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Na^+/H^+ Exchangers, Structure, and Function: Role in Human Health and Disease

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Synonyms

Membrane proteins; Na^+/H^+ exchanger; pH regulation

Definition

Na^+/H^+ exchangers are a family of membrane proteins that exchange sodium for protons across lipid bilayers. They are widely distributed in all living cell types and are critical in several human diseases. The best known type of Na^+/H^+ exchanger in multicellular species is the mammalian Na^+/H^+ exchanger isoform 1 (NHE1). It is a plasma membrane protein that regulates intracellular pH by removing one intracellular hydrogen ion in exchange for one extracellular sodium ion. NHE1 regulates intracellular pH, but is also involved several diseases in the myocardium and in cancer, in addition to its role in cell growth, movement and differentiation. This review summarizes current knowledge of Na^+/H^+ exchangers, with emphasis on the most well characterized human Na^+/H^+ exchanger, the NHE1 isoform.

Introduction, Na^+/H^+ Exchangers

Na^+/H^+ exchangers are a class of membrane proteins that exchange Na^+ for H^+'s across lipid bilayers. The prokaryotic and eukaryotic genes that encode this monovalent cation proton antiporter (CPA) superfamily were recently reviewed (Brett et al. 2005). Briefly, the superfamily includes the CPA1, CPA2, and NaT-DC (Na^+-transporting carboxylic acid decarboxylase) families, each of which has unique bacterial ancestors. The CPA1 family includes bacterial NhaP transporters. It also includes many well studied Na^+/H^+ exchangers including from fungi, plants and mammals, and the human NHE1–NHE10 isoforms, the SLC9A paralogous genes. The CPA2 family shares its origins with prokaryotic NhaA. The *E. coli* form of NhaA is structurally, the most well studied Na^+/H^+ exchanger and its crystal structure has been deduced. However, many other genes of this family are not well studied. This family also includes HsNHA1 and HsNHA2 which are two relatively recently characterized forms of human Na^+/H^+ exchanger that may be involved in hypertension (Brett et al. 2005). The NaT-DC family is a smaller family that mediates transmembrane export of 1–2 Na^+ in exchange for an extracellular H^+ (Brett et al. 2005).

Human NHE1–10

1. *General* – Human NHE1–NHE10 are membrane proteins that transport hydrogen ions in exchange for sodium ions. Human NHE1 is ubiquitously expressed in mammalian cells and is widespread throughout the animal kingdom. NHE1 is critical

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in intracellular pH (pH_i) regulation, protecting cells from acidification, as well as regulating cell volume and sodium fluxes (reviewed in). Of the family of ten isoforms, the NHE1 isoform is ubiquitous and is on the plasma membrane of mammalian cells. NHE1 is important in the myocardium as it is implicated in the diseases ischemia/reperfusion injury, and in heart hypertrophy (see below and reviewed in Fliegel 2008). NHE1 has a 500 amino acid membrane domain that transports ions and a cytosolic regulatory domain of approximately 315 amino acids that modulates activity of the membrane domain and are a target regulation by proteins and phosphorylation (Fliegel 2008).

2. *Subtypes* – Human NHE1 is an 815 amino acid protein. The family of mammalian NHE-like proteins includes ten isoforms (NHE1–10). Each are the product of a different gene and with different tissue distributions and physiological roles. The first type cloned was named NHE1. NHE2–4 are principally expressed in the kidney and gastrointestinal tract. NHE5 is located in the brain. NHE6–9 are found in intracellular organelle membranes, such as endosomes, mitochondria, and the golgi apparatus, NHE10 is found in osteoclasts. The identity of the various isoforms varies from 25% to 70%; however, all have a similar predicted secondary structure (see below) (Fliegel 2008).

Structure

1. *Topological models of NHE1* – Currently, the high-resolution structure of NHE1 has not yet been solved, due to the difficulty in expressing and crystallizing membrane proteins. The first 500 residues are predicted to be 12 transmembrane spanning segments, and the remaining 315 residues constitute a cytosolic intracellular regulatory domain (Fliegel 2008). Most of the information on structure of the membrane domain comes from topology models of NHE1, some biochemical studies, a low resolution electron diffraction envelope, a crystal structure of a bacterial homologue and NMR studies of fragments of the NHE1 protein. Our laboratory produced and purified the full length NHE1 protein (Fliegel 2008). The EM structure showed that NHE1 exists as a homodimers which was confirmed by intermolecular cross-linking (Fliegel 2008; Lee et al. 2011).

   Two initial studies investigated the topology of the membrane domain (Landau et al. 2007; Wakabayashi et al. 2000) and vary somewhat between each other and are reviewed in detail in (Lee et al. 2011). The topology was first examined in detail by Wakabayashi et al. (Wakabayashi et al. 2000) and using substituted cysteine accessibility they suggested that NHE1 contains 12 TM helices with both the N- and C-terminal in the cytoplasm, and the N-linked glycosylation site on the extracellular side (Fig. 1). They also suggested that two intracellular loops and one extracellular loop may be pore or membrane associated.

   A more recent model for the topology of NHE1 was developed by Landau et al. (Landau et al. 2007) and was based on computational methods, including evolutionary conservation analyses and fold alignment methods with *E. coli* NhaA. This model has some significant differences from that of Wakabayashi et al. (Wakabayashi et al. 2000). The model of Landau suggests that the first two helices predicted by the Wakabayashi et al. (2000) are removed and function only as a signal sequence (Fig. 1). The next 6 TM segments of amino acids 127–315, have the same topology in both models. Amino acids 328–398, vary in their assignments between the two models. The last three transmembrane segments, TM 10–12 (amino acids 410–500) are very similar in both models.

   More recently, Nygaard et al. (Nygaard et al. 2010) followed up with further structural modeling of NHE1, based on the structure of NhaA. Their 3D model principally was similar to the model of Wakabayashi et al. (Wakabayashi et al. 2000) in basic transmembrane topology. However, their conclusions were not in agreement with some biochemical data and their EPR results did not distinguish between the two earlier models, so that the structure of the NHE1 protein remains unresolved and controversial (Lee et al. 2011).

2. *Structural Studies on Human NHE1* – Our laboratory has used a "divide and conquer" approach to study the NHE1 protein (Lee et al. 2011). We studied individual TM (transmembrane) segments, which are components of the larger multi-TM domain. This avoids the difficulties that have

plagued researchers in producing multi-TM spanning proteins. Studies on individual TM helices and loops have shown that the structures are often very similar to the structures of helices found in crystal structures of entire TM proteins (Lee et al. 2011). We determined the structures of several isolated TM helices of NHE1 using NMR. These were TM IV (residues 155–180), TM VI (229–236), TM VII (250–275), TM IX (338–365), and TM XI (447–472). Each putative TM segment had unique structures and none were complete, perfect helices. Often, discontinuity in the helices was in the middle of the TM segments and near or containing functionally important residues. Very briefly, TM IV was irregularly structured, not resembling a canonical alpha-helix. Residues 165–168, were an extended region containing two proline residues. For TM VI, residues 229–236 contained two helical regions oriented at approximately right angles to each other (residues 229–236 and 239–250) surrounding a central unwound region. TM VII (residues 250–275) was helical over residues 255–260 and 264–272, with the central residues at 261–263 non helical. TM IX (residues 338–365) contained two regions containing alpha helix structure at residues 340–344 and 353–359, with a 90° kink at Ser^{351} between the two regions which could provide flexibility. TM XI also contains two helical regions, residues 447–454 and 460–471 that are linked by an extended region of residues 455–459 (Lee et al. 2011).

NHE1 Physiological Roles

NHE1 has many roles in different cell types (Brett et al. 2005; Fliegel 2008). Knockout of NHE1 from cells demonstrates a role in growth, which is pronounced in more acidic media. Similarly, NHE1-knockout mice demonstrated decreased growth and also ataxia and epileptic-like seizures. NHE1 is additionally permissive in cell differentiation and in cell cycle progression. NHE1 modifies apoptosis. NHE1 is activated by trophic factor withdrawal in mouse β-cells, leading to cellular alkalinization and progression of apoptosis. This activation is through p38 dependent phosphorylation of the NHE1 tail (Malo and Fliegel 2006).

Pathological Roles

1. *NHE1 in transformed cells* – The role of NHE1 is cellular transformation and metastasis is significant. NHE1-dependent alkalinization plays a pivotal role in the development of a transformed phenotype of malignant cells, and inhibition of NHE1 prevents or reduces such development (Stock et al. 2008). This is particularly well studied in breast cancer. Metastasis is the leading cause of fatality in breast cancer. In breast cancer cells, NHE1 activation contributes to cell invasion (Stock et al. 2008). Early work showed that serum deprivation stimulated NHE1 activity in breast epithelial cell lines, in contrast to inhibiting it in non-tumor cells. Reshkin and coworkers championed the idea that a RhoA/p160ROCK/p38MAPK signaling pathway is responsible for mediating activation of NHE1 through serum deprivation (Stock et al. 2008).
   The mechanism by which NHE1 activity enhances invasion by breast cancer cells is not only by raising pH_i, but also by acidification of the extracellular microenvironment of tumor cells. The extracellular acidification is thought to be necessary for protease activation which facilitates the digestion and remodeling of the extracellular matrix (Stock et al. 2008), critical in metastasis.
2. *NHE1 and heart disease* – NHE is important in pH_i regulation in the myocardium. However, it also has a key role to play in several myocardial pathologies. The best known of these is the role of NHE1 in ischemia/reperfusion damage in the myocardium. During ischemia, anaerobic glycolytic metabolism is elevated resulting in increased proton production. This decreases pH_i and serves to activate NHE1, which leads to a rapid accumulation of sodium in the cell (Fliegel 2008). The high sodium concentration drives an increase in Ca^{2+} via reversal of the activity of the Na^+/Ca^{2+} exchanger and this triggers various deleterious pathways leading to cell death. Many preclinical studies have shown that NHE1 inhibition protects the myocardium from this calcium overload (Fliegel 2008). Activation of NHE1 regulatory pathways is also important in NHE1-mediated damage to the myocardium and this results in further detrimental activity of the NHE1 protein (Fliegel 2008).
3. *NHE1 in cardiac hypertrophy* – NHE1 is also important in cardiac hypertrophy (Fliegel 2008).

Studies have shown that NHE1 inhibition prevents cardiac hypertrophy in several different models of hypertrophy. We also demonstrated that the effect of the hypertrophic agonist aldosterone can be blocked by NHE1 inhibition. A possible mechanism by which NHE1 inhibition prevents hypertrophy is by prevention of increases in intracellular Na^+. The expression level and activity of NHE1 are elevated in a variety of cardiovascular diseases, including in hypertensive, hypertrophied, or diabetic myocardium (Fliegel 2008) which may accentuate the effects of NHE1 in these diseases.

Other cardiovascular conditions – NHE1 inhibitors have been useful in inhibiting diabetic vascular hypertrophy and they also prevent alterations in coronary endothelial function in streptozotocin-induced diabetes. NHE1 inhibitors also have antifibrillatory and antiarrhytmic effects in dogs and rats. Inhibition of NHE1 has also been shown to be protective in cardiac resuscitation models and NHE1 inhibition was beneficial in improving the outcome in a canine transplantation model. NHE1 inhibition may additionally be useful in treatment of circulatory. Overall, NHE1 inhibition has many and varied beneficial cardiovascular effects (Karmazyn et al. 2003).

Clinical Use of NHE1 Inhibitors

Trials with NHE1 inhibitors have not been very successful. Large-scale studies with various inhibitors have given mostly disappointing results (see (Avkiran et al. 2008) for review). In a small trial of patients with myocardial infarction who received coronary angioplasty, the NHE1 inhibitor cariporide had some beneficial effects on ejection fraction, wall-motion abnormalities and enzyme release. The NHE1 inhibitor eniporide was tested in a larger scale two-stage trial of myocardial infarction. It showed a dose dependent effect to reduce enzyme release, indicating reduced infarction. However, in a second later stage of the trial there was no beneficial effect and an overall negative effect. The GUARDIAN trial tested patients undergoing coronary artery bypass graft surgery. Analysis of subgroups of the trial showed that cariporide was beneficial possibly because treatment with the inhibitor in this trial was early (Avkiran et al. 2008). The EXPEDITION trial tested if the inhibitor cariporide reduces myocardial injury in patients with coronary artery bypass graft surgery. It was successful for this purpose; however, it also had adverse side effects and increasing cerebrovascular events significantly (Avkiran et al. 2008).

Summary

Na^+/H^+ exchangers are an important superfamily of cation transporting membrane proteins. There are several families of the monovalent cation proton antiporter (CPA) superfamily. Mammalian NHE1-NHE10 are part of the CPA1 family. They comprise ten distinct isoforms of NHE protein. NHE1 is the best studied. It is a plasma membrane pH regulatory protein that extrudes one intracellular proton in exchange for an extracellular Na^+. It is comprised of a 500 amino acid membrane domain and a 315 amino acid cytosolic regulatory domain. The topology of NHE1 is in dispute, though the structures of regions of the transmembrane domain have been elucidated. NHE1 is involved heart disease and cancer and is critical in cell growth and differentiation. To date, NHE1 inhibitors have shown great promise in preclinical studies on the myocardium, but these have not translated to beneficial clinical results. Future improvements in the design and use of inhibitors may result in clinically useful treatments.

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Cross-References

▶ Calmodulin
▶ Role of Na Ions in Biological Systems
▶ Sodium Transport in Eucaryotic Cells
▶ Sodium: Physical and Chemical Properties
▶ Types of Na-Binding Sites in Proteins

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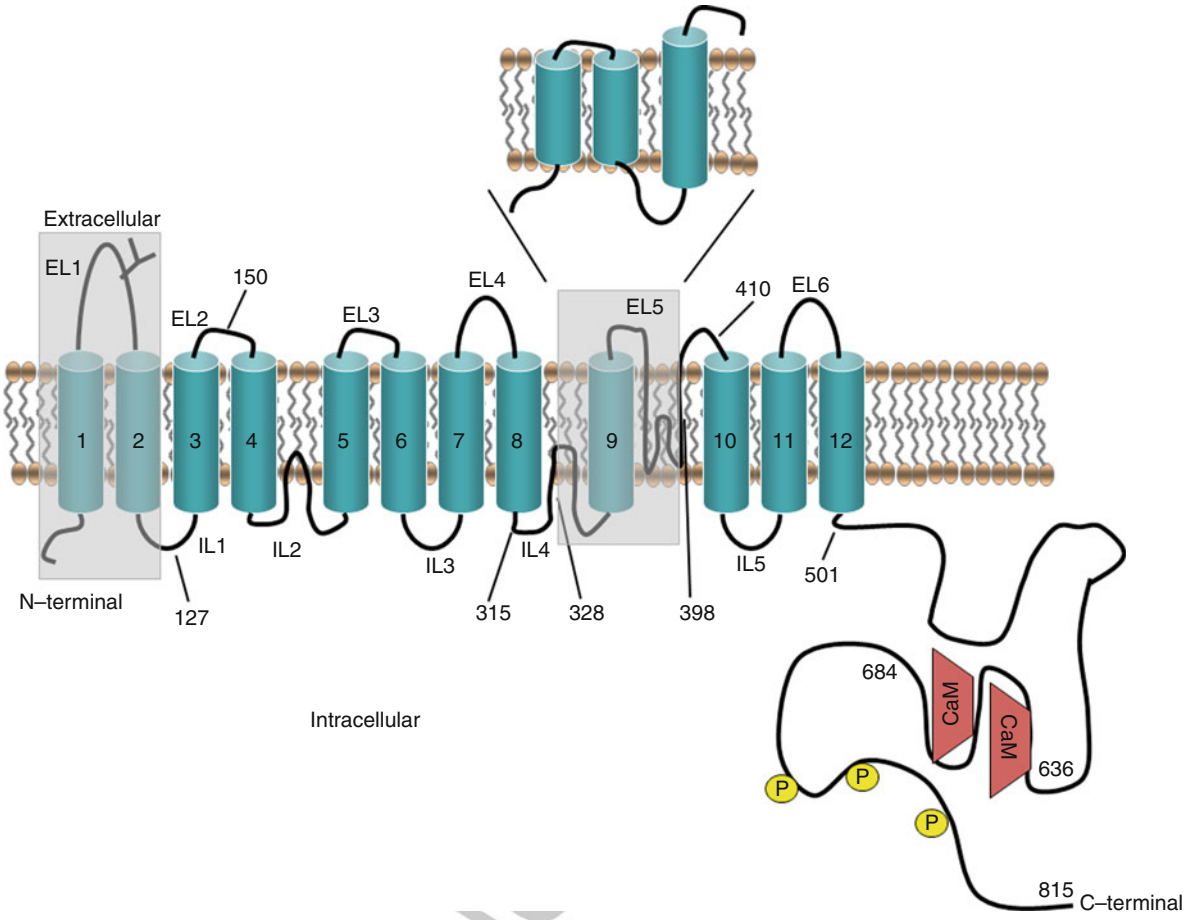

Na^+/H^+ Exchangers, Structure, and Function: Role in Human Health and Disease, Fig. 1 *Schematic diagram of different versions of the two dimensional topology of NHE1*. Alternative two dimensional models of NHE1 topology according to Wakabayashi et al. (2000) and Landau et al. (2000). TM segments according to Wakabayashi et al. (2000) are indicated by 1–12. The carboxyl and amino termini are indicated. Shaded TM's 1–2 are proposed to be deleted by Landau et al. (2000). The region with varying predicted topology of amino acids 328–398 is indicated by shading and the alternative topology proposed by Landau et al. (2000) is shown above. *P* indicates putative regulatory phosphorylation sites. CaM indicates regulatory calmodulin binding region. *EL* and *IL* refer to intracellular and extracellular loops according to Wakabayashi et al. (2000)

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