See related article, pages 776–782

NHE-1 and NHE-6 Activities
Ischemic and Reperfusion Injury

H.E. Cingolani, I.L. Ennis, S.M. Mosca

The study[1] published in this issue of *Circulation Research* showing that a null mutation of NHE-1 improves the tolerance of the heart to ischemia and reperfusion (I/R) is an important contribution for the following reasons: (1) In the animals with null mutation, contracture during the ischemic period was less and ATP levels were preserved compared with wild-type animals. This observation, on the one hand, provides evidence that protection by downregulation of NHE-1 during the ischemic period itself is indeed possible and, on the other hand, it argues against the suggestion that the exchanger is inactive during this same period.[2] (2) In contrast with chronic blockade of the NHE-1 by pharmacological interventions,[3] the long-term absence of the exchanger does not elicit major compensatory changes that, in turn, might negate the cardioprotective effect of blocking its activity for a relative short term. This point is related to a recent publication[3] showing that long-term treatment with the NHE-1 blocker cariporide is followed by an upregulation of the functional units of the exchanger in a similar way to the well-known tolerance phenomenon following β-adrenergic receptor blockade. The absence of such upregulation negates possible hypersensitivity to ischemia upon withdrawal of the medication. The risk is evident in hearts with upregulation of NHE-1, which gain Na^+_i more rapidly during ischemia, and show impaired recovery after reperfusion.[4] (3) No additional protection was obtained by adding the NHE-1 blocker eniporide to the NHE-1 null mice, suggesting that there is not another NHE isoform that can be blocked with this compound to add additional protection; the findings additionally hint that the attenuation of the injury obtained by the absence of the sarcolemmal NHE-1 is maximal and, therefore, no further beneficial effect will be detected by blocking the mitochondrial NHE (MNHE).

The classical explanation of the mechanism by which the NHE-1 blockade protects against I/R is as follows. During ischemia, a cytosolic acidosis of approximately 1 pH unit occurs in about 10 minutes. This cytosolic acidosis stimulates the NHE-1, increasing its activity and augmenting cytosolic Na^+ and Ca^{2+} levels.[5,6] Although other mechanisms could contribute to the increase in intracellular Na^+ (Na^+_i)[7] during ischemia, it has been shown that blockade of the NHE-1 before ischemia abolishes the increase in Na^+_i during this period[5,8] and diminishes the increase in cytosolic Na^+ and Ca^{2+} during reperfusion.[5,6] The increase in Ca^{2+} secondary to the increase in Na^+ seems to be caused by the Na^+-Ca^{2+} exchanger (NCX) working in reverse mode,[9] and cytosolic Ca^{2+} overload is a necrotic and apoptotic signal. Although with some contradictory results, it has been possible to obtain protection from I/R by blocking the NHE-1 or the NCX only after the onset of reperfusion,[10–12] suggesting that there is protection against the reperfusion injury induced by these mechanisms in addition to protection from the ischemic injury.

Although other mechanisms can be operative,[13] the activation of NHE-1 at the onset of reperfusion has been linked to the increase in reactive oxygen species (ROS).[14–16] This exchanger reaches its maximal activity early after reperfusion. It has been proposed that the increase in ROS leads to activation of the mitogen-activated protein kinase pathway, phosphorylating ERK1/2 and the cytosolic tail of the NHE-1, increasing the exchanger activity.[17] On the other hand, it has been also reported that ERK1/2 mediates activation of the Na^+-HCO_3^- cotransport and that blockade of this mechanism protects against I/R injury.[18]

The available evidence, therefore, establishes that the cytosolic Ca^{2+} overload induced by the NCX working in its reverse mode can be prevented by blocking the NHE-1, decreasing Na^+_i. A recent publication[19] makes things more difficult to interpret. In this study, the authors showed that cariporide preserves mitochondrial proton gradient and delays ATP depletion in mouse-derived myocytes (HL-1) after simulated ischemia and suggested that these mitochondrial changes are not secondary to changes in the cytosol. They concluded that, during ischemia, cariporide acts at the mitochondrial level, delaying mitochondrial matrix acidification and preserving ATP levels. They also suggested that the prevention of mitochondrial Ca^{2+} overload was not cariporide's mechanism of protection.

Mitochondrial NHE (MNHE) is apparently encoded by NHE-6, with a molecular structure similar to that of NHE-1.[20–22] However, there are still some concerns about the identification of the NHE-6 with the MNHE.[21,22] The MNHE can be blocked by several NHE blockers including cariporide[19,23,24]; therefore, this drug blocks both the NHE-1 (sarcolemmal) and the NHE-6 (mitochondrial) gene products.

In a more recent study,[25] the effect of cariporide on cell death induced by oxidative stress was examined in cultured neonatal cardiomyocytes. The inhibitor suppressed cytosolic Na^+ and Ca^{2+} accumulation and the loss of mitochondrial

From the Centro de Investigaciones Cardiovasculares, La Plata, Argentina.

Correspondence to Horacio E. Cingolani, MD, Centro De Investigaciones Cardiovasculares, Facultad de Ciencias Medicas, Universidad Nacional de Le Plata-60 y 120, La Plata 1900, Argentina. E-mail cicmes@infovia.com.ar

(*Circ Res.* 2003;93:694-696.)

***Circulation Research* is available at http://www.circresaha.org**
DOI: 10.1161/01.RES.0000097926.29243.96

membrane potential induced by H_2O_2, while also decreasing the mitochondrial Ca^{2+} overload induced by H_2O_2. Despite the decrease in cytosolic and mitochondrial Ca^{2+} overload, their results suggested a contribution of two Ca^{2+}-independent pathways: calcium-independent cell death and calcium-independent cytochrome *c* release induced by the blocker. These results suggest that NHE-1 blockade preserves mitochondrial integrity after oxidant stress. These actions were not blunted by mitochondrial K_{ATP} channel blockers, revealing different mechanisms of protection from those operative in ischemic preconditioning. Although some studies have suggested that NHE-1 inhibition and ischemic preconditioning are linked phenomena, this[25] and other studies[26–29] provide evidence that this is not the case. Moreover, experiments in isolated rat hearts comparing ischemic preconditioning with NHE inhibition showed that, although after reperfusion protection was similar, only the NHE inhibition was able to decrease the ischemic contracture.[26]

How can we reconcile the evidence that null mutation of the NHE-1 induces protection from ischemia/reperfusion and that cariporide also protects against ischemia/reperfusion but acting on another isoform? The simplest explanation is that, although the downregulation of the NHE-1 by its ablation by genetic techniques protects the heart from ischemia/reperfusion, additional protection can be obtained by the blockade of MNHE (NHE-6) with the compound. However, the investigators did not accomplish additional protection from I/R when a blocker of the NHE-1, eniporide, was added to the animals with the null mutation of the NHE-1. Among the possibilities to understand this discrepancy, we should consider the following: first, that NHE-1 is not only the sarcolemmal isoform of the NHE but also the mitochondrial one; second, that the genetic manipulation of these animals produced an unintentional downregulation or modification of the MNHE, making it "inactive" for the ischemic/reperfusion injury; third, that the attenuation of the injury obtained with the blockade of the NHE-1 is maximal and so no further beneficial effect will be detected blocking the MNHE.

New avenues of research begin with the novel contributions of these studies about the reperfusion injury process. Should we consider the existence of two protections, one during ischemia and the other one during reperfusion, each mediated by a different NHE isoform? Is the acidification of the cytosol a change that attenuates the reperfusion injury, in contrast to the same change in the mitochondrial matrix? What is the role of NCX in cytosol and mitochondrial Ca^{2+} overload? May Ca^{2+} overload in one compartment protect the other? Should we consider the downregulation of the heart NHE by genetic techniques a gene therapy possibility? All are questions that should be answered in the near future.

In contrast with the promising results coming from studies in several animal species and NHE inhibitors, clinical trials have not shown unequivocal evidence of this protection. The GUARDIAN,[30] ESCAMI,[31] and EXPEDITION[32] trials are examples of such disappointment. The latter was started in 2001 and stopped 6 months later for reasons unreleased to the public. The strikingly beneficial effects of NHE inhibition in well-controlled experimental systems give reason to hope that future drug trials may prove the clinical utility of this therapeutic principle.

References

1. Wang Y, Meyer JW, Ashraf M, Shull GE. Mice with a null mutation in the NHE1 Na^+-H^+ exchanger are resistant to cardiac ischemia-reperfusion injury. *Circ Res*. 2003;93:776–782.
2. Allen DG, Xiao XH. Role of the cardiac Na^+/H^+ exchanger during ischemia and reperfusion. *Cardiovasc Res*. 2003;57:934–941.
3. Camilion de Hurtado MC, Ennis IL, Perez NG, Chiappe de Cingolani GE, Morgan P, Cingolani HE. Upregulation of myocardial Na^+/H^+ exchanger induced by chronic treatment with a selective inhibitor. *J Mol Cell Cardiol*. 2002;34:1539–1547.
4. Bak MI, Ingwall JS. Contribution of Na^+/H^+ exchange to Na^+ overload in the ischemic hypertrophied hyperthyroid rat heart. *Cardiovasc Res*. 2003;57:1004–1014.
5. An J, Varadarajan SG, Camara A, Chen Q, Novalija E, Gross GJ, Stowe DF. Blocking Na^+/H^+ exchange reduces $[Na^+]_i$ and $[Ca^{++}]_i$ load after ischemia and improves function in intact hearts. *Am J Physiol*. 2001;281: H2398–2409.
6. Strömer H, de Groot MCH, Horn M, Faul C, Leupold A, Morgan JP, Scholz W, Neubauer S. Na^+/H^+ exchange inhibition with HOE 642 improves postischemic recovery due to attenuation of Ca^{2+} overload and prolonged acidosis on reperfusion. *Circulation*. 2000;101:2749–2755.
7. Baetz D, Bernard M, Pinet C, Tamareille S, Chattou S, El Banani H, Coulombe A, Feuvray D. Different pathways for sodium entry in cardiac cells during ischemia and early reperfusion. *Mol Cell Biochem*. 2003; 242:115–120.
8. ten Hove M, van Emous JG, van Echteld CJ. Na^+ overload during ischemia and reperfusion in rat hearts: comparison of the Na^+/H^+ exchange blockers EIPA, cariporide and eniporide. *Mol Cell Biochem*. 2003;205:47–54.
9. Schafer C, Ladilov Y, Inserte J, Schafer M, Haffner S, Garcia-Dorado D, Piper HM. Role of the reverse mode of the Na^+/Ca^{2+} exchanger in reoxygenation-induced cardiomyocyte injury. *Cardiovasc Res*. 2001;51: 241–250.
10. Rohmann S, Weygandt H, Minck K-O. Preischaemic as well as postischaemic application of a Na^+/H^+ exchange inhibitor reduces infarct size in pigs. *Cardiovasc Res*. 1995;30:945–951.
11. Hurtado C, Pierce GN. Inhibition of Na^+/H^+ exchange at the beginning of reperfusion is cardioprotective in isolated, beating adult cardiomyocytes. *J Mol Cell Cardiol*. 2000;32:1897–2007.
12. Matsumoto T, Miura T, Miki T, Genda S, Shimamoto K. Blockade of the Na^+-Ca^{2+} exchanger is more efficient than blockade of the Na^+-H^+ exchanger for protection of the myocardium from lethal reperfusion injury. *Cardiovasc Drugs Ther*. 2002;16:295–301.
13. Le Prigent K, Lagadic-Gossmann D, Mongodin E, Feuvray D. HCO_3^- dependent alkalinizing transporter in adult rat ventricular myocytes: characterization and modulation. *Am J Physiol*. 1997;273:H2596–H2603.
14. Sabri A, Byron KL, Samarel AM, Bell J, Lucchesi PA. Hydrogen peroxide activates mitogen-activated protein kinases and Na^+-H^+ exchange in neonatal rat cardiac myocytes. *Circ Res*. 1998;82: 1053–1062.
15. Myers ML, Farhangkhoee P, Karmazyn M. Hydrogen peroxide induced impairment of post-ischemic ventricular function is prevented by the sodium-hydrogen exchange inhibitor HOE 642 (cariporide). *Cardiovasc Res*. 1998;40:290–296.
16. Snabaitis AK, Hearse DJ, Avkiran M. Regulation of sarcolemmal Na^+/H^+ exchange by hydrogen peroxide in adult rat ventricular myocytes. *Cardiovasc Res*. 2002;53:470–480.
17. Rothstein EC, Byron KL, Reed RE, Fliegel L, Lucchesi PA. H_2O_2-induced Ca^{2+} overload in NRVM involves ERK1/2 MAP kinases: role for an NHE-1-dependent pathway. *Am J Physiol*. 2002;283:H598–H605.
18. Baetz D, Haworth RS, Avkiran M, Feuvray D. The ERK pathway regulates Na^+-HCO_3^- cotransport activity in adult rat cardiomyocytes. *Am J Physiol*. 2002;283:H2102–H2109.
19. Ruiz-Meana M, Garcia-Dorado D, Pina P, Inserte J, Agulló L, Soler-Soler J. Cariporide preserves mitochondrial proton gradient and delays ATP depletion in cardiomyocytes during ischemic conditions. *Am J Physiol*. 2003;285:H999–H1006.
20. Numata M, Petrecca K, Lake N, Orlowski J. Identification of a mitochondrial Na^+/H^+ exchanger. *J Biol Chem*. 1998;273:6951–6959.
21. Miyazaki E, Sakaguchi M, Wakabayashi S, Shigekawa M, Mihara K. NHE6 protein possesses a signal peptide destined for endoplasmic

reticulum membrane and localizes in secretory organelles of the cell. *J Biol Chem*. 2001;276:49221–49227.
22. Brett CL, Wei Y, Donowitz M, Rao R. Human Na^+/H^+ exchanger isoform 6 is found in recycling endosomes of cells, not in mitochondria. *Am J Physiol*. 2002;282:C1031–C1041.
23. Hotta Y, Ishikawa N, Ohashi N, Matsui K. Effects of SM-20550, a selective Na^+-H^+ exchange inhibitor, on the ion transport of myocardial mitochondria. *Mol Cell Biochem*. 2001;219:83–90.
24. Kapus A, Lukacs GL, Cragoe EJ Jr, Ligeti E, Fonyo A. Characterization of the mitochondrial Na^+-H^+ exchange: the effect of amiloride analogues. *Biochim Biophys Acta*. 1988;944:383–390.
25. Teshima Y, Akao M, Jones SP, Marbán E. Cariporide (HOE 642), a selective Na^+-H^+ exchange inhibitor, inhibits the mitochondrial death pathway. *Circulation*. In press.
26. Mosca SM, Cingolani HE. Comparison of the protective effects of ischemic preconditioning and the Na^+/H^+ exchanger blockade. *Naunyn Schmiedebergs Arch Pharmacol*. 2000;362:7–13.
27. Gumina RJ, Beier N, Schelling P, Gross GJ. Inhibitors of ischemic preconditioning do not attenuate Na^+/H^+ exchange inhibitor mediated cardioprotection. *J Cardiovasc Pharmacol*. 2000;35:949–953.
28. Gumina RJ, Terzic A, Gross GJ. Do NHE inhibition and ischemic preconditioning convey cardioprotection through a common mechanism?. *Basic Res Cardiol*. 2001;96:318–324.
29. Gumina RJ, Buerger E, Eickmeier C, Moore J, Daemmgen J, Gross GJ. Inhibition of the Na^+/H^+ exchanger confers greater cardioprotection against 90 minutes of myocardial ischemia than ischemic preconditioning in dogs. *Circulation*. 1999;100:2519–2526.
30. Theroux P, Chaitman BR, Danchin N, Erhardt L, Meinertz T, Schroeder JS, Tognoni G, White HD, Willerson JT, Jessel A. Inhibition of the sodium-hydrogen exchanger with cariporide to prevent myocardial infarction in high-risk ischemic situations: main results of the GUARDIAN trial. *Circulation*. 2000;102:3032–3038.
31. Zeymer U, Suryapranata H, Monassier JP, Opolski G, Davies J, Rasmanis G, Linssen G, Tebbe U, Schroder R, Tiemann R, Machnig T, Neuhaus KL, ESCAMI Investigators. The Na^+/H^+ exchange inhibitor eniporide as an adjunct to early reperfusion therapy for acute myocardial infarction: results of the evaluation of the safety and cardioprotective effects of eniporide in acute myocardial infarction (ESCAMI) trial. *J Am Coll Cardiol*. 2001;38:1644–1650.
32. Scholz W, Beier N. Development of the NHE inhibitors for cardiovascular therapeutics. In: Karmazyn M, Avkiran M, Fliegel L, eds. *The Sodium-Hydrogen Exchanger*. Boston, Mass: Kluwer; 2003:255–262.

KEY WORDS: myocardial infarction ■ NHE-1 ■ NHE-6 ■ apoptosis ■ necrosis