The Na\(^+\)-Ca\(^{2+}\) exchanger (NCX) links transmembrane movements of Ca\(^{2+}\) ions to the reciprocal movement of Na\(^+\) ions. It normally functions primarily as a Ca\(^{2+}\) efflux mechanism in excitable tissues such as heart, but it can also mediate Ca\(^{2+}\) influx under certain conditions. Na\(^+\) and Ca\(^{2+}\) ions exert complex regulatory effects on NCX activity. Ca\(^{2+}\) binds to two regulatory sites in the exchanger’s central hydrophilic domain, and this interaction is normally essential for activation of exchange activity. High cytosolic Na\(^+\) concentrations, however, can induce a constitutive activity that bypasses the need for allosteric Ca\(^{2+}\) activation. Constitutive NCX activity can also be induced by high levels of phosphatidylinositol-4,5-bisphosphate (PIP\(_2\)) and by mutations affecting the regulatory calcium binding domains. In addition to promoting constitutive activity, high cytosolic Na\(^+\) concentrations also induce an inactivated state of the exchanger (Na\(^+\)-dependent inactivation) that becomes dominant when cytosolic pH and PIP\(_2\) levels fall. Na\(^+\)-dependent inactivation may provide a means of protecting cells from Ca\(^{2+}\) overload due to NCX-mediated Ca\(^{2+}\) influx during ischemia.

Na\(^+\)/Ca\(^{2+}\) exchange (NCX) is a carrier-mediated transport process that translocates Ca\(^{2+}\) ions across membranes in an obligatory exchange for Na\(^+\) ions. In excitable tissues such as heart and nerve, it functions primarily as a plasma membrane Ca\(^{2+}\) efflux mechanism, although it can also mediate Ca\(^{2+}\) influx given the appropriate thermodynamic gradients. NCX activity is regulated principally by the ions that comprise the major determinants of the NCX driving forces, i.e., cytosolic Na\(^+\) and Ca\(^{2+}\). Increases in cytosolic Na\(^+\) down-regulate NCX activity by inducing an inactive state of the exchanger (Na\(^+\)-dependent inactivation), whereas increases in cytosolic Ca\(^{2+}\) upregulate activity (through allosteric Ca\(^{2+}\) activation). Here we will briefly review the history and molecular biology of Na\(^+\)/Ca\(^{2+}\) exchange, describe the major characteristics of Na\(^+\)-dependent inactivation and allosteric Ca\(^{2+}\) activation, and discuss issues related to the possible physiological roles of these regulatory mechanisms. We will focus mainly on the cardiac isoform of the Na\(^+\)/Ca\(^{2+}\) exchanger (NCX1.1). The Na\(^+\)/Ca\(^{2+}\) exchange family of transporters has been the subject of several recent reviews.1-3

### A Brief History of Na\(^+\)/Ca\(^{2+}\) Exchange

The existence of a transporter linking oppositely-directed movements of Na\(^+\) and Ca\(^{2+}\) across the plasma membrane was first described 40 years ago by two groups working independently and respectively with squid giant axons\(^4\) and guinea pig atria.\(^5\) It was immediately recognized that this novel transporter could be crucial to the understanding of the inotropic effects of cardiac glycosides. Experimental work in the two decades that followed, done mostly with internally dialyzed squid axons or barnacle muscle, did much to establish the basic features of Na\(^+\)/Ca\(^{2+}\) exchange and its regulation by ATP and by cytosolic Ca\(^{2+}\) (see review by Blaustein and Lederer\(^6\)).

In 1979, the introduction of plasma membrane vesicles for exchange studies provided an important biochemical tool for further characterization of exchange activity. The stoichiometry of the exchanger was demonstrated to be 3Na\(^+\)/1Ca\(^{2+}\) in null-point studies with vesicles where the electrical potential as a driving force for exchange activity was offset by an oppositely directed Na\(^+\)-gradient.\(^7\) This value is still generally accepted although there have recently been indications from exchange current measurements of higher stoichiometries.\(^8,9\) The situation is complicated by the existence of a Na\(^+\)-Ca\(^{2+}\) co-transport mode of the exchanger which provides an electrogenic Na\(^+\)-leak current when Na\(^+\) plus Ca\(^{2+}\) exchanges for Ca\(^{2+}\) alone.\(^10\)

The use of plasma membrane vesicles also provided a route for the purification and identification of the exchanger protein using detergent solubilization, protein purification and vesicle reconstitution techniques. Philipson and his colleagues\(^11\) succeeded in cloning the cardiac exchanger (NCX1) in 1990. The Philipson group later described additional genes coding for NCX2 and NCX3 (both expressed primarily in brain and skeletal muscle). The cDNA for the Na\(^+\)/Ca\(^{2+}\) exchanger in squid axons, in which so much early work was carried out, was cloned in 1998;\(^12\) the squid axon exchanger (NCX-SQ1) showed 58% identity to mammalian NCX1.

Electrical currents due to Na\(^+\)/Ca\(^{2+}\) exchange activity were first demonstrated definitively by Kimura et al.\(^13\) Electrophysiological studies of Na\(^+\)/Ca\(^{2+}\) exchange were markedly enhanced by the use of giant membrane patches, initially from cardiac myocytes and later from Xenopus oocytes expressing the exchanger.\(^14,15\) The giant patch technology allowed the fluid composition on both sides of the membrane to be controlled and provided access to the cytosolic membrane surface for biochemical modification.
The use of retinal rod outer segments in exchange studies, first described by Schnetkamp,16 provided insight into a different kind of exchange process, one that had an absolute requirement for the co-transport of Ca\(^{2+}\) plus K\(^{+}\).17,18 The stoichiometry of this exchanger (NCKX) was determined to be 4 Na\(^{+}\) per 1 Ca\(^{2+}\) plus 1 K\(^{+}\). The retinal rod exchanger was cloned in 1992 by Cook and his colleagues19 and showed little homology to the cardiac (NCX) family of exchangers, except in two short regions called the α-repeats (see below). Subsequent studies established that the retinal rod exchanger (NCKX1) was the founding member of a group of at least 5 different K\(^{+}\)-dependent exchangers.20 The NCX and NCKX exchangers are themselves members of a much larger family of cation/Ca\(^{2+}\) exchangers.21 The functional properties of most of these exchangers have not yet been determined.

**Molecular Architecture of the Cardiac Na\(^{+}/Ca^{2+}\) Exchanger**

The mature form of the cardiac Na\(^{+}/Ca^{2+}\) exchanger (NCX1.1) is a protein of 938 amino acids containing nine transmembrane segments (TMS) (Fig. 1). An initial transmembrane segment is cleaved as part of a signal sequence and is not present in the mature protein.22,23 By convention, numbering of the amino acid residues of NCX1 starts after the signal sequence cleavage site (32 residues after the start site). The exchange protein can be divided into three separate domains: an N-terminal region containing five transmembrane segments, a cytosolically disposed central hydrophilic domain, 544 residues in length, and a C-terminal domain containing the remaining four transmembrane segments. The topology of the exchanger was firmly established using cysteine-mutagenesis combined with accessibility to permeable and non-permeable sulfhydryl reagents.24,25

Two regions, called the α-repeats, are homologous to each other and are highly conserved in all members of the exchanger family, including the NCKX exchangers. Within the α-repeat regions of NCX1 are two re-entrant loops in which some residues appear to be accessible from both sides of the membrane. The two α-repeat regions lie close to one another in the protein, as shown by cysteine-based cross-linking studies (Fig. 2).26 The re-entrant loops were initially suggested to be involved in ion translocation, by analogy to the P-loops of ion channels, but, at least for the α-1 repeat, mutagenesis studies did not support this hypothesis.27 Mutations in TMS segments 2 and 3, however, showed marked defects in NCX function. It was suggested that residues in TMS 3 are involved in binding of Na\(^{+}\) ions, but not of Ca\(^{2+}\) ions.27 NCX activity was also strongly inhibited by certain mutations in TMS 7 and within the re-entrant loop of the α-2 repeat.28

There are two additional regions, termed the β-repeats, that show close homology to each other and are highly conserved among members of the NCX family, although they are not represented in the NCKX family.29 The β-repeats form the primary backbone of the two calcium-binding domains, which will be discussed in more detail below.

The central hydrophilic domain contains several regions of structural and functional interest. At the very beginning of the hydrophilic domain, the region designated XIP (Fig. 1) comprises 19 amino acids highly enriched in basic and hydrophobic residues. Given its resemblance to a calmodulin-binding autoinhibitory domain, Philipson et al.,30,31 prepared a peptide corresponding to this region and found that it strongly inhibited NCX activity, hence the designation eXchange Inhibitory Peptide for XIP. Although calmodulin was found to have no effect on NCX activity, subsequent work established that XIP strongly interacts with phosphatidylinositol-4,5-bisphosphate (PIP\(_2\)) and appears to be an important determinant of the regulatory effects of Na\(^{+}\) on NCX activity (see below).

Following XIP is a bi-partite region which has been designated the catenin-like domain (CTL; residues 217–370 and 651–705).32 This region has modest (~22–24%) homology to the adherens junction protein catenin; it also shows homology to proteins involved in nuclear protein import (e.g., transportin 1 and karyopherin). These homologies suggest that this region may be important in protein-protein interactions, although this hypothesis has yet to be tested.

Two tandem calcium-binding domains (CBD-1 and -2) separate the two portions of the CTL (Fig. 1). The regions mediate the allosteric effects of cytosolic Ca\(^{2+}\) on NCX activity (see below). The structures of both domains have been determined by NMR spectroscopy32 and by X-ray crystallography;33,34 the crystallographic structures are shown in Figure 3. Both domains display 7 beta strands arranged in two anti-parallel β sheets, a common arrangement known as the immunoglobulin fold. The Ca\(^{2+}\) binding regions are formed by loops connecting the beta strands at the top of the figures. CBD1 binds four calcium ions while CBD2 binds only two. Isothermal titration calorimetry32 yielded apparent K\(_d\) values for Ca\(^{2+}\) of 120 nM and 240 nM for CBD1, while the affinity of CBD2 was much lower (K\(_d\) 820 nM and 8.6 μM). Importantly, removal of Ca\(^{2+}\) induced major structural re-arrangements of CBD1, but the CBD2 structure changed only slightly.32,33 The structural stability of CBD2 in the absence of Ca\(^{2+}\) probably reflects the presence of a stabilizing salt-bridge between Lys-585 and two acidic residues in CBD2, an arrangement that is absent in CBD1.

Tissue specific splice variants of NCX1 are found in the C-terminal region of CBD2 and the distal portion of the CTL. Two exons, A and B, are mutually exclusive and are followed by four additional exons (C–F), which may or may not be expressed. The A exon is expressed primarily in excitable tissues, while the B exon...
is expressed in non-excitable tissues such as the kidney (but also in vascular smooth muscle). Comparison of the properties of NCX1.4 (exons AD, expressed in brain) and NCX1.3 (exons BD, kidney, smooth muscle) revealed significant differences in their detailed regulatory responses to Na⁺ and Ca²⁺, although the essential attributes of Na⁺- and Ca²⁺-dependent regulation were similar in both.35

New evidence from the Philipson laboratory demonstrates that NCX1.1 forms dimers.36 The functional consequences of dimerization are unknown but this is certain to become an interesting avenue of investigation.

**Allosteric Regulation of NCX Activity by Ca²⁺**

In experiments with dialyzed squid axons in 1979, Dipolo found that Na⁺-driven Ca²⁺ influx (“reverse” mode exchange) required the presence of μM levels of cytosolic Ca²⁺, indicating that Ca²⁺ is an allosteric activator of NCX.37 Subsequent experiments have demonstrated that this requirement holds for all tested gene products and splicing variants of NCX, with the exception of the exchanger from Drosophila (CalX) (see below). On the other hand, no allosteric Ca²⁺ activation has yet been described for any of the K⁺-dependent exchangers (NCKX).

The site of allosteric Ca²⁺ activation has been localized through mutational and structural analysis to the calcium binding domains (CBD-1, -2) described above. Both CBD1 and CBD2 are essential for allosteric Ca²⁺ activation. Removal of portions of the cytosolic loop containing the CBD’s, either by mutation or by treatment with proteases, results in constitutive behavior, i.e., exchange activity no longer requires allosteric Ca²⁺ activation. Mutations in CBD1 that lower its affinity for Ca²⁺ produce a corresponding increase in the concentration of Ca²⁺ required to activate the exchanger.38 Thus, the conformational changes in CBD1 that are linked to Ca²⁺ binding (see above) appear to be an essential signaling event in allosteric Ca²⁺ activation. In contrast to this behavior, mutations that disrupt Ca²⁺ binding in CBD2 yield an exchanger that no longer requires allosteric Ca²⁺ activation.33 Thus, CBD2 is essential for normal allosteric Ca²⁺ activation, although how it impinges on the signaling role of CBD1 remains a mystery. A role for the catenin-like domain in transmitting the signal from the CBD’s is suggested by a recent finding from our lab that deletion of the proximal portion of the CTL also yields constitutive behavior.

The exchanger from Drosophila, CalX1, behaves in a rather bizarre fashion in comparison to other NCX family members: in CalX1, activity does not require allosteric Ca²⁺ activation and, in fact, cytosolic Ca²⁺ inhibits exchange activity.39 Overall, CalX1 is 47% identical to NCX1.1 with 66% similarity. The Ca²⁺ binding domains exhibit a similar level of homology and the residues that directly interact with Ca²⁺ are completely conserved. A chimera was constructed in which CBD1 of NCX1 (plus small flanking regions on either side) replaced the corresponding region of CalX1 (39% identity, 58% similarity). This construct showed activation of exchange activity by cytosolic Ca²⁺ rather than inhibition.40 Thus, relatively modest differences in the sequence of CBD1 can invert the normal Ca²⁺ regulatory properties of the exchanger. Since the overall structure of the CalX CBD1 is not likely to be dramatically different from the NCX CBD1 (although this remains to be seen), there must be profound differences between the two exchangers in the molecular interactions of the Ca²⁺ binding domains with the remainder of the protein.

For the cardiac isoform of NCX (NCX1.1), most reports indicate that allosteric activation of activity occurs within the range of 150–400 nM. This is nicely situated within the range of the normal cardiac [Ca²⁺] transient. The time dependence of allosteric Ca²⁺ activation and de-activation is of particular importance with respect to its physiological function. While most observers, with one exception,41 find that high concentrations of cytosolic Ca²⁺ activate exchange activity very rapidly (<50 ms42,43), concentrations of 200–300 nM appear to activate activity much more slowly.44,45 It is unclear where physiological changes of cytosolic Ca²⁺ (e.g., 100–1,000 nM) fall within this spectrum of responses, but the high local sub-membrane Ca²⁺ concentrations generated during Ca²⁺ release from the sarcoplasmic reticulum46 suggest that NCX activation is more likely than not to be a rapid event (but see Section V).

Deactivation of activity upon removal of cytosolic Ca²⁺ requires several seconds. This was evident in early experiments by Hilgemann and his colleagues in which exchange currents were measured in excised “giant” patches. The simultaneous application of cytosolic Na⁺ (to induce outward (reverse-mode) exchange currents) and the
removal of cytosolic Ca\(^{2+}\) (to deactivate exchange activity) led to a transient exchange current that decayed to zero within 10 s. Other experiments showed that the decay of the activated state following Ca\(^{2+}\) removal was prolonged or even eliminated in patches treated with the signaling lipid PIP\(_2\). In transfected Chinese hamster ovary cells, time-dependent deactivation of NCX was manifest as the retention of exchange activity for tens of seconds following the decay of a [Ca\(^{2+}\)] transient even though cytosolic [Ca\(^{2+}\)] had declined far below the level required to activate the exchanger. This was termed “persistent Ca\(^{2+}\) activation” and was initially thought to reflect elevated concentrations of Ca\(^{2+}\) beneath the plasma membrane. Now, however, it appears more likely that alloteric Ca\(^{2+}\) activation displays hysteresis, such that the rate of deactivation is slow unless cytosolic [Ca\(^{2+}\)] is reduced far below physiological levels. Experiments in cardiac myocytes show that following allosteric activation of exchange activity by Ca\(^{2+}\), little or no deactivation of the exchanger was found during tens of seconds at rest. The mechanism underlying this effect remains unknown, but the results raise perplexing questions about the possible physiological role of allosteric Ca\(^{2+}\) activation in cells such as cardiac myocytes that undergo regularly spaced [Ca\(^{2+}\)] transients (see Section V. Physiological Perspectives below).

Ablation of the requirement for alloteric Ca\(^{2+}\) activation can be produced by mutation, as discussed above, but also by simply elevating the cytosolic Na\(^{+}\) concentration. This behavior surfaced during our investigations of Chinese hamster ovary cells expressing a CBD-1 mutant, D447V, that had a markedly reduced affinity for regulatory Ca\(^{2+}\) (K\(_h\) ~ 1.8 \(\mu\)M) compared to the wild-type NCX1 (0.3 \(\mu\)M). At low concentrations of cytosolic Na\(^+\) and Ca\(^{2+}\) (<20 mM and <100 nM, respectively) reverse-mode exchange activity was immeasurably low, as expected. However, activity progressively increased as cytosolic Na\(^+\) increased although the initial cytosolic Ca\(^{2+}\) remained <100 nM. We concluded that exchange activity became independent of allosteric Ca\(^{2+}\) activation at the higher cytosolic Na\(^+\) concentrations. Since this was not the case when exchange activity was measured in excised patches, we suggested that the high levels of PIP\(_2\) in the intact cells might have promoted the constitutive activity seen in our experiments. Indeed, in excised patches, exchange activity became independent of allosteric Ca\(^{2+}\) activation when PIP\(_2\) levels were increased.

The various conditions that induce constitutive behavior of the exchanger are summarized in Figure 4. Clearly, allosteric Ca\(^{2+}\) activation is a complex regulatory process that is not completely understood at present.

**Inactivation of NCX by Cytosolic Na\(^{+}\)**

It has long been known that Na\(^{+}\) and Ca\(^{2+}\) compete for transport by NCX. Our early experiments with sarcolemmal vesicles showed that at high concentrations, Na\(^{+}\) competed with Ca\(^{2+}\) in a 2:1 ratio. Therefore we suggested that one of the translocation sites on the exchanger can bind either 1 Ca\(^{2+}\) ion or 2 Na\(^{+}\) ions (A site) while a second (B) site binds the 3\(^{rd}\) Na\(^{+}\) ion involved in Na\(^+\)/Ca\(^{2+}\) exchange. Since the ionic radii of Na\(^{+}\) and Ca\(^{2+}\) are very similar, the Na\(^{+}\) bound conformation of the translocation site would necessarily differ substantially from the Ca\(^{2+}\) bound conformation. We speculate that this conformational difference may underlie the induction of constitutive behavior noted above, as well as a Na\(^{+}\)-dependent inactivation process that will be discussed in this section.
Ionic regulation of Na/Ca exchange

agents for the treatment of cardiac ischemia and essential hypertension. SEA0400 acts at submicromolar concentrations and has few non-specific effects on channel activity. SEA0400 appears to inhibit NCX activity by promoting Na+-dependent inactivation as shown by the following observations: SEA0400 inhibits steady-state outward NCX currents more effectively than peak currents and it is a more effective inhibitor at high Na+ concentrations than at low concentrations. Moreover, sensitivity to inhibition by SEA0400 correlates with susceptibility to Na+-dependent inactivation in various XIP mutants. Thus, F223E, which is highly susceptible to Na+-dependent inactivation, is hypersensitive to SEA0400, while the resistant K229Q mutant is also resistant to SEA0400.

It has been noted that these agents are more effective inhibitors of the reverse (Ca2+ influx) mode of NCX activity than of the forward (Ca2+ efflux) mode of activity, a property that would appear to violate the laws of thermodynamics. These findings, however, are likely to be a consequence of the ionic conditions during NCX activity assays. Thus, cytosolic Na+-concentrations are generally kept low in assays for the forward mode while they are substantially higher when assaying for the reverse mode. Under conditions where both modes of activity can be assayed at similar cytosolic Na+ concentrations, there is little or no difference in inhibitory potency between the two modes. This linkage between inhibition by SEA0400 and Na+-dependent inactivation is highly desirable for an NCX-based therapeutic agent. Thus, one would expect SEA0400 to have minimal effects on NCX activity (and Ca2+ homeostasis) under normal physiological conditions but strongly inhibit activity when Na+-dependent inactivation is promoted by cytosolic Na+-overload, as in ischemia.

A dramatic illustration of the possible therapeutic efficacy of SEA0400 comes from studies of hypertension in transgenic mice. Mice overexpressing NCX1.3 in vascular smooth muscle showed increased hypertension in response to induced salt-retention compared to wild-type mice, and administering SEA0400 blocked this hypersensitivity. However, SEA0400 was ineffective under the same conditions in mice overexpressing a SEA-resistant mutant of NCX1.3. Incidentally, SEA0400 had no effect on blood pressure in control animals. The experiments provided strong support for the Blaustein hypothesis, which envisions salt-sensitive hypertension as arising from NCX-mediated Ca2+ influx in vascular smooth muscle secondary to Na+/K+-ATPase inhibition (more details in ref. 68).

How does XIP influence NCX activity? Although the mechanism has not been definitively established, it is thought that the XIP region acts as an autoinhibitory domain and blocks activity when it interacts with a docking site elsewhere on the exchange protein (Fig. 6). Presumably, this interaction would be promoted by the conformational state induced by high concentrations of cytosolic Na+. This docking site has not yet been identified, although there are indications that it might lie within residues 562–679 of the central hydrophilic domain. According to this view, PIP2 disrupts the autoinhibitory interaction by binding to the XIP region (Fig. 6). This is not necessarily a highly specific interaction, however, since phosphatidyl serine and anionic amphiphiles such as dodecylsulfate also antagonize Na+-dependent inactivation.

The actions of certain NCX inhibitors are tied closely to Na+-dependent inactivation. Benzylxoyphenyl derivatives such as SEA0400 have attracted much attention recently as possible therapeutic

Figure 5. Na+-dependent inactivation of NCX1.1. (A) Typical current trace from excised patch experiment. Application of cytosolic Na+ leads to peak of outward current followed by decay to steady state value (left). The decay phase is eliminated by treating patch with chymotrypsin (right). Data reprinted with permission from Hilgemann. (B) ATP reverses Na+-dependent inactivation; activity persists after ATP removal. (C) ATP is ineffective in patches treated with PI-specific phospholipase C but addition of PIP2 leads to reversal of inactivation. Figure modified with permission from Hilgemann and Ball.

Figure 6. Hypothetical scheme illustrating mechanism of Na+-dependent inactivation and effect of PIP2. See text for details.
Physiological Perspectives

The work summarized above characterizes the two major mechanisms regulating the activity of the cardiac Na⁺/Ca²⁺ exchanger. There are troubling conceptual issues, however, suggesting that neither of these mechanisms would be very effective in regulating NCX activity in normally functioning myocardial muscle. As for Na⁺-dependent inactivation, it seems unlikely in cardiac cells. As for Na⁺-dependent inactivation, it seems unlikely that the exchanger would be nearly fully activated at the peak of the [Ca²⁺] transient (>1,000 nM). Due to the persistence of the activated state during the declining phase of the [Ca²⁺] transient, the exchanger would probably remain fully activated in the interval between beats, implying that exchange activity is continuously “on” in regularly contracting myocytes. In this setting, the rate of the forward (Ca²⁺ efflux) mode of NCX activity would be determined solely by the binding of [Ca²⁺] to the translocation sites (Kₘ ~ 5 μM), and not by allosteric Ca²⁺ activation.

Na⁺-dependent inactivation also seems an unlikely physiological regulator of NCX activity, since in normal functioning cells ATP and PIP₂ levels are high, and cytosolic Na⁺ levels are low. In our laboratory, we have recently completed studies of Na⁺-dependent inactivation in transfected Chinese hamster ovary cells expressing the cardiac NCX1.1 and various mutants. We found that the wild-type NCX was quite resistant to Na⁺-dependent inactivation, even when cytosolic [Na⁺] was increased to 140 mM and cellular PIP₂ was depleted. However, inactivation of the wild-type NCX could readily be demonstrated when the cytosol was acidified. We therefore suggested that this inactivation process was unlikely to be an important regulator of NCX activity under normal physiological conditions, but could serve a protective function to limit NCX-mediated Ca²⁺ influx under conditions such as ischemia in which the cytosol is acidified, cytosolic Na⁺ levels are elevated and ATP/PIP₂ levels are low.

Given these considerations, what functions could allosteric Ca²⁺ activation and Na⁺-dependent inactivation serve under normal physiological conditions? We recently reported that the exchanger interacts with the cytoskeleton, particularly F-actin, and that this interaction imposes a constraint on allosteric Ca²⁺ activation, possibly leading to hysteresis in the activation process. We speculate that this could provide a mechanism for the gradual activation and deactivation of the exchanger, so that the exchanger would respond to the time-average of [Ca²⁺] transients over multiple beats in cardiac cells. As for Na⁺-dependent inactivation, it seems unlikely to us that this process has evolved solely as a means of protecting cells against Ca²⁺ overload during ischemic events. Our experiments suggest that increases in cytosolic Na⁺ have dual regulatory effects on NCX activity, promoting constitutive activity under normal physiological conditions but leading to inactivation when cytosolic ATP and pH levels fall. The constitutive state induced by Na⁺ obviously provides a means of by-passing allosteric Ca²⁺ activation as a regulatory pathway and, in the context of gradual activation/deactivation process discussed above, these two processes could provide subtlety and flexibility in regulating the exchange process in cardiac cells.

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