REVIEW ARTICLE

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The Myocardial Na^+/H^+ Exchanger

A Potential Therapeutic Target for the Prevention of Myocardial Ischaemic and Reperfusion Injury and Attenuation of Postinfarction Heart Failure

Morris Karmazyn, John V. Sostaric and *Xiaohong Tracey Gan*

Department of Pharmacology and Toxicology, University of Western Ontario, Medical Sciences Building, London, Ontario, Canada

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Abstract

The myocardial Na^+/H^+ exchange (NHE) represents a major mechanism for pH regulation during normal physiological processes but especially during ischaemia and early reperfusion. However, there is now very compelling evidence that its activation contributes to paradoxical induction of cell injury. The mechanism for this most probably reflects the fact that activation of the exchanger is closely coupled to Na^+ influx and therefore to elevation in intracellular Ca^{2+} concentrations through the Na^+/Ca^{2+} exchange. The NHE is exquisitely sensitive to intracellular acidosis; however, other factors can also exhibit stimulatory effects via phosphorylation-dependent processes. These generally represent various autocrine and paracrine as well as hormonal factors such as endothelin-1, angio-

tensin II and α_1-adrenoceptor agonists, which probably act through receptor-signal transduction processes.

Thus far, 6 NHE isoforms have been identified and designated as NHE1 through NHE6. All except NHE6, which is located intracellularly, are restricted to the sarcolemmal membrane. In the mammalian myocardium the NHE1 subtype is the predominant isoform, although NHE6 has also been identified in the heart. The predominance of NHE1 in the myocardium is of some importance since, as discussed in this review, pharmacological development of NHE inhibitors for cardiac therapeutics has concentrated specifically on those agents which are selective for NHE1.

These agents, as well as the earlier nonspecific amiloride derivatives have now been extensively demonstrated to possess excellent cardioprotective properties, which appear to be superior to other strategies, including the extensively studied phenomenon of ischaemic preconditioning. Moreover, the salutary effects of NHE inhibitors have been demonstrated using a variety of experimental models as well as animal species suggesting that the role of the NHE in mediating injury is not species specific.

The success of NHE inhibitors in experimental studies has led to clinical trials for the evaluation of these agents in high risk patients with coronary artery disease as well as in patients with acute myocardial infarction (MI). Recent evidence also suggests that NHE inhibition may be conducive to attenuating the remodelling process after MI, independently of infarct size reduction, and attenuation of subsequent postinfarction heart failure. As such, inhibitors of NHE offer substantial promise for clinical development for attenuation of both acute responses to myocardial as well as chronic postinfarction responses resulting in the evolution to heart failure.

Changes in intracellular pH (pH_i) can have profound effects on cardiac contractility through complex mechanisms. It is therefore critical that the cell possesses mechanisms by which pH_i is regulated, especially after intracellular acidosis as a consequence of myocardial ischaemia. Although the regulation of pH_i is very complex and reflects a net balance of alkalinising and acidification processes, the two major alkalinising exchangers which are important for controlling intracellular acidosis are the Na^+/H^+ exchanger (NHE) and a Na-HCO_3-symport. The NHE represents one of the key mechanisms for restoring pH_i following ischaemia-induced acidosis by extruding protons concomitantly with Na^+ influx in an electroneutral process. At present, 6 NHE isoforms have been identified (termed NHE1 to NHE6) with the NHE1 subtype representing the ubiquitous isoform, although it appears that it is the primary one found in the mammalian heart. When NHE is activated, the simultaneous entry of Na^+ during NHE activation probably represents an important route for increasing intracellular Na^+ concentrations during various conditions. In the ischaemic cell particularly, the activation of NHE by intracellular proton generation and the resultant entry of Na^+ results in a potentially disastrous consequence as the excess Na^+ cannot be extruded because of depressed Na^+/K^+ adenosine triphosphate (ATP)ase activity. As a result, the reduction in the transmembrane Na^+ gradient will result in increased intracellular Ca^{2+} levels via the Na^+/Ca^{2+} exchanger producing intracellular Ca^{2+} overload and cell death. Pharmacological studies with NHE inhibitors, in particular, have extensively and repeatedly demonstrated protective effects in a large number of experimental models. Inhibition of NHE as a therapeutic tool has now entered the clinical arena as reflected by substantial effort by the pharmaceutical industry to develop potent NHE1-specific inhibitors with poten-

tial as effective therapeutic agents in patients with coronary artery disease (CAD). Indeed, some of these agents have either undergone or are currently in the process of clinical evaluation. It is interesting that in addition to its potential role in mediating ischaemic and reperfusion injury, NHE appears to also contribute to the postinfarction hypertrophic and remodelling process, which can lead to the eventual development of heart failure. As such, a potential added benefit of NHE1 inhibitors may include attenuation of the evolution of infarcted myocardium to failure. The aim of this review is to summarise our current knowledge of NHE1 in the heart in terms of its structure and regulation, and of particular relevance, the importance of NHE1 in cardiac pathology. We also discuss the pharmacology of NHE inhibitors and the development of novel and specific NHE1 inhibitors for cardiac therapeutics. The pharmacology of NHE inhibitors can be readily separated into two periods of study and development, the first representing an era where amiloride or its analogues represented the primary pharmacological tools to probe the exchanger. These studies have provided important evidence as well as mechanisms for the role of NHE in cardiac injury.[1-3] It is clear however that the therapeutic benefits of this research will probably materialise with more recently developed NHE1 specific inhibitors aimed at clinical development, of which 2 have already entered clinical trials. Accordingly, this review will focus almost exclusively on these compounds that are at present in preclinical or clinical development.

1. Na^+/H^+ Exchanger (NHE) Isoforms

As mentioned in the introduction, to date 6 isoforms of NHE have been identified in mammalian cells. They represent distinct gene products and exhibit distinct differences in their primary structures, patterns of tissue expression, membrane localisation, the number of transmembrane spanning regions, functional properties, physiological roles, and sensitivities to pharmacological inhibition.[4,5] Briefly, NHE1 to NHE5 share approximately 34 to 60% amino acid homology, whereas NHE6 shares only 20% homology with the other isoforms. NHE1 is expressed in virtually all-mammalian cells, whereas NHE2 to NHE5 show a more restricted pattern of expression. NHE6 is intracellularly localised[6,7] and could be an important modulator of intramitochondrial Na^+ and H^+ levels as well mitochondrial Ca^{2+} levels, particularly in pathological conditions.[6] However, substantial research is necessary in order to delineate the potential role of NHE6 in the heart either under normal or pathological conditions.

The various isoforms show marked differences in their sensitivities to pharmacological inhibition by amiloride and its derivates, which represent the prototypical NHE inhibitors and which are generally not specific for NHE subtypes.[8] Evidence to date indicates that NHE1 is the primary form identified in the mammalian myocardium[9] and represents the major subtype discussed in this review. Accordingly, as is discussed in this review, this characteristic has aided the development of therapeutic agents which are aimed exclusively at inhibiting this particular NHE subtype.

2. Structure and Cellular Localisation of NHE1

As depicted in figure 1, NHE1 contains 815 amino acids and can be separated into 2 distinct functional domains: a 500-amino acid transmembrane domain, made up of 12 transmembrane spanning segments and a 315-amino acid hydrophilic cytoplasmic carboxy terminal domain.[4,5] The 500-amino acid transmembrane domain is primarily responsible for proton extrusion,[10] and the 315-amino acid C terminal domain is responsible for modulation of NHE1 activity, primarily via phosphorylation-dependent reactions.[4,11] Although the predicted molecular weight of the exchanger is 91kDa, the actual weight is 110kDa since it is glycosylated, although this does not appear to be essential for transport function.[12]

Immunohistochemical studies have revealed that NHE1 is predominantly localised at the intercalated disk region of atrial and ventricular myocytes in close proximity to the gap junction protein,

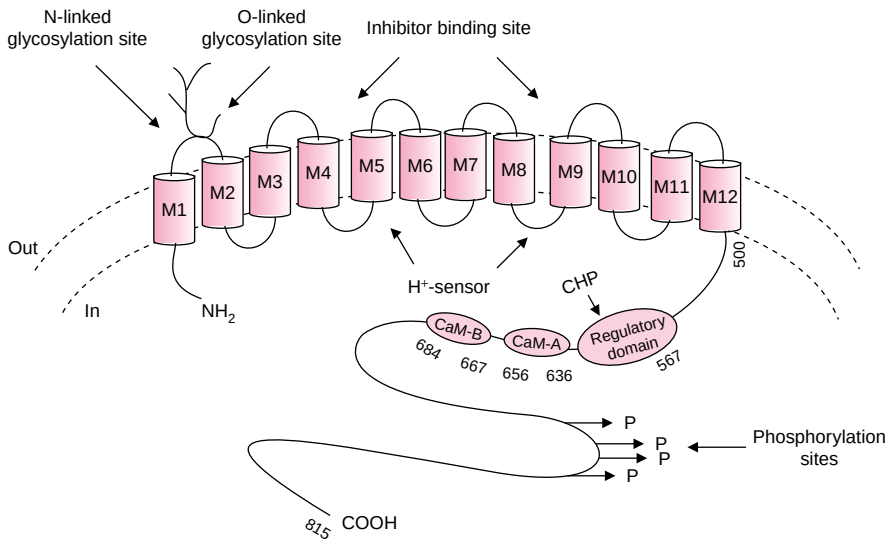


Fig. 1. Putative topological model of 815 amino acid Na^+/H^+ exchange (NHE) isoform NHE1, showing 12 transmembrane spanning segments and hydrophilic carboxyl terminus with indications of proposed regulatory sites. The transmembrane domain represents the site of functional ion exchange whereas the cytoplasmic region represents the major sites for regulation via phosphorylation-dependent and -independent reactions. The regulatory domain is the major determinant of the set-point of the exchanger and involves regulation by growth factors as well as osmotic stress and which is possibly regulated by calcineurin homologous protein (CHP). Both high (CaM-A) and low (CaM-B) affinity calmodulin binding sites on the C-terminal domain of NHE1 have been identified and which are linked to NHE1 activation. Stimulation of these binding sites are thought to represent the basis for the reversal of the autoinhibitory state of the antiporter which normally exists under unstimulated conditions when intracellular calcium levels are low.

connexin 43, and to a lesser extent, along the transverse tubular system.[13] Connexin 43 and the sarcoplasmic reticulum Ca^{2+} release channel (i.e. ryanodine receptor) are highly pH_i-sensitive. Thus it has been speculated that because of its apparent localisation, NHE1 regulates the pH microenvironment of these pH_i-sensitive proteins, and thereby influences cell-to-cell ion-dependent communication and intracellular Ca^{2+} levels.[13]

3. Regulation of NHE1 Activity

3.1 Role of Intracellular pH

The major stimulus that regulates NHE1 activity under normal physiological conditions is pH_i.[14] This concept is depicted in figure 2 which shows the theoretical relationship between pH_i and NHE1 activity. Within the normal physiological pH range (pH 7.1 to 7.3), NHE1 activity is negligible, but as pH_i decreases, the exchanger becomes rapidly activated. The reason for this rapid activation is because of the so-called H^+ sensor, which is found on the cytoplasmic surface of the exchanger and accounts for the sensitivity of the exchanger to pH_i. Although the exact nature of the molecular mechanisms involved in activation by the H^+ sensor is poorly understood, it is believed that binding of H^+ to this site induces a conformational change of the NHE oligomer resulting in an increase in NHE activity.[10] Extrinsic factors such as hormones, growth factors, cytokines, and autocrine/paracrine regulators modulate NHE1 activity by increasing the sensitivity of the H^+ sensor to pH_i, thus causing a shift of NHE1 activity towards an alkaline range; that is, NHE1 activity increases at a less acidic pH_i (fig. 2). This shift in pH_i dependence is accomplished mainly via phosphorylation reactions of the C terminal domain of the exchanger, which is responsi-

ble for determining the pH_i set point value of the H^+ sensor.[11,15]

3.2 Activation by Paracrine and Autocrine Factors

Various signalling pathways can modulate cardiac NHE1 activity, including endothelin-1,[16,17] angiotensin II,[18,19] α_1-adrenergic agonists,[20,21] thrombin,[22] and growth factors.[15,23,24] Accordingly, it is important to stress that these stimulatory factors are modulators of normal cardiac activity and are also potential contributors to cardiac pathology. The effects of these agonists generally involve phosphoinositide hydrolysis and activation of kinases such as protein kinase C (PKC) resulting in NHE1 activation.[4,11,24,25] In addition, cardiotoxic ischaemic metabolites such as hydrogen peroxide[26] and lysophosphatidylcholine (LPC)[27] have also been demonstrated to stimulate NHE1 activity, a phenomenon which probably contributes to the cardiotoxic effects of these factors (see section 5).

3.3 Role of Phosphorylation

Structure-function studies have indicated that NHE1 contains consensus sequences for mitogen-activated protein (MAP) kinases, which have been implicated in NHE1 phosphorylation and activation[26,28] and it has been established, using rabbit skeletal muscle, that MAP kinases can directly phosphorylate the C terminal domain of NHE1.[28] Recently, a role for $p90^{rsk}$ in MAP-kinase dependent phosphorylation of NHE1 has been demonstrated in rat myocardium.[29] In addition, hypoxia, hypoxia with reoxygenation,[30] hydrogen peroxide[26] and other reactive oxygen species[31] have been known to stimulate the MAP-kinase signalling pathway, which can contribute to NHE1 activation.

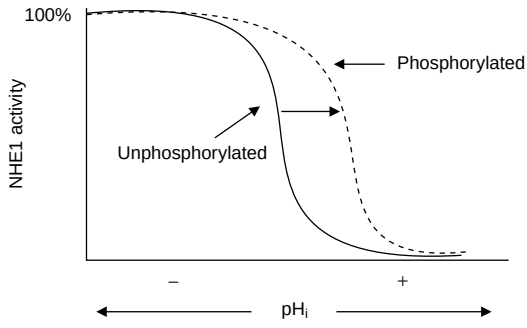


Fig. 2. Illustration of the theoretical relationship between intracellular pH (pH_i) and Na^+/H^+ exchange (NHE) isoform NHE1 activity. Note the steep relationship between pH changes and NHE1 activity as well as the ability of various factors which can phosphorylate NHE1 to shift the pH activity rightwards. Although actual pH_i values are not shown, NHE1 activity at normal pH values (e.g. 7.2) is minimal (approximately 20% of maximum).

3.4 Phosphorylation-Independent Regulation

NHE1 activity can also be regulated via phosphorylation-independent mechanisms.[32,33] For example, deletion of the cytoplasmic C terminal domain at residue 635 removes all phosphorylation sites although this reduces growth factor activation of NHE1 by only 50%.[28] In addition, NHE1 activity can be completely eliminated following deletion of residues 567-635, while preserving mitogen-stimulated phosphorylation.[33] These studies therefore strongly implicate factors other than phosphorylation which may be involved in NHE1 activation. Bertrand et al.[32] reported that the cytoplasmic C terminal tail of NHE1 contained 2 domains capable of binding calmodulin with either high (CaM-A residues 636-656) or low (CaM-B residues 667-684) affinity. The high affinity CaM-A site is believed to be important in transport regulation. Deletions of residues 636-656 render NHE1 constitutively active, as if cytosolic Ca^{2+} levels were continuously elevated. Based on these observations, it was suggested that at basal intracellular Ca^{2+} levels, the unoccupied CaM-A binding domain exerts an autoinhibitory effect that is relieved upon Ca^{2+}/calmodulin binding.[33] Although this has yet to be demonstrated in the myocardium, it nevertheless suggests an alternative method for NHE1 activation in pathological conditions in which intracellular Ca^{2+} levels are elevated.

3.5 Role of Adenosine Triphosphate

ATP has also been demonstrated to regulate NHE1 activity. Depletion of cytoplasmic ATP results in reduced transport activity of the exchanger[34,35] although it appears that this is unlikely to be related to changes in the phosphorylation state of the exchanger.[35] It has been hypothesised that a yet-to-be-identified ancillary protein may mediate the effect of ATP depletion on NHE1 activity. It is believed that an ATP-dependent reversible association of a cofactor may regulate the exchanger, and that upon binding of ATP, this cofactor will dissociate from the exchanger and remove its inhibitory effect.[35] Whether this has relevance to the regulation of NHE1 in the ischaemic myocardium is not known, particularly since ATP depletion during ischaemia is a relatively slow process. However, the possibility exists that very low levels of the nucleotide in ischaemic myocytes could potentially counter the stimulatory effect of intracellular acidosis on NHE1 activity.[2]

3.6 Activation by G-proteins

It is also worth mentioning that a number of G-proteins can modulate NHE1 activity, although they have only been demonstrated to do so in non-cardiac tissue. The mechanisms by which G-proteins stimulate NHE1 activity are very complex, and vary depending on the G-protein type. For example, $G_{\alpha q}$ and $G_{\alpha 12}$ have been shown to regulate NHE1 activity primarily via a PKC-dependent pathway, whereas $G_{\alpha 13}$ mediates NHE1 activity via a PKC-independent pathway.[36-38] $G_{\alpha 13}$ utilises a distinct kinase cascade using the Rho family of guanine triphosphate (GTP)ases (Cdc42 and RhoA) to activate NHE1 through MAP/extracellular signal-regulated kinase kinase 1 (MEKK1)-dependent (Cdc42) and –independent (RhoA) pathways.[39]

A phosphoprotein termed calcineurin homologous protein (CHP) has been reported to inhibit both serum- and GTPase-activated NHE1 activity by binding to the regulatory domain of the C terminal.[40] It is not known at present what the role of CHP is regulating NHE1 activity in the myocardium under normal or pathological conditions.

4. Regulation of NHE1 Expression

Another important level of regulation of NHE1 deals with the number of available exchanger units available on the plasma membrane. Although the majority of the studies published on the regulation of NHE1 have focused on regulation of activity, only a few studies have focused on the regulation of expression of the exchanger. This primarily reflects the fact that the promoter region of NHE1 has only been recently cloned. Kolyada et al.[41] utilised footprinting analysis to identify the existence of 4 protected sites that were able to bind to hepatic proteins and that two of these binding regions (B and C) contained a binding site for the activator protein-2 (AP-2) or the AP-2-like transcription factor.[41] Although the AP-2 site contributes to the transcriptional regulation of NHE1 in cardiomyocytes, a majority of NHE1 promoter activity has been demonstrated to be due to elements distal to the AP-2 site, primarily a poly(dA · dT)-rich region.[42,43] Irrespective of the exact mechanism for transcriptional regulation of NHE1, it is nonetheless relevant that many factors which produce cell injury, including myocardial ischaemia or the direct administration of cardiotoxic compounds including LPC or hydrogen peroxide, can all increase tissue levels of NHE1 mRNA suggesting that the exchanger may be stimulated by both increased activity as well as turnover.[44]

5. Mechanisms Underlying NHE Involvement in Myocardial Ischaemic and Reperfusion Injury

A scheme for NHE1 involvement for ischaemic- and reperfusion-induced injury is shown in figure 3, which also depicts the potential role of the antiporter in chronic postinfarction responses which are discussed below in section 9. The primary basis for NHE1 involvement in acute injury reflects the inability of the ischaemic cardiac cell to extrude Na^+ because of Na^+/K^+ ATPase inhibition, which occurs in concert with NHE1 activation, the latter oc-

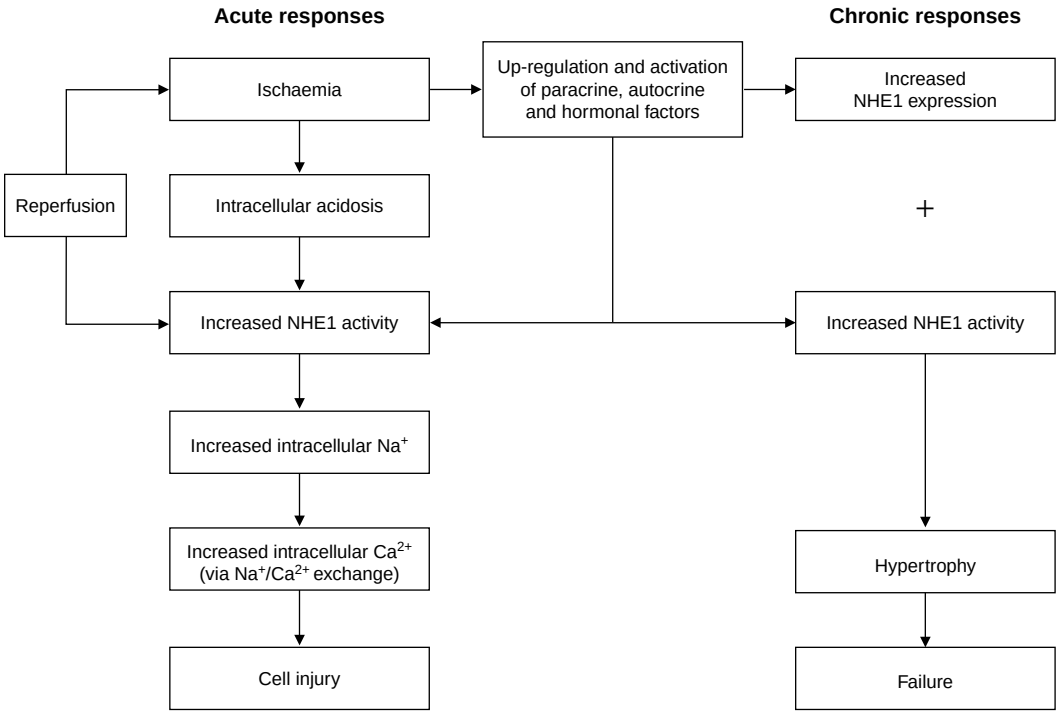


Fig. 3. Simplified schematic demonstration of the potential pivotal role of Na^+/H^+ exchange (NHE) in mediating acute and chronic postinfarction responses. See text for detailed description.

curring as a consequence of increased proton generation during ischaemia. Indeed, it could be stated that inhibition of Na^+/K^+ ATPase is a *prerequisite* for NHE1 involvement in ischaemic and reperfusion injury and that in the absence of such inhibition NHE1 activation would be unlikely to represent a deleterious influence on the myocardium. In addition, as noted in section 3, NHE1 is further activated by various hormonal, autocrine, or paracrine factors as well as metabolites produced either extracellularly or intracellularly during myocardial ischaemia including hydrogen peroxide and LPC. Thus, the net result is a multifactorial stimulation of NHE under pathological conditions, not only due to increased intracellular acidosis but also to activation by external factors. Such marked NHE1 stimulation increases an elevation in intracellular Na^+ levels which in turn increases intracellular Ca^{2+} levels *via* Na^+/Ca^{2+} exchange resulting in cell injury. Recent evidence suggests that the Na^+/Ca^{2+} exchanger may actively contribute to calcium overload via reverse-mode Ca^{2+} entry since transgenic mice overexpressing this exchanger show an increased sensitivity to ischaemic injury, which would not be expected if elevated Ca^{2+} levels occurred primarily via reduced efflux.[45] It is interesting that this increased sensitivity was observed in male, but not female animals.[45]

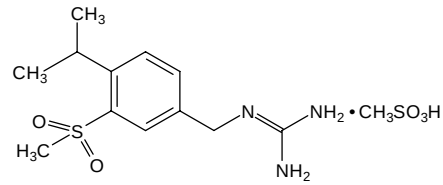
It should be noted that an alternative concept regarding a reperfusion-induced NHE-dependent injury through Ca^{2+}-independent mechanisms has also been proposed, which has been termed the pH paradox. This hypothesis proposes that the reduction in intracellular ATP levels during myocardial ischaemia results in phospholipase and protease activation that would normally produce cell mem-

brane injury; however, because these enzymes possess pH optima in the alkaline range, their detrimental effects are attenuated by ischaemia-induced acidosis. Upon reperfusion the rapid restoration of pH_i reverses the suppression of proteases and other enzymes seen during the ischaemic period and results in cell death.[46] In addition, the restoration of pHi stimulates the formation of the mitochondrial membrane permeability transition which results in depression of ATP resynthesis via oxidative phosphorylation pathways.[46] The relative contribution of this process to NHE1-dependent cardiac injury is however not certain but is supported by studies utilising individual myocytes illustrating a protective effect of NHE inhibition against reoxygenation, which can be dissociated from intracellular Ca^{2+} levels.[47] It is possible that this mechanism may contribute specifically to reperfusion injury *per se* but obviously would not account for the potential role of NHE1 inhibition during ischaemia in the absence of reperfusion where the exchanger plays a critical role.

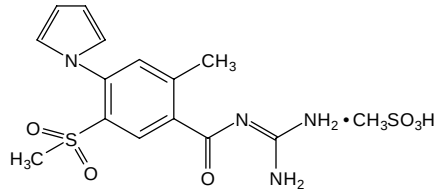
6. Pharmacological Modulation of NHE Activity

The first series of drugs that have been demonstrated to inhibit NHE are the potassium-sparing diuretic amiloride and, more specifically, the *N*-5 disubstituted derivatives of amiloride which exhibit greater potency and specificity than the parent compound.[48] Despite the ability of these agents to inhibit NHE, their eventual therapeutic development has been restricted by various nonspecific actions, lack of selectivity against NHE1 and relatively low potency. This has led to the development of novel benzoylguanidine compounds targeted specifically against NHE1, thereby increasing potential for treatment in patients with CAD.[49-51] The first such compound was 3-methylsulphonyl-4-piperidinobenzoyl-guanidine methanesulphonate (HOE-694) which was followed by 4-isopropyl-3-methylsulphonylbenzoyl-guanidine methanesulphonate (HOE 642, cariporide); the latter, as discussed in section 8.1, recently undergoing clinical trials in high risk patients with acute coronary syndromes. Cariporide is of particular interest as it appears to be a selective inhibitor of the cardiac-specific NHE1 isoform rendering it particularly attractive for therapeutic interventions for cardiac disorders while minimising the potential for adverse effects (see section 7). The mechanism of action of NHE1 inhibitors is not known precisely, although their effects on the antiporter involve binding to sites on the lipophilic transmembrane region. Following construction of a variety of chimeric NHE constructs, Orlowski and Kandasamy[8] demonstrated potential sites on a number of transmembrane units to which these drugs can bind, with the M4 and M9 regions of particular importance. Using site-directed mutagenesis, Wang and colleagues[52] have shown that the histidine 349 residue may be of particular importance for interaction of NHE1 with inhibitors, at least of the amiloride series, although it is not known whether this can be extended to the more recently developed NHE1 inhibitors.

The structures of cariporide and eniporide (EMD-96785), two NHE1 inhibitors currently in clinical development, are shown in figure 4.



Cariporide



Eniporide

Fig. 4. Structures of 2 Na^+/H^+ exchange (NHE) isoform NHE1 specific inhibitors currently in clinical development for the treatment of patients with coronary artery disease.

7. Myocardial Protection by NHE Inhibitors

The extensive documentation demonstrating cardioprotective effects of NHE inhibitors has strongly supported the concept of the antiporter's involvement in cardiac injury, especially under conditions of ischaemia and reperfusion. The earlier studies utilised amiloride or amiloride analogues to demonstrate cardioprotective properties; however, more recent data utilising drugs targeted for clinical development reported excellent and consistent protection in a wide variety of experimental models and animal species, which is probably unmatched in the cardioprotection literature. As summarised in table I, a number of such agents are either in clinical or preclinical development, and all have been shown to protect the myocardium against either ischaemia or reperfusion or against the direct deleterious effects of cardiotoxic compounds produced by the ischaemic myocardium. Moreover, there appear to be no discrepant results that fail to show protective effects of NHE1 inhibitors. In addition, as table I illustrates, the protective effects of NHE1 inhibitors appear to be species-independent. A particularly striking feature of NHE1 inhibitors is their ability to protect against various forms of dysfunctions including reduced mortality, limitation of infarct size, improvement of functional recovery after reperfusion, reduction of arrhythmias, attenuation of calcium and sodium dyshomeostasis, reduction of apoptosis as well as preservation of metabolic status such as attenuation of high energy phosphate depletion.

Many of the newer drugs have been tested for their ability to protect the myocardium when administered only at reperfusion, a property that would be important in terms of treatment of patients who present with acute myocardial infarction (MI). As summarised in table I, most agents do indeed demonstrate protective effects when administered at this period although it should be stated that, in general, such protection is less than that seen with pre-ischaemia drug administration. From a mechanistic perspective, these findings are not surprising since NHE1 activation during ischaemia contributes substantially to the sodium and calcium overloading conditions and resultant cell injury with further NHE1 activation occurring immediately upon reflow. As such, *optimum* protective effects of NHE1 inhibitors will probably be realised when treatment can be maintained during both ischaemia and reperfusion.

It is also interesting to note that the potential for toxicity or untoward adverse effects of NHE1 inhibitors is relatively low in view of the specificity of newer agents. Moreover, it is important to point out that drugs targeted at NHE1 inhibition have limited potential for disruption of normal cell homeostasis since NHE1 activity is generally restricted under normal conditions:[81] thus, these drugs have the potential for selectivity by inhibiting a process associated primarily with pathology. Indeed, clinical evaluation of both cariporide and eniporide has so far revealed excellent tolerance.

7.1 Comparison of NHE1 Inhibitors with Other Cardioprotective Strategies

A critical consideration in designing novel cardioprotective strategies is to assess whether such new approaches surpass currently available modes of protection. Extensive head-to-head comparisons between NHE1 inhibitors and other strategies have not been reported, although some of the earlier evidence with cariporide demonstrated reduction of arrhythmias in the infarcted myocardium which were generally refractory to classical antiarrhythmic agents.[53] From a general perspective, it can be confidently stated that studies with NHE1 inhibitors are unique in the sense that no contradictory data have been reported and, as summarised in table I, the approach appears to be effective against various forms of injury and in a variety of experimental models, properties not seen with other cardioprotective approaches.

7.1.1 NHE Inhibition versus Ischaemic Preconditioning as Cardioprotective Strategies

It is likely that the most widely studied cardioprotective strategy is ischaemic preconditioning, a phenomenon in which multiple episodes of brief ischaemia confers protection against sub-

Table I. Summary of some recent studies demonstrating cardioprotective effects of Na^+/H^+ exchange (NHE) isoform NHE1 inhibitors in preclinical or clinical development against various models of cardiac injury. The table summarises major findings published in peer-reviewed literature utilising drugs that are presently in preclinical or clinical development and is not meant to represent a complete listing of all studies demonstrating protective effects of NHE inhibitors

Drug (manufacturer)	Model	Species	Parameters attenuated	Effective when given only at reperfusion?	Reference
Cariporide[a] (Aventis)	CAL	Rat	Arrhythmias	Not determined	53
	CAL	Rat	Mortality/arrhythmias	Not determined	54
	CAL	Pig	Rigor formation/ IS/arrhythmias	Limited antiarrhythmic effect	55
	CAL	Rat	IS/arrhythmias	Not determined	56
	CAL	Rat	Arrhythmias/mortality	Yes	57
	CAL	Rat	Arrhythmias/metabolic disturbances	Yes	58
	CAL	Rabbit	IS/LVEDP	Yes	59
	CAL	Rabbit	IS	No	60
	CAL	Pig	IS/contractile dysfunction	Not determined	61
	CAL	Dog	Ventricular fibrillation	Not determined	62
	Anoxic myocytes	GP	Na^+ overload	Not applicable	63
	IR isolated heart	Rat	Arrhythmias/HEP depletion/glycogen depletion/enzyme efflux	Not determined	53
	IR isolated heart	GP	Contractile dysfunction/Na^+ overload/HEP depletion	Not determined	64
	IR isolated heart	Rat	Electrical disturbances/ATP depletion/LVEDP	Not determined	65
	IR isolated heart	Rat	Contractile dysfunction/enzyme efflux	Not determined	66, 67
	IR isolated heart	Rat	Contractile dysfunction, HEP depletion	Not determined	68, 69
	IR isolated heart	Rat	Apoptosis	Not determined	70
	HR isolated heart	Rat	Oedema/enzyme efflux	Not determined	71
	LPC isolated heart	Rat	Contractile dysfunction/HEP depletion/glycogen depletion/ultrastructural injury	Not applicable	27
	H_2O_2 isolated heart	Rat	Contractile dysfunction/HEP depletion/glycogen depletion	Not applicable	72
EMD-85131[a] (Merck KGaA)	CAL	Dog	IS	Yes	73
BIIB-513 (Boehringer)	CAL	Dog	IS	Not determined	74
	CAL	Dog	IS/arrhythmias	Yes	75
SM-15681 (Sumitomo)	IR isolated heart	Rat	Contractile dysfunction/enzyme efflux/Ca^{2+} overload	Not determined	76
SM-20550 (Sumitomo)	CAL	Dog	IS/microvascular protection	Yes	77
MS-31038 (Mitsui)	CAL	Rat	IS	Yes	78
FR-183998 (Fujisawa)	CAL	Rat	IS/arrhythmias/mortality	Yes (arrhythmias only)	79
FR-168888 (Fujisawa)	CAL	Rat	IS/arrhythmias	Not determined	80

a Indicates drug undergoing clinical trials (see text). Note that EMD-85131 is the hydrochloride analogue of eniporide (EMD-96785).

CAL = coronary artery ligation (*in vivo*); **GP** = guinea pig; **HEP** = high energy phosphate; **HR** = hypoxic and reoxygenated; **IR** = ischaemic and reperfused; **IS** = infarct size; **LPC** = lysophosphatidylcholine; **LVEDP** = left ventricular end diastolic pressure.

sequent prolonged ischaemic insult.[82] The mechanism of ischaemic preconditioning is not completely understood but is probably distinct from NHE1 inhibition, indeed NHE1 inhibitors offer added protection when administered to preconditioned hearts.[66] Protection by ischaemic preconditioning is indeed impressive and it has generally been considered for many years as the most effective known cardioprotective strategy. This has recently been challenged, however, in a study that demonstrated comparable protection by NHE1 inhibition in terms of infarct size reduction in dogs subjected to coronary artery ligation for 60 minutes. However, when the period of occlusion was extended to 90 minutes, preconditioning failed to exert salutary effects, although NHE1 inhibition reduced infarct size by approximately 70%. This indicates that NHE1 inhibition confers superior protection to ischaemic preconditioning, at least in this model.[74]

8. Clinical Evaluation of NHE1 Inhibitors in Patients with Coronary Heart Disease

8.1. Cariporide: The GUARDIAN Study

Clinical development and testing of NHE1 inhibitors in cardiac disease states has been relatively rapid, possibly reflecting the consistent and excellent protection with these agents demonstrated in animal studies. The first such study, termed the GUARDIAN (Guard During Ischaemia Against Necrosis) was an ambitious combined phase II/phase III double-blind, randomised, placebo-controlled study of more than 11 590 patients to assess different doses of cariporide in individuals with acute coronary syndromes with outcomes evaluated at 36 days.[83] The population of patients included (i) those with unstable angina pectoris/non–Q-wave MI (ii) those undergoing high risk percutaneous transluminal coronary angioplasty and (iii) those undergoing high risk coronary artery bypass surgery (CABG). The GUARDIAN study revealed that cariporide is well tolerated although it failed to demonstrate an overall significant attenuation (10%) of the 2 primary events, mortality and incidence of MI. However, favourable effects among the 3 major subgroups were observed, especially a significant reduction in event rate in patients undergoing CABG receiving the highest dose of 120mg intravenously every 8 hours. This dose appeared to be effective in reducing the overall incidence of Q-wave MI by about 40%. It should be noted that the GUARDIAN study carried some inherent risks in view of the heterogeneity of patients recruited, some of which were apparently not subjected to reperfusion protocols, thus precluding any potential myocardial salvaging benefit of cariporide. Moreover, as a dose-finding study and the observation that only the highest dose exerted any benefit, it is likely that optimal dosages and plasma therapeutic levels were not achieved in this study.

Additionally, results with cariporide in a relatively small clinical trial with 100 patients were recently presented and indicate improved left ventricular function when administered prior to balloon angioplasty in patients with acute MI.[84]

8.2 Eniporide: The ESCAMI Study

A larger (approximately 1300 patients) clinical evaluation for acute MI is currently underway in which the NHE1-selective inhibitor eniporide is being investigated in a phase II placebo-controlled, multicenter European trial, the Evaluation of the Safety and the Cardioprotective effects of Eniporide in Acute Myocardial Infarction (ESCAMI) study, in which the drug is administered prior to angioplasty or thrombolysis. An interim analysis based on more than 400 patients was performed in late May 1999 with what appeared to be favourable results leading to a continuation of the study. The final results of the ESCAMI study have not, at time of publication, been made public.

Taken together, the above initial clinical evaluations of NHE1 inhibition are promising and overall support the concept that NHE1 inhibition represents a well tolerated therapeutic approach for cardioprotection.

9. Potential of NHE1 Inhibitors in Chronic Postinfarction Responses and Heart Failure

In addition to myocardial protection, emerging evidence suggests that NHE1 inhibition may offer other beneficial effects in the postinfarcted myocardium in terms of cardiac hypertrophy, remodelling and heart failure which represent major and clinically important consequences after MI. Although precise mechanisms need to be fully elucidated, it is nonetheless evident that NHE inhibitors block hypertrophic responses to various stimuli, which may be important to the chronic postinfarction remodelling process in the surviving myocardium. For example, stretch-induced stimulation in protein synthesis in neonatal cardiac myocytes as well as stretch-induced alkalinisation in feline papillary muscles can be blocked by NHE inhibitors,[85,86] as can norepinephrine-induced protein synthesis in cultured rat cardiomyocytes.[87] In earlier studies, orally administered amiloride reduced fibre diameter in rat coronary ligation[88] and murine dilated cardiomyopathy models.[89] We have recently found that dietary administration of cariporide completely abrogates the increased length of surviving myocytes after one week following coronary artery occlusion and ameliorates contractile dysfunction in the absence of afterload or infarct size reduction, thus suggesting a direct effect of NHE1 inhibition on surviving myocytes.[90]

Although, the mechanism for NHE1 involvement in the remodelling heart failure process is not known, it is relevant to recall that numerous endogenous paracrine, autocrine and hormonal factors which have been implicated in the ventricular remodelling/heart failure process also activate NHE1 through receptor-dependent signal transduction processes (see section 3). Indeed, as summarised in figure 3, these are upregulated as a consequence of ischaemia and may induce chronic adaptive remodelling processes which can contribute to ultimate heart failure. In our initial animal studies with NHE1 inhibitors, attenuation of early remodelling occurred in the absence of any afterload reduction, suggesting a direct role of NHE1 in myocardial responses.[90] Recently, we have reported that the salutary effects of cariporide are maintained 3 months after myocardial infarction, as demonstrated by improved haemodynamics and diminished hypertrophy in surviving myocytes.[91] Further studies are clearly necessary to ascertain whether NHE1-specific inhibitors represent useful adjunct therapies for the treatment of congestive heart failure, particularly in combination with approaches aimed at distinct mechanistic targets which can produce potential additive effects.

10. Conclusion

The past 12 years have seen substantial progress and advances with respect to the understanding of NHE in the heart, particularly its role in mediating myocardial ischaemia and reperfusion injury, and more recently, its potential role in long term myocardial adaptation and the development of heart failure. In terms of ischaemic injury and cardiac protection, these advances have lead, relatively rapidly, to the establishment of clinical trials aimed at determining whether selective NHE1 inhibition protects high risk patients with CAD or those with acute MI. It is very likely and hopeful that new therapeutic strategies will emerge, based on both the clinical trials which have been or are currently being undertaken, as well as the obvious rapid development of a large number of new NHE1 inhibitors. Nonetheless, much work needs to be done in this regard, particularly in terms of understanding of the regulation of NHE1 in chronic responses and how the system can be ideally modulated for therapeutic strategies, either alone or as adjunctive therapy with other treatment modalities, for the treatment of heart disease.

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Correspondence and offprints: Dr *Morris Karmazyn,* Department of Pharmacology and Toxicology, University of Western Ontario, Medical Sciences Building, London, Ontario N6A 5C1, Canada.
E-mail: mkarm@uwo.ca