Role of Acid/Base Transporters in the Male Reproductive Tract and Potential Consequences of Their Malfunction

Acid/base transporters play a key role in establishing an acidic luminal environment for sperm maturation and storage in the male reproductive tract. Impairment of the acidification capacity of the epididymis, via either genetic mutations or exposure to environmental factors, may have profound consequences on male fertility.

Impairment of the acidification capacity of the epididymis, via either genetic mutations or exposure to environmental factors, may have profound consequences on male fertility. Of the large number of couples that are affected by infertility, ~30% of the problems result from a male fertility defect. Between 30 and 50% of these male infertility cases are idiopathic. Although a significant number of these cases are likely to have a genetic basis (90), environmental factors are also significant contributors (118). Several studies have shown a progressive decrease in sperm counts and semen quality over the last few decades, although this issue is the subject of considerable current debate (76, 90). In addition to an inability to produce an adequate number of spermatozoa, a major cause of male infertility is the production of sperm with reduced function, including low motility and poor interaction with the oocyte (41).

The generation of competent sperm is a complex process that includes the production of a large number of spermatozoa by the testis, followed by several maturation steps that occur in the efferent duct. The efferent duct of the male reproductive tract is composed of highly heterogeneous tissues, including the efferent ducts, epididymis, and vas deferens. Spermatozoa acquire their ability to become motile and to fertilize an egg in the lumen of the tubules that form the epididymis (68, 90). Therefore, failure of the epididymis to provide an adequate environment for sperm maturation and storage in the male reproductive tract can have profound effects on male fertility. One critical feature of the epididymal luminal fluid is that it is maintained acidic (84, 85). Low pH and HCO₃⁻ concentration help to keep spermatozoa in a quiescent, immotile state while they mature and are stored in the epididymis (1, 32, 57, 68). In this review, the expression and regulation of key players in acid/base transport in the epididymis will be described. The proximal epididymis is connected to the testis via the efferent ducts (FIGURE 1). In the rat, the epididymis is composed of one long convoluted tubule that is divided into several regions, including the initial segments and caput, located in the proximal epididymis (FIGURE 1), the corpus, and the cauda, which connects into the vas deferens.
B are masking the apical pole of narrow cells in this con-

The long stereociliae of principal cells stained for NHE3 identified by their positive immunoreactivity for the E

strong apical staining for NHE3 (green). Narrow cells are: principal cells in the distal initial segment show a

A exchanger AE2 in the epididymis

BCECF showed that EIPA-dependent Na+/H+ exchange accounts for ~50% of apical acid extru-

B, confocal image shows the absence of NHE3 from a narrow cell stained for the E subunit of the V-ATPase (V-ATPase; yellow).

The long stereociliae of principal cells stained for NHE3 are masking the apical pole of narrow cells in this con-

ventional microscopy image. B: confocal image shows the absence of NHE3 from a narrow cell stained for the

E subunit of the V-ATPase (red) and its presence in prin-

cipal cell stereociliae (green). C and D: all epithelial cells of the initial segment show intense basolateral staining for

NHE3-1-A (C) and AE2 (D).

The acidification of the luminal fluid (30, 84, 85, 136). This is accompanied by a significant

net water, Na+, Cl–, and HCO3– reabsorption, K+ secretion, and luminal acidification (4, 84, 85, 127).

Several lines of evidence indicate the occurrence of significant transepithelial acid-base transport by

the epididymal epithelium. The luminal concentra-

tion of HCO3– becomes significantly lower than that of blood as the efferent duct fluid transits through the proximal regions of the epididymis (85).

Luminal HCO3– concentration then remains low in the more distal parts of the epididymis and the vas deferens (85). This is accompanied by a significant acidification of the luminal fluid (30, 84, 85, 136).

**HCO3– Reabsorption in the Proximal Epididymis**

Previous studies have measured significant water reabsorption, coupled to Na+ and HCO3– reabsorp-

tion, in the efferent ducts and in the initial seg-

ments (28, 49, 85, 140). These tissues are related embryologically to the kidney, and they share simi-

lar transport mechanisms (58). In the kidney, the Na+/H+ exchanger NHE3 plays a major role in acid-

base balance and Na-fluid homeostasis (113). It is highly expressed in the apical membrane of kidney proximal tubules, where it is involved in Na3 and HCO3– reabsorption (62, 96, 135). In the male repro-
ductive tract, NHE3 is highly expressed in the api-
cal membrane of nonciliated cells of the efferent
ducts and principal cells of the epididymis and it is absent from narrow and clear cells (6, 72, 186) (**FIGURE 2, A AND B**). Interestingly, the level of expression of NHE3 varies in different regions of the rat epididymis (6). In the proximal parts, NHE3 is most abundant in the initial segment and in the proximal caput epididymidis. In the distal regions of the epididymis, NHE3 is expressed in the prox-
imal cauda only, being absent from the distal cauda and vas deferens. In addition, another Na+/H+ exchanger, NHE2, has been described in the apical membrane of principal cells from the caput, corpus, and cauda epididymidis but was shown to be absent from the initial segments (36).

Functional studies using epididymal tubules perfused in vitro and loaded with the intracellular pH indicator BCECF showed that EIPA-dependent Na+/H+ exchange accounts for ~50 of apical acid extru-
sion in the nominal absence of extracellular HCO3– (6). Because the initial segments were reported to be negative for NHE2 (36), NHE3 was the most like-
ly isoform to be involved in this region. However, EIPA894, used at a concentration that was expected to inhibit most NHE2 activity but not NHE3 (37), inhibited all of the EIPA-sensitive intracellular pH recovery. This unexpected result indicates that either 2) NHE2 is present in the initial segments but has so far remained undetected, 2) an NHE3 splice variant, sensitive to HOE694, is expressed in the epididymis; or 3) epidydal posttranslational modification confers HOE694 sensitivity to NHE3. The two latter possibilities were proposed to explain a similar unanticipated inhibition of NHE3 by HOE694 in the initial segment of the epididymis, while NHE2 is not present.

**FIGURE 2.** Immunofluorescence localization of the Na+/H+ exchanger NHE3, the Na+/HCO3– cotransporter NBCe1-A, and the Cl–/HCO3– exchange AE2 in the epididymis.

A: principal cells in the distal initial segment show a strong apical staining for NHE3 (green). Narrow cells are

identified by their positive immunoreactivity for the E

subunit of the V-ATPase (V-ATPase; yellow).

The long stereociliae of principal cells stained for NHE3 are masking the apical pole of narrow cells in this con-

ventional microscopy image. B: confocal image shows the absence of NHE3 from a narrow cell stained for the E subunit of the V-ATPase (red) and its presence in prin-
cipal cell stereociliae (green). C and D: all epithelial cells of the initial segment show intense basolateral staining for

NHE3-1-A (C) and AE2 (D).
by HOE694 in the main pancreatic duct (82). It is, therefore, conceivable that, as in the pancreatic duct, an HOE694-sensitive NHE3 is expressed in the initial segments of the epididymis. Additional experiments using NHE2, NHE3, and double-knockout mice will be required to answer these questions.

In addition, immunofluorescence studies showed that the electroneutral Na\(^+\)-HCO\(_3\) cotransporter NBCe1-A (originally called NBC) and the Cl:\HCO\(_3\) exchange transporter AE2 are expressed in the basolateral membrane of all epididymal cells, whereas AE1 is not present in this tissue (65, 66) (FIGURE 2, C AND D). The levels of expression of NBCe1-A and AE2 were higher in the proximal regions of the epididymis, where a low luminal concentration of HCO\(_3\) is established (84, 85), indicating their potential role in HCO\(_3\) reabsorption. Epididymal principal cells also show strong cytosolic carbonic anhydrase (CA) activity, especially in the proximal regions (40) and CA-I is expressed in their apical and basolateral membranes (73, 101) (see also FIGURE 3). More recently, additional CA isoforms have been identified in kidney and epididymal epithelial cells. In the kidney, CA-XII is present in the basolateral membrane of epithelial cells of the thick ascending limb of Henle, distal convoluted tubule, and collecting duct (78, 102), and membrane-bound CA-XIV message has been found in proximal tubules (74, 92). In human epididymis, CA-XII colocalizes with CA-II in apical mitochondria-rich cells (which correspond to rat narrow and clear cells) (71). More recently, a complex pattern of CA expression was reported in the rat epididymis (54). CA-XII was located in the basolateral membrane of narrow cells of initial segments and in principal cells of the corpus and cauda epididymis, and CA-XIV was located apically and basolaterally in principal cells.

In summary, principal cells of the proximal regions of the epididymis are fully equipped for net HCO\(_3\) reabsorption and use transport mechanisms that are very similar to those used by the kidney proximal tubule (48). In the initial segments of the epididymis, the apical Na\(^+\)/H\(^+\) exchanger NHE3 or an “NHE3-like” exchanger secretes protons into the lumen (FIGURE 4). These H\(^+\) ions combine with luminal HCO\(_3\) to form CO\(_2\) and H\(_2\)O under the enzymatic activity of CA-IV and CA-XIV. Newly formed CO\(_2\) then diffuses into the cell through the apical membrane and is hydrated by cytosolic CA-II to form H\(^+\) and HCO\(_3\). The protons recycle back into the lumen via NHE3, whereas HCO\(_3\) would diffuse passively across the basolateral membrane via the Na\(^+\)/HCO\(_3\) cotransporter NBCe1-A or the anion exchange transporter AE2. The result of these processes is net HCO\(_3\) reabsorption with no net proton secretion. Basolateral CAs were proposed to facilitate Na\(^+\)-HCO\(_3\) cotransport by preventing the development of an alkaline disequilibrium pH in the interstitium (116, 128).

**Net Proton Secretion in the Distal Epididymis: Role of the Vacular H\(^+\)-ATPase**

The luminal fluid of the distal epididymis is maintained at the acidic pH of 6.8 (84, 85), indicating that active proton secretion takes place in this segment. Previous studies have shown that: 1) narrow and clear cells express high levels of the vacuolar H\(^+\)-ATPase (V-ATPase) in their luminal plasma membrane, as well as in intracellular vesicles (14, 22, 25, 50, 53, 104, 120); 2) CA-II is highly expressed in narrow and clear cells in the epididymis and vas deferens (11, 14, 15) (FIGURE 5); and 3) the bulk of proton secretion in the vas deferens is inhibited by the specific V-ATPase inhibitors bafilomycin and concanamycin (14). Coexpression of the V-ATPase and CA-II in the same subpopulation of cells indicated the participation of this CA in net proton secretion. It has to be noted that an absence of CA-II in clear cells has been reported previously (54). Whether this apparent discrepancy is attributed to different fixation protocols (a fixation solution containing paraformaldehyde, lysine, and periodate (PLP) gave positive results, whereas Bouin’s or St-Marie’s fixatives gave negative results) or different primary antibodies (an antibody provided by William S. Sly, St. Louis University Medical Center and School of Medicine, gave positive results, whereas Boutin’s or St-Marie’s fixatives gave negative results) still remains to be elucidated. Au and Wong have shown a marked reduction in the rate of luminal
AE2. Basolateral CA-IV is proposed to facilitate HCO₃⁻ transport. In primary cultures of cauda epididymis (83). In the isolated vas deferens, the rate of net proton secretion is independent of Cl⁻ but is strongly inhibited by SITS under normal and Cl⁻-free conditions (11). Low expression of AE2 and NBCe1-A was detected in the basolateral membrane of epithelial cells lining the cauda epididymis and vas deferens (65, 66). However, because net proton secretion is independent of Cl⁻ in this segment (11), AE2 does not appear to be involved, and NBCe1-A is the most likely candidate for basolateral HCO₃⁻ transport.

Thus net luminal acidification in the cauda epididymis and vas deferens is achieved by the V-ATPase, which works in conjunction with basolateral HCO₃⁻ transporters to couple proton secretion and HCO₃⁻ reabsorption. The V-ATPase is a complex enzyme that is composed of several subunits (20, 65, 93, 118, 129). It is divided into two distinct sectors, the V₀ and V₁ sectors. The V₀ sector is composed of transmembrane subunits (subunits a, b, and c) as well as subunit d, which is closely associated with the membrane. The V₁ sector forms a unique clathrin-free ribosome-binding site (46). A family of soluble n-ethylmaleimide-sensitive factor attachment protein receptors (SNARPs) is implicated in the regulation of CA-II targeting to the AP-1 clathrin-coated vesicles (18, 81, 89, 101). The SNARPs are also negatively regulated by SNAREs. Brown, uncoupling in primary cultures of clear cells resulted in the loss of the unique clathrin-free AP-1 coating sites and the formation of an extended cytoplasmic coat, which is associated with targeting of the V-ATPase to the endosome.

**FIGURE 4.** Model for HCO₃⁻ reabsorption in the initial segments of the epididymis

Protons are transported out of the cell via apical Na⁺/H⁺ exchanger NHE3. The driving force for proton extrusion is provided by basolateral Na⁺/K⁺-ATPase, which maintains a low intracellular Na⁺ concentration. K⁺ is recycled back into the cell by basolateral Na⁺-K⁺-ATPase. The driving force for proton extrusion is facilitated by the basolateral Na⁺-HCO₃⁻ co-transporter, a reaction that is catalyzed by cytosolic CA-II. Newly formed HCO₃⁻ is transported through the basolateral membrane by facilitating HCO₃⁻ dissociation in the interstitium.

**FIGURE 5.** Immunofluorescence localization of V-ATPase (E subunit) and CA-II in a cryostat section of the cauda epididymis

Clear cells show strong apical staining for V-ATPase (yellow) and intense cytoplasmic staining for CA-II (red). No CA-II or V-ATPase staining is detected in adjacent principal cells.
cytosolic complex of eight subunits (subunits A-H) and is associated with the membrane via its interaction with the V$_2$ sector. Several subunits of the V$_1$ and V$_2$ sectors exist in more than one isoform. The exact function of each of these isoforms is the subject of intensive research. These isoforms are expressed by distinct cell types and are located in different compartments of the cells, and it has been proposed that the assembly of a particular set of subunit isoforms may determine the intracellular targeting of the V-ATPase (intracellular organelles vs. plasma membrane) and may control its function.

**Regulation of Proton Secrecion via Recycling of V-ATPase**

Similarly to renal type A intercalated cells, α-CARich cells of the turtle bladder, and osteoclasts, proton secretion in clear cells is regulated via recycling of V-ATPase-containing vesicles to and from the apical membrane (8, 13, 103). This active recycling is reflected by a very high rate of endocytosis (2, 18, 19, 24, 26, 55, 89, 91, 114, 121). In these cells, an increase in V-ATPase surface expression closely correlates with an increase in proton secretion (8, 18, 81, 103, 121). The molecular mechanisms responsible for the regulation of V-ATPase recycling are still poorly characterized. In renal intercalated cells, V-ATPase-containing vesicles possess an extensive cytoplasmic coat consisting largely of V-ATPase subunits (19). Previous studies have shown that these vesicles are devoid of clathrin (21, 23) and are still poorly characterized. In renal intercalated cells, V-ATPase-labeled microvilli were detected (46). In addition, the B1 subunit of the V-ATPase is present in aquaporin 2 (AQP2)-containing endosomes isolated from kidney principal cells (111). Because these endosomes do not contain other subunits of the V-ATPase and do not acclify their lumen, it is possible that the B1 subunit has a function independent of proton-pumping activity. Therefore, some subunits of the V-ATPase may be involved, in a novel way, in the recycling and targeting machinery underlying the delivery of the V-ATPase itself, as well as some other membrane proteins, to their target membrane. Previous reports have shown interaction between the V-ATPase and members of the SNARE family, including syntaxin 1A and SNAP23 in inner medullary collecting duct cultured cells (7, 86, 94). In the epididymis, we have shown that cellubrevin, a vesicle-associated SNARE protein (v-SNARE), also known as B-SNARE (35), is highly expressed in clear cells of the epididymis and vas deferens that cleavage of cellubrevin by tetanus toxin markedly inhibited bafilomycin-dependent proton secretion in isolated vas deferens (13). Because tetanus toxin did not directly inhibit the V-ATPase pumping activity in endosomes isolated from rat kidney cortex, we proposed that inhibition of proton secretion by tetanus toxin resulted from a decrease in the number of membrane-inserted V-ATPase molecules, due to an impairment of the endocytic process. SNAP23 is also expressed in epididymal clear cells, where it partially colocalizes with the V-ATPase (39). However, we did not detect syntaxin 1A mRNA or protein in epithelial cells of the epididymis, and the target membrane T-SNARE that would interact with the V-ATPase in the male reproductive tract is still unknown.

**HCO$_3$-regulated soluble adenylyl cyclase modulates V-ATPase recycling**

Soluble adenylyl cyclase (sAC) is a chemo sensor that mediates HCO$_3$-dependent elevation of cAMP (34). It is distinct from transmembrane adenylyl cyclases and is directly stimulated by HCO$_3$ ions. We have shown that sAC is highly expressed in clear cells of the epididymis (103). V-ATPase recycling in epididymal clear cells is strongly dependent on luminal pH or luminal HCO$_3$ concentration. In vas deferens perfused in vivo with a physiological solution containing HCO$_3$ and adjusted to pH 7.1, clear cells exhibited numerous, well-developed V-ATPase-labeled microvilli. Under these conditions, very little endocytosis of the V-ATPase was detected. In contrast, when vas deferens was perfused in a HCO$_3$-free solution kept at the same pH value, a larger portion of V-ATPase molecules were present in subapical endosomes and shorter and fewer V-ATPase-labeled microvilli were detected (46). A similar response was observed when the vas deferens was perfused at very alkaline pH values (pH 7.8) in the absence of HCO$_3$ (103). The sAC inhibitor 2-hydroxyestradiol
A: In the presence of luminal HCO₃⁻, the V-ATPase is mainly located in well-developed apical microvilli. B: In the absence of HCO₃⁻, the V-ATPase is distributed between apical microvilli and subapical vesicles. The arrows indicate the frontier between apical microvilli and the subapical region of the cell.

FIGURE 6. Effect of luminal HCO₃⁻ on V-ATPase localization in clear cells. Confocal images showing clear cells from cauda epididymis perfused luminally in vitro with modified Hanks buffer containing 12 mM HCO₃⁻ (pH 7.1, 5% CO₂/VA) or PBS adjusted to pH 7.1 (B). Endocytosis was detected by adding horseradish peroxidase (HRP) into the lumen solutions. Double staining for V-ATPase (green) and HRP (red) was performed. A: In the presence of luminal HCO₃⁻, the V-ATPase is mainly located in well-developed apical microvilli. B: In the absence of HCO₃⁻, the V-ATPase is distributed between apical microvilli and subapical vesicles. The arrows indicate the frontier between apical microvilli and the subapical region of the cell.

FIGURE 7. Modulation of the actin cytoskeleton via gelsolin participates in the regulation of V-ATPase recycling. A: In the presence of luminal HCO₃⁻, the V-ATPase is mainly located in well-developed apical microvilli. B: In the absence of HCO₃⁻, the V-ATPase is distributed between apical microvilli and subapical vesicles. The arrows indicate the frontier between apical microvilli and the subapical region of the cell.
Several membrane transporters (105, 107, 117, 118, 119) are currently investigated the possibility that the actin skeleton and/or the V-ATPase are involved in the trafficking of the V-ATPase still remains to be elucidated. However, modulation of the actin skeleton by cAMP might exert its action via both an increase in V-ATPase exocytosis and a decrease in V-ATPase endocytosis (103). Thus cAMP might exert its action via both an increase in V-ATPase exocytosis and a decrease in V-ATPase endocytosis, would induce the apical accumulation of V-ATPase in epididymal clear cells. We have also shown that cAMP can induce V-ATPase apical membrane accumulation of cell types that express the V-ATPase in their plasma membrane and recycling vesicles, including kidney intercalated cells, osteoclasts, and spermatogenesis (10, 27, 88). Therefore, modulation of the actin cytoskeleton by this severing and capping protein may represent a common regulatory mechanism for proton secretion in these cells.

Summary

The mechanisms responsible for the apical membrane accumulation of V-ATPase in response to various stimuli are still not fully characterized. Whereas some recycling membrane proteins, such as AQP2 and CFTR, are directly phosphorylated by PKA (9, 17), none of the subunits of the V-ATPase are phosphorylated by PKA (122, 129). This indicates that an indirect mechanism might be involved in the cAMP-induced apical accumulation of the pump. Modulation of the actin cytoskeleton by cAMP is a key step in the regulation of several membrane transporters (105, 107, 117, 123, 124, 134). It is, therefore, conceivable that depolymerization of the actin cytoskeleton by a cAMP-dependent mechanism, leading to inhibition of V-ATPase endocytosis, would induce the apical accumulation of V-ATPase in epididymal clear cells. Whether cAMP acts via modulation of the actin cytoskeleton and/or via PKA phosphorylation of an intermediate protein involved in the trafficking of the V-ATPase still remains to be elucidated.

Impairment of Acidification and Consequences for Male Fertility

Mutations of V-ATPase subunits

As mentioned above, the V-ATPase is a very complex enzyme that is composed of many distinct subunits. Two of its subunits, B and C, are of particular interest because mutations of one of their isoforms in humans lead to several disease states. The B subunit is part of the V1 sector and has four isoforms, a1, a2, a3, and a4. Mutations of the Atp6v1b1 gene coding for the B1 subunit and of the Atp6v0a4 gene coding for the a4 subunit cause recessive distal renal tubular acidosis,
due to impairment of proton secretion by collecting duct intercalated cells (129). The exact mechanisms by which these mutations cause a dysfunction of the V-ATPase remain to be elucidated. For example, it is not known whether the mutated B1 and a4 subunits can assemble in the V-ATPase complex and, if so, whether they may affect its targeting to the apical membrane. Interestingly, whereas the B1 subunit is expressed in kidney intercalated cells and is absent from proximal tubules, the a4 subunit is present in both cell types. However, no apparent defect in proximal tubule acid/base transport has been detected in patients harboring mutations of the a4 subunit. Similarly, the presence of other subunits (a1, a2, or a3) might compensate for the lack of functional a4 subunit in the proximal tubule or in the inner ear.

Both B1 and a4 subunits have been described in the male reproductive tract, where they are highly expressed in the apical plasma membrane of epididymal narrow and clear cells (14, 22, 120). Because luminal acidification is achieved in part by the V-ATPase, it is possible that fertility might be altered in patients harboring B1 and a4 subunit mutations. Several such patients were diagnosed recently, and they were all younger than 6 years old, with the majority presenting before the first year of age. Long-term clinical follow-up of these young patients will determine whether or not their fertility will be impaired.

**V-ATPase B1 subunit-deficient mice**

To better characterize the role of the B1 isoform in the function of the V-ATPase, mice deficient in the murine V-ATPase B1 subunit homolog (Atp6v1b1−/− mice) were generated (44). Interestingly, in contrast to human patients with B1 mutations, Atp6v1b1−/− mice appeared normal, as long as they were fed a normal diet (44). However, their urine was significantly more alkaline, and when challenged with an oral acid load, they developed a more pronounced metabolic acidosis compared with their wild-type littermates. These findings indicate that although some V-ATPase function is retained in the absence of B1, the pump cannot increase its activity after an acid challenge. Expression of the B2 subunit, together with the B1 subunit, has recently been described in renal intercalated cells (104). Under baseline conditions, the B2 isoform was detected mainly in intracellular vesicles, but under some conditions, including CA inhibition, it was also detected in the apical membrane of proton-secreting type A intercalated cells. These results indicate that the B2 isoform can compensate, at least partially, for the lack of B1 in the function of the V-ATPase. Interestingly, Atp6v1b1−/− mice were reported to be fertile. In the male reproductive tract, B2 is also expressed in clear cells together with B1, but in normal mice and rats, it is located mainly in subapical vesicles and is absent from apical microvilli (104), where other subunits of the V-ATPase predominate. However, we have recently detected a significant redistribution of the B2 subunit from intracellular vesicles to the apical plasma membrane in clear cells of Atp6v1b1−/− mice compared with wild-type mice (29). In this way, the B2 subunit could partially compensate for the absence of B1 and help maintain the epididymal luminal pH within the acid range compatible with fertility (Da Silva N, Paunescu TG, Brown D, and Breton S, unpublished observation). Thus the B2 isoform may serve as a potential backup for the active role played by the B1 subunit in luminal acidification of the epididymis and the kidney.

It will be interesting to determine whether the presence of mutated B1 in human patients might prevent the assembly of B2 in the V-ATPase holoenzyme, which would result in a more complete inhibition of V-ATPase function. In Atp6v1b1−/− mice, the absence of B1 (or the presence of a significantly truncated and dysfunctional B1 protein) might allow for the incorporation of the B2 isoform into the holoenzyme, which might preserve a better V-ATPase function.

**Cadmium inhibits the V-ATPase**

Several studies conducted in different populations of the world have suggested a significant decrease in male fertility over the past 50 years (76, 118). Although such a decline is still a matter of debate and might be attributed to methodological factors and regional differences, there is some evidence that environmental substances, including estrogenic and antianimal agents, fungicides, pesticides, and heavy metals, have a detrimental effect on male fertility (5, 56, 67, 69, 75, 125). Exposure to environmental pollutants, including cadmium, is known to affect luminal acidification in the epididymis (29, 52) and induce a reduction in male fertility (108, 125). The heavy metal cadmium is of particular interest in male fertility because it is present in tobacco smoke and food, and it accumulates in the environment due to human contamination by mining, smelting, and use in industry. Cadmium exposure induces a reduction in the size of testis, epididymis, and seminal vesicles (29, 79, 80), a decrease in sperm concentration (79, 80, 99, 112), and a decrease in plasma testosterone concentration (42, 43). Interestingly, a significant alkalization of the luminal fluid of the epididymis was observed in adult male rats treated with cadmium (29). In addition, treatment of rats with cadmium for 2 wk induced a significant decrease in the epididymal sperm count and a marked decrease in luminal fluid pH (31, 106). Cadmium also inhibits the V-ATPase at the plasma membrane of spermatozoa (139). Cadmium also reduces the expression of the V-ATPase and decreases its activity in epididymal spermatozoa (31, 106). These findings indicate that cadmium might be responsible for the lack of functional B1 in the function of the V-ATPase remaining in spermatozoa. Cadmium also inhibits the V-ATPase in epididymal spermatozoa from cadmium-exposed animals (31, 106). In summary, both the epididymal sperm and the epididymal cells appear to play an important role in male fertility. NHE3 is abundant in the epididymal epithelium, where few V-ATPase-positive proton transporters are present. In contrast, the epididymal sperm head contains a significant number of V-ATPase-positive vesicles. Despite this, the epididymal sperm are highly sensitive to CA inhibition, which suggests that the epididymal sperm may use NHE3 as a proton transporter. Further studies need to be performed to determine whether NHE3 is responsible for the pH decrease observed in epididymal spermatozoa.
for 2 wk induced a regression of the morphology of the epididymis to a prepubertal or castrated phenotype and a marked redistribution of the V-ATPase from the apical pole of clear cells into intracellular vesicles scattered throughout the cytoplasm (52). Cadmium also directly inhibited basolateral ATPase activity in preparations of epididymal plasma membranes and inhibited basolateral-sensitive proton secretion in isolated, cut-open vas deferens, indicating a direct inhibition of the V-ATPase by this heavy metal (52). Thus cadmium may act as a potential causative factor for several environmental and occupational pollutants that have been implicated in the reduction of fertility in men and animals. The cadmium-induced internalization of the V-ATPase in clear cells may be at least partially responsible for the luminal alkalinization that occurs after cadmium intoxication (29). In kidney proximal tubules, cadmium-metallothionein induces a depolymerization of microtubules, leading to the internalization of a variety of apical membrane proteins, including the V-ATPase (110). Interestingly, a significant increase in blood cadmium was observed in smokers (70), a condition known to significantly reduce male fertility (77, 139). The reduction in male fertility in smokers was associated in part with a reduction in sperm quality and motility. Whereas the exact component of the cigarette smoke responsible for this adverse effect is still not known, it is tempting to propose that cadmium, by altering the luminal pH of the epididymis, may contribute to at least partially to this defect. In this respect, it is important to note that compared with several parts of the reproductive tract, the epididymis, together with the kidney, are among the organs that accumulate cadmium most efficiently (97).

Conclusion

In summary, transepithelial acid/base transport in the epididymis is achieved by distinct sets of transport proteins located in principal cells and clear cells. In the proximal epididymis, the Na+/H+ exchanger NHE3 or an “NHE3-like” transporter appears to be involved in HCO3− reabsorption. NHE3 is abundantly expressed in the apical membrane of principal cells in the initial segments, where few V-ATPase-rich narrow cells are present. In contrast, NHE3 is not detectable in the distal cauda epididymidis, where V-ATPase-rich clear cells are numerous. In the distal cauda epididymidis, where spermatozoa are stored, clear cells are poised to play a central role in the final steps of luminal acidification. Another Na+/H+ exchanger, NHE2, is also present in the apical membrane of principal cells and may participate in luminal acidification (36). These apical proton-secreting proteins work in conjunction with cytosolic CA-II, membrane-bound CA-IV and CA-XIV, and basolateral HCO3− transporters, including the electrogenic Na+/HCO3− cotransporter NBCe1-A and the Cl−/HCO3− exchanger AE2, to secrete protons and reabsorb HCO3−.

Net proton secretion by clear cells is modulated via active endocytosis and exocytosis of V-ATPase-containing vesicles. This recycling mechanism represents a possibly unique clathrin- and caveolin-independent process. V-ATPase recycling depends on an intact microtubule network, is highly regulated via modulation of the actin cytoskeleton, and requires the participation of members of the SNARE protein family. In addition, accumulation of V-ATPase in the apical membrane is induced by either an increase in luminal pH or HCO3− concentration, following a sAC-dependent elevation of cAMP. Ongoing studies in our laboratory are aimed at examining the downstream effectors of cAMP in regulating V-ATPase recycling, as well as other potential extracellular stimuli that may regulate proton secretion in the epididymis. A protein that is central to establishing a suitable acidic luminal environment in which sperm mature and are stored in a quiescent state.

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References


9. Bertrand CA and Frizzell RA. The role of regulat-

19. Brown D and Breton S. Mitochondria-rich, proton-

18. Brown D and Breton S. H(+)V-ATPase-dependent

17. Brown D. The ins and outs of aquaporin-2 traf-

14. Breton S, Smith PJS, Lui B, and Brown D. 

2. Al-Awqati Q. Plasticity in epithelial polarity of

5. Auger J, Eustache F , Andersen AG, Irvine DS,

8. Beaulieu V, Da Silva N, Pastor-Soler N, Brown CR,

16. Breton S, Wiederhold T, Marshansky V, Nsumu

1. Al-Awqati Q. Plasticity in epithelial polarity of

10. Balskus EP, Ohlson P. New insights into the roles of


11. Breton S, Gluck S, and Hartwig J. Structure of the


15. Breton S, Smith P, and Brown D. Mitochondria-rich,

18. Brown D and Breton S. H(+)V-ATPase-dependent

17. Brown D. The ins and outs of aquaporin-2 traf-

16. Breton S, Wiederhold T, Marshansky V, Nsumu

1. Al-Awqati Q. Plasticity in epithelial polarity of

10. Balskus EP, Ohlson P. New insights into the roles of


11. Breton S, Gluck S, and Hartwig J. Structure of the


15. Breton S, Smith P, and Brown D. Mitochondria-rich,

18. Brown D and Breton S. H(+)V-ATPase-dependent

17. Brown D. The ins and outs of aquaporin-2 traf-

16. Breton S, Wiederhold T, Marshansky V, Nsumu

1. Al-Awqati Q. Plasticity in epithelial polarity of

10. Balskus EP, Ohlson P. New insights into the roles of


11. Breton S, Gluck S, and Hartwig J. Structure of the


15. Breton S, Smith P, and Brown D. Mitochondria-rich,


124 Smira GM, Klussmann E, Procino G, Svelto M, Tas S, Lauwerys R, and Lison D. Occupational haz-

125 spel on kidney collecting duct principal cells and increases from synthes

126 Soderlund A, Gluck S, and Brown D. Apical endosomes iso-

127 Soderlund A, Gluck S, and Brown D. Apical endosomes iso-


