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INVITED REVIEW

Role of Na^+/H^+ exchanger 3 in the acidification of the male reproductive tract and male fertility

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SUMMARY

1. Male fertility is a complex process that is dependent on sex hormones and the normal function of the reproductive organs. Defects of these organs result in abnormal sperm production and function, which, in turn, lead to infertility.

2. Spermatozoa released from the testis are unable to move and fertilize with eggs. These features, known as sperm maturation, are acquired during their transit through the epididymis.

3. Among several processes that take place in the epididymis, absorption and acidification of the luminal fluid are essential for sperm maturation, sperm storage and fertility. Currently, the mechanism by which acidification occurs in the epididymis is still not fully understood.

4. The epididymis is fully equipped with the proteins required for acid/base transport, such as Na^+/H^+ exchanger 3 (NHE3, SLC9A3), vacuolar-type adenosine triphosphatase (V-ATPase) and various isoforms of enzyme carbonic anhydrase (CA).

5. Most studies, so far, have focused on the role of V-ATPase on H^+ secretion and acidification of the epididymis. The involvement of NHE3 in creating the acidic environment of the epididymal spermatozoa receives little attention.

6. This review presents evidence for and discusses the role of NHE3 in the acidification of the male reproductive tract and its requirement for male fertility.

Key words: acidification, male fertility, male reproductive tract, Na^+/H^+ exchanger.

INTRODUCTION

Approximately 15% of couples of reproductive age are infertile. Of these, up to 20% is attributed to male infertility and 30–40% is attributed to infertility of both partners.[1,2] Male infertility is defined by abnormalities of the semen, which include low sperm number, abnormal sperm morphology and low sperm motility. Among these parameters, sperm motility and concentration provide more accurate indices for male infertility.[3] It is now well documented that spermatozoa released from the seminiferous tubules, even after completion of normal spermatogenesis, are functionally immature. They acquire the ability to move and to fertilize with eggs, the process known as sperm maturation, during their transit through the epididymis.[4,5] Studies in several animal species, including humans, show that fertilizing capacity of spermatozoa begins in the proximal to mid-region of the caput epididymidis, and that spermatozoa gain their full fertilizing capacity in the cauda epididymidis.[5,6] Development of the fertilizing capacity of spermatozoa depends on their exposure to the special environment created by the epididymis.[5,6] Therefore, failure to provide an adequate milieu for sperm maturation and storage would have a profound negative effect on male fertility. Several components of the epididymal fluid, such as ionic concentration, pH, osmolality, proteins and lipids, appear to play a role on this phenomenon.[4,5,7] In the present review, we will focus on the role of luminal pH on sperm maturation and male fertility. The transport function of the epididymis is usually considered to mirror that in the renal tubule, the tissue that shares the same embryonic precursor.[8] Acidification of the epididymal fluid, therefore, depends on the activities of Na^+/H^+ exchangers (NHE) and/or vacuolar H^+-ATPase (V-ATPase). The role of the latter (V-ATPase) has been extensively reviewed,[9–12] whereas the role played by Na^+/H^+ exchangers receives little attention or is considered to mainly involve fluid[13] or HCO_3^- absorption.[10] The present review attempts to bring up available evidence for the acid/base function of Na^+/H^+ exchanger isoform 3 (NHE3, SLC9A3) in the epididymis and its impact on male fertility. The review consists of four parts: (i) the role of the acidic environment in the epididymal lumen on sperm maturation and storage; (ii) the distribution of acid/base transporters that are possibly involved in luminal acidification in the epididymis of rats, mice and humans; (iii) the evidence supporting the role of NHE3 in luminal acidification and male fertility; and (iv) a proposed model for luminal acidification by NHE3 in the epididymis compared with the acid/base transport in the proximal tubule.

LUMINAL PH IN VARIOUS REGIONS OF THE EPIDIDYMIS AND SPERM MATURATION

By using the micropuncture technique and pH microelectrodes, Levine and colleagues[14,15] were the first to show that the rat

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epididymis secretes H^+ and absorbs HCO_3^-, resulting in acidic luminal pH along its length from the proximal to distal segments. In fact, pH in the caput is lower than that of the rat cauda epididymidis.[14] This finding was later confirmed by other groups investigating pH in the epididymis of rats,[16] mice[17] and boars.[18] The role of acidic pH of the luminal fluid on sperm maturation is not fully known; however, it is believed that acidic pH might play a role in suppressing sperm motility[19–21] and sperm metabolism during their transit through and storage in the epididymis.[22] Changes in sperm surface by additions, deletions and modifications of membrane proteins have been shown to occur during epididymal transit.[23–27] These processes require enzyme reactions, which are pH dependent. For instance, adsorption of prostasomes (multilamella lipoprotein membrane particles) in the epididymal fluid to the bovine caput sperm surfaces needs an optimal pH of 6.0–6.5, suggesting that the acidic environment might be essential for sperm maturation.[28,29] In addition, β-D-galactosidase, which is required for glycosylation of sperm surface glycoproteins, is functionally active between 5.6 and 6.8 pH.[30] All together, these findings suggest that acidification of the epididymal fluid is essential for alterations of the sperm surface proteins, which is a feature of sperm maturation. Furthermore, there is strong evidence that sperm motility is suppressed by acidic pH[19–21]. Sperm storage is, therefore, favoured under this condition.

ACID/BASE TRANSPORTERS IN THE EPIDIDYMIS

Because of the similarity in embryonic origin between the epididymis and the kidney,[8] several mechanisms by which the epididymis secretes acid, such as proton secretion and bicarbonate reabsorption, both of which account for luminal acidification, are conferred from those of the renal tubules. Numerous acid/base transporters have been identified to be localized in the male reproductive tract of rats,[31–36] mice[13,17,37,38] and humans.[39,40] Expressions of these proteins in different segments of the male reproductive tract are summarized in Table 1.

Proton pump (H^+-ATPase)

It is well established that, in the rat epididymis, the vacuolar H^+-ATPase (V-ATPase) is highly expressed in the apical membrane of nonciliated cells of the efferent ducts,[41] in the apical/narrow cells of the initial segment and the caput,[17,41,42] and in the clear cells, but not in the principal cells, of the corpus and cauda epididymidis.[31,33,41] Expression of V-ATPase in the mouse epididymis has a similar pattern to that of the rat, except that the mouse efferent ducts lack this protein.[42] In human epididymis, V-ATPase is known to express in the narrow cells and the apical mitochondria-rich cells.[40] Narrow cells are located exclusively in the initial segment, whereas clear cells are found in all regions of the epididymis, except in the initial segment.[5] Clear cells, which are believed to be analogous to the intercalated cells of the kidney, are ascribed as acid-secreting cells and are responsible for acidification of the luminal fluid.[9] The role of these cells and V-ATPase on the generation of acidic pH of the epididymal fluid has been extensively reviewed.[9–12]

Na^+/H^+ exchangers

Na^+/H^+ exchangers (NHE) are cell membrane proteins that transport H^+ in exchange with Na^+ in a stoichiometry of one-to-one, resulting in secretion of H^+ and absorption of Na^+. This type of transporter is ubiquitously present in every cell and is responsible for intracellular pH regulation, cell volume homeostasis and absorption of NaCl in epithelial tissues.[43] Currently, nine isoforms of Na^+/H^+ exchanger (NHE1-NHE9) have been identified in mammalian tissues.[44] Just three isoforms of Na^+/H^+ exchangers (NHE1, NHE2, NHE3) have been identified in the male reproductive tract. So far, there are few studies on NHE1 and NHE2 in the epididymis, perhaps as a result of the potential role of these NHE isoforms on sperm maturation and male fertility not being clear. By contrast, the role of NHE3 has been extensively studied in many species. This NHE isoform has been implicated in Na^+ and fluid absorption in the efferent ducts and infertility.[13]

NHE1

NHE1 was the first Na^+/H^+ exchanger protein cloned from human genomic DNA[45] and its mRNA can be found in almost all mammalian cells.[46,47] It is, therefore, considered a 'house keeper', which has an important role in intracellular pH homeostasis, cell volume regulation and probably in cell proliferation.[43] In the rat, NHE1 is present in the testis,[46] the basolateral membrane of the efferent ducts and in all segments of the epididymis.[48]

NHE2

The expression of NHE2 mRNA is more restricted compared with that of NHE1. NHE2 mRNA is found in the intestine, kidney, adrenal gland, and, to a lesser extent, the trachea and skeletal muscle.[49] NHE2 protein is known to express on the apical membrane of the epithelial cells in all regions of the rat epididymis, except in the initial segment.[48] Although the physiological role of NHE2 is not well defined, it has been suggested that this NHE isoform is involved in volume regulation of the inner medullary collecting duct cells[50] and in Na^+ reabsorption of the epididymis.[48]

NHE3

In the rat, NHE3 mRNA was found in the kidney, stomach and intestine, but not in the testis.[46,47] Although expression of NHE3 protein was detected on the apical surface of the epididymal epithelium of both rats[33,35] and humans,[39,40] localization of this protein in a specific region of the rat epididymis is still controversial. Using reverse transcription polymerase chain reaction and immunohistochemistry, Kaunisto *et al.*,[35,51] Leung *et al.*[36] and our group (unpublished observation) found that NHE3 was expressed in the efferent ducts and in all regions of the rat epididymis. In addition, the intensity of expression increases from the caput towards the cauda epididymidis.[35] In contrast, the other groups showed that this protein was highly expressed in the initial segment[34] and the caput,[33] and the level of expression decreased towards the proximal cauda, whereas virtually no expression was detected in the distal cauda epididymidis of rats.[33,34] The discrepancy might, in part, be a result of differences in the technique of tissue fixation or the specificity of the antibodies used for immunostaining, which were either polyclonal[35] or monoclonal[33,34] antibodies against rat[35] or rabbit[33,34] NHE3. It is also conceivable that a splice variant of NHE3 might exist. Hence, ethylisopropyl amiloride (EIPA, an inhibitor of NHE3) or HOE694 (an inhibitor of NHE2) partially blocked luminal acidification of the

Table 1 Distributions of proteins involved in luminal acidification of the male reproductive tract epithelia in rats, mice and humans

Reproductive tract	Rats		Mice		Humans	
	Proteins	References	Proteins	References	Proteins	References
Testis	NHE1	46	No CA IV	37	No CA II	60
Efferent ducts	NHE1	36	NHE3	13	NHE3	39
	NHE2	36			NHERF1	40
	NHE3	34–36			CFTR	39
	CA II, CA III, CA XII, CA XIV	59			SLC26A2	40
	H^+-ATPase	41			SLC26A6	40
					CA IX, CA XII	61
Initial segment	NHE1	48	NHE3	17	H^+-ATPase	40
	NHE3	35	H^+-ATPase	42		
	CA II, CA III, CA XII, CA XIV	59	NBC1	17		
	H^+-ATPase	41	CA II, CA XIV	37		
	AE2	54	AE2	54		
Caput epididymis	NHE1	48	H^+-ATPase	42	NHERF1	40
	NHE2	48	CA II, CA XIV	37	H^+-ATPase	40
	NHE3	33–35	AE2	54	CFTR	39,40
	H^+-ATPase	32–34,41,62			CA II, CA XII	61
	NBC1	53,54				
	NBC3	33				
	CA II, CA III, CA XII, CA XIV	59,62				
	AE2	54				
Corpus epididymis	NHE1	48	H^+-ATPase	42	NHERF1	40
	NHE2	48	CA II, CA IV	37	H^+-ATPase	40
	NHE3	33–35			CFTR	39,40
	H^+-ATPase	32–34,41,62			CA II	40,60
	NBC1	53,54				
	NBC3	33				
	CA II, CA III, CA XII, CA XIV	59,62				
Cauda epididymis	NHE1	48	H^+-ATPase	38,42	H^+-ATPase	40
	NHE2	48	CA IV	37	CFTR	39,40
	NHE3	34,35	AE2	54	CA II	40,60
	H^+-ATPase	32–34,41,62				
	NBC1	53,54				
	NBC3	33				
	CA II, CA III, CA XII, CA XIV	59,62				
	AE2	54				
Vas deferens	NHE1	48			CA II	60
	NHE2	48				
	H^+-ATPase	32,41,62				
	AE2	54				

AE2, anion exchanger isoform II; CA II, (enzyme carbonic anhydrase isoform II; CA III, enzyme carbonic anhydrase isoform III; CA IV, enzyme carbonic anhydrase isoform IV; CA XII, enzyme carbonic anhydrase isoform XII; CA XIV, enzyme carbonic anhydrase isoform XIV; CFTR, cystic fibrosis transmembrane conductance regulator; H^+-ATPase, vacuolar-type adenosine triphosphatase; NBC1, electrogenic Na^+-bicarbonate co-transporter; NBC3, Na^+-bicarbonate co-transporter; NHE1, Na^+/H^+ exchanger isoform I; NHE2, Na^+/H^+ exchanger isoform II; NHE3, Na^+/H^+ exchanger isoform III; NHERF1, Na^+/H^+ exchanger regulating factor 1; SLC26A2, solute carrier family 26 member 2; SLC26A6, solute carrier family 26 member 6.

perfused distal initial segments of rats *in vitro*.[34] Furthermore, the addition of HOE694 after EIPA did not induce additional inhibition. Among many possible explanations for the unexpected results, the authors proposed that a splice variant of NHE3 sensitive to HOE694 might be present in the rat epididymis.[34] Future studies are, therefore, required to clarify this issue.

So far, data on the distribution of NHE3 in the mouse epididymis are sparse. Nevertheless, expression of this protein in the efferent ducts[13] and initial segment[17] has been reported. In view of the recent studies in which specific gene knockout mice were used to investigate the role of this protein in male fertility,[13,17] normal distribution of NHE3 protein in all regions of the mouse epididymis warrants further investigation.

Sodium bicarbonate co-transporter and anion exchangers

In the renal proximal tubule, H^+ secretion is tightly associated with HCO_3^- and NaCl reabsorption through the Cl^-/HCO_3^- exchanger and Na^+/HCO_3^- co-transporter.[52] HCO_3^- reabsorption is also highly dependent on brush border and cytosolic carbonic anhydrase enzyme. By analogy to the kidney, electrogenic Na^+/HCO_3^- co-transporter (NBC1) and Cl^-/HCO_3^- exchanger (anion exchanger isoform 2, AE2) are localized on the basolateral membrane of all epididymal cells.[53,54] Furthermore, the neutral Na^+/HCO_3^- co-transporter (NBC3) is expressed on the apical membrane of the narrow cells of the caput and clear cells of the corpus and cauda epididymidis of rats.[33] This transporter is, however, absent in the principal cells of all

regions of the epididymis. Interestingly, NBC3 is co-localized with V-ATPase in the clear cells.[33] It is, therefore, proposed that NBC3 plays a role in HCO_3^- absorption and activation of H^+ secretion through V-ATPase in the clear cells of the epididymis.[10]

Carbonic anhydrase

The presence of carbonic anhydrase enzyme (CA) in specific cell types of the rat epididymis was described by Cohen *et al.*,[55] but its role in sperm maturation has not been recognized until recently. In mammals, 16 isoforms of α-CA have been identified,[56] but only six isoforms (CA II, III, IV, IX, XII and XIV) were detected in the rat,[42,57–59] mouse[37] and human epididymis.[40,60,61] In the rat, cytosolic CA III was expressed in all cells of the efferent ducts, in principal cells of the initial segment and that of all segments of the epididymis,[59] whereas CA I was detected only in the narrow cells of the initial segment and the intermediate zone,[42] and in the principal cells, but not in the clear cells, of all epididymal regions.[59] In contrast, the plasma membrane-associated CA XII and CA XIV were detected in the basolateral membrane of the non-ciliated cells of the efferent ducts and in the narrow cells (CA XII) or principal cells (CA XIV) of the initial segment.[59] Principal cells of the proximal caput showed staining for CA XII with increasing intensity towards the proximal cauda epididymidis.[59] In contrast to CA XII, CA XIV was expressed in the apical membrane of the principal cells of the initial segment and in the proximal caput, whereas this enzyme was localized in both apical and basal membranes of the principal cells in the distal caput, the corpus and the cauda.[59] Notably, clear cells and basal cells of the entire epididymis were devoid of all isoforms of CA.[42,59] It is, however, suggested that CA might be expressed in the clear cells of the cauda epididymidis.[32,57,62] The abundance and redundant expression of multiple isoforms of CA might suggest an important role of this enzyme in H^+ secretion and HCO_3^- reabsorption in the male reproductive tract.

Only isozyme II of cytosolic CA has been reported in the mouse and its distribution is similar to that in the rat.[37,42] Of note, in this species, clear cells of the cauda epididymidis showed weak staining of CA II.[37] The membrane-associated CA IV was detected only on the microvilli of the principal cells of the distal caput and corpus, whereas the apical and basolateral membrane of these cells of the cauda epididymidis were stained for the protein.[37] In humans, however, the apical mitochondrial rich cells and the narrow cells of the epididymal duct were stained for CA II. Both types of cells co-expressed CA II with H^+-ATPase.[40]

EVIDENCE FOR THE ROLE OF NHE3 ON LUMINAL ACIDIFICATION AND MALE FERTILITY

Although the notion that proper luminal milieu of the epididymis is essential for sperm maturation and storage is well accepted,[4–7] the influence of luminal pH receives only little attention. More recent work, however, has unveiled the important role of this factor. Despite the data showing that the epididymis is fully equipped with several transport systems necessary for acid/base regulation of its milieu, V-ATPase has been considered to play a central role, whereas the role played by NHE has been underscored.[10] However, there is growing evidence showing the close association between the defects in NHE3, changes in the epididymal fluid pH, and male fertility.[17,63] It is conceivable that, in addition to V-ATPase in the narrow cells and clear cells, NHE3 in the principal cells might also play a crucial role in the acidification of epididymal fluid. This hypothesis is based on the following lines of evidence. First, most studies showed that NHE3 was expressed in the principal cells, which are most abundant in all regions of the epididymis, and the level of expression correlated with the luminal pH profile in the epididymis. Second, CA, which is required for hydration of CO_2 and subsequent generation of intracellular H^+, and the proteins that assist HCO_3^- absorption, such as NBC3 and AE2, were also detected in the principal cells. Hence, the principal cells are fully equipped with the proteins that are involved in acid secretion and bicarbonate absorption. Third, studies in knockout mice, such as estrogen receptor alpha knockout (αERKO), NHE3 knockout (NHE3KO), aquaporin-1 knockout (AQP1KO) and CA II knockout (CAIIKO) mice, showed that only those mice with NHE3 deficiency showed defects in epididymal acidification and infertility.

Expression of NHE3 in the principal cells of the epididymis correlates with its luminal fluid pH

It is important to note that NHE3 is expressed on the apical membrane of the principal cells in all regions of the epididymis.[35,36,51] These cells contribute to approximately 70–80% of total epididymal cells in rats,[5,7] In contrast, V-ATPase is localized only in the narrow cells in the initial segment and clear cells in the corpus and cauda epididymidis.[31] The narrow cells only occupy 3% of the initial segment, whereas clear cells constitute 5–40% of the epithelial cells of the epididymis with an increasing contribution from the caput towards the cauda segment.[5] Acidification of the luminal fluid, however, takes place in the initial segment, with no further decrease in pH in the distal regions.[14,15] The profile of luminal fluid pH, therefore, correlates well with the expression of NHE3 in the principal cells. Support to the involvement of NHE3 in luminal acidification is also obtained from the *in vitro* perfusion of the rat cauda epididymidis, where acid secretion is sodium dependent, which can be inhibited by acetazolamide.[64] In a more recent study, luminal acidification of the *in vitro* perfused rat distal initial segment and proximal cauda epididymidis was Na^+ dependent and was inhibited (~48%) by ethylisopropyl amiloride (EIPA), a NHE3 inhibitor, showing the functional importance of NHE3 in acidification of the luminal fluid.[34] Furthermore, data from that study suggest that luminal acidification occurred in the initial segment and proximal cauda epididymidis is, at least in part, attributable to the activity of NHE3.

NHE3 and CA are co-expressed in the principal cells of the epididymis

In the renal tubules, acidification of the tubular fluid involves the secretion of H^+ through the apical membrane, which is the main mechanism, and the reabsorption of HCO_3^- through the basolateral membrane of the tubular cells. Three mechanisms are responsible for the H^+ secretion across the apical membrane; that is, NHE, V-ATPase and the H–K exchange pump. The secretion of H^+ is enhanced by the interconversion of CO_2 to H_2CO_3, which is accelerated by the enzyme CA. In contrast, reabsorption of HCO_3^- takes place by the NBC3 and Cl–HCO_3 exchanger.[65] In the epididymis, all proteins involved in acid and/or bicarbonate transport are expressed in the principal cells. Thus, cytosolic and plasma membrane, both apical and basal, CA are detected in the principal cells of all regions of the

rat epididymis.[42,57–59] However, the expression of CA in clear cells that co-express V-ATPase in the distal region of the epididymis is still in dispute. Breton *et al.*[32] showed that clear cells of the cauda epididymidis expressed CA, but other groups failed to confirm the finding.[42,57,59] Inhibition of CA by acetazolamide suppressed acid secretion by the perfused cauda epididymidis, suggesting the important role of this enzyme in luminal acidification.[64]

NHE3-deficient mice showed defects in acidification of the epididymal fluid and fertility

In 2001, Zhou *et al.*[13] compared fertility of four different gene-deficient male mice, αERKO, NHE3KO, AQP1KO and CA II deficient. They found that only NHE3KO and αERKO were infertile. According to the authors, the infertility was attributed to the failure to reabsorb fluid in the efferent ducts of both KO mice. Recently, it has become clear that infertility of αERKO mice is associated with alterations of the luminal fluid pH of the epididymis, that is, αERKO mice failed to acidify the epididymal fluid, and consequently reduced sperm motility.[17] It is noteworthy that αERKO mice have reduced expression of several transporters including NHE3, CA XIV and NBC1 proteins, whereas expression of V-ATPase proteins was unaffected.[13,17] In addition, male mice treated with ICI 182780, which is a specific αER/βER blocker, showed normal fertility,[66] suggesting that infertility of the αERKO mice is not a result of deficiency of αER, but rather a result of lacking NHE3 expression. In support of this notion, the same group of investigators later showed that long-term treatment (125 days) with ICI 182780, which abolished the expression of NHE3 in the efferent ducts, decreased male fertility.[67] Unfortunately, the expression of NHE3 in the epididymis was not reported in that study.

In the c-ros KO mouse, the males were infertile, although they produced normal sperm and had no mating defect.[68] It was previously thought that the infertility was a result of the defects in sperm volume regulation during their journey in the female reproductive tract.[69,70] More recent work, however, has shown that the infertility of the c-ros KO mouse was associated with increased pH of the cauda epididymal fluid and decreased expression of NHE2 and NHE3 proteins in the caput and cauda epididymidis, respectively. Notably, the expression of V-ATPase in all regions of the epididymis was not changed.[63] All together, the evidence from targeted gene deletion studies, either the NHE3 gene or others that eventually suppress NHE3 protein expression in the epididymis, which leads to alteration of luminal acidification, result in male infertility.

A more recent study, however, has shown that the forkhead transcription factor Foxi 1 is required for the expression of the B1 subunit of V-ATPase and CA II, and is associated with male fertility[71]. Foxi 1-null male mice failed to express the B1 subunit of V-ATPase, Cl^-/HCO_3^- transporter pendrin (SCL26A4), and CA II in the narrow and clear cells. However, CA II expression in the corpus and cauda segments was independent of Foxi 1. Of note, epididymal fluid of the infertile Foxi 1-null mice had a slight, but significant, alkaline pH compared with that of the wild type (pH 6.9 vs 6.4).[71]

PROPOSED MECHANISMS FOR LUMINAL ACIDIFICATION BY NHE3 IN THE PRINCIPAL CELLS

Based on the aforementioned experimental evidence, we propose that the molecular model for acidification of the luminal fluid in the epididymis is comparable with that in the renal proximal tubule, in which H^+ is secreted by NHE3 and V-ATPase on the apical membrane combines with either HCO_3^-, resulting in bicarbonate reabsorption, or with NH3, creatinine or phosphate to form titratable acids (Fig. 1a)[72]. However, some differences in the detail mechanisms for acidification of the luminal fluid exist between the two organs.

According to the transport model of the epididymis (Fig. 1b), a neutral NHE3 transporter on the apical membrane of the principal cells secretes H^+ in exchange with Na^+. The latter is then extruded together with HCO_3^- through the basal membrane electrogenic Na^+/HCO_3^- co-transporter (NBC1) into the blood.[53,54] Meanwhile,

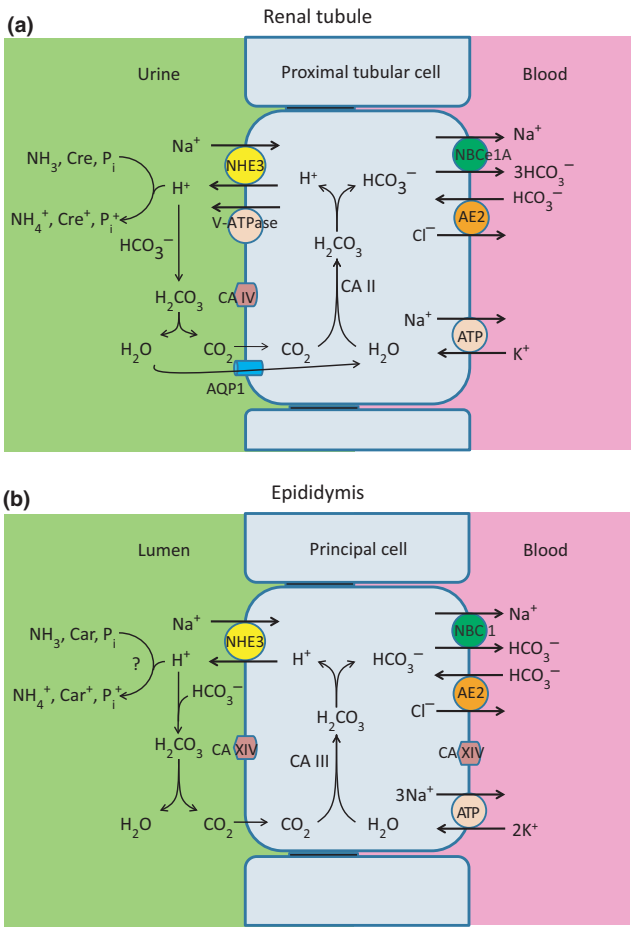

Fig. 1 Cellular models for luminal acidification in (a) the renal tubule compared with (b) the epididymis. (a) In the proximal renal tubule, H^+ is extruded into the lumen by both Na^+/H^+ exchanger 3 (NHE3) and vacuolar-type adenosine triphosphatase (V-ATPase), then combines with either HCO_3^- to form H_2CO_3 and subsequently water and CO_2, which is assisted by the apical membrane carbonic anhydrase (CA IV) or with NH_3, creatinine (Cre) or phosphate (P_i), resulting in titratable acids. Bicarbonate is then transported through the basolateral membrane into the blood through an electrogenic Na^+/HCO_3^- co-transporter (NBCe1-A). (b) In the principal cells of the epididymis, H^+ is secreted into the lumen in exchange with Na^+ absorption by NHE3. Combinations of H^+ and HCO_3^-, or NH_3, carnitine (Car) or phosphate (P_i) result in HCO_3^- absorption or net acid secretion, respectively. Generation of intracellular H_2CO_3 is facilitated by carbonic anhydrase enzyme (CA III). Basolateral CA XIV also maintains intracellular H_2CO_3. The presence of basolateral electrogenic Na^+/HCO_3^- co-transporters (NBC1) account for absorption of HCO_3^- into the blood, whereas Cl^-/HCO_3^- exchangers (AE2) account for NaCl absorption.

H^+ in the lumen combines with HCO_3^- to form carbonic acid, which, in turn, dissociates to yield CO_2 that diffuses into the principal cells. In the presence of intracellular CA III,[59] H^+ is regenerated from the hydration of CO_2. This process results in absorption of luminal HCO_3^-. In addition, the secreted H^+ might combine with luminal carnitine and/or phosphate, which are present at high concentrations in the epididymal fluid.[73,74] Proton trapping by NH_3 to form NH_4^+ in the lumen, as in the renal tubule, would also lead to net acid secretion. Whether or not epididymal cells secrete NH_3 awaits future studies.

In conclusion, recent studies have provided more insight into the role of the luminal milieu of the epididymis on sperm maturation, sperm storage and male fertility. The concept has now received experimental support for the influence of luminal acidification, and sperm motility and fertility. The epididymis is fully equipped with the transport systems necessary for acidification of its luminal fluid, as in the renal tubule. By analogy to the kidney in which tubular acid secretion is mediated by several transporters, such as NHE3, V-ATPase, NBC and AE2, these systems are likely to also play a role in acidification in the epididymis. In the present review, we have presented the evidence and brought up the role of NHE3, which has been previously de-emphasized. The extent of the contribution by these mechanisms to luminal acidification in the epididymis requires future investigation.

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