Elevated Na^+–H^+ Exchanger Expression and Its Role in Myocardial Disease

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Abstract

The mammalian Na^+–H^+ exchanger isoform 1 (NHE1) is a plasma membrane protein that regulates intracellular pH in the myocardium by removing one intracellular hydrogen ion in exchange for one extracellular sodium ion. While NHE1 regulates intracellular pH, it is also involved in the damage that occurs to the myocardium with ischemia and reperfusion. Additionally, NHE1 levels are elevated in cardiac diseases such as hypertrophy, and NHE1 inhibition can reduce ischemia–reperfusion damage and prevent heart hypertrophy in animal models. Recently, it has been demonstrated that elevation of NHE1 levels occurs in several kinds of hearts disease. Surprisingly, the effect of elevation of these levels is varied, sometimes having beneficial and sometimes detrimental effects.

Keywords

Acidosis • Apoptosis • Hypertrophy • Ischemia • MAP kinase • Membrane • NHE1 • Na^+–H^+ exchanger • pH regulation

Introduction

The mammalian Na^+–H^+ exchanger (NHE) is a membrane protein that removes one intracellular hydrogen ion in exchange for one extracellular sodium ion. It is ubiquitously expressed in mammalian cells and is widespread throughout the animal kingdom. NHE plays a critical role in intracellular pH (pH*i*) regulation protecting cells from acidification as well as regulating cell volume and sodium fluxes (reviewed in [1]). One family of ten isoforms of NHE exist; however, the NHE isoform 1 (NHE1) is the only plasma membrane isoform expressed in the myocardium [2–7]. NHE1 is of special importance in the myocardium as it is implicated in both myocardial damage from ischemia–reperfusion injury, and heart hypertrophy (see below and reviewed in [8]). NHE1 consists of a membrane domain that transports ions and a cytosolic domain that modulates activity of the

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membrane domain and is a target of various forms of regulation (reviewed in [9]).

NHE Structure and Subtypes

Human NHE1 is an 815 amino acid protein. The first 500 residues are predicted to be 12 transmembrane spanning segments, and the remaining residues constitute a cytosolic intracellular regulatory domain [10–12] (Fig. 1). It should be noted, however, that there is recently some doubt about the transmembrane organization [13]. NHE1 forms homodimers in vivo, although the individual protein subunits can function independently of each other [14, 15]. The structure of a bacterial sodium hydrogen antiporter has been determined [16], but it is not very homologous with NHE1 and is part of a different NHE family with different characteristics including electrogenic transport [17]. The family of mammalian NHE-like proteins includes ten commonly known isoforms (NHE1-10) each the product of a different gene and with different tissue distributions and physiological roles (reviewed in [9, 18]). The first type cloned was named NHE1 [19], and it is ubiquitously expressed in mammalian cells. NHE1 was also identified by our laboratory as the predominant plasma membrane isoform in the myocardium [3]. It has been shown to be concentrated along intercalated disks and transverse tubule system of heart cells [20]. Heart cells lack the plasma membrane isoforms NHE2–5. NHE2–4 are expressed mainly in the kidney and gastrointestinal tract [21–23]. NHE5 is found in the brain [24], while NHE6–9 are localized to intracellular organelle membranes, such as endosomes, mitochondria, and the Golgi apparatus [25, 26], NHE10 is expressed in osteoclasts [27]. The protein identity of the various isoforms varies from 25 to 70%; however, all share a common predicted secondary structure [7]. A more distant family of two mammalian cation proton antiporters is made of NHA1 and NHA2, and these proteins may be involved in mediating hypertension [28, 29]. Similarly, a splice variant of NHE1 has also been suggested to be important in hypertension [30].

Importance of NHE and pH Regulation in the Myocardium

NHE is important in pH*i* regulation in the myocardium. Myocardial energy production generates protons and the negative membrane potential of the plasma membrane tends to accumulate protons within the cytosol (Fig. 2). Decreased pH*i* inhibits contractility. NHE1 removes these

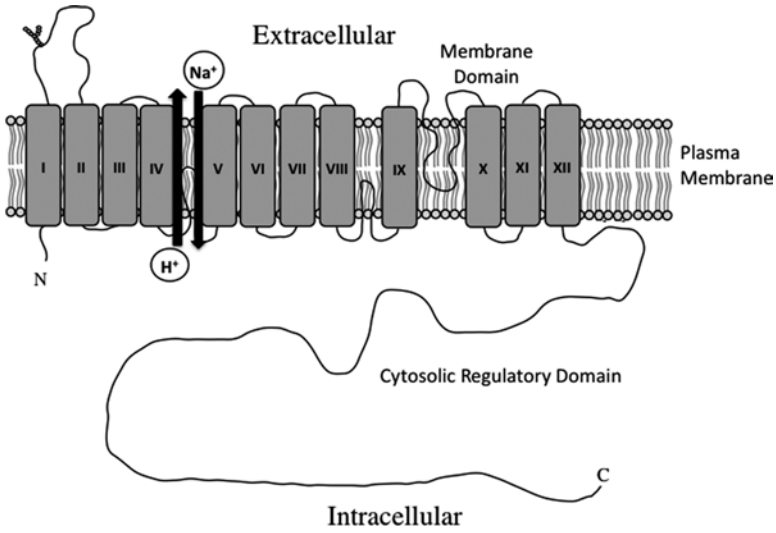

Fig. 1 Simplified schematic diagram of the Na^+–H^+ exchanger. The membrane domain of approximately 500 amino acids catalyzes the exchange of one intracellular H^+ in exchange for one extracellular Na^+. The intracellular cytosolic domain of approximately 315 amino acids regulates the membrane domain

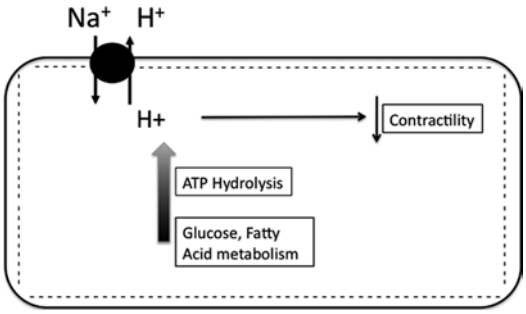

Fig. 2 Illustration of the role of the Na^+–H^+ exchanger in myocardial metabolism. The Na^+–H^+ exchanger removes protons generated as a result of metabolism or ATP hydrolysis. Excess protons are inhibitory to cardiac contractility. The negative membrane potential tends to retain intracellular protons

protons. At low pH*i* (pH ≤ 6.5), the exchanger is maximally active and at higher pH's activity is reduced or negligible. However, the pH dependence can be shifted toward the more alkaline range by α1-adrenergic stimulation and by hormones such as endothelin [31, 32]. Our laboratory has shown that MAP kinase-dependent pathways are important in this response [33, 34]. NHE1 is normally the key mechanism of proton removal with HCO_3-based transporters contributing to a lesser degree from recovery from intracellular acidosis [35–41].

NHE1 Physiological and Pathological Roles (in Other Tissues)

NHE1 has several roles in many cell types (see [7, 9, 17, 42] for reviews). Knockout of NHE1 from cells shows that NHE1 plays a role in growth, especially in the presence of more acidic media [43]. Similarly, NHE1-deficient mice demonstrated decreased postnatal growth and ataxia and epileptic-like seizures [44, 45]. NHE1 is also important in cell cycle progression [46, 47], while NHE1 is additionally permissive in cell differentiation which we [48] and others [49] demonstrated. The involvement of NHE1 in cell growth and differentiation suggests that the protein is important in normal developmental processes. NHE1 also modifies apoptosis. In mouse β-cells, trophic factor withdrawal triggers pH*i* dysregulation and apoptosis. NHE1 is activated leading to cellular alkalinization and progression of apoptosis [50]. We showed that this activation is through p38-dependent phosphorylation of the NHE1 tail [50]. 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 [51–53]. Additionally, in breast cancer cells NHE1 activation is key in their cell invasion activity [54–56].

Pathological Roles of NHE1 in the Myocardium

NHE1 contributes to several types of myocardial disease. The best known is the role of NHE1 in ischemia–reperfusion damage in the myocardium [57–60]. During ischemia, anaerobic glycolysis occurs, resulting in the increased production of protons. These serve to activate NHE1. The activated NHE1 exchanges the more H^+i for extracellular Na^+. This leads to a rapid accumulation of sodium in the cell [57–60]. The high sodium concentration drives an increase in Ca^{2+} via reversal of the activity of the Na^+–Ca^{2+} exchanger. This results in an increased level of intracellular Ca^{2+}, which triggers various pathways leading to cell death (Fig. 3). Hundreds of preclinical studies have shown that inhibition of NHE1 during ischemia and reperfusion protects the myocardium from calcium overload (see [60, 61] for reviews). In various animal models, NHE1 inhibitors such as cariporide, amiloride, and EMD 85131 have proven to be cardioprotective [62–64]. 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 [65].

NHE1 Is Important in Cardiac Hypertrophy

Preclinical studies have shown directly that NHE1 inhibition prevents cardiac hypertrophy including in vivo in rats subjected to myocardial

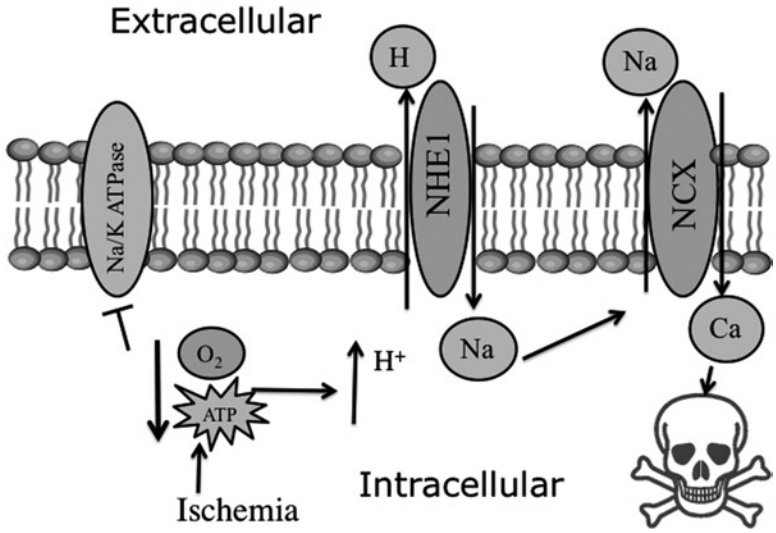

Fig. 3 Series of events leading to myocardial injury through the Na^+–H^+ exchanger. Ischemia leads to an increase in intracellular protons and decrease in ATP levels. This causes an increase in intracellular protons and the decrease in cellular ATP levels is inhibitory to the Na^+–K^+ ATPase. Excess intracellular protons are removed by the Na^+–H^+ exchanger, which results in an increase in intracellular sodium. This is removed by the reverse mode of the Na^+–Ca^{2+} exchanger, resulting in an increase in intracellular calcium. This leads to cell damage including necrosis and apoptosis

infarction [66, 67], in mice with guanylyl cyclase-A receptor knockout [68] and in vitro in isolated cardiomyocytes [69]. NHE1 is activated by MAP kinases and protein kinase C-dependent pathways, which are important in hypertrophic and remodeling processes [34, 70]. We demonstrated that the effect of the hypertrophic agonist aldosterone can be blocked by NHE1 inhibition [69] as can stretch-induced hypertrophy [71]. Prevention of increases in intracellular Na^+ is a possible mechanism by which NHE1 inhibition prevents hypertrophy [72, 73].

NHE1 Is Important in Apoptosis in the Myocardium

Studies in animals and humans have shown that in addition to necrosis, apoptosis significantly contributes to myocyte loss following myocardial infarction [74–77] including results suggesting that apoptosis is a critical form of cell death in infarcted human myocardium [78]. Much of the detrimental effects of NHE1 in hypoxia-reoxygenation are mediated through apoptosis. Inhibition of NHE1 activity via the specific inhibitor cariporide results in decreased apoptosis in isolated cardiomyocytes [79] and in fibroblasts [80]. In addition, inhibition of NHE1 activity before ischemia has been shown to reduce myocardial apoptosis in isolated rat hearts [81, 82], in mouse hearts [83] and in pacing-induced heart failure in rabbits [84]. Humphreys et al. [85] also reported that in an ischemic rat model, the NHE1 inhibitor cariporide reduced apoptosis and this was associated with a significantly higher ratio of (antiapoptotic) Bcl-2 to (pro-apoptotic) Bax [83, 85]. Regulation of NHE1 has been implicated in NHE1 induced apoptosis in the myocardium [86, 87]. Recently, we have shown that amino acids Ser^{726} and Ser^{729} are involved in critical regulation of NHE1 causing apoptosis [88]; however, in cardiac myocytes we showed that amino acids Ser^{770} and Ser^{771} are more important in regulation of NHE1 [65].

NHE1 Inhibition for Treatment of Other Cardiovascular Conditions

Aside from the general conditions of hypertrophy and ischemia–reperfusion damage, a number of specific cardiovascular conditions have been examined and are briefly summarized. NHE1 inhibitors have for example, been useful in inhibiting diabetic vascular hypertrophy [89, 90] and prevent alterations in coronary endothelial function in streptozotocin-induced diabetes [91]. NHE1 is also known to play an important role in ischemia–reperfusion injury in the central nervous system where NHE1 inhibition is beneficial [92]. Studies have shown the NHE1 inhibition has potent antifibrillatory and antiarrhythmic effects in dogs and rats [93, 94]. These effects extend to other species where NHE1 inhibition has been shown to an effective intervention for resuscitation from ventricular fibrillation [95]. In addition, NHE1 inhibition has been shown to be a better protective agent than ischemic preconditioning in some trials [96, 97]. It has been suggested that NHE1 inhibitors may be of use during cardiac surgery, including being of use in hearts subjected to prolonged hypothermic storage [98]. Kim et al. [99, 100] also showed that NHE1 inhibition was beneficial in improving the outcome in a canine transplantation model. Inhibition of NHE1 has also been shown to be protective in models of cardiac resuscitation [101]. NHE1 inhibition may additionally be useful in treatment of circulatory shock as NHE-1 inhibition attenuated ischemic myocardial hypercontracture and cardiovascular decompensation, and delayed the onset of hypovolemic circulatory shock in a porcine model of circulatory shock [102]. Overall, it can be said that inhibition of NHE1 activity has many and varied beneficial cardiovascular effects in a large number of models.

Clinical Trials

Despite all the success with inhibition of NHE1 in preclinical experiments, trials with NHE1 inhibitors have not been very successful. Large-scale studies with several inhibitors in various types of myocardial infarctions and treatments have given mostly disappointing results (see [87] for review). Rupprecht et al. [103] tested effects of cariporide in a small trial of patients with myocardial infarction who received coronary angioplasty. They found some beneficial effects on ejection fraction, wall-motion abnormalities and enzyme release. A larger scale two-stage trial [104] with eniporide treatment of myocardial infarction 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 reason for the discrepancy between the beneficial effects in pre-clinical trials and the negative effects in clinical trials may be because NHE inhibition is required early in ischemia, rather than in reperfusion as was the case in the clinical trials [87]. The GUARDIAN trial [105], had some more positive results. In patients undergoing coronary artery bypass graft surgery analysis of subgroups showed that cariporide was beneficial. Treatment with the inhibitor in this trial was early, which may account for its beneficial effect in this study [87]. The EXPEDITION trial tested if pre-ischemic inhibition of NHE1 by cariporide reduces myocardial injury in patients with coronary artery bypass graft surgery. Cariporide reduced myocardial infarction but it also had adverse side effects and increasing cerebrovascular events significantly. This resulted in the study being terminated early [87, 105]. Another study [106] tested the efficacy of the NHE inhibitor zoniporide on reducing cardiovascular events in patients undergoing non-cardiac vascular surgery. There was no beneficial effect and this has been attributed to a lack of myocardial reperfusion, which is required for beneficial NHE inhibitors to access the myocardium [87, 106].

NHE1 Is Elevated in Myocardial Disease

A variety of studies have shown that NHE1 protein expression and activity increases in the myocardium in response to stimuli that occur in the disease

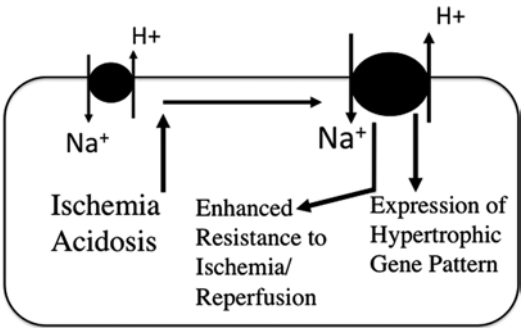

Fig. 4 Chronic stimuli increase Na^+–H^+ exchanger activity. Chronic stimulation by ischemia, acidosis, or other factors can cause an elevation in Na^+–H^+ exchanger messenger RNA levels, activity, and protein levels. This can lead to expression of a hypertrophic gene pattern but can also lead to enhanced resistance to ischemia–reperfusion damage

state. Early experiments showed that NHE1 message levels are elevated in hearts subjected to ischemia followed by reperfusion. In addition, treatment of primary cultures of neonatal rat myocytes with low external pH increased NHE1 activity [107]. Further studies showed that ischemia with or without reperfusion increase NHE1 levels in the disease state [108]. Interestingly, human sarcolemmal NHE1 activity was elevated in recipient hearts with chronic end stage heart failure, even though protein abundance was not increased [109] suggesting that the protein is activated (Fig. 4).

Aside from ischemia–reperfusion, the expression level and activity of NHE1 are elevated in a variety of cardiovascular diseases, including in hypertensive, hypertrophied, or diabetic myocardium [69, 110–112]. Both NHE1 message levels and protein levels have been shown to be elevated in these models. In aldosterone-induced hypertrophy, inhibition of NHE1 activity prevented the effect of aldosterone on inducing cardiac hypertrophy [69].

Transgenic Models of Elevated NHE1 Show Hypertrophy, but Surprisingly, Resistance to Ischemia Reperfusion

Because of the known elevation in the levels of NHE1 that occur in myocardial disease, a number of studies have examined transgenic mice with elevation of the levels of the NHE1 protein. We first examined the effect of NHE1 overexpression in mice hearts subjected to subjected to 20 min of ischemia followed by 40 min of reperfusion. Surprisingly, contractility after ischemia reperfusion, improved in NHE1-overexpressing hearts (Fig. 4). In addition, NMR spectroscopy revealed that NHE1 overexpressor hearts contained higher ATP levels during early reperfusion and there was no difference in Na^+ accumulation during between transgenic and WT hearts. Cariporide, the NHE1 inhibitor, equivalently protected both WT and NHE1-overexpressing hearts. Similar results were later shown by Cook et al. [113] who also showed that NHE1 overexpression induced an ER stress response in mouse myocardium, which might afford protection against ischemia–reperfusion-induced injury. We also suggested that a possible explanation for the beneficial effects is that basal activity of NHE1 is not rate-limiting in causing damage during ischemia–reperfusion, therefore increasing the level of NHE1 does not enhance injury and can have some small protective effects [114].

While overexpression of NHE1 may have some beneficial effects with ischemia and reperfusion, it has other detrimental effects. In a different transgenic model, Nakamura et al. [115] demonstrated that overexpression of an activated NHE1 led to cardiac hypertrophy and eventually to heart failure. Intracellular Na^+ levels were elevated, as were both diastolic and systolic calcium levels. This study found that the Ca^{2+}-dependent prohypertrophic molecules calcineurin and CaMKII were highly activated in these transgenic hearts. More recently we examined transgenic mice that had an elevation of either wild-type NHE1 protein or had an elevation of hyperactive NHE1 protein [116]. We found that mice with hyperactive NHE1 developed hypertrophy, including elevated heart weight-to-body weight ratio and increased cross-sectional area of the cardiomyocytes, interstitial fibrosis, as well as depressed cardiac function. Mice which expressed only wild-type NHE1 had modest changes in gene expression whereas mice expressing hyperactive NHE1 had a very strong transcriptional response. The most significant changes in gene expression were elevations in message levels of genes involved in cardiac

hypertrophy, cardiac necrosis/cell death, and cardiac infarction. Secreted phosphoprotein 1 and its signaling pathways were notably upregulated in mice with hyperactive NHE1. This study demonstrated that expression of activated NHE1 elicits specific pathways of gene activation in the myocardium that lead to cardiac hypertrophy, cell death, and infarction (Fig. 4).

Conclusions

It is clear that NHE1 has a number of detrimental effects on the myocardium and is involved in the cardiac pathologies of ischemia–reperfusion damage and cardiac hypertrophy. NHE1 inhibition has not yet proven to be a useful clinical tool. Whether more specific NHE1 inhibitors can be developed, which could be useful clinically for treatment of ischemic heart disease, has still to be determined. It is of interest that elevation of NHE1 levels has some cardioprotective effects. Whether this is due to elevation of ER stress proteins or through other cardioprotective mechanisms should be investigated. Animal models have been able to achieve almost total inhibition of NHE1 protein activity, while this may be difficult to obtain in humans with treatment with inhibitors. Is it possible that clinical studies have achieved only partial inhibition of the Na^+–H^+ exchanger in humans and that the inhibition is both not effective enough to prevent calcium overload and also abrogates some other beneficial activities of the protein? Further studies are required to answer this question. The detrimental effects of elevation of NHE1 levels, in causing cardiac hypertrophy, are of significant interest. Since NHE1 inhibitors prevent myocardial hypertrophy in preclinical studies, NHE1 inhibition for treatment of myocardial hypertrophy remains a potential clinical target.

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