The Myocardial Na\(^{+}\)-H\(^{+}\) Exchange
Structure, Regulation, and Its Role in Heart Disease

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Abstract—The Na\(^{+}\)-H\(^{+}\) exchange (NHE) is a major mechanism by which the heart adapts to intracellular acidosis during ischemia and recovers from the acidosis after reperfusion. There are at least 6 NHE isoforms thus far identified with the NHE1 subtype representing the major one found in the mammalian myocardium. This 110-kDa glycoprotein extrudes protons concomitantly with Na\(^{+}\) influx in a 1:1 stoichiometric relationship rendering the process electroneutral, and its activity is regulated by numerous factors, including phosphorylation-dependent processes. There is convincing evidence that NHE mediates tissue injury during ischemia and reperfusion, which probably reflects the fact that under conditions of tissue stress, including ischemia, Na\(^{+}\)-K\(^{+}\) ATPase is inhibited, thereby limiting Na\(^{+}\) extrusion, resulting in an elevation in [Na\(^{+}\)]. The latter effect, in turn, will increase [Ca\(^{2+}\)], via Na\(^{+}\)-Ca\(^{2+}\) exchange. In addition, NHE1 mRNA expression is elevated in response to injury, which may further contribute to the deleterious consequence of pathological insult. Extensive studies using NHE inhibitors have consistently shown protective effects against ischemic and reperfusion injury in a large variety of experimental models and has led to clinical evaluation of NHE inhibition in patients with coronary artery disease. Emerging evidence also implicates NHE1 in other cardiac disease states, and the exchanger may be particularly critical to postinfarction remodeling responses resulting in development of hypertrophy and heart failure.

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Key Words: Na\(^{+}\)-H\(^{+}\) exchange ▪ ischemia/reperfusion ▪ remodeling ▪ hypertrophy ▪ heart failure

Intracellular pH regulation in the cardiac myocyte is dependent on 4 primary membrane transporters, which are Na\(^{+}\)-H\(^{+}\) exchange (NHE) and Na\(^{+}\)-HCO\(_3\) symport, both of which are activated after acid loading and mediate acid efflux, as well as a Cl\(^{-}\)-HCO\(_3\) and Cl\(^{-}\)-OH\(^{-}\) exchanger, which are stimulated by intracellular alkalosis and are involved in proton influx.\(^1\) NHE plays a pivotal role in the regulation of pH\(_i\) by removing protons that are continuously generated under normal cellular homeostatic processes, as well as during ischemia. NHE1, the so-called housekeeping isoform is ubiquitously distributed in most tissues and is the primary NHE subtype found in the mammalian cardiac cell.\(^2-4\) There are at present 5 known NHE isoforms found in the plasma membrane in mammalian cells.\(^2-4\) A yeast NHE homologue that is intracellularly localized has been identified. Although initially proposed as a mitochondrial NHE in various tissues including heart,\(^5\) recently endosomal localization has been proposed.\(^6\) Cardiac cells lack NHE2 to NHE5 isoforms, although, under prolonged (12-hour) autoradiographic exposure, very faint levels of NHE3 mRNA were detected in rat heart.\(^7\) The function of NHE, or at least isoforms NHE1 to NHE5, is the regulation of pH\(_i\) and cell volume through extrusion of protons in exchange for sodium influx in a 1:1 stoichiometric relationship rendering the process electroneutral.\(^8\) Although NHE is a major regulator of pH\(_i\), it shares this function with a number of other cellular homeostatic pH regulatory systems, as referred to above. Whereas the localization of the other NHE isoforms appears to be restricted to the plasma membrane, the possibility that NHE6 is a mitochondrial isoform suggests that this subtype may regulate mitochondrial function particularly with respect to intramitochondrial Na\(^{+}\) and H\(^{+}\) levels.\(^5\) Because mitochondrial calcium levels can be regulated by Na\(^{+}\) and H\(^{+}\) gradients, NHE6 could represent an important modulator of mitochondrial calcium, particularly under pathological conditions.

Individual NHE isoforms represent distinct gene products and differ by tissue distribution, molecular structures, sensitivities to pharmacological inhibitors, and isoform-dependent distinct roles in cell regulation. Although NHE6 shares only a \(~20\%

amino acid similarity compared with the other currently known NHE isoforms found in the plasma membrane, amino acid identity between these (NHE1 to NHE5) subtypes is markedly greater, ranging from 34% to 60%. Different sensitivities of NHE isoforms to pharmacological inhibition represents a rather fortuitous characteristic that has aided in the development of therapeutic agents, such as newly developed NHE1-specific inhibitors including 4-isopropyl-3-methylsulphonylbenzoyl-guanidine methanesulphonate (HOE 642) (cariporide) or 2-methyl-5-methylsulphonyl-1-(1-
This review will focus primarily on NHE1, which has a major role in the regulation of cardiac function, particularly under pathological conditions and, as discussed below, is emerging as a potential downstream mediator in complex cell signaling, especially in response to receptor activation by a variety of endobiotics. A number of more general overviews of NHE can be recommended.

**NHE1 Structure and Cellular Localization**

Figure 1 represents the putative topological model for the mammalian NHE1, which consists of the following 2 principal domains: a 500–amino acid transmembrane domain and a 315–amino acid highly hydrophilic carboxyl-terminus cytoplasmic domain. The number of membrane-spanning units differs according to NHE isoform type, although NHE1 contains 12 such spanning regions that are critical for the maintenance of NHE1 function in terms of proton extrusion. The hydrophilic cytoplasmic region plays an important role in modulation of the exchanger, especially through phosphorylation-dependent reactions. This region is NHE isoform specific, which likely accounts for differential regulation by diverse factors or stimuli. Although the predicted molecular mass of NHE1 is 91 kDa, because the protein is glycosylated its apparent molecular mass is 110 kDa.

It has recently been suggested that NHE1 is localized primarily in the intercalated disk region of atrial and ventricular myocytes in close proximity to connexin 43, and, to a lesser degree, at the transverse tubular systems. Such localization of NHE1 may implicate the antiporter in cell-to-cell ion-dependent communication via gap junctions, as well as the regulation of \([\text{Ca}^{2+}]\) levels through proton-dependent modulation of \(\text{Ca}^{2+}\) channels in transverse tubules. It is not known whether the apparent specialized localization of NHE1 in the normal cardiomyocyte is preserved when hearts are subjected to pathological factors such as ischemia.

**Regulation of NHE1 Activity**

Intracellular acidosis is a major stimulus for NHE1 activation. Although \(\text{Na}^{+}\) can also affect NHE1, it is unlikely to be a major regulator within a physiological range (7 to 16 mmol/L) of \([\text{Na}^{+}]\). Approximately 60% of proton removal capability of the cardiac cell is accomplished via NHE. NHE1 is regulated by hormones, paracrine/autocrine regulators, and mechanical stimuli such as hypoxic stress, mostly via phosphorylation reactions (reviewed in Reference 16), resulting in increased affinity of the so-called \(\text{H}^+\) sensor (Figure 1). Although the nature of this sensor, which accounts for the exquisite sensitivity of NHE1 to changes in pH, is poorly understood, there is evidence (reviewed in Reference 17) that proton binding triggers a protein conformational change within an NHE oligomer resulting in NHE activation. The sensitivity of the \(\text{H}^+\) sensor is greatly regulated by distinct regions of the C-terminal, and...
agonists that activate NHE1 via phosphorylation reactions shifts the pH$_i$-NHE1 curve toward the alkaline range, that is, there is greater activity at less acidic pH. Studies using C-terminal deletion constructs have demonstrated that when the C terminus beyond position 698 is deleted, the serum-stimulated phosphorylation was no longer observed, whereas with elimination of the C terminus beyond position 635, constitutive phosphorylation was abolished.18

As summarized in Figure 2, various signaling pathways can stimulate cardiac NHE1. These represent modulators of cardiac activity as well as potential contributors to cardiac pathology, including endothelin-1,19–22 angiotensin II,23–25 α$_1$-adrenergic agonists,26–28 thrombin,29 and growth factors.17,30 In addition, cardiotoxic ischemic metabolites, such as hydrogen peroxide31 and lysophosphatidylcholine,32 stimulate NHE1 activity. The effects of these agonists generally reflect the stimulation of phosphoinositide hydrolysis and subsequent activation of kinases such as protein kinase C (PKC), which then phosphorylate and activate NHE1.2,15,33

NHE1 has consensus sequences for mitogen-activated protein (MAP) kinase, and the enzyme has been implicated in NHE1 phosphorylation and activation.31,34 In skeletal muscle, phosphorylation occurs in the 178 amino acids of the C terminus, although removal of this residue still resulted in 50% growth factor activation of the exchanger.34 Recently, a role for p90rsk in MAP kinase-dependent phosphorylation of NHE1 has been demonstrated using rat myocardium.35 MAP kinase-dependent regulation of NHE1 may be important in cardiac disease, as stimulation of the Ras/MAP kinase pathway by cytokines or growth factors may contribute to a participatory role of NHE1 in long-term adaptive responses (see below). Moreover, this system is stimulated by hypoxia, which may contribute to NHE1 activation.36

NHE1 activity can also be regulated by phosphorylation-independent mechanisms.16,17,30,37 For example, complete removal of sites that are phosphorylated in response to thrombin/insulin does not abolish NHE1 activity. Conversely, NHE1 activity can be completely eliminated by deletion of positions 567 and 635, which still preserves mitogen-stimulated phosphorylation.37 There appear to be a number of important regulators of NHE1 that act through such phosphorylation-independent mechanisms. For example, both high- and low-affinity calmodulin binding sites on the C-terminal domain of NHE1 have been identified as resulting in NHE activation and are thought to represent a reversal of the autoinhibitory state of the antiporter that exists under unstimulated conditions.37–39 If this occurs in the heart, it may suggest an alternate mechanism for NHE1 activation under pathological conditions when [Ca$^{2+}$], levels are elevated.

ATP may also regulate NHE1, given that cytoplasmic ATP depletion leads to reduced transport activity.39,40 Independently of phosphorylation and even with truncated NHE1 mutants lacking phosphorylation sites,41 a yet-to-be-identified cofactor may mediate the effect of ATP depletion on NHE1 activity, possibly as a consequence of the inability of this cofactor to interact with NHE1 under conditions of low ATP levels.41,42 Although speculative, it is possible that very low ATP levels in the ischemic myocytes counter the stimulatory effect of intracellular acidosis on NHE1 activity.

In noncardiac tissue, a number of G proteins, including Go$_{q}$, Go$_{12}$, and Go$_{13}$, can modulate NHE activity.43–46 The mechanisms for G protein–induced stimulation appear to be very complex and heavily dependent on G protein type. For example, Go$_{12}$ and Go$_{q}$ have been shown to activate NHE1 via a PKC-dependent mechanism, whereas the effect of Go$_{13}$ is most likely PKC independent.43 It appears that Go$_{13}$ utilizes a distinct kinase cascade using the Rho family of GTPases (Cdc42 and RhoA) to activate NHE1 through MAP/extracellular signal–regulated kinase kinase 1 (MEKK1)–dependent (Cdc42) and –independent (RhoA) pathways.46

Transcriptional Regulation of NHE1

There have been relatively few studies dealing with regulation of expression of the antiporter. Such research has become fruitful after the initial cloning of the promoter region of the NHE1 gene from several species. Kolyada et al37 utilized footprinting analysis to identify the existence of 4 protected sites that were able to bind hepatic proteins. Two of these binding regions (B and C) contain the binding site for activator protein-2 (AP-2) or an AP-2–like transcription factor.48 Although this site contributes to the transcriptional regulation of NHE1 in cardiomyocytes, it appears that 75% of the NHE1 promoter activity in these cells is due to elements distal to the AP-2 site.39 Another element consisting of 15- to 20-dT residues, which may play a role in promoter regulation in various cell types, has been characterized,50 although transcriptional regulation in the cardiac cell remains to be determined.

NHE1 in the Ischemic and Reperfused Myocardium

NHE1 Activity in Ischemia and Reperfusion

Undoubtedly, the most widely studied area in terms of the role of NHE in cardiac pathology pertains to its role in the ischemic and reperfused myocardium. Ischemia-induced acidosis represents a major stimulus for NHE activation with further stimulation by ischemic metabolites such as hydrogen peroxide and lysophosphatidylcholine, as well as paracrine and autocrine factors acting through phosphorylation-dependent processes.

NHE1 mRNA Expression in Ischemia and Reperfusion

In addition to activity, NHE1 mRNA levels are also increased in both the ischemic myocardium as well as in hearts exposed to cardiotoxic ischemic metabolites,51,52 including hydrogen peroxide and lysophosphatidylcholine,52 suggesting that cardiac insult increases NHE1 expression. In contrast, it is interesting that ischemic preconditioning reduces NHE1 mRNA levels and prevents the ability of either ischemia or chemical cardiotoxic agents to increase expression.52

Stimulation of NHE1 mRNA expression is also observed in left ventricular myocardium after coronary artery ligation with or without reperfusion, which interestingly does not occur in animals treated with the NHE1 inhibitor cariporide.53 The basis for the latter is not known; however, because cariporide did not affect basal NHE1 mRNA levels, it is possible that it reflects an inhibition of secondary compo-
Mechanistic Basis for NHE Involvement in Myocardial Ischemic and Reperfusion Injury

General Concepts

Because NHE activation is associated with Na\(^+\) influx, the exchanger may also regulate [Na\(^+\)]\(_i\), under some conditions. Indeed, activation of the NHE in the cardiac myocyte accounts for up to 50% of the basal membrane permeability to Na\(^+\), which may explain the mechanistic basis for the ability of amiloride to decrease the cardiac effects of digitalis glycosides.\(^{55,56}\)

Increasing [Na\(^+\)]\(_i\) will also affect [Ca\(^{2+}\)]\(_i\), levels in the cardiac cell that will affect cardiac function, especially under ischemia and reperfusion. As illustrated in Figure 3, the basis for NHE involvement in myocardial ischemic and reperfusion injury reflects a close interaction between ion-regulatory processes found in the cardiac cell, especially NHE, Na\(^+\)-Ca\(^{2+}\) exchange, and the Na\(^+\)-K\(^+\) ATPase; indeed, inhibition of the latter during ischemia is an important prerequisite for NHE involvement in ischemic and reperfusion injury and forms the basis for a Na\(^+\)-dependent elevation in [Ca\(^{2+}\)]\(_i\), levels resulting in cell injury. It is known that changes in pH\(_i\) and in cytosolic Ca\(^{2+}\) levels are closely related.\(^{57,58}\) Most likely because Na\(^+\) entering via NHE activation is exchanged for Ca\(^{2+}\) via Na\(^+\)-Ca\(^{2+}\) exchange, leading to an increase in Ca\(^{2+}\), a concept supported by various studies.\(^{59-61}\) For example, amiloride attenuated the ability of ouabain to elevate [Na\(^+\)]\(_i\), and to increase the rate and extent of Ca\(^{2+}\) entry through the Na\(^+\)-Ca\(^{2+}\) exchange. Other investigators showed that total cell Ca\(^{2+}\) during and after an NH\(_4\)\(^+\)-induced acid loading in chick heart muscle cells.\(^{62}\) During exposure to NH\(_4\)\(^+\), [Ca\(^{2+}\)]\(_i\) declined by \(\approx\)50%, whereas changing to a NH\(_4\)\(^+\)-free solution, which results in intracellular acidification and stimulation of the NHE, produced an increase in [Ca\(^{2+}\)]\(_i\), back to control values. The net uptake of Ca\(^{2+}\) and net Na\(^+\) extrusion were temporarily correlated, leading the authors to suggest that both the Na\(^+\)-Ca\(^{2+}\) exchange and the Na\(^+\)-K\(^+\) exchange were important in re-establishing the Na\(^+\) gradient subsequent to pH\(_i\) regulation. Duan and Moffat\(^{63}\) have previously demonstrated that accumulation of [Ca\(^{2+}\)]\(_i\), by isolated ventricular myocytes during realkalinization after a brief period of lactate acidosis was inhibited by hexamethylen amiloride in a concentration-dependent fashion, although this was completely dependent on cell stimulation and did not occur in quiescent cells during the initial 4 minutes of realkalinization.\(^{64}\) This observation suggested that the Na\(^+\)-K\(^+\) ATPase by itself was able to dissipate the acidosis-induced Na\(^+\) load in the absence of Na\(^+\) entry through the voltage-dependent channels. This represents an important difference in the cellular response to NHE activation under normoxic versus ischemic conditions in that in the latter Na\(^+\)-K\(^+\) ATPase inhibition would preclude an effective removal of Na\(^+\) ions, thereby causing Ca\(^{2+}\) overloading conditions. Indeed, ouabain enhances injury in reoxygenated myocytes, as evidenced by enhanced cytosolic Ca\(^{2+}\) oscillations, which is attenuated by NHE inhibition.\(^{64}\)

NHE Activation During Reperfusion

It has been proposed\(^{57}\) that activation of the NHE at the time of reflow represents a major component of reperfusion-associated dysfunction and cell injury. This would contribute to restoration of pH\(_i\); however, on the basis of the concepts discussed in the preceding section, the concomitant Na\(^+\) influx would increase [Ca\(^{2+}\)]\(_i\), through the Na\(^+\)-Ca\(^{2+}\) exchanger. Na\(^+\)-Ca\(^{2+}\) exchange involvement in ischemia and reperfusion injury has recently been reinforced by the finding that such injury is enhanced in hearts of mice overexpressing this exchanger, although, interestingly, the phenomenon was not observed in female animals.\(^{65}\) The fact that injury was exacerbated in animals that overexpress Na\(^+\)-Ca\(^{2+}\) exchange could also be taken to suggest that this exchanger actively participates in injury possibly by increasing Ca\(^{2+}\) influx through a reverse mode process.

An alternate concept regarding a reperfusion-induced NHE-dependent injury through Ca\(^{2+}\)-independent mechanisms has also been proposed. This hypothesis, termed the pH paradox, suggests that during ischemia, the loss in intracellular ATP results in phospholipase and protease activation, which results in cell membrane injury; however, because these enzymes possess pH optima in the alkaline range, their detrimental effects are attenuated by ischemia-induced acidosis. However, on reperfusion the rapid restoration of pH\(_i\) reverses the suppression of proteases and other enzymes seen during the ischemic period and results in cell death.\(^{66}\) In addition, the restoration of pH\(_i\) stimulates the formation of the
Cardioprotective Effects of NHE Inhibitors

Very strong support for NHE involvement in cardiac injury has originated from studies that have used drugs that inhibit the antiporter. Indeed, such evidence is very compelling, with protective effects of NHE inhibitors being extensively demonstrated in numerous studies. As summarized in the Table, these effects have been shown with respect to a large number of parameters of cardiac function, including enhanced contractility, reduced contracture, and a decrease in the incidence of arrhythmias, as well as in clinical studies. In addition, improvements in biochemical and ultrastructural indices have been extensively demonstrated with NHE inhibition. The first study demonstrating protection was reported from our laboratory,71 which showed that amiloride, the prototypical NHE inhibitor, enhanced ventricular recovery, and diminished enzyme efflux from repurposed ischemic isolated rat hearts. We have also shown that the protective effect of amiloride is associated with reduced incidence of arrhythmias and preservation of ultrastructural integrity.72 Early studies have shown that this protection is associated with diminished tissue contents of Na⁺ and Ca²⁺, in support of a close association between NHE and Na⁺-Ca²⁺ exchange activity.73 More recent studies have used more specific and potent benzoylguanidine NHE inhibitors, such as the HOE compounds, to demonstrate protection and implicate NHE involvement. For example, the first such agent, HOE 694, has been shown to exert protective effects in terms of virtually all parameters studied both under in vitro conditions and in animals subjected to coronary artery occlusion and reperfusion.74–80 Interestingly, HOE 694 and ethylisobutylamiloride were equally effective as antiarrhythmic agents when administered only at reperfusion.79 It has been proposed that buffer composition may explain some of the controversy concerning the locus of action of NHE inhibitors (ie, during ischemia or reperfusion [see below]), because NHE inhibitors exerted protective effects irrespective of time of addition when hearts were perfused with bicarbonate-free medium, whereas addition before ischemia was a prerequisite for protection with bicarbonate-containing medium.80

HOE 642 (cariporide), a potent NHE1-selective inhibitor, exerts marked protective effects in various experimental models and in terms of numerous parameters under both in vitro and in vivo situations.53,81–89 This may explain the relatively rapid development of this drug for clinical use as a cardioprotective strategy (see below). Likewise, the benzoylguanidine compound EMD 85131 has recently been shown to exert potent infarct-reducing effects in a canine occlusion-reperfusion model, which has also resulted in its entry into the clinical arena.90

Locus of Protection

Although it is not completely certain whether prereperfusion treatment with NHE inhibitors is necessary for myocardial protection, most studies suggest that this is indeed a major requirement. We initially reported that amiloride failed to improve recovery of isolated ischemic rat hearts when administered only at reflow.71 In addition, HOE 694 was ineffective both in blood-perfused isolated ischemic rabbit hearts75 and in porcine hearts76 when it was administered.
solely at reperfusion. In other studies, amiloride or amiloride analogues exerted diminished protection but were not completely ineffective in protecting the reperfused heart when added only during reperfusion compared with protection seen with drug pretreatment before ischemia. Others have shown good protective effects of a variety of amiloride analogs in reperfused right ventricular tissue irrespective of drug addition protocols, which are independent of whether the agents were administered before ischemia or on reperfusion, a finding that is in agreement with the theoretical concept that maximum activation of NHE occurs at reperfusion. Indeed, in posthypoxic reoxygenated rat ventricular myocytes, HOE 694 added at reoxygenation attenuated the development of contracture and Ca$^{2+}$ oscillations by prolonging intracellular acidosis during this period. Although specific NHE1 inhibitors, including cariporide and EMD 85131, reduce infarct size when administered in late ischemia, protection is markedly greater when given before coronary occlusion. A recent report, however, showed a lack of protection when cariporide was administered immediately before reperfusion after coronary artery occlusion in the pig. The superior protection with NHE inhibitors present during ischemia reflects the ability of these agents to inhibit injury in the ischemic nonreperfused myocardium, as discussed above. It can therefore be concluded that NHE inhibitors protect when administered before reperfusion, although for optimal protection, drug administration during both ischemia and reperfusion appears to be a critical prerequisite.

### NHE and Apoptosis

Increasing evidence that apoptosis is an important response of the myocardium to ischemia, which is rapid, precedes cell necrosis, and appears to contribute the overall sequel of cardiac injury [reviewed in Reference 94]. NHE inhibitors can attenuate apoptosis in a variety of models, including myocytes subjected to metabolic inhibition, and ischemia and reperfused isolated hearts, and in vivo coronary artery occlusion; however, the precise mechanism for this effect remains to be determined.

### NHE and Ischemic Preconditioning

One of the characteristics of ischemic preconditioning is diminished acidosis during ischemia, which is associated with reduced [Na$^+$] and [Ca$^{2+}$] content. It has been proposed that reduced acidosis reflects stimulated NHE, and in a calcium-induced preconditioning model dimethylamiloride blocks the cardioprotective effects. However, these findings represent a paradoxical argument against the numerous studies showing benefits of NHE inhibitors. Moreover, inhibition of NHE in the preconditioned myocardium does not abolish the reduced acidosis, which suggests that the latter is NHE independent. Furthermore, NHE inhibition fails to alter the cardioprotective effects of ischemic preconditioning and offers additive protective effects, and the protection by NHE inhibition occurs via a mechanism different from preconditioning. Further evidence that the protective effect of NHE inhibitors differs from that produced by ischemic preconditioning reflects the apparent superior ability produced by the former approach. For example, a recent study demonstrated that the salutary effects of preconditioning in terms of infarct size reduction are overcome with prolonged (90 minutes) coronary artery occlusion of the canine myocardium, whereas protection with NHE inhibition is still maintained.

### Role of NHE in Cardiac Injury Produced by Ischemic Metabolites

In addition to NHE activation due to acidosis, further antiport activation could occur because of direct stimulation by metabolites produced by the ischemic myocardium. For example, endothelin-1 (ET-1) levels are elevated in myocardial ischemia, which can produce deleterious effects on the reperfused myocardium in terms of inducing both diastolic and systolic abnormalities. ET-1 is a potent NHE activator, a property that may account for the positive inotropic effects of the peptide. Indeed, the toxicity produced by ET-1 can be attenuated by NHE inhibition, suggesting an important role of the antiporter in mediating the detrimental effect of the peptide on the ischemic and reperfused myocardium. NHE activation may also represent an important mechanism for development of arrhythmogenesis in the reperfused myocardium, particularly under conditions of elevated catecholamine levels. For example, $\alpha_1$-adrenergic agonists enhance ventricular arrhythmias in the reperfused ischemic myocardium, which can be markedly decreased by NHE inhibition.

In addition to a direct protective effect of NHE inhibitors on the ischemic and reperfused heart, we have also demonstrated that the NHE inhibitors may also explain the initial observation by Neely and Grotyohann that inhibition of glycolytic flux by glycogen depletion results in improved postischemic ventricular recovery, presumably as a result of reduction in the lactate burden at the end of ischemia, given that exogenous lactate reversed the protection. Whether the role of lactate is proton dependent remains uncertain, particularly as the contribution of lactate to intracellular acidosis in the ischemic myocardium is controversial. However, it is interesting that inhibition of lactate accumulation in the ischemic myocardium mimics the effects of amiloride in terms of postischemic contractile recovery as well as Na$^+$ and Ca$^{2+}$ accumulation after reperfusion. Moreover, lactate-induced reduction in contractile recovery after reperfusion is reversed by NHE inhibitors. Whereas these observations are suggestive of NHE involvement in the lactate-induced depression in postischemic recovery, it should be noted that pH$_i$ was not measured in these studies to confirm enhanced ischemia-induced acidosis by lactate. Therefore, the relationship among lactate-induced attenuation of postischemic recovery, pH$_i$, and NHE activity remains to be determined.

We recently reported that lysophosphatidylcholine is a potent NHE activator in the cardiac cell and that the cardio-toxic effects, at least at low concentrations of this amphiphile, can be markedly attenuated by NHE inhibitors. Moreover, we have demonstrated that direct toxicity produced by hydrogen peroxide or the ability of low concentrations of hydrogen peroxide to depress postischemic recovery can be attenuated by NHE inhibition.
Clinical Development and Evaluation of NHE Inhibitors in the Treatment of Coronary Heart Disease

The preliminary results of the first clinical evaluation of NHE inhibition were recently presented at the 48th Annual Scientific Session of the American College of Cardiology. The GUARDIAN (Guard During Ischemia Against Necrosis) study, a Phase II/Phase III double-blind, randomized placebo-controlled study of >11 500 patients, assessed different doses of cariporide in individuals with acute coronary syndromes. The study failed to demonstrate an overall significant attenuation (10%) of the 2 primary events, mortality and incidence of myocardial infarction; however, favorable effects among the 3 major subgroups were observed in those patients receiving the highest dose (120 mg every 8 hours) of the drug, including a significant event rate reduction in high-risk patients undergoing coronary artery bypass surgery. These results are therefore encouraging, especially given that the study also represented a dose-finding component, and overall supports the concept that NHE inhibition represents a safe, therapeutic approach for cardioprotection that undoubtedly deserves future attention. Indeed, the NHE1-selective inhibitor eniporide (EMD 96785) developed by Merck KGaA has been shown to be well tolerated in Phase I trials and is currently being investigated in a Phase II 800 to 1200 patient placebo-controlled trial, the ESCAMI (Evaluation of the Safety and the Cardioprotective Effects of Eniporide in Acute Myocardial Infarction) study in which the drug is administered before angioplasty or thrombolysis. An interim analysis based on >400 patients was performed in late May 1999, at which time it was concluded that the study will continue. Additionally, results with cariporide in a relatively small clinical trial with 100 patients indicate that the drug has the ability to improve left ventricular function when administered before balloon angioplasty in patients with acute myocardial infarction.111 Taken together, there has been a rather rapid evolution of progress in the development of novel therapeutic strategies for treating coronary heart disease, although further clinical assessment is still required.

NHE and Other Cardiac Disease States

Diabetic Myocardium

Myocardial NHE1 activity in diabetes appears to be depressed, possibly because of reduced calcium/calmodulin II–dependent phosphorylation of the antiporter,112 and recovery from ischemia-induced acidosis in the diabetic heart is less dependent on NHE and more on bicarbonate-dependent processes.113 Depressed NHE activity may explain increased resistance of diabetic hearts to ischemia and reperfusion.114,115

Cardiac Hypertrophy and Heart Failure

The ability of pH i to affect protein synthesis renders NHE a potentially important target for the regulation of cell proliferation and hypertrophy through pharmacological approaches. Indeed, there is now compelling evidence from studies using a variety of tissues that cell growth and proliferation may be regulated by NHE and that NHE inhibitors can block such responses. Of particular interest are reports that neo-intimal thickening and smooth muscle cell proliferation after carotid artery balloon injury are mitigated by NHE inhibitors.116,117 Moreover, angiotensin II–induced vascular smooth muscle cell hypertrophy appears to be mediated by NHE.118 Thus, NHE inhibitors could be potentially useful for the attenuation of atherosclerotic lesion development and restenosis.

As shown in Figure 2, NHE represents a key downstream factor activated by a variety of hypertrophic stimuli. Indeed, in cardiac cells, NHE inhibitors block hypertrophic responses to various stimuli. 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,119,120 as can norepinephrine-induced protein synthesis in cultured rat cardiomyocytes.121 Orally administered amiloride, a nonspecific NHE inhibitor, reduces fiber diameter in rat coronary ligation122 and murine dilated cardiomyopathy models.123 We have recently found that dietary administration of cariporide completely abrogates the increased length of surviving myocytes after 1 week after coronary artery occlusion and ameliorates contractile dysfunction in the absence of afterload or infarct size reduction.124 Thus, it appears that numerous endogenous factors that have been implicated in the ventricular remodeling/heart failure process also activate NHE. It is unlikely that the antiport is the only intracellular messenger mediating such responses, although it has been suggested that NHE may therefore serve as an important downstream regulator contributing to remodeling in response to various hypertrophic factors.125 Interestingly, however, it has recently been shown that the ability of both endothelin-1 and angiotensin II to activate NHE is impaired in rat cardiac myocytes from hypertrophied hearts caused by aortic banding, a finding attributed to defective coupling between PKC and NHE activation.21 A similar observation, at least with respect to angiotensin II, has been demonstrated in surviving ventricular myocytes from chronically infarcted rabbit myocardium, suggesting that this defective coupling may not be dependent on species or hypertrophic model.126 The relevance of these findings remains to be determined, although it is attractive to suggest that they may reflect potential protective compensatory responses aimed at attenuating the potential deleterious effect of stimulated NHE activation.

Conclusions

The past 11 years have seen impressive progress in the understanding of the regulation of NHE in terms of activity and transcriptional regulation. In the domain of cardiac research, there has emerged virtually undisputable evidence that NHE activation in the ischemic and reperfused heart plays a major role in restoring pH i, which at the same contributes to tissue damage. Accordingly, NHE inhibition has been demonstrated in numerous experimental studies to protect the myocardium, which has translated into initial clinical evaluation. In addition to its role in acute ischemia and reperfusion, the antiporter is likely of importance in other cardiac disease states, the most compelling of which appear to be postinfarction myocardial remodeling, hypertrophy, and evolution to heart failure. Much work needs to be done in this regard, particularly in terms of understanding of the regula-
tion of NHE in chronic responses and how the system can be ideally modulated for therapeutic strategies, either alone or as adjunctive therapy with other treatment modalities.

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