The Molecular Determinants of Ionic Regulatory Differences between Brain and Kidney Na\(^+\)/Ca\(^{2+}\) Exchanger (NCX1) Isoforms*

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The Na\(^+\)/Ca\(^{2+}\) exchanger gene NCX1 undergoes alternative splicing leading to several isoforms that differ in a small portion of the large cytoplasmic loop. This loop is involved in many regulatory processes of NCX1, including ionic regulation by the transported substrates Na\(^+\) and Ca\(^{2+}\). High intracellular Ca\(^{2+}\) can alleviate intracellular Na\(^+\)-dependent inactivation in exon A (NCX1.4)-containing isoforms but not in those containing the mutually exclusive exon B (NCX1.3). Giant excised patches from Xenopus oocytes expressing various NCX1 constructs were used to examine the specific amino acids responsible for these observed regulatory differences. Using a chimeric approach, the region responsible was narrowed down to the small central part of exon A (IDDEEYKNRT). Replacing the second aspartic acid of this sequence with arginine (the corresponding amino acid in exon B) in an exon A background completely prevented the effect of Ca\(^{2+}\) on intracellular Na\(^+\)-dependent inactivation. Mutating the second lysine to cysteine (exon B) had a similar, but only partial, effect. The converse double mutant, but neither single mutation alone, introduced into an exon B background (arginine to aspartic acid and cysteine to lysine) was able to restore the NCX1.4 regulatory phenotype. These data demonstrate that aspartic acid 610 and lysine 617 (using the rat NCX1.4 numbering scheme) are critical molecular determinants of the unique Ca\(^{2+}\) regulatory properties of NCX1.4.

The Na\(^+\)/Ca\(^{2+}\) exchanger (NCX) is an integral membrane protein found in nearly every cell type of the body where it plays an important role in Ca\(^{2+}\) homeostasis. Normally the NCX serves as a Ca\(^{2+}\) extrusion mechanism driven by the Na\(^+\) electrochemical gradient. In cardiac muscle, for example, Ca\(^{2+}\) entering through L-type channels during the action potential is subsequently extruded by the NCX. However, it has also been shown that Ca\(^{2+}\) influx through the exchanger can occur under certain conditions (1). The NCX family consists of three genes, NCX1, NCX2, and NCX3. These genes, with the exception of NCX2, undergo alternative splicing leading to many different isoforms (2–5). In the case of NCX1, 12 different alternatively spliced isoforms have been observed, several of which are expressed in a tissue-specific manner (6, 7). To date, the prototypical canine cardiac exchanger NCX1.1 has been most widely studied with respect to structure, function, and regulation (8, 9).

Alternative splicing of the NCX1 gene produces transcripts that differ in a segment corresponding to a small region of the protein in the large cytoplasmic loop, 600 amino acids from the beginning of the open reading frame (see Fig. 1). The splicing process results in the mutually exclusive use of exons denoted A or B followed by the inclusion or exclusion in any combination of the cassette exons C to F (5). Cardiac muscle expresses the NCX1.1 isoform encoded by a transcript containing alternatively spliced exons A, C, D, E, and F. The two alternatively spliced isoforms used in this study are NCX1.3 and NCX1.4. NCX1.3, which is encoded by a transcript containing alternatively spliced exons B and D, is the predominant isoform expressed abundantly in the kidney and ubiquitously elsewhere. NCX1.4, encoded by transcripts including alternatively spliced exons A and D, and NCX1.5 (which contains exons A, D, and F) are the major isoforms expressed in neurons of the brain. Exon A encodes 35 amino acids (residues 601–635), while exon B encodes one less at 34 (residues 601–634). Exon D is relatively small encoding for 6 amino acids (GGFTLT). The specific physiological functions of the NCX1 alternatively spliced products are largely unknown.

NCX activity is regulated in many different ways including by the ions it transports. Overall the rate of exchange is limited by the extent to which the different transport substrates saturate the ion translocation binding sites. However, NCX is also regulated allosterically by both transport substrates. Intracellular sodium (Na\(^+\)) regulates the NCX by inducing an inactive conformation in a process referred to as Na\(^+\)-dependent or I\(_1\) inactivation (10). In the absence of cytoplasmic Ca\(^{2+}\) (Ca\(^{2+}\)\(_i\)), the exchanger enters an inactive state in a process referred to as Ca\(^{2+}\)-dependent or I\(_2\) inactivation (11). These two regulatory processes show interaction. For example, in the cardiac exchanger NCX1.1, I\(_1\) inactivation is alleviated if Ca\(^{2+}\)\(_i\) is sufficiently high.

Recent studies using recombinant expression systems and in vitro assays have identified functional differences in the regulatory properties of NCX1 alternatively spliced isoforms. He et
al. (12) compared rat NCX1.4 (containing alternatively spliced exons A and D) and NCX1.3 (containing exons B and D) in Xenopus oocytes. They observed an increase of 39% in the activity of NCX1.4 upon activation by protein kinase A that was not seen for NCX1.3. Also two alternatively spliced NCX isoforms of Drosophila melanogaster, Calx1.1 and Calx1.2, which differ by five amino acids at a position corresponding to the alternative splicing site of mammalian NCX1, show significant differences in their regulatory responses to Ca$^{2+}$, and Na$^+$ (13).

One of our laboratories has recently identified ionic regulatory differences between the two NCX1 isoforms NCX1.4 and NCX1.3 (14). Dyck et al. (14) used outward exchange currents measured in giant excised patches from Xenopus oocytes to demonstrate that Na$^+$-dependent inactivation was alleviated by high Ca$^{2+}$, in NCX1.4 but not in NCX1.3.

In this study, we used the rat isoforms NCX1.3 and NCX1.4 to determine which amino acids contribute to the different regulatory properties of these exchangeers. Using electrophysiological recordings we compared the functional properties of different isoforms and mutants and were able to identify two residues that are critical in conferring the unique regulatory properties for each isoform.

**MATERIALS AND METHODS**

The rat NCX1.3 and NCX1.4 cDNAs were constructed as follows. The coding region of the NCX1.7 clone F1 (7) was excised using the MunI and Bst1107I restriction sites located at nucleotides 24 and +2913, respectively, where +1 is the start of the NCX1 open reading frame. This fragment was made blunt-ended using the Klenow fragment of DNA polymerase and ligated into SmaI-digested pBluescript SKII– (Stratagene). The region encoding the large cytoplasmic loop of kidney NCX1.5 and brain NCX1.4 was obtained using reverse transcription–coupled PCR of kidney and brain mRNA, respectively. A BstI fragment (+812 to +995) of this large cytoplasmic loop, which contained the NCX1.3- or NCX1.4-specific alternatively spliced region, was subsequently ligated into the BstI-digested pBluescript– NCX1.7 construct. The exchanger coding region was confirmed by sequencing and moved to the pcDNA3.1+ mammalian expression vector (Invitrogen) using HindIII and BamHI restriction sites.

The products were produced by PCR overlap extension. The outer primer pair was named cd1 and cd2. Cd1 consisted of the sequence 5′-TTCGCTGGGTTGTCGACAGC-3′ from position +733 to +752. Cd2 was the reverse primer consisting of 5′-TGATACGATGATTACAAAACCAG3′ from position +2288 to +2267. The chimERIC constructs A/B and B/A were prepared using NCX1.4 and NCX1.3 as templates. Specifically the A fragment in the A/B chimera was produced using cd1 primer and an reverse primer with the exon A tail. These two fragments were amplified using the outermost primers that had overhanging ends that were of the exon A sequence. The B fragment was similarly made using the cd2 reverse primer and a forward primer of exon B sequence with a 5′ exon A tail. These two fragments were annealed, extended, and then amplified using the outer primer pair. The B/A chimera was similarly prepared. The BAB chimera was produced using cd1 and cd2 primers that had overhanging ends that were of the exon B sequence. These two fragments were annealed to each other with the innermost primers overlapped each other with the site of chimerism and a 5′ tail of exon B sequence. The cd1 outermost primer was used with a reverse inner primer of exon A sequence and an exon B overhang matching the sequence of exon B at the A1B junction of the BAB1 chimera. The cd2 outermost primer was used with a forward inner primer consisting of the exon B sequence with an overhang matching the exon A sequence at the A1B junction using NCX1.3 as template. These two fragments were then combined and allowed to anneal and extend for four PCR cycles followed by amplification using the outermost primers. The constructs with the individual point mutations were produced by an overlapping PCR technique where the innermost primers overlapped each other with the mutation included in the primer-overlapped region. The outermost primers were the same as those previously mentioned. All of the constructs that were produced by PCR were digested using AflII (nucleotide +988) and EcoI (nucleotide +1993 for NCX1.4 or nucleotide +1990 for NCX1.3) and ligated into the pcBluescript SKII–NCX1.4 or -NCX1.3 constructs, which were similarly digested. All constructs were confirmed by sequencing and then moved into the mammalian expression vector pcDNA3.1+ with HindIII and BamHI digestion.

All the constructed cDNAs in pcDNA3.1+ were linearized by digestion with the SmaI restriction enzyme, which cuts at a position 1396 nucleotides downstream of the end of the exchanger open reading frame in the pcDNA3.1+ vector. Following digestion, the linearized constructs were purified using the Qiagroup PCR Cleaning kit (Qiagen). cRNA was produced using the T7 mMessage mMachine kit (Ambion) according to the manufacturer’s instructions. cRNAs were dissolved in 30–40 μL of RNA-grade water yielding a final concentration of ~1 μg/μL. Xenopus laevis oocytes were then injected with 20–50 ng of the individual cRNAs.

The oocytes were prepared as previously described (14). Follie-free stage V-VI oocytes were selected and incubated at 16 °C in secondary water for 1 day after injection and examined the next day for expression. They were used for electrophysiological recordings for 3–6 days following injection.

The phenotypes of the different chimeras and site-specific mutants were compared by obtaining current traces using the giant excised patch-clamp technique as previously described (15–17). Briefly, borosilicate glass pipettes were pulled, and the tips were fire-polished to internal diameters of 20–30 μm. To increase seal stability and reduce electrical noise, the tips were coated in a Polyfilm™-mineral oil mixture. Prior to patching, the vitelline layer was dissected from the oocyte by placing them in a shrinking solution containing 100 mM potassium aspartate, 100 mM KOH, 100 mM MES, 20 mM HEPES, 5 mM EGTA, 5 mM MgCl2, pH 7.0 at room temperature for 15 min. Once the vitelline layer had been removed the oocytes were placed into a solution containing 100 mM KOH, 100 mM MES, 20 mM HEPES, 5 mM EGTA, 5–10 mM MgCl2, pH 7.0 at room temperature. Gigahm seals were formed with gentle suction, and the inside-out patches were removed carefully from movements of the micromanipulator. A custom-built 20-channel computer-controlled solution switcher was used for rapid solution switching. Data were acquired and analyzed using an Axopatch 200A amplifier (Axon Instruments) with Axotape and pClamp 6. To measure outward exchange currents the pipette solution contained 100 mM n-methyl-g-glucamine, 100 mM MES, 30 mM HEPES, 30 mM tetraethylammonium hydroxide, 8 mM CaCl2, 16 mM sulfamic acid, 6 mM KOH, 0.25 mM ouabain, 0.1 mM nifedipic acid, 0.1 mM flufenamic acid, pH 7.0 with MES at room temperature. To obtain outward exchange currents, the bath solutions were switched between Na$^+$- and Li$^+$- containing solutions: 100 mM Na$^+$ or Li$^+$ aspartate, 20 mM MOPS, 20 mM tetraethylammonium hydroxide, 20 mM CsOH, 10 mM EGTA, 0–9.91 mM CaCl2, 1–1.5 mM Mg(OH)2, adjusted to pH 7.0 with MES or LiOH. Ca$^{2+}$ and Mg$^{2+}$ concentrations were adjusted using MAXC software (18) to yield free concentrations of 0–10 μM and 1.0 μM, respectively. Experiments were conducted at 30 ± 1°C. Data are mean ± S.E. Fss, the fractional steady-state current, was calculated from the ratio of theoretical current level at infinite time (obtained from fitting current traces to a single exponential decay function) to the initial peak current. Fss values were compared, following a logarithmic conversion used to transform the data to a normal distribution, using one-way analysis of variance with the Turkey–Kramer procedure for post-test multiple comparisons (Instat 3.0a, GraphPad Software Inc.).

Peptides were produced in the peptide synthesis core facility at the University of Calgary corresponding to the sequence of exon A from the chimeras NCXBA1B and the corresponding region from exon B. These were called peptide A and B, respectively. The amino acid sequence of peptide A was IDDEEYKKNKTF and of peptide B was FDRDEEYEKCESF. Both peptides were found to be 98% pure by analytical high performance liquid chromatography. The peptides were applied in the bath, i.e. to the cytoplasmic surface of the exchanger, at a concentration of 25 μM.
RESULTS

The objective of this study was to identify the amino acids responsible for the functional differences in ionic regulation observed between the two \( \text{Na}^+/\text{Ca}^{2+} \) exchanger isoforms NCX1.3 and NCX1.4. Specifically, regulatory \( \text{Ca}^{2+} \) can alleviate \( \text{Na}^+ \)-dependent inactivation in exon A-containing isoforms but not in those containing exon B as previously shown for canine NCX1.3 and NCX1.4. Here we examined the same isoforms from rat that also differ only in the region of alternative splicing due to distinct mutually exclusive exons A and B. These two exons contain numerous identities and similarities as shown in Fig. 1 with the majority of identical amino acids occurring in the first half. Several constructs were generated, and the corresponding cRNA was injected into \( \text{Xenopus} \) oocytes. The giant excised patch-clamp technique was used to compare phenotypes between wild-type and mutant constructs.

Fig. 2 shows the alleviation of \( \text{Na}^+ \)-dependent inactivation at higher regulatory \( \text{Ca}^{2+} \) concentrations for the wild-type rat NCX1.4 but not for the NCX1.3 isoform. This illustrates the typical NCX1.4 phenotype, i.e., high exchange activity at high regulatory \( \text{Ca}^{2+} \). These effects of regulatory \( \text{Ca}^{2+} \), were also seen with the rat cardiac isoform NCX1.1 (data not shown). The current traces shown are representative single measurements. Summary data from several identical experiments for each construct are numerically compared in Fig. 4 using the fraction of steady-state to peak current (\( F_{ss} \)) values. The \( F_{ss} \) values obtained for the wild-type NCX1.4 and NCX1.3 isoforms at high (10 \( \mu \text{M} \)) \( \text{Ca}^{2+} \) were significantly different (0.84 \( \pm 0.02 \) versus 0.09 \( \pm 0.02 \), respectively, \( p < 0.001 \)). At 1 \( \mu \text{M} \) \( \text{Ca}^{2+} \), the \( F_{ss} \) values obtained for NCX1.4 and NCX1.3 were 0.16 \( \pm 0.02 \) versus 0.09 \( \pm 0.01 \), respectively (no significant difference).

Our first approach was to determine whether the residues involved in this functional difference were found in either the first or last half of exon A. Hence, two chimeras were produced. Chimera A/B consisted of the first 15 amino acids of exon A followed by the last 19 amino acids of exon B, and chimera B/A consisted of the first 15 amino acids of exon B followed by the final 20 amino acids of exon A. The current traces obtained from giant patches of oocytes injected with these mutant cRNAs are shown in Fig. 2. Neither chimera exhibited full exchange activity when exposed to high \( \text{Ca}^{2+} \), i.e., both chimeras underwent Na\(^+\)-dependent inactivation to an extent not different from NCX1.3 and significantly greater than that observed for NCX1.4 (Fig. 4).

This result suggested at least two possibilities. First, part of each half of exon A may be necessary for \( \text{Ca}^{2+} \)-mediated alleviation of Na\(^+\)-dependent inactivation. Alternatively, the crit-
Fig. 3. Representative outward exchange current traces obtained for single mutants and the double mutant constructs. Outward exchange currents are shown elicited by application of 100 mM Na\(^+\) at different [Ca\(^{2+}\)], to excised patches from oocytes injected with the indicated mutant NCX1 cRNA. The single mutant NCX1.4D10R illustrates the conversion of the NCX1.4 phenotype to the corresponding NCX1.3 one, whereas the NCX1.3R10D mutant does not result in the converse change. The individual mutants are numbered relative to the first amino acid of exon A or B. See legend to Fig. 2 for further details.

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NCX1.4 D10R

NCX1.4 K17C

NCX1.3 R10D

NCX1.3 C17K

NCX1.3 RDCK

NCX1.4 G24E, and NCX1.4K1 (removal of final lysine in exon A). None of these mutations significantly changed the NCX1.4 phenotype as is clear from the Fss data of Fig. 4. These results indicate that the observed switch in phenotype was not due to just a change in the overall charge of this region but rather was due to specific residues within the central small portion. Thus, of the eight NCX1.4 exon A mutants tested, six had no effect, and the remaining two fully (D10R) or partially (K17C) converted NCX1.4 to an NCX1.3 phenotype.

As phenotypic conversion occurred for the aspartic acid at position 10 and the lysine at position 17, the converse mutations were made in NCX1.3. The currents obtained using the arginine to aspartic acid at position 10 (NCX1.3R10D) point mutant were similar to those of NCX1.3 (Fig. 3), and the Fss values were not significantly different from those obtained with NCX1.3 (Fig. 4). Mutation of the cysteine at position 17 of exon B to lysine in exon A (NCX1.3C17K) also resulted in currents and Fss values that were slightly different from NCX1.3 at least at 10 \(\mu\)M Ca\(^{2+}\). However, the R10D,C17K double mutant in NCX1.3 (NCX1.3RDCK) yielded a phenotype with an Fss value at 10 \(\mu\)M Ca\(^{2+}\), identical to that of NCX1.4 and significantly different from NCX1.3. Thus mutating just two residues of exon B restored the ability of high Ca\(^{2+}\) to suppress the process of Na\(^+\)-dependent inactivation.

Interestingly the value of Fss for NCX1.3RDCK at 1 \(\mu\)M Ca\(^{2+}\), was significantly higher than that of NCX1.4 (\(p < 0.001\)).

NCX1.3 RDCK

To test the hypothesis that interaction of the critical portion of exon A with another site on the exchanger might be involved in the Ca\(^{2+}\)-mediated alleviation of Na\(^+\)-dependent inactivation, two peptides were produced corresponding to the small central region of exon A or B (amino acids 8–19; exon A, IDDEYEKNKTF; exon B, FDREEYKCSF). These peptides were added to the bath perfusate (cytosolic side of the patch) at 25 \(\mu\)M. Continual application of peptides A or B to either NCX1.3- or NCX1.4-expressing oocyte patches had no detecta-
The NCX1 isoforms are regulated in several ways including by the ions that they exchange (10, 11, 19–21). One of these ionic regulatory mechanisms observed is mediated by Na$^+$, when activity of the NCX is measured in excised oocyte patches, activation of the exchanger by rapid application of cytoplasmic Na$^+$ results in the rapid activation of exchange current followed by a slow inactivation process, ultimately resulting in much lower steady-state current levels. This type of inactivation is termed Na$^+$-dependent or I$_1$ inactivation (10). It is analogous to the use-dependent inactivation observed for many voltage-gated ion channels and appears to involve a redistribution of exchangers into active and inactive conformations (22).

In this study, the brain and kidney NCX1 isoforms NCX1.4 and NCX1.3, respectively, were used to explore the molecular determinants of the functional differences previously observed between them. Dyck et al. (14) examined these isoforms from dog and found that Na$^+$-dependent inactivation was alleviated by high levels of Ca$^{2+}$, in NCX1.4 but not in NCX1.3. These exchangers differ only by the presence of sequences encoded by mutually exclusive alternate exon A or B, respectively (Fig. 1). As is illustrated in Fig. 2, the phenomenon previously observed in dog was also evident in rat NCX1.4 and NCX1.3. The conservation of regulatory differences across species is suggestive of a physiologically relevant role for this phenomenon.

A chimeric approach was used first to narrow down the region of exon A required to observe Ca$^{2+}$-dependent phenotype as seen in Fig. 4, while NCX1.4K17C made, six had no effect on the current traces characteristic of the NCX1.4 phenotype. The result of the NCX1.3RDCK double mutant indicates that these two amino acids, aspartic acid 10 and lysine 17, are critical molecular determinants required for the unique Ca$^{2+}$-binding properties conferred by exon A. Interestingly there are seven amino acids conserved between exons A and B in the small region of exon A present in the BA1B chimera. Of these seven, four are negatively charged, three of which surround the aspartate at position 10, as illustrated in Fig. 1. It is possible that these conserved and negatively charged residues also play a role in the Ca$^{2+}$-dependent phenotype of NCX1.4.

There are several regions within the large intracellular loop of NCX1 that have been extensively studied with respect to ion regulation. A short positively charged and amphipathic amino acid segment resembling a calmodulin-binding motif is present at the beginning of the loop. Addition of an exogenous peptide corresponding to this sequence to the cytoplasmic surface causes inhibition of the exchanger and led to the moniker exchanger inhibitory peptide, or XIP (23). Thus, it has been hypothesized that the XIP sequence interacts with another region of the exchanger in an intramolecular fashion to cause inhibition of activity. Although it has been demonstrated that XIP can interact with a peptide corresponding to amino acids 445–455 of NCX1, it seems unlikely that this interaction causes inhibition as recent studies using split exchangers lacking amino acids 265–671 maintained XIP inhibition (24, 25). The XIP region is thought to mediate the activating influence of acidic phospholipids, such as phosphatidylinositol 4,5-bisphosphate, which are modeled to bind directly to XIP, hence preventing its inhibitory interactions with the second exchanger region (26, 27). Studies examining exchangers in which the XIP region has been altered by mutagenesis have also revealed that this region is intimately involved in mediating Na$^+$-dependent inactivation (28–31).

Two clusters of acidic amino acids at positions 445–455 and 498–509 in the cytosolic loop are thought to bind the regulatory Ca$^{2+}$, that is an absolute requirement for full exchanger activity (19, 32, 33). This process has been termed Ca$^{2+}$-dependent or I$_2$ regulation (11). Analysis of exchangers with mutations in these Ca$^{2+}$-binding regions has revealed both alterations in Ca$^{2+}$-activation properties and also changes to Na$^+$-dependent inactivation (21). Overall, ionic regulation of the exchanger is complex and must involve at least a functional interaction between the XIP and Ca$^{2+}$-binding domains. Moreover, it is
now clear that a third region, namely the alternative splicing region, plays a prominent role in ionic regulation.

Our study focused on the ability of elevated cytosolic Ca2+ to suppress Na+-dependent inactivation. The data reported here have implicated two specific amino acids within a cluster of acidic residues near the middle of mutually exclusive alternate exons A and B. Disruption of the sequence DDEEYE found in exon A by replacement of Asp-10 with Arg in exon B (DREEYE) resulted in loss of Ca2+-mediated suppression of Na+-dependent inactivation. It is possible that this region contributes to a Ca2+-binding site (perhaps in concert with the previously defined Ca2+-binding sites involved in Ca2+-dependent (I2) regulation of the exchanger) that when occupied prevents the inhibitory intramolecular interaction between XIP and its binding site elsewhere on the exchanger. Since two amino acid changes in exon B (i.e. the R10D,C17K double mutant) are required for NCX1.3 to gain Ca2+-mediated suppression of Na+-dependent inhibition, it is possible that constraining the acidic stretch in the correct conformation is essential to forming an effective Ca2+-binding site.

The experiments using peptides from the alternatively spliced region were performed in an effort to gain further insight into potential mechanisms for these regulatory phenotypes. We postulated that the addition of peptide A or B to the intracellular side of the giant excised patch expressing NCX1.4 might block the effect of the high Ca2+ either by interacting with Ca2+ itself or by interfering with some conformational change brought about by Ca2+ binding. Conversely it was hoped that the addition of peptide A or B to patches expressing NCX1.3 might lead to an NCX1.4 phenotype. Neither of the peptides, however, had any effect on the patches expressing the NCX1.4 or NCX1.3 isoforms.

Philipson’s group (32) used glutathione S-transferase fusions of different parts of the NCX1 cytoplasmic loop and found that only those constructs containing the acidic clusters showed any measurable Ca2+ binding. One of their constructs contained the region of alternative splicing without the calcium binding region(s), and this did not bind Ca2+ under their experimental conditions. It is possible that the acidic cluster within exon A contributes to a Ca2+-binding site but does not constitute one alone. Future Ca2+ binding experiments using longer fragments of the cytoplasmic loop encompassing the different potential Ca2+ binding regions may reveal differences in Ca2+ binding between exons A and B.

Analysis of tissue-specific expression of NCX1 alternatively spliced isoforms indicates that excitable cells such as myocytes and neurons express predominantly NCX1 transcripts containing exon A (isoforms NCX1.1, NCX1.4, and NCX1.5). On the other hand, non-excitable cells such as astrocytes or kidney epithelial cells express transcripts containing exon B (isoforms NCX1.3 and NCX1.7). Excitable cells experience much larger fluctuations in membrane potential and intracellular concentrations of Na+ and Ca2+ (particularly close to the face of the membrane) than do non-excitable cells. It seems likely that the interacting influence of Ca2+ and Na+ on NCX1 activity found in exon A-containing isoforms, but not in exon B-containing ones, is an adaptation designed to provide control over exchanger function in a cellular environment that experiences such large ionic fluctuations.

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