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Critical Review

# The $Na^+/H^+$ Exchanger and pH Regulation in the Heart

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## Abstract

The mammalian $Na^+/H^+$ exchanger isoform 1 (NHE1) is an integral membrane protein ubiquitously expressed in mammalian cells. It is made up of two domains: a 500-amino acid membrane domain that is responsible for transport and removes protons, and a regulatory intracellular cytosolic domain made up of 315 amino acids. NHE1 is the major isoform found in the myocardium where it plays an important role in the regulation of intracellular pH by exchanging one intracellular proton for one extracellular sodium. Although NHE1 normally fulfills this important physiological role, aberrant regulation and overactivation of NHE1 contribute to heart disease, including acute ischemia reperfusion damage and cardiac hypertrophy. This review summarizes past and current knowledge of the role and regulation of NHE1 in the myocardium. © 2014 IUBMB Life, 00(00):000–000, 2014

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## Introduction

The $Na^+/H^+$ exchanger (NHE) is a membrane transport protein present in virtually all living organisms. In mammals, it functions primarily to protect cells from intracellular acidification by catalyzing the electroneutral removal of one intracellular $H^+$ in exchange for one extracellular $Na^+$. Ten isoforms of NHE are currently known to exist (NHE1–10) with distinct tissue expression, cellular localization, and physiological roles (1). Based on their subcellular localization, NHE1–5 are classified as plasma membrane proteins, and NHE6–9 are organellar NHEs being present in intracellular membranes such as Golgi; although NHE8 is also expressed in the apical membrane of polarized epithelial cells. The more recently identified NHE10 appears to be exclusively expressed in osteoclasts (1,2). The ubiquitous isoform, NHE1, was the first isoform discovered (1). It is also the only plasma membrane isoform present in the myocardium where it localizes to the intercalated disks and transverse tubules (2,3). NHE1 is made up of 815 amino acids separated into two domains: an N-terminal transmembrane (TM) domain where ion transport is catalyzed and where pH sensing occurs, and a C-terminal cytosolic domain that regulates the ion transport activity (4).

NHE1 exists as a homodimer in the plasma membrane with dimeric interactions shown both at the membrane and cytoplasmic domains (1,2). Although the complete structure of NHE1 has yet to be determined, models of the topology of NHE1 have been proposed. Using substituted cysteine accessibility analysis, a 12-TM model with a glycosylation site between TM1 and TM2 was proposed by Wakabayashi et al. (Fig. 1; ref. 5). Subsequently, a slightly different model was F1
proposed by Landau et al., which also had 12 TM segments, but excludes the first two TM segments presented in the Wakabayashi model. The N-terminus was suggested to contain a signal sequence with a possible cleavage site occurring just behind TM1 (6). Although this model had its proponents, it has been demonstrated that glycosylation is seen consistently in immunoblots of NHE1 (4) and that the only N-linked glycosylation site is present on the first extracellular loop consistent with the model of Wakabayashi et al. (5). However, further study is necessary to clarify this and other differences between these models. Other conflicting three-dimensional models of NHE1 have also been suggested (6,7), and one suggests that TM IV and TM XI are involved in ion translocation and inhibitor binding (7). However, these molecular models have not been verified at depth by experimental data (reviewed in


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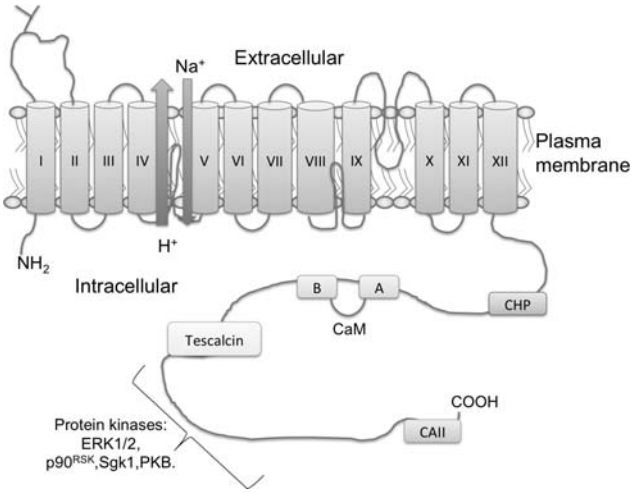


**FIG 1**

*Schematic model of NHE1 structure. NHE1 consists of two main domains: the 12-transmembrane segment domain where ion exchange takes place (TMI-TMXII) and the regulatory cytoplasmic domain. The cytoplasmic region is phosphorylated by various kinases and interacts with a number of proteins that modulate NHE1 activity at the plasma membrane. Regulatory proteins of particular importance in the myocardium are illustrated at approximate locations of action including calcineurin homologous proteins (CHPs), calmodulin (CaM; A: high-affinity binding site; B: intermediate-affinity binding site), carbonic anhydrase II (CAII), extracellular signal regulated protein 1/2 (ERK1/2), ribosomal protein S6 kinase ($p90^{RSK}$), serum- and glucocorticoid-inducible kinase (Sgk1), and protein kinase B (PKB).*

ref.4). Although the complete structure of human NHE1 is not known, our laboratory has made several reports on the deduced NMR structures of TM segments of NHE1. These have shown that NHE1 contains several discontinuous TM segments that are comparable with those deduced in the crystal structure of the *E. coli* NHE NhaA (reviewed in ref. 4). These results suggest that NHE1 may have a transport mechanism similar to that present in NhaA.

The primary function of NHE1 is the regulation of intracellular pH ($pH_i$); however, it is also involved in cell volume regulation, cell differentiation, cell proliferation, cytoskeletal organization, and cell migration. NHE1 has also been associated in apoptosis but with differing roles in various cells. In renal cells, it has been shown to provide a protective effect against apoptosis, whereas in mouse $\beta$-cells and cardiomyocytes, it has been implicated in the progression of apoptosis (reviewed in ref. 8). In transformed cells, alkalinization mediated by NHE1 plays an important role in the development of the transformed phenotype, and this is prevented when NHE1 is inhibited (9). NHE1 also contributes to cell invasion in breast cancer cells, and its inhibition induces apoptosis in these cells (2,10). A NHE1-null phenotype in mice results in decreased postnatal growth and increased mortality, ataxia, and epileptic seizures (8), and we have recently demonstrated that homozygous expression of a defective NHE1 gene in humans results in severe disease, with a phenotype including ataxia and deafness (11).

## Pathological Role of NHE1 in the Myocardium

Being the major plasma membrane NHE isoform in the heart, NHE1 plays an important role in normal cardiac function. However, NHE1 has also been implicated in various pathological conditions in the myocardium particularly in ischemia/reperfusion (I/R) injury, cardiac hypertrophy, and apoptosis. During ischemia, the myocardial cells switch to anaerobic metabolism resulting in lactic acidosis and consequently a decrease in $pH_i$. The reduction in $pH_i$ stimulates NHE1 activity causing an accumulation of sodium ions in the cell. This leads to the reversal of activity of the $Na^+/Ca^{2+}$ exchanger and results in an increase in intracellular calcium, triggering deleterious pathways that eventually lead to cell death (10). Further support for the involvement of NHE1 in myocardial I/R injury is based on evidence from various studies that genetic ablation and/or pharmacological inhibition of NHE1 in animal models and patients protects the myocardium from I/R injury (10,12). Our laboratory has also been able to demonstrate an increase in the activity of NHE1 regulatory kinases in response to ischemia and reperfusion in the myocardium, which leads to activation of the protein in the disease state (13).

A link between NHE1 and cardiac hypertrophy has also been established with increased expression and/or activity of NHE1 demonstrated in various animal models of hypertrophy. Inhibition of NHE1 was, however, able to prevent or abrogate hypertrophy in these animals (14). Although the mechanism by which this occurs is not entirely known, some studies have provided insights into how NHE1 may induce cardiac hypertrophy. For example, blocking receptors of hormones such as phenylephrine and aldosterone have been shown to prevent cardiac hypertrophy in neonatal rat ventricular myocytes (15). These hormones are known hypertrophic agents and are also regulators of NHE1 activity. In addition, the inhibition of carbonic anhydrase, which is another regulator of NHE1, has been shown to prevent and reverse cardiac hypertrophy in adult mouse cardiomyocytes (16). Elevated expression of activated forms of NHE1 in transgenic mice has been shown to result in induction of hypertrophy (see below; refs. 17 and 18). Cardiac hypertrophy is often accompanied by mitochondrial dysfunctions such as opening of the mitochondrial permeability transition pore and imbalance in the expression of mitochondrial fission and fusion proteins. Studies have demonstrated the presence of NHE1 in heart mitochondria, and pharmacological inhibition and gene silencing of NHE1 attenuated the opening of the mitochondrial permeability transition pore and attenuated the elevated expression of mitochondrial fission and fusion proteins. This suggested that NHE1-induced cardiac

hypertrophy may be at least, in part, be mediated by the mitochondria (19,20).

Apoptosis plays a critical role in cardiac pathology by contributing significantly to myocardial loss and cell death following myocardial infarction (2). Apoptosis can also be induced by ischemia and hypoxia/reoxygenation. Studies from our laboratory have demonstrated that increased NHE1 expression in isolated rat cardiomyocytes predisposes them to apoptosis following I/R (21). As noted above, I/R has been demonstrated to activate NHE1 in the myocardium (13), and the effects of I/R might be accentuated by increased levels of the protein. Inhibition of NHE1 has also been shown to prevent and/or reduce apoptosis in rat cardiomyocytes subjected to ischemia or hypoxia/reoxygenation. Studies also suggest that the decrease in apoptosis via NHE1 inhibition is due to an increase in mitochondrial $Ca^{2+}$ concentration, a reduction in caspase-3 activity, and a higher ratio of antiapoptotic protein Bcl-2 to proapoptotic protein Bax (22). These results provide an insight into the mechanism of NHE1 involvement in cardiac pathology.

## Clinical Evaluation of NHE1 Inhibitors

Despite several studies showing that inhibition of NHE1 provided cardioprotection in various animal models, clinical studies in human subjects have unfortunately met with very limited success (reviewed in ref. 23). A small-scale study tested the effect of cariporide, an NHE1-specific inhibitor, on patients with myocardial infarction receiving coronary angioplasty. Patients who received cariporide showed higher ejection fractions, reduced end-systolic volume, and significant improvement in wall motion abnormalities. A larger scale, two-stage clinical trial, the ESCAMI study, was then carried out to test the efficacy of eniporide, another NHE1-specific inhibitor, on patients with myocardial infarctions. However, the overall conclusion from this study was that eniporide neither reduced infarct size nor improved clinical outcome. The GUARDIAN study investigated the effects of cariporide on three subgroups of patients with different heart conditions. The study showed no overall evidence of cardioprotection except for patients with coronary artery bypass graft (CABG) treated with high dose (120 mg/8 h) of cariporide. The EXPEDITION study was a phase 3 clinical trial testing the cardioprotective effect of cariporide in patients undergoing CABG. Although NHE1 inhibition by cariporide did result in the reduction of myocardial infarction, the study had to be terminated early due to increased mortality caused by an increase in cerebrovascular events.

Results from these clinical trials, however disappointing, indicate that the idea of NHE1 inhibition for cardioprotection is valid in theory. However, the key question is whether NHE1 inhibition still remains a viable treatment strategy for cardiovascular diseases or whether the adverse affects of cariporide are a class effect shared by NHE1 inhibitors. Karmazyn (23) argued that NHE1 inhibition remains an effective potential clinical treatment despite the failure of the EXPEDITION study. He noted that the EXPEDITION study used a different administration protocol than the GUARDIAN study with significantly increased doses (nearly double), which may have promoted thrombosis. The argument is made that the high doses and method of administration may have unmasked a deleterious property of cariporide, which may be specific to this single type of NHE1 inhibitor and not due to NHE1 inhibition. Clearly, more study is needed in this area as NHE1 inhibition by several different types of inhibitors has remained overwhelmingly beneficial in numerous preclinical studies of various types of cardiovascular disease without any earlier noted side effects. Another key point is that most beneficial effects of NHE1 inhibition have been shown in studies in which it is administered prior to ischemic insult, although administration postinsult is less effective (23). It may be that NHE1 inhibition will only prove clinically useful where its use can be anticipated prior to ischemic episodes such as in CABG surgery.

## NHE1 and pH Regulation in the Heart

The regulation of $pH_i$ is crucial in myocardial cells for proper functioning of the heart. Under certain physiological and pathological conditions, changes in $pH_i$ occur in the heart, and compensatory mechanisms are required to restore the cells back to resting pH (7.1–7.4). In the absence of these regulatory mechanisms, a fall in $pH_i$ would lead to a reduction in cardiac contractility, disruption of intracellular $Ca^{2+}$ signaling, and, in some cases, arrhythmia (1,10). Following a decline in $pH_i$ in myocytes, two ion transporters are primarily activated, NHE and $Na^+$–$HCO_3^-$ cotransporter. However, evaluation of the contribution of each transporter to acid extrusion showed that NHE1 is the dominant transporter for proton efflux following intracellular acid load (24). In addition, the $Na^+$–$HCO_3^-$ cotransporters are more active at alkaline $pH_i$ (>7.0), thus making NHE1 the key pH-regulatory protein in the myocardium at acidic $pH_i$ (2).

## Regulation of NHE1 in the Myocardium

Normally quiescent, NHE1 is activated in response to specific stimuli such as growth factors, hormones, osmotic stress, and intracellular acidosis. This occurs via phosphorylation of the cytoplasmic domain and by phosphorylation-independent mechanisms such as the binding of various regulatory proteins. Regulation of NHE1 has been studied in both myocardial and nonmyocardial cells (see reviews in refs. 1 and 10); however, this review will be restricted to regulation in cardiomyocytes.

## Growth Factor and Hormonal Regulation of NHE1

Various hormones and growth factors, many of which are known modulators of cardiac activity and contributors to cardiac pathology, regulate NHE1 activity. These include angiotensin II, thrombin, endothelin-I, α-adrenergic agonists, and epidermal growth factor. Hormonal regulation often results in the activation of protein kinases that activate NHE1.

Angiotensin II has been shown to increase NHE1 activity following intracellular acidosis in rat ventricular myocytes. This stimulatory action occurs via the $AT_1$ receptor and is mediated by PKC and the EGF receptor. The $AT_2$ receptor mediates an opposing counteracting inhibition of NHE1 (25). The inotropic effect of endothelin on the myocardium has also been partly attributed to its stimulation of NHE1 activity (26). $\alpha_1$-Adrenergic agonists, such as phenylephrine, stimulate NHE1 activity by shifting its $pH_i$ sensitivity to a more alkaline range, and this is mediated by the $\alpha_{1A}$-adrenoceptor subtype and the ERK pathway of the MAPK cascade (27,28). The stimulation of NHE1 by $\alpha_1$-adrenergic agonists may also play a role in the exacerbation of reperfusion-induced arrhythmias (29).

## Phosphorylation

Phosphorylation of the cytoplasmic domain is also a mechanism of NHE1 regulation, and this occurs in the 180-amino acid distal region of the carboxyl terminal tail (1,10). It accounts for about half of the growth factor-induced regulation of NHE1. The MAPK signaling pathway has been shown to play an important role in the regulation of NHE1 in the myocardium. Studies from our laboratory demonstrated that ERK1/2 and $p90^{RSK}$ directly phosphorylate and activate NHE1 in healthy rat myocardium (30). Stimulation of NHE1 activity in isolated cardiomyocytes by sustained intracellular acidosis is mediated by the Ras signaling pathway and subsequent activation of ERK 1/2 (31). Protein kinase-mediated regulation of NHE1 by ERK1/2 and $p90^{RSK}$ has also been implicated in the cardiac I/R injury (13). Using mass spectrometry, we identified the phosphorylation sites for ERK 1/2 in NHE1 (32). These were grouped into four regions as follows: 1, S693; 2, T718,S723/726/729; 3, S766/770/771; and 4, T779,S785 (2). Further studies from our laboratory demonstrated that phosphorylation of $Ser^{770}$ and $Ser^{771}$ of region 3, by ERK1/2, mediates the activation of NHE1 by sustained intracellular acidosis in cardiomyocytes (33). Recently, the serum- and glucocorticoid-inducible kinase has been shown to phosphorylate myocardial NHE1 at $Ser^{703}$ (34). Interestingly, $Ser^{703}$ has also been shown to be phosphorylated in vascular smooth muscle cells by $p90^{RSK}$ and has been suggested to be important in I/R injury in the myocardium (35). Protein kinase B (PKB or Akt) has also been identified as a regulatory kinase of cardiac NHE1. It phosphorylates NHE1 at $Ser^{648}$ and is the only kinase known so far to have an inhibitory effect on NHE1. Following intracellular acidosis in cardiac myocytes, PKB binds to and phosphorylates NHE1 at $Ser^{648}$, which is within the calmodulin (CaM) high-affinity binding region (see below).

Although the phosphorylation of NHE1 has been well studied, there has been much less work on the requisite dephosphorylation of NHE1. Protein phosphatases 1 and 2A (PP1 and PP2A) have been suggested to directly associate with NHE1 in myocardial cells (36,37). The calcineurin A subunit also binds to NHE1 (38); however, the significance of this binding in dephosphorylation of the protein is not yet established, and this binding may be involved in NHE1-induced translocation of NFAT and the progression of myocardial hypertrophy (38).

## Regulatory Proteins

The binding of accessory proteins to its cytoplasmic tail also regulates NHE1. This regulatory mechanism accounts for about 50% of the hormonal regulation of NHE1 but has not been well studied in myocardial cells (for reviews, see refs. 2 and 10). However, a few proteins that regulate cardiac NHE1 activity have been identified and are summarized as follows.

Carbonic anhydrase II (CAII) catalyzes the hydration of carbon dioxide to produce bicarbonate ions and protons. We have demonstrated that CAII binds to NHE1 *in vivo*, at the penultimate 13-amino acid region of the cytoplasmic tail, with $Ser^{796}$ and $Asp^{797}$ forming part of the binding site. We also showed that this association is dependent on NHE1 phosphorylation at a region upstream the CAII-binding site (39,40). Recent studies have suggested an increase in CAII interaction with NHE1 during myocardial stretch, and this increase is mediated by the phosphorylation of NHE1 by $p90^{RSK}$. In addition, inhibition of CAII prevented the slow-force response caused by myocardial stretch, indicating that CAII/NHE1 interactions may play a role in cardiac hypertrophy (41).

Calmodulin is a calcium-binding second messenger protein known to mediate the $Ca^{2+}$-induced activation of NHE1. It binds two regions on the cytoplasmic tail of NHE1, a high-affinity region (amino acids 637–656) and an intermediate-affinity region (amino acids 657–700), in the presence of $Ca^{2+}$ (reviewed in refs. 2 and 10). CaM modulates NHE1 activity by binding to the high-affinity region of the NHE1 tail preventing this autoinhibitory domain from acting on the membrane domain. PKB phosphorylates NHE1 at $Ser^{648}$, which is within the CaM high-affinity binding region. This prevents CaM binding to NHE1 and results in a reduction in NHE1 activity by preventing CaM from blocking the autoinhibitory site on the NHE1 tail. This may serve as a cardioprotective mechanism during I/R injury (42).

Calcineurin B homologous proteins (CHPs) are $Ca^{2+}$-binding proteins with EF-hand motifs, which coordinate $Ca^{2+}$ ions similar to CaM. They exist in three isoforms: CHP1, CHP2, and CHP3 (or tescalcin). CHP1 is expressed in many tissues including the heart, whereas the expression of CHP2 is more restricted to intestinal epithelial cells and malignant tumor cells. CHP3, which was initially detected in mouse testis, is also expressed in the heart, brain, stomach, and hematopoietic cells. CHP1 is an important cofactor of NHE1 that binds to the cytoplasmic tail at amino acids 518–537. Preventing CHP1 binding to NHE1, either by deletion or mutation of the CHP-binding domains, severely reduced NHE1 activity (43). In addition, such NHE1 mutants exhibit shorter half-lives and reduced cell surface expression (44). CHP3 also binds to NHE1 in a $Ca^{2+}$-dependent manner, and its N-myristoylation is essential for NHE1 stability and activity at the plasma membrane (45). However, although both CHP1 and CHP3 are expressed in the

myocardium, their specific role in regulating NHE1 in the myocardium has not been studied.

## The role of elevated NHE1 Expression in Diseased Myocardium

As NHE1 expression and activity have been shown to increase in many myocardial disease models, it became imperative to study the effects of NHE1 overexpression in the heart. This would ascertain if indeed NHE1 activity significantly ~~contributed~~ to cardiac pathology and would also elucidate the molecular and cellular events involved.

Thus, the effects of cardiac-specific NHE1 overexpression in transgenic mice hearts subjected to ischemia and reperfusion were studied in our laboratory. Results from our study surprisingly showed that NHE1 overexpression did not enhance I/R injury but in fact provided some cardioprotection. In addition, inhibition of NHE1 provided similar protection for both WT and NHE1-overexpressing heart. Furthermore, transgenic hearts had higher ATP levels during early reperfusion, and there was no difference in $Na^+$ accumulation between transgenic and WT heart during I/R (46). These studies were, however, carried out with glucose being the only substrate, which did not completely represent physiological conditions that occur during I/R. We therefore carried out similar studies, but under more physiological conditions, using both glucose and fatty acids as energy substrates. We obtained similar results to our earlier study except that only the overexpression of NHE1 provided cardioprotection when compared with wild-type hearts (47). It is not yet completely clear why elevated expression of NHE1 provides protection against I/R injury, but NHE1-induced endoplasmic reticulum stress response and increased diastolic $Ca^{2+}$ loading are thought to be involved (48,49). It appears as though NHE1 activity has a biphasic effect on myocardial function. Total blockage of activity provides a beneficial effect, but overexpression also provides cardioprotection.

Although elevated expression of NHE1 seems to have some beneficial effects during I/R, it appears to have other detrimental effects, particularly during cardiac hypertrophy. Transgenic mice overexpressing an activated form of NHE1 developed cardiac hypertrophy and heart failure as a result of activated $Ca^{2+}$-dependent prohypertrophic pathways. Intracellular $Na^+$ levels were also increased, as well as diastolic and systolic $Ca^{2+}$ levels (17). Treatment with the NHE1 inhibitor cariporide abrogated these effects. We also found that transgenic mice overexpressing hyperactive NHE1 developed cardiac hypertrophy, as well as increased heart weight-to-body weight ratio and reduced cardiac function. There was also marked elevation in the expression of genes involved in cardiac hypertrophy, cardiac necrosis, and cardiac infarction. Further studies in our laboratory showed that these changes are not merely due to elevated expression of NHE1 alone but also due to increased activation of NHE1 (18). We also observed that elevated expression and activity had no significant effect on other pH-regulatory proteins correlating with a previous study which suggested that NHE1 activation is sufficient enough to induce hypertrophy (17,50). Overexpression of active NHE1 also makes cardiomyocytes more susceptible to apoptosis caused by hypoxia/reoxygenation (21).

A key point of these recent studies was that simple elevation of the level of wild-type NHE1 protein is not strongly hypertrophic and that activation of the protein is a key component of the early induction of myocardial hypertrophy (18). NHE1 is regulated by $pH_i$ such that it is inactive at more alkaline $pH_i$s (1,10). Thus, the simple presence of more NHE1 protein, which is either inactive or nearly so, may be insufficient to cause deleterious effects on the myocardium. The presence of more NHE1 proteins, combined with activation of NHE1, may be a key in eliciting a myocardial hypertrophic pathway. Thus, regulation of NHE1 may be an important area to address in future attempts to limit damage caused by increased NHE1 expression.

## Conclusions

It is apparent that NHE1 plays a significant role in myocardial $pH_i$ regulation and also in cardiac pathologies, including I/R injury and cardiac hypertrophy. There is also a large body of evidence showing that inhibiting NHE1 is effective against heart failure in various animal models of heart disease. Unfortunately, this has not translated into successful treatment of myocardial infarction in humans as revealed in the clinical trials. Whether the undesirable side effects observed in one clinical trial are due to NHE1 inhibition outside of the myocardium or are a direct consequence of side effects of the specific inhibitor and dose and administration method used require further investigation. A greater understanding of this area might pave the way for developing more effective inhibitors of NHE1 or more appropriate doses or routes of administration. Could it be that certain patients may benefit more from alteration of regulation of NHE1 activity rather than direct inhibition? This question can be answered by studies geared toward a deeper insight into regulation of NHE1 in cardiomyocytes under normal and pathological conditions. This might also provide novel approaches to therapeutic intervention at the regulatory level, which would preserve basal NHE1 activity while treating heart conditions caused by altered NHE1 activity.

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