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Physiological and Metabolic Implications of V-ATPase Isoforms in the Kidney

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Not all vacuolar-type H^+-ATPases (V-ATPases) are alike; those responsible for H^+ movement across plasma membranes contain some different, tissue-specific subunit isoforms. This brief review outlines those that have special relevance to the kidney, and illustrates their importance by describing various human diseases where loss of local proton pump function not only confers a severe phenotype, but has revealed related tissues where these same isoforms are expressed, signifying their physiological importance.

KEY WORDS: Kidney; V-ATPase; renal tubular acidosis.

INTRODUCTION

In higher organisms, the vacuolar-type H^+-ATPases (V-ATPases) that are ubiquitous in nature subserve the essential function of regulating proton flux at various sites, both intra- and extracellular (Nishi and Forgac, 2002). While the general structure of V-ATPases is similar wherever they are found, their composition is subtly different at sites where there is a specific requirement for acid flux across the plasma membrane. These include specialized cells in kidney, bone, inner ear, testis, sperm and brain (Fig. 1a). Dysfunction of any one of the particular V-ATPases found at these sites might be expected to cause human disease, whereas mutation in ubiquitously present intracellular pumps might well be incompatible with life.

All V-ATPases are comprised of a V_1 or hydrolytic domain containing 3A, 3C, one each of B, C, D, E, F, H and two G subunits, loosely linked to the membrane-anchored V_0 domain, which comprises single copies of the a, c, d and e subunits with multiple numbers of c and c″ subunits, the latter two types in a ring structure. It is generally accepted that organellar V-ATPases are comprised of the A, B2, C1, D1, E1, F, G1, H, a1, c, c″, d1 and e1 subunit isoforms, all encoded by separate genes. Importantly, for those designated with a numerical suffix, there are separately encoded homologues that vary depending on the site and/or tissue in question.

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α-INTERCALATED CELL (AIC) FUNCTION

In mammals, most of the bicarbonate filtered by the glomerulus is reabsorbed in the proximal segment, with little net acid secretion, while the majority of fine regulation of systemic pH by the kidney is carried out by α-IC located in the distal portion of the nephron, particularly in the cortical collecting duct (CD). All of the epithelial cells that form the renal tubule are highly polarized because one surface is in contact with the urine and the other with interstitial fluid. The strict requirement for vectorial transport of ions and other molecules across both apical and basolateral plasma membranes is necessary to allow the kidney to fulfil its wide variety of homeostatic, secretory and resorptive functions. Thus, the cell surface-resident proteins via which this transport occurs are different on the opposite sides of each cell, and their repertoires also differ in the different cell types found in different nephron segments.

Therefore in the α-IC (Fig. 2), protons, which are produced by the catalytic activity of cytosolic carbonic anhydrase II, are secreted actively across the apical surface

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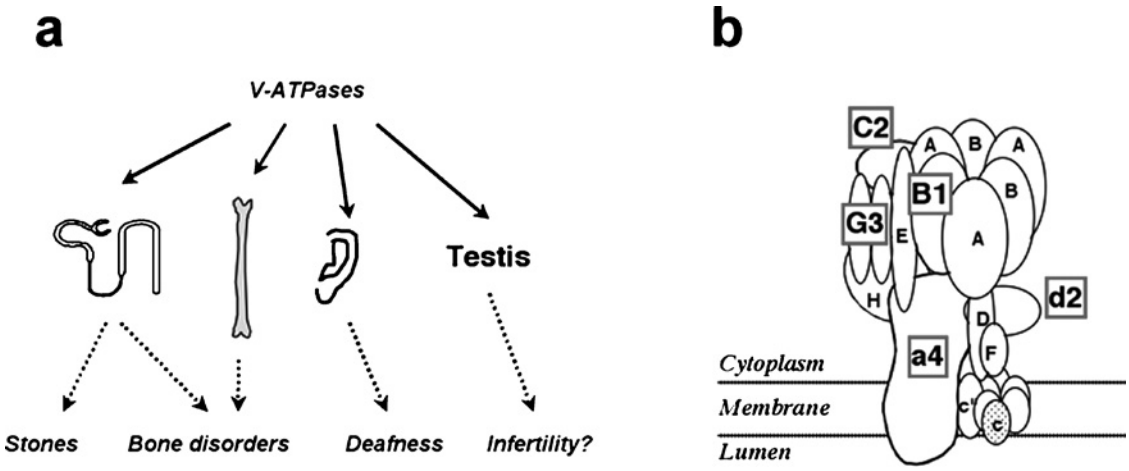

Fig. 1. (a) Specialized sites at which proton pumping across the plasma membrane is essential, and the disorders resulting from V-ATPase malfunction. (b) The renal apical proton pump. Of the (at least) 13 subunits, 5 are the product of alternate genes (boxed) that are either solely or mainly expressed in the kidney.

of the cell into the CD lumen by V-ATPases present at very high density in the plasma membrane. There is also a P-type ATPase in the apical membrane that exchanges H^+ for K^+, together with other ion transporters. Secretion of H^+ ions into the urine is coupled to the reclamation of HCO_3^- across the basolateral surface, in exchange for Cl^-, via the Cl^-/HCO_3^- exchanger, AE1. AE1 is not expressed anywhere else in the nephron, whereas apical V-ATPases are also found proximally, though their exact composition is probably different, partly because one reason for their presence is to participate in endocytic recycling to facilitate protein and solute reabsorption.

In the human distal nephron, most of the acid–base regulatory cells are α-ICs, because of the net acid load generated by our food intake (70–100 mmol H^+ daily on an omnivorous diet). However, in some circumstances,

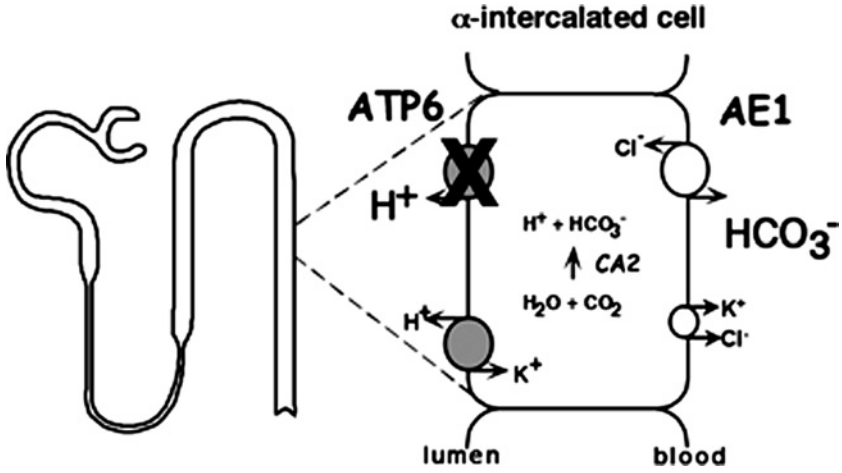

Fig. 2. Polarized function of renal α-intercalated cell and molecular contributors to dRTA. In α-IC, acid–base balance is tightly regulated by proton pumps on the apical surface of the cell that actively secrete acid into the duct. This process is coupled to the reclamation of bicarbonate ions into the interstitium, via the chloride–bicarbonate exchanger AE1, whose expression in the kidney is confined to the basolateral surface of these cells. Other molecular residents are also illustrated, with the exception of the ubiquitously expressed Na/K-ATPase. The KCC4 potassium chloride co-transporter has to date only been localized in rodents.

e.g. in the rodent kidney, a significant proportion of the cells are of the opposite morphology, termed β-IC, and are responsible for HCO_3^- extrusion into urine. Their resident membrane proteins are similar but not always identical to those of the α-IC. To date, no human disorders have been associated with non-α-ICs.

DISTAL RENAL TUBULAR ACIDOSIS

Distal RTA (dRTA) arises when the CD fails to remove the excess acid load of a normal diet into the urine, i.e. when α-IC function fails. It was first reported in the mid-1930s (Butler *et al*., 1936; Lightwood, 1935). It is characterised by failure of the kidney to produce an appropriately acid urine either in the presence of systemic metabolic acidosis, or following acid loading. It can be diagnosed biochemically if urine pH is greater than 5.3 when systemic pH is less than 7.34 (with normal anion gap and renal function), or following the administration of NH_4Cl as an oral acid challenge; 100 mg/kg body weight should reduce urine pH to less than 5.3 over the next 6 h (Wrong and Davies, 1959).

Of the inherited forms, dominant dRTA in general (but not always) presents later (sometimes not until adulthood) and with milder phenotype, than does the recessive form. Sensorineural hearing loss (SNHL) is a common accompaniment to dRTA, but is only ever a feature of recessive disease. Primary dRTA is relatively rare in Western populations, but occurs more commonly in areas of the world where rates of parental consanguinity are high (Karet, 2002).

In its severest form, affected infants with dRTA are very acidotic and volume-depleted, with marked hypokalemia but otherwise normal renal excretory function. Growth is poor, and rickets are common in untreated cases. Nephrocalcinosis begins at a very early age, and may lead to accelerated renal impairment. Urinary citrate is low in dRTA, because citrate reabsorption is up-regulated in the proximal tubule to provide new HCO_3^- (1 citrate = 2 HCO_3^-). Abnormal calcium deposition in dRTA is mainly attributed to this hypocitraturia.

DRTA is treated with oral alkali replacement (1–3 mg/kg per day of citrate or HCO_3), which can reverse most of the biochemical abnormalities and the associated bone disease in both dominant and recessive dRTA (Peces, 2000; Santos and Chan, 1986). Unfortunately, the progressive SNHL is not improved by alkali therapy (Zakzouk *et al*., 1995). K^+ salts are considered preferable to Na^+ as the latter can exacerbate hypokalemia.

The dRTAs could in theory result either from loss of function of the essential transporters involved in acid–base homeostasis, or from abnormalities of the machinery that gets them to their proper site of action. In fact, two recessively inherited forms of dRTA are now known to be the direct result of malfunction of the apical proton pump.

KIDNEY-SPECIFIC V-ATPase SUBUNIT GENES THAT CAUSE DISEASE

Genome-wide searches to localize genes for recessive dRTA in cohorts of largely consanguineous kindreds separated by the presence or absence of hearing loss at diagnosis revealed linkage to two loci, on 2p and 7q. We have identified both the responsible genes, which perhaps predictably encode different kidney-specific subunits of the apical V-ATPase. These account for RTA Types 1b (early deafness) and 1c (milder hearing loss) respectively.

THE B1 SUBUNIT

ATP6V1B1, encoding the B1-subunit of V-ATPase, is on chromosome 2. This gene was of particular interest at the time, because, in man, expression of the B1 isoform had been demonstrated at significant levels only in kidney and male genital tract epithelia (van Hille *et al*., 1994). We found 15 different mutations in kindreds where almost all the affected individuals had SNHL at a young age (Karet *et al*., 1999). The majority of these mutations are predicted to lead to loss of function of this specialized form of the proton pump.

RT-PCR of material from the human inner ear, and immunolocalization in mouse tissue, showed that B1 mRNA and protein are both expressed within the cochlea and endolymphatic sac (Karet *et al*., 1999). This is of interest in view of the unique ionic composition of endolymph, the fluid that fills these compartments, which has a high $[K^+]$ of some 150 mM. In the context of a pH of $\leq$7.4 (rather than the predicted alkalinity), this implies a requirement for proton pumping into this fluid. Although it remains speculative in the absence of a suitable animal model, the idea is that defective V-ATPase function leads to raised endolymph pH and eventual irremediable functional loss of hair cells, which would explain why alkali therapy is of no help to the deafness of recessive dRTA.

B1 subunit expression is also reported in the eye, localizing to ciliary epithelium in rat and rabbit at least (Wax *et al*., 1997), though no human studies exist. Topical application of bafilomycin A1 reduced intraocular pressure in rabbits, indicating an essential role of the V-ATPase in ciliary epithelial ion transport.

A mouse lacking *Atp6v1b1* has been made, but escapes the severe phenotype seen in human disease

(Finberg *et al*., 2005). Although urine pH is higher in −/− animals, their systemic pH, K^+ levels and hearing are normal, and renal tract calcification is absent. However, oral acid challenge does reveal a urine acidification defect. The nature of the compensation in these animals is unknown; however, expression of the B2 subunit has recently been observed at the apical surface of IC in rodents (Paunescu *et al*., 2004).

ATP6V0A4

By a similar linkage approach in a cohort of dRTA kindreds where hearing was essentially normal at diagnosis, the second defective gene was found, and named *ATP6V0A4* because it proved to encode a newly identified kidney-specific isoform of the proton pump's 116 kDa 'accessory' a-subunit, designated a4 (Smith *et al*., 2000). It had previously been debated whether V-ATPases in kidney even contained an a-subunit (Gillespie *et al*., 1991; Gluck and Caldwell, 1988). Although the genetics showed that in kidney at least, the a-subunit must be essential for proper V-ATPase function, its role within the multi-subunit pump structure remains somewhat unclear. Yeast studies suggest that it may assist not only in H^+ translocation, but also in assembling and stabilizing the molecular complex (Leng *et al*., 1998).

Apart from hearing status at diagnosis, there did not initially appear to be major differences between patients with *ATP6V1B1* and *ATP6V0A4* mutations, and treatment is identical. However, screening of a larger cohort later revealed that a4 mutations are indeed associated with SNHL. In general, this begins later and is milder, though it can still give rise to profound deafness (Stover *et al*., 2002). As predicted from these observations, the a4 subunit has also been found within the cochlea (Dou *et al*., 2004; Stover *et al*., 2002).

The various a-subunit isoforms share the same general bipartite structure, in which the first ∼420 N-terminal residues form a hydrophobic domain located on the cytosolic side of the membrane (the same side as the pump's V_1 domain). This part probably interacts with several other subunits, including G (unpublished observations). The C-terminal half, approximately 400 residues, is arranged in a series of transmembrane segments, the number of which has been debated and may vary among species. In humans, we have shown that at least the renal a4 and the ubiquitously expressed a1 subunit interact via their C-termini with the rate-limiting glycolytic enzyme PFK-1 (Su *et al*., 2003). This places the C-terminus of the a-subunit on the cytosolic side, and implies an even number of transmembrane helices.

Interestingly, the E subunit interacts with aldolase, the next enzyme down the glycolytic cascade (Lu *et al*., 2001, 2004). Together, these findings suggest a direct link between proton pump function and glycolysis, which is interesting given that glucose is necessary for pump assembly (Kane, 1995).

ADDITIONAL HETEROGENEITY AND THE DISCOVERY OF FURTHER 'KIDNEY-SPECIFIC' GENES

A few recessive families do not link to either *ATP6V1B1* or *ATP6V0A4* (Smith *et al*., 2000). We hypothesized that other subunits may have as yet unrevealed novel isoforms in kidney, which would be candidate genes. By similarity searching of the human genome, we have discovered three novel genes that encode human V-ATPase subunit isoforms solely or mainly renally expressed, designated C2, G3 and d2 (Smith *et al*., 2002), and each has a murine orthologue (Sun-Wada *et al*., 2003b). However, to date we have found no disease-attributable mutations.

C2, G3 AND D2 EXPRESSION

C2 subunit mRNA is found in kidney, lung and placenta. The gene's open reading frame of 1143 bp encodes a 381 amino acid protein with a predicted molecular weight of 43.9 kDa. Hydropathy analysis predicts that like C1, the C2 subunit is a hydrophilic protein with no apparent transmembrane segments. Among the 12 coding exons, two possible alternative splicing patterns are present. In mouse these proteins have been designated C2a (46 amino acids longer) and C2b, the former appearing in lung, specifically in the lamellar bodies of type II alveolar cells, and the latter the plasma membrane of α- and β-IC in murine kidney (Sun-Wada *et al*., 2003a).

G3 meanwhile, is kidney specific, whereas the d2 subunit is present in both renal IC and osteoclasts in bone. Co-localization studies suggest that d2 participates in the same pumps as the a4 and a3 subunits respectively (Smith *et al*., 2002). Thus, the factorial number of potentially different V-ATPases is increasing, and studying these may shed light on specialized function.

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