Na\(^+\)/Ca\(^{2+}\) Exchange Inhibitors: Potential Drugs to Mitigate the Severity of Ischemic Injury

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Each year more than a million Americans suffer heart attacks and strokes. Most of these events result from the sudden thrombotic occlusion of a coronary or cerebral artery at the site of an atherosclerotic plaque (Corti et al., 2002). Tissue solely supplied by the occluded artery becomes hypoxic/anoxic. Oxygenation of surrounding tissue with collateral blood supply may also be compromised. At the cellular level, acute hypoxia/anoxia induces a switch from oxidative metabolism to glycolysis. This diminishes the energy supply in the affected cells and increases acid production, initiating processes leading to cell injury or death. A major goal of medicine today is to prevent the initial thrombotic event or, failing that, to minimize the resulting tissue damage. There are two aspects to minimizing tissue damage. One is to reduce the metabolic effects of ischemia on the tissue surrounding the anoxic region (that is, to reduce infarct size by preserving peripheral tissue). The second is to reperfuse the occluded vessel by thrombolysis or angioplasty to prevent anoxic cell death (Corti et al., 2002). During reperfusion, however, formation of reactive oxygen species in the ischemic tissue can also cause cellular damage (Li and Jackson, 2002; Zeitz et al., 2002). Thus, understanding the physiological basis of ischemic- and reperfusion-induced cell injury and death is critical to identifying new therapeutic modalities. The elevation of intracellular calcium after ischemia and reperfusion is a major mediator of subsequent cellular injury and death (Banasiak et al., 2000; Orrenius et al., 2003). The Na\(^+\)/Ca\(^{2+}\) exchanger (NCX) plays a central role in elevating intracellular calcium during ischemia and reperfusion in cardiac, neural and renal tissue (Stys et al., 1992; Blaustein and Lederer, 1999; Tomes and Agrawal, 2002; Zeitz et al., 2002; Yamashita et al., 2003; Craner et al., 2004). As such, the NCX is a promising new target for drugs to reduce hypoxic cell injury (Mochizuki and Jiang, 1998; Shigekawa and Iwamoto, 2001). In this issue of Molecular Pharmacology, Iwamoto et al. (2004) report the initial characterization of a new Na\(^+\)/Ca\(^{2+}\) exchange inhibitor, SN-6. They report the tantalizing result that when administered at the time of reoxygenation (in a cell culture model, at least), SN-6 reduces subsequent cell injury/death.

The human genome contains three genes encoding Na\(^+\)/Ca\(^{2+}\) exchangers (NCX1, NCX2, NCX3) that undergo extensive alternative splicing (Philipson et al., 2002). Expression of these three NCX isoforms is tissue-specific. NCX1 is highly expressed in the heart, brain, and kidney and at lower levels in most other tissues. NCX2 and NCX3 are both almost exclusively expressed in brain and skeletal muscle (Blaustein and Lederer, 1999; Gibney et al., 2002; Philipson et al., 2002). An NCX1 knockout is embryonic lethal, whereas an NCX2 knockout increases hippocampal long-term potentiation and improves performance on learning and memory tests (Reuter et al., 2002b; Jeon et al., 2003). The NCX family is part of a larger gene superfamily that also includes K\(^+\)-dependent Na\(^+\)/Ca\(^{2+}\) exchangers, bacterial Na\(^+\)/Ca\(^{2+}\) exchangers, and bacterial Ca\(^{2+}\)/K\(^+\) exchangers (Blaustein and Lederer, 1999; Philipson et al., 2002).

The NCX ion translocation pathway is formed by nine transmembrane (TM) segments and two reentrant loops, referred to as \(\alpha\) repeats (Fig. 1A) (Philipson et al., 2002). The two \(\alpha\) repeats seem to be nearby in the folded protein structure because cysteines engineered into the TM segments flanking the N-terminal repeat form disulfide bonds with cysteines engineered into the TMs flanking the C-terminal \(\alpha\) repeat (Qiu et al., 2001).

Inactivation processes decrease the NCX transport rate.

ABBREVIATIONS: NCX, Na\(^+\)/Ca\(^{2+}\) exchanger; TM, transmembrane; PIP\(_2\), phosphatidylinositol 4,5-bisphosphate; XIP, exchange inhibitory peptide.
much like inactivation or desensitization in ion channels. NCX inactivation is regulated by intracellular Na\(^{+}\) and Ca\(^{2+}\) levels and by the phosphatidylinositol 4,5-bisphosphate (PIP\(_2\)) content in the cytoplasmic plasma membrane leaflet (He et al., 2000; Philipson and Nicoll, 2000). These ions and lipids interact with regions in the large cytoplasmic loop connecting TM5 and TM6 that contains the exchange inhibitory peptide (XIP) and Ca\(^{2+}\)-binding domains (Fig. 1A). A 20-amino acid peptide encoding the XIP sequence inhibits Na\(^{+}/Ca^{2+}\) exchange, and mutations in the XIP domain strongly influence the rate of onset and the extent of inactivation (Li et al., 1991; He et al., 2000). Elevated intracellular Na\(^{+}\) increases inactivation through interactions involving the XIP domain (Philipson and Nicoll, 2000). PIP\(_2\) binds to the XIP region, reducing inactivation (He et al., 2000). Cardiac myocytes can regulate the membrane PIP\(_2\) content. Thus, modulation of PIP\(_2\) concentration may be a mechanism for regulation of NCX activity and cardiac cell excitability and contractility (Nasuhoglu et al., 2002; Hilgemann, 2003).

The NCX transporter works in a bidirectional manner. Four factors determine the direction of net transport: the transport cycle stoichiometry, the transmembrane Na\(^{+}\) concentration gradient, the transmembrane Ca\(^{2+}\) concentration gradient, and the transmembrane electrical potential. The predominant transport mode exchanges three Na\(^{+}\) ions for one Ca\(^{2+}\); minor transport modes with different stoichiometries, however, do occur (Hilgemann et al., 1991; Blaustein and Lederer, 1999; Kang and Hilgemann, 2004). During the normal cardiac action potential, NCX mainly runs in the “forward” direction, transporting Na\(^{+}\) into the cell, down its electrochemical gradient, while transporting Ca\(^{2+}\) out of the cell, up its electrochemical gradient (Fig. 1B). Thus, NCX helps to clear Ca\(^{2+}\) from the cytoplasm. This facilitates diastolic relaxation in cardiac myocytes or the cessation of Ca\(^{2+}\) signaling in neurons and other cells. NCX, however, can function equally well in “reverse” mode if the electrochemical gradient for transport is reversed (Fig. 1C).

The pathological role of NCX in hypoxic cell injury is caused by Ca\(^{2+}\) influx via “reverse” mode transport (Stys et al., 1992; Cross et al., 1998; Imahashi et al., 1999; Banasiak et al., 2000). During hypoxia, the intracellular Na\(^{+}\) concentration rises because of increased influx and decreased efflux. Na\(^{+}\) influx increases because H\(^{+}\) generation by glycolysis activates the Na\(^{+}/H^{+}\) exchanger and also possibly because of activation of voltage-dependent Na\(^{+}\) channels (Stys et al., 1992; Imahashi et al., 1999; Craner et al., 2004). Furthermore, reduced ATP generation slows Na\(^{+}\) efflux via the Na\(^{+}/K^{+}\)-ATPase. In addition, the cell membrane potential polarizes during hypoxia because of a combination of direct effects on K\(^+\) channels and rundown of the transmembrane K\(^+\) gradient (Haddad and Liu, 2000; Baczko et al., 2003). These forces combine to reverse the driving force on the Na\(^{+}/Ca^{2+}\) exchanger, inducing “reverse” mode transport that brings Ca\(^{2+}\) into the cell. This raises the intracellular Ca\(^{2+}\) concentration and initiates cellular injury.

Experiments with transgenic mice support the hypothesis that inhibition of NCX may protect from hypoxic injury. Although homozygous NCX1 knockouts are embryonic lethal, NCX1-knockout heterozygotes are viable. They have reduced NCX protein levels, a smaller rise in intracellular calcium after hypoxia and reduced susceptibility to ischemic injury (Yamashita et al., 2003; Ohtsuka et al., 2004).

Because “reverse” mode transport is a critical step in ischemia-induced cytoplasmic calcium elevation, inhibition of NCX, particularly inhibition of “reverse” mode transport, should protect against ischemic injury. In theory, NCX inhibitors administered shortly after the onset of a heart attack or stroke might reduce infarct size by limiting damage to endangered tissue at the periphery of the anoxic region. Whether NCX inhibitors will also be able to reduce reperfusion injury depends on whether the Ca\(^{2+}\) entry occurs during the ischemia or after reperfusion. As a cautionary note, in human clinical trials of Na\(^{+}/H^{+}\) exchange inhibitors, they were only effective at reducing ischemic damage if they were administered before the onset of ischemia. No therapeutic benefit was observed when they were administered at the time of reperfusion (Avirkar and Marber, 2002). For NCX inhibitors, at least in tissue culture systems and in animal models, the extent of ischemic injury is diminished whether they are administered before the ischemic episode or at the time of reoxygenation (Ladilov et al., 1999; Elias et al., 2001; Matsuda et al., 2001; Matsumoto et al., 2002; Tomes and Agrawal, 2002; Baczko et al., 2003; Magee et al., 2003). Whether clinical trials in humans will demonstrate a similar therapeutic effect remains an intriguing and exciting possibility.

So far, only two specific NCX inhibitors, KB-R7943 and SEA0400, have been available. In this issue, Iwamoto and colleagues describe the characterization of a new NCX inhibitor, SN-6, a derivative of KB-R7943. As reported recently for SEA0400 (Bouchard et al., 2004), SN-6 seems to act by accelerating Na\(^{+}\)-dependent inactivation, thereby preferentially inhibiting the “reverse” mode transport that will occur.
References


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