MINIREVIEW / MINISYNTHÈSE

The cardiac Na-H exchanger: a key downstream mediator for the cellular hypertrophic effects of paracrine, autocrine and hormonal factors[1]

Larry Fliegel and Morris Karmazyn

Abstract: The major mechanism by which the heart cell regulates intracellular pH is the Na^+–H^+ exchanger (NHE) with the NHE-1 isoform as the primary cardiac subtype. Although NHE-1 has been implicated in mediating ischemic injury, more recent evidence implicates the antiporter as a key mediator of hypertrophy, which is produced by various autocrine, paracrine and hormonal factors such as endothelin-1, angiotensin II, and α_1 adrenoceptor agonists. These agonists activate the antiporter via phosphorylation-dependent processes. NHE-1 inhibition is likely conducive to attenuating the remodelling process after myocardial infarction. These effects probably occur independently of infarct size reduction and involve attenuation of subsequent postinfarction heart failure. As such, inhibitors of NHE offer substantial promise for clinical development that will attenuate acute responses to myocardial postinfarction and chronic postinfarction, which evolve toward heart failure. The regulation of NHE-1 is discussed as is its potential role in mediating cardiomyocyte hypertrophy.

Key words: NHE-1, cardiac hypertrophy, heart failure, myocardial remodelling.

Résumé : Le mécanisme principal par lequel le pH intracellulaire est régulé dans la cellule cardiaque implique l'échangeur Na^+–H^+ ou NHE, l'isoforme NHE-1 étant considérée comme principal sous-type cardiaque. Quoique NHE-1 ait été impliqué comme médiateur du dommage ischémique, des évidences plus récentes impliquent cet antiport comme médiateur clé dans l'hypertrophie produite par divers facteurs autocrines, paracrines et hormonaux comme l'endothéline-1, l'angiotensine II et les agonistes α_1-adrénergiques qui activent l'antiport par des processus dépendant de la phosphorylation. L'inhibition de NHE-1 peut probablement mener à une atténuation du processus de remodelage après un infarctus du myocarde, indépendamment de la réduction de la taille de l'infarctus, et à l'atténuation de l'insuffisance cardiaque subséquente à un infarctus. Ainsi, les inhibiteurs de NHE offrent d'importantes promesses de développement clinique, afin d'atténuer tant les réponses aiguës du myocarde que les réponses chroniques post-infarctus qui évoluent vers l'insuffisance cardiaque. La régulation de NHE-1 et son rôle potentiel comme médiateur de l'hypertrophie des cardiomyocytes sont discutés.

Mots clés : NHE-1, hypertrophie cardiaque, insuffisance cardiaque, remodelage myocardique.

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L. Fliegel. Department of Biochemistry, University of Alberta, Edmonton, AB T6G 2H7, Canada
M. Karmazyn.[2] Department of Physiology and Pharmacology, University of Western Ontario, Medical Sciences Building, London, ON N6A 5C1, Canada.

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[2]Corresponding author (email: Morris.Karmazyn@fmd.uwo.ca).

Introduction

Heart failure is a major challenge of the 21st century. The incidence of heart failure has risen. It continues to rise dramatically, with death rates from heart failure more than doubling in the past 10 years. Currently, the 5-year mortality rate for heart failure is about 50 percent. In addition to mortality from pump failure, these patients exhibit an incidence of sudden cardiac death at 6–9 times the rate of the general population. Heart failure is not a disease per se but rather a complex clinical syndrome. It is the final common pathway of numerous cellular and molecular defects caused by many instigating factors. As a result of advances in molecular and

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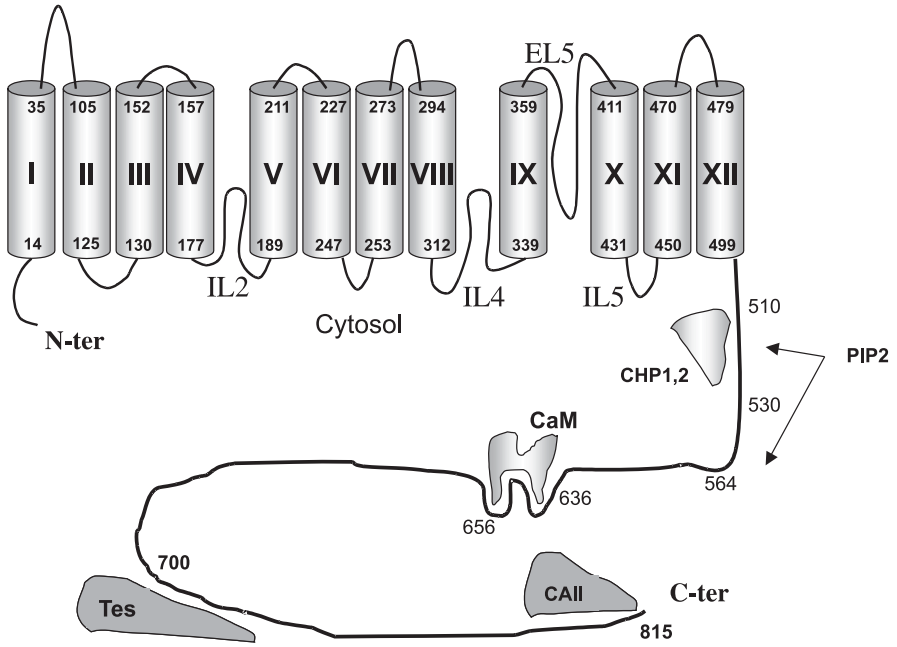
Fig. 1. Proposed model of the NHE-1 isoform of the Na^+/H^+ exchanger. CAII, putative carbonic anhydrase II binding site; CaM, calmodulin; CHP, calcineurin homologous protein binding site; PIP2, phosphatidylinositol 4,5-bisphosphate binding site; Tes, putative tescalcin binding site. IL and EL refer to intracellular and extracellular loops respectively. C-ter, C-terminal; N-ter, N-terminal.



cellular biology, it is now known that heart failure extends beyond abnormal heart function and organ physiology but involves numerous intracellular defects. Moreover, the complexity of the heart failure process is well-known, particularly in view of the numerous cellular and molecular changes that are seen in heart failure, many of which appear to be interrelated. Two important components underlying heart failure include the initial adaptive hypertrophic response which follows myocardial injury and the second, the subsequent evolution to heart failure. Indeed, inhibition of the early (mal)adaptive hypertrophy is an important therapeutic component, which can result in an attenuation of the heart failure response. NHE-1 therefore represents not only a key intracellular pH regulatory process in the cardiac cell after induction of acidosis but emerging evidence suggests that it may play an important role in cell growth.

NHE-1 as a mediator of the hypertrophic phenotype

There are a number of lines of evidence suggesting that NHE-1 may represent a key factor mediating hypertrophic responses, especially after myocardial infarction. This suggests that the exchanger could be an important cellular target for attenuation of both the hypertrophic responses as well as heart failure. From a theoretical perspective, it is important to indicate that NHE-1 stimulation can occur through receptor-dependent mechanisms. This reflects the fact that the antiporter is the target of multiple signalling pathways, such as those activated by various kinases and G protein-coupled receptors which mediate hypertrophic responses to numerous paracrine, autocrine, and hormonal factors. Thus, NHE-1 activation appears to be a common downstream target for many hypertrophic factors important in the etiology of myocardial hypertrophy, remodelling, and heart failure. As suggested below, kinase activation may represent an important mechanism for NHE-1 dependent hypertrophic responses, particularly in response to various paracrine and autocrine factors such as endothelin-1, angiotensin II, and α_1 adrenergic agonists.

NHE-1 structure

The Na^+–H^+ exchanger (NHE) is composed of 2 major domains, a transmembrane domain and a large intracellular cytoplasmic domain (Fig. 1). The N-terminal membrane domain of approximately 500 amino acids represents the region responsible for cation transport. It contains a proton-sensing site that regulates activity of the protein (Wakabayashi et al. 1992). The membrane domain has 12 integral membrane segments (Wakabayashi et al. 2000) — intracellular loops 2 and 4 may invaginate into the lipid bilayer and extracellular loop 5 is thought to be a re-entrant loop. The large C-terminal cytoplasmic tail of approximately 315 amino acids regulates the membrane domain and influences activity of NHE (Wakabayashi et al. 1992; Orlowski and Grinstein 1997; Counillon and Pouyssegur 2000). It is phosphorylated by protein kinases and binds regulatory proteins and co-factors. The detailed physical structure of the protein is not yet known, though it is known to be a dimer (Fliegel et al. 1993).

Regulation of NHE-1 by proteins and cofactors

NHE-1 is one of the most closely regulated transporters currently, which occurs by a combination of proteins, co-factors, and phosphorylation-dependent reactions involving a

number of protein kinases including ERK and $p90^{rsk}$ (Fliegel 1999; Moor et al. 2001). Phosphorylation likely accounts for approximately half of the growth factor-dependent stimulation of activity. Only 50% of the growth factor stimulation of NHE activity occurs when the phosphorylatable distal region of the cytoplasmic domain is removed (Bianchini et al. 1995; Noel and Pouyssegur 1995; Counillon and Pouyssegur 2000) (Fig. 1). NHE-1 is also regulated by a variety of proteins including calmodulin, tescalcin, calcineurin homologous protein, and carbonic anhydrase, as reviewed below.

Regulation by calmodulin

The proximal cytosolic domain (Fig. 1) between amino acids 636 to 700 contains high and low affinity calmodulin binding sites (Bertrand et al. 1994; Wakabayashi et al. 1994). The high affinity site acts as an endogenous inhibitor of the protein. Increases in intracellular Ca^{2+} relieve this inhibition and stimulate the antiporter. Mutations to the calmodulin binding site that prevent calmodulin binding cause a hyperactive Na^+/H^+ exchanger (Wakabayashi et al. 1994).

Regulation by CHP

Another regulatory protein of the NHE is calcineurin homologous protein (CHP). CHP exists in two isoforms, CHP1 and CHP2 (Pang et al. 2001, 2002). CHP1 is homologous to both calmodulin and calcineurin (regulatory subunit). Overexpression of CHP1 prevents stimulation of NHE-1 activity in some cell types (Lin and Barber 1996). CHP1 binds to the tail of the antiporter between amino acids 510 to 530 (Pang et al. 2001; Lin and Barber 1996) and acts as an inhibitor that is released by growth factor stimulation (Fig. 1). CHP2 shares 61% amino acid identity with CHP1, and is expressed at a much lower level in most human tissues. It is not detectable in human myocardium, but has been identified in malignant cells (Pang et al. 2002).

Regulation by tescalcin

Tescalcin is a newly discovered calcium-binding protein that binds to the C-terminus of NHE (Li et al. 2003; Mailander et al. 2001). Tescalcin is homologous to CHP and calmodulin. However, it does not bind to the same region of the NHE-1 tail as these 2 proteins, but rather binds directly to the NHE C-terminal 182 amino acid residue (Li et al. 2003). Cells stably transfected with tescalcin exhibit a marked inhibition in NHE-1 activity following acid loading, suggesting that the protein exerts an endogenous NHE-1 inhibitory property (Li et al. 2003).

Regulation by PIP2

The most proximal region of the cytoplasmic tail of NHE-1 next to the membrane contains 2 binding motifs at amino acids 509–516 and 552–560 for phosphatidylinositol 4,5-bisphosphate [PIP_2] (Aharonovitz et al. 2000) (Fig. 1). Like other polyphosphoinositides, PIP_2 is a ubiquitous constituent of animal plasma membranes, which exerts regulatory effects on K^+ channels and Na^+/Ca^{2+} antiporters (Hilgemann and Ball 1996; Baukrowitz et al. 1998). Reduction of PIP_2 or depletion of ATP in the cell have both been shown to inhibit NHE-1 activity (Aharonovitz et al. 2000).

Regulation by carbonic anhydrase II (CAII)

Carbonic anhydrases catalyze the hydration of CO_2 to produce HCO_3^- and H^+. They exert their effects by binding to anion exchange proteins at acidic residues (Vince and Reithmeier 1998, 2000). In the case of CAII, this facilitates bicarbonate transport forming a metabolon, which moves ions more efficiently (Vince and Reithmeier 1998, 2000). Interestingly, carbonic anhydrases also bind to the cytosolic tail of NHE-1 and improve efficiency of proton export. CAII binds to the C-terminal 178 amino acids of the cytoplasmic tail. The CAII binding process is augmented by NHE-1 phosphorylation, with the net result of increasing NHE-1 activity (Li et al. 2002). The significance of these findings needs to be studied further, although this attests to the complex nature of NHE-1 regulation. Moreover, the findings suggest a potential additional approach for inhibiting NHE-1 by blocking CAII activity. Indeed, of relevance to the present review, CAII inhibition has recently been shown to exert an antihypertrophic effects in cultured cardiomyocytes through a mechanism associated with NHE-1 downregulation (Alvarez et al. 2004).

Cytosolic domain structure of NHE-1

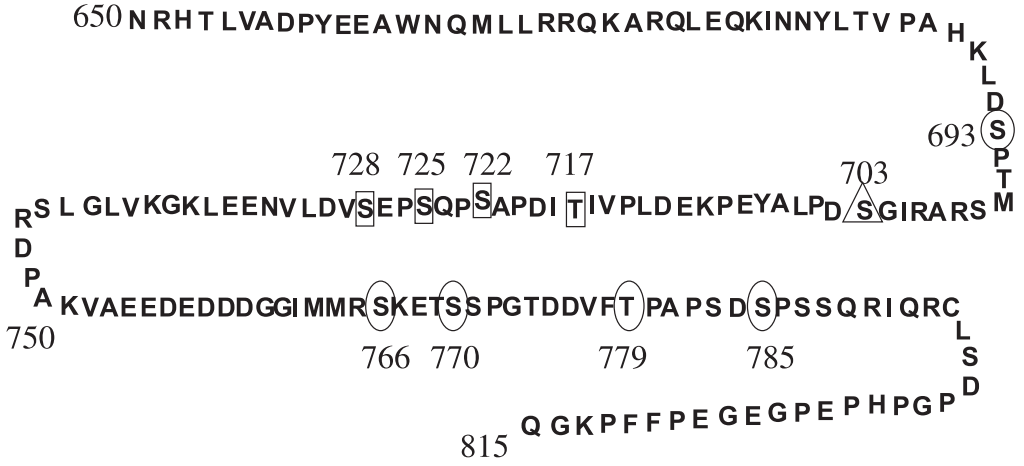
Gebreselassie et al. 1998 examined the structure of amino acids 516–815 and found that it contained approximately 35% α-helix, 17% β-turn, and 48% random coil. It has also been shown that amino acids 633–815 are primarily monomeric, undergo pH dependent changes in conformation, and are folded in nature with very little α-helical structure but a preponderance of β-structure (Li et al. 2003; Rieder and Fliegel 2003). Overall, the results suggest that amino acids near the membrane (516–633) must contain significant amount of α-helix, while those more distal are predominantly of β-structure. Since a decrease in pH affects the structure of the C-terminal region and regulates the membrane domain in a pH-dependent manner (Li et al. 2003), this may account for some of the pH sensitivity of NHE.

Regulation by phosphorylation (non-myocardial tissues)

About 50% of the growth factor stimulation of NHE-1activity occurs at the phosphorylatable distal region of the NHE tail (Bianchini et al. 1995; Counillon and Pouyssegur 2000; Noel and Pouyssegur 1995). Briefly, for non-cardiac tissue, it has been shown that a variety of hormones such as thrombin and epidermal growth factor stimulate phosphorylation of the cytosolic domain. These agents shift the set point such that NHE is more active at more alkaline pHs (Sardet et al. 1990).

The exact protein kinases involved are still under investigation, although ERK1/2 from skeletal muscle has been shown to directly phosphorylate the cytosolic domain of NHE-1 (Moor and Fliegel 1999; Wang et al. 1997; Wei et al. 2001; Kusuhara et al. 1998). Moreover, transfecting Chinese

Fig. 2. Molecular model of C-terminal 165 amino acids of the NHE-1 isoform of the Na^+/H^+ exchanger. The amino acid sequence (1-letter abbreviation) of amino acids 650 to 815 is illustrated. Square indicates amino acids that have been shown to be phosphorylated by p38 whereas ovals indicate amino acids phosphorylated by ERK, and triangle indicates an amino acid phosphorylated by $p90^{rsk}$.



hamster ovary cells with an inducible ERK dominant-negative mutant caused a reduction in serum-stimulated alkalinization, suggesting a physiological function for the kinase (Wang et al. 1997).

A kinase downstream of ERK, $p90^{rsk}$, has been reported to phosphorylate the cytosolic domain of NHE-1 at amino acid 703 in smooth muscle cells (Phan et al. 1997; Takahashi et al. 1997). Another kinase, $p160^{ROCK}$, also mediates activation of the NHE in non-myocardial tissue (Tominaga et al. 1998), and plays a role in cytoskeleton organization (Tominaga et al. 1998). p38 has additionally been suggested to negatively regulate NHE-1 in smooth muscle (Kusuhara et al. 1998). However, p38 has also been shown to phosphorylates NHE-1 and stimulate activity in pro-b cell lines (Khaled et al. 2001). NIK, a recently identified Nck-interacting kinase, binds to, activates, and phosphorylates NHE-1 in response to platelet-derived growth factor in human embryonic kidney cells (HEK293) and fibroblasts (Yan et al. 2001). It has also been demonstrated that Ca^{2+}/calmodulin-dependent kinase II can phosphorylate the C-terminal domain of NHE-1 in vitro (Fliegel et al. 1992).

Several protein kinases are known to stimulate activity of NHE-1 through indirect mechanisms such as other regulatory proteins. For example, protein kinase C (PKC) pathways are activated by phorbol esters such as 12-myristate 13-acetate (PMA), and stimulate NHE-1 activity (Orlowski and Shull 1996). In addition, in vascular smooth muscle down-regulation of PKC and inhibitors of PKC pathways block aldosterone-induced activation of NHE-1 (Ebata et al. 1999). However, it appears that PKC is unable to phosphorylate NHE-1 directly and the antiporter does not express consensus sites for phosphorylation by PKC (Fliegel et al. 1992; Wang et al. 1997).

NHE-1 activity can also be mediated by protein kinase A (PKA), even though NHE-1 does not contain any consensus sites for PKA and PKA does not phosphorylate NHE-1 directly. The activation of NHE-1 by PKA is likely cell specific. For example, human and rabbit NHE-1 expressed in PS120 (NHE-1-deficient) cells do not show any response to cAMP analogs (Borgese et al. 1992) whereas human NHE-1 expressed in opossum kidney cells is inhibited by PKA (Helmle-Kolb et al. 1993). In contrast, cAMP stimulates NHE-1 activity in primary rat hepatocytes (Moule and McGivan 1990), rat osteoblastic UMR-106 cells (Gupta et al. 1994), and AP1-NHE-1 cells (Kandasamy et al. 1995).

Localization of phosphorylation sites

The exact amino acids phosphorylated by protein kinases has been partially elucidated but this area is still in need of further investigation. We have shown that p38 phosphorylation that occurs in pro-b cell lines (Khaled et al. 2001) is at 4 amino acids in NHE-1: Thr 717, Ser 722, Ser 725, and Ser 728. These were important in activation of the NHE that mediates apoptosis (Khaled et al. 2001). NIK, a recently identified Nck-interacting kinase, binds to, activates, and phosphorylates NHE-1 in response to platelet-derived growth factor in human embryonic kidney cells (HEK293) and fibroblasts. The phosphorylation sites of NIK are distal to amino acid 638 and are distinct from the NIK-binding site on NHE-1 (between amino acids 538 and 638) (Yan et al. 2001). We have earlier shown that Ca^{2+}–calmodulin-dependent kinase II phosphorylation is on the C-terminal domain of NHE-1 on the last 178 amino acids, but the exact amino acids were not identified (Fliegel et al. 1992). Recently (Liu et al. 2004) we have shown that in vitro ERK phosphorylates Ser 693, Ser 766, Ser 770, Thr 779, and Ser 785. Earlier work has also suggested that amino acid Ser703 is phosphorylated by $p90^{rsk}$ (Takahashi et al. 1999). Figure 2 illustrates the amino acids of the cytosolic tail and the amino acids that have been identified as phosphorylated by protein kinases. The exact site of phosphorylation for the other protein kinases including NIK, $p160^{Rock}$, and calmodulin-dependent kinase II has not been elucidated.

Receptor-mediated regulation of myocardial NHE-1

Key autocrine, paracrine, and hormonal factors are important in cardiac pathology, especially maladaptive growth (dis-

cussed in detail see below), which generally involve G-protein coupled receptors, such as catecholamine-induced NHE-1 activation via α_1-adrenergic receptor stimulation (Wallert and Frohlich 1992). This stimulation raises steady state pHi and enhances the rate of NHE mediated recovery from an acute acid load (Terzic et al. 1982; Wallert and Frohlich 1992). It appears that this effect requires ERK but not p38 (Snabaitis et al. 2000). The effect is mediated by the α_{1A}-adrenorecptor subtype (Yokoyama et al. 1998) through ERK and protein kinase C - dependent pathways (Snabaitis et al. 2000).

Angiotensin II (Ang II) is a peptide hormone which stimulates cardiomyocyte growth and is a potent NHE-1 activator in intact hearts and rat cardiomyocytes (Grace et al. 1996; Ito et al. 1997). The stimulation occurs through the AT1 receptor and is antagonized by AT2 receptors (Gunasegaram et al. 1999).

NHE-1 is also stimulated by the 21-amino acid vasoactive peptide endothelin-1 (ET-1) (Kramer et al. 1991; Meyer et al. 1996). In isolated myocytes it accelerates pHi recovery from an acid load (Ito et al. 1997) and increases resting pH (Moor and Fliegel 1999), which may be dependent on ERK dependent pathways (Moor and Fliegel 1999). The role of PKC in this activation is uncertain because diverse effects have been reported with PKC inhibitors (Kramer et al. 1991; Wu and Tseng 1993).

A number of other less well-characterized receptor-mediated pathways also modulate myocardial NHE-1 activity. For example, thrombin and muscarinic receptor agonists activate NHE-1 through a PKC dependent pathway (Yasutake et al. 1996; Wu and Vaughan-Jones 1994) and adenosine inhibit NHE-1 via A_1 receptor activation (Avkiran and Yokoyama 2000).

Aldosterone has been shown to activate NHE-1 activity in cardiac cells after prolonged treatment (Gekle et al. 2002; Korichneva et al. 1995), although a recent study reported significantly increased NHE-1 expression in cultured cardiomyocytes after 24-hour exposure to aldosterone (Karmazyn et al. 2003).

Protein kinase - mediated regulation of NHE-1 in the myocardium

Specific protein kinases that are involved in regulation and phosphorylation of NHE-1 are most likely downstream of cardiac receptors. One study has shown that protein kinase D contributes to regulation of the NHE but likely does not phosphorylate the protein directly (Haworth et al. 1999). The ability of immunoprecipitated $p90^{rsk}$ and ERK1/2 to directly phosphorylate and regulate NHE-1 in the myocardium and the enhancement of this effect has previously been reported an effect which was abolished by the MEK inhibitor, PD98059 (Moor and Fliegel 1999). An important role for ERK has recently been reported for acidosis-induced NHE-1 activation in cardiomyocytes (Haworth et al. 2003).

A number of other protein kinases and pathways are also likely involved in the regulation of NHE-1 in the myocardium although these have not been well characterized. They include novel protein kinases of molecular mass 55, 44, and 40 kDa, which were able to phosphorylate the antiporter. These protein kinases contrast with JNK, MEK or p38, where significant direct phosphorylation by JNK, MEK or p38 was not observed (Moor et al. 2001). We have also shown that in isolated myocytes, CaM kinase II inhibition reduced NHE-1 activity and that myosin light chain kinase is important in osmotic regulation of the NHE in the myocardium (Moor et al. 2000).

NHE-1 as a mediator of cardiac pathology

NHE-1 in myocardial ischemic and reperfusion injury

It was first demonstrated in 1988 that NHE inhibition could represent a potentially effective approach towards limiting myocardial ischemic and reperfusion injury (Karmazyn 1988). Since that initial report, extensive evidence has been presented implicating NHE-1 in myocardial, ischemic, and reperfusion injury (Karmazyn et al. 1999, 2001; Avkiran and Marber 2002). Its role in such injury reflects the inability to extrude Na by the ischemic cardiac cell due to Na-K ATPase inhibition occurring in concert with NHE-1 activation. Thus, the net result is an elevation in intracellular sodium levels, which in turn increases intracellular Ca levels via Na-Ca exchange and results in cell injury. Numerous studies have shown robust protection by NHE-1 inhibitors against various forms of dysfunction including reduced mortality, limitation of infarct size, improvement of functional recovery after reperfusion, reduction of arrhythmias, attenuation of Ca and Na dyshomeostasis, and reduction of apoptosis as well as preservation of metabolic status (Karmazyn et al. 1999, 2001). The protection appears to be superior to other cardioprotective strategies, including ischemic preconditioning (Gumina et al. 1999; Gumina and Gross 1999; Haist et al. 2003).

Clinical development and testing of NHE-1 inhibitors in cardiac disease has been quite rapid, possibly reflecting the consistent and excellent protection these agents demonstrate in animal studies. A number of clinical studies have been carried out with various degrees of success (Théroux et al. 2000; Karmazyn 2000; Rupprecht et al. 2000; Zeymer et al. 2001). The most recent study, the EXPEDITION study, has evaluated the NHE-1 specific inhibitor cariporide in high risk coronary artery bypass grafting patients. The primary endpoints were myocardial infarction or death 1 week and 6 months after surgery. The study revealed a highly significant relative risk reduction by cariporide. Indeed, this represented the first study that demonstrated clinical efficacy of a cardioprotective strategy (Mentzer and the EXPEDITION Study Investigators 2003). Unfortunately and unexpectedly, the use of cariporide was also associated with a significantly greater incidence of strokes, thus negating the overall benefit of this treatment. The mechanistic basis for increased strokes in these patients is unknown and requires further studies to resolve.

NHE-1 as a key downstream mediator of cardiac hypertrophy and heart failure

Experimental evidence for NHE-1 involvement in cardiac cell hypertrophy

As noted, NHE-1 represents a key downstream factor activated by a variety of hypertrophic stimuli, a property which is likely not shared by any other cellular regulatory process.

The ability of a large number of hypertrophic stimuli to activate NHE-1 does not necessarily confirm or prove cause- and-effect relationships. However, this concept has been reinforced in studies which have utilized pharmacological inhibitors of the antiporter.

A number of investigators have used cultured neonatal cardiac myocytes or isolated tissues to demonstrate NHE-1 involvement in hypertrophy. These in vitro approaches are advantageous in that one can study precisely the direct hypertrophic responses to relevant stimuli in the absence of other contributing factors. Moreover, the study of the cellular and molecular basis for hypertrophy is facilitated by these types of investigations. The primary limitation of using in vitro methodology is that the complete picture of the complex underlying mechanisms contributing to both the hypertrophy and the subsequent development of heart failure cannot be fully addressed. In addition, the use of neonatal cells could be problematic. Results using such cells should be interpreted cautiously, since these cells likely possess different relative receptor subtypes or cell signalling mechanisms from adult tissues. Nonetheless, the use of cultured neonatal cardiac myocytes have provided useful and important information for understanding mechanisms of hypertrophic responses.

Studies using cardiac cells have consistently demonstrated that NHE-1 inhibitors block hypertrophic responses to various stimuli. For example, stretch-induced stimulation in protein synthesis in neonatal cardiac myocytes as well as stretch-induced alkalinization in feline papillary muscles can be blocked by NHE inhibitors (Cingolani et al. 1998; Yamazaki et al. 1998), as can norepinephrine-induced protein synthesis in cultured rat cardiomyocytes (Hori et al. 1990). We recently demonstrated that aldosterone induces hypertrophy in cultured neonatal ventricular myocytes through a NHE-1 dependent pathway (Karmazyn et al. 2003). Thus, the fact that NHE inhibitors can block the hypertrophic response to a wide variety of stimuli is strongly suggestive of the antiporter as a common key downstream mediator.

Experimental evidence for NHE involvement in heart failure

Infarction-induced heart failure

Research into the potential role of NHE, or more specifically, NHE-1, in the development of heart failure have used in vivo approaches and well-defined heart failure models. Initial experiments in this area have used relatively nonspecific inhibitors of the antiporter, such as amiloride. Indeed, it has been shown that orally administered amiloride reduces fibre diameter in both the rat coronary ligation (Hasegawa et al. 1995) and murine dilated cardiomyopathy models (Taniguchi et al. 1996). The former is of particular usefulness in that it is characterized in a well-defined series of postinfarction adaptive responses culminating in heart failure similar to that seen in the clinical setting. We (Yoshida and Karmazyn 2000; Kusumoto et al. 2001) and others (Spitznagel et al. 2000) have used this model to identify the potential beneficial effects of cariporide (the NHE-1 selective inhibitor) on both early and late postinfarction-induce heart failure. Orally administered cariporide completely abrogated the increased length of surviving myocytes after 1 week after coronary artery occlusion and improved contractile dysfunction (Yoshida and Karmazyn 2000). It is important to note that these effects occurred in the absence of afterload reduction. Moreover, improved hemodynamics was associated with an almost complete abrogation or left ventricular hypertrophy. These studies were further expanded to a more chronic (3-month) follow-up period where both adaptive, i.e., hypertrophic, and heart failure responses are more pronounced. As with the 1 week study, cariporide significantly attenuated left ventricular dysfunction, which included a marked attenuation of left ventricular end-diastolic pressures (Kusumoto et al. 2001). This was associated with diminished LV dilation and cell hypertrophy, and improved shortening of surviving myocytes. Because infarct size was unaffected, the results likely reflect the ability to decrease the hypertrophic–remodelling process by NHE-1 inhibition. Using another potent NHE-1 inhibitor, EMD 87580, it has recently been demonstrated that this agent can reverse the postinfarction remodelling process when administered 4 weeks after the onset of coronary artery ligation (Chen et al. 2004). Although the cellular and molecular basis for reversal of remodelling needs to be determined, these findings are of immense clinical importance as they suggest that NHE-1 inhibition could exert beneficial effects and reverse the pathological process after NHE-1 inhibition has been established.

Heart failure secondary to pulmonary hypertension

The salutary effects of NHE-1 inhibitors are not restricted to infarction-induced heart failure. For example, it has also been observed that NHE-1 inhibition attenuates right ventricular hypertrophy in a model of pulmonary hypertension produced by acute monocrotaline administration to rats. In that study, monocrotaline produced severe pulmonary artery remodelling and intimal thickening, which resulted in compensatory right ventricular hypertrophy (Chen et al. 2001). Cariporide had no effect on vascular responses but significantly attenuated the right ventricular parameters and improved hemodynamic parameters, suggesting a direct antihypertrophic effect of NHE-1 inhibition in this model (Chen et al. 2001).

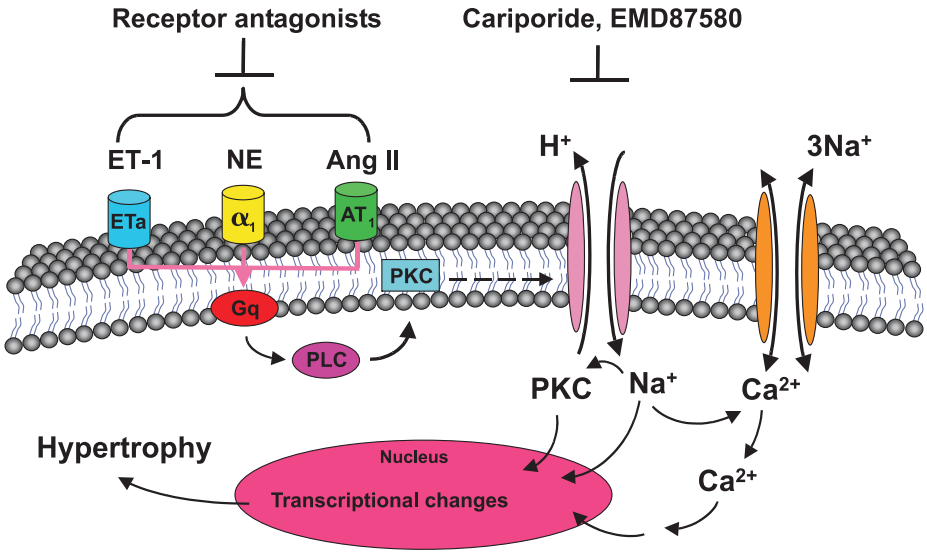
Left ventricular pressure overload-induced heart failure

NHE-1 inhibitors have also been shown to reduce LV remodelling due to pressure overload. For example cariporide administration to mice significantly reduced LV hypertrophy and systolic dysfunction (Marano et al. 2004). Related to this finding are a number of studies showing regression of LV hypertrophy secondary to hypertension by NHE-1 inhibitors (Cingolani et al. 2003) or hypertrophy produced by isoproterenol administration (Ennis et al. 2003).

Genetic models of heart failure

Engelhardt and colleagues have recently demonstrated a nearly total prevention of ventricular remodelling in transgenic mice overexpressing the β1 cardiac adrenenoceptor by the NHE-1 inhibitor cariporide (Engelhardt et al. 2002). Indeed, the beneficial effects were dramatic in that these were associated with a virtual complete prevention in hemodynamic abnormalities, cardiomyocyte hypertrophy, and collagen deposition.

Fig. 3. Summary of potential mechanisms underlying NHE-1 dependent cadiomyocyte hypertrophy. NHE-1-activation via a Gq protein dependent phospholipase C (PLC) activation and subsequent sodium influx is likely a major contributor to hypertrophy produced by various agonists including endothelin-1 (ET-1), norepinephrine (NE) or angiotensin II (Ang II) acting on their receptors, ETa, α_1 and AT1, respectively. Sodium entry may have direct effects on transcriptional regulation or secondary effects via either protein kinase C (PKC) activation or influx of calcium via reverse mode sodium-calcium exchange activity. Accordingly, the accompanying reduction in sodium entry subsequent to NHE-1 inhibition by specific receptor antagonists (but more effectively by NHE-1 inhibitors including cariporide or EMD 87580) may represent the major basis for salutary effects of NHE-1 inhibition on hypertrophy and heart failure.



Potential mechanisms for NHE-1 involvement in cardiomyocyte hypertrophy

The precise mechanism of NHE-1 involvement in the hypertrophic response or heart failure remains be determined. It is unlikely to be related to intracellular pH because its other intracellular pH-regulatory processes would most likely be recruited to assure intracellular pH homeostasis. Thus, in view of the multiplicity of intracellular pH-regulatory mechanisms in the cardiac cell, it is doubtful that intracellular acidosis would be markedly greater in hearts treated with NHE-1 inhibitors, although this needs to be determined with certainty.

In essence, the precise mechanism for NHE-1 involvement in hypertrophy and remodelling is unknown, although a number of potential possibilities have been put forth. A potentially interesting hypothesis involves sodium ions that are important mediators of cell hypertrophy (Gu et al. 1998; Hayasaki-Kajiwara et al. 1999). A reduction in sodium entry accompanies the inhibition of NHE-1. Accordingly, the reduction in sodium entry may explain why the effects of NHE-1 inhibition on hypertrophy and heart failure are salutary. Indeed, in a recent study which utilized neonatal rat ventricular myocytes exposed to various hypertrophic agents including phenylephrine, endothelin-1, or a phorbol ester, it was proposed that NHE-1-dependent sodium influx is a major contributor to their hypertrophic effects which appeared to be dependent on sodium-induced activation of PKC isoforms, especially PKCδ and PKCε (Hayasaki-Kajiwara et al. 1999). Inhibitors of PKC were found to reduce the hypertrophic response, whereas the NHE-1 inhibitors HOE-694 decreased both hypertrophy and PKC activation, thus reinforcing the link between PKC and NHE-1. However, other cell signalling mechanisms may also participate. For example, stretch-induced cardiac cell hypertrophy was also associated with Raf-1 and MAP kinase activation, both of which were blocked by the NHE-1 inhibitor HOE-694 (Yamazaki et al. 1998). Taken together, these findings suggest NHE-1 involvement in the activation of various kinases. As a result of the cell activation, there is cell growth, although NHE-1, too, may be a direct influence on the hypertrophic process.

Because calcium can also produce hypertrophy via a number of mechanisms, it is attractive to suggest that enhanced calcium entry via reverse mode sodium-calcium exchange activity could mediate the pivotal role of NHE-1 in producing hypertrophy. This concept requires further studies to elucidate it, especially as it is not totally certain whether enhanced calcium levels occur in nonischemic cells. One would expect that removing Na via the Na-K ATPase would occur under these conditions which would preclude a Na-dependent reverse mode Na-Ca exchange activity.

A summary of potential mechanisms underlying NHE-1 dependent cadiomyocyte hypertrophy is provided in Fig. 3.

Concluding remarks

NHE-1 is a fascinating protein, and likely the most carefully regulated membrane transporter currently known. Much of NHE-1 regulation involves phosphorylation-dependent processes involving various kinases. Although substantial progress has been achieved in terms of understanding the mechanisms of NHE-1 regulation by phosphorylation, what has not yet been clarified is how the many phosphorylation sites work together on the protein. In addition, in most cases, it remains to be elucidated which sites are phosphorylated and which kinases carry out the phophorylation. It is also evident that regulation by the kinases varies from 1 cell type

to another, such that a complete analysis will require comparisons in different tissues. Further, the requisite dephosphorylation of the NHE-1 by phosphatases has not yet been studied in detail.

Recent evidence suggests that NHE-1 is also a major downstream mediator to various hypertrophic factors. Thus, NHE-1 is an attractive potential candidate for targeted intervention in terms of attenuation of the remodelling and hypertrophic processes which contributes to heart failure. Experimental studies have shown that NHE-1 inhibitors attenuate cardiomyocyte hypertrophy induced by various factors and reduce heart failure in vivo, independently of infarct size reduction. Although the precise cellular mechanisms for NHE-1 involvement remain to be elucidated, current data suggest a potentially effective new therapeutic approach for the treatment of heart failure via NHE-1 inhibition.

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