

REVIEW**Na⁺-Ca²⁺ Exchanger: Physiology and Pharmacology**Toshio Matsuda, Kazuhiro Takuma[#] and Akemichi Baba*Department of Pharmacology, Faculty of Pharmaceutical Sciences, Osaka University, 1-6 Yamada-oka, Suita, Osaka 565, Japan**Received February 7, 1997*

ABSTRACT—The Na⁺-Ca²⁺ exchanger in the plasma membrane is a bidirectional electrogenic ion transporter that couples the translocation of Na⁺ in one direction with that of Ca²⁺ in the opposite direction. This system is involved in regulation of intracellular Ca²⁺ concentration via the forward mode (Ca²⁺ extrusion) or the reverse mode (Ca²⁺ influx). There are two types of the plasma membrane Na⁺-Ca²⁺ exchanger in an animal, and they are called the cardiac type and rod outer segment type. In addition, there is an electroneutral Na⁺-Ca²⁺ exchanger present in mitochondria. Recent studies by the molecular biology technique show that there are at least 8 isoforms of the cardiac type (NCX1), and there are two other exchangers in the brain (NCX2 and NCX3). Due to new techniques of molecular biology and electrophysiology, much evidence is accumulating with respect to the structure, mechanism, regulation, and physiological and pathological roles of the Na⁺-Ca²⁺ exchanger. This review summarizes recent progress in this research field that is of pharmacological interest.

Keywords: Na⁺-Ca²⁺ exchanger, Transporter, Ca²⁺ signaling, Inhibitor, Astrocyte

| | | | |
|------------------------------|---|------------------------------|----|
| I. Introduction | 1 | D. Astrocytes | |
| II. Structure | 2 | E. Others | |
| III. Mechanism | 3 | VI. Pathological roles | 9 |
| IV. Regulation | 3 | A. Cardiac muscle | |
| A. Substrate regulation | | B. Smooth muscle | |
| B. ATP and phosphorylation | | C. Brain and neurons | |
| C. Others | | D. Astrocytes | |
| V. Physiological roles | 7 | VII. Inhibitors | 11 |
| A. Cardiac muscle | | A. Peptides | |
| B. Smooth muscle | | B. Chemicals | |
| C. Brain and neurons | | VIII. Conclusions | 12 |

I. Introduction

Na⁺-Ca²⁺ antiport (exchange) was first reported in squid axons (1) and guinea pig heart (2) and then found in

various tissues including the rod outer segment (ROS) (3–7) and in mitochondria (8–10). The exchange in ROS differs fundamentally from that observed in the heart and in most other tissues (3–7). Na⁺-Ca²⁺ exchange in the plasma membrane is electrogenic and is considered to contribute to regulation of intracellular Ca²⁺ concentration ([Ca²⁺]_i). On the other hand, Na⁺-Ca²⁺ exchange in the mitochondria is electroneutral and has a role in main-

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taining a low intramitochondrial Ca^{2+} concentration by extruding Ca^{2+} . In the cardiac muscle, much attention was focused on the exchange as a possible entry route for contractile Ca^{2+} on a beat-to-beat basis, as a mechanism for extrusion of Ca^{2+} from the cell and as a mediator of the inotropic effects of cardiac glycoside (11–15). Due to the physiological and pathological importance of intracellular Ca^{2+} , Na^+ - Ca^{2+} exchange has been extensively studied at both physiological and biochemical levels. However, a number of problems interfered with the studies. First, there are multiple Ca^{2+} transport pathways in most cells. Second, the protein responsible for Na^+ - Ca^{2+} exchange is of low abundance in most tissues. Third, there was no specific inhibitor, drug or affinity ligands to label the protein and manipulate its exchange activity (16).

In the 1990s, modern molecular biology techniques have been applied to the Na^+ - Ca^{2+} exchange research, and much information has been accumulating about structure-function relationships and the presence of diverse isoforms of the Na^+ - Ca^{2+} exchanger. The cardiac type Na^+ - Ca^{2+} exchanger (NCX1) was first cloned from canine cardiac tissues in 1990 (17). Subsequently, NCX1 and several splicing isoforms of NCX1 have been cloned from several species and tissues (18–29). The ROS type Na^+ - Ca^{2+} exchanger was also cloned from bovine rod outer segments in 1992 (30). Furthermore, two isoforms of the Na^+ - Ca^{2+} exchanger, designated as NCX2 and

NCX3, were cloned from rat brain in 1994 (31) and 1996 (32), respectively. The expression of NCX2 and NCX3 seems to be restricted to the brain and skeletal muscle. The nonmammalian Na^+ - Ca^{2+} exchanger isoforms have also been cloned (33, 34). The mitochondrial Na^+ - Ca^{2+} exchanger still remains to be cloned. These studies certainly contribute to the advanced research on the regulation and roles of the Na^+ - Ca^{2+} exchanger in mammalian cells. In addition, an antisense strategy has now been applied to this field (35–40). In this review, we will attempt to review recent progress of the Na^+ - Ca^{2+} exchanger research, especially the physiological and pathological roles of the Na^+ - Ca^{2+} exchanger, including our studies on the exchanger in astrocytes. A more comprehensive background on this topic has been given in recent books (41, 42).

II. Structure

A group of the Na^+ - Ca^{2+} exchangers comprises products of three mammalian genes. NCX1 is expressed at a high level in cardiac tissue, although it can be detected in several tissues (19, 25). NCX1 is proposed to be a glycosylated multipass membrane protein that contains a cleavable leader peptide (43, 44) followed by 5 putative transmembrane regions at the N-terminus and 6 transmembrane regions at the C-terminus with a large intracellular loop (sometimes called loop f) in between (Fig. 1)

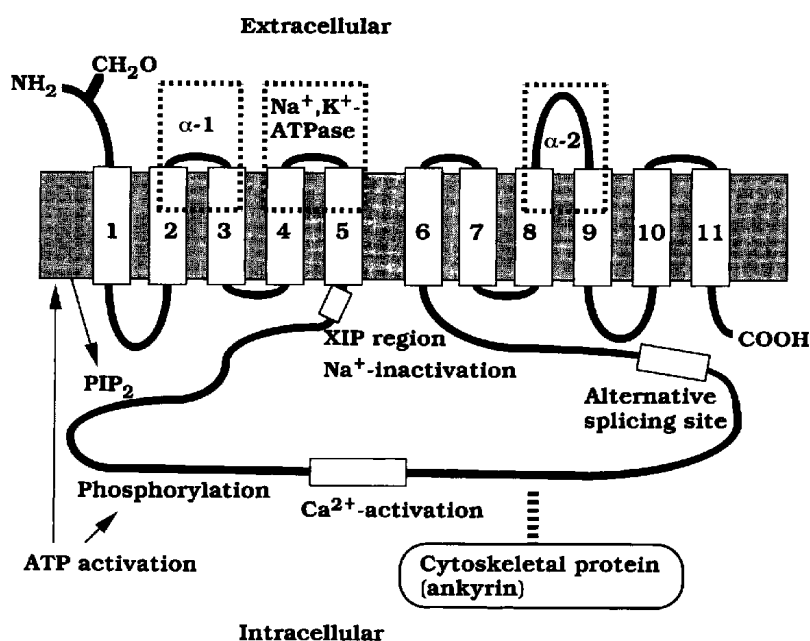


Fig. 1. A model of the mammalian NCX1. The deduced sequence predicts 11 putative transmembrane domains and a long intracellular loop that is more than half the size of the total protein. The loop contains the XIP (exchanger inhibitory peptide) region and the Ca^{2+} binding domain that is responsible for the Ca^{2+} regulation. CH_2O represents the known site of glycosylation. Regions corresponding to the α -1, α -2 repeats and Na^+ , K^+ -ATPase are enclosed by dotted lines.

(45–47). NCX2 has generally similar functional properties to NCX1 and has 65% amino acid sequence homology to NCX1. Eight isoforms for NCX1 with high homology ($\geq 90\%$) have been identified and are called NACA1...NACA8 (48). The only structural diversity among the NCX1 isoforms lies in a small region toward the end of the cytoplasmic loop, as a consequence of alternative splicing (25). NCX2 and NCX3 seem to be restricted to the brain and skeletal muscle (31, 32). NCX3 has 73% amino acid sequence homology with NCX1 and 75% with NCX2. The ROS $\text{Na}^+-\text{Ca}^{2+}$ exchanger contains little sequence homology to NCX1, but its architecture with 11 putative transmembrane domains and a large cytoplasmic loop is similar to NCX1.

The $\text{Na}^+-\text{Ca}^{2+}$ exchanger has two internal repeats called the α - and β -repeats (32) and a region of similarity to Na^+, K^+ -ATPase (Na^+, K^+ pump region) (17). The α -1 and α -2 motifs are in putative transmembrane segments 2–3 and 8–9, respectively. The β -1 and β -2 motifs are in loop f. The Na^+, K^+ pump region is in putative transmembrane segments 4–5. The loop f has a high-affinity Ca^{2+} binding site that regulates the exchange activity (called the secondary Ca^{2+} regulation as described below (see IV. Regulation)) (49, 50). Mutation experiments on NCX1 demonstrate that the α -repeat regions, Na^+, K^+ -pump region and some of the acidic residues may be important for the $\text{Na}^+-\text{Ca}^{2+}$ exchange function (50–52).

III. Mechanism

The $\text{Na}^+-\text{Ca}^{2+}$ exchanger transports Ca^{2+} across the membrane in exchange for Na^+ ions in a bidirectional way. The cardiac $\text{Na}^+-\text{Ca}^{2+}$ exchanger exchanges three Na^+ ions for each Ca^{2+} ion (53), and the ROS $\text{Na}^+-\text{Ca}^{2+}$ exchanger exchanges four Na^+ ions for each Ca^{2+} ion plus each K^+ ion (5, 54, 55), indicating that the exchange reactions are electrogenic (56–58). The cardiac

$\text{Na}^+-\text{Ca}^{2+}$ exchanger can also catalyze the $\text{Ca}^{2+}-\text{Ca}^{2+}$ and Na^+-Na^+ exchanges (46, 59, 60). The $\text{Na}^+-\text{Ca}^{2+}$ exchange cycle and its partial reactions can be described as separate movements of Na^+ and Ca^{2+} through the exchanger (consecutive or Ping-Pong mechanism) (Fig. 2). Kinetic studies using reconstituted proteoliposomes (61, 62) and an electrophysiological technique (60, 63, 64) showed that the apparent affinity of the exchanger for one ion increased as the concentration of the trans ion decreased, indicating a characteristic property of a consecutive reaction cycle. Ion binding sites can be assumed to have a net charge of -2 . The apparent ion affinities of the exchanger depend on ion concentrations on the opposite membrane side as expected for a consecutive exchange cycle. Approximately one net positive charge is moved across the membrane electrical field during the translocation of three Na^+ ions, while Ca^{2+} translocation is largely electrically silent: voltage-dependence resides in the binding and release of extracellular Na^+ . The $\text{Na}^+-\text{Ca}^{2+}$ exchanger will enter an inactive state when three Na^+ ions are bound on the cytoplasmic side. The Na^+ -dependent inactivation reaction (called “transporter gating”) is disturbed by limited proteolysis from the cytoplasmic side, indicating that the site is the intracellular membrane surface (64, 65). With respect to the rate-limiting reaction of the $\text{Na}^+-\text{Ca}^{2+}$ exchanger, it is suggested that the voltage-sensitive Na^+ efflux limits $\text{Na}^+-\text{Ca}^{2+}$ exchange, while the Ca^{2+} efflux limits $\text{Ca}^{2+}-\text{Ca}^{2+}$ exchange at physiological pH in the cardiac sarcolemmal preparation (66).

IV. Regulation

The regulation of the $\text{Na}^+-\text{Ca}^{2+}$ exchanger has been extensively studied in cardiac sarcolemmal vesicles, squid giant axons, whole-cell voltage clamped myocytes and giant excised patches of sarcolemma. There is little regulatory information on the ROS $\text{Na}^+-\text{Ca}^{2+}$ exchanger.

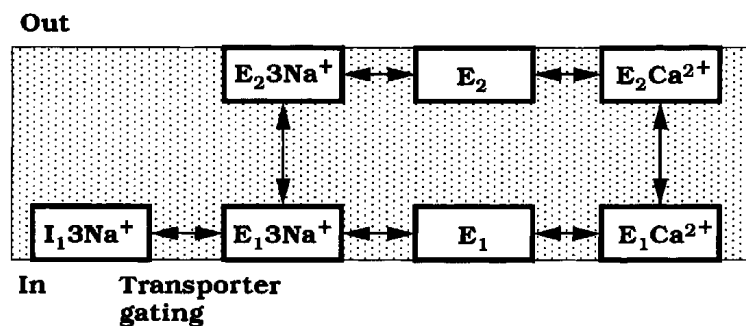


Fig. 2. A model of the ion transport cycle of the NCX1. E_1 and E_2 are different states of the $\text{Na}^+-\text{Ca}^{2+}$ exchanger: the ion binding sites are facing the cytoplasmic side (E_1) and extracellular side (E_2). The exchanger can enter an inactive state (I_1) whenever the binding sites facing the cytoplasmic side are loaded with three Na^+ molecules.

Table 1. Factors affecting Na^+ - Ca^{2+} exchange activity and the Na^+ - Ca^{2+} exchanger mRNA expression

| Factors or conditions | Activity | mRNA | Tissues or cells | References |
|------------------------|-------------|------|-----------------------|------------|
| Phosphorylation | | | | |
| PKA (cAMP) | ↓ | | chromaffin cells | 91 |
| | ↓ | ↓ | arterial myocytes | 92 |
| | ↓ | | frog cardiac myocytes | 93 |
| | ↓ | | chromaffin cells | 112 |
| | ↓ | | bone cells | 207 |
| PKC | ↓ | | VSMCs | 87 |
| | ↓ | | NCX1-expressed cells | 88 |
| | ↓ | ↓ | renal cells | 89 |
| | ↓ | | chromaffin cells | 91 |
| | ↓ | | bone cells | 207 |
| CaMK | ↓ | | adrenal cells | 90 |
| Unknown kinase | ↓ | | squid axons | 79 |
| Glucocorticoids | ↓ | ↓ | VSMCs | 104 |
| Ovarian steroid | ↓ | | synaptosomes | 114 |
| Vasopressin | ↓ | | synaptosomes | 115 |
| Thyroid hormone | ↓ (protein) | | heart | 103 |
| PTH | ↓ | | distal tube | 187, 189 |
| | ↓ | | bone cells | 207 |
| cGMP | ↑ | | VSMCs | 107 |
| | ↓ | | mesangial cells | 108 |
| NO/cGMP | ↓ | | astrocytes | 109 |
| | ↓ | | synaptosomes | 109 |
| Ascorbic acid | ↓ | | synaptic membrane | 105 |
| | ↓ | | astrocytes | 106 |
| IP_3 | ↓ | | synaptosomes | 110 |
| | ↓ | | renal cells | 111 |
| Adrenomedullin | ↓ | | chromaffin cells | 113 |

PKA, cAMP-dependent protein kinase; PKC, protein kinase C; CaMK, Ca^{2+} /calmodulin-dependent protein kinase; IP_3 , inositol 1,4,5-trisphosphate; PTH, parathyroid hormone; VSMCs, vascular smooth muscle cells.

The factors affecting the Na^+ - Ca^{2+} exchange activity and the Na^+ - Ca^{2+} exchanger mRNA expression are summarized in Table 1, and some of the regulation mechanisms such as Ca^{2+} -activation, Na^+ -inactivation and ATP-activation are also shown in Fig. 1.

A. Substrate regulation

The Na^+ - Ca^{2+} exchanger has a relatively high affinity Ca^{2+} transport site at the intracellular membrane surface and a low-affinity Ca^{2+} transport site at the extracellular surface. The K_m values of the exchanger for intracellular Ca^{2+} were reported to be about 1–40 μM . The wide variety of the K_m value may reflect different regulatory states of the exchanger. For example, ATP and Na^+ affect the affinity for intracellular Ca^{2+} . In the squid axon, the K_m values for Ca^{2+} are about 12 μM and 1–3 μM in the absence and presence of ATP, respectively (67).

The K_m value in the giant excised sarcolemmal patch is variable from about 1 to 10 μM depending on the extracellular Na^+ concentration (46). On the other hand, the K_m value for extracellular Ca^{2+} of the Na^+ - Ca^{2+} exchanger is in the mM range (68–70), which is much higher than that for intracellular Ca^{2+} . This property contrasts with that of the ROS Na^+ - Ca^{2+} exchanger which has similar K_m values in the 1–2 μM range at both surfaces (5). The K_m value for intracellular Ca^{2+} is substantially above resting $[\text{Ca}^{2+}]_i$. Then, the important question arises whether the Na^+ - Ca^{2+} exchanger has a role in Ca^{2+} efflux at low $[\text{Ca}^{2+}]_i$. In the cardiac muscle, the Ca^{2+} pump is considered to be a main pathway of Ca^{2+} extrusion at low $[\text{Ca}^{2+}]_i$. However, the Na^+ - Ca^{2+} exchanger alone is the only Ca^{2+} efflux mechanism in ROS (7, 71, 72). That is, the Na^+ - Ca^{2+} exchanger alone is clearly capable of lowering $[\text{Ca}^{2+}]_i$ to physiological levels

in ROS. We have recently demonstrated that the Na^+ - Ca^{2+} exchanger plays a role in Ca^{2+} efflux when $[\text{Ca}^{2+}]_i$ is raised to submicromolar concentrations in cultured astrocytes (39). It is likely that there is enough exchange activity to lower $[\text{Ca}^{2+}]_i$ to a level significantly below the K_m value.

The Na^+ - Ca^{2+} exchanger has K_m values of 20–30 mM for Na^+ at the intra- and extracellular surfaces, with Hill coefficients of 2–3; and its value is almost symmetric at intracellular and extracellular surfaces (46, 47). The Na^+ transport site is specific for Na^+ : no other monovalent cation can substitute for Na^+ in the Na^+ - Ca^{2+} exchange reaction. Thus, the Na^+ - Ca^{2+} exchanger in the plasma membrane contrasts with the mitochondrial one (10), which transports Li^+ as well as Na^+ .

The reverse mode of the Na^+ - Ca^{2+} exchanger is activated by intracellular Ca^{2+} (46, 47). That is, the exchanger has a high-affinity Ca^{2+} regulatory site at the intracellular surface, in addition to having a transport site for Ca^{2+} (49, 50). The secondary regulation by internal Ca^{2+} was first observed in the squid axon (73) and then demonstrated in various intact preparations, but not in isolated plasma membrane vesicles (74). The K_d value for Ca^{2+} at the regulatory site varies in the different preparations (in dialyzed myocytes, about 50 nM; in giant excised sarcolemmal patches, about 1 μM). The affinity is also influenced by ATP: the K_d values in the absence and presence of ATP are about 12 and 2 μM , respectively, in the squid axon. Due to the experimental difficulty, it has not been demonstrated whether the cytosolic Ca^{2+} regulatory site modulates the forward mode of the Na^+ - Ca^{2+} exchanger. Although the physiological importance of the Ca^{2+} regulatory site is not well-understood, the implication is reported in the cardiac system (13, 14). Ca^{2+} entry through the Ca^{2+} channel upon depolarization could increase Ca^{2+} binding to the exchanger regulatory site and activate Ca^{2+} influx through the exchanger during the early phase of the action potential, while the exchanger may be inactivated to block further Ca^{2+} efflux when Ca^{2+} is reduced below about 100 nM. That is, the Ca^{2+} regulatory site may act as a safety valve to prevent the exchanger from lowering $[\text{Ca}^{2+}]_i$ too far. A recent experiment in transfected CHO cells expressing the Na^+ - Ca^{2+} exchanger shows that the Na^+ - Ca^{2+} exchanger undergoes a regulatory activation during Ca^{2+} release from intracellular stores (75).

Cytosolic Na^+ promotes an inactive state of the Na^+ - Ca^{2+} exchanger: binding to Na^+ at the cytoplasmic membrane surface promotes a time-dependent entry of the exchanger into an inactive state as described above (in III. Mechanism) (64, 65). The Na^+ -dependent inactivation is reversible upon removal of Na^+ and is specific for the Na^+ -bound configuration. Furthermore, the

inactivation is exacerbated at acidic cytosolic pH (76) and can be attenuated by increasing intracellular Ca^{2+} and by ATP (77).

B. ATP and phosphorylation

ATP stimulates Na^+ - Ca^{2+} exchange activity with an increase in the affinity of the exchanger for its substrates such as Na^+ and Ca^{2+} in the giant axon and heart cells (46, 47, 78). The effect of ATP in the axon requires internal Ca^{2+} and hydrolyzable ATP, suggesting the involvement of a Ca^{2+} -dependent protein kinase. The importance of the phosphorylation/dephosphorylation process in the ATP effect is supported by the experiment using vanadate in squid axon (79). The outward Na^+ - Ca^{2+} exchange current in sarcolemmal patches is also stimulated by ATP, but the mechanism seems to contrast with that for the ATP effect observed in squid axon, since the effect is not mediated by protein kinases (74). One hypothesis is that the effect of ATP on the Na^+ - Ca^{2+} exchanger is due to an indirect effect on the phospholipid asymmetry of the sarcolemmal membrane (80, 81). That is, ATP generates acidic lipids on the cytoplasmic side of the membrane in parallel with a stimulation of Na^+ - Ca^{2+} exchange activity (81, 82). Hilgemann and Ball (83) have recently provided evidence that the ATP effect is mediated via phosphatidylinositol-4,5-bisphosphate (PIP_2) generated from phosphatidylinositol (PI). In giant cardiac membrane patches, the effect of ATP is abolished by a PI-specific phospholipase C and is mimicked by exogenous PIP_2 . A positively charged cytoplasmic regulatory domain of the cardiac Na^+ - Ca^{2+} exchanger is considered to be a PIP_2 binding site (51, 84). They (83) also showed that K_{ATP} channels, but not Na^+ , K^+ pump and Na^+ channels, had the similar sensitivity to PIP_2 . These observations indicated that it is likely that surface membrane ion transporters and channels may be regulated by PI signaling pathways. Alternatively, ATP-dependent regulation of Na^+ - Ca^{2+} exchange activity may be in part explained by interaction of the exchanger with the cellular cytoskeleton because treatment with cytochalasin D mimics the effects of ATP depletion in transfected CHO cells (85). This interaction might be mediated by ankyrin, a cytoskeletal protein that has been shown to bind to the Na^+ - Ca^{2+} exchanger (86); however, this interpretation cannot explain the effect of ATP in giant sarcolemmal patches since cytoskeletal elements appear to be absent in these patches.

There are several reports with respect to regulation by phosphorylation. Iwamoto et al. (87) demonstrated that physiological ligands such as platelet-derived growth factor and α -thrombin or phorbol esters phosphorylated serine residues of the Na^+ - Ca^{2+} exchanger in a protein kinase C-dependent way and enhanced the exchange

activity in smooth muscle cells. They (88) further showed that the $\text{Na}^+\text{-Ca}^{2+}$ exchanger was up-regulated by endothelin-1, acidic fibroblast growth factor, phorbol esters and okadaic acid, indicating the involvement of protein kinase C-catalyzed phosphorylation in the regulation. This finding implies that the $\text{Na}^+\text{-Ca}^{2+}$ exchanger may play an important role in the previously reported negative inotropic actions of phorbol esters and other PKC-activating agents. In contrast, Smith et al. (89) reported that phorbol esters down-regulated the expression of the $\text{Na}^+\text{-Ca}^{2+}$ exchanger in renal epithelial cells. It is conceivable that there is a difference in the regulation mechanism of the $\text{Na}^+\text{-Ca}^{2+}$ exchanger among the isoforms. Isosaki et al. (90) reported that $\text{Na}^+\text{-Ca}^{2+}$ exchange activity was inhibited by the Ca^{2+} /calmodulin-dependent protein kinase inhibitor staurosporine in isolated bovine adrenal medullary cells. In contrast, Lin et al. (91) found that $\text{Na}^+\text{-Ca}^{2+}$ exchange activity was inhibited by stimulation of protein phosphorylation in chromaffin cells. In this relation, down-regulation of the $\text{Na}^+\text{-Ca}^{2+}$ exchanger by activation of adenylate cyclase was demonstrated in arterial myocytes (92) and frog ventricular myocytes (93).

C. Others

Many anionic lipid components and the membrane sterol component stimulate $\text{Na}^+\text{-Ca}^{2+}$ exchange activity (46, 94). The dependence of the $\text{Na}^+\text{-Ca}^{2+}$ exchanger on membrane lipid environment has been shown using phospholipases, a variety of amphipathic molecules and reconstitution techniques (80, 95, 96). The anionic lipids

at the cytoplasmic surface and specific interaction between the $\text{Na}^+\text{-Ca}^{2+}$ exchanger and cholesterol are important for the exchange activity (97).

It is reported that proton markedly inhibits $\text{Na}^+\text{-Ca}^{2+}$ exchange activity by competing at the Ca^{2+} translocation site and also possibly at the Na^+ translocation site (98, 99). The sites of proton interaction are disrupted by partial proteolysis from the cytoplasmic site and thus may be located in the intracellular loop of the $\text{Na}^+\text{-Ca}^{2+}$ exchanger. Relief of proton inhibition may be responsible for the stimulatory effect of proteolysis on $\text{Na}^+\text{-Ca}^{2+}$ exchange activity observed in previous studies (74, 100). In addition, the Na^+ -dependent component of proton inhibition may also underlie intrinsic Na^+ -dependent inactivation of the exchanger (76).

K^+ is obligatory for the $\text{Na}^+\text{-Ca}^{2+}$ exchanger in ROS, but not in other tissues as described above. Dahan et al. (101) reported that $\text{Na}^+\text{-Ca}^{2+}$ exchange activity was moderately activated by K^+ in brain synaptosomes. We (70) also observed the similar K^+ activation of $\text{Na}^+\text{-Ca}^{2+}$ exchange in cultured astrocytes.

Recent studies show that the expression of the $\text{Na}^+\text{-Ca}^{2+}$ exchanger mRNA is regulated by several factors such as cAMP (92), phorbol ester (89), thyroid hormone (102, 103) and glucocorticoid (104). Moreover, there are several substances affecting $\text{Na}^+\text{-Ca}^{2+}$ exchange activity. Ascorbic acid decreases $\text{Na}^+\text{-Ca}^{2+}$ exchange activity in brain synaptic membrane (105) and cultured astrocytes (Fig. 3) (106). The effect is not related to lipid peroxidation, although the exact mechanism for the effect is not

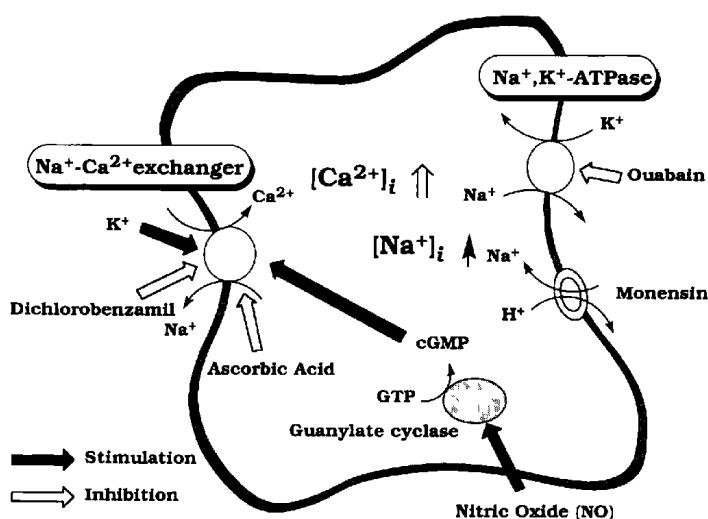


Fig. 3. The $\text{Na}^+\text{-Ca}^{2+}$ exchanger in astrocytes. $\text{Na}^+\text{-Ca}^{2+}$ exchange activity, the exchanger protein and its mRNA are present in cultured astrocytes. The activity of the $\text{Na}^+\text{-Ca}^{2+}$ exchanger in the reverse mode is determined by Ca^{2+} uptake or an increase in $[\text{Ca}^{2+}]_i$ in the presence of ouabain and monensin. Monensin causes an increase in intracellular pH that affects $\text{Na}^+\text{-Ca}^{2+}$ exchange activity, but it is likely that the monensin-induced increase in Ca^{2+} uptake is due mainly to an increase in $[\text{Na}^+]_i$ (unpublished).

known. The experiment in cultured astrocytes suggests that ascorbic acid acts at the intracellular sites and the inhibitory control on the exchange activity is probably indirect (106). cGMP stimulates the $\text{Na}^+\text{-Ca}^{2+}$ exchange activity in smooth muscle cells (107), while it decreases the activity in cultured human mesangial cells (108). The stimulation induced by cGMP in smooth muscle cells may be involved in nitric oxide (NO)-induced vasodilation. We (109) demonstrated that NO/cGMP stimulates $\text{Na}^+\text{-Ca}^{2+}$ exchange activity in neuronal preparations and cultured astrocytes (Fig. 3). There is no evidence that the cGMP effect on the $\text{Na}^+\text{-Ca}^{2+}$ exchanger is mediated by cGMP-dependent phosphorylation (107, 109). In contrast to cGMP, a cAMP analog did not affect $\text{Na}^+\text{-Ca}^{2+}$ exchange activity in astrocytes. Other factors such as inositol 1,4,5-trisphosphate (110, 111), pituitary adenylate cyclase-activating polypeptide (112), adrenomedullin (113), ovarian steroid (114), vasopressin (115) and oxidative stress (116) are also reported to affect $\text{Na}^+\text{-Ca}^{2+}$ exchange activity.

V. Physiological roles

The $\text{Na}^+\text{-Ca}^{2+}$ exchanger is present in various cells and tissues, and its forward mode (Ca^{2+} efflux) or the reverse mode (Ca^{2+} influx) is involved in regulation of $[\text{Ca}^{2+}]_i$.

A. Cardiac muscle

It is known that a small rise in Ca^{2+} concentration at the vicinity of the sarcoplasmic reticulum (SR) activates the Ca^{2+} -release channels (ryanodine receptors) in the SR to cause a much larger release of Ca^{2+} ions from the SR (referred to as Ca^{2+} -induced Ca^{2+} release, CICR) which then bind to troponin and activates contraction. The origin of the Ca^{2+} that triggers SR Ca^{2+} release has been investigated. While it is clear that L-type Ca^{2+} channels (117) located in the T-tubular system can trigger SR Ca^{2+} release, Ca^{2+} entry via the $\text{Na}^+\text{-Ca}^{2+}$ exchanger is possibly involved in CICR (98, 118–120). Since the first demonstration of Ca^{2+} -current-independent SR Ca^{2+} release (121), evidence is accumulating that the $\text{Na}^+\text{-Ca}^{2+}$ exchanger can trigger SR Ca^{2+} release (13, 122, 123). However, the importance of the $\text{Na}^+\text{-Ca}^{2+}$ exchanger in triggering SR Ca^{2+} release under physiological conditions remains to be clarified (124). There are four Ca^{2+} transport systems that compete to remove intracellular Ca^{2+} for ventricular relaxation. These are the SR Ca^{2+} -ATPase, the $\text{Na}^+\text{-Ca}^{2+}$ exchanger, the sarcolemmal Ca^{2+} -ATPase and the mitochondrial Ca^{2+} uniporter. Recent studies suggest a coordinate control of the cellular Ca^{2+} transport processes mediated by the $\text{Na}^+\text{-Ca}^{2+}$ exchanger and SR Ca^{2+} -ATPase (102, 125). During the normal cardiac contraction-relaxation cycle, the SR Ca^{2+} -ATPase

and the $\text{Na}^+\text{-Ca}^{2+}$ exchanger are clearly the most important (56, 126–129), although the relative roles vary in a species-dependent manner (130). The elementary events of SR Ca^{2+} release are known as Ca^{2+} sparks, and they take place at the T-tubular system-ryanodine receptor junction (119, 120). Thus, just as excitation-contraction coupling in the heart arises under local control of SR Ca^{2+} release, the $\text{Na}^+\text{-Ca}^{2+}$ exchanger is well-placed to extrude the elevated $[\text{Ca}^{2+}]_i$ (131–136).

Iwakura et al. (137) showed that the $\text{Na}^+\text{-Ca}^{2+}$ exchanger might be functionally coupled with the $\text{Na}^+\text{-H}^+$ exchanger in cardiac myocytes. α_1 -Adrenergic stimulation enhances $\text{Na}^+\text{-H}^+$ exchange, allowing intracellular alkalization, and that subsequent activation of $\text{Na}^+\text{-Ca}^{2+}$ exchange may increase Ca^{2+} influx. In addition, Urcelay et al. (138) suggested that a functional coupling between the plasma membrane $\text{Na}^+\text{-H}^+$ exchanger and the $\text{Na}^+\text{-Ca}^{2+}$ exchanger might lead to enhanced Ca^{2+} influx and stimulation of Ca^{2+} -dependent processes during α_1 -adrenoceptor activation in liver cells. A functional coupling between the $\text{Na}^+\text{-Ca}^{2+}$ exchanger and $\text{Na}^+\text{,K}^+\text{-ATPase}$ was also reported in the heart: these proteins changed reciprocally in a number of models known to have altered $\text{Na}^+\text{,K}^+\text{-ATPase}$ abundance (103).

B. Smooth muscle

Several lines of evidence indicate that the $\text{Na}^+\text{-Ca}^{2+}$ exchanger operates in the Ca^{2+} efflux mode to reduce $[\text{Ca}^{2+}]_i$ and cell tension in vascular smooth muscle cells. The relative contribution of the $\text{Na}^+\text{-Ca}^{2+}$ exchanger, under physiological conditions, in regulation of $[\text{Ca}^{2+}]_i$ and cell tension remains unknown. Fay and coworkers (139–141) suggested that the $\text{Na}^+\text{-Ca}^{2+}$ exchanger might be involved in β -adrenergic relaxation of smooth muscle cells. The β -adrenergic receptor stimulation by isoproterenol activates adenylate cyclase and increases cAMP production. cAMP directly or indirectly activates the K^+ channels which hyperpolarizes the cell and, in addition, stimulates the Na^+ pump. The pump stimulation both hyperpolarizes the cell and decreases $[\text{Na}^+]_i$, and these effects lead to operation of the forward mode (Ca^{2+} extrusion) of the $\text{Na}^+\text{-Ca}^{2+}$ exchanger. Contraction in response to agents, such as acetylcholine which release Ca^{2+} from internal stores, is therefore inhibited. Interestingly, they (142) investigated the distribution of the $\text{Na}^+\text{,K}^+\text{-ATPase}$, the $\text{Na}^+\text{-Ca}^{2+}$ exchanger, calsequestrin, a marker of the SR, and vinculin, a marker for regions of the attachment of the contractile machinery to the cell membrane, in single isolated smooth muscle cells. The $\text{Na}^+\text{,K}^+\text{-ATPase}$ and the $\text{Na}^+\text{-Ca}^{2+}$ exchanger are co-localized in the same macrodomain on the cell membrane, which is distinct from regions occupied by vinculin, and they appear to exist close to regions occupied by calse-

questrin. It is likely that the $\text{Na}^+\text{-Ca}^{2+}$ exchanger may have preferred access to Ca^{2+} from the SR, and it may be functionally coupled with $\text{Na}^+\text{,K}^+\text{-ATPase}$.

C. Brain and neurons

Neuronal $\text{Na}^+\text{-Ca}^{2+}$ exchange has been investigated mainly in brain synaptosomes and synaptic plasma membrane vesicles (143–147) and recently in cultured cells (148–150). The studies using antibodies raised against the dog heart $\text{Na}^+\text{-Ca}^{2+}$ exchanger showed that cultured neurons possessed the $\text{Na}^+\text{-Ca}^{2+}$ exchanger and that the $\text{Na}^+\text{-Ca}^{2+}$ exchanger might be concentrated at presynaptic nerve terminals (149). Furthermore, the immunohistochemical study in a coronal section of rat hippocampus showed that the regions containing most of the neuronal cell bodies were only sparsely labeled with antibodies raised against the $\text{Na}^+\text{-Ca}^{2+}$ exchanger, but the synaptic regions were intensely labeled (150). In this relation, Kennedy and Thomas (151) reported that $\text{Na}^+\text{-Ca}^{2+}$ exchange activity is not present in the cell body where $[\text{Ca}^{2+}]_i$ is regulated by the Ca^{2+} pump in snail neurons. With respect to the regional distribution of the $\text{Na}^+\text{-Ca}^{2+}$ exchanger in the brain, *in situ* hybridization and Northern blot studies revealed the corresponding mRNA of the $\text{Na}^+\text{-Ca}^{2+}$ exchanger was present in numerous brain regions and to a lesser extent in the striatum (152). Juhaszova et al. (150, 153) have shown that the $\text{Na}^+\text{-Ca}^{2+}$ exchanger is much more prevalent in neurons than in astrocytes or vascular smooth muscle cells. The localization of the $\text{Na}^+\text{-Ca}^{2+}$ exchanger implies that the $\text{Na}^+\text{-Ca}^{2+}$ exchanger as well as plasma membrane $\text{Ca}^{2+}\text{-ATPase}$ is involved in the regulation of $[\text{Ca}^{2+}]_i$. Although the $\text{Na}^+\text{-Ca}^{2+}$ exchanger has a relatively low affinity for cytosolic Ca^{2+} , it may influence the numerous processes that depend on the endoplasmic reticulum Ca^{2+} release as described in the cardiac muscle section. The $\text{Na}^+\text{-Ca}^{2+}$ exchanger also may mediate net Ca^{2+} influx during nerve terminal depolarization. The depolarization and increase in $[\text{Na}^+]_i$ as well as the transient rise in $[\text{Ca}^{2+}]_i$ may enhance Ca^{2+} entry via the reverse mode of the $\text{Na}^+\text{-Ca}^{2+}$ exchanger (154–156). The idea is supported by the recent observation in the synaptic membranes suggesting that the $\text{Na}^+\text{-Ca}^{2+}$ exchanger is involved in Ca^{2+} inflow into the synaptic terminal upon stimulation (157). Gleason et al. (158, 159) also reported that the $\text{Na}^+\text{-Ca}^{2+}$ exchanger may play a role in transmitter release in retinal amacrine cells. Furthermore, pharmacological evidence is accumulating that the $\text{Na}^+\text{-Ca}^{2+}$ exchanger may be involved in transmitter release (160–163). In this connection, evidence is reported that $\text{Na}^+\text{-Ca}^{2+}$ exchange is involved in stimulation of PI hydrolysis induced by Na^+ channel opening agents and depolarization (164–167). The possible roles of the $\text{Na}^+\text{-Ca}^{2+}$ exchanger in regula-

tion of $[\text{Ca}^{2+}]_i$ and hormone secretion are also reported in adrenal chromaffin cells (168–171) and pancreatic B cell (172–175).

D. Astrocytes

In 1994, Goldman et al. (176) and we (70) demonstrated that the $\text{Na}^+\text{-Ca}^{2+}$ exchanger is present in cultured astrocytes (Fig. 3). Several ligands such as glutamate, noradrenaline and ATP induce the Ca^{2+} signal in astrocytes (177). We observed that NO/cGMP stimulate $\text{Na}^+\text{-Ca}^{2+}$ exchange activity (109) and attenuate the Ca^{2+} signal induced by glutamate, noradrenaline and ATP in astrocytes (39). NO modulation of elevated $[\text{Ca}^{2+}]_i$ was also reported in PC12 cells, and the sites for the modulation were suggested to be voltage-sensitive Ca^{2+} channels (178) and phospholipase C (179). However, in experiments using antisense deoxynucleotides, we (39) demonstrated that the $\text{Na}^+\text{-Ca}^{2+}$ exchanger is a target of NO modulation of the agonist-induced Ca^{2+} signal in astrocytes (Fig. 4). The studies also suggest that the $\text{Na}^+\text{-Ca}^{2+}$ exchanger plays a role in the recovery of elevated $[\text{Ca}^{2+}]_i$ to the resting level and imply that the exchanger may modulate the intracellular Ca^{2+} signaling induced by agonists in astrocytes. The idea is consistent with the recent observation in the transfected CHO cells (75) that Ca^{2+} efflux via the $\text{Na}^+\text{-Ca}^{2+}$ exchanger limits the rise in $[\text{Ca}^{2+}]_i$ during sustained Ca^{2+} entry. Although the usefulness of an antisense strategy for inhibition of exchange activity was reported in cultured myocytes (35–37) and in renal epithelial cells (40), our result (38, 39) was the first demonstration that the antisense oligomer to the sequence encoding the NXC1 decreased selectively the expression of the gene product. Golovina et al. (180) have recently showed that the Ca^{2+} content in intracellular Ca^{2+} stores can be regulated by the $\text{Na}^+\text{-Ca}^{2+}$ exchanger in cultured mouse astrocytes.

E. Others

The kidney, like the heart and brain, is one of the richest sources of the $\text{Na}^+\text{-Ca}^{2+}$ exchanger protein in an animal (19). The $\text{Na}^+\text{-Ca}^{2+}$ exchanger is involved not only in transcellular Ca^{2+} reabsorption in the distal and connecting tubule of the nephron (181–184) but also in maintenance and regulation of $[\text{Ca}^{2+}]_i$ in the cortical thick ascending limb of the loop of Henle (185, 186). Previous studies show that $\text{Na}^+\text{-Ca}^{2+}$ exchange in renal cells is regulated by parathyroid hormone (187), vitamin D (188) and cAMP (189), and it may be modulated by phosphorylation events (190, 191).

The $\text{Na}^+\text{-Ca}^{2+}$ exchanger is very widely distributed. Although this review concerns the exchangers only in the selected tissues or cells, the presence and roles of $\text{Na}^+\text{-Ca}^{2+}$ exchange activity are reported in other tissues or

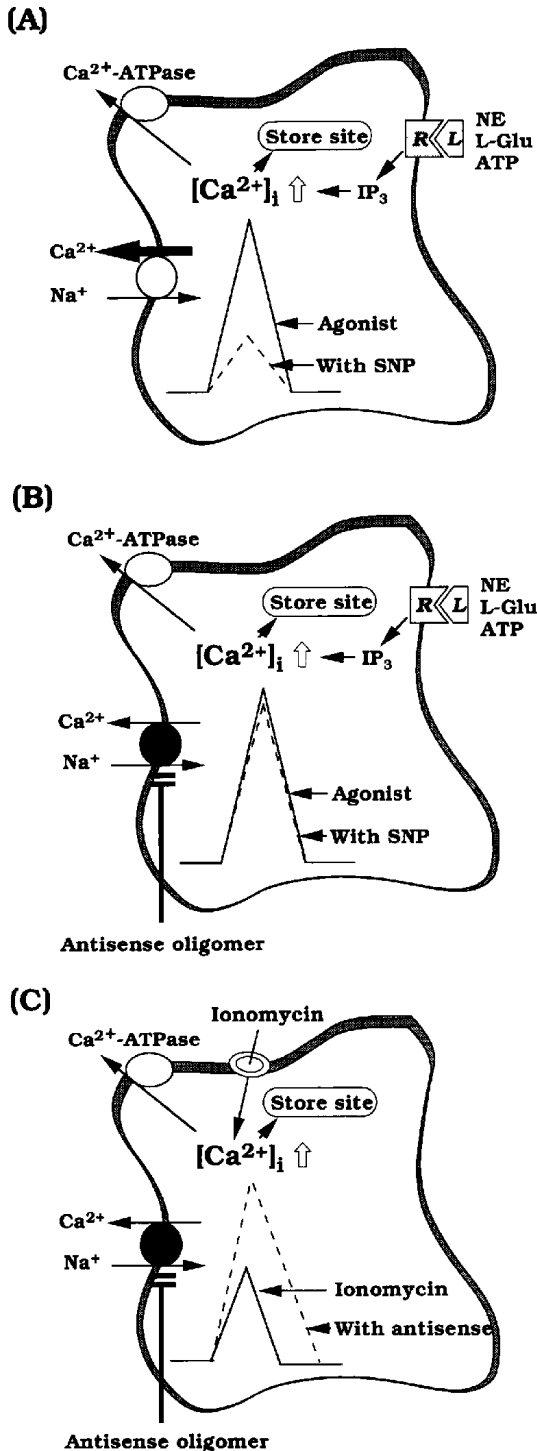


Fig. 4. Physiological role of the $\text{Na}^+-\text{Ca}^{2+}$ exchanger in astrocytes. Under physiological conditions, the $\text{Na}^+-\text{Ca}^{2+}$ exchanger operates in the forward mode (Ca^{2+} extrusion). A: Agonist stimulation and ionomycin cause an increase in $[\text{Ca}^{2+}]_i$. The Ca^{2+} signal is attenuated by the NO generator sodium nitroprusside (SNP) which stimulates the exchange activity. B: The NO modulation is blocked by pretreatment of the cells with an antisense oligomer to the sequence encoding the $\text{Na}^+-\text{Ca}^{2+}$ exchanger. C: In the cells treated with the antisense oligomer, the ionomycin-induced Ca^{2+} signal is enhanced. NE and L-Glu represent norepinephrine and L-glutamate, respectively.

cells such as skeletal muscle (192–194), hepatocytes (195–197), inner hair cells (198, 199), blood cells (200–203), NG108-15 cells (204) and osteoblasts (205–207). It should be noted that the mitochondrial $\text{Na}^+-\text{Ca}^{2+}$ exchanger, unlike the cardiac and ROS exchangers, is electroneutral (10, 208). The exchanger excludes intramitochondrial Ca^{2+} with Na^+ uptake into mitochondria. Since the activities of the mitochondrial enzymes pyruvate dehydrogenase, isocitrate dehydrogenase and α -ketoglutarate dehydrogenase are stimulated by Ca^{2+} (209), the mitochondrial Ca^{2+} transport systems such as the exchanger and Ca^{2+} uniporter are considered to play an important role in the overall rate of oxidative ATP synthesis. That is, an antagonist of the mitochondrial $\text{Na}^+-\text{Ca}^{2+}$ exchanger would be expected to increase the intramitochondrial Ca^{2+} concentration and thereby stimulate the ATP synthesis. The pharmacological significance of the mitochondrial $\text{Na}^+-\text{Ca}^{2+}$ exchanger is discussed (9, 210–213).

VI. Pathological roles

A. Cardiac muscle

The $\text{Na}^+-\text{Ca}^{2+}$ exchanger has a role in the initiation of arrhythmias produced by ectopic beats generated in regions of Na^+ and Ca^{2+} overload (214). Each rise in internal Ca^{2+} activates the inward membrane current, either by activating the inward mode of the $\text{Na}^+-\text{Ca}^{2+}$ exchanger or by activating nonspecific cation channels. In ventricular cells, the evidence is that the major component of such oscillatory current is generated by the $\text{Na}^+-\text{Ca}^{2+}$ exchanger. If this inward current is sufficiently large, it may trigger ectopic beats. Expression of the cardiac $\text{Na}^+-\text{Ca}^{2+}$ exchanger changes in pathological states: it decreases in global myocardial ischemia (215) and increases in animal models of cardiac hypertrophy (216) and failing human heart (217, 218). The involvement of the $\text{Na}^+-\text{Ca}^{2+}$ exchanger in pathological states such as ischemia is also shown by pharmacological and physiological experiments (118, 219–222). The increased expression and functional activity of the $\text{Na}^+-\text{Ca}^{2+}$ exchanger in failing hearts may in part compensate for depressed SR Ca^{2+} -ATPase function, thereby limiting intracellular diastolic Ca^{2+} overload (217, 223). The increase in the $\text{Na}^+-\text{Ca}^{2+}$ exchanger could also result in an increased Ca^{2+} influx and might be involved in the well-known increased incidence of arrhythmias in patients with severe chronic heart failure (217).

B. Smooth muscle

Pharmacological studies indicate that blockade of $\text{Na}^+-\text{Ca}^{2+}$ exchange activity in the vascular smooth muscle increases $[\text{Ca}^{2+}]_i$, resulting in vasoconstriction,

increased peripheral resistance, and hypertension (224–227). Thompson et al. (228) reported that the activity of the Na^+ - Ca^{2+} exchanger was depressed in tail arteries of stroke-prone spontaneously hypertensive rats and suggested that the change in resistance vessels would contribute to the enhanced vascular tone present in hypertension. Nakanishi et al. (229) showed that Na^+ - Ca^{2+} exchange activity, unlike Na^+ , K^+ -ATPase activity, in sarcolemmal vesicles from hypertrophied hearts was stimulated compared with the control vesicles and suggested that the alteration in the Ca^{2+} transport activity might serve as an adaptive mechanism during the development of cardiac hypertrophy. Furthermore, Smith and Smith (104) reported that glucocorticoids down-regulated the Na^+ - Ca^{2+} exchanger mRNA and activity in aortic myocytes. The effect may result in an inhibition of net Ca^{2+} efflux evoked by vasoconstrictors and may be involved in glucocorticoid-induced hypertension (230, 231). Blaustein and Hamlyn (224) proposed that Na^+ , K^+ -ATPase inhibition by endogenous circulating ouabain or a digitalis-like compound (232, 233) may cause Ca^{2+} entry into nerve endings and vascular smooth muscle via the Na^+ - Ca^{2+} exchangers in both cells, resulting in vasoconstriction and hypertension.

C. Brain and neurons

Stys and co-workers (234–237), using an anoxic optic nerve model, found that anoxic injury in the central nervous system white matter critically depended on extracellular Ca^{2+} , suggesting that Ca^{2+} entry might be responsible for the injury. Several lines of evidence indicate that during anoxia, Ca^{2+} enters the axoplasm via the reverse mode of the Na^+ - Ca^{2+} exchanger (238–241). Anoxia produces cellular energy depletion, leading to failure of Na^+ , K^+ -ATPase. The increase in intracellular Na^+ and membrane depolarization both act to drive the Na^+ - Ca^{2+} exchanger in the reverse mode, leading to intracellular Ca^{2+} overload. Interrupting the cascade at the Na^+ influx process with Na^+ channel blockers results in protection against anoxic injury (236, 237, 242, 243). The Na^+ - Ca^{2+} exchanger blockers are also effective in protection against anoxic injury (236). In this relation, one hypothesis is that Na^+ -dependent Ca^{2+} extrusion is important in protecting against neuronal excitotoxicity. Mattson et al. (244) showed that removal of extracellular Na^+ reduced the threshold for glutamate neurotoxicity in hippocampal neurons. On the other hand, Andreeva et al. (148) reported that blockade of the exchange activity enhanced the development of glutamate-induced delayed neuronal death in primary cerebellar granule cell cultures. In this case, the Na^+ - Ca^{2+} exchanger seems to operate as the forward mode (Ca^{2+} extrusion), and then its inhibition leads to enhancement of the injury.

Colvin et al. (245, 246) reported that Na^+ - Ca^{2+} exchange activity was significantly elevated in the plasma membranes purified from Alzheimer's disease brain tissue. The observation seems to be consistent with the Ca^{2+} hypothesis of aging and dementia, although an age-related change in Na^+ - Ca^{2+} exchange activity is not confirmed (247, 248). With regard to Alzheimer's disease, Mark et al. (249) demonstrated that amyloid- β -induced neuronal death might be due to a decreased activity of Na^+ , K^+ -ATPase, but not the Na^+ - Ca^{2+} exchanger: they could not detect any change in Na^+ - Ca^{2+} exchange activity.

D. Astrocytes

When astrocytes are exposed to Ca^{2+} -free medium for 10–30 min, a persistent increase in $[\text{Ca}^{2+}]_i$ is observed immediately after reperfusion with Ca^{2+} -containing medium, and the number of surviving cells decreased 3–5 days later (38). The similar cell toxicity was reported in human astrocytoma cells (250, 251). This phenomenon is similar to Ca^{2+} paradox injury that is well-known in the heart (252). Previous studies in the heart suggested that the Na^+ - Ca^{2+} exchanger might be involved in the injury, but conclusive evidence supporting this idea was not shown. We (38) first demonstrated using an antisense technique that the reperfusion-induced increase in $[\text{Ca}^{2+}]_i$ is due to operation of the reverse mode of the Na^+ - Ca^{2+} exchanger and the Ca^{2+} overload results in cell toxicity (Fig. 5). The delayed glial death is protected against by blocking Na^+ - Ca^{2+} exchange activity with taurine, ascorbic acid and 3,4-dichlorobenzamil and is enhanced by stimulating the exchange with NO (38, 253,

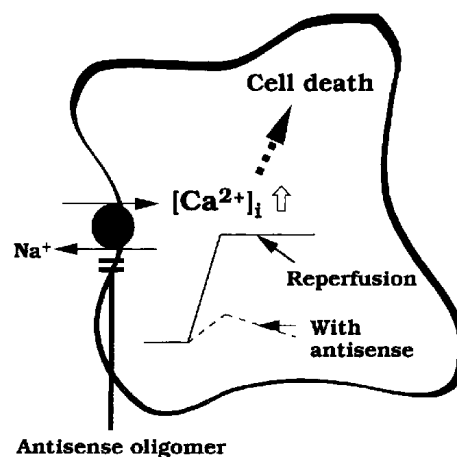


Fig. 5. Pathological role of the Na^+ - Ca^{2+} exchanger. Reperfusion of astrocytes in Ca^{2+} -containing medium after Ca^{2+} depletion causes an increase in $[\text{Ca}^{2+}]_i$ via the reverse mode of the Na^+ - Ca^{2+} exchanger, resulting in delayed cell death. The antisense blocks the increase in $[\text{Ca}^{2+}]_i$ and protects the cell from reperfusion injury.

254). We also found that NO is partly involved in the toxicity (255) and that this astrocyte toxicity, like neuronal death observed in ischemic brain, is attenuated by induction of heat shock proteins that may affect processes down stream of the increase in $[\text{Ca}^{2+}]_i$ (256). The Ca^{2+} paradox-like reaction in nervous tissue occurs in pathological states such as ischemia (257, 258), but it remains to be determined whether the reperfusion-induced glial cell toxicity observed in the in vitro experiment indeed occurs under the in vivo situation.

VII. Inhibitors

The previous search for inhibitors has consisted principally of determining whether various inorganic cations, compounds with proven cardiovascular activities, or known blockers of other transport systems will inhibit $\text{Na}^+\text{-Ca}^{2+}$ exchange. A number of compounds has been reported as inhibitors (16, 259), but none of them is specific for inhibition of $\text{Na}^+\text{-Ca}^{2+}$ exchange activity. On

the other hand, studies in the 1990s showed that several peptides and an isothiourea derivative are more potent inhibitors of the $\text{Na}^+\text{-Ca}^{2+}$ exchanger. Structures of the typical inhibitors are shown in Fig. 6.

A. Peptides

As noted above, the $\text{Na}^+\text{-Ca}^{2+}$ exchanger contains a large regulatory intracellular loop (45, 118). A 20-amino acid sequence, corresponding to residues 251–270 of the NCX1, was identified on the intracellular loop as a possible calmodulin-binding domain with an autoinhibitory potency (XIP region in Fig. 1) (17). The calmodulin-binding autoinhibitory domain of the $\text{Na}^+\text{-Ca}^{2+}$ exchanger is similar to that of the plasma membrane Ca^{2+} pump in inhibiting the SR and plasma membrane Ca^{2+} pumps (260). The sequence is similar to that previously found in a number of calmodulin-binding proteins (261), but there are no reports of a direct effect of calmodulin on the $\text{Na}^+\text{-Ca}^{2+}$ exchanger. Li et al. (262) synthesized a family of peptides corresponding to this region and demonstrated that the peptide inhibited the Na^+ -dependent Ca^{2+} uptake in a noncompetitive manner with respect to both Na^+ and Ca^{2+} with an IC_{50} value of about $1.5\ \mu\text{M}$. This peptide (RRLIFYKYVYKRYRAGKQRG) (called XIP) has an apparent specific binding site on the intracellular surface of the exchanger (263). XIP also inhibits $\text{Na}^+\text{-Ca}^{2+}$ exchange current in ventricular cells (264) and $\text{Na}^+\text{-Ca}^{2+}$ exchange activity in squid axon (265). However, it is not known whether the XIP segment of the $\text{Na}^+\text{-Ca}^{2+}$ exchanger interacts with this same binding site to exert a regulatory influence. Shannon et al. (84) suggested that the XIP domain may associate with the phospholipid bilayer under conditions in which $\text{Na}^+\text{-Ca}^{2+}$ exchange activity is stimulated. A structural homology was suggested among the cardiac, rat and human brain $\text{Na}^+\text{-Ca}^{2+}$ exchangers in the XIP binding domain (266). XIP is more potent and specific than dichlorobenzamil (a potent amiloride derivative), but its application for physiological experiments is limited because it acts intracellularly and may interact with other calmodulin-binding proteins.

Khananshvili et al. (267) found that the tetrapeptide (FMRFa) and its analogs inhibited $\text{Na}^+\text{-Ca}^{2+}$ exchange activity in the cardiac sarcolemmal vesicles with IC_{50} values of 10^{-6} – 10^{-3} M. Furthermore, they (268) tested the inhibitory potency of synthetic cyclic hexapeptides (FRCRCFa) on $\text{Na}^+\text{-Ca}^{2+}$ exchange activity in view of the chemical model studies showing that the intramolecular cyclization of linear peptide inhibitors might restrict a conformational flexibility of the structure. FRCRCFa inhibits the Na^+ -dependent Ca^{2+} uptake in the sarcolemmal vesicles with an IC_{50} value of $10\ \mu\text{M}$. FRCRCFa inhibits Na^+ -dependent Ca^{2+} efflux (IC_{50} value of $2\text{--}3\ \mu\text{M}$) more potently than Na^+ -dependent Ca^{2+} influx. This

A. Peptides

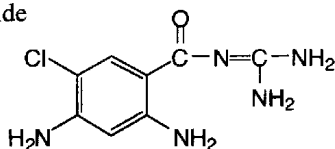
XIP RRLIFYKYVYKRYRAGKQRG

FMRFa (tetrapeptide) FMRF-CONH₂

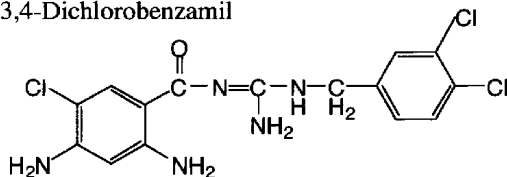
FRCRCFa (cyclic hexapeptide) FRCRCF-CONH₂

B. Chemicals

Amiloride



3,4-Dichlorobenzamil



No.7943

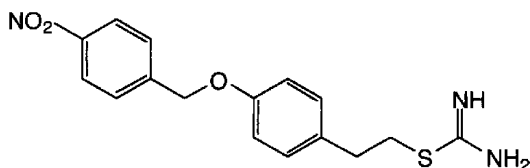


Fig. 6. Inhibitors of the $\text{Na}^+\text{-Ca}^{2+}$ exchanger.

contrasts with XIP that is a more potent inhibitor of the Na^+ -dependent Ca^{2+} influx than the Na^+ -dependent Ca^{2+} efflux (262). These studies not only suggest that FRCRCFa and XIP may bind to distinct inhibitory sites but also imply that the forward and reverse modes of the Na^+ - Ca^{2+} exchanger have distinct properties.

B. Chemicals

Amiloride and its derivatives have been identified as relatively effective inhibitors of the Na^+ - Ca^{2+} exchanger (16, 259). Na^+ - Ca^{2+} exchange activity is also inhibited by several drugs such as alcohol (269), tricyclic antidepressants (270), calmodulin antagonists (271) and taurine (272). The inhibitor has a positive inotropic effect (273), antiarrhythmic activity (274) and blocking effect on taurocholate-induced relaxation of guinea pig ileum smooth muscle (275). However, their application is strictly limited for most biomedical experiments because they inhibit a number of other Na^+ transport systems and the voltage-gated Na^+ and Ca^{2+} channels (16, 259).

A newly synthesized compound, 2-[2-[4-(4-nitrobenzyloxy)phenyl]ethyl]isothiourea (designated No. 7943), has recently been reported as a potent and selective inhibitor of the Na^+ - Ca^{2+} exchanger by Iwamoto et al. (276) and Watano et al. (277). No. 7943 inhibits Na^+ -dependent Ca^{2+} uptake in cardiomyocytes, smooth muscle cells and Na^+ - Ca^{2+} exchanger-transfected fibroblasts with a IC_{50} of 1.2–2.4 μM , and it does not affect other transport systems at 10 μM . It may be of interest that the effect is more potent in the Ca^{2+} influx mode than in the Ca^{2+} extrusion mode of the Na^+ - Ca^{2+} exchanger in intact cells. This means that there is a distinct difference in properties between the forward and reverse modes of the Na^+ - Ca^{2+} exchanger, as noted in the peptide inhibitors. They (276, 277) suggested that No. 7943 could have therapeutic potential as a selective blocker of excessive Ca^{2+} influx mediated via the Na^+ - Ca^{2+} exchanger under pathological conditions, which include cardiac ischemia/reperfusion, hypoxia/reoxygenation, and possibly some forms of essential hypertension.

VIII. Conclusions

The Na^+ - Ca^{2+} exchanger has been attractive to pharmacologists because the exchanger may be one of molecular targets for improvement of Ca^{2+} -mediated pathology. The most interesting point at the beginnings of Na^+ - Ca^{2+} exchanger research was that the exchanger might be responsible for the pharmacological effect of cardiac glycosides, and its inhibitors had a positive inotropic effect in isolated guinea pig atria (11, 12). However, there is no inhibitor of the Na^+ - Ca^{2+} exchanger that is clinically used (42). Due to advances in

the Na^+ - Ca^{2+} exchanger research at both molecular and physiological levels in the 1990s, much is now known about the structure, mechanism, regulation, and functional roles of the exchanger. These studies indicate that the Na^+ - Ca^{2+} exchanger has indeed a key role of the regulation of $[\text{Ca}^{2+}]_i$ and may be involved in Ca^{2+} -mediated pathological states as described above. They also raise several questions. How are the isoforms of the Na^+ - Ca^{2+} exchanger expressed in a tissue-specific manner? Do the isoforms have the different functions and regulation mechanisms? Are there endogenous factors affecting the Na^+ - Ca^{2+} exchange activity, like the case of the Na^+ , K^+ -ATPase? In the pharmacological respect, it seems worthwhile to investigate the drugs that modulate the Na^+ - Ca^{2+} exchanger in isoform- and mode-specific manners. The previous studies (262, 268, 276, 277) indicating that the forward mode and reverse mode of the Na^+ - Ca^{2+} exchanger have different affinity to the inhibitors open the possibility for development of such a drug. We hope future studies will address the above questions and certainly they will contribute to the development of new drugs targeting the Na^+ - Ca^{2+} exchanger.

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