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Larry Fliegel

Regulation of myocardial Na^+/H^+ exchanger activity

Abstract The Na^+/H^+ exchanger is a plasma membrane protein, present in the myocardium, which removes intracellular protons and exchanges them with extracellular Na^+. The protein comprises an N-terminal, hydrophobic, integral membrane domain that transports the ions and a C-terminal, hydrophilic region that regulates the N-terminal domain. The C-terminal domain has several sub-domains, including one region that binds calmodulin and another that is phosphorylated by protein kinases. The Na^+/H^+ exchanger is activated by angiotensin, endothelin and α_1-adrenergic stimulation. These effectors increase phosphorylation of the C-terminal domain by protein kinases, and G proteins have been implicated in this, but their role remains to be defined. It has recently been shown that ischemia and other stimuli lead to an increased expression of the Na^+/H^+ exchanger in the myocardium. The role of this increased expression in the pathology of ischemia and reperfusion-mediated myocardial damage has yet to be determined. Recent evidence suggests that the Na^+/H^+ exchanger may play a key role in hypertrophy of the myocardium, and that its activation through G protein-coupled receptors may be important in mediating its effects.

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Dr. L. Fliegel (✉)
Departments of Biochemistry
Faculty of Medicine, University of Alberta
347 Medical Science Building
Edmonton, Alberta, Canada, T6G 2H7
E-Mail: lfliegel@gpu.srv.ualberta.ca

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Introduction

The Na^+/H^+ exchanger is a ubiquitous, integral membrane protein that is present in all mammalian cell types. In higher eukaryotes, it removes an intracellular H^+ and exchanges it for an extracellular Na^+ (8, 22), protecting cells from intracellular acidification. In addition, Na^+/H^+ exchange is involved in the regulation of sodium fluxes and in the regulation of cell volume after osmotic shrinkage (8, 22). The Na^+/H^+ exchanger is of vital importance in the myocardium, since it prevents the intracellular acidosis that inhibits contractility (8). In mammals, it also plays a key role in the development of myocardial damage during ischemia and reperfusion (11). In all cell types, Na^+/H^+ exchange is regulated via multiple mechanisms, which include G protein-coupled receptors and phosphorylation by protein kinases, and it appears that the hydrophilic, cytoplasmic domain of the exchanger modifies the activity of the N-terminal domain (22). This review summarizes our current knowledge concerning the biochemistry, molecular biology and regulation of the Na^+/H^+ exchanger.

Structure of the myocardial Na^+/H^+ exchanger

Presently, six isoforms of the Na^+/H^+ exchanger are known and they are designated NHE1–NHE6. NHE1 is the predominant isoform found in myocardial plasma membranes (9), and its sequence is identical to that of the NHE1 isoform present in other tissues (9). The deduced amino acid sequence of the human protein comprises

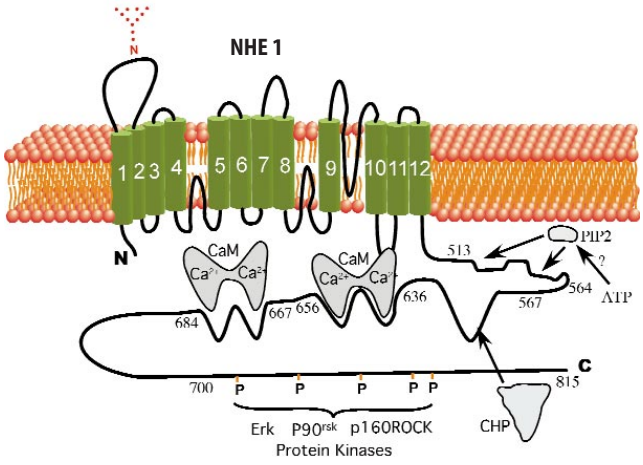

Fig. 1 Schematic diagram illustrating basic structure and regulatory elements of the Na^+/H^+ exchanger. *CaM* Calmodulin; *CHP* calcineurin homologous protein; *PIP2* phosphatidylinositolbisphosphate. See text for detailed description.

815 residues. The sequence encompasses an N-terminal, hydrophobic, membrane-associated domain, which contains about 500 amino acids, and a C-terminal, hydrophilic domain that contains about 315 amino acids (Fig. 1). The membrane-associated domain has 12 transmembrane segments and one membrane-associated segment (30) and is responsible for ion transport. The C-terminal, hydrophilic domain is contained in the cell cytoplasm (Fig. 1) where it interacts with a variety of other proteins, including protein kinases (30). This domain regulates the ion transport mediated by the integral membrane domain (22).

The cytoplasmic, C-terminal domain of the exchanger can be divided into distinct sub-domains (Fig. 1). The first sub-domain (nearest the membrane) is involved in ATP-dependent regulation of the protein. Two regions of the exchanger, between amino acids 513 and 564, account for at least part of this regulation. While the Na^+/H^+ exchanger does not use ATP directly, depletion of ATP in the cell is known to decrease Na^+/H^+ exchanger activity. This effect may be mediated by phosphatidylinositol 4,5-bisphosphate (1). The next sub-domain contains a binding site, located between amino acids 567 and 637, for an inhibitory protein called calcineurin homologous protein (CHP) (18) (see below). A third sub-domain, which binds calmodulin, contains both high- and low-affinity calmodulin binding sites (amino acids 636-656 and 657-700, respectively). Deletion of the high affinity binding site yields an "activated" protein (29). The fourth sub-domain of the Na^+/H^+ exchanger's cytoplasmic region, which maps to amino acids 700 to 815, is the phosphorylation domain (Fig. 1). Protein kinases activated by growth factors are known to phosphorylate this region of the exchanger and to stimulate its activity in the myocardium (20).

Hormonal regulation of the Na/H exchanger

The Na^+/H^+ exchanger is maximally active at low intracellular pH (pH < 6.5) and its activity declines as the pH increases. However, hormones can shift the pH-dependence into a more alkaline range, via phosphorylation of the exchanger's cytosolic domain. In the myocardium the Na^+/H^+ exchanger is subject to complex hormonal regulation. For example, via activation of the α_1-adrenergic receptor, catecholamines stimulate exchanger activity. This causes both alkalinization of steady-state intracellular pH and an enhanced rate of recovery from an acid load (8). Also, the 21-amino acid vasoactive peptide endothelin (ET-1) stimulates Na^+/H^+ exchange in cardiac myocytes (19). Further, angiotensin II (21) and thrombin (35) both activate the Na^+/H^+ exchanger. We have recently demonstrated (20) that, *in vivo*, the Na^+/H^+ exchanger is phosphorylated in response to hormonal stimulation, including stimulation by ET-1. The protein kinases that mediate this phosphorylation have not yet been identified. However, recent studies show that in mammalian myocardial and skeletal muscle, mitogen-activated protein kinase (MAPK), specifically ERK1 and 2, phosphorylates the cytosolic domain of the Na^+/H^+ exchanger with a stoichiometry of 1 mole of phosphate per mole of protein (17, 20, 31). In smooth muscle cells and in the heart, $p90^{rsk}$ also phosphorylates the exchanger's cytosolic domain (20, 26). Furthermore, it appears that the protein kinase p38 may inhibit the Na^+/H^+ exchanger in smooth muscle tissues (17).

It is likely that several protein kinases regulate Na^+/H^+ exchanger activity, even if some of them exert their effects only indirectly (Fig. 1). For example, protein kinase D activity inhibits the Na^+/H^+ exchanger yet does not phosphorylate it directly (15). Similarly, protein kinase C (PKC) regulates the activity of the protein (24) but does not appear to phosphorylate it directly (10, 31).

G protein regulation of the Na^+/H^+ exchanger

While it is known that α_1-adrenergic agonists, endothelin, and angiotensin II can activate the Na^+/H^+ exchanger, the mechanism of this activation is not resolved. These particular agonists are all known to be coupled to G (guanine-nucleotide binding) proteins through G protein-coupled receptors (GPCR) (4). All GPCRs share a common architecture – seven transmembrane helices, with an extracellular domain involved in ligand binding and an intracellular domain involved in the recognition and activation of G proteins (Fig. 2) (32). The G proteins, which are heterotrimers made up of 3 distinct subunits, α, β and γ, transduce ligand binding to the GPCR into an intracellular response. The four main classes of the G proteins are te following: Gs, which acti-

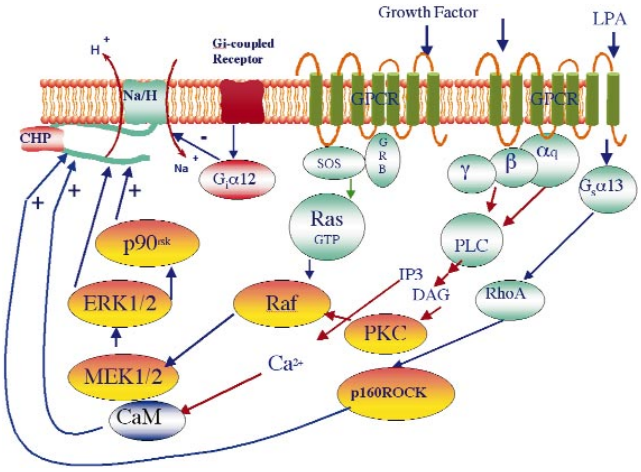

Fig. 2 G protein-coupled pathways in regulation of the Na^+/H^+ exchanger. Hypothetical and established pathways illustrating how G protein-coupled receptors may affect the Na^+/H^+ exchanger. (Some intermediate proteins are omitted for clarity.) *CaM* calmodulin; *CHP* calcineurin homologous protein; *GPCR* G protein-coupled receptor; *LPA* lysophosphatidic acid; *PKC* protein kinase C. One pathway may involve GPCR and heterotrimeric G proteins. The G proteins may activate protein kinase C which activates Raf kinase, or they may increase intracellular calcium levels, thereby affecting calmodulin. Another pathway may involve GPCR and Ras, where Ras may activate Raf kinase which can then activate MEK1/2. RhoA may also activate the Na^+/H^+ exchanger via activation of LPA receptors and via the kinase p160ROCK. Inhibitory receptors might decrease activation of the Na^+/H^+ exchanger and G proteins might affect the cytoplasmic domain of the Na^+/H^+ exchanger via CHP. See text for further details.

vates adenylyl cyclase; Gi, which inhibits adenylyl cyclase; Gq, which activates phospholipase C; and G_{12} and G_{13}, which are of unclear function (14). There has been little study of the role of either these proteins or the GPCR in regulation of myocardial Na^+/H^+ exchanger activity. One recent study has shown that blockage of the GiPCR receptor inhibits α_1-adrenergic activation of the Na^+/H^+ exchanger by phenylephrine (2). Studies in other tissues have shown varying effects (23).

As discussed above, mitogen activated protein kinases (MAPK), specifically ERK1 and 2, and $p90^{rsk}$, phosphorylate the Na^+/H^+ exchanger in the myocardium and in other tissues (20, 25). A summary of the possible steps involved in this phosphorylation, through GPCR, is shown in Fig. 2. Briefly, when the GPCR is activated by agonists, the proteins Grb2 and SOS are recruited. This allows a small GTP-binding protein, Ras, to interact with SOS. Ras is another GTP-binding protein that has GTPase activity. When Ras interacts with SOS it converts from an inactive form which binds GDP to an active form which binds GTP. Ras-GTP recruits Raf kinase, which is then activated through a complex mechanism. Activated Raf kinase phosphorylates MEK1/2, which can then activate ERK1/2 (12). ERK 1/2 may act directly on the Na^+/H^+ exchanger or through $p90^{rsk}$ (20). While ERK1/2 has been shown to phosphorylate the myocardial Na^+/H^+ exchanger (20), the intermediate steps in this pathway have yet to be confirmed.

GPCR and the heterotrimeric G proteins can activate ERK1/2 via another pathway. In this pathway, ligand binding causes the GPCR to interact with a heterotrimeric G protein. GDP bound to the α subunit of the G protein is then released and replaced with GTP. This promotes dissociation of the trimer and allows Gaq to activate phospholipase C. Phospholipase C releases diacylglycerol and phosphatidylinositol trisphosphate (IP_3) from membrane phospholipids. Diacylglycerol can activate protein kinase C, which activates Raf kinase, which activates ERK1/2 as described above (12) (Fig. 2). Alternatively, IP_3 causes intracellular calcium concentrations to increase and this may activate the Na+/H+ exchanger via its interaction with calmodulin. It is not clear which pathway dominates in regulation of the Na^+/H^+ exchanger in the myocardium. However, it has been suggested that during activation of the myocardial exchanger by angiotensin II, it is protein kinase C rather than Ras that plays a critical role in the activation of Raf-1 kinase (39). It has also recently been demonstrated that inhibition of protein kinase C blocks activation of the Na^+/H^+ exchanger by α_1-adrenoreceptor agonists (24).

As noted earlier, one sub-domain within the cytoplasmic region of the Na^+/H^+ exchanger, amino acids 567 to 637, contains a binding site for calcineurin homologous protein (CHP) (18). This protein is homologous to both calmodulin and calcineurin. Over-expression of CHP inhibits serum-mediated and GTPase-mediated stimulation of Na^+/H^+ exchanger activity. Normally, CHP may bind to the cytoplasmic domain of the exchanger and inhibit its activity until it is released in response to cellular stimulation by growth factors. CHP is present in the myocardium but has not been studied there (18).

Recent work has suggested that p160ROCK (the Rho-associated ser/thr protein kinase required for stress fiber and focal adhesion formation) may also play a role in regulation of the Na^+/H^+ exchanger (28). It was suggested that p160ROCK might activate NHE1 via lysophospholipids and RhoA. Indeed, activation of p160ROCK stimulates the GTPase RhoA and expression of the p160ROCK inhibitor Y-27632 blocks RhoA activation of the Na^+/H^+ exchanger. p160ROCK also phosphorylates the C-terminal domain of the Na^+/H^+ exchanger, but the effects of this on regulation of the myocardial Na^+/H^+ exchanger have not yet been studied.

It must be noted that angiotensin II, endothelin and the α_1-adrenergic receptor have all been implicated in the development of cardiac hypertrophy (38). The role of MAP kinases and G proteins in this pathway is still under investigation: however, it is clear that the activation of MAPK cascades can induce a hypertrophic response (4). It has already been suggested that the Na^+/H^+ exchanger is involved in the molecular mechanisms of cardiac hypertrophy (27) and, recently, an exciting study has

shown that the early adaptive hypertrophic response of myocytes is dependent on the Na^+/H^+ exchanger. Specifically, blockage of the Na^+/H^+ exchanger attenuated hypertrophy in the myocardium of rats subjected to coronary artery ligation (37).

Regulation of expression of the Na^+/H^+ exchanger

We have examined transcriptional regulation of the NHE1 gene in primary cultures of isolated cardiomyocytes. By transfecting cardiomyocytes with the NHE1 promoter (directing the luciferase reporter gene) we showed that a proximal region of the gene with an AP-2 binding site is involved in regulating myocardial expression of the protein (33). Other regions of the gene have not been examined in detail in the myocardium. However, it is known that both a novel poly (ddA:dAT) region of the gene (34) and the transcription factor COUP (7) are important in regulating its expression. Also, serum stimulates expression of the NHE1 gene in the myocardium, and this is likely mediated via growth factors (33).

Levels of mRNA for the Na^+/H^+ exchanger, and of the protein itself, vary during development and in response to environmental stimuli. It has been demonstrated that amounts of NHE1 message are greater in newborn heart than in adult (3, 16). We examined the expression and activity of the Na^+/H^+ exchanger following ischemia and reperfusion of the myocardium. We found that in isolated perfused hearts low-flow ischemia elevates NHE1 message levels. In addition, we demonstrated that acidosis in isolated cardiomyocytes increases NHE1 activity (5, 6). Subsequent studies have confirmed this observation (13). It has also recently been shown that sarcolemmal NHE activity is significantly greater in recipient hearts with chronic end-stage heart failure than it is in donor hearts (36).

Overall, it is clear that levels of the Na^+/H^+ exchanger vary in response to a number of environmental stimuli. It is unfortunate that ischemia, acidosis, heart failure and hypertrophy might all increase levels of expression, since activity of the Na^+/H^+ exchanger is detrimental to the myocardium during ischemia followed by reperfusion. Future experiments are required to explore the possibility that hearts with elevated NHE1 levels are more susceptible to injury in a variety of pathophysiological circumstances.

Conclusion and future perspectives

While a great deal of information about the Na^+/H^+ exchanger and its expression has been gathered, there are still many things that are not understood. We have little detailed knowledge regarding regulation of the myocardial exchanger. It is also unclear how expression of the protein is regulated in response to ischemia, and how alterations in its expression affect its role in health and disease. Our ultimate goal is a more complete picture of these elements, which will lead to better understanding and manipulation of the protein in the healthy and diseased myocardium.

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