Minireview

Physiology of Na\(^+\)/H\(^+\) Exchangers in the Male Reproductive Tract: Relevance for Male Fertility\(^1\)

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**ABSTRACT**

The maintenance of pH homeostasis in the male reproductive tract is kept through the involvement of several mechanisms, among which is included the transmembranous movement of H\(^+\) ions. Na\(^+\)-H\(^+\) exchangers (SLC9, solute carrier 9 family members) are among the membrane transporters known to participate in intracellular and extracellular pH regulation but also have important roles in salt and water absorption across epithelia and in the regulation of cell volume. The presence of several Na\(^+\)-H\(^+\) exchangers has been reported in the male reproductive tract. Their involvement in the processes that ensure the correct pursuance of the spermatogenetic event and spermatogenesis maturation has been suggested. Indeed, the formation of mature spermatid is highly dependent on the maintenance of adequate ductal luminal milieu pH and ionic balance. Perturbations in these processes result in reduced male reproductive potential and consequently male subfertility and/or infertility. Thus, it is imperative to understand H\(^+\) transport dynamics in order to identify and counteract possible alterations associated with reduced male fertility caused by pathological conditions. Herein, we will discuss the expression pattern and physiological roles of SLC9 family members in the cells of the male reproductive tract as well as the molecular basis of H\(^+\) transport and its involvement in male reproductive potential.

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**INTRODUCTION**

The maintenance of intracellular pH (pH\(_i\)) within physiological values is a fundamental aspect of cell physiology. Thus, establishing a proper pH\(_i\) control is vital for a stable operation of multiple cellular events and to the establishment of cellular interactions, controlling cell survival and function. Indeed, in the absence of regulation, the cytosol would become acidic as an effect of the continuous increase of metabolic acidic activity [1].

There are several mechanisms involved in pH\(_i\) regulation. This cellular parameter is kept mainly through the net balance between production and elimination of protons as well as by the action of intracellular buffers [2]. Cells possess in their plasma membrane a wide range of ion transporters that participate in pH\(_i\) regulation, among which are the basic and acidic ions membrane transporters [2]. These transporters, directly involved in the movement of basic and acidic ions across the membrane, are classified as acid exudators or acid loaders. Two main categories of transmembrane proteins are involved in mammalian cells pH regulation: 1) proton carriers, which transport hydrogen ions, and 2) bicarbonate carriers, which transport bicarbonate. Proton carriers include transporters of the solute carrier 9 (SLC9) family, which are highly effective in pH\(_i\) regulation and widely expressed. These cation exchangers constitute a family of membrane transporters commonly termed Na\(^+\)/H\(^+\) exchangers (NHEs) or antiproters (NHAs). NHEs are classified as secondary active transporters because their catalytic driving force is not directly coupled to ATP hydrolysis, being instead derived from the electrochemical gradient established for one of the solutes, which drives the countertransport of the other. In addition to the movement of H\(^+\) ions, the transmembrane influx of Na\(^+\) directly contributes to the absorption of salt and water across various epithelia and to the regulation of cell volume (for extensive review, see Fuster and Alexander [3]). Herein we propose to briefly present an overview of Na\(^+\)/H\(^+\) exchanger’s general structure and function and then discuss their expression throughout the male reproductive tract and their relevance to male fertility.

**NHE ISOFORMS: FROM STRUCTURE TO FUNCTION**

The SLC9 family is composed by three subgroups of transporters that encompass: 1) the SLC9A plasma membrane isoforms NHE-1 to NHE-5 (SLC9A1-5) and the predominantly intracellular isoforms NHE-6 to NHE-9 (SLC9A6-9); 2) the two recently cloned isoforms of the SLC9B subgroup, NHA-1...
and NHA-2 (SLC9B1 and SLC9B2, respectively); and 3) the SLC9C subgroup isoforms, which consist of a sperm-specific plasma membrane NHE (sNHE, SLC9C1, or SLC9A10) and a putative NHE-11 (SLC9C2) (for review, see Fuster and Alexander [3]; Fig. 1). Each NHE isoform corresponds to a distinct gene product and has a different molecular structure, sensitivity to pharmacological inhibitors, and tissue distribution. In fact, the different sensitivities of the NHE isoforms to pharmacological inhibition represent a complementary characteristic that has been very useful in the development of therapeutic agents (for review, see Orlowski and Grinstein [4]).

There are several NHE isoforms that vary in length from 500 to 1200 amino acids and share a distinctive similarity in primary structure. Indeed, NHE-1 to NHE-5 isoforms share about 70%–80% amino acid similarity with each other, while the other NHE isoforms share only about 20% similarity (for review, see Orlowski and Grinstein [4]). Based on their primary structure, an analogous membrane topology was predicted for all isoforms, with 10–12 membrane-spanning regions at the N-terminus and a large cytoplasmic region at the C-terminus, which exhibits a minor degree of similarity between isoforms [5]. Published data suggest that these NHE isoforms exist in the membrane as homodimers, although their exact topological organization remains a matter of debate [6].

The NHE-1 isoform is located in the plasma membrane and is ubiquitously expressed in virtually all cells [7]. It plays a key role in pH homeostasis, cell adhesion, and migration as well as in cell volume regulation, being also involved in tumor growth [7]. The isoforms NHE-2 to NHE-4 have a tissue-specific pattern of expression, being more abundantly found in kidneys and in some regions of the gastrointestinal tract [3]. These isoforms may fulfill overlapping functions with NHE-1, particularly in the kidney, which lacks detectable levels of NHE-1 [3]. They are located primarily in the plasma membrane, being also present in recycling endosomes (Fig. 1). The intracellular location of the isoforms seems to be directly related to the regulation of NHEs activity, with changes in cellular ion composition stimulating net translocation of the NHEs between plasma and endosomal membranes [8]. Whether these NHE isoforms contribute to endosomal acidification awaits confirmation. The NHE-5 isoform is also tissue specific and is abundantly expressed in neuron-rich regions of the brain, regulating activity-dependent local pH changes and dendritic spine growth on neuronal activation, being also located in intracellular vesicles [9]. The isoforms NHE-6 to NHE-9 are found predominantly in intracellular membranes [3]. While NHE-6 and NHE-9 are ascribed primarily to sorting and recycling endosomes, while NHE-7 is localized mostly in the trans-Golgi network and NHE-8 in mid-trans-Golgi stacks.

**REGULATION OF NHE FUNCTION**

The activity of NHEs has been found to have a strong dependence on external Na\(^+\) concentration, exhibiting a Michaelis-Menten kinetics [14]. For most NHE isoforms, the affinity of Na\(^+\) for its external binding site (30–60 mM) is approximately three times below the physiological extracellular Na\(^+\) concentration (135–145 mM) [15–17]. It has been reported that higher Na\(^+\) concentrations may activate NHE-1 [15] but have an opposite effect on NHE-2 and NHE-3 [18, 19]. Interestingly, NHE-4 shows a completely distinctive feature with respect to its affinity for Na\(^+\), as its binding site for that cation is not selective entirely for Na\(^+\) but also for H\(^+\) and Li\(^+\) [20, 21] through a competitive binding process. On the other hand, K\(^+\) ions, although not transported by NHEs, since they are of greater dimensions than Na\(^+\), may inhibit NHE-1 while having no effect on NHE-2 or NHE-3 [22]. Furthermore, NHEs activity is extremely sensitive to pH, [23]. Once pH falls below a certain threshold, these exchangers are activated by intracellular H\(^+\) concentration to promote a rise of pH\(_i\). This H\(^+\) sensitivity varies between isoforms, being more pronounced in NHE-1 to NHE-3 isoforms [24, 25]. Some NHE isoforms are also responsive to increases in cytosolic Ca\(^{2+}\) concentration and to changes in cell volume [26]. Additionally, several trophic factors (e.g., interleukins 3 and 7) and pharmacological agents (e.g., inhibitors of Janus kinases) known to directly interact with protein tyrosine kinases and also some agonists of serine/threonine (Ser/Thr) protein kinases (e.g., calyculin A) have been described as modulators of NHEs activity [27, 28]. The regulation of NHE activity may be...
explained by their direct phosphorylation. Their primary sequence reveals the existence of consensus sites for phosphorylation by agonists of Ser/Thr kinases, like phospho-fructokinase A and/or C, as well as several sites that are appropriate substrates for Ca^{2+}/calmodulin-dependent protein kinases and for proline-directed Ser/Thr kinases. The latter include the mitogen-activated protein kinases, which have been implicated in NHE activation [29]. Moreover, H^+ pumping by NHEs may also be regulated by the recruitment of transporters from endomembrane stores (a pool of membrane transporters present in the membrane of intracellular vesicles/organelles) to the plasma membrane when specific stimuli are triggered, particularly in the case of NHE-3, which is thought to also reside in intracellular vesicles. In fact, agents and conditions that induce or modulate NHE activity are known to change the rates of endo- or exocytosis or affect the redistribution of vesicles within cells [30]. Since spermatogenesis is highly dependent on the metabolic and ionic establishment of adequate luminal fluids, all these mechanisms related to NHEs functioning are crucial to the normal male reproductive health.

**NHE FUNCTION AND EXPRESSION ARE CRUCIAL FOR MALE FERTILITY**

Mammalian spermatozoa are produced in the testis and undergo a maturation process, while traveling to the cauda epididymis, in order to acquire their fertilizing capacity and motility. Most of the fluid that leaves the testis is reabsorbed by efferent ducts and leads to a high concentration of spermatozoa in the epididymis, where the fluid composition is further modified (for review, see Rato et al. [31]). Indeed, the various processes that occur along the male reproductive tract of mammals, namely, spermatogenesis and sperm maturation, are affected by pH establishment in the several luminal fluids. Disturbances of acid-base homeostasis in the reproductive tract have been associated with male infertility/subfertility in mammals (for review, see Pastor-Soler et al. [32]). The luminal pH reaches its highest value in the seminiferous tubules (pH ≈ 7.2–7.4) and the lowest in the caput epididymis (pH ≈ 6.4–6.5) and then slightly rises in the cauda epididymis and vas deferens (pH ≈ 6.5–6.8) [33, 34]. These pH-controlling processes are fulfilled by a number of transport systems that translocate ions, organic solutes, and macromolecules as well as water across the epithelia lining the male reproductive tract (for review, see Rato et al. [31]). For that, the epithelial walls of the male reproductive tract actively transport acid-base equivalents to tightly control the luminal fluids pH.

Several members of the NHE family have been described in cells of the male reproductive tract (Fig. 2). NHE-1 has been detected in the basolateral membrane of epithelium lining the rat epididymis [35] (Fig. 2) and in cultured epithelial cells of the efferent duct [36]. Due to its functions, such as maintenance of pH, regulation of cell volume, and cell proliferation, this isoform is generally known as “housekeeping” NHE, and it is thought that NHE-1 participates in the homeostatic control of cells [37]. Besides, NHE-1 may also be implicated in transepithelial electrolyte transport, namely, in the efferent duct and cauda epididymis. Together with the Na^+/K^+ pump, Na^+/K^+/Cl^- symport, and basolateral K^+ channels it also functions to promote intracellular accumulation of Cl^- and HCO_3^- [36]. The presence of NHE-2 has also been reported along the male reproductive tract. NHE-2 has been detected in the apical membrane of principal cells from the caput, corpus, and cauda epididymis, but it was shown to be absent from the initial segments (Fig. 2). Therefore, it was suggested that NHE-2 participates in Na^+ reabsorption in the epididymis [35].

**FIG. 2.** A) Localization of the Na^+/H^+-exchangers (NHE) throughout the mammalian epididymis. The epididymis is composed of three main segments: caput, corpus, and cauda. NHEs contribute to both luminal fluid and intracellular pH maintenance. The presence of three distinct NHE isoforms has been reported throughout the epididymis. NHE-2 has been described in all three section of the epididymis, while NHE-1 and NHE-3 were localized only in caput and cauda. B) Subcellular localization of NHE isoforms in mammalian spermatozoa. The mature spermatozoa are highly specialized cells composed of the head and the flagellum. The flagellum is composed of three segments: the mitochondria-rich midpiece, the principal piece, and the end piece. NHEs could contribute to the export of H^+, which is also essential for intracellular pH maintenance. The presence of three distinct NHE isoforms has been reported in spermatozoa. NHE-1 and NHE-5 were localized in the spermatozoa midpiece. The sperm-specific NHE (NHE-10 or sNHE) was described in the caudal epididymal spermatozoa and was located on the principal piece of the sperm flagellum.
Additionally, this NHE is also present in the testis, particularly in Sertoli cells [38] and in ciliated cells of the efferent duct, where its function remains unknown [36]. Likewise, NHE-3 has also been described in cells of the male reproductive tract. Leung et al. [36] demonstrated the presence of NHE-3 in cultured epithelial cells of the efferent duct, where this isoform not only represents approximately 25% of the total NHE activity but also may have an important role in regulating the fluidity and pH of the epididymal fluid [36]. NHE-3 is the major NHE isoform expressed in the brush border membrane of the proximal tubule, where it plays a significant role in Na⁺ and fluid reabsorption. NHE-3 is also a likely candidate to be involved in Na⁺ and fluid reabsorption by the efferent duct. Indeed, more than 95% of the testicular fluid is reabsorbed by the efferent duct. This fluid reabsorption is Na⁺ dependent and decreases by 70% when NHEs are blocked, illustrating that Na⁺ reabsorption by the efferent duct is mediated mainly through NHEs specifically located in the apical membrane [39]. NHE-3 is also expressed in rat epididymis, and its level of expression varies in different regions of the epididymis [40]. NHE-3 is most abundant in the initial segment, in the proximal caput epididymis, and in the proximal cauda (Fig. 2). In the initial segments of the epididymis, the apical NHE-3 exchanger secretes protons into the lumen. These H⁺ ions combine with luminal HCO₃⁻ to form CO₂ and H₂O under the enzymatic activity of carbonic anhydrase. The produced CO₂ then diffuses into the cell through the apical membrane and is hydrated by cytosolic carbonic anhydrase to form H⁺ and HCO₃⁻. The protons recycle back into the lumen via NHE-3 [32]. Additionally, NHE-3 is known to be expressed in the apical membrane of testicular cells, particularly in Sertoli cells [41, 42]. Although its function in these cells has not been fully elucidated, it has been reported that this transporter is crucial to the overall testicular functioning. Indeed, Zhou et al. [41] demonstrated that NHE-3-knockout mice exhibit tubular fluid accumulation with associated loss of fertility. Moreover, Goyal et al. [43] reported a strong expression of NHE isoform 8 in the testis of mice. However, the authors did not exploit the specific testicular expression pattern of this Golgi-located NHE. Thus, its function in this tissue remains unclear. It is noteworthy that NHEs regulation is essential to the formation and maturation of spermatozoa.

**NHEs ARE INVOLVED IN SPERMATOZOA FORMATION AND MATURATION**

The concentration of specific ions, particularly important to pH establishment, in the tubular fluids of the male reproductive tract or in the seminal fluid is crucial for several aspects of fully competent male gamete formation (for review, see Liu et al. [44]). Furthermore, sperm pHᵢ has been proposed as an important player in determining sperm quality. Hence, it has been suggested that several NHE isoforms may mediate key processes in mammalian spermatozoa [45]. In fact, three NHEs have been identified in spermatozoa, namely, NHE-1, NHE-5, and sNHE (sperm-specific NHE) [46] (Fig. 2). NHE-1 was located in the spermatozoa midpiece, and a putative functional role for this NHE in controlling the local concentration of intracellular H⁺ has been suggested [45]. Nevertheless, male mice lacking NHE-1 are fully fertile, suggesting that this NHE may not be critical for sperm function [47]. On the other hand, little information is available on the role of NHE-5 on male fertility or sperm motility. It has been reported that NHE-5 is colocalized with NHE-1 in the sperm midpiece, highlighting the possible importance of NHE-5 on sperm physiology, since there is evidence that points to a negligible role of NHE-1 in spermatozoa functioning.

The sperm-specific member of the mammalian NHE superfamily was described in the caudal epididymal spermatozoa of mouse and was located on the principal piece of the sperm flagellum [48]. Disruption of sNHE gene causes male infertility and absolute loss of sperm motility without an apparent effect on spermatogenesis or morphological alterations in sperm flagellum, midpiece, or head [49, 48]. These effects appear to be the outcome of an interaction between sNHE and soluble adenylyl cyclase (sAC) in spermatozoa [49, 50]. A comparison of two knockout mouse models for sNHE and for sAC showed several similarities in sperm phenotypes. The lack of sNHE greatly reduces adenylyl cyclase activity in spermatozoa, providing an explanation for the resemblances in sperm phenotypes between these knockout models [49, 50]. In mammalian spermatozoa, sAC family members are responsible for the control of cAMP synthesis. Wang et al. [46] described that the addition of cAMP analogues was an effective way of reversing the sperm motility failure observed in the sNHE knockout model, being that spermatozoa acquire motility levels comparable with wild-type animals. Hence, a stable function of the sAC protein in mouse epididymal spermatozoa requires sNHE expression, suggesting that these two proteins are components of a signaling pathway that is essential for sperm motility [50]. It has also been proposed that sNHE plays a restricted role in controlling localized pHᵢ changes, mainly in the flagellar principal piece [50]. Thus, sNHE may also contribute to the regulation of sAC activity as a result of the pH dependence of this enzyme, regulating sperm motility.

The final sperm maturation events (known as capacitation and acrosome reaction) do not occur within the male reproductive tract. It is noteworthy that these events also require the participation of the sperm-specific sNHE isoform (for review, see Visconti et al. [51]). After ejaculation, when spermatozoa are transferred to the female tract, the extracellular fluid HCO₃⁻ concentration increases, activating the onset of capacitation, which also involves the activation of sperm motility. The hyperpolarization of the membrane stimulates H⁺ extrusion, causing an increase in pHᵢ. This increase in pHᵢ plays a dominant role in these final maturation processes [50]. As discussed before, it has been proposed that pHᵢ regulation during these events relies on sNHE activity, as shown by the sterile phenotype of sNHE-null mice. However, inhibition of sNHE-dependent pHᵢ regulation in capacitated spermatozoa is not sufficient to block the initiation of acrosome reaction [52]. Other functions, such as the regulation of capacitation or sperm motility, are nonetheless affected by inhibition of this pHᵢ regulatory mechanism [46]. Overall, these studies point toward a crucial role for NHEs, not only in spermatozoa formation but also in their maturation, particularly in their ability to acquire motility.

**CONCLUSION**

Transmembrane H⁺ transport is important not only for the maintenance of pHᵢ but also for the establishment of the proper pH of the fluids in the various body compartments. The molecular mechanisms underlying H⁺ homeostasis in the male reproductive tract remain largely unknown. Indeed, members of the NHE family are specifically and widely expressed in the various cells throughout that tract, suggesting a relevant contribution of these transporters to the maintenance of male fertility. Furthermore, the expression of a specific NHE isoform in spermatozoa and the reported infertility in the absence of this protein highlights the importance that H⁺ transport dynamics...
have on sperm maturation physiology and provides an attractive target for the development of a male contraceptive.

Nevertheless, further studies will be needed to provide a more detailed comprehension of the role of NHE transporters in male fertility. A deeper knowledge regarding NHE expression, function, and operation throughout the male reproductive tract will greatly improve our understanding of the molecular mechanisms behind pathophysiological conditions of male infertility. Finally, it may shed important light on the clinical therapy for infertile male individuals.

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REFERENCES


