SLC9C1 (NHE10/sNHE) in Male Fertility

Subjects: Physiology

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The SLC9C1 gene encodes the NHE10 protein (also known as sNHE). This protein has been shown to be essential for male fertility in both mice and humans and therefore there has been much interest in studying this protein. What is known about NHE10 and its role in male fertility is highlighted.

Na+/H+ exchangers (NHEs) SLC9 sperm pH regulation male fertility

1. Molecular Genetics and Expression Patterns

SLC9C1 (NHE10/sNHE) is located on human Chromosome 3 and is comprised of 29 exons. Both the mouse and human NHE10 (also known as sNHE) proteins are expected to contain 17 total transmembrane domains: the first thirteen transmembrane domains are predicted to encode an *N*-terminal NHE domain, and the last four transmembrane domains are predicted to encode a voltage sensing domain (VSD). The C-terminus of NHE10 is predicted to encode an intracellular cyclic nucleotide binding domain (CNBD) [1]. The predicted structure of NHE10 suggests that the NHE transport activity of the protein may be regulated by cyclic nucleotides and/or sperm membrane potential (E_m). Additionally, recent work from researchers' lab suggests that mouse, rat, and human *SLC9C1* possess conserved, and unique, methylation-sensitive DNA regulatory elements that contribute towards maintaining testis/sperm-specific expression [2] .

Seminal work from the Garbers group [1] identified the first sperm-specific NHE and demonstrated its critical importance in mouse sperm motility and male fertility. The NHE10 (originally termed sNHE) cDNA was cloned from a mouse spermatid enriched cDNA library and the transcript was found to be exclusively expressed in testis in mouse and the NHE10 protein was found to localize to the principal piece of the mature mouse sperm flagellum [1].

2. Sperm Physiology and Fertility

NHE10 knockout (KO) mice exhibit normal testis and sperm morphologies but are completely infertile due to immotile sperm [1]. Interestingly, the cell permeable weak base NH_3 is able to rescue motility in about 20% of the NHE10 KO mouse sperm and partially rescue their ability to fertilize zona pellucida-free eggs during in vitro fertilization (IVF) experiments, suggesting that the immotility phenotype is caused, at least partially, by an acidic intracellular pH [1]. However, there is ample evidence that lack of cAMP also underlies the loss of sperm motility and infertility in the NHE10 KO mouse. cAMP analogs rescue motility of NHE10 KO mouse sperm and partially restore their ability to fertilize zona pellucida-free eggs in IVF experiments [1]. NHE10 knockout sperm do not undergo normal protein tyrosine phosphorylation under capacitating conditions, but the addition of the cAMP

analog Sp-cAMP along with the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX) restore protein tyrosine phosphorylation levels back to the wildtype levels [4]. NHE10 KO mouse sperm have reduced basal levels of cAMP and HCO_3^- sensitive cAMP synthesis is undetectable. Immunoblotting revealed that the full-length soluble adenylyl cyclase (sAC) protein is absent in NHE10 KO sperm, but levels of truncated sAC protein are similar in NHE10 KO and wild-type sperm. NHE10 appears to regulate the expression of full-length sAC at the protein level because the NHE10 KO and wild-type testes have comparable levels of full-length sAC mRNA. Cell culture experiments validated this hypothesis; co-transfection of NHE10 and sAC increased the expression of both the full length and truncated isoforms of sAC. It was also shown that expression of the carboxy terminus of NHE10 physically interacts with truncated sAC when coimmunoprecipitation studies were performed [4].

Optogenetics, using a transgenic mouse model that expresses a photoactivated adenylyl cyclase (bPAC) in the sperm, was used to further examine the functional relationship between sAC and NHE10 [5]. When bPAC mouse sperm are exposed to blue light, there is a rapid increase in intracellular cAMP synthesized by the transgenic photoactivated adenylyl cyclase. This bPAC mouse was bred with the NHE10 KO mouse background. When bPAC/NHE10 KO mouse sperm are exposed to blue light, there is a rapid increase in cAMP, sperm motility is restored, and the sperm are able to fertilize zona pellucida-intact oocytes in vitro [5]. All of this is evidence that in mice, NHE10 is not necessary for sperm motility and fertilization following an increase in cAMP via sAC. However, NHE10 is necessary for proper sAC expression and subsequent cAMP synthesis in mouse sperm. It is still unclear exactly what physiological role NHE10 has upstream of the increase in cAMP signaling events, although a recent study suggests that NHE10 expression is necessary for mediating the hyperpolarization-mediated alkalization observed in the mouse sperm flagellum [6]. Of note, addition of cAMP analogs is only able to partially rescue in vitro fertility, ~50%, and cAMP synthesis via pBAC is only able to rescue in vitro fertility ~30% [5]. This partial rescue of fertility in the absence of NHE10 protein suggests that NHE10 plays other roles in sperm to support motility and fertility.

3. NHE10 Transport Activity

Initial attempts to characterize the transport activity of the NHE10 protein failed due to the inability to express the NHE10 protein in cultured cells. Eventually, the Garbers group was able to show very weak expression of mouse NHE10 in a human cell line (HEK 293F) that was co-transfected with a truncated isoform of mouse sAC [4]. In an effort to increase NHE10 expression, the same group created a chimeric NHE1–NHE10 protein in which the first transmembrane domain of the mouse NHE10 protein was replaced with the first three transmembrane domains of the mouse NHE1 protein. When transfected into HEK293F cells, this chimeric NHE1–NHE10 protein was expressed and localized to the plasma membrane. Expression of the chimeric NHE1–NHE10 protein in a NHE1-null fibroblast cell line conferred resistance to acid-loading selection and the chimeric transporter was shown to possess weak Na^+/H^+ exchange activity [4]. However, since it is not clear whether the minimal NHE activity reported for the NHE1–NHE10 chimeric protein [4] is present without the contribution of the NHE1 components, characterization of the native mammalian NHE10 proteins is critical to understand how the transport activity of NHE10 influences mammalian sperm physiology.

Unfortunately, no unmodified mammalian NHE10 has been shown to possess NHE activity to date. However, sea urchins express a single SLC9C protein (originally identified as NHE10) that has recently been shown to be a bona fide NHE whose activity is not affected by the NHE inhibitors amiloride, EIPA, cariporide, and phloretin [7]. Mammals possess two SLC9C proteins (NHE10 and NHE11 [8]) and the sea urchin protein is no more similar to mammalian NHE10 than mammalian NHE11 [9], making it impossible to determine whether it is a specific ortholog of either. Therefore, researchers will refer to the sea urchin isoform as the sea urchin SLC9C (suSLC9C) protein from here on.

Immunolocalization demonstrated that the suSLC9C protein resides in the plasma membrane of the entire sea urchin sperm flagellum as well as in part of the sperm head, potentially the acrosome [7]. The analyses of the suSLC9C protein also demonstrated that its NHE activity is enhanced in response to the egg peptide speract [7], confirming earlier work suggesting a voltage sensitive Na^+/H^+ exchange in sea urchin spermatozoa [10]. In addition, mass spectrometry revealed ~54,000 suSLC9C protein molecules in the flagellum of sea urchin *Arbacia punctulata* sperm, one of the most abundant proteins found in this research [11]. Such a concentration of suSLC9C protein molecules found in the flagellum would allow this sperm to rapidly fine tune pH_i in response to various cell signaling conditions.

The overall domain structure of the suSLC9C protein is predicted to be similar to the structure predicted for the mouse NHE10 in that they both contain an *N*-terminal NHE domain, followed by a VSD, and then a C-terminal CNBD; each domain containing many conserved functional residues [7]. Electrophysiological characterization of suSLC9C protein expressed in cultured cells revealed that this NHE is an electroneutral Na^+/H^+ exchanger that can exchange in either direction, depending on the ion gradient [7]. The VSD of the suSLC9C protein produces gating currents, suggesting that it is a functional voltage sensing domain. In addition, the NHE activity of the suSLC9C protein is voltage dependent with a $V_{1/2} < -70.9$ mV and cyclic nucleotides modulated the $V_{1/2}$ of its activity: cAMP shifted the $V_{1/2}$ of exchange activity to −56.8 mV and cGMP shifted the $V_{1/2}$ of exchange activity to a lesser degree, to −67.8 mV [7]. These findings suggest that binding of cAMP to the CNBD of suSLC9C protein affects NHE activity by shifting the voltage dependence of activation. Although the suSLC9C protein has been confirmed to be a voltage and cyclic nucleotide-sensitive NHE, it was noted that specific amino acid residues in the suSLC9C protein VSD and CNBD, thought to be important for function, are not conserved in the mouse and human NHE10 orthologs [7]. Further studies are required to determine the activity of the mammalian proteins to determine whether mammalian NHE10 is an NHE that responds to changes in membrane potential and cyclic nucleotides to regulate sperm function similar to the suSLC9C protein in sea urchin sperm.

A recent study found that NHE10 KO mouse sperm are unable to alkalize their principal piece in response to valinomycin-induced hyperpolarization in the same manner that WT mouse sperm can [6]. These experiments suggest that NHE10 is responsible for the hyperpolarization-induced alkalization in mouse sperm and that the NHE10 protein is likely able to regulate pH and is sensitive to membrane potential. However, it was found that human sperm do not alkalize in response to valinomycin-induced hyperpolarization [6]. It is therefore possible that the human NHE10 activity is not regulated by membrane potential, but further characterization of the function of the VSD of human NHE10 is necessary.

4. NHE10 and Human Fertility

Although few specifics about the transport activity of mammalian NHE10 are known, evidence points to human NHE10 having a critical role in male fertility. A recent clinical study found significantly less NHE10 protein in sperm from asthenozoospermic compared to normozoospermic men and that NHE10 expression was positively correlated with higher sperm motility parameters [12]. It was noted in this research that NHE10 is specifically localized to the principal piece of the human sperm flagellum [12], similar to what is seen in mouse sperm [1]; however, human NHE10 is also reported to localize to the entire sperm flagellum [13]. Even more recent analysis of an infertile male presenting with asthenozoospermia found that this patient bares a homozygous mutation in *SLC9C1* [13]. The patient's sperm displayed severely impaired motility as well as characteristics indicating membrane and flagellar fragility. Transmission electron microscopy (TEM) analysis revealed that the sperm exhibits midpiece defects but no acrosomal or axonemal structural abnormalities were observed. The specific mutation caused aberrant splicing around exon 22 which is predicted to cause an in-frame deletion of 33 amino acids in the consensus cyclic nucleotide binding domain of NHE10. The mutant NHE10 protein is still produced and was reported to localize, similar to the wild-type NHE10, to the entire length of the sperm flagellum [13]. It should be noted that this patient also bears mutations in two other genes and therefore the possibility that the infertile phenotype is due to a combination of these mutations cannot be excluded. In addition, since the level of sAC was not assessed in these mutant sperm, it is not clear whether the infertility of this patient is due to defective transport resulting from the mutant NHE10 protein, or if sAC protein expression is affected as is thought to occur in the mouse model completely lacking NHE10 [4]. While these findings highlight the importance of NHE10 in human male fertility, more work is needed to dissect the exact functions and interactions of human NHE10.

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