0026-895X/04/6601-8–10$20.00
MOLECULAR PHARMACOLOGY
Copyright © 2004 The American Society for Pharmacology and Experimental Therapeutics
Mol Pharmacol 66:8–10, 2004

Vol. 66, No. 1
232/1156921
Printed in U.S.A.

PERSPECTIVE

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

Myles H. Akabas

Departments of Physiology & Biophysics and Neuroscience, Albert Einstein College of Medicine of Yeshiva University, Bronx, New York

Received March 8, 2004; accepted March 23, 2004

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+}/H^+ 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 α repeats (Fig. 1A) (Philipson et al., 2002). The two α 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 α repeat (Qiu et al., 2001).

Inactivation processes decrease the NCX transport rate

Article, publication date, and citation information can be found at http://molpharm.aspet.org.
DOI: 10.1124/mol.104.000232

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).

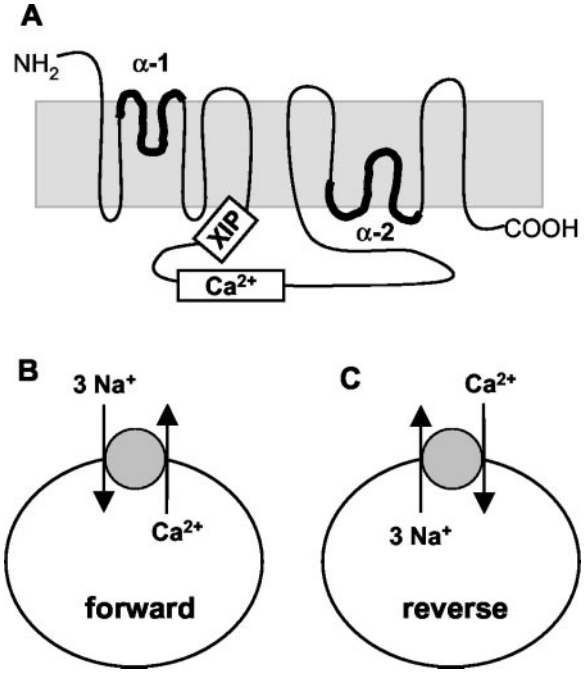

Fig. 1. Diagrams illustrating the proposed NCX TM topology (A) and the forward (B) and reverse (C) transport modes of the exchanger. A, the membrane is indicated by the light gray rectangle. The α repeat reentrant loops are indicated by the thick black line. The regulatory XIP and Ca^{2+}-binding domains are indicated by boxes in the cytoplasmic loop between TM5 and TM6. B, forward mode transport. NCX is represented as the gray circle. The large circle represents the cell membrane. C, reverse mode transport that brings Ca^{2+} into the cell after hypoxic injury.

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 depolarizes 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 (Avkiran 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

with elevated intracellular Na^+ during hypoxia. SN-6 is reportedly specific for NCXs. The extent to which it is specific for NCX remains uncertain because both KB-R7943 and SEA0400 displayed similar specificity on an initial screen of receptors and channels (Iwamoto et al., 1996; Matsuda et al., 2001); however, both drugs depressed Ca^{2+} transients in myotubes from NCX1 knockout mice, suggesting that they are not completely specific for NCX (Reuter et al., 2002a). This may be an important caveat for experimental uses of these drugs but may not affect their therapeutic potential in humans. In an initial experiment, SN-6 applied either at the start of hypoxia or at the start of reoxygenation reduced cell injury in a renal cell culture hypoxic injury model. Given these promising initial results, SN-6 joins a small but growing class of experimental Na^+/Ca^{2+} exchange inhibitors with major therapeutic potential to mitigate the tissue damage that results from acute ischemic events such as heart attacks and strokes.

References

Avkiran M and Marber MS (2002) Na^+/H^+ exchange inhibitors for cardioprotective therapy: progress, problems and prospects. *J Am Coll Cardiol* **39:**747–753.

Baczko I, Giles WR, and Light PE (2003) Resting membrane potential regulates Na^+-Ca^{2+} exchange-mediated Ca^{2+} overload during hypoxia-reoxygenation in rat ventricular myocytes. *J Physiol* **550:**889–898.

Banasiak KJ, Xia Y, and Haddad GG (2000) Mechanisms underlying hypoxia-induced neuronal apoptosis. *Prog Neurobiol* **62:**215–249.

Blaustein MP and Lederer WJ (1999) Sodium/calcium exchange: its physiological implications. *Physiol Rev* **79:**763–854.

Bouchard R, Omelchenko A, Le HD, Choptiany P, Matsuda T, Baba A, Takahashi K, Nicoll DA, Philipson KD, Hnatowich M, et al. (2004) Effects of SEA0400 on mutant NCX1.1 Na^+-Ca^{2+} exchangers with altered ionic regulation. *Mol Pharmacol* **65:** 802–810.

Corti R, Farkouh ME, and Badimon JJ (2002) The vulnerable plaque and acute coronary syndromes. *Am J Med* **113:**668–680.

Craner MJ, Hains BC, Lo AC, Black JA, and Waxman SG (2004) Co-localization of sodium channel Nav1.6 and the sodium-calcium exchanger at sites of axonal injury in the spinal cord in EAE. *Brain* **127:**294–303.

Cross HR, Lu L, Steenbergen C, Philipson KD, and Murphy E (1998) Overexpression of the cardiac Na^+/Ca^{2+} exchanger increases susceptibility to ischemia/reperfusion injury in male, but not female, transgenic mice. *Circ Res* **83:**1215–1223.

Elias CL, Lukas A, Shurraw S, Scott J, Omelchenko A, Gross GJ, Hnatowich M, and Hryshko LV (2001) Inhibition of Na^+/Ca^{2+} exchange by KB-R7943: transport mode selectivity and antiarrhythmic consequences. *Am J Physiol* **281:**H1334–H1345.

Gibney GT, Zhang JH, Douglas RM, Haddad GG, and Xia Y (2002) Na^+/Ca^{2+} exchanger expression in the developing rat cortex. *Neuroscience* **112:**65–73.

Haddad GG and Liu H (2000) Different O_2-sensing mechanisms by different K^+ channels. *Adv Exp Med Biol* **475:**441–452.

He Z, Feng S, Tong Q, Hilgemann DW, and Philipson KD (2000) Interaction of PIP_2 with the XIP region of the cardiac Na/Ca exchanger. *Am J Physiol* **278:**C661–C666.

Hilgemann DW (2003) Getting ready for the decade of the lipids. *Annu Rev Physiol* **65:**697–700.

Hilgemann DW, Nicoll DA, and Philipson KD (1991) Charge movement during Na^+ translocation by native and cloned cardiac Na^+/Ca^{2+} exchanger. *Nature (Lond)* **352:**715–718.

Imahashi K, Kusuoka H, Hashimoto K, Yoshioka J, Yamaguchi H, and Nishimura T (1999) Intracellular sodium accumulation during ischemia as the substrate for reperfusion injury. *Circ Res* **84:**1401–1406.

Iwamoto T, Inoue Y, Ito K, Sakaue T, Kita S, and Katsuragi T (2004) The XIP region-dependent inhibition of Na^+/Ca^{2+} exchange (NCX1) by SN-6, a novel benzyloxyphenyl derivative. *Mol Pharmacol* **66:**45–55.

Iwamoto T, Watano T, and Shigekawa M (1996) A novel isothiourea derivative selectively inhibits the reverse mode of Na^+/Ca^{2+} exchange in cells expressing NCX1. *J Biol Chem* **271:**22391–22397.

Jeon D, Yang YM, Jeong MJ, Philipson KD, Rhim H, and Shin HS (2003) Enhanced learning and memory in mice lacking Na^+/Ca^{2+} exchanger 2. *Neuron* **38:**965–976.

Kang TM and Hilgemann DW (2004) Multiple transport modes of the cardiac Na^+/Ca^{2+} exchanger. *Nature (Lond)* **427:**544–548.

Ladilov Y, Haffner S, Balser-Schafer C, Maxeiner H, and Piper HM (1999) Cardioprotective effects of KB-R7943: a novel inhibitor of the reverse mode of Na^+/Ca^{2+} exchanger. *Am J Physiol* **276:**H1868–H1876.

Li C and Jackson RM (2002) Reactive species mechanisms of cellular hypoxia-reoxygenation injury. *Am J Physiol* **282:**C227–C241.

Li Z, Nicoll DA, Collins A, Hilgemann DW, Filoteo AG, Penniston JT, Weiss JN, Tomich JM, and Philipson KD (1991) Identification of a peptide inhibitor of the cardiac sarcolemmal Na^+-Ca^{2+} exchanger. *J Biol Chem* **266:**1014–1020.

Magee WP, Deshmukh G, Deninno MP, Sutt JC, Chapman JG, and Tracey WR (2003) Differing cardioprotective efficacy of the Na^+/Ca^{2+} exchanger inhibitors SEA0400 and KB-R7943. *Am J Physiol* **284:**H903–H910.

Matsuda T, Arakawa N, Takuma K, Kishida Y, Kawasaki Y, Sakaue M, Takahashi K, Takahashi T, Suzuki T, Ota T, et al. (2001) SEA0400, a novel and selective inhibitor of the Na^+-Ca^{2+} exchanger, attenuates reperfusion injury in the in vitro and in vivo cerebral ischemic models. *J Pharmacol Exp Ther* **298:**249–256.

Matsumoto T, Miura T, Miki T, Genda S, and Shimamoto K (2002) Blockade of the Na^+-Ca^{2+} exchanger is more efficient than blockade of the Na^+-H^+ exchanger for protection of the myocardium from lethal reperfusion injury. *Cardiovasc Drugs Ther* **16:**295–301.

Mochizuki S and Jiang C (1998) Na^+/Ca^{++} exchanger and myocardial ischemia/reperfusion. *Jpn Heart J* **39:**707–714.

Nasuhoglu C, Feng S, Mao Y, Shammat I, Yamamato M, Earnest S, Lemmon M, and Hilgemann DW (2002) Modulation of cardiac PIP2 by cardioactive hormones and other physiologically relevant interventions. *Am J Physiol* **283:**C223–C234.

Ohtsuka M, Takano H, Suzuki M, Zou Y, Akazawa H, Tamagawa M, Wakimoto K, Nakaya H, and Komuro I (2004) Role of Na^+-Ca^{2+} exchanger in myocardial ischemia/reperfusion injury: evaluation using a heterozygous Na^+-Ca^{2+} exchanger knockout mouse model. *Biochem Biophys Res Commun* **314:**849–853.

Orrenius S, Zhivotovsky B, and Nicotera P (2003) Regulation of cell death: the calcium-apoptosis link. *Nat Rev Mol Cell Biol* **4:**552–565.

Philipson KD and Nicoll DA (2000) Sodium-calcium exchange: a molecular perspective. *Annu Rev Physiol* **62:**111–133.

Philipson KD, Nicoll DA, Ottolia M, Quednau BD, Reuter H, John S, and Qiu Z (2002) The Na^+/Ca^{2+} exchange molecule: an overview. *Ann NY Acad Sci* **976:**1–10.

Qiu Z, Nicoll DA, and Philipson KD (2001) Helix packing of functionally important regions of the cardiac Na^+-Ca^{2+} exchanger. *J Biol Chem* **276:**194–199.

Reuter H, Henderson SA, Han T, Matsuda T, Baba A, Ross RS, Goldhaber JI, and Philipson KD (2002a) Knockout mice for pharmacological screening: testing the specificity of Na^+-Ca^{2+} exchange inhibitors. *Circ Res* **91:**90–92.

Reuter H, Henderson SA, Han T, Ross RS, Goldhaber JI, and Philipson KD (2002b) The Na^+-Ca^{2+} exchanger is essential for the action of cardiac glycosides. *Circ Res* **90:**305–308.

Shigekawa M and Iwamoto T (2001) Cardiac Na^+-Ca^{2+} exchange: molecular and pharmacological aspects. *Circ Res* **88:**864–876.

Stys PK, Waxman SG, and Ransom BR (1992) Ionic mechanisms of anoxic injury in mammalian CNS white matter: role of Na^+ channels and Na^+-Ca^{2+} exchanger. *J Neurosci* **12:**430–439.

Tomes DJ and Agrawal SK (2002) Role of Na^+-Ca^{2+} exchanger after traumatic or hypoxic/ischemic injury to spinal cord white matter. *Spine J* **2:**35–40.

Yamashita J, Kita S, Iwamoto T, Ogata M, Takaoka M, Tazawa N, Nishikawa M, Wakimoto K, Shigekawa M, Komuro I, et al. (2003) Attenuation of ischemia/reperfusion-induced renal injury in mice deficient in Na^+/Ca^{2+} exchanger. *J Pharmacol Exp Ther* **304:**284–293.

Zeitz O, Maass AE, Van Nguyen P, Hensmann G, Kogler H, Moller K, Hasenfuss G, and Janssen PM (2002) Hydroxyl radical-induced acute diastolic dysfunction is due to calcium overload via reverse-mode Na^+-Ca^{2+} exchange. *Circ Res* **90:**988–995.

Address correspondence to: Dr. Myles Akabas, Department of Physiology and Biophysics, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461. E-mail: makabas@aecom.yu.edu