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Molecules in focus

The Na^+/H^+ exchanger isoform 1

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Abstract

The Na^+/H^+ exchanger (NHE) isoform 1 is a ubiquitously expressed integral membrane protein which regulates intracellular pH in mammalian cells. Nine isoforms of the Na^+/H^+ exchanger have been identified. The isoform first discovered has two domains: an N-terminal membrane domain containing approximately 500 amino acids and a C-terminal regulatory domain containing approximately 315 amino acids. The exchanger, which resides in the plasma membrane, exchanges an intracellular proton for an extracellular sodium, thereby regulating intracellular pH. It is involved in cell growth and differentiation, cell migration, and regulation of sodium fluxes. The Na^+/H^+ exchanger plays an important role in myocardial damage during ischemia and reperfusion and has recently been implicated as a mediator of cardiac hypertrophy. Inhibitors of the Na^+/H^+ exchanger, which may prove useful in the clinical treatment of these conditions, are currently being developed and clinical trials are underway.

Keywords: Cation transport; Membrane; Na^+/H^+ exchanger; pH regulation

1. Introduction

The Na^+/H^+ exchanger (NHE) is a ubiquitous protein in mammalian cells. It is an integral membrane protein which exchanges one intracellular H^+ ion for an extracellular Na^+ ion, thereby protecting cells from intracellular acidification. In addition, NHE participates in regulation of sodium fluxes and cell volume (Orlowski & Grinstein, 2003).

NHE was originally cloned in 1989 by Dr. J. Pouyssegur (Sardet, Franchi, & Pouysségur, 1989). Its activity is stimulated by growth factors. It contains 815 amino acids which are arranged in two domains, an integral membrane domain and a cytosolic "tail." Since its discovery, eight other isoforms have been identified (NHE2-NHE9). These isoforms each have a more restricted distribution and some, such as NHE6 and NHE7, are located within the cell rather than in the plasma membrane (Orlowski & Grinstein, 2003). The human NHE1 gene is found on chromosome 1 and homology among species for this isoform is extremely high. For the other isoforms, the degree of homology to NHE1 varies from 25 to 70%. NHE is sensitive to inhibition by amiloride derivatives (Orlowski & Grinstein, 2003).

Abbreviations: AngII, angiotension II; EGF, epidermal growth factor; ERM, ezrin, radixin and moesin; NCE, Na^+/Ca^{2+} exchanger; NHE, Na^+/H^+ exchanger; NHE1-8, Na^+/H^+ exchanger isoforms 1–8; TM, transmembrane segment

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2. Structure

The N-terminal integral membrane domain of the Na^+/H^+ exchanger (Fig. 1) is responsible for cation movement. This domain has 12 integral membrane segments (Wakabayashi, Pang, Su, & Shigekawa, 2000a), two intracellular loops which might fold into the lipid bilayers (2 and 4) and an extracellular loop (6) which might be a re-entrant loop. Several of the transmembrane (TM) segments are important in the protein's function.

2.1. TM IV

This segment is crucial in NHE1 function. The residues Phe161, Phe162, Leu163, and Gly173 all affect NHE's affinity for Na^+ and/or its resistance to inhibitors (Counillon, Franchi, & Pouyssegur, 1993; Counillon, Noel, Reithmeier, & Pouyssegur, 1997; Touret, Poujeol, & Counillon, 2001). We have recently demonstrated that Pro167 and Pro168 are critical in NHE1 activity (Slepkov et al., 2004).

2.2. TM VI and VII

In TM VII, Glu262 and Asp267 are critical for activity (Murtazina, Booth, Bullis, Singh, & Fliegel, 2001). Mutation of these residues from charged to uncharged eliminates NHE activity, whereas mutations that conserve the charge have little effect on the activity. Residues with the same relative position and the same charge were not critical in TM VI.

2.3. TM IX

TM IX is important in conferring sensitivity to antagonists. Specifically, a hybrid NHE1, in which TM IX has been replaced with TM IX from the amiloride-resistant NHE3, is resistant to amiloride (Orlowski & Kandasamy, 1996). In addition, Wang et al. (Wang, Balkovetz, & Warnock, 1995) have shown that alteration of His349 in TM IX reduces the protein's sensitivity to amiloride compounds.

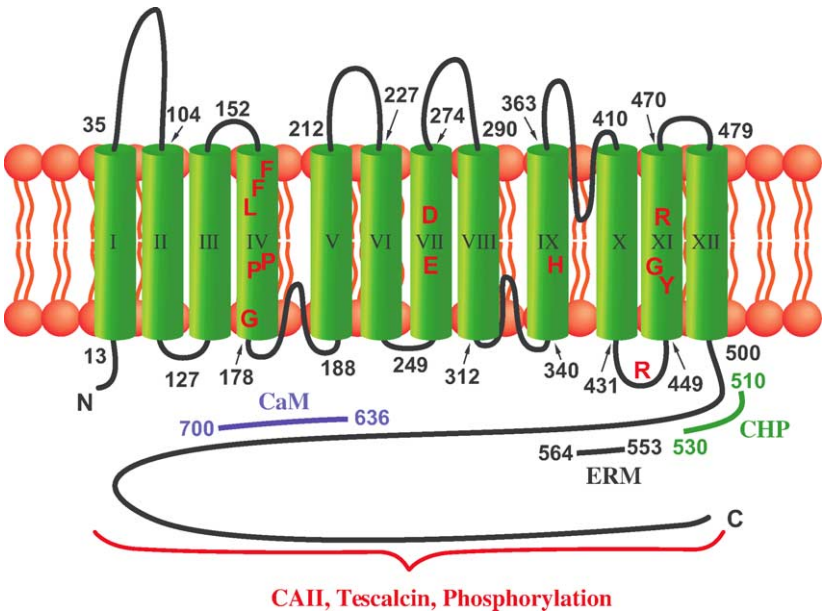

Fig. 1. Topological model of NHE1 (Wakabayashi et al., 2000a). Amino acid numbers at the aqueous-membrane interface are noted. Only one amino acid number in the middle of short loops is labeled. Amino acids in red (F161, F162, L163, G174, E262, D267, H349, Y454, G455, R440 and R458) are critical to NHE1 function. The approximate sites of binding of the proteins calcineurin homologous protein (CHP), calmodulin (CaM), carbonic anhydrase II (CAII), ERM proteins and tescalcin are noted. The region of phosphorylation by regulatory kinases is indicted.

2.4. TM XI

Mutation of amino acids Tyr454 and Arg458 has shown that both are essential in targeting NHE1 to the cell surface (Wakabayashi, Pang, Su, & Shigekawa, 2000b). TM XI and its neighboring intracellular loop (IL5) are also implicated in pH sensing. Mutation of Gly455 causes alkaline shifts in the pH-dependence of NHE activity but does not affect the protein's affinity for Na^+ or H^+ ions. In contrast, mutation of Arg440 in IL5 (Fig. 1) has the opposite effect, and removal of the positive charge impairs pH sensing (Wakabayashi, Hisamitsu, Pang, & Shigekawa, 2003).

The large, hydrophilic *cytosolic domain* of NHE1 (amino acids 500–815), which regulates the activity of the integral membrane domain, is a target for phosphorylation by protein kinases and for the binding of regulatory proteins (Fig. 1). Several protein kinases are thought to phosphorylate NHE1, including Erk1/2, $p90^{rsk}$, p160ROCK, p38, and a Nck-interacting kinase (Khaled et al., 2001; Moor, Karmazyn, & Fliegel, 2001; Tominaga, Ishizaki, Narumiya, & Barber, 1998). Phosphorylation shifts the pH-dependence of NHE1 activity, making the exchanger more active at a more alkaline pH. A number of regulatory proteins bind to the cytosolic domain of the Na^+/H^+ exchanger, including calmodulin, calcineurin homologous protein (CHP), tescalcin (Li, Liu, Kay, Muller-Esterl, & Fliegel, 2003), and carbonic anhydrase II (CAII) (Li, Alvarez, Casey, Reithmeier, & Fliegel, 2002). These proteins alter the pH-dependence of NHE1 activity, calmodulin and CAII (Li et al., 2002; Putney, Denker, & Barber, 2002) have stimulatory roles while tescalcin is inhibitory (Li et al., 2003).

3. Biological functions

The major function of NHE1 is the regulation of intracellular pH (Fig. 2A) and so, primarily, NHE1 is

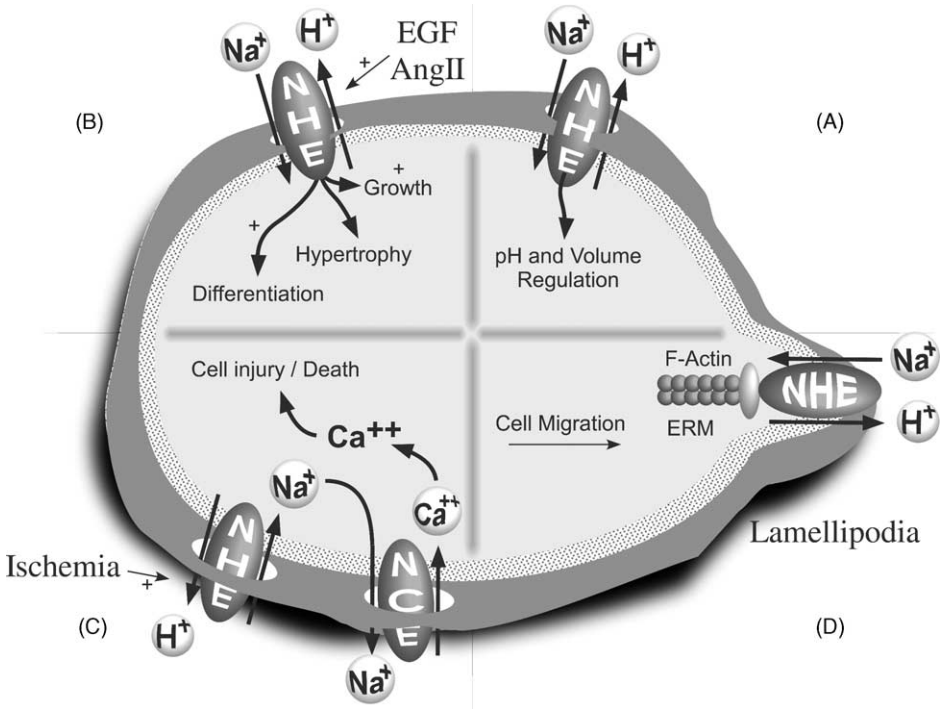

Fig. 2. Physiological functions of NHE1. (A) steady state pH regulation by NHE1. (B) hormones such as epidermal growth factor (EGF) and angiotension II (AngII) activate NHE1. This leads to increased cell growth and cell differentiation. In the myocardium, this can lead to hypertrophy. (C) activation of NHE1 during ischemia and reperfusion leads to increased intracellular Na^+ that results in increased intracellular calcium through the Na^+/Ca^{+2} exchanger (NCE) and ultimately cell damage and cell death. (D) in some cells NHE1 is found in lamellipodia. There cytoskeletal protein binding through ERM proteins and its activity are important in cell migration.

activated by decreases in intracellular pH. NHE1 activity is also stimulated by numerous hormones which activate protein kinases, and can act through auxiliary proteins.

The activation of NHE is associated with a variety of downstream events, including *cell proliferation*. Cell proliferation is markedly decreased in NHE-deficient cells, which have delayed transit through the G2-M checkpoint (Putney et al., 2002) (Fig. 2B). NHE1 also plays a role in *cell differentiation* (Fig. 2B), such that deletion/inhibition of NHE1 can impair differentiation pathways (Wang, Singh, & Fliegel, 1997). In some cell types NHE1 may play a role in *apoptosis*, since cells with active NHE1 are more resistant to apoptotic stimuli (Putney et al., 2002).

NHE1 knockout mice survive, but they show defective growth and reduced survival rates (Bell et al., 1999; Cox et al., 1997). In addition they exhibit severe neurological defects, including slow wave epilepsy, and ataxia with neuronal degradation (Bell et al., 1999; Cox et al., 1997; Putney et al., 2002).

NHE1 is also important in *cytoskeletal organization and cell migration* (Fig. 2D). In some cell types NHE1 is localized in lamellipodia, where its tail (amino acids 553–564) acts as an anchor for actin filaments via binding of ezrin, radixin and moesin (ERM) proteins. Disruption of the cytoskeletal binding site by mutation of these amino acids or inhibition of NHE1 activity, prevents formation of focal adhesions and inhibits cell migration (Denker & Barber, 2002; Putney et al., 2002).

4. Possible medical applications

NHE has several important biological functions and plays a role in the pathology of many diseases. In the myocardium, activation of NHE results in the accumulation of intracellular sodium. This, in turn, increases intracellular calcium levels, via action of the Na^+/Ca^{2+} exchanger, resulting in intracellular calcium overload and cell death (Fig. 2C). NHE inhibitors can block this cycle of cell damage. Many companies are currently developing and testing potent NHE inhibitors as potential therapeutic agents for the treatment of coronary artery disease. Some of these inhibitors are already involved in clinical studies (Karmazyn, 2003a).

Congestive heart failure is a major clinical problem in North America, and hypertrophy is an early maladaptive response. Very recently, NHE1 has been implicated in cardiac hypertrophy and heart failure. Specifically, hypertrophy can be prevented by blocking NHE activity (Fig. 2B). The mechanism by which this occurs is not known, but it could result from excess sodium accumulation due to NHE activity, followed by elevated protein kinase C activity and transcriptional changes resulting in hypertrophy (Karmazyn, 2003b).

5. Summary

The Na^+/H^+ exchanger is a protein that regulates cellular pH, but which has other physiological functions. NHE1 is located in the plasma membrane and affects intracellular pH, and cell growth, migration, differentiation, and apoptosis. The Na^+/H^+ exchanger also plays a significant role in the pathology of heart failure and, consequently, attempts are underway to develop effective therapeutic NHE inhibitors.

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References

Bell, S. M., Schreiner, C. M., Schultheis, P. J., Miller, M. L., Evans, R. L., & Vorhees, C. V. et al., (1999). Targeted disruption of the murine Nhe1 locus induces ataxia, growth retardation, and seizures. *American Journal of Physiology*, *276*, C788–C795.

Cox, G. A., Lutz, C. M., Yang, C.-L., Biemesderfer, D., Bronson, R. T., & Fu, A. et al., (1997). Sodium/hydrogen exchanger gene defect in slow-wave epilepsy mice. *Cell*, *91*, 139–148.

Counillon, L., Franchi, A., & Pouyssegur, J. (1993). A point mutation of the Na^+/H^+ exchanger gene (NHE1) and amplification of the mutated allele confer amiloride resistance upon chronic acidosis. *Proceedings of the National Academy of Sciences of the United States of America*, *90*, 4508–4512.

Counillon, L., Noel, J., Reithmeier, R. A. F., & Pouyssegur, J. (1997). Random mutagenesis reveals a novel site involved in inhibitor interaction within the fourth transmembrane segment of the Na^+/H^+ exchanger-1. *Biochemistry*, *36*, 2951–2959.

Denker, S. P., & Barber, D. L. (2002). Cell migration requires both ion translocation and cytoskeletal anchoring by the Na-H exchanger NHE1. *The Journal of Cell Biology*, *159*, 1087–1096.

Karmazyn, M. (2003a). Mechanisms underlying NHE-1 involvement in myocardial ischemic and reperfusion injury. In M. Karmazyn, M. Avkiran, & L. Fliegel (Eds.), *The Na^+/H^+ exchanger, from molecular to its role in disease* (pp. 221–237). Boston, Dordrecht, London: Kluwer Academic Publishers.

Karmazyn, M. (2003b). Role of NHE-1 in cardiac hypertrophy and heart failure. In M. Karmazyn, M. Avkiran, & L. Fliegel (Eds.), *The Na^+/H^+ exchanger, from molecular to its role in disease* (pp. 211–219). Boston, Dordrecht, London: Kluwer Academic Publishers.

Khaled, A. R., Moor, A. N., Li, A., Kim, K., Ferris, D. K., & Muegge, K. et al., (2001). Trophic factor withdrawal: p38 mitogen-activated protein kinase activates NHE1, which induces intracellular alkalinization. *Molecular and Cellular Biology*, *21*, 7545–7557.

Li, X., Alvarez, B., Casey, J. R., Reithmeier, R. A., & Fliegel, L. (2002). Carbonic anhydrase II binds to and enhances activity of the Na^+/H^+ exchanger. *Journal of Biological Chemistry*, *277*, 36085–36091.

Li, X., Liu, Y., Kay, C. M., Muller-Esterl, W., & Fliegel, L. (2003). The Na(+)/H(+) exchanger cytoplasmic tail: Structure, function, and interactions with tescalcin. *Biochemistry*, *42*, 7448–7456.

Moor, A., Karmazyn, X. T. G. M., & Fliegel, L. (2001). Protein kinase mediated regulation of the Na^+/H^+ exchanger isoform 1 (NHE1) in ischemic and ischemic-reperfused rat heart. *Journal of Biological Chemistry*, *27*, 16113–16122.

Murtazina, B., Booth, B. J., Bullis, B. L., Singh, D. N., & Fliegel, L. (2001). Functional analysis of polar amino acid residues in membrane associated regions of the NHE1 isoform of the Na^+/H^+ exchanger. *European Journal of Biochemistry*, *268*, 1–13.

Orlowski, J., & Grinstein, S. (2003). In M. Karmazyn, M. Avkiran, & L. Fliegel (Eds.), *The Na^+/H^+ exchanger, from molecular to its role in disease* (pp. 17–34). Boston, Dordrecht, London: Kluwer Academic Publishers.

Orlowski, J., & Kandasamy, R. A. (1996). Delineation of transmembrane domains of the Na^+/H^+ exchanger that confer sensitivity to pharmacological antagonists. *Journal of Biological Chemistry*, *271*, 19922–19927.

Putney, L. K., Denker, S. P., & Barber, D. L. (2002). The changing face of the Na^+/H^+ exchanger, NHE1: Structure, regulation, and cellular actions. *Annual Review of Pharmacology and Toxicology*, *42*, 527–552.

Sardet, C., Franchi, A., & Pouysségur, J. (1989). Molecular cloning, primary structure, and expression of the human growth factor-activatable Na^+/H^+ antiporter. *Cell*, *56*, 271–280.

Slepkov, E. R., Chow, S., Lemieux, M. J., & Fliegel, L. (2004). Proline resides in transmembrane segment IV are critical for activity, expression, and membrane targeting of the Na^+/H^+ exchanger isoform 1. *Biochem. J.*, *379*, 31–38.

Tominaga, T., Ishizaki, T., Narumiya, S., & Barber, D. L. (1998). p160ROCK mediates RhoA activation of Na^+-H^+ exchange. *The EMBO Journal*, *17*, 4712–4722.

Touret, N., Poujeol, P., & Counillon, L. (2001). Second-site revertants of a low-sodium-affinity mutant of the Na^+/H^+ exchanger reveal the participation of TM4 into a highly constrained sodium-binding site. *Biochemistry*, *40*, 5095–5101.

Wakabayashi, S., Pang, T., Su, X., & Shigekawa, M. (2000a). A novel topology model of the human Na^+/H^+ exchanger isoform 1. *Journal of Biological Chemistry*, *275*, 7942–7949.

Wakabayashi, S., Pang, T., Su, X., & Shigekawa, M. (2000b). Second mutations rescue point mutant of the Na(+)/H(+) exchanger NHE1 showing defective surface expression. *FEBS Letters*, *487*, 257–261.

Wakabayashi, S., Hisamitsu, T., Pang, T., & Shigekawa, M. (2003). Mutations of Arg 440 and Gly455/Gly456 oppositely change pH sensing of Na^+/H^+ exchanger 1. *Journal of Biological Chemistry*, *278*, 11828–11835.

Wang, D., Balkovetz, D. F., & Warnock, D. G. (1995). Mutational analysis of transmembrane histidines in the amiloride-sensitive Na^+/H^+ exchanger. *American Journal of Physiology*, *269*, C392–C402.

Wang, H., Singh, D., & Fliegel, L. (1997). The Na^+/H^+ antiporter potentiates growth and retinoic-acid induced differentiation of P19 embryonal carcinoma cells. *Journal of Biological Chemistry*, *272*, 26545–26549.