Na^+/H^+ Exchangers

Molecular Diversity and Relevance to Heart

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ABSTRACT: During the last several years, significant advances have been made in our understanding of the molecular, cellular, and physiological diversity of mammalian Na^+/H^+ exchangers. This transporter forms a multigene family of at least six members (NHE1–NHE6) that share ~20–60% amino acid identity. NHE1 is the most predominant isoform expressed in heart and it contributes significantly to myocardial pH_i homeostasis, which is important for maintaining contractility. However, hyperactivation of NHE1 during episodes of cardiac ischemia and reperfusion disrupts the intracellular ion balance, leading to cardiac dysfunction and damage in several animal models, but which can be prevented by pharmacological antagonists of NHE1. Molecular studies have indicated that the predicted transmembrane segments M4 and M9 contain several residues involved in drug sensitivity. Molecular dissection of the drug binding region should facilitate the rational design of more potent and isoform-specific drugs that may provide therapeutic benefit in the prevention of cardiac ischemia and reperfusion injuries.

ROLES OF THE Na^+/H^+ EXCHANGER IN CARDIAC PHYSIOLOGY AND PATHOPHYSIOLOGY

Myocardial function is greatly influenced by changes in intracellular pH (pH_i). For example, myocardial acidosis results in marked decreases in contractility,[1] which is associated with reduced myosin-ATPase activity,[2] diminished binding of Ca^{2+} to troponin C of the contractile apparatus,[3] decreased ion currents through voltage-activated Na^+ and Ca^{2+} channels,[4–6] and reductions in gap junction conductance.[7] Hence, regulation of pH_i is of critical importance for maintaining cardiac function.

At least three different ion transporters contribute to myocardial pH_i regulation; the Cl^-/HCO_3^- exchanger,[8,9] the Na^+-HCO_3^- cotransporter,[10,11] and the Na^+/H^+ exchanger.[12–14] An increase in pH_i activates the Cl^-/HCO_3^- exchanger, which extrudes intracellular HCO_3^- for extracellular Cl^-. By contrast, intracellular acidification activates both the Na^+-HCO_3^- cotransporter and the Na^+/H^+ exchanger, with the latter being the predominant mechanism for restoring myocardial pH_i to the neutral range (pH_i 7.1 to 7.3).[10,11,13]

Aside from its role in normal myocardial pH_i homeostasis, accumulating evidence points to the Na^+/H^+ exchanger as a contributing factor in the pathophysiology of cardiac ischemia and reperfusion injuries. During cardiac ischemia, ATP stores

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are depleted as a consequence of depressed mitochondrial activity and lactic acid levels are increased due to anaerobic metabolism of glucose. This results in a rapid decrease in both intracellular and extracellular pH, which precipitates a series of other changes in myocardial ion homeostasis, primarily Na^+_i and Ca^{2+}_i overloads, which then lead to cardiac dysfunction and tissue damage (for further details[15–19]). Ischemia elevates Na^+_i by two mechanisms: the acidosis that occurs during the first few minutes of ischemia leads to an influx of Na^+ by activation of the Na^+/H^+ exchanger, and the diminished ATP levels cause depression of Na^+,K^+-ATPase activity, which normally extrudes Na^+_i. Indeed, the Na^+/H^+ exchanger accounts for as much as 50% of the cardiac membrane's basal permeability to Na^+ following intracellular acidification.[20–22] Furthermore, the reduction in ATP levels also causes a decrease in the pH_i sensitivity (or threshold for activation) of the Na^+/H^+ exchanger, thereby impairing its ability to fully restore pH_i to neutral.[23] The net result is a chronic state of cellular acidosis.

The elevation of Na^+_i reduces the transmembrane Na^+ gradient, thereby inhibiting Na^+/Ca^{2+} exchangers that, under normal conditions, extrude Ca^{2+}_i in exchange for Na^+_o. Moreover, if Na^+_i increases sufficiently the Na^+/Ca^{2+} exchanger could reverse and mediate Ca^{2+} influx. This Ca^{2+}_i overload is associated with cardiac arrhythmias and, if untreated, contributes to contractile failure. Reperfusion of the failing heart with physiological fluids to restore pH_i is the standard approach to rescuing the tissue, but may lead to further tissue damage. This has been referred to as the "pH paradox." Rapid removal of the acidic extracellular fluid generates a large transmembrane pH gradient that drives Na^+/H^+ exchange. This further augments Na^+_i and markedly elevates Ca^{2+}_i, causing reperfusion arrhythmias, contractile failure, and cellular necrosis. Thus, the Na^+/H^+ exchanger appears to play a central role in injuries caused by ischemia and reperfusion.

The involvement of Na^+/H^+ exchangers in ischemia- and reperfusion-induced injuries, however, is most convincingly demonstrated by animal studies showing that treatment with amiloride, a relatively weak inhibitor of the Na^+/H^+ exchanger, significantly reduces Na^+ and Ca^{2+} overload and is cardioprotective.[24] Similar protective effects are obtained with amiloride analogues,[25–33] and benzoyl guanidinium compounds such as HOE694,[34–38] HOE642 (cariporide),[39–43] and compound 246,[44] which are more potent and selective antagonists of the Na^+/H^+ exchanger. The beneficial effects of these compounds are obtained only during the early stages (within several minutes) of ischemia and reperfusion when the Na^+/H^+ exchanger is most active. The antiarrhythmic action of amiloride has also been demonstrated in human clinical studies, where it suppressed inducible ventricular tachycardia[45] and spontaneous ventricular premature beats.[46] Thus, these observations strongly implicate overactivation of the Na^+/H^+ exchanger as a central factor in ischemia- and reperfusion-induced injuries.

EXPRESSION AND LOCALIZATION OF Na^+/H^+ EXCHANGER ISOFORMS IN HEART

In mammals, at least six Na^+/H^+ exchanger isoforms (NHE1 to NHE6) are known to exist and they exhibit distinct differences in their primary structures (~20–60%

amino acid identity), patterns of tissue expression, membrane localization, functional properties, and physiological roles.[47,48]

Mammalian cardiac tissue expresses predominantly the NHE1 mRNA,[49–52] albeit minor amounts of NHE2 mRNA are also detected in some species.[50] Indeed, NHE1 is present in virtually all tissues and most cell types examined, consistent with its proposed "housekeeping" role to maintain intracellular pH and cell volume.[49,53,54] Thus, the functional characteristics of the cardiac sarcolemmal Na^+/H^+ exchanger described in numerous studies are most likely those of NHE1.

Recent immunological studies have demonstrated that NHE1 is localized predominantly at the intercalated disc regions and to a lesser extent along the transverse tubular systems of both atrial and ventricular muscle cells.[81] Unexpectedly, NHE1 was not detected along the lateral sarcolemmal membranes. This distribution differs somewhat from the Cl^-/HCO_3^- exchangers which accumulate mainly at the lateral sarcolemma and transverse tubules of isolated adult rat ventricular myocytes.[55] The location of the other major cardiac pH regulatory protein, the Na^+-HCO_3^- cotransporter, is currently unknown. The physiological relevance of the high density of NHE1 at the intercalated discs is unclear. While speculative, in this region it may serve to regulate the opening of gap junction channels, which are highly sensitive to minor fluctuations in pH_i within the physiological range,[7,56] and thereby to influence impulse conduction between myocytes.

The heart also expresses NHE6, but it is localized to the mitochondria inner membrane[57] where it is responsible for extruding Na^+ from the alkaline matrix of respiring mitochondria[58] and, as such, may contribute to organellar volume homeostasis.[59] This process may also be functionally coupled to the efflux of Ca^{2+} from the mitochondria by the recycling of Na^+ between the mitochondrial Na^+/H^+ and Na^+/Ca^{2+} exchangers.[59,60] There are indications that the mitochondrial NHE is also responsible for mediating transport of NH_4^+ from the mitochondrial matrix.[61]

STRUCTURAL AND FUNCTIONAL FEATURES OF THE Na^+/H^+ EXCHANGER

The NHE isoforms exhibit similar membrane topologies, with 12 predicted membrane-spanning (M) regions at the N-terminus and a large cytoplasmic region at the C-terminus. The most highly conserved regions of the NHE isoforms are the membrane-spanning segments, which probably mediate cation transport and drug binding. The C-terminal regions are highly hydrophilic and exhibit a lower degree of similarity among isoforms. Structural studies indicate that this latter region is involved in regulation by growth factors and other mitogens, consistent with it being oriented towards the cytoplasmic side of the membrane.[47,48]

Even less is known about the tertiary or quaternary structure of Na^+/H^+ exchangers, although recent evidence suggests that they exist in the membrane as homodimers.[62,63] The site of interaction between the monomers resides in the putative transmembranous region,[63] possibly linked by disulfide bonding,[62] although the precise location(s) of contact have yet to be defined.

As mentioned above, the NHE is a known target for inhibition by the diuretic compound amiloride and its analogues.[64] Amiloride analogues containing hydro-

phobic substituents on the 5-amino group of the pyrazine ring have higher affinity and specificity for the NHE relative to other ion transporters. Using a heterologous expression system, NHE isoforms exhibit a wide range of affinities for amiloride and its analogues, which span over two orders of magnitude and show the following order of sensitivity: NHE1 ≥ NHE2 ≫ NHE3.[65,66] Recently, HOE694[67,68] and its related compound HOE642[39] have also been found to inhibit the isoforms with a similar rank order as the amiloride compounds, but over a larger concentration range (three to four orders of magnitude). Other pharmacological agents, such as cimetidine, clonidine, and harmaline, also exhibit differential affinities for the NHE isoforms.[65,66] While these compounds are chemically unrelated to amiloride or HOE694, they possess either an imidazoline or guanidinium moiety and hence bear some structural similarity to these compounds.

Biochemical analyses indicate that inhibition by amiloride compounds, cimetidine,[73] and HOE694[67] is reduced by high external Na^+. This competitive inhibition suggests they bind near the external Na^+ transport site and may also share a common site. However, under different anionic buffer conditions, amiloride and its derivatives also inhibit transport noncompetitively, suggesting that the external Na^+- and amiloride-binding sites may not be identical.[74,75] Furthermore, the extracellular Na^+- and amiloride-binding sites can be altered independently of each other using genetic selection techniques.[76] Taken together, these data indicate that amiloride and other antagonists probably interact with multiple sites on the exchanger.

Consistent with this idea, recent molecular studies of human NHE1 have shown that two predicted membrane-associated domains are targets for interaction with amiloride and its analogues. Residues in the fourth (Phe^{161}, Leu^{163}, Gly^{174})[77,78] and the ninth (His^{349})[79] transmembrane segments appear to contribute to amiloride sensitivity without affecting Na^+ affinity. Likewise, mutagenesis of a homologous residue in the fourth transmembrane domain of rabbit NHE2 ($Leu^{143} \rightarrow Phe^{143}$) also reduced its sensitivity to amiloride compounds.[80] However, mutations at each of these sites produced only modest changes in drug sensitivity and did not confer the degree of drug resistance observed for the NHE3 isoform.[65,67,77] Thus, other residues of the exchanger are presumably involved in determining drug sensitivity.

A recent analysis of chimeric NHE1 and NHE3 proteins identified a 66 amino acid segment containing the putative ninth transmembrane (M9) domain and its adjacent loops that significantly influences drug sensitivity.[68] Homologous substitution of this region between isoforms caused a reciprocal change in the drug sensitivities of NHE1 and NHE3 by one to three orders of magnitude, depending on the drug. The greatest changes in affinity were for ethylisopropylamiloride and HOE694. These alterations differ from those caused by mutations at His^{349} in the putative M9 domain of human NHE1, where either a modest twofold increase ($His^{349} \rightarrow$ Tyr or Phe) or twofold decrease ($His^{349} \rightarrow$ Gly or Leu) in amiloride sensitivity was observed, whereas other amino acid substitutions at this position had no effect.[79] This suggests that other residues within this region serve as major determinants in conferring drug sensitivity. Further molecular dissection of the drug binding region could be helpful in developing more potent and isoform-specific drugs that may be of therapeutic benefit in the prevention of cardiac ischemia and reperfusion injuries.

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