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The Cellular Physiology of Carbonic Anhydrases

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Summary

Carbonic anhydrases are zinc metalloenzymes that catalyze the reversible hydration of CO_2 to form HCO_3^- and protons according to the following reaction: $CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- + H^+$. The first reaction is catalyzed by carbonic anhydrase and the second reaction occurs instantaneously. The carbonic anhydrase (CA) gene family includes ten enzymatically active members, which are major players in many physiological processes, including renal and male reproductive tract acidification, bone resorption, respiration, gluconeogenesis, signal transduction, and formation of gastric acid [1]. The newly identified CA IX (previously called MN) and CA XII are related to cell proliferation and oncogenesis [2, 3, 4]. Carbonic anhydrase isozymes have different kinetic properties and they are present in various tissues [1] and in various cell compartments. CA I, II, III and VII are cytoplasmic, CA V is mitochondrial, and CA VI is present in salivary secretions. CA IV, IX, XII and XIV are membrane proteins: CA IV is a glycosyl-phosphatidylinositol-anchored protein, and CA IX, XII and XIV are transmembrane proteins. The present work will focus on the roles of CA II and CA IV in transepithelial proton secretion and bicarbonate reabsorption processes. The localization of these isoforms in selected epithelia that are involved in net acid/base transport, such as kidney proximal tubules and collecting ducts, and tubules from the male reproductive tract will be reviewed.

Kidney Acidification

CA II is highly expressed in kidney intercalated cells, and it is expressed at lower levels in kidney proximal tubules, loop of Henle and collecting duct principal cells [5, 6]. It is the only soluble form of carbonic anhydrase in renal epithelial cells. CA IV is present on the apical brush-border membrane and on the basolateral membrane of proximal tubule cells [7, 8]. More recently, additional isoforms have been identified in kidney epithelial cells. CA XII is present in the basolateral membrane of thick ascending limb of Henle and distal convoluted tubules, and in collecting duct principal cells [9]. In addition, membrane-bound CA XIV message has been found in proximal tubules [10].

The kidney reabsorbs virtually all the bicarbonate that is filtered by the glomeruli. 70-85% of bicarbonate reabsorption takes place in the proximal tubule, and 10-20% in the thick ascending limb of Henle. In these segments, the activity of both intracellular CA II and apical CA IV contribute to net transepithelial bicarbonate transport. In the proximal tubule, an apical Na^+/H^+ exchanger, NHE3, and the vacuolar H^+ATPase secrete protons into the

lumen. These H^+ ions combine with luminal HCO_3^- to form CO_2 and H_2O under the enzymatic action of CA IV. Newly formed CO_2 then diffuses into the cell through the apical membrane and is hydrated under the influence of cytosolic CA II to form H^+ and HCO_3^-. The protons recycle back into the lumen *via* NHE3 and the vacuolar H^+ATPase, while HCO_3^- diffuses passively across the basolateral membrane *via* a Na^-/HCO_3^- cotransporter. The result of these processes is net bicarbonate reabsorption with no net proton secretion. Basolateral CA IV was proposed to facilitate Na^-/HCO_3^- co-transport by preventing the development of an alkaline disequilibrium pH in the interstitium [11, 12]. The thick ascending limb, which also contains both CA II and CA IV, reabsorbs filtered bicarbonate. This is achieved *via* apical NHE3 and vacuolar H^+ATPase, and possibly *via* a basolateral Cl^-/HCO_3^- exchanger [13].

Collecting duct intercalated cells are responsible for the remaining HCO_3^- reabsorption and net proton secretion that occurs in the urinary tubule [14, 15]. In this case, only CA II is involved. Apical proton extrusion occurs in type A intercalated cells *via* vacuolar H^+ATPase and an H^+-K^+-ATPase. Bicarbonate exits the cells across the basolateral membrane *via* the Cl^-/HCO_3^- exchanger AE1. Intracellular CA II provides proton and bicarbonate from the hydration of metabolic CO_2. In cortical collecting ducts, another subtype of intercalated cell exists, the type B intercalated cell, which secretes bicarbonate and reabsorbs protons. This is achieved *via* an apical Cl^-/HCO_3^- exchanger, recently identified as a novel anion exchanger (AE) isoform, AEIV [16], basolateral vacuolar H^+ATPase and cytosolic CA II.

Epididymis/Vas Deferens Acidification

A low bicarbonate concentration and an acidic pH in the luminal fluid of the epididymis and vas deferens help to maintain sperm in a quiescent state during maturation and storage in these organs [17, 18]. Active bicarbonate reabsorption takes place in the proximal regions of the epididymis (initial segments, caput epididymidis) and net proton secretion occurs in the distal portions of the epididymis (cauda epididymidis) and vas deferens. The epithelium lining the epididymis and vas deferens is composed of two major cell types, narrow cells and principal cells in the initial segments, and clear cells and principal cells in the caput and cauda epididymidis.

Narrow and clear cells express high levels of the vacuolar type H^+ATPase on their apical membrane and sub-apical vesicles [19, 20, 21, 22], and high levels of cytosolic CA II [20, 21, 23], indicating that these cells are involved in proton secretion coupled to bicarbonate reabsorption. In addition, we have recently shown that the Na^+/H^+ exchanger, NHE3, is expressed in the apical membrane of principal cells of the proximal regions of the epididymis [24]. The Cl^-/HCO_3^- exchanger, AE2 [25], and the electrogenic Na^-/HCO_3^- cotransporter, NBC [26], are present on the basolateral membrane of epididymal epithelial cells. Epididymal principal cells also show apical carbonic anhydrase activity [27] and CA IV is expressed in their apical membrane [23, and Breton, Sly and Brown: unpublished data].

Previous studies on the effect of acetazolamide on luminal acidification have resulted in conflicting results. *In vivo* microperfusion of the cauda epididymal duct showed a marked inhibition of the rate of luminal acidification by acetazolamide [28]. Subsequent work examining more proximal portions of the epididymis (caput, corpus and proximal cauda epididymis) showed no effect of acetazolamide on luminal pH [29]. More recently, we have shown a marked inhibition of net proton secretion by acetazolamide in isolated vas deferens [20]. Altogether, these results suggest a role for carbonic anhydrase in the luminal acidification of the distal segments of the male reproductive tract (cauda epididymis and vas

deferens), whereas the proximal segments (caput and corpus epididymis) may rely on different acidification mechanisms. Alternatively, the lack of effect of acetazolamide in the proximal regions of the epididymis, where principal cells express apical CA IV, might reflect a lower sensitivity of this isozyme for acetazolamide. The marked inhibition of proton secretion by acetazolamide in the vas deferens, together with the colocalization of CA II and H^+ATPase in clear cells, strongly indicate that cytosolic carbonic anhydrase plays a major role in luminal acidification in this segment. It, therefore, appears that CA II is involved in net proton secretion by clear cells in the distal portion of the epididymis and the vas deferens and that CA IV participates in bicarbonate reabsorption by principal cells of the proximal regions.

CA II-Deficient Mice and Acetazolamide-Treated Rats as Models for the Role of Carbonic Anhydrase

The physiological role for carbonic anhydrases has been illustrated by the study of various pathological conditions. Both kidney CA II and CA IV expression is upregulated during metabolic acidosis [30, 31]. CA II deficiency in humans is an autosomal recessive disease characterized by renal tubular acidosis, osteopetrosis, cerebral calcification, and growth retardation [32]. CA II-deficient mice were produced by an induced mutation that reproduced partially the human disease [33]. These mice presented a syndrome that includes renal tubular acidosis and growth retardation. We have shown that the kidneys of these mice were virtually devoid of medullary collecting duct intercalated cells and that only a few residual cortical collecting duct intercalated cells were present [34]. Intercalated cells are present in newborn CA II-deficient mice [35], indicating that at some point during post-natal development, intercalated cells are selectively deleted from the medullary collecting ducts and are replaced by principal cells. These results suggest a potential role for CA II in regulating cell-type diversity in kidney collecting ducts. We have tested this hypothesis by treating rats with the carbonic anhydrase inhibitor acetazolamide using osmotic mini-pumps [36]. We showed that a significant remodeling of the cellular profile of collecting ducts occurs in adult rats after two weeks of acetazolamide treatment. The percentage of type B intercalated cells significantly decreased whereas the percentage of type A intercalated cells (IC) increased, without any change in the number of total intercalated cells *vs.* principal cells. In the inner stripe of the outer medulla, the percentage of IC increased significantly and the percentage of principal cells (PC) decreased. Interestingly, the number of IC was significantly reduced in the proximal part of the inner medulla, a result that partially reproduced the CA II-deficient mouse model. These results suggest that carbonic anhydrase activity is important for the establishment of the differentiated phenotype of kidney collecting duct epithelial cells.

More recently, additional isoforms of carbonic anhydrase were suggested to participate in cell proliferation. Whereas CA IX and XII are expressed in some normal tissues, these isozymes are also present in tumors of several tissues in which they are not normally expressed [2, 9, 37]. CA IX is present in normal stomach [38] and in several human carcinomas [4]. It is also expressed in normal human gut, where it is restricted to the basolateral membrane of the epithelial cells that have the greatest proliferative activity, consistent with its proposed role in cell proliferation [37]. CA XII is expressed in both normal human kidney and in renal cancer cells [9], whereas CA IX is present in renal carcinomas only [39]. Ivanov and collaborators [40] proposed that plasma membrane carbonic anhydrases may be functionally involved in the invasion process. In favor of this hypothesis, Parkkila *et al.* showed that acetazolamide reduced the

invasion capacity of renal cancer cells by 18-74%, depending on the cell line studied [41].
In summary, while carbonic anhydrases participate in net acid/base transepithelial transport in various epithelia including renal and epididymal tubules, some isoforms, present in the basolateral membrane, might serve as regulators of cell proliferation. These isozymes might, therefore, be important players in the development of specific cell types during normal tissue maturation, as well as in the cascade of events responsible for the aberrant cell proliferation that takes place in cancer.

Key words Epididymis; Ion Transport; Kidney; Vas Deferens

Abbreviations AE: anion exchanger; CA: carbonic anhydrase; IC: intercalated cells; NHE: Na^+/H^+ exchanger; PC: principal cells

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