Increased ammoniogenesis and the renal tubular effects of potassium depletion

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SUMMARY The cause of the morphological changes and functional defects in the renal tubule seen in patients with severe potassium depletion is unknown. In man and animals potassium status is a major factor regulating ammonia synthesis in the kidney and urinary ammonium excretion. A primary effect of potassium depletion is to cause an increase in ammoniogenesis by the renal tubular cells. It is proposed that the vacuolation of the renal tubular cells and the functional defects of tubular proteinuria, polyuria, resistance to arginine vasopressin, renal resistance to the action of parathyroid hormone, and increased urinary excretion of N-acetyl- β -glucosaminidase found in potassium depletion are secondary effects caused by high concentrations of ammonia in the renal tubular cells.

It has been recognised since 1937 that potassium depletion is associated with vacuolation of the renal tubular cells.¹ It was subsequently shown in 1956 by Relman and Schwartz² that this vacuolation, most prominent in the proximal convoluted tubule, is reversed in man by potassium repletion. More recently, experimental studies in the rat kidney have shown that this is lysosomal vacuolation.³ This vacuolation is associated with defects in tubular function which have received a lot of attention. Polyuria and a reversible resistance to the antidiuretic hormone arginine vasopressin have been noted in man.² In the rat kidney cyclic AMP production in response to arginine vasopressin and parathyroid hormone is also impaired.^{4,5} In man there is reversible tubular proteinuria,⁶ and more recently an increase in the urinary excretion of the lysosomal enzyme N-acetyl- β -glucosaminidase, (E.C.3.2.1.52 NAG) has been found.⁷

Renal glycosuria has been described as a late feature in two patients with potassium depletion secondary to chronic nephritis associated with increased urinary potassium loss.^{8,9} Both patients were complex, and potassium depletion was secondary to, rather than the cause of, their renal disease. Apart from these patients, renal glycosuria has not been commented on in potassium depletion. Renal glycosuria does not seem to occur in potassium depleted animals.¹⁰ Aminoaciduria is not usually a feature of potassium depletion. It was present in one

of the patients referred to above with glycosuria,⁸ and aminoaciduria has also been reported during the postoperative period in two patients with potassium depletion.¹¹ In these patients other causes of aminoaciduria cannot be excluded. When specifically looked for in patients with potassium depletion due to primary hyperaldosteronism aminoaciduria was not present.¹² The evidence that potassium depletion causes renal glycosuria and aminoaciduria is tenuous.

Potassium depletion and renal ammoniogenesis

Patients who are potassium depleted (as a result of excessive gastrointestinal¹³ or renal¹⁴ potassium losses) show increased urinary ammonia excretion. This also occurs in experimentally induced potassium depletion.^{15,16} Conversely, the administration of potassium supplements decreases urinary ammonia excretion.¹⁷ This indicates that there is an inverse relation between potassium status and urinary ammonia excretion. That this is related to the rate of ammonia production in the renal tubular cells is shown by increased ammoniogenesis in tissue slices¹⁸ and isolated mitochondria¹⁹ prepared from the kidneys of potassium depleted animals.

Glutamine is the major substrate for renal ammoniogenesis and the mitochondria are the major site of ammonia production.²⁰ At a molecular level potassium depletion stimulates the entry of glutamine into mitochondria²¹ and causes an increase in the activity of both phosphate dependent

glutaminase (L-glutamine amidohydrolase, E.C.3.5.1.2.) and glutamate dehydrogenase (L-glutamate: NAD⁺ oxidoreductase, E.C.1.4.1.2.), thus increasing mitochondrial ammoniogenesis.¹⁹ Potassium depletion also stimulates cytosolic ammoniogenesis from glutamine by increasing the activity of glutamine transaminase [L-glutamine 2-oxo-acid aminotransferase, E.C.2.6.1.15].²² Ammonia production from aspartate is also increased as a result of increased synthesis and activity of adenylosuccinate synthetase (IMP:L-aspartate ligase, E.C.6.3.4.4.) and adenylosuccinase (adenylosuccinate AMP-lyase, E.C.4.3.2.2.), thus stimulating the purine nucleotide cycle.²³ The synthesis of glutamine from glutamate is inhibited in potassium depletion, thus decreasing ammonia consumption by the cell.²⁴ Conversely, an increased potassium concentration inhibits ammoniogenesis *in vitro* in rat and canine renal tissue slices.²⁵ Clearly, potassium depletion increases ammonia production by the kidney.

The effects of increased ammoniogenesis on renal tubular function have received some attention. It is of considerable importance for potassium homeostasis that the increase in urinary ammonia excretion, resulting from increased renal ammoniogenesis, is directly associated with a decrease in urinary potassium excretion.²⁶ The effect of increased ammoniogenesis enables an increase in acid excretion by the kidney in the absence of a low urinary pH.²⁷ Other investigators maintain, however, that in addition to stimulating ammoniogenesis potassium depletion impairs the ability of the distal nephron to generate and maintain a pH gradient across the tubular cells.²⁸ Other effects of ammonia and ammonium on cell morphology and function have recently been the subject of much research. This work, which for technical reasons has to be performed on cells in tissue culture, is relevant to many of the effects of potassium depletion on renal tubular cell morphology and function.

Ammonia, ammonium, and cell function

In the biological published work the terms ammonia and ammonium are often used synonymously. This is especially true when reference is being made to the measurement of ammonia or ammonium in biological fluids. The methods used to measure blood or plasma ammonia in fact measure total ammonium plus ammonia, but the result is referred to as being a blood or plasma ammonia level.²⁹⁻³⁴ This loose use of terminology suggests that the biological properties of ammonia and ammonium are similar, if not identical, which is not so.

Ammonia (NH₃) is lipophilic and diffuses rapidly

throughout cells and through biological membranes. Ammonium (NH₄⁺) is hydrophilic and does not cross biological membranes, except in some bacteria, fungi, plants, and ammoniotelic aquatic animals which have an ammonium transport mechanism.³⁵ The dissociation constant for NH₄⁺ is 1.26 × 10⁻⁹ (pK = 8.90) in aqueous solutions at 37°C, so that in biological systems the concentration of the ammonium is far greater than that of ammonia.³⁶

Vacuolation of nucleated cells by ammonia was shown by Heinz in 1890³⁷ and this has subsequently been shown to be due to lysosomal swelling and vacuolation.³⁸ The mechanism for this is that in the lysosome ammonia is protonated to the ammonium ion, and as a result it is trapped within the acid lysosomes where it exerts an osmotic effect leading to swelling and vacuolation (Fig. 1). Amines with this property of ammonia/ammonium are called lysosomotropic amines.³⁹ Cells in tissue culture become vacuolated with concentrations of ammonium in the culture medium of 1.0 to 10 mmol/l when the culture medium pH is 7.6⁴⁰; this corresponds to ammonia concentrations of 48 to 480 μmol/l, respectively.

Other effects of ammonia are to inhibit endocytosis by cells and the degradation of protein by the lysosome. An extracellular source of

Fig. 1 Ammonia (NH₃) rapidly diffuses into the lysosomes, and acidic endosomes, where it is converted to ammonium (NH₄⁺), which does not readily cross biological membranes. In an attempt to maintain the acidic pH of the lysosomes, hydrogen ions (H⁺) are actively pumped into the lysosomes, thus causing the accumulation of ammonium in the lysosomes. This results in osmotic swelling and eventually vacuolation of the lysosomes. Because of the large volume of culture medium relative to the volume of cells in tissue culture systems, the accumulation of ammonium in the lysosomes has an unmeasurable effect on the concentration of ammonium in the medium, and provided the pH remains constant the concentration of ammonia will be unchanged. At a steady state the lysosomal pH will depend on the balance resulting from the rapid diffusion of ammonia and the active transport of hydrogen ions into the lysosomes and the slow leakage of ammonium ions out of the lysosomes.

ammonia is not required as intracellular ammonia production will inhibit lysosomal protein degradation in cells if it is allowed to accumulate.⁴¹ Endocytosis and lysosomal protein degradation are virtually completely inhibited at a concentration of ammonium in the medium of 10 mmol/l when the pH is 7.6, and there is about 75% and 30% inhibition with concentrations of 6.0 and 2.0 mmol/l respectively.^{42,43} The explanation for these observations is the effect of ammonia on lysosomal pH (see Fig. 1). Using fluorescein labelled dextran the stable pH of lysosomes has been found to be 4.75 ± 0.06 in cultured cells. The addition of ammonium to the culture medium, with a pH of 7.6 and at a concentration of 10.0 mmol/l, results in an ammonia concentration in the medium of 480 $\mu\text{mol/l}$ and causes the lysosomal pH to rise within one minute to over 6.3; it will stabilise at about 6.1 after 4–5 min. If the concentration of ammonium in the culture medium is 1.0 mmol/l, this will result in an ammonia concentration of 48 $\mu\text{mol/l}$, and the pH will rise to over 5.7 and plateau at 5.5.^{44,45} This phenomenon is reversible within 5 min. The increase in the intralysosomal pH will cause inhibition of the lysosomal acid hydrolases, thus decreasing lysosomal protein degradation. This disruption of lysosomal function coupled with the failure to acidify the endosomes adequately will disrupt endocytosis.

Ammonia and other lysosomotropic amines decrease the number of receptors for many ligands on the surfaces of cells.⁴⁶ Peptide hormones, and some other ligands, interact with specific receptors on the surfaces of the cells of their target tissues. These receptor-ligand complexes are endocytosed and, in the acidic environment of the endosome and lysosome, the receptor-ligand complexes dissociate, allowing the receptors to be recycled to the surface of the cell for further usage, while the ligand is degraded in the lysosome. Lysosomotropic amines, by increasing the pH of the lysosomes and acidic endosomes, prevent receptor-ligand dissociation and lead to an accumulation of receptor-ligand complexes intracellularly, with a resulting depletion of the number of cell surface receptors. This effect of ammonia on hormone receptors has been shown for insulin and epidermal growth factor.^{47,48}

Ammonia increases the release of N-acetyl- β -glucosaminidase (E.C.3.2.1.52) and other lysosomal enzymes from the cell without causing the release of the cytoplasmic enzyme lactate dehydrogenase (E.C.1.1.1.27).⁴⁹ Lysosomal enzyme precursors are synthesised in the Golgi apparatus. They possess a 6-phosphomannose residue which binds to a membrane receptor which transports the precursor enzymes to the lysosomes. In the lysosomes the 6-phosphomannose residue is hydrolysed and the

precursor enzymes are proteolytically converted to the mature enzyme. Ammonia inhibits the maturation of lysosomal enzymes; the enzyme precursors, which have catalytic activity, are directed to the cell surface, where they become dissociated from the cell and can be measured as secreted enzymes.^{50,51}

Ammonia, urinary ammonium, and renal tubular cell function

It is to be expected from the above that if the concentration of ammonia in the renal tubular cells is high enough it will have the following effects. The cells will be vacuolated. The endocytosis of proteins filtered at the glomerulus will be inhibited, thus causing tubular proteinuria. The number of receptors on the surface of the cells will be decreased, which explains the resistance to arginine vasopressin and parathyroid hormone. The release of catalytically active lysosomal enzyme precursors from the renal tubular cells will result in an increase in urinary N-acetyl- β -glucosaminidase activity. These are some of the major characteristic effects of potassium depletion on the renal tubule. I am not aware of any reports that ammonia or ammonium alters the uptake of amino acids or glucose by cells. However, aminoaciduria and glycosuria do not appear to be features of the renal tubular effects of potassium depletion.

In potassium depleted human subjects or animals the concentration of ammonia has not been directly measured in intrarenal fluids—namely, renal tubular fluid, renal blood, or interstitial fluid; however, the urine pH and the concentration of urinary ammonium have been measured (such urinary ammonium measurements are invariably the sum of the total urinary ammonium concentration plus the total urinary ammonia concentration). Urinary ammonium is formed from ammonia which is produced in the renal tubular cells and passively diffuses into the tubular fluid. As hydrogen ions secrete into the tubular fluid ammonium ions are formed, which cannot diffuse back into the renal tubular cells and are excreted in the urine.⁵¹

Because ammonia diffuses rapidly through renal tissue, the urinary ammonium concentration in conjunction with the urinary pH, the pK of ammonium and applying the law of mass action, can be used to estimate the concentration of ammonia in the renal tubular cells. This has been verified experimentally by isotope dilution measurements in dogs.⁵² Micropuncture studies in rats have shown that the partial pressure of ammonia in the first, second, and final thirds of the proximal renal tubule are the same as in the distal tubule and equal to that of the renal venous blood. Concentration of ammonium, how-

Fig. 2 The partial pressure of ammonia ($p\text{NH}_3$) in the fluid of the first, second, and final thirds of the proximal renal tubule is the same as that in the distal tubule and equal to that of the renal venous blood. As the ammonia in urine and renal venous blood originates from the renal tubular cells, it is not unreasonable to deduce that this concentration of ammonia will be equal to, or perhaps even slightly less, than that in the renal tubular cell cytoplasm and lysosomes.

Unlike ammonia the concentration of ammonium increases in the tubular fluid as it proceeds along the nephron, but it does so in accordance with changes in the pH and, as predicted from the pK of ammonium, the concentration of ammonia and the Law of Mass Action. These data indicate that the urine pH and ammonium concentration may be used to estimate the concentration of ammonia in the renal tubular cells.

ever, increases along the renal tubule but does so in relation to the pH of the tubular fluid⁵³ (Fig. 2).

Cells exposed to 6.0 mmol/l ammonium in culture media at pH 7.6 become vacuolated, and endocytosis and lysosomal protein degradation are both inhibited by about 75%.^{39,41,42} The same intracellular ammonia concentration in the renal tubular cell would result in a urinary ammonium concentration of 38 mmol/l when the urine pH was 6.8 or 6.0 mmol/l at pH 6.6. In healthy subjects such high urinary ammonium concentrations associated with these relatively high urinary pH values are not encountered. In potassium depletion the concentration of ammonia in the renal tubular cells is high enough to result in urinary ammonium concentrations of this magnitude with the urine pH at such relatively high levels.¹³ I therefore propose that the morphological changes and some of the functional defects in the renal tubule seen in patients with severe potassium depletion are caused by high concentrations of ammonia in the kidney. The high concentrations of ammonia are due to a primary increase in renal ammoniagenesis caused by potassium depletion.

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