

Sodium-Hydrogen Exchanger, Cardiac Overload, and Myocardial Hypertrophy

Horacio E. Cingolani, MD; Irene L. Ennis, MD

Overload of neonatal and adult cardiomyocytes and multicellular myocardial preparations, which include whole hearts, are accompanied by an enhanced activity of the Na^+/H^+ exchanger 1 (NHE-1). Exogenous administration of prohypertrophic agents such as angiotensin II (Ang II), endothelin-1 (ET-1), and α_1 -adrenergic agonists also stimulates NHE-1 activity, which leads to an increased concentration of intracellular Na^+ ($[\text{Na}^+]_i$). Moreover, inhibition of NHE-1 activity prevents the increase in $[\text{Na}^+]_i$, induces the regression of cardiac hypertrophy, and exerts beneficial effects in experimental heart failure.

The present review summarizes the current knowledge of the causative factors and pathophysiological correlation of cardiac overload and NHE-1 activity.

The Na^+/H^+ Exchanger

NHE is an integral membrane glycoprotein expressed ubiquitously in mammalian cells, and it electroneutrally exchanges intracellular H^+ for extracellular Na^+ (1:1) to regulate intracellular pH (pH_i) and the concentration of $[\text{Na}^+]_i$. Nine isoforms of this exchanger have been described: NHE-1 to NHE-9. NHE-1, the first isoform to be cloned, is expressed ubiquitously in the plasma membrane and is considered to be the cardiac-specific isoform.¹ The inward gradient of Na^+ , produced mainly by the Na^+/K^+ ATPase, provides a constant driving force for H^+ extrusion and Na^+ influx through the NHE.

NHE-1 (Figure 1) is a protein of 815 amino acid residues with a predicted molecular mass of 85 kDa that can be separated into an N-terminal membrane-associated domain (≈ 500 amino acid residues) and a long C-terminal cytoplasmic tail. The membrane domain, composed of 12 transmembrane regions, is associated with ionic transport, and it contains the allosteric H^+ sensor site that determines the exquisite sensitivity of the exchanger to intracellular H^+ . The cytoplasmic domain (≈ 300 amino acid residues) is involved in the regulation of the activity of the exchanger by several mechanisms. Removal of the distal region of the cytosolic tail causes a shift of the pH_i sensitivity to the acidic side and an important inhibition of NHE-1 activation by growth factors.¹

NHE-1 is constitutively phosphorylated, and further phosphorylation increases its activity. The phosphorylation sites

are targets of extracellular signal-regulated kinases 1 and 2 (ERK1/2), p90^{rsk} , and other protein kinases such as Rho kinase and Nck-interacting kinase.^{1,2} It has been demonstrated that Ca^{2+} /calmodulin (CaM)-dependent kinase II (CaMKII) can phosphorylate the C-terminal domain of the NHE-1 in vitro. Some other protein kinases such as protein kinase C (PKC) and protein kinase D are able to influence NHE-1 activity in response to neurohormonal stimulation, although they are not believed to phosphorylate the exchanger directly. NHE-1 is susceptible to dephosphorylation by protein phosphatases I and 2A.^{3,4} Other mechanisms of regulation of NHE-1 activity involve phosphorylation and/or dephosphorylation of regulatory proteins or interaction with cofactors. The binding proteins CaM, calcineurin B homologous protein 1, and tescalcin exert an inhibitory effect on the exchanger activity that is released when they bind Ca^{2+} . Overexpression of calcineurin B homologous protein 1 prevents the activation of NHE-1, and this effect is abolished by growth factor stimulation. Cells transfected with tescalcin exhibit a marked inhibition of NHE-1 activity after an acid load. The cytosolic tail, in its region proximal to the membrane domain, also includes 2 putative inositol 4,5-bisphosphate binding sites involved in ATP regulation of NHE-1. Although the exchanger does not use ATP directly, ATP depletion decreases transport activity significantly.^{1,2}

Another enzyme that has recently been reported to influence NHE-1 activity is carbonic anhydrase II.⁵ Carbonic anhydrase II catalyzes the hydration of CO_2 to HCO_3^- and H^+ . The enzyme binds to the cytosolic domain, and the binding is a function of NHE-1 phosphorylation.⁵ Inhibition of carbonic anhydrase II decreases NHE-1 activity.⁵

The actin-binding proteins of the ezrin, radixin, and moesin family form important links between the cytoskeleton and integral proteins of the plasma membrane, and they interact with the cytosolic tail of NHE-1.² Therefore, NHE-1 functions as a scaffold for recruitment of signaling complexes.

Enhanced Function of the NHE-1: $[\text{Na}^+]_i$ or pH_i ?

Stimulation of heptahelical receptors coupled to G α_q protein by Ang II, ET, and α_1 -adrenergic agonists, as well as reactive oxygen species (ROS) and intracellular acidosis, are well-known activators of the NHE-1, at least in part, through

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From the Centro de Investigaciones Cardiovasculares, Facultad de Ciencias Médicas, Universidad Nacional de La Plata, La Plata, Argentina.

Correspondence to Dr Horacio E. Cingolani, Centro de Investigaciones Cardiovasculares, Facultad de Ciencias Médicas, Universidad Nacional de La Plata, Calle 60 y 120, 1900 La Plata, Argentina. E-mail cicmes@infovia.com.ar (*Circulation*. 2007;115:1090-1100.)

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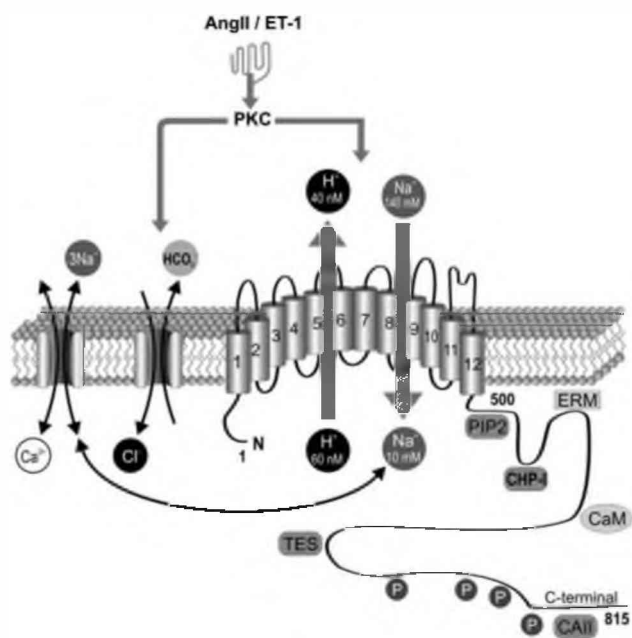


Figure 1. Ang II and ETs, as well as other guanine nucleotide-binding protein α -q couple receptor agonists, activate PKC and the NHE-1 and Na⁺-independent Cl⁻/HCO₃⁻ exchanger (AE). Moreover, neither Ang II nor ET stimulation of NHE-1 induces intracellular alkalization when bicarbonate is present in the medium because of the simultaneous activation of AE through a PKC-dependent phosphorylation.^{7,10b} The increased H⁺ extrusion by NHE-1 activity is therefore offset by the H⁺ equivalent influx through the AE. On the contrary, because of the Na⁺-independence of AE, the influx of Na⁺ through NHE-1 is not offset by the AE, and therefore its intracellular concentration increases. Several sites of interaction with factors potentially involved in the regulation of the exchanger activity in the cytosolic tail of NHE-1 have been described. PIP2 indicates inositol 4,5-bisphosphate; CHP-I, calcineurin homologous protein I; ERM, ezrin, radixin, and moesin actin binding family of proteins; CaM, calmodulin; TES, tescalcin; and CAII, carbonic anhydrase II.

mitogen-activated protein kinase (MAPK) activation.⁶ Other autocrine, paracrine, and hormonal factors, such as epidermal growth factor, aldosterone, adenosine, thrombin, and osmolarity also modulate the exchanger activity.⁶ Although NHE-1 hyperactivity is often thought to be associated with an increase in pH_i, this is not necessarily correct. In the presence of bicarbonate, stimulation of NHE-1 by Ang II or ET-1 does not induce intracellular alkalosis, because a bicarbonate-dependent acidifying mechanism, the Cl⁻/HCO₃⁻ exchanger, is activated simultaneously through a PKC-dependent mechanism.⁷ We would like to emphasize this point because the absence of changes in pH_i in bicarbonate media after growth factor stimulation is not yet widely recognized, although it was reported by Ganz et al⁸ in 1988 in mesangial cells and there was a call for attention to this fact in a letter to *Nature* by Thomas a year later (Figures 1 and 2).⁹

NHE-1 Inhibition as a Potential Therapeutic Strategy

Strong evidence exists that the harmful effect of NHE-1 hyperactivity is the result of the increase in [Na⁺]_i that leads to Ca²⁺ overload through the Na⁺/Ca²⁺ exchanger (NCX), myocardial dysfunction, hypertrophy, apoptosis, and fail-

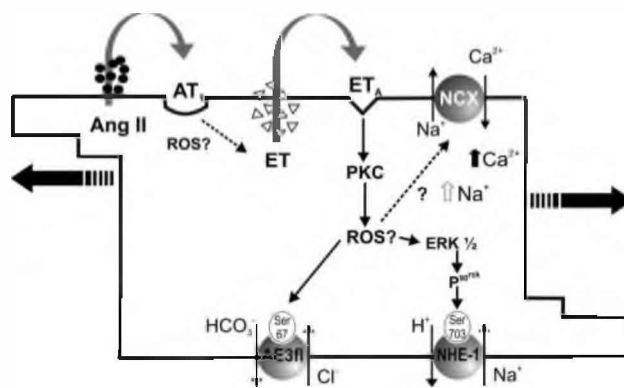


Figure 2. A representation of the proposed autocrine/paracrine cascade of events that follow myocardial stretch. Endogenous Ang II is released from the myocytes, which activates AT₁ receptors in an autocrine fashion. Stimulation of AT₁ induces the release/formation of ET, which simultaneously activates NHE-1 and AE through the ET_A receptors. Activation of the AE prevents the expected intracellular alkalization caused by activation of NHE-1 but does not prevent the rise in [Na⁺]_i. The increase in [Na⁺]_i drives NCX in the reverse mode, and this, together with a probable direct action on the exchanger, leads to the increase in Ca²⁺ transient. Abbreviations as in Figure 1.

ure.^{10–14} Consequently, inhibition of its hyperactivity emerges as a therapeutic strategy to prevent the deleterious consequences of increases in [Na⁺]_i and intracellular Ca²⁺ concentration ([Ca²⁺]_i), activation of many intracellular pathways, and their effects: ischemia/reperfusion injury, hypertrophy, necrosis, and apoptosis.

The pharmacological evidence that supports the beneficial effect of NHE-1 inhibition in ischemia/reperfusion injury was confirmed in mice hearts with genetic ablation of NHE-1 (NHE-1-null mutant, Nhe1^{-/-}) that exhibited enhanced resistance to ischemic and reperfusion injury.¹⁵ Furthermore, a recent report showed that NHE-1 hyperactivity can be blunted by a decrease in its phosphorylation level at serine 703 on the C terminus by generation of dominant negative-p90 ribosomal S6 kinase transgenic (DN-RSK-TG) mice, which showed reduced cardiomyocyte apoptosis and ischemia/reperfusion injury after 45 minutes of left coronary occlusion.¹⁶

NHE-1 Inhibitors

Several NHE-1 inhibitors were developed with the goal of cardioprotection after exchanger activation by prevention of [Na⁺]_i and [Ca²⁺]_i accumulation. Amiloride, a sparing K⁺ diuretic, was the first compound proposed to inhibit the exchanger. NHE-1 and NHE-2 are the isoforms most sensitive to this drug, which also inhibits Na⁺ channels and NCX. Amiloride derivatives were synthesized to improve the potency and selectivity of the NHE-1 isoform. Double substitution of the nitrogen atom of the 5-amino group yielded several compounds, of which ethylisopropil amiloride was one of the most frequently used. Its IC₅₀ was ≈100 times less than that of amiloride and with less action on Na⁺ channels and NCX. Further investigations led to benzoylguanidines such as HOE-694, HOE-642 (cariporide), EMD96785 (eniporide), BIIB-513, BIIB 723, EMD 87580, and other chemically related compounds such as SM-20550 and zoniporide

(CP-597396). The IC₅₀ of most of the newly developed compounds is of the same order of magnitude as that of ethylisopropil amilorode, but with much higher selectivity for the NHE-1. These compounds differ in their solubility, the possible routes of administration, and persistence of the effect after drug washout.¹⁷

Clinical Perspectives

In broad terms, NHE-1 inhibition appears to be a new pharmacological tool for the treatment of ischemia/reperfusion injury, myocardial infarction, apoptosis, heart failure, pathological cardiac hypertrophy, and remodeling. Evidence also exists that NHE inhibition provides benefits after cerebrovascular ischemia by prevention of activation of phospholipases during reperfusion.¹⁷ As described for the heart and brain, renal ischemia enhances the activity of the NHE, especially the NHE-3 isoform, and its inhibition has beneficial effects.¹⁷ Unfortunately, the promising results obtained in experimental animal protocols of ischemia/reperfusion injury could not be reproduced in clinical trials. In 2 large clinical trials (Guard During Ischemia Against Necrosis [GUARDIAN] and Evaluation of the Safety and Cardioprotective Effects of Eniporide in Acute Myocardial Infarction [ESCAMI]), no overall clinical benefit was detected after NHE-1 inhibition in acute coronary syndromes.^{18,19} A cardioprotective effect was detected after eniporide only in a smaller trial (100 patients) in individuals with acute myocardial infarction who underwent percutaneous transluminal coronary angioplasty.²⁰ The most recent study, the Sodium-Hydrogen Exchange Inhibition to Prevent Coronary Events in Acute Cardiac Conditions (EXPEDITION) Study, revealed myocardial benefits of cariporide treatment in patients with ischemic heart disease, but unfortunately and unexpectedly the treatment was associated with significantly greater incidence of strokes.¹ Whether these pharmacological adverse effects are common to all NHE-1 inhibitors or are peculiar to cariporide is not apparent to us at present. The possibility of a deleterious effect of NHE-1 inhibition under basal conditions in contrast to a beneficial effect, when inhibition occurs only after an acid load, has been proposed recently.^{4,46} Therefore, although at first glance this new therapeutic strategy may appear difficult to practice, it deserves further study.

Cardiac Overload and NHE-1

We will focus especially on the concentric hypertrophy characterized by an increase in the thickness of the ventricular wall caused by parallel addition of sarcomeres with re-expression of a fetal gene program and little or no chamber dilatation. This cardiac phenotype can be triggered by systemic hypertension, aortic stenosis, and myocardial infarction. Concentric left ventricular hypertrophy is the predominant form of cardiac hypertrophy, and it very frequently precedes cardiac dilation and failure. We will exclude from this review a discussion of the mechanisms involved in physiological cardiac hypertrophy. The relationship of NHE-1 activation to this interesting hypertrophic pathway, which involves the PI3K/Akt/GSK-3 β and mammalian target of rapamycin-dependent pro-growth factors, has not been explored. It would be interesting to explore the level of activation of the NHE-1 and the effect of its inhibition on

hypertrophy in Akt-1 knockout mice, in which ET-1 induces exaggerated cardiac hypertrophy, but the cardiac growth response to exercise is blunted.²¹ Pathological cardiac hypertrophy can be the result of multiple intracellular signaling pathways triggered through the stimulation of membrane receptors by neurohumoral factors and/or by myocardial stretch. The mechanical stress imposed by the increase in wall tension is the primary event sensed in the transduction of the mechanical load. Integrins and the associated cytoskeletal proteins interact with the extracellular matrix, and possibly they sense mechanical stress and trigger intracellular prohypertrophic signaling pathways. Integrins are well-known mechanotransducers that have been shown to increase their expression in both physiological and pathological cardiac hypertrophy.²² These proteins form the costameres, the junctional complex localized at the Z lines that connect the sarcomeres to members of the Z disc proteins, such as α -actin, vinculin, talin, and melusin, and to the extracellular matrix. Interestingly, this zone is rich in NHE-1 and NCX units. However, the link between the costameres and the activity of the exchangers deserves future research.

In an attempt to mimic cardiac overload under controlled conditions, isolated neonatal and adult rat cardiac myocytes,^{23–38} multicellular preparations such as papillary muscles from different species,^{33,39–47} and even whole rat and pig hearts^{48–51} have been stretched to induce mechanical deformation and activation of the intracellular prohypertrophic signaling pathways. Considering all these results together, and despite some discrepancies about the intracellular cascade activated by myocardial stretch, the findings can be summarized by the scheme presented in Figure 2. Myocardial stretch induces the release of preformed Ang II that, through AT₁ receptor stimulation, induces the release/formation of ET, which binds to ET_A receptors and activates NHE-1, possibly by phosphorylation of serine 703 by the ERK1/2-p90^{msk} pathway.⁵² The exchanger activation is followed by an augmentation of [Na⁺]_i that modifies the thermodynamic conditions for the NCX that leads to an increase in [Ca²⁺]_i caused by a decrease in Ca²⁺ efflux (forward mode of operation of the NCX) and/or an increase in Ca²⁺ influx (reverse mode). Increased production of ROS as a result of Ang II/ET stimulation of nicotinamide adenine dinucleotide phosphate oxidase may be one of the pathways involved in the activation of ERK 1/2-p90^{msk}.^{53,54} However, ROS may be involved in several other steps of the intracellular signaling pathway triggered by myocardial stretch as depicted in Figure 2.^{31,54,55} The initial step of this cascade, the release of Ang II, was proposed first by Sadoshima et al²⁵ in isolated neonatal rat ventricular myocytes. In fact, this group of researchers found that the culture medium of the stretched cardiac myocytes (conditioned medium) caused induction of immediate-early genes and activation of prohypertrophic signaling pathways in nonstretched myocytes.⁵⁶ In line with these results is the work by van Wamel et al,³² which showed that the conditioned medium from stretched myocytes contained Ang II and ET-1, which are involved in the cardiomyocyte hypertrophic response. Moreover, Ito et al⁵⁷ found in neonatal rat cardiomyocytes that Ang II promoted the release/formation of ET-1, and that the hypertrophic effect of Ang II

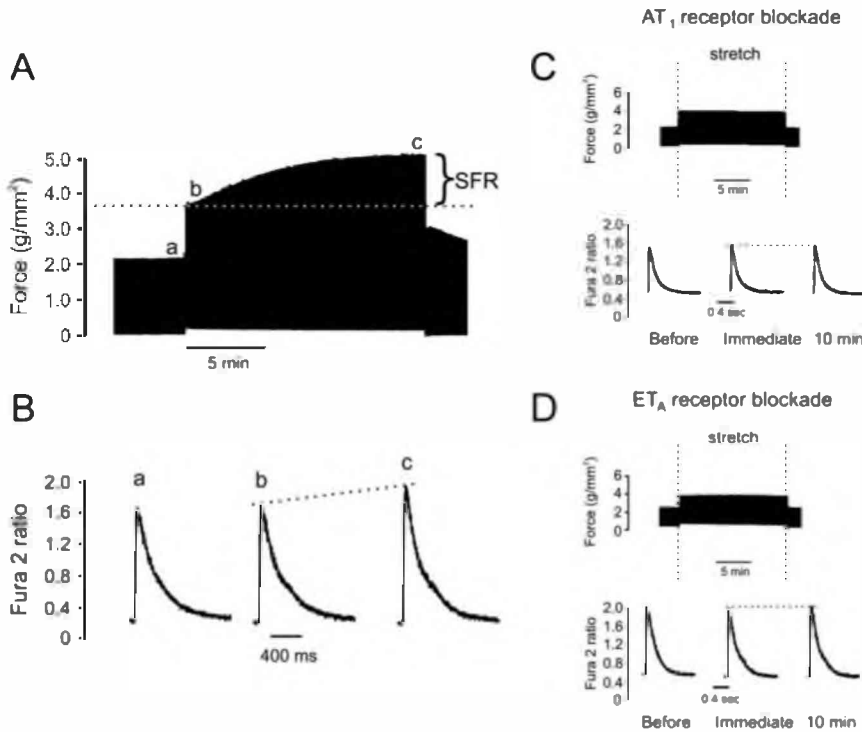


Figure 3. The stretch of a papillary muscle produces a rapid initial increase in force (A, a and b), caused by an increase in myofilament Ca²⁺ responsiveness. Immediately after and during the next 10 to 15 minutes, a progressive increase in force develops: the SFR (A, b and c) that is caused by an increase in Ca²⁺ transients (B). AT₁ and ET_A blockers blunt both the SFR and the increase in Ca²⁺ transients (C and D). Modified from Alvarez et al,⁴⁰ with permission of the publisher. Copyright © 1999, the American Heart Association.

was blocked by an ET_A receptor antagonist or by the introduction of an antisense sequence against the coding region of preproET-1 mRNA, which demonstrated that ET-1 was an autocrine/paracrine factor in the mechanism of Ang II-induced cardiac hypertrophy. Yamazaki et al²⁸ reported that stretch promoted an increase of the constitutively secreted ET-1 into the culture medium together with an increase in MAPKs and NHE-1 activities in neonatal rat cardiomyocytes. Interestingly, in this study NHE-1 inhibition partially inhibited activation of MAPKs. Therefore, in isolated neonatal cardiac myocytes, the results are in agreement with the cascade of events, starting with the release of Ang II and ET followed by activation of NHE-1, thus increasing [Na⁺]_i and, by this mechanism promoting the rise in [Ca²⁺]_i through the NCX. However, the results obtained from experiments performed in neonatal cells can be problematic because these cells possess different relative receptor subtypes and cell-signaling mechanisms, and the participation of NCX in the excitation-contraction coupling is much greater than in adult cells.^{1,58} Nevertheless, the experiments performed in isolated cardiomyocytes, multicellular preparations, and even whole hearts from adult animals that we discuss next are consistent with those of neonates and confirm the chain of events depicted in Figure 2.

The mechanical counterpart that follows the stretch of multicellular myocardial preparations, such as cat papillary muscles (Figure 3), represents a useful model to visualize the role played by the local renin-angiotensin-endothelin system in the chain of events depicted in Figure 2. When muscle length is increased, corresponding rapid and slow increases in developed force can be observed.^{14,59} The rapid change in force is thought to be the basis of the Frank-Starling mechanism and is induced by an increase in myofilament Ca²⁺ sensitivity. On the other hand, the slow force response (SFR)

is caused by a progressive increase in the Ca²⁺ transient amplitude without changes in myofilament responsiveness, a finding first reported by Allen and Kurihara in 1982⁶⁰ and later by several other laboratories (Figure 3).^{40,59,60} According to the proposed chain of events, the SFR is also abolished by inhibition of ET-converting enzyme by phosphoramidon,⁴² NHE-1 blockade, and NCX reverse mode inhibition with KB-R7943.^{42,43,47}

The pivotal role of NHE-1 activation in this autocrine/paracrine chain of events has been reported by several investigators.^{14,33,39,40,42–46} The role of ET as the activator of the NHE-1 after myocardial stretch has been confirmed by Calaghan and White⁴¹ in ferret papillary muscles but not by von Lewinsky et al^{44,45} in rabbit papillary muscle or in failing human myocardium. Despite confirming the activation of NHE-1, von Lewinsky et al were unable to detect the role of Ang II or ET in its activation. Differences between species in the extent of the stretch or in the ET isoform released by stretch may account for these discrepancies. In connection with this, the stretch of cat papillary muscles induced the upregulation of preproET-3 mRNA, whereas the stretch of pig and rat myocardium upregulated ET-1.³⁶ Nevertheless, the 3 ET isoforms are equipotent in their ability to stimulate NHE-1 activity.⁴⁶ The possible role of stretch-operated channels (SOC) in the myocardial response to mechanical stress is also controversial. Sadoshima et al²⁴ and Yamazaki et al³⁰ did not find participation of these channels in the hypertrophic response of neonatal rat cardiac myocytes to stretch. Von Lewinsky et al^{44,45} tested the possible role of SOC in the mechanical counterpart that follows myocardial stretch, the SFR, in nonfailing rabbit myocardium and in failing human myocardium. No effect of gadolinium or streptomycin, 2 SOC inhibitors, was detected on the response to stretch.^{44,45} However, Calaghan and White³³ reported that the activation

of both SOC and NHE-1 underlie the SFR in isolated myocytes and in multicellular muscle preparations from adult rat heart. Isenberg et al⁶¹ proposed that myocardial stretch increases $[Na^+]$ and $[Ca^{2+}]$ in the cytosol and in cell organelles partly by their influx through the SOC, but they were unable to prevent the increase in $[Na^+]_i$ by cariporide.

Ang II, ET-1, and α_1 -adrenergic agonists activate conventional heterotrimeric G protein-dependent and unconventional G protein-independent intracellular pathways. Moreover, recent evidence suggests that G protein coupled receptors can trigger intracellular signals independent of G protein activation.⁶² Ang II/ET and α_1 -adrenergic agonists by generation of anion superoxide activate the ERK 1/2-p90^{msk} pathway that phosphorylates and consequently activates the NHE-1 that triggers intracellular signaling pathways.⁵²

In line with the concepts illustrated by Figures 2 and 3 are experiments that show that the SFR can be mimicked by small amounts of exogenous Ang II (1 nmol/L).⁶³ The positive inotropic effect seen in a multicellular preparation such as cat papillary muscle, as well as in isolated cat ventricular myocytes, is abrogated completely by AT₁ or ET_A receptors blockade, NHE-1-specific inhibition, or inhibition of the reverse mode of the NCX.^{40,42,43}

In vivo studies in pig heart showed that the first molecular response to pressure overload was the disappearance of the granular staining that corresponded to Ang II stores in the cardiomyocyte cytoplasm after 30 minutes of the imposed overload.⁵⁰ These findings were followed by an increase in the mRNA of preproET-1 and angiotensinogen, changes that were abrogated by AT₁ receptor blockade.⁵⁰ Furthermore, in situ mRNA hybridization revealed that ventricular myocytes were the source of ET-1 in the hypertrophied hearts in vivo.⁶⁴

Cardiomyocyte-specific disruption of the ET-1 gene in mice reduced the hypertrophic response to tri-iodothyronine, which suggests that myocyte-produced ET-1, which acts in a paracrine/autocrine manner, is an important signal for myocardial hypertrophy development in response to thyroid hormone.⁶⁵ Thus, converging pieces of evidence coming from experiments in neonatal rat ventricular myocytes, adult myocytes, multicellular preparations, and whole hearts support the theory that the activation of NHE-1 is an early event after myocardial stretch triggered by the activation of the local renin-angiotensin-endothelin system and leads to an increase in $[Ca^{2+}]_i$ through the NCX. This chain of events can be mimicked also by applying small amounts of any ET isoform.^{11,46,63} Furthermore, as we discuss in the next section of this review, the NHE-1 has been demonstrated to be activated in several models of cardiac hypertrophy, and its specific inhibition resulted in prevention/regression of hypertrophy.

The NHE-1 in Myocardial Hypertrophy

Enhanced activity of NHE-1 in the hypertrophied myocardium of spontaneously hypertensive rats (SHR) was reported in 1995.^{66,67} The hyperactivity of NHE-1 has been described for several other tissues from hypertensive humans and other animals.⁶⁸ Experiments in our laboratory showed that the hyperactivity of NHE-1 in the hypertrophied myocardium of SHR was not accompanied by an increase in pH_i because simultaneous activation of the acidifying Cl/HCO_3^- ex-

changer occurred (Figure 1).⁶⁷ The increased activity of NHE-1 was, in this model, the result of a PKC-dependent posttranslational modification of the exchanger.⁶⁹ It was further hypothesized that inhibition of the antiporter activity could regress and/or prevent the development of the hypertensive hypertrophy. Camili6n de Hurtado et al⁷⁰ reported that the myocardial hypertrophy of SHR regressed with cariporide treatment with no change in arterial pressure. Another feature of the hypertensive cardiac hypertrophy is the enhancement of interstitial fibrosis, which was decreased by long-term blockade of NHE-1, although this effect took longer than the regression of myocyte size,⁷¹ possibly as a reflection of the lower turnover rate of collagen metabolism.⁷²

The link between NHE-1 activity and myocardial growth has been established for several other neurohormonal models of cardiac hypertrophy in addition to hypertensive hypertrophy. Kusumoto et al⁷³ showed that NHE-1 was upregulated after myocardial infarction and that the specific inhibition of this exchanger with cariporide decreased hypertrophy and remodeling in these hearts. Upregulation of NHE-1 was reported for the cardiac hypertrophy and failure model of the β_1 -adrenergic receptor transgenic mice.⁷⁴ In this model, NHE-1 inhibition prevented the development of cardiac hypertrophy and fibrosis, and NHE-1 inhibition normalized the expression of the exchanger, which suggests that its upregulation is essential for the detrimental cardiac effects of long-term β_1 -receptor stimulation in the heart.⁷⁴ Similarly, the cardiac hypertrophy induced in rats by repeated isoproterenol administration was prevented by the inhibition of NHE-1.⁷⁵ The mechanism(s) by which β -adrenergic receptor stimulation induces upregulation at both the translational and post-translational levels of NHE-1 in the myocardium is not clear.

Hypertrophied hyperthyroid hearts showed enhanced NHE-1 activity, and when exposed to acute ischemia they accumulate more Na^+ than the control nonhypertrophied hearts; these changes were prevented by NHE-1 inhibition.¹⁰ Moreover, it has been demonstrated that thyroid hormone, by the interaction of its receptor with the NHE-1 promoter, increases the expression of the NHE-1 protein.⁷⁶

In patients with end-stage renal disease and secondary hyperparathyroidism, as well as in patients with primary hyperparathyroidism, a strong correlation between cardiac hypertrophy and serum parathyroid hormone levels has been reported.^{77,78} Although the observation is controversial,^{79,80} a stimulatory effect of parathyroid hormone over NHE-1 has been described; therefore, it is tempting to speculate about the possible involvement of the antiporter in the signaling pathway evoked by parathyroid hormone in the genesis of cardiac hypertrophy.

In neonatal rat ventricular myocytes, aldosterone stimulation induced a hypertrophic response accompanied by upregulation of NHE-1 at both the mRNA and protein levels accompanied by increased $[Na^+]_i$. Both hypertrophy and elevated $[Na^+]_i$ were prevented by the NHE-1-specific inhibitor EMD87580 as well as the aldosterone antagonist spironolactone.⁸¹ Similar results were obtained in uninephrectomized rats exposed to deoxycorticosterone acetate/salt, in which treatment with cariporide completely inhibited hypertrophy and NHE-1 upregulation.⁸²

Recently, a very interesting study reported that cardiac hypertrophy of atrial natriuretic peptide receptor-deficient mice was accompanied by an increased activity of NHE-1 with increased [Ca²⁺]_i.⁸³ Long-term treatment with the NHE-1 inhibitor cariporide normalized these alterations.⁸⁰ These results are in line with a report by Tajima et al, who demonstrated that atrial natriuretic peptide inhibits NHE-1 activity.⁸⁴

Leptin, a protein encoded by the obesity gene, has been reported to activate NHE-1 through a PKC-dependent pathway,⁸⁵ and emerging evidence indicates that this hormone is linked to cardiac hypertrophy.^{86–88} It has been reported that leptin elevates ET-1 and ROS levels, which results in hypertrophy of neonatal rat cardiac myocytes.⁸⁷ Therefore, it seems reasonable to speculate that NHE-1 hyperactivity is involved in this prohypertrophic signaling pathway. More recently, Karmazyn's group⁸⁷ implicated leptin as an autocrine mediator of the hypertrophic effects of Ang II and ET-1 in cultured neonatal ventricular myocytes.⁸⁸ Whether leptin is upstream and/or downstream of ET-1 secretion appears to be controversial.^{87,88} Myocardial NHE-1 mRNA abundance was enhanced in right ventricular hypertrophy caused by monocrotaline-induced pulmonary artery injury. Both hypertrophy and NHE-1 upregulation were abrogated by treatment with cariporide.⁸⁹

In a preliminary report, carbonic anhydrase II inhibition was reported to reverse cardiac hypertrophy, an effect probably related to its stimulatory effect on NHE-1 as mentioned before.⁹⁰ When the sarcolemmal NHE-1 activity from normal human donor hearts was compared with that of hearts with chronic end-stage heart failure that exhibited varying degrees of hypertrophy, a significantly greater NHE-1 activity was detected in the hypertrophied myocytes.⁹¹ This report, along with others, opens the possibility of considering the inhibition of NHE-1 as a new therapeutic strategy for cardiac failure. We should keep in mind that NHE-1 is one of the main pathways for Na⁺ entry into the cardiac cell.⁹²

In the Table, we summarize the cardiac hypertrophy models in which NHE-1 activation may be involved. As we mentioned before, an enhanced activity of NHE-1 may be the result of an increased expression of the exchanger, an increased turnover of the functional units, or a combination of both alternatives. In line with this view, the models reviewed clearly exhibited cases of enhanced NHE-1 activity caused by upregulation of the exchanger expression, posttranslational modification, or a combination of both. In any case, the hyperactivity of NHE-1 was linked to cardiac hypertrophy. Interestingly, whereas long-term NHE-1 inhibition by cariporide in the whole animal induces upregulation of the exchanger,¹⁰³ the normalization of its previously augmented expression has also been reported after long-term NHE-1 inhibition.^{74,75,83,89} Several aspects require further investigation to clarify the precise mechanism by which NHE-1 is involved in the development of cardiac hypertrophy and failure and its possible link with other intracellular signaling pathways. A disparate negative result about the antihypertrophic effect of NHE-1 inhibition in rats was reported by Loennechen et al,¹⁰⁴ who described a salutary effect of

Models of Cardiac Hypertrophy in Which NHE-1 Plays a Role

Cardiac Hypertrophy Model	References
Cardiac overload	12, 63, 64, 67, 93
Stretch of rat neonatal cardiac myocytes	25, 27
Ang II stimulation	27, 49
ET-1 stimulation	25, 27, 54, 94
Postmyocardial infarction	70, 95–97
Leptin stimulation	82–85
Human heart failure	89
Monocrotaline-induced	86
Hyperparathyroidism	77, 98
Hyperthyroidism	170
Hamster model hereditary cardiomyopathy	13
Mineralocorticoid stimulation	78, 79
Atrial natriuretic peptide receptor-deficient mice	80
Carbonic anhydrase inhibition	5, 88
β-Adrenergic stimulation	71, 72, 99
α-Adrenergic stimulation	94, 100, 101
Pacing-induced	102

losartan on postinfarction remodeling and gene expression that was not shared by cariporide.¹⁰⁴

Probable Mechanisms by Which NHE-1 Inhibition Induces an Antihypertrophic Effect

The multiple hypertrophic signals appear to converge on a common set of nuclear transcription factors that will interact to activate or repress transcription of certain cardiac genes. Research into the effect of NHE-1 inhibition on the activity of these transcription factors is lacking. Because modifications of [Na⁺]_i secondary to NHE-1 activity are believed to be followed by changes in [Ca²⁺]_i, it seems reasonable to assume that the changes in [Ca²⁺]_i (diastolic, systolic, or restricted to certain intracellular spaces) will be a downstream step from NHE-1 inhibition in its antihypertrophic effect. However, the possibility that NHE-1 inhibition decreases ROS formation makes the interpretation of the mechanism more complicated because it is possible that >1 hypertrophic pathway is targeted by NHE-1 blockade. NHE-1 inhibition in adult SHR during 1 month was followed by regression of cardiac hypertrophy with normalization of calcineurin Aβ expression and nuclear abundance of nuclear factor of activated T cells.¹⁰⁵ If we consider the results reported by Kilic et al, the calcineurin/nuclear factor of activated T cells pathway is probably not the only pathway involved in myocardial hypertrophy triggered by pressure overload.⁸³ Kilic et al reported an increased activity of NHE-1, augmented [Ca²⁺]_i, and activation of the 4 prohypertrophic signaling pathways examined: the MAPKs, the serine-threonine kinase Akt, calcineurin, and CaMKII. However, the regression of cardiac hypertrophy induced by the treatment with cariporide normalized only 2 of these signaling pathways, the CaMKII and the Akt.

Changes in pH_i

After the report that fertilization of sea urchin eggs was followed by a rise in pH_i, it has become widely accepted that

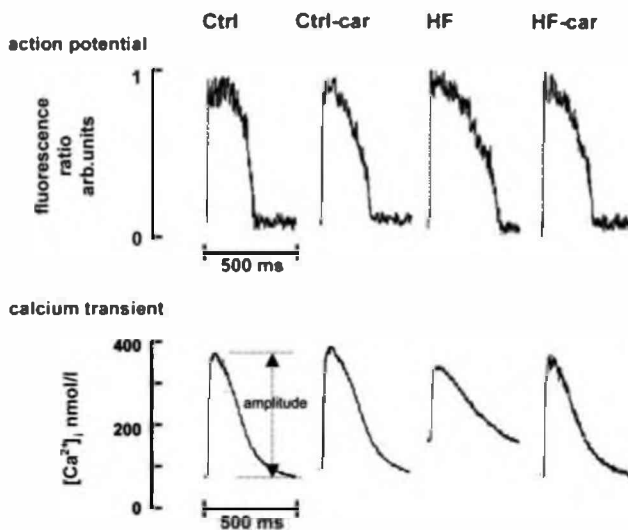


Figure 4. When rabbits with cardiac hypertrophy and failure (caused by combined pressure and volume overload) were treated repeatedly with the NHE-1 inhibitor cariporide for 3 months, prevention of cardiac hypertrophy and normalization of the previously high diastolic levels of Ca^{2+} were observed. This figure shows representative examples of action potential (top) and calcium transients (bottom) in myocytes of the control group (Ctrl), control treated with cariporide group (Ctrl-car), the heart failure group (HF), and the heart failure treated with cariporide (HF-car) groups. Reproduced from Baartscheer et al¹² with permission of the publisher. Copyright © 2005, the European Society of Cardiology.

intracellular alkalization caused by enhanced NHE-1 activity was responsible for the increase in protein synthesis and cell growth.^{106,107} Therefore, if this notion is correct, the inhibition of NHE-1 would reverse and prevent cardiac hypertrophy by normalization of pH_i . However, in SHR hypertrophy the enhanced activity of NHE-1 is not accompanied by intracellular alkalosis, as we have already mentioned.⁶⁷ Furthermore, in a medium that contains bicarbonate, Ang II and ET-1 do not increase pH_i .^{14,39,40,42,43,65,108} Therefore, keeping in mind this observation, we should expect that NHE-1 inhibition will not change pH_i significantly when bicarbonate-dependent mechanisms are operative. In agreement with this, a recent publication demonstrates that long-term treatment with cariporide, without significantly altering pH_i , induces the regression of cardiac hypertrophy, prevention of heart failure, and normalization of elevated $[\text{Na}^+]_i$ and $[\text{Ca}^{2+}]_i$ (Figure 4).¹² We should therefore be cautious as we extrapolate the absence of pH_i changes in spite of NHE-1 activation to conditions other than myocardial stretch and Ang II/ET stimulation.⁸³

Changes in $[\text{Na}^+]_i$

An increase in $[\text{Na}^+]_i$ is detected after NHE-1 stimulation by Ang II, ET-1, or myocardial stretch. Although the magnitude of the increase in $[\text{Na}^+]_i$ reported by different authors and for different species is somewhat variable, it ranges from 3 to 6 mmol/L.^{12,40,47} It is known that the increase in $[\text{Na}^+]_i$ can induce an increase in $[\text{Ca}^{2+}]_i$ through the NCX as a result of a decrease in Ca^{2+} efflux (decreased forward mode) and/or an increase in Ca^{2+} entry (increased reverse mode). The increase

in $[\text{Na}^+]_i$ induced by stretch and by exogenous Ang II or ET-1, at doses that produce similar increases in force to the SFR, is prevented by blocking NHE-1.^{11,40,42,65} This increase in $[\text{Na}^+]_i$ shifts the reversal potential of the NCX (E_{NCX}) to a more negative potential to give more time for the NCX to operate in reverse mode during the action potential and promotes Ca^{2+} influx to the cell, which determines the increase in force. As reported by Bers et al,⁹⁷ cardiomyocytes have a limited capacity to buffer increases in $[\text{Na}^+]_i$, and NCX is more sensitive than the Na^+/K^+ ATPase pump to a change in $[\text{Na}^+]_i$ of this magnitude.

Recently, Baartscheer et al¹² showed that long-term treatment with cariporide of rabbits with combined pressure and volume overload cardiac hypertrophy and failure attenuated hypertrophy and decreased the previously augmented diastolic $[\text{Ca}^{2+}]_i$ without significant alteration of systolic $[\text{Ca}^{2+}]_i$. Recent experiments performed in isolated neonatal rat ventricular myocytes by Dulce et al⁹⁴ showed that the hypertrophic response to ET-1 was accompanied by increases in $[\text{Na}^+]_i$, $[\text{Ca}^{2+}]_i$, cell surface area, phenylalanine incorporation, and atrial natriuretic peptide mRNA expression. These effects were diminished by the blockade of NHE-1 with cariporide and by the inhibition of the reverse mode of NCX with KB-R7943.⁹⁴ Experiments performed by Karmazyn's group showed that the decrease in Na^+ influx caused by the NHE-1 inhibitor EMD87580 attenuates and reverses myocardial hypertrophy and heart failure after myocardial infarction.¹⁰⁹ Similar results were reported by Chahine et al,¹³ who used the hereditary cardiomyopathic hamster model of cardiac hypertrophy and failure. In this case, the blockade of NHE-1 by EMD87580 prevented the development of hypertrophy and necrosis by preventing the increase in Na^+ influx through the NHE-1.¹³

Changes in $[\text{Na}^+]_i$ may trigger intracellular signals independently of altering the reverse potential of the NCX.¹¹ It has been reported that an increase in $[\text{Na}^+]_i$ can stimulate PKC activity¹¹⁰ and perhaps it may stimulate production of ROS.¹¹¹ Furthermore, additional factors of the NHE-1-mediated increase in $[\text{Na}^+]_i$ exist that need to be considered in the autocrine/paracrine loop triggered by myocardial stretch that may contribute to the rise in $[\text{Ca}^{2+}]_i$: the increase in action potential duration¹¹² and the increased ROS production that targets the NCX.¹¹³ Both effects are probably caused by Ang II/ET-1 stimulation of nicotinamide adenine dinucleotide phosphate oxidase and anion superoxide formation.

Experiments performed in NCX knockout mice challenged the possible role of this exchanger in the determination of cardiac hypertrophy development.⁹⁶ Further experiments are necessary to rule out the possibility that NCX knockout mice develop adaptive compensatory mechanisms responsible for the hypertrophic response.

Mitochondrial Effects and ROS

Emerging evidence indicates that NHE-1 can be activated by ROS. This pathway of activation involves ERK1/2- and p90^{rsk} -dependent phosphorylation.^{52,114} In connection with this, the positive inotropic effect of 1 nM Ang II assessed by sarcomere shortening is blunted by the scavenger *N*-(2-mercapto-propionyl) glycine in isolated myocytes.⁵⁵ Less

well explored is the possibility that the increased ROS production, in addition to its role upstream in the cascade of activation of NHE-1, may be induced by the rise in [Na⁺]_i secondary to NHE-1 hyperactivity. In connection with this, and as was mentioned before, the results of the experiments reported by Yamazaki et al²⁸ showed that myocyte stretch induced NHE-1 and MAPK activation, and that specific blockade of NHE-1 partially decreases the activation of MAPK. Furthermore, Javadov et al¹¹⁵ showed recently in rats with myocardial infarction that NHE-1 inhibition was able to prevent cardiac hypertrophy completely and decrease the vulnerability of mitochondria to Ca²⁺. NHE-1 inhibition also increased mitochondrial respiratory function, especially at the level of complex I and complex II. Javadov and colleagues attributed the antihypertrophic effect of NHE-1 inhibition to the decreased generation of mitochondrial-derived ROS.^{115,116}

As to the putative relationship between NHE-1 activity and oxidative stress and the possibility that ROS has a role downstream from in addition to upstream of the exchanger activation, we should remember that the granulocyte superoxide production induced by Ang II has been reported to be reduced under NHE-1 inhibition,¹¹⁷ and that Ang II-induced increased cardiac production of ROS in in vivo experiments is abrogated by a low-Na⁺ diet.¹¹¹ Moreover, the decrease in infarct size and level of tissue lipoperoxidation (assessed by thiobarbituric acid reactive substances), induced by ROS scavengers administered during the reperfusion, can be mimicked by specific blockade of NHE-1.¹¹⁸

It seems possible that ROS may participate in several steps of the intracellular prohypertrophic pathway triggered by myocardial stretch. A recent study reports that ROS can mediate the upregulation of NHE-1 gene expression and thus increase cell resistance to death.¹¹⁹

Mitochondrial NHE can be blocked by several NHE blockers, which include cariporide^{120–122}; therefore, these drugs block both NHE-1 (sarcolemmal) and mitochondrial NHE.

In a recent study, the effect of cariporide on cell death induced by oxidative stress was examined in cultured neonatal cardiomyocytes.¹²³ Despite the fact that the NHE-1 inhibitor suppressed the cytosolic Na⁺ and Ca²⁺ accumulation nearly completely and prevented the loss of mitochondrial membrane potential induced by H₂O₂, the beneficial effect on the apoptosis markers evaluated was only partial, which suggests a contribution of Ca²⁺-independent death pathways, such as cytochrome *c* release. Furthermore, an action of cariporide that delays mitochondrial matrix acidification and preserves ATP levels has been suggested.¹²⁰

Karmazyn's group also reported that the opening of the mK_{ATP} channels by diazoxide blunted the α-adrenergic-induced hypertrophy and NHE-1 upregulation in neonatal rat cardiomyocytes.¹⁰¹ Because a decrease in the amount of mitochondrial ROS release by opening the mK_{ATP} channels has been described,¹²⁴ a relationship between mK_{ATP}, ROS, NHE-1 and cardiac hypertrophy seems probable. Therefore, NHE-1 inhibition may exert its beneficial effects by decreasing [Na⁺]_i and/or ROS production. Both [Na⁺]_i and ROS target the NCX to modify its activity, and therefore target

[Ca²⁺]_i either in the bulk of the cytosol or in more restricted spaces.

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Disclosures

None.

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