Pushing and Pulling the Cardiac Sodium/Hydrogen Exchanger

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The strangest animal in Dr Dolittle’s zoo was a Pushmi-pullyu (pronounced push-me-pull-you), a mythical chimera of 2 antelopes, with a head at each end. Sometimes it looked and moved forwards, sometimes backwards. The direction could be coaxed by Dr Dolittle (played originally by Rex Harrison in the Hollywood movie), who spoke to the animal in its own language. A report from the laboratory of Avkiran, published in this issue of Circulation Research, shows that there is something strangely reminiscent of the Pushmi-pullyu in the cardiac sodium–hydrogen exchanger (NHE1), a ubiquitous, dimeric glycoprotein, intimately involved with the regulation of intracellular pH (pHi) (reviewed elsewhere). In cardiac myocytes, the NHE1 protein product is expressed at the sarcolemma. It is a secondary active transporter expelling one H⁺ ion in exchange for the entry of one Na⁺ ion, and its activity is governed acutely by the intracellular H⁺ ion concentration (ie, pHi). Activity increases steeply when pH falls from its resting level of ≈7.2. This is why the protein is so effective as a pH regulator.

But why the resemblance to a Pushmi-pullyu? It is becoming apparent that NHE1 in the cardiac cell can be both stimulated and inhibited directly by intracellular signaling cascades. Like Dr Dolittle, these talk to the transporter, and the chorus determines whether cellular H⁺ extrusion during intracellular acidosis is stimulated or inhibited. It has long been known that the pHᵢ sensitivity of NHE1 can be enhanced through a coupling of the transport protein to surface membrane receptors, operating via endogenous intracellular ligands. Many of the pathways involved have been highlighted in other tissues, but, in the cardiac myocyte, typical extracellular receptor agonists that stimulate NHE1 activity are angiotensin II (acting on AT₁ receptor), catecholamines, or maneuvers that elevate intracellular cAMP, PKG has not been established. Interestingly, extracellular adenosine can inhibit NHE1, but this is via the action of a phosphatase enzyme (PPA2) that dephosphorylates the CD, possibly at the stimulatory site targeted by extracellular ligands on NHE1 activity have been documented previously (these are highlighted for the cardiac myocyte in the Figure [left]), but, in many cases, the mechanisms of action, at least in the wild-type cardiac cell, are either unknown or only partly elucidated. Thus, β₁ adrenoceptor activation by catecholamines, or maneuvers that elevate intracellular cAMP, result in a slowing of cardiac NHE1 activity. Similarly, the nitric oxide donor sodium nitroprusside or elevation of intracellular cGMP also slows cardiac NHE1 activity. However, whether the effect involves direct phosphorylation of the transport protein by a canonical kinase such as PKA or PKG has not been established. Interestingly, extracellular adenosine can inhibit NHE1, but this is via the action of a phosphatase enzyme (PPA₂) that dephosphorylates the CD, possibly at the stimulatory site targeted by extracellular signal-regulated kinase/RSK, an example of deactivation rather than inactivation of the transporter (Figure). The phosphorylation of serine 648 by PKB/Akt is, therefore, the first evidence of a direct link between an endogenous regulator kinase and cardiac NHE1 inhibition.

The involvement of PKB/Akt in the regulation of cardiac NHE1 activity is of particular interest, because isoforms Akt1 and -2 are intimately involved in the control of cell survival, glucose uptake, and glucose metabolism (reviewed elsewhere). The coinvolvement of a transport protein that helps to discharge the acidic waste of metabolism is therefore not likely to be merely fortuitous. PKB/Akt is also intimately involved with the regulation of intracellular pH (pHi) (reviewed elsewhere). Common endogenous agents include mitogen-activated protein kinases, including extracellular signal-regulated kinase (ERK), which can regulate RSK (p90 ribosomal S6 kinase). The stimulant effect of Ca²⁺ is mediated through interaction with calmodulin (CaM), which activates calmodulin kinase II (CaMkII) but can also bind directly to the NHE1 protein. Various relevant ligands and receptors, and their relationship with the target NHE1 protein, are summarized schematically in the Figure (right). Although the details of action of many ligands are unknown, the stimulatory effect of some results in phosphorylation of the transporter. This occurs at sites on its cytoplasmic carboxyl-terminal domain (CD). Interaction of the CD with the transmembrane domain (TMD) then upregulates H⁺ efflux acutely during acidosis. This is the “pushmi” side of the NHE protein. The article by Snaibatis et al now focuses on the “pullyu” side. In an elegant and carefully executed series of molecular and functional studies, they have shown, for the first time, that direct CD phosphorylation (principally at serine 648) by protein kinase B (PKB) (also known as Akt1/2) acutely decreases pHᵢ sensitivity of NHE1. This decreases NHE-mediated H⁺ extrusion from a mammalian ventricular myocyte (elements of this scheme are illustrated in the Figure [left]). Thus, NHE1 can be both stimulated and inhibited by phosphorylation, making it a true Pushmi-pullyu protein.

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involved in the downstream effects of insulin receptor activation in the cardiac myocyte. Indeed, Snabaitis et al show in their work that a novel Akt1/2 inhibitor (Akti-1/2), which enhances NHE activation by intracellular $\text{H}^+$ ions, also blocks insulin-induced phosphorylation of another Akt target protein, glycogen synthase kinase 3β. Because insulin activates Akt, one may ask whether it is also an acute inhibitory controller of cardiac NHE1 activity. It is intriguing, for example, that the Goto–Kakizaki rat model of insulin-resistant type 2 diabetes appears to be associated with powerful upregulation of cardiac NHE1 expression and activity.12 Surprisingly, the possible effect of exogenous insulin on cardiac NHE is not particularly well documented in the literature, although insulin appears to stimulate NHE activity in other cell types, including skeletal muscle and vascular smooth muscle. In fact, application of insulin can alkalize resting pH, in cardiac myocytes,13 an effect that is inhibited by cariporide (a selective NHE inhibitor), suggesting a possible stimulation rather than inhibition of cardiac NHE1 activity. Postreceptor insulin signaling, however, is complex, and may involve activation of both Akt and mitogen-activated protein kinase–mediated pathways.11 Because the latter can stimulate NHE1 (Figure), a possibility is that inhibitory and excitatory phosphorylation of NHE1 results, on balance, in a modest stimulation. Snabaitis et al may, in the future, be able to tell us whether this is correct.

An exciting clinical possibility emerging from the work of Snabaitis et al is the possibility of manipulating the pushmi and pullyu effects on NHE1 of endogenous ligands, in favor of inhibition. Selective stimulation of PKB/Akt may offer a novel means for achieving this. Despite disappointing clinical trials,14 there is ample evidence from animal models of the cardioprotective effects of NHE1-selective inhibitors in pathophysiological circumstances, such as postischemic reperfusion of the heart. Similarly, in various animal models of maladaptive cardiac hypertrophy, chronic application of pharmacological NHE1 inhibitors, such as cariporide, may ameliorate or even reverse the hypertrophic phenotype and slow its progression toward heart failure.15 The pathophysiological effects of NHE1 activity are believed to be secondary to the associated rise of intracellular $\text{Na}^+$.16 By acting on sarcolemmal $\text{Na}^+\text{-Ca}^{2+}$ exchange, this raises $\text{Ca}^{2+}$, which may then underpin mechanisms of myocardial injury and hypertrophy. It is perhaps pertinent that many of the ligands and receptors arranged on the right-hand side of the Figure have been invoked in these pathological events, whereas some of the agents on the left-hand side, such as NO, cGMP, and now PKB/Akt may, under the right circumstances, be cardioprotective. It is therefore satisfying that Snabaitis et al have discovered that phosphorylation of NHE1 by PKB/Akt is associated with a reduced binding of $\text{Ca}^{2+}$–calmodulin. PKB activation may thus abrogate one of the key possible mechanisms for the hypertrophic influence of $\text{Ca}^{2+}$, its stimulation of NHE1.

Speculation on the possible clinical benefits of PKB activation should be tempered by a warning. Although it slows NHE1 activity, the effects of PKB on other key pH regulatory transport proteins expressed at the cardiac sarcolemma,17,18 such as $\text{Na}^+\text{-HCO}_3^-$ cotransport (NBC), $\text{Cl}^-\text{-HCO}_3^-$ exchange, $\text{Cl}^-\text{-OH}^-$ exchange, and $\text{H}^+$-lactate co-transport, remain to be assessed. It is noteworthy, for example, that acute inhibition of cardiac NHE1 activity by a β-adrenoceptor agonist can be paralleled by a coordinate stimulation of acid extrusion (and hence $\text{Na}^+$ influx) on NBC.19 The challenge now, perhaps, will be to assess possible pushmi-pullyu effects of PKB on parallel acid and base extrusion pathways. The far-sighted work of Snabaitis et al and the laboratory of Avkiran have robustly laid down this challenge. It would appear that Dr Dolittle has more than a little to do.

**Figure.** Regulation of NHE1 activity by surface membrane receptors (italicized) and endogenous/exogenous ligands (nonitalicized). Kinase enzymes highlighted in gray. Center, transport domain (TMD) of NHE1 protein and its cytoplasmic domain (CD), showing phosphorylation sites (stars); green arrows, stimulation; red arrows, inhibition. $A_1$ indicates adenosine receptor; $AC$ indicates adenylate cyclase; $AT$, angiotensin II; $ET$, endothelin; $GC$, guanylate cyclase; $M_2$, muscarinic receptor; ROS, reactive oxygen species.
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References

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