explain the activation of H-K-ATPase. Specifically, similar changes in

Fig. 8 Effects of renal ammonia metabolism in addition to its primary role in systemic acid–base homeostasis. *BP* Blood pressure
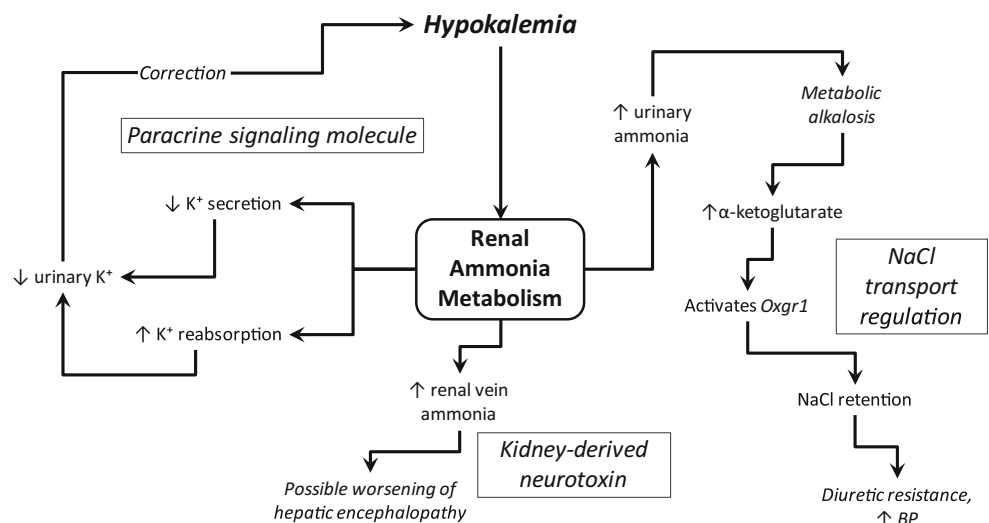
specific receptor protein(s) through which ammonia initiates these effects in the intercalated and principal cells of the collecting duct has not been identified.

Indirect effects of ammonia metabolism on sodium chloride transport

A third effect of ammonia metabolism other than in acid–base homeostasis has to do with the indirect effects of ammonia on renal sodium chloride transport. For this consideration, I return to a non-acid–base-mediated stimulation of ammonia metabolism, hypokalemia. Hypokalemia and/or potassium deficiency is well known to cause sodium chloride retention, mild intravascular volume expansion, increases in blood pressure and diuretic resistance [73–75]. In addition, hypokalemia often results in mild metabolic alkalosis [76, 77] that results in large part from the increased ammonia metabolism and urinary ammonia excretion (discussed in preceding sections in detail).

This ammonia-induced effect to stimulate metabolic alkalosis may have significant effects on renal sodium chloride transport. Human clinical studies show that metabolic alkalosis decreases diuretic responsiveness [78]. This effect may occur, at least in part, through a 2-oxoglutarate-dependent pathway. Metabolic alkalosis increases renal excretion of the organic anion, 2-oxoglutarate [79–82], and 2-oxoglutarate stimulates sodium and chloride reabsorption [79]. Detailed studies have shown that luminal 2-oxoglutarate acts through a specific G-protein-coupled receptor, Oxgr1, expressed in the apical membrane of pendrin-positive collecting duct cells, i.e. non-A non-B cells and Type-B intercalated cells [79]. Activation of Oxgr1 by luminal 2-oxoglutarate then stimulates sodium and chloride reabsorption through activation of sodium transporting protein NDCBE (sodium-dependent chloride bicarbonate exchanger) and the chloride reabsorbing protein pendrin [79]. By increasing sodium chloride reabsorption, this mechanism can explain a component of the hypokalemia-induced sodium chloride retention, volume expansion, increased blood pressure and diuretic resistance.

Another important mechanism through which hypokalemia stimulates salt retention involves the DCT and does not involve ammonia. Hypokalemia increases phosphorylation of the Na^+–Cl^- cotransporter (NCC) [83], which increases its activity [84], leading to increased NaCl reabsorption. This likely occurs because hypokalemia causes hyperpolarization of DCT cells resulting from K^+ transport through the basolateral K^+ channel KCNJ10 (Kir4.1) [85]. The hyperpolarization presumably increases basolateral Cl^- exit, presumably via the basolateral Cl^- channel ClC-Kb in association with its accessory subunit barttin [86], decreasing intracellular Cl^-. Decreased intracellular Cl^- activates the signaling protein WNK4, increasing SPAK phosphorylation, which then phosphorylates and activates the apical Na^+–Cl^- cotransporter, NCC [83, 87]. At this

time, the relative roles of the ammonia-dependent pathway and ammonia-independent pathway in hypokalemia-dependent increases in NaCl reabsorption and intravascular volume expansion have not been studied specifically.

Summary

The aim of this review was to discuss the roles of renal ammonia metabolism other than its traditionally considered role as a component of net acid excretion. The intention was not to diminish the importance of the role of ammonia in acid–base homeostasis, but rather to highlight additional roles of renal ammonia metabolism (Fig. 8). First, the kidneys produce ammonia, and under normal circumstances they excrete ~50 % of the ammonia so produced into the urine. The remaining proportion of ammonia produced in the kidney is added to the systemic circulation as ammonia of renal origin. In patients at risk of hyperammonemia, such as those with acute or chronic liver disease, this addition of ammonia of renal origin to the systemic circulation can lead to hyperammonemia and subsequent precipitation and/or exacerbation of hepatic encephalopathy. Second, ammonia is a paracrine signaling molecule that is produced in response to changes in extracellular potassium, which can then regulate potassium transport by the collecting duct through its effects on both potassium secretion by principal cells and potassium reabsorption by intercalated cells. Finally, stimulation of renal ammonia metabolism, particularly in response to hypokalemia, can be, albeit indirectly, related to its effect in causing generation of metabolic alkalosis by stimulating sodium reabsorption by the collecting duct through a 2-oxoglutarate–2-oxoglutarate receptor 1–sodium-driven chloride/bicarbonate exchanger (NDCBE) and pendrin-mediated mechanism.

Compliance with ethical standards

Conflict of interest The author declares that he has no conflict of interest.

References

1. Gil-Pena H, Mejia N, Santos F (2013) Renal tubular acidosis. J Pediatr 164:691–698

2. Mitch WE (2006) Metabolic and clinical consequences of metabolic acidosis. J Nephrol 19:S70–S75

3. Weiner ID, Verlander JW (2015) Renal acidification mechanisms. In: Tall MW, Chertow GM, Marsden PA, Skorecki K, Yu AS, Brenner BM (eds) Brenner and Rector's the kidney, 10th edn. W.B. Saunders Press, New York, pp 234–257

4. Hamm LL, Nakhoul N, Hering-Smith KS (2015) Acid–base homeostasis. Clin J Am Soc Nephrol 10:2232–2242

5. Weiner ID, Mitch WE, Sands JM (2014) Urea and ammonia metabolism and the control of renal nitrogen excretion. Clin J Am Soc Nephrol 10:1444–1458

6. Weiner ID, Verlander JW (2013) Renal ammonia metabolism and transport. Compr Physiol 3:201–220

7. Halperin ML, Dhadli SC, Kamel KS (2006) Physiology of acid–base balance: links with kidney stone prevention. Semin Nephrol 26:441–446

8. Unwin RJ, Capasso G, Shirley DG (2004) An overview of divalent cation and citrate handling by the kidney. Nephron Physiol 98:15–20

9. Eriksson LS, Broberg S, Bjorkman O, Wahren J (1985) Ammonia metabolism during exercise in man. Clin Physiol 5:325–336

10. Elkinton JR, Huth EJ, Webster GD Jr, McCance RA (1960) The renal excretion of hydrogen ion in renal tubular acidosis. Am J Med 36:554–575

11. Curthoys NP, Moe OW (2014) Proximal tubule function and response to acidosis. Clin J Am Soc Nephrol 9:1627–1638

12. Wright PA, Knepper MA (1990) Phosphate-dependent glutaminase activity in rat renal cortical and medullary tubule segments. Am J Physiol 259:F961–F970

13. Wright PA, Knepper MA (1990) Glutamate dehydrogenase activities in microdissected rat nephron segments: effects of acid–base loading. Am J Physiol 259:F53–F59

14. Nagami GT (2004) Ammonia production and secretion by S3 proximal tubule segments from acidotic mice: role of ANG II. Am J Physiol Renal Physiol 287:F707–F712

15. Nagami GT, Sonu CM, Kurokawa K (1986) Ammonia production by isolated mouse proximal tubules perfused in vitro: effect of metabolic acidosis. J Clin Invest 78:124–129

16. Nagami GT (2008) Role of angiotensin II in the enhancement of ammonia production and secretion by the proximal tubule in metabolic acidosis. Am J Physiol Renal Physiol 294:F874–F880

17. Weiner ID, Verlander JW (2014) Ammonia transport in the kidney by Rhesus glycoproteins. Am J Physiol Renal Physiol 306:F1107–F1120

18. Weiner ID, Verlander JW (2011) Role of NH3 and NH4+ transporters in renal acid–base transport. Am J Physiol Renal Physiol 300:F11–F23

19. Owen EE, Tyor MP, Flanagan JF, Berry JN (1960) The kidney as a source of blood ammonia in patients with liver disease: the effect of acetazolamide. J Clin Invest 39:288–294

20. Haussinger D, Lamers WH, Moorman AF (1992) Hepatocyte heterogeneity in the metabolism of amino acids and ammonia. Enzyme 46:72–93

21. Haussinger D (1989) Glutamine metabolism in the liver: overview and current concepts. Metabolism 38:14–17

22. Kaiser S, Gerok W, Haussinger D (1988) Ammonia and glutamine metabolism in human liver slices: new aspects on the pathogenesis of hyperammonaemia in chronic liver disease. Eur J Clin Invest 18:535–542

23. Haussinger D (1987) Structural-functional organization of hepatic glutamine and ammonium metabolism. Biochem Soc Trans 15:369–372

24. Haussinger D (1986) Regulation of hepatic ammonia metabolism: the intercellular glutamine cycle. Adv Enzyme Regul 25:159–180

25. Verlander JW, Chu D, Lee HW, Handlogten ME, Weiner ID (2013) Expression of glutamine synthetase in the mouse kidney: localization in multiple epithelial cell types and differential regulation by hypokalemia. Am J Physiol Renal Physiol 305:F701–F713

26. Koen H, Okuda K, Musha H, Tateno Y, Fukuda N, Matsumoto T, Shisido F, Rikitake T, Iinuma T, Kurisu A, Arimizu N (1980) A dynamic study of rectally absorbed ammonia in liver cirrhosis using ammonia and a positron camera. Dig Dis Sci 25:842–848

27. Cooper AJ (1990) Ammonia metabolism in normal and portacaval-shunted rats. Adv Exp Med Biol 272:23–46

28. Conn HO (1972) Studies of the source and significance of blood ammonia. IV. Early ammonia peaks after ingestion of ammonium salts. Yale J Biol Med 45:543–549

29. Gumz ML, Rabinowitz L, Wingo CS (2015) An Integrated view of potassium homeostasis. N Engl J Med 373:60–72

30. Unwin RJ, Luft FC, Shirley DG (2011) Pathophysiology and management of hypokalemia: a clinical perspective. Nat Rev Nephrol 7:75–84

31. Weiner ID, Wingo CS (1997) Hypokalemia—consequences, causes and correction. J Am Soc Nephrol 8:1179–1188

32. Gabuzda GJ, Hall II (1966) Relation of potassium depletion to renal ammonium metabolism and hepatic coma. Medicine (Baltimore) 45:481–489

33. Shear L, Gabuzda GJ (1970) Potassium deficiency and endogenous ammonium overload from kidney. Am J Clin Nutr 23:614–618

34. Baertl JM, Sancetta SM, Gabuzda GJ (1963) Relation of acute potassium depletion to renal ammonium metabolism in patients with cirrhosis. J Clin Invest 42:696–706

35. Han KH, Lee HW, Handlogten ME, Bishop JM, Levi M, Kim J, Verlander JW, Weiner ID (2011) Effect of hypokalemia on renal expression of the ammonia transporter family members, Rh B glycoprotein and Rh C glycoprotein, in the rat kidney. Am J Physiol Renal Physiol 301:F823–F832

36. Busque SM, Wagner CA (2009) Potassium restriction, high protein intake, and metabolic acidosis increase expression of the glutamine transporter SNAT3 (Slc38a3) in mouse kidney. Am J Physiol Renal Physiol 297:F440–F450

37. Hossain SA, Chaudhry FA, Zahedi K, Siddiqui F, Amlal H (2011) Cellular and molecular basis of increased ammoniagenesis in potassium deprivation. Am J Physiol Renal Physiol 301:F969–F978

38. Nagami GT (1990) Effect of bath and luminal potassium concentration on ammonia production and secretion by mouse proximal tubules perfused in vitro. J Clin Invest 86:32–39

39. Adam WR, Koretsky AP, Weiner MW (1986) 31P-NMR in vivo measurement of renal intracellular pH: effects of acidosis and K+ depletion in rats. Am J Physiol 251:F904–F910

40. Jones B, Simpson DP (1983) Influence of alterations in acid–base conditions on intracellular pH of intact renal cortex. Ren Physiol 6:28–35

41. Schoolwerth AC, Culpepper RM (1990) Measurement of intracellular pH in suspensions of renal tubules from potassium-depleted rats. Miner Electrolyte Metab 16:191–196

42. Olde Damink SW, Jalan R, Deutz NE, Redhead DN, Dejong CH, Hynd P, Jalan RA, Hayes PC, Soeters PB (2003) The kidney plays a major role in the hyperammonemia seen after simulated or actual GI bleeding in patients with cirrhosis. Hepatology 37:1277–1285

43. Bounoure L, Ruffoni D, Muller R, Kuhn GA, Bourgeois S, Devuyst O, Wagner CA (2014) The role of the renal ammonia transporter Rhcg in metabolic responses to dietary protein. J Am Soc Nephrol 25:2040–2052

44. Brosnan JT, McPhee P, Hall B, Parry DM (1978) Renal glutamine metabolism in rats fed high-protein diets. Am J Physiol 235:E261–E265

45. Lee HW, Osis G, Handlogten ME, Guo H, Verlander JW, Weiner ID (2015) Effect of dietary protein restriction on renal ammonia metabolism. Am J Physiol Renal Physiol 308:F1463–F1473

46. Inoue K, Takahashi T, Yamamoto Y, Suzuki E, Takahashi Y, Imai K, Inoue Y, Hirai K, Tsuji D, Itoh K (2015) Influence of glutamine synthetase gene polymorphisms on the development of hyperammonemia during valproic acid–based therapy. Seizure 33:76–80

47. Elhamri M, Ferrier B, Martin M, Baverel G (1993) Effect of valproate, sodium 2-propyl-4-pentenoate and sodium 2-propyl-2-

pentenoate on renal substrate uptake and ammoniagenesis in the rat. J Pharmacol Exp Ther 266:89–96

48. Martin G, Durozard D, Besson J, Baverel G (1990) Effect of the antiepileptic drug sodium valproate on glutamine and glutamate metabolism in isolated human kidney tubules. Biochim Biophys Acta 1033:261–266

49. Doval M, Culebras M, Lopez-Farre A, Rengel M, Gougoux A, Vinay P, Lopez-Novoa JM (1989) Effect of valproate on lactate and glutamine metabolism by rat renal cortical tubules. Proc Soc Exp Biol Med 190:357–364

50. Marini AM, Zaret BS, Beckner RR (1988) Hepatic and renal contributions to valproic acid-induced hyperammonemia. Neurology 38:365–371

51. Rengel M, Doval M, Culebras M, Gougoux A, Vinay P, Lopez-Novoa JM (1988) Ammoniagenesis and valproic acid in the rat in vivo: role of the kidney. Contrib Nephrol 63:132–135

52. Imler M, Chabrier G, Marescaux C, Warter JM (1986) Effects of 2, 4-dinitrophenol on renal ammoniagenesis in the rat. Eur J Pharmacol 123:175–179

53. Warter JM, Brandt C, Marescaux C, Rumbach L, Micheletti G, Chabrier G, Krieger J, Imler M (1983) The renal origin of sodium valproate-induced hyperammonemia in fasting humans. Neurology 33:1136–1140

54. Warter JM, Marescaux C, Brandt C, Rumbach L, Micheletti G, Chabrier G, Imler M, Kurtz D (1983) Sodium valproate associated with phenobarbital: effects on ammonia metabolism in humans. Epilepsia 24:628–633

55. Warter JM, Imler M, Marescaux C, Chabrier G, Rumbach L, Micheletti G, Krieger J (1983) Sodium valproate-induced hyperammonemia in the rat: Role of the kidney. Eur J Pharmacol 87:177–182

56. Tannen RL (1977) Relationship of renal ammonia production and potassium homeostasis. Kidney Int 11:453–465

57. Tannen RL (1970) The effect of uncomplicated potassium depletion on urine acidification. J Clin Invest 49:813–827

58. Tannen RL, Terrien T (1975) Potassium-sparing effect of enhanced renal ammonia production. Am J Physiol 228:699–705

59. Jaeger P, Karlmark B, Giebisch G (1983) Ammonium transport in rat cortical tubule: relationship to potassium metabolism. Am J Physiol 245:F593–F600

60. Hamm LL, Gillespie C, Klahr S (1985) NH4Cl inhibition of transport in the rabbit cortical collecting tubule. Am J Physiol 248:F631–F637

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

62. Muto S (2001) Potassium transport in the mammalian collecting duct. Physiol Rev 81:85–116

63. Frank AE, Wingo CS, Weiner ID (2000) Effects of ammonia on bicarbonate transport in the cortical collecting duct. Am J Physiol Renal Physiol 278:F219–F226

64. Chalfant ML, Denton JS, Berdiev BK, Ismailov II, Benos DJ, Stanton BA (1999) Intracellular H+ regulates the alpha-subunit of ENaC, the epithelial Na+ channel. Am J Physiol 276:C477–C486

65. Konstas AA, Mavrelos D, Korbmacher C (2000) Conservation of pH sensitivity in the epithelial sodium channel (ENaC) with Liddle's syndrome mutation. Pflugers Arch 441:341–350

66. Nakhoul NL, Hering-Smith KS, Abdulnour-Nakhoul SM, Hamm LL (2001) Ammonium interaction with the epithelial sodium channel. Am J Physiol Renal Physiol 281:F493–F502

67. Greenlee MM, Lynch IJ, Gumz ML, Cain BD, Wingo CS (2010) The renal H, K-ATPases. Curr Opin Nephrol Hypertens 19:478–482

68. Weiner ID, Linus S, Wingo CS (2010) Disorders of potassium metabolism. In: Johnson RJ, Fluege J, Feehally J (eds)

69. Frank AE, Wingo CS, Andrews PM, Ageloff S, Knepper MA, Weiner ID (2002) Mechanisms through which ammonia regulates cortical collecting duct net proton secretion. Am J Physiol Renal Physiol 282:F1120–F1128

70. Good DW (1990) Ammonium transport by the loop of Henle. Miner Electrolyte Metab 16:291–298

71. Wall SM, Davis BS, Hassell KA, Mehta P, Park SJ (1999) In rat tIMCD, NH4+ uptake by the Na+, K+-ATPase is critical to net acid secretion during chronic hypokalemia. Am J Physiol 277:F866–F874

72. Frank AE, Weiner ID (2001) Effects of ammonia on acid–base transport by the B-type intercalated cell. J Am Soc Nephrol 12:1607–1614

73. Barri YM, Wingo CS (1997) The effects of potassium depletion and supplementation on blood pressure: a clinical review. Am J Med Sci 314:37–40

74. Krishna GG, Kapoor SC (1991) Potassium depletion exacerbates essential hypertension. Ann Intern Med 115:77–83

75. Krishna GG (1990) Effect of potassium intake on blood pressure. J Am Soc Nephrol 1:43–52

76. Hernandez RE, Schambelan M, Cogan MG, Colman J, Morris RC, Sebastian A (1987) Dietary NaCl determines severity of potassium depletion-induced metabolic alkalosis. Kidney Int 31:1356–1367

77. Cremer W, Bock KD (1977) Symptoms and course of chronic hypokalemic nephropathy in man. Clin Nephrol 7:112–119

78. Loon NR, Wilcox CS (1998) Mild metabolic alkalosis impairs the natriuretic response to bumetanide in normal human subjects. Clin Sci (Lond) 94:287–292

79. Tokonami N, Morla L, Centeno G, Mordasini D, Ramakrishnan SK, Nikolaeva S, Wagner CA, Bonny O, Houillier P, Doucet A, Firsov D (2013) a-Ketoglutarate regulates acid–base balance through an intrarenal paracrine mechanism. J Clin Invest 123:3166–3171

80. Martin M, Ferrier B, Baverel G (1989) Transport and utilization of alpha-ketoglutarate by the rat kidney in vivo. Pflugers Arch 413:217–224

81. Ferrier B, Martin M, Baverel G (1985) Reabsorption and secretion of alpha-ketoglutarate along the rat nephron: a micropuncture study. Am J Physiol 248:F404–F412

82. Balagura S, Pitts RF (1964) Renal handling of a-ketoglutarate by the dog. Am J Physiol 207:483–494

83. Terker AS, Zhang C, Erspamer KJ, Gamba G, Yang CL, Ellison DH (2016) Unique chloride-sensing properties of WNK4 permit the distal nephron to modulate potassium homeostasis. Kidney Int 89:127–134

84. Hadchouel J, Ellison DH, Gamba G (2016) Regulation of renal electrolyte transport by WNK and SPAK-OSR1 kinases. Annu Rev Physiol 78:367–389

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

86. Subramanya AR, Ellison DH (2014) Distal convoluted tubule. Clin J Am Soc Nephrol 9:2147–2163

87. Bazua-Valenti S, Chavez-Canales M, Rojas-Vega L, Gonzalez-Rodriguez X, Vazquez N, Rodriguez-Gama A, Argaiz ER, Melo Z, Plata C, Ellison DH, Garcia-Valdes J, Hadchouel J, Gamba G (2015) The effect of WNK4 on the Na+−Cl- cotransporter is modulated by intracellular chloride. J Am Soc Nephrol 26:1781–1786

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