See related article, pages 814–819

Na^+-H^+ Exchanger Inhibition
A New Antihypertrophic Tool

Horacio E. Cingolani, María Cristina Camilión de Hurtado

Cardiac hypertrophy (CH) is a major risk factor for cardiac death and commonly precedes the development of heart failure (HF). This is motivating the search for novel pharmacological strategies to prevent the development and/or regress CH. Although the signaling pathways leading to myocardial hypertrophy are complex, one important set of pathways involves the mitogen-activated protein kinases (MAPKs).[1] MAPKs phosphorylate numerous substrates, including nuclear transcription factors that activate the expression of different genes. The Na^+-H^+ exchanger (NHE) is a common downstream effector of this cascade[2–4] and has been implicated in different models of hypertrophy, such as "hypertensive" myocardium, aortic constriction-induced hypertrophy, and postinfarction myocardial hypertrophy.[5–8] Interestingly, stretch-induced hypertrophy of cultured neonatal cardiomyocytes is also accompanied by an increase in MAPK activity[6,8] and NHE activation. Furthermore, stretch-induced MAPK stimulation is partially prevented by inhibition of NHE activity.[6]

The article published in this issue of *Circulation Research* by Engelhardt et al[9] reports another example of a link between NHE activity and cellular growth, employing a different experimental model of CH induced by overexpression of β_1-adrenergic receptors in transgenic mice. CH, fibrosis, and failure induced by this model were prevented by NHE inhibition. The overstimulation of β_1-adrenergic receptors with isoproterenol is perhaps a similar experimental model of hypertrophy that has been extensively studied before[10–13] and reported to be mediated by p41/p42-MAPK activation.[13] Karmazyn's group[7] reported that the inhibition of NHE activity attenuated the hypertrophy that follows myocardial infarction. Camilión de Hurtado et al[14] recently reported that chronic NHE blockade in vivo induces regression of CH in spontaneously hypertensive rats (SHR) without a significant decrease in arterial pressure. The in vivo blockade of NHE also decreases the cellular proliferation detected in vessels from diabetic rats.[15] Taken together, these findings provide compelling evidence of a common role played by NHE in different models of myocardial hypertrophy.

Which would be the relationship between NHE activity and myocardial hypertrophy? Perhaps the most convincing demonstration of a signaling role for NHE activation in cellular growth is found in sea urchin eggs, where fertilization is followed by NHE activation, and cellular growth is precluded if NHE activation is prevented.[16] Because NHE exchanges intracellular H^+ for extracellular Na^+ in a one-by-one stoichiometry, the intracellular ionic changes resulting from its activation will be a decrease in $[H^+]_i$ and an increase in $[Na^+]_i$. However, whereas the activation of NHE may result in nonsignificant changes in pH_i (under physiological conditions where the bicarbonate-dependent mechanisms are active),[17–19] the increase in $[Na^+]_i$ is always detected.[20] This rise in $[Na^+]_i$ might be responsible for an increase in $[Ca^{2+}]_i$ levels mediated by Na^+-Ca^{2+} exchange (NCX). The increase in $[Ca^{2+}]_i$ is recognized as a cell growth signal.[21] NHE can carry ≈50% of the Na^+ entering the cells.[22] We should, therefore, learn to think more of increases of $[Na^+]_i$ than in increases of pH_i when NHE is activated by "growth factors." Conversely, blockade of NHE activity will promote the decrease of $[Na^+]_i$ levels.

Recently, a new factor has been identified to contribute to the NHE hyperactivity-hypertrophy relationship: the anion exchanger (AE).[23] Some isoforms of the AE are upregulated in the hypertrophied myocardium.[24] This exchanger is, even at steady pH_i, continuously loading the cell with acid.[25] This acid load is balanced by other acid extruder mechanisms, and under normal conditions, pH_i is kept within normal limits. AE hyperactivity, by increasing $[H^+]_i$, will stimulate the NHE leading to the increase of $[Na^+]_i$. Interestingly, β_1-adrenergic receptor stimulation enhances AE activity.[26] It is possible that isoproterenol (or overexpression of β_1 receptors), through AE stimulation, increases $[H^+]_i$, thereby causing NHE activation. The possible linkage between NHE and other ionic membrane transport mechanisms is depicted in the Figure.

It has been previously demonstrated that $[Na^+]_i$ increases in hypertrophied myocardium.[27] Furthermore, in isolated myocytes and vascular smooth muscle cells, Na^+ has a direct effect of increasing protein synthesis and decreasing protein degradation.[28] Epidemiological and basic studies support the notion that Na^+ is an independent factor for the development of salt-induced CH.[29,30] Frohlich and colleagues reported that a high Na^+ diet increased not only cardiac enlargement in SHR but also cardiac mass in normotensive rats without detectable change of arterial pressure.[31] Therefore, given the fact that NHE is a pathway for Na^+ entry, its pharmacological blockade appears to be an intervention capable of reducing Na^+ influx.

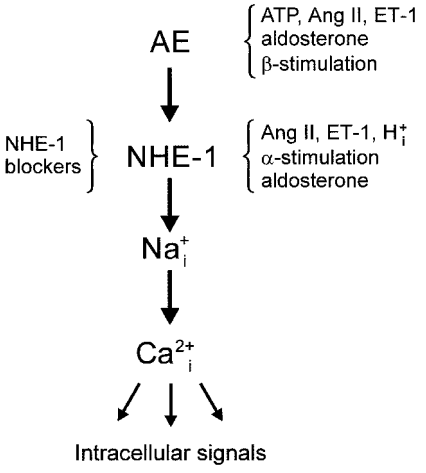
An increased activity of the NHE has been detected in HF[32] and cariporide has been shown to have antiapoptotic effect

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

Correspondence to Dr H.E. Cingolani, Centro de Investigaciones Cardiovasculares, Facultad de Ciencias Médicas, calle 60 y 120, La Plata, 1900, Argentina. E-mail cicmes@infovia.com.ar

(*Circ Res.* 2002;90:751-753.)

***Circulation Research* is available at http://www.circresaha.org**
DOI: 10.1161/01.RES.0000016836.24179.AE



$[Ca^{2+}]_i$ is known to be a hypertrophic signal either acting through kinases such as PKC and/or the Ca^{2+}/calmodulin-activated phosphatase, calcineurin. $[Ca^{2+}]_i$ is modified by $[Na^+]_i$ through modulation of NCX function. Because ≈50% of Na^+ entry is due to NHE activity, we should expect a rise of $[Na^+]_i$ when NHE activity increases and, conversely, a decrease in $[Na^+]_i$ when it decreases. NHE activity can be increased by several interventions. β_1-Receptor stimulation, which itself does not increase NHE function, can through the activation of AE activity induce NHE stimulation leading to the rise of $[Na^+]_i$ and, consequently, of $[Ca^{2+}]_i$.

that, as proposed by Humpreys et al,[33] could be of help in HF. It is not evident to us why, in the article by Engelhardt et al,[9] the regression of hypertrophy was accompanied by normalization (through a translational mechanism) of NHE activity after the blockade. This finding deserves further investigation but it is interesting that the regression of CH after antihypertensive treatment is also accompanied by normalization (through a posttranslational mechanism) of NHE activity in SHR.[34]

Engelhardt et al[9] also reported fibrosis in accompanying CH in the mice overexpressing β_1-adrenergic receptors. However, neither fibrosis nor increases in myocardial stiffness has been detected in previous experiments[10–13] in which hypertrophy is induced by isoproterenol. The possibility of "reparative fibrosis" instead of "reactive fibrosis" that characterizes the hypertensive hypertrophy was raised by the authors. Further studies about possible changes in diastolic compliance, collagen cross-linking, and/or accumulation of different collagen subtypes[35] will be required to properly characterize this model of hypertrophy.

References

1. Clerck A, Sugden PH. Activation of protein kinase cascades in the heart by hypertrophic G protein–coupled receptor agonists. *Am J Cardiol*. 1999;83:64H–69H.
2. Bianchini L, L'Allemain G, Pouysségur J. The p42/p44 mitogen-activated protein kinase cascade is determinant in mediating activation of the Na^+-H^+ exchanger (NHE1 isoform) in response to growth factors. *J Biol Chem*. 1997;272:271–279.
3. Moor AN, Fliegel L. Protein kinase-mediated regulation of the Na^+-H^+ exchange in the rat myocardium by mitogen-activated protein kinase-dependent pathways. *J Biol Chem*. 1999;274:22985–22992.
4. Snabaitis AK, Yokoyama H, Avkiran M. Roles of mitogen-activated protein kinases and protein kinase C in α_{1A}-adrenoceptor-mediated stimulation of the sarcolemmal Na^+-H^+ exchanger. *Circ Res*. 2000;86:214–222.
5. Pérez NG, Alvarez BV, Camilión de Hurtado MC, Cingolani HE. pH_i regulation in myocardium of the spontaneously hypertensive rat: compensated enhanced activity of the Na^+-H^+ exchanger. *Circ Res*. 1995;77:1192–1120.
6. Takewaki S, Kuro-o M, Hiroi Y, Yamazaki T, Noguchi T, Miyagishi A, Nakahara K, Aikawa M, Manabe I, Yazaki Y, et al. Activation of Na^+-H^+ antiporter (NHE-1) gene expression during growth, hypertrophy and proliferation of the rabbit cardiovascular system. *J Mol Cell Cardiol*. 1995;27:729–742.
7. Yoshida H, Karmazyn M. Na^+-H^+ exchange inhibition attenuates hypertrophy and heart failure in 1-wk postinfarction rat myocardium. *Am J Physiol Heart Circ Physiol*. 2000;278:H300–H304.
8. Yamazaki T, Komuro I, Kudoh S, Zou Y, Nagai R, Aikawa R, Uozumi H, Yazaki Y. Role of ion channels and exchangers in mechanical stretch-induced cardiomyocyte hypertrophy. *Circ Res*. 1998;82:430–437.
9. Engelhardt S, Hein L, Keller U, Klämbt K, Lohse MJ. Inhibition of Na^+-H^+ exchange prevents hypertrophy, fibrosis, and heart failure in β_1-adrenergic receptor transgenic mice. *Circ Res*. 2002;90:814–819.
10. Nagano M, Higaki J, Nakamura F, Higashimori Koichi, Nagano N, Mikami H, Ogihara T. Role of cardiac angiotensin II in isoproterenol-induced left ventricular hypertrophy. *Hypertension*. 1992;19:708–712.
11. Golomb E, Abassi ZA, Cuda G, Stylianou M, Panchal VR, Trachewsky D, Keiser HR. Angiotensin II maintains, but does not mediate, isoproterenol-induced cardiac hypertrophy in rats. *Am J Physiol Heart Circ Physiol*. 1994;267:H1496–H1506.
12. Leenen FHH, White R, Yuan B. Isoproterenol-induced cardiac hypertrophy: role of circulatory versus cardiac renin-angiotensin system. *Am J Physiol Heart Circ Physiol*. 2001;281:H2410–H2416.
13. Zou Y, Komuro I, Yamazaki T, Kudoh S, Uozumi H, Kadowaki T, Yazaki Y. Both G_s and G_i proteins are critically involved in isoproterenol-induced cardiomyocyte hypertrophy. *J Biol Chem*. 1999;274:9760–9770.
14. Camilión de Hurtado MC, Portiansky EL, Pérez NG, Rebolledo OR, Cingolani HE. Regression of cardiomyocyte hypertrophy in SHR following chronic inhibition of the Na^+-H^+ exchanger. *Cardiovasc Res*. In press.
15. Jandeleit-Dahm K, Hannan KM, Farrelly CA, Allen TJ, Rumble JR, Gilbert RE, Cooper ME, Little PJ. Diabetes-induced vascular hypertrophy is accompanied by activation of Na^+-H^+ exchange and prevented by Na^+-H^+ exchange inhibition. *Circ Res*. 2000;87:1133–1149.
16. Moolenaar WH, Bierman AJ, de Laat SW. Effects of growth factors on Na^+-H^+ exchange. In: Grinstein S, ed. *Na^+-H^+ Exchange*. Boca Raton, Fla: CRC Press Inc; 1998:227–234.
17. Boron WF, Boyarski G, Ganz M. Regulation of intracellular pH in renal mesangial cells. *Ann N Y Acad Sci*. 1989;574:321–332.
18. Camilión de Hurtado MC, Alvarez BV, Pérez NG, Ennis IL, Cingolani HE. Angiotensin II activates Na^+-independent Cl^--HCO_3^- exchange in ventricular myocardium. *Circ Res*. 1998;82:473–481.
19. Camilión de Hurtado MC, Alvarez BV, Ennis IL, Cingolani HE. Stimulation of myocardial Na^+-independent Cl^--HCO_3^- exchanger by angiotensin II is mediated by endogenous endothelin. *Circ Res*. 2000;86:622–627.
20. Pérez NG, Camilión de Hurtado MC, Cingolani HE. Reverse mode of the Na^+-Ca^{2+} exchange following myocardial stretch: underlying mechanism of the slow force response. *Circ Res*. 2001;88:376–782.
21. Marbán E, Koretsune Y. Cell calcium, oncogenes, and hypertrophy. *Hypertension*. 1990;15:652–658.
22. Frelin C, Vigne P, Lazdunski M. The role of the Na^+-H^+ exchange system in cardiac cells in relation to the control of the internal Na^+ concentration. *J Biol Chem*. 1984;259:8880–8885.
23. Alvarez BV, Fujinaga J, Casey JR. Molecular basis for angiotensin II–induced increase of chloride/bicarbonate exchange in the myocardium. *Circ Res*. 2001;89:1246–1253.
24. Chiappe de Cingolani G, Morgan P, Mundiña-Weilenmann C, Casey J, Fujinaga J, Camilión de Hurtado MC, Cingolani HE. Hyperactivity and altered mRNA isoform expression of the Cl^-/HCO_3^- anion-exchanger in the hypertrophied myocardium. *Cardiov Res*. 2001;51:71–79.
25. Leem CH, Lagadic-Gossman D, Vaughan-Jones RD. Characterization of intracellular pH regulation in the guinea-pig ventricular myocyte. *J Physiol*. 1999;517:159–180.
26. Vassort G, Pucéat M, Désilets M. Chloride dependence of pH modulation by β-adrenergic agonist in rat cardiomyocytes. *Circ Res*. 1994;75:862–869.
27. Gray PR, McIntyre H, Sheridan DS, Fry CH. Intracellular sodium and contractile function in hypertrophied human and guinea-pig myocardium. *Pflügers Arch*. 2001;442:117–123.

28. Gu JW, Anand V, Shek EW, Moore MC, Brady AL, Kelly WC, Adair TH. Sodium induces hypertrophy of cultured myocardial myoblasts and vascular smooth muscle cells. *Hypertension*. 1998;31:1083–1087.
29. Du Calair G, Ribstein J, Daures J-P, Minran A. Sodium and left ventricular mass in untreated hypertensive and normotensive subjects. *Am J Physiol*. 1992;263:H177–H181.
30. Schmieder RE, Messerli FH, Garavaglia GE, Nunez BD. Dietary salt intake: a determinant of cardiac involvement in essential hypertension. *Circulation*. 1988;78:951–956.
31. Frohlich ED, Apstein C, Chobanian A, Devereux R, Dustan H, Dzau V, Fauad-Tarazi F, Horan M, Marcus M, Massie B, Pfeffer M, Re R, Roccella E, Savage D, Shub C. The heart in hypertension. *N Engl J Med*. 1992;327:998–1008.
32. Yokoyama H, Gunasegaram S, Harding SE, Avkiran M. Sarcolemmal Na^+-H^+ exchanger activity and expression in human ventricular myocardium. *J Am Coll Cardiol*. 2000;36:534–540.
33. Humpreys RA, Haist JV, Chakrabarti S, Feng Q, Arnold JM, Karmazyn M. Orally administered NHE1 inhibitor cariporide reduces acute responses to coronary occlusion and reperfusion. *Am J Physiol*. 1999; 276:H749–H757.
34. Ennis IL, Alvarez BV, Camilión de Hurtado MC, Cingolani HE. Enalapril induces regression of cardiac hypertrophy and normalization of pH_i regulatory mechanisms. *Hypertension*. 1998;31:961–967.
35. Woodiwiss AJ, Tsotetsi OJ, Sprott S, Lancaster EJ, Mela T, Chung ES, Meyer TE, Norton GR. Reduction in myocardial collagen cross-linking parallels left ventricular dilatation in rat models of systolic chamber dysfunction. *Circulation*. 2001;103:155–160.

KEY WORDS: Na^+-H^+ exchanger ■ cardiac hypertrophy ■ NHE1 inhibitors