Na+/Ca++ Exchanger and Myocardial Ischemia/Reperfusion

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SUMMARY

Myocardial hypoxia and ischemia are characterized by the depletion of ATP and the development of intracellular acidosis, which alter cellular ionic homeostasis. Specifically, elevated cytosolic free Ca++ concentrations cause cellular injury during hypoxia/ischemia and lead to irreversible myocardial damage during reoxygenation/reperfusion. An increase in the intracellular Na+ concentration has been shown to correlate with Ca++ overload. Although inhibition of Na+/K+ exchange because of decreased ATP production may be involved, it is more likely that intracellular acidosis drives Na+ into the cells via Na+/H+ exchange. Experimental evidence supports the notion that Na+/H+ exchange is primarily responsible for Na+ influx during hypoxia/ischemia. The accumulation of intracellular Na+ may then activate the Na+/Ca++ exchanger causing Ca++ overload. Therefore, the Na+/Ca++ exchanger plays a crucial role in cellular injury during hypoxia/ischemia and in cell death during reoxygenation/reperfusion. In the past few years, the Na+/Ca++ exchanger has been cloned and the structure/function relationship studied intensively. Agents which inhibit the Na+/Ca++ exchanger may have therapeutic potential for the treatment of ischemic heart disease. These advances will greatly accelerate the understanding of the cellular and molecular mechanisms underlying the role of the Na+/Ca++ exchanger in the development of myocardial damage during hypoxia/ischemia and reoxygenation/reperfusion. (Jpn Heart J 1998; 39: 707–714)

Key words: Na+/Ca++ exchange, Ischemia/reperfusion, Anoxia/reoxygenation, Na+/H+ exchange, Myocyte, Adenosine triphosphate

The cytoplasmic-free Ca++ concentration plays a crucial second-messenger function in the regulation of excitation-contraction coupling. The Na+/Ca++ exchanger is expressed at high levels in the cardiac sarcolemma and regulates intracellular Ca++ concentrations by transporting Ca++ out of the cardiac myocyte. The stoichiometry for Na+/Ca++ exchange across the sarcolemma is three Na+ molecules for one Ca++ molecule. Therefore, the exchanger is electro-
genic and voltage-sensitive, transporting Ca\(^{++}\) either in or out of the cytosol, depending on the electrochemical Na\(^{+}\) gradient.

Under physiologic conditions, the cardiac Na\(^{+}/Ca\(^{++}\) exchanger exchanges intracellular Ca\(^{++}\) ions for extracellular Na\(^{+}\) ions and restores the low intracellular Ca\(^{++}\) concentration present during diastole. However, changes in the intracellular and extracellular environment, such as local increases in the intracellular Na\(^{+}\) concentration in the subsarcolemmal space, may activate the exchanger to transport Ca\(^{++}\) into myocytes.\(^1\) The role of the Na\(^{+}/Ca\(^{++}\) exchanger in the development of myocardial damage during hypoxia/ischemia and reoxygenation/reperfusion has been the subject of intense investigation. With the cloning of the Na\(^{+}/Ca\(^{++}\) exchanger,\(^2\) studies have been performed to understand the regulation of the exchanger and its role at the molecular level in physiologic and pathophysiologic processes.

**Molecular Structure and Regulation of the Na\(^{+}/Ca\(^{++}\) Exchanger**

The Na\(^{+}/Ca\(^{++}\) exchanger is a member of the cation exchanger superfamily. The cardiac sarcolemmal Na\(^{+}/Ca\(^{++}\) exchanger was cloned and characterized in 1990.\(^1\) Since then, several splicing isoforms have been cloned from different species and tissues. It has become clear that the isoforms of the protein are the products of two genes. One of these genes produces eight isoforms which differ both in the 5\(^{\prime}\) untranslated region and within the large putative cytoplasmic loop of the protein. The open reading frames of the cloned human heart Na\(^{+}/Ca\(^{++}\) exchanger code for a protein consisting of 973 amino acids.\(^4\) The proposed topology of the exchanger, based on the hydropathy plot, suggests that the protein can be divided into three regions: a hydrophobic amino-terminal portion containing five potential membrane-spanning segments, a long hydrophilic region, and a hydrophobic carboxy-terminal portion containing six potential membrane-spanning segments. There are potential glycosylation sites in the extracellular domain as well as in the putative cytoplasmic loops. The transmembrane segments form an ion translocation channel and the regulatory sites for the protein are located in the large intracellular loop.

The Na\(^{+}/Ca\(^{++}\) exchanger transports Ca\(^{++}\) but also is regulated by the Ca\(^{++}\) concentration by a high affinity Ca\(^{++}\) binding site that is separate from the Ca\(^{++}\) transport site.\(^5,6\) Earlier electrophysiologic studies using inside-out membrane patches have shown that the addition of Na\(^{+}\) to the cytoplasmic side of the exchanger initiates the exchange of Na\(^{+}\) for Ca\(^{++}\). This initiation requires the presence of a trace amount of Ca\(^{++}\) that is not transported. Further studies have demonstrated that regulation of the exchanger by Ca\(^{++}\) can be eliminated if the large intracellular loop of the exchanger is deleted. However, deletion of this
intracellular domain does not decrease Ca\(^{2+}\) translocation. \(^{45}\)Ca\(^{2+}\) overlay techniques show the smallest peptide fragment that binds Ca\(^{2+}\) contains the amino acids 371–525 of the exchanger.\(^7\)-\(^9\) This region has two highly acidic sequences, each containing three consecutive aspartic acid residues. The Ca\(^{2+}\) affinity of the regulatory site markedly decreases if these aspartates are mutated.\(^10\) Mutations at these sites cause altered Ca\(^{2+}\) regulation of the exchanger resulting in complex changes in enzyme kinetics.

In addition to regulation by Ca\(^{2+}\), the Na\(^{+}/Ca\(^{2+}\) exchanger exhibits intrinsic regulation by intracellular Na\(^{+}\). Upon application of Na\(^{+}\) to the cytoplasmic surface, three Na\(^{+}\) ions bind at the intracellular transport sites. The exchanger then either translocates Na\(^{+}\) across the plasma membrane to the external surface or enters an inactivated state. The Na\(^{+}\)-dependent inactivation is mediated by a region of the exchanger with the same amino acid sequence as the exchanger inhibitory peptide.\(^11\) This region is located at the amino end of the large intracellular loop. The deletion of the first four amino acids of this loop is sufficient to abolish Na\(^{+}\)-dependent inactivation without affecting Ca\(^{2+}\) regulation.\(^7\)-\(^9\)

Adenosine triphosphate (ATP) has been found to play a role in the regulation of the Na\(^{+}/Ca\(^{2+}\) exchanger. In rat cardiac myocytes, ATP depletion of Na\(^{+}\)-loaded myocytes results in a strong inhibition of the Na\(^{+}/Ca\(^{2+}\) exchanger, as evidenced by inhibition of intracellular Na\(^{+}\)-dependent Ca\(^{2+}\) uptake.\(^12,13\) It has been shown that the half maximal rate of Ca\(^{2+}\) uptake occurs at an intracellular ATP content of 1.96 nmol/mg, which is about 10% of the normal ATP content. In addition, depletion of intracellular ATP reduces the degree of activation of the exchanger by Ca\(^{2+}\). However, it is not clear how the exchanger senses changes in the intracellular ATP concentration.

**Na\(^{+}/Ca\(^{2+}\) EXCHANGER AND CARDIAC EXCITATION-CONTRACTION COUPLING**

The resting intracellular free Ca\(^{2+}\) concentration is approximately 10,000 times lower than the extracellular Ca\(^{2+}\) concentration. In addition, the interior of the cell is negatively charged relative to the extracellular space. Therefore, the electrochemical gradient favors Ca\(^{2+}\) movement into the cell. Upon depolarization, extracellular Ca\(^{2+}\) enters the cardiac myocyte through voltage-gated Ca\(^{2+}\) channels, which triggers the release of Ca\(^{2+}\) from the sarcoplasmic reticulum via activation of the Ca\(^{2+}\) release channel (ryanodine receptors). The cardiac myocyte contracts in response to the increase in intracellular Ca\(^{2+}\).

During relaxation, reuptake of Ca\(^{2+}\) from the sarcoplasm is mediated by the sarcoplasmic reticulum Ca\(^{2+}\)-ATPase. In addition, in order to restore the resting intracellular Ca\(^{2+}\) concentration, Ca\(^{2+}\) which entered the cell from the extracellular space during excitation, is transported from the myocyte by the Na\(^{+}/Ca\(^{2+}\)
exchanger and the sarcolemmal Ca\(^{++}\)-ATPase. About 15\% of the intracellular Ca\(^{++}\) that is pumped out of the cell is removed by the Na\(^{+}/Ca\(^{++}\) exchanger, and the remainder of the cytoplasmic Ca\(^{++}\) is transported by the Ca\(^{++}\) ATPase into the sarcoplasmic reticulum.

The Na\(^{+}/Ca\(^{++}\) exchanger may be responsible for Ca\(^{++}\) entry into cardiac myocytes during the action potential. Based on theoretical thermodynamic calculations, the Na\(^{+}/Ca\(^{++}\) exchanger can mediate Ca\(^{++}\) influx when the reversal potential is exceeded during the early portion of the action potential.\(^{14}\) Experimental results suggest that at a normal intracellular Na\(^{+}\) concentration the contribution of the exchanger to Ca\(^{++}\) influx is minimal (less than 1\%). However, as the intracellular Na\(^{+}\) concentration increases, the contribution of the exchanger to total Ca\(^{++}\) influx increases.\(^{15}\)

**THE Na\(^{+}/Ca\(^{++}\) EXCHANGER AND MYOCARDIAL ISCHEMIA/HYPOXIA**

Myocardial hypoxia and ischemia are characterized by a rapid decrease in contractile force, depletion of ATP, intracellular acidosis, and, in the case of ischemia, the accumulation of metabolites.\(^{16}\) It is well known that the ionic homeostasis of cardiac myocytes is altered by ATP depletion and intracellular acidosis. Further, changes in the intracellular ionic homeostasis play a significant role in cellular injury. In particular, the cytosolic Ca\(^{++}\) concentration in cardiac myocytes increases in response to ATP depletion and intracellular acidosis caused by hypoxia or ischemia.\(^{17}\) As a result, cytosolic Ca\(^{++}\) overload causes cellular injury.\(^{18,19}\)

Several mechanisms that are involved in regulating Ca\(^{++}\) homeostasis have been shown to contribute little to the accumulation of cytosolic Ca\(^{++}\) in the setting of ischemia. Conventional L-type channel blockers, such as nifedipine, have no effect on the increase in cytosolic Ca\(^{++}\) in rat ventricular myocytes exposed to substrate-free anoxia. Treatment with agents that inhibit Ca\(^{++}\) reuptake by the sarcoplasmic reticulum, such as caffeine and thapsigargin, does not alter the magnitude or time course of Ca\(^{++}\) overload.\(^{20}\) In addition, removal of external Ca\(^{++}\) reduces the increase in cytosolic Ca\(^{++}\) in response to hypoxia, suggesting that sarcolemmal Ca\(^{++}\) influx, rather than efflux, accounts for Ca\(^{++}\) overload.\(^{11,20}\)

An increase in the intracellular Na\(^{+}\) concentration during hypoxia/ischemia\(^{21}\) has been documented by the direct measurement of the intracellular Na\(^{+}\) concentration and is believed to mediate the increase in the cytosolic free Ca\(^{++}\) concentration via activation of the Na\(^{+}/Ca\(^{++}\) exchanger. One possible mechanism underlying the accumulation of intracellular Na\(^{+}\) is the inhibition of Na\(^{+}/K^{+}\) ATPase due to diminished ATP production. However, this is unlikely to be the primary mechanism because the time course for ATP depletion lags be-
hind that of intracellular Na⁺ accumulation. Furthermore, there is no correlation between ATP content and intracellular Na⁺ concentration.

Na⁺/H⁺ exchange represents a major mechanism for the regulation of intracellular pH after an acid load by virtue of the ability of the Na⁺/H⁺ exchanger to extrude H⁺ in exchange for Na⁺. Although the Na⁺/H⁺ exchanger can be activated through various mechanisms including phosphorylation, the greatest activation occurs with the development of a transmembrane H⁺ gradient in association with intracellular acidosis. During hypoxia/ischemia, the production of lactate through anaerobic glycolysis and hydrolysis of ATP results in intracellular acidosis. Acidosis decreases the sensitivity of myofilaments to cytosolic free Ca²⁺ through changes in the binding of Ca²⁺ to the troponin complex. Higher cytosolic free Ca²⁺ concentrations are therefore required to produce the same amount of force in the setting of ischemia/hypoxia. The decrease in force generation may protect cardiac muscle from ATP depletion. However, a decrease in the intracellular pH drives Na⁺ into the cells through Na⁺/H⁺ exchange. The Na⁺/H⁺ exchanger compensates for intracellular acidosis by pumping protons from the cell, but at the expense of decreasing the normal Na⁺ gradient. Experiments using Na⁺/H⁺ exchange inhibitors support the hypothesis that Na⁺/H⁺ exchange is the main pathway for Na⁺ influx during hypoxia/ischemia. Further, inhibition of Na⁺/H⁺ exchange by amiloride significantly reduces the accumulation of intracellular Na⁺.

There are several lines of evidence suggesting that the increase in cytosolic Ca²⁺ is coupled to the rise in cytosolic Na⁺ during myocardial hypoxia/ischemia. First, in some studies the increase in cytosolic Na⁺ clearly precedes the rise in cytosolic Ca²⁺. However, in other studies, the time course for the increase in cytosolic Na⁺ is similar to the time course for the rise in the Ca²⁺ concentration. These observations suggest that the increase in the cytosolic Na⁺ concentration can stimulate Na⁺-Ca⁺⁺ exchange and thereby produce an increase in cytosolic Ca⁺⁺ during hypoxia/ischemia. Second, removal of extracellular Na⁺ attenuates Ca⁺⁺ overload during hypoxia. Finally, the presence of amiloride or nickel during hypoxia/ischemia attenuates the rise in cytosolic Ca⁺⁺ and Na⁺.

Several studies have suggested that Na⁺/Ca⁺⁺ exchange may decrease during hypoxia and metabolic inhibition. These studies have demonstrated that both forward and reverse Na⁺/Ca⁺⁺ exchange and the processes leading to release of Ca⁺⁺ from the sarcoplasmic reticulum are inhibited by hypoxia and metabolic inhibition. These changes occur because Na⁺/Ca⁺⁺ exchanger activity requires ATP and is inhibited by intracellular acidosis. Rapid cellular acidification in the setting of ischemia may cause Ca⁺⁺ influx through coupling of Na⁺/H⁺ and Na⁺/Ca⁺⁺ exchange. In contrast, the decrease in ATP does not significantly affect the activity of the exchanger because the half maximal rate of Ca⁺⁺ uptake...
occurs at an ATP content that is about 10% of the normal cell ATP level.

**The Na⁺/Ca²⁺ Exchanger and Reperfusion/Reoxygenation**

Successful reoxygenation/reperfusion of reversibly injured myocytes is associated with partial or complete restoration of metabolic processes. However, it has been hypothesized that some cardiac myocytes that are injured during hypoxia/ischemia are still viable immediately before reoxygenation/reperfusion, but are killed by deleterious factors present during reoxygenation/reperfusion. Proposed mechanisms for reperfusion injury include direct myocyte injury caused by free radicals or rapid cellular swelling.²⁹ However, the intracellular accumulation of Ca²⁺ is the most crucial factor responsible for irreversible myocardial injury. Specifically, Ca²⁺ overload impairs mitochondrial respiration, activates endogenous proteases and phospholipases, and initiates cell contracture.³⁰,³¹

The role of the Na⁺/Ca²⁺ exchanger in the development of Ca²⁺ overload during reoxygenation/reperfusion has been studied in various models. In rat hearts, intracellular Na⁺ increases continuously during ischemia and increases further during the first 2 minutes of reperfusion. Amiloride and preischemic glycogen depletion significantly reduce Na⁺ accumulation during reperfusion, suggesting that the uptake occurs through Na⁺/H⁺ exchange. The cytosolic Na⁺ concentration at the end of ischemia and after 2 minutes of reperfusion are correlated with Ca²⁺ uptake, suggesting that Ca²⁺ uptake during reperfusion occurs by Na⁺/Ca²⁺ exchange.³² In perfused rat hearts, amiloride prevents Ca²⁺ overload during reoxygenation.³³ Kawada et al.³⁴ have reported that dichlorobenzamil, an inhibitor of the Na⁺/Ca²⁺ exchanger, significantly attenuates Ca²⁺ accumulation. These studies have demonstrated that Na⁺/Ca²⁺ exchange is at least partially responsible for reperfusion-induced Ca²⁺ overload.

The mechanism responsible for augmented Na⁺/Ca²⁺ exchange during reperfusion is not well understood. It is possible that cytoplasmic acidification and Na⁺ accumulation are not reversed during prolonged hypoxia followed by reperfusion. At the same time, Na⁺/Ca²⁺ exchange is inhibited or attenuated by ATP depletion. As a result, Ca²⁺ accumulation slows or stops. Upon reoxygenation/reperfusion, renewed production of ATP allows Na⁺/Ca²⁺ exchanger activity to return to normal.

**Therapeutic Implications of Na⁺/Ca²⁺ Exchanger Regulators**

The role of the Na⁺/Ca²⁺ exchanger in the development of Ca²⁺ overload during hypoxia/ischemia and reoxygenation/reperfusion provides a point for therapeutic intervention. Direct inhibition of the Na⁺/Ca²⁺ exchanger or reduc-
tion of Na⁺/H⁺ exchanger activity and cytosolic Na⁺ concentration have been shown to protect the myocardium from reperfusion-induced cellular injury. Amiloride delays or reduces the ischemia-induced rise in the cytosolic Ca²⁺ concentration and improves the recovery of mechanical function. Other studies have demonstrated that amiloride reduces the incidence of reperfusion-induced ventricular arrhythmias.

REFERENCES


