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Modulation of the Cardiac Na^+-Ca^{2+} Exchanger by Cytoplasmic Protons: Molecular Mechanisms and Physiological Implications

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Abstract

A precise temporal and spatial control of intracellular Ca^{2+} concentration is essential for a coordinated contraction of the heart. Following contraction, cardiac cells need to rapidly remove intracellular Ca^{2+} to allow for relaxation. This task is performed by two transporters: the plasma membrane Na^+-Ca^{2+} exchanger (NCX) and the sarcoplasmic reticulum (SR) $Ca^{2+}-$ ATPase (SERCA). NCX extrudes Ca^{2+} from the cell, balancing the Ca^{2+}entering the cytoplasm during systole through L-type Ca^{2+} channels. In parallel, following SR Ca^{2+} release, SERCA activity replenishes the SR, reuptaking Ca^{2+} from the cytoplasm.

The activity of the mammalian exchanger is fine-tuned by numerous ionic allosteric regulatory mechanisms. Micromolar concentrations of cytoplasmic Ca^{2+} potentiate NCX activity, while an increase in intracellular Na^+ levels inhibits NCX via a mechanism known as Na^+-dependent inactivation. Protons are also powerful inhibitors of NCX activity. By regulating NCX activity, Ca^{2+}, Na^+ and H^+ couple cell metabolism to Ca^{2+} homeostasis and therefore cardiac contractility. This review summarizes the recent progress towards the understanding of the molecular mechanisms underlying the ionic regulation of the cardiac NCX with special emphasis on pH modulation and its physiological impact on the heart.

Graphical abstract

Impact of cytoplasmic acidosis on proteins mediating excitation-contraction coupling

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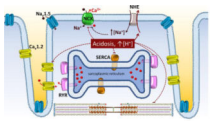
Author Contributions Section

N/A



Keywords

Na^+-Ca^{2+} exchanger; protons; pH regulation; calcium binding domains; excitation contraction coupling; Ca^{2+} dynamics

1. Introduction

Cytoplasmic protons play pivotal roles in cardiac cells: from powering mitochondrial respiration to modulating the activities of enzymes, contractile proteins, ion channels and transporters. Because of the multifaceted effects of protons, intracellular pH is tightly regulated within a narrow range as any untoward alterations in proton levels can lead to drastic changes in metabolism, enzyme function and ionic balances that power both the contractile and electrical activities of cardiac cells. Whereas small fluctuations of intracellular pH frequently occur during changes in cardiac workload [1], drastic changes in intracellular pH are seen during respiratory and metabolic acidosis, and myocardial ischemia [2, 3]. These conditions can be associated with decreased heart rate and contractile force, and if left untreated can ultimately lead to arrhythmia [2–7]. The detrimental effects of these pathological conditions are in large part due to the accompanying cellular acidosis and the direct interaction of protons with many of the Ca^{2+} handling proteins in the cell [7]. Of particular interest is the effects that pH exerts on the plasma membrane transporter: the Na^+-Ca^{2+} exchanger (NCX) [8], the activity of which is strongly inhibited by changes in cytoplasmic protons within the physiological range [9–12].

NCX is considered the main Ca^{2+} extrusion mechanism in cardiomyocytes [13–16]. It accomplishes this task by moving three Na^+ into the cells while transporting one Ca^{2+} in the opposite direction. This results in an electrogenic transport that can be measured as ionic current. By extruding Ca^{2+} from the cell in exchange for Na^+ influx, NCX helps restoring intracellular [Ca^{2+}] after each contraction to its diastolic levels. Although the normal mode of the exchanger is to extrude Ca^{2+} (forward mode) [13, 14, 17, 18], in certain instances set by Na^+ and Ca^{2+} gradients and membrane potential, NCX can promote Ca^{2+} influx. This “reverse mode” seems relevant during pathophysiological situations such as ischemia-reperfusion [19–21].

Due to the powerful inhibitory effect that pH exerts on NCX, intracellular protons are important regulators of Ca^{2+} dynamics. This review summarizes our current knowledge on the regulatory properties of NCX with emphasis on NCX pH regulation: the molecular determinants underlying its regulation; its effects on NCX activity; and the potential physiological impact of NCX pH regulation on heart function. We will focus on the cardiac isoform of the exchanger (NCX1.1) as most studies have been conducted using this exchanger.

2. Topology of the cardiac Na^+-Ca^{2+} exchanger

The cardiac Na^+-Ca^{2+} exchanger (NCX1.1) is composed by 970 amino acids with the first 32 cleaved off as part of an N-terminal signal sequence, which is not required for function [8, 22]. The current model predicts these residues to be organized into two groups of 5 transmembrane segments (10 total TMSs) separated by a large cytoplasmic loop (Figure 1). This membrane topology is based on the crystal structure of the archaebacterial homolog NCX_Mj [23] and biochemical studies conducted on the cardiac isoform [24, 25].

Among the transmembrane segments of special interest are TMSs 2, 3, 7 and 8 as they contain two sequences known as α-repeats (Figure 1). These regions, which span residues 97–150 in TMSs 2–3 (α 1-repeat) and 799–849 in TMSs 7–8 (α 2-repeat) of the cardiac isoform, show intramolecular homology, suggestive of gene duplication [26]. Moreover, they are highly conserved among other exchangers and are regarded as the signature sequence of the cation/Ca^{2+} transporter superfamily [26, 27]. There is a great deal of evidence to support their involvement in ion transport as mutations at these sites have been shown to dramatically affect the activity of eukaryotic exchangers [28–37]. The crystal structure of the archaebacterial exchanger (NCX_Mj) has further solidified their role in ion translocation by showing that the α-repeats contain 12 residues that coordinate the transported ions [23]. These amino acids are highly conserved between the archaebacterial and mammalian homologs [23] and they share functional similarity [30, 33, 35, 38–40].

Between TMSs 5 and 6 is a ~540 amino acid cytoplasmic loop, which confers extensive regulatory properties to NCX. At its N-terminus lies a stretch of 20 hydrophobic and positive amino acids (aa 219–238) termed the exchanger inhibitory peptide (XIP) region, as a peptide with identical sequence inhibits NCX activity when exogenously applied to the cytoplasmic surface of the exchanger [41]. It is well established that this region is involved in the regulation of NCX by intracellular Na^+ [41, 42] (see Section 3.1) and PIP2 [43, 44].

A recent NMR study showed the presence of a structured domain downstream of XIP. This region (aa 284–322) has been identified as a two helix bundle domain (THB) since it forms two tandem α-helices connected by a short linker [45]. Despite the conserved amino acid sequence of this domain, the functional role of THB remains to be determined.

Forty-nine amino acids downstream of THB lies two sequential regions responsible for Ca^{2+} regulation. They are identified as Ca^{2+}-binding domain 1 (CBD1; aa 371–501) and Ca^{2+}-binding domain 2 (CBD2; aa 501–678). These domains share the same β-sandwich architecture (Figure 2A) and form conjoint structures connected by a short linker within the full length exchanger (Figure 1). Several CBDs structures are available including the conjunct domains from the drosophila exchanger and the mammalian CBD1 mutated at position E454 [46–51].

CBD1 coordinates four Ca^{2+} ions, while CBD2 undergoes alternative splicing providing different Ca^{2+} coordinating properties [48–55]. Evidence shows that CBD2 expressing exon A coordinate two Ca^{2+} ions (Figure 2A), while exchangers expressing exon B in CBD2 do not bind Ca^{2+} [56]. The latter splice variant is mainly expressed in non-excitable cells, while the Ca^{2+} coordinating CBD2 isoforms are found in excitable cells, including the heart [57].

This suggests that the Ca^{2+}-binding domain properties of CBD2 are tailored to meet the specific needs of the cells where they are expressed. Several extensive reviews have covered this subject and the reader is referred to these for further information [48, 56, 58–60].

Finally, within the large cytoplasmic loop distal to the CBDs is an amphipathic α-helix encompassing residues 740–756. This region is found to be essential for palmitoylation of NCX at residue C739 [61–63].

As an atomic structure of a mammalian exchanger is not available, the three-dimensional organization of these regulatory regions as well as their molecular interactions with TMSs 1–5 and TMSs 6–10 remain unknown. Any such interactions are likely to translate the signal from the large regulatory loop to the transport sites embedded within the TMS to control the activity of NCX. This intramolecular level of regulation may be complicated by evidence indicating that the mammalian NCX exists at least as a dimer [64, 65] and that the large cytoplasmic loops of the adjacent proteins undergo conformational changes induced by cytoplasmic Ca^{2+} [64]. Clearly this information depicts a much more complex organization for the mammalian exchangers when compared to its archaebacterial homolog and it suggests that the mammalian NCX has evolved a complex regulatory system to ensure dynamic control of Ca^{2+} inside excitable cells in general and cardiac cells in particular, vital for efficient and timely contraction.

3. Allosteric regulation of the cardiac Na^+-Ca^{2+} exchanger

The most extensively studied regulatory mechanisms of NCX are its modulation by cytosolic ions: Na^+, Ca^{2+} and protons. Other non-ionic modulators have been shown to affect NCX activity which will be briefly mentioned herein. Among them are calmodulin [66], phosphorylation [67–69] and calpain which, by cleaving NCX within the large cytoplasmic loop, either activates [70] or inhibits [71] NCX activity, in a NCX isoform-dependent manner. Strong evidence also demonstrates that NCX activity is enhanced by lipids [72–74], while more recent findings show that Cys 739 within the large cytoplasmic loop of NCX is palmitoylated and that this is a prerequisite process for the Na^+-dependent inactivation to take place [63].

Together these regulatory mechanisms portray a multilevel and intricate control system to modulate NCX activity. This complicates determining the extent of NCX activity in both physiological and pathophysiological settings and more investigations are necessary to determine the relative contributions of each modulation to cardiac function.

3.1 Regulation by cytoplasmic Na^+ and Ca^{2+}

In addition to being transported, cytoplasmic Na^+ and Ca^{2+} allosterically regulate NCX activity. High concentrations of intracellular Na^+ (half inactivation ~15–20 mM) results in a slow decay of exchanger current (Figure 2B), via a process known as Na^+-dependent inactivation [42, 75]. The current model predicts that Na^+ bound to its cytoplasmic transport sites triggers conformational changes that inhibit activity [75]. The XIP region is strongly implicated in these molecular rearrangements as point mutations within this stretch of amino acids drastically alter the extent of Na^+-dependent inactivation, as shown in Figure 2C [41,

42]. Since high levels of Na^+ [42, 75, 76] are required to initiate the inactivation of NCX, its physiological relevance has been questioned [77]. Thus, experimental studies directly addressing the possible impact of this regulation in cardiac function are needed.

In contrast to the inhibitory effect of intracellular Na^+, an elevation of cytoplasmic Ca^{2+} stimulates NCX activity. The site for this "regulatory" Ca^{2+} is distinct from that of transported Ca^{2+}, as it binds to two domains within the large cytoplasmic loop (see Section 2). The binding of Ca^{2+} at these sites increases the NCX turnover rate and attenuates any inactivation caused by cytoplasmic Na^+ (Figure 2B) [42, 78–80].

Experiments conducted with the isolated conjoint Ca^{2+}-binding domains indicates a Ca^{2+} affinity in the lower nanomolar range (~10 nM) [64, 81], while the apparent Ca^{2+} affinity obtained from the full length exchanger tends to be higher (22 to 800 nM) [78, 80, 82, 83]. In spite of this large variability, electrophysiological studies in isolated cardiomyocytes provided strong evidence that NCX is regulated by cytoplasmic Ca^{2+} under physiological settings [84].

Although cytosolic Ca^{2+} is generally known as an activator of NCX, in certain instances it can act as a suppressor of exchanger activity. This is observed in mammalian cells in which repetitive Ca^{2+} influx results in decreased NCX1 activity [85], which seems to be due to a Ca^{2+}-dependent endocytotic process, i.e. removal of NCX from the plasma membrane [86].

NCX1 shares significant sequence and functional similarity with its two isoforms NCX2 and NCX3, including regulation by intracellular Ca^{2+}. As NCX2 and NCX3 are not the focus of this review, their Ca^{2+} regulatory properties are only briefly mentioned herein. Electrophysiological studies indicate that NCX2 currents are less sensitive to Ca^{2+} when compared to NCX1 [87], while the Ca^{2+} dependent regulation of NCX3 ionic currents is poorly characterized. This is mainly due to the limited expression of this protein in *Xenopus* oocytes which has prevented a detailed analysis of its biophysical properties. NCX3 appears to have a dual response to cytoplasmic Ca^{2+} by showing an initial activation, as seen in NCX1 [88], followed by a Ca^{2+}-driven inactivation process [85, 89]. This inactivation process may be responsible for the decline in NCX3 ionic currents observed upon repetitive Ca^{2+} influx [85].

Studies conducted with the isolated Ca^{2+} binding domains indicated that the properties of CBD1 are quite conserved among the three exchanger isoforms. Instead, CBD2 shows distinct features between the three isoforms, which likely contribute to the different responses of these exchangers to Ca^{2+} [48, 90]. We refer the reader to other references for a more detailed analysis of this subject [48, 90–92].

3.2 Modulation by cytoplasmic protons

Proton regulation of NCX was first identified in squid axons more than twenty years ago [93]. Subsequently, using highly purified sarcolemmal vesicles isolated from canine ventricles, Philipson and his collaborators demonstrated that Na^+-dependent Ca^{2+} uptake was inhibited at pH 6 and stimulated at pH 9 [12], providing the first demonstration that protons act directly on NCX activity. However, as the isolated vesicles contained both

outside-out (extracellular surface- outward) and inside-out (cytoplasmic surface-outward), it was not possible to definitively assign the sidedness of action of protons on NCX. Thus, Philipson further characterized NCX proton modulation by analyzing the effects of pH exclusively on inside-out vesicles. It was established that protons interact with the cytoplasmic portion of the cardiac exchanger, inhibiting its forward mode [12].

With the development of the giant excised patch technique and its application to NCX [94], it was possible to directly monitor the regulation of NCX by cytoplasmic pH. Using this approach, Hilgemann and colleagues observed [95] that half inhibition of the exchanger outward current occurred at physiologically relevant pH levels. Moreover, it was shown that alkaline pH could relieve the Na^+-dependent inactivation process, thereby increasing NCX activity. This result provided the first insight into a potential molecular process governing NCX pH regulation: namely demonstrating a link between proton and Na^+ regulation. This concept was further developed by Lederer's group which by performing giant patch excised experiments on adult guinea pig ventricular cardiac myocytes investigated how intracellular Na^+ influenced NCX pH modulation [96]. It was demonstrated that NCX proton inhibition could be partially relieved by removing cytoplasmic Na^+ [11, 96]. As protons were not competing with Na^+ at its transport sites, it was concluded that the Na^+-dependent inactivation of NCX may be at least in part due to Na^+ acting as cofactor in proton block and therefore enhancing proton inhibition. This hypothesis was supported by two additional lines of evidence: first, proton inhibition was largely insensitive to changes in intracellular Ca^{2+}, ruling out the regulatory Ca^{2+} binding sites as potential proton targets and second, that the exposure of the cytoplasmic side of NCX to α-chymotrypsin not only abolished Na^+ and Ca^{2+} regulation, but also proton inhibition. As α-chymotrypsin cleaves a portion of the large cytoplasmic loop, the removal of both Na^+-dependent inactivation and pH regulation further supported an intimate connection between these two regulatory mechanisms.

This working hypothesis was, however, disputed by subsequent electrophysiological studies showing that acidic pH decreased the sensitivity of NCX current measured from cardiac myocytes to cytoplasmic Ca^{2+} [10]. These findings shifted the focus of pH regulation to the Ca^{2+} regulatory domains instead of the Na^+-dependent inactivation process. To validate the role of the CBDs in pH regulation, Boyman and colleagues directly measured Ca^{2+} binding to the purified CBDs at different pH values [10]. Results demonstrated that protons significantly decreased the Ca^{2+} sensitivity of the isolated Ca^{2+} regulatory domains, further supporting their role in NCX pH regulation. Based on these results, it was proposed that protons act by displacing Ca^{2+} ions from the coordinating sites of CBD1 and CBD2 resulting in an inhibition of NCX transport activity [10].

To gain further understandings into the molecular mechanisms that govern NCX pH modulation, a detailed analysis utilizing mutagenesis and giant patch technologies was conducted on the cardiac exchanger [9]. The approach was to use site-specific mutagenesis to remove individually, or in combination, the Na^+- or Ca^{2+}-regulatory mechanism of NCX, allowing the investigation of their respective roles in NCX pH sensitivity.

Strikingly, these investigations revealed that proton block persisted after replacement of key residues coordinating Ca^{2+} within both of the Ca^{2+}-binding domains. This demonstrated that

protons were interacting with exchanger regions outside of the Ca^{2+}-binding domains [9]. Moreover, it was shown that an exchanger lacking the Na^{+}-dependent inactivation was still sensitive to cytoplasmic pH, although with a significantly reduced sensitivity [9]. Two important conclusions were made: first, Ca^{2+} and Na^{+} regulation are not required for proton block to occur, indicating that NCX pH regulation is a distinct molecular process; and second, intracellular Na^{+} is an important cofactor in pH modulation as it enhances the sensitivity of NCX for protons, as previously reported [96].

To hunt down the residues responsible for protein inhibition extensive single site mutagenesis was undertaken. It was revealed that two amino acids significantly contributed to NCX pH regulation: namely, histidine 124 and 165. Individual replacement of these two histidines decreased the apparent affinity of NCX for protons by 2 and 4 fold, respectively, underlying their relevance in proton regulation [9]. As shown in Figure 3, the concomitant replacement of these two histidines, with alanine, almost completely abolished NCX sensitivity to pH.

Histidine 124 is located in the linker between TMSs 2 and 3 within the α1-repeat (Figure 1), a region previously characterized as important for ion binding and transport [34, 35, 97]. This region of cardiac exchanger has been modeled to form a reentrant loop as a mutant with a cysteine placed at position 125 generated an ionic current that was subsequently blocked by cytoplasmically applied membrane impermeant sulfhydryl reagents [29, 30]. In contrast, the corresponding region in the archaebacterial homolog is extracellular. Given this discrepancy between the two exchangers, whether His 124 affects NCX pH regulation by being directly protonated, which would require access from the cytoplasmic side, remains to be established. Another possibility is that histidine 124 perturbs other regions involved in pH inhibition. Indeed, previous investigations reported that replacement of His 124 with an asparagine, significantly slowed down the Na^{+}-dependent inactivation [35]. As previously discussed, Na^{+} regulation enhances pH inhibition, and as such a mutation at this site may affect the affinity of NCX for protons indirectly.

Histidine 165 is located at the cytoplasmic end of TMS 4 (Figure 1) within a short sequence particularly enriched with positively charged amino acids (^{161}RKIKHLR). Replacement of this histidine with alanine removed both Na^{+} and Ca^{2+} regulation and drastically decreased the apparent affinity of NCX for cytoplasmic protons and the cooperativity of proton binding [9]. Undoubtedly, this residue is highly strategic and essential for NCX ionic allosteric regulation. A speculative role for this residue/region is to transduce the regulatory signals from the large cytoplasmic loop to the to the transmembrane segments responsible for ion transport and protonation may be an effective way of altering this transfer of information. Independently of the mechanisms by which these residues control NCX pH regulation, these findings reveal their essential contribution to NCX pH regulation. These two histidines are highly conserved within the exchanger family by being present across vertebrate species, further highlighting their role in NCX regulation.

The studies highlighted in this section clearly depict conflicting mechanisms of action of protons on NCX activity, calling for further investigations to fully decipher how pH modulates NCX. Similarly, the physiological impact of this regulation remains elusive as its

investigation remains technically challenging in native tissue. Nonetheless, in the following section we review and discuss how inhibition of NCX by cytosolic protons may impact cardiac function.

4. NCX role in cardiac function

4.1 Role of the Na^+-Ca^{2+} exchanger in excitation-contraction coupling

Cardiac myocytes express only one exchanger isoform, NCX1.1 [57, 98, 99]. In mammals this isoform is the dominant Ca^{2+} efflux mechanism. This is particularly relevant during excitation-contraction coupling when NCX helps to remove the excess intracellular Ca^{2+}, therefore maintaining contractility [13, 14, 100].

Because of the essential role that NCX plays in Ca^{2+} dynamics, alterations in its activity or expression are associated with various cardiac pathologies including systolic heart failure [101–105] and post-ischemic cardiac injury [19, 20, 106]. The increased NCX expression and function observed in some studies during the course of heart failure could have two major effects. Depending on intracellular Na^+ concentration, it could promote Ca^{2+} efflux, which will act over time to deplete the sarcoplasmic reticulum Ca^{2+} content, thereby causing decreased contractility. Concomitantly, the increased depolarizing current generated via NCX can induce delayed afterdepolarizations (DAD). Such aberrant electrical activity is known to contribute to the arrhythmogenicity frequently associated with heart failure [107–111]. The effect of NCX on DADs and triggered arrhythmias and has been well demonstrated in several studies using genetically modified mice [112, 113]. Conversely, Ca^{2+} influx via NCX acting in the reverse mode has been shown to be a major contributor to Ca^{2+} overload during reperfusion after an ischemic event, which can ultimately lead to myocyte death [19, 20, 106].

Numerous reviews have covered the role of NCX in excitation-contraction coupling, ischemia-reperfusion injury and arrhythmia and the reader is referred to them [16, 20, 105, 114–118].

4.2 Contribution of NCX to the detrimental effects linked to cardiac acidosis

The detrimental effects of acidosis on cardiac performance have long been known. Isaac Newton in the 17th century noticed that the heart of an eel stopped beating when exposed to a drop of vinegar [119]. Pathophysiologically, numerous conditions cause alterations in the acid-base balance of cardiac muscle: metabolic and respiratory acidosis, malignant hyperthermia and hypercapnia, sleep apnea/hypopnea syndrome, and most notably ischemia. Many of these conditions have been linked to decreased contractility and heart rate, increased cell depolarization, ventricular fibrillation and propensity for arrhythmia [3, 5, 6, 120]. Acidosis can be transient or persistent and the heart responds differently to the two insults. Within a few seconds of the onset of acidosis there is a reduction in contractility. Although the increased levels of protons inhibits key proteins involved in Ca^{2+} handling such as ryanodine receptors, (RyRs) [121], L-type Ca^{2+} channels [121–123], the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) [124–126], and NCX itself, this initial decrease in contractility is mainly due to the effects of protons on the contractile proteins

themselves, namely by reducing the binding of Ca^{2+} to troponin C (TnC) (Graphical Abstract) [127–131].

A different scenario is observed in conditions of persistent acidosis. As an adaptive response, both diastolic and systolic Ca^{2+} levels increase to compete off the protons from TnC, thereby partially recovering heart contractility. Among the mechanisms proposed for this rise in intracellular Ca^{2+} is a functional coupling between NCX and the myocardial Na^+-H^+ exchanger (NHE) [3, 132]. With acidosis NHE is activated, increasing intracellular Na^+ levels leading to two potential outcomes regarding NCX: the increased Na^+ reduces the Na^+ driving force, which will act to decrease the Ca^{2+} efflux; or NCX is pushed into the reverse mode, thereby leading to Ca^{2+} entry. While such an interaction between NHE and NCX is generally accepted and theoretically plausible, some studies provide a contrarian viewpoint. Allen and Xiao have shown that although intracellular Ca^{2+} rises during late acidosis, it occurs independently of changes in intracellular Na^+ [133]. Possibly, the elevated intracellular Na^+ and protons may both act on NCX to suppress its activity via their regulatory roles, leading to increased diastolic Ca^{2+}. Further investigations are needed to dissect the contribution of NCX to the Ca^{2+} aberrations triggered by acidosis and the role of the direct inhibition of NCX activity by cytoplasmic protons.

In addition to contributing to the Ca^{2+} imbalance seen during acidosis, NCX may also play a role in acidosis induced arrhythmia by triggering DADs [6, 134]. These abnormal depolarizations are more prominent during recovery from acidosis since it coincides with the complete relief of NCX proton block. In this condition, the Ca^{2+} released from the overloaded sarcoplasmic reticulum [134, 135] triggers NCX depolarizing current, thereby creating the opportunity for ectopic beats.

Although such evidence makes NCX an important player in the detrimental effects linked to cardiac acidosis, it has to be underscored that experimentally it is difficult to assess its direct role in these processes due to the lack of specific blockers or potential adaptations encountered in transgenic animals. New strategies are needed to determine the contribution of NCX to cardiac function in both physiological and pathophysiological settings.

5. Conclusions

In this review, we have summarized the current knowledge of allosteric regulation of NCX focusing upon ionic regulation in general and pH regulation in particular. We have highlighted the progress towards understanding NCX pH regulation and its potential physiological and pathophysiological implications. As one of the most prominent controlling mechanisms for Ca^{2+} dynamics, NCX has the ability to greatly influence cardiac contractility and excitability. With its relatively high sensitivity to protons and the frequent association of acidosis with detrimental effects on cardiac function, NCX could be a vital point of pharmacological intervention in reducing the negative effects of such conditions. It is therefore essential that we continue to tease apart the complex interplay that ionic regulations play in the mechanistic understanding of NCX under both normal and pathological settings.

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HIGHLIGHTS

- The cardiac Na^+-Ca^{2+} exchanger (NCX) controls Ca^{2+} homeostasis
- Cytoplasmic Ca^{2+} enhances NCX activity via two cytoplasmic Ca^{2+}-binding domains
- Intracellular Na^+ inactivates NCX and this process involves the XIP region
- Intracellular protons strongly inhibit NCX activity
- NCX pH regulation involves histidines 124 and 165 and two Ca^{2+}-binding domains

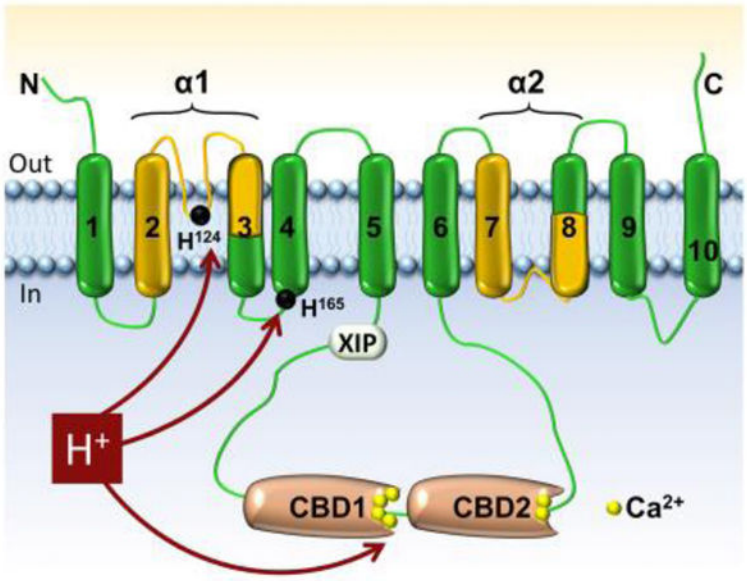

Figure 1: Schematic topology of NCX1.1

Topology of the cardiac exchanger NCX1.1 as modeled by Ren and coworkers [24]. The transmembrane segments (TMS) involved in ion transport are shown in yellow. These regions, which are highly conserved among the Cation/Ca^{2+} exchanger superfamily, are named α-repeats [26] and host the 12 residues that coordinate the transported Na^{+} and Ca^{2+} ions (as demonstrated in NCX_Mj by [23], not shown). Between TMSs 5 and 6 there is a large cytoplasmic loop which includes the XIP region and two Ca^{2+}-binding domains (CBD). The XIP region controls the inactivation of NCX due to high levels of cytosolic Na^{+} [75], while the Ca^{2+}-binding domains confer Ca^{2+} regulation to NCX. Protons can displace Ca^{2+} from these sites decreasing NCX activity [10]. The two histidines found relevant for NCX pH regulation are indicated. Mutations at these sites decrease the sensitivity of NCX to cytoplasmic protons [9].

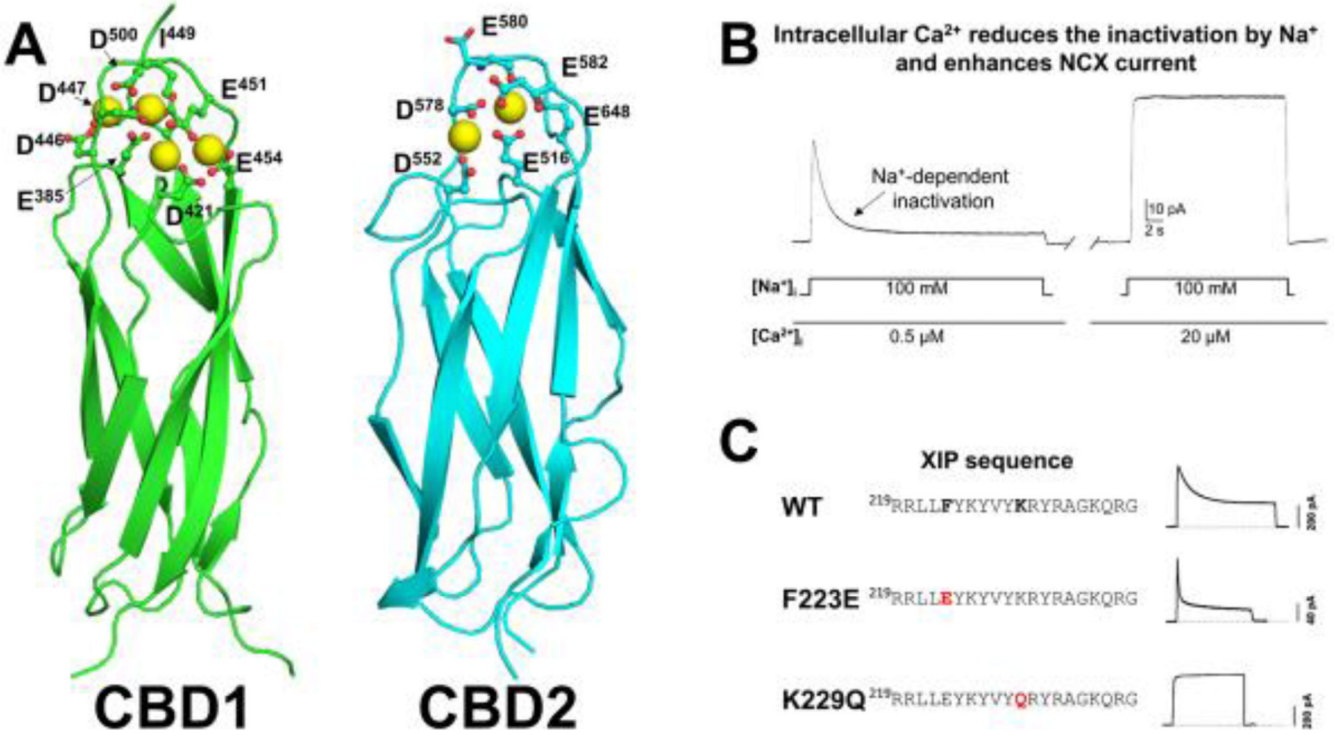

Figure 2: Cytoplasmic Ca^{2+} and Na^+ regulate the cardiac Na^+-Ca^{2+} exchanger

A. Structure of NCX1 Ca^{2+} regulatory domains (CBD1–2DPK and CBD2–2QVM) [52, 136]. Ca^{2+} bound to either CBD1 or CBD2 is not transported, but activates NCX by increasing the turnover rate and removing the Na^+-dependent inactivation [78]. B. Typical outward current recorded using the giant excised patch technique. cRNA encoding for the cardiac exchanger (NCX1.1) is injected into *Xenopus laevis* oocytes. Four to five days after injection, exchange currents are recorded using the giant patch technique (20–30 μm tip diameter) in the inside out mode. 8 mM Ca^{2+} is present within the pipette (external side) while the cytoplasmic side of the patch is bathed either in 100 mM Cs^+ or Na^+ and the indicated cytoplasmic free Ca^{2+} concentrations. Cs^+ cannot bind to the transport sites, preventing activity. Replacement of Cs^+ with Na^+ generates an outward current (reverse mode) that peaks and then declines over several seconds due to the Na^+-dependent inactivation. This process is not observed in the presence of micromolar Ca^{2+} concentrations. C. The amino acid sequence of the XIP region of NCX1.1 is shown on the left. Two single-site substitutions within this domain either accelerate (F223E) or abolish (K229Q) the Na^+-dependent inactivation, as shown by the corresponding exchanger outward current traces (right) (modified from [42]).

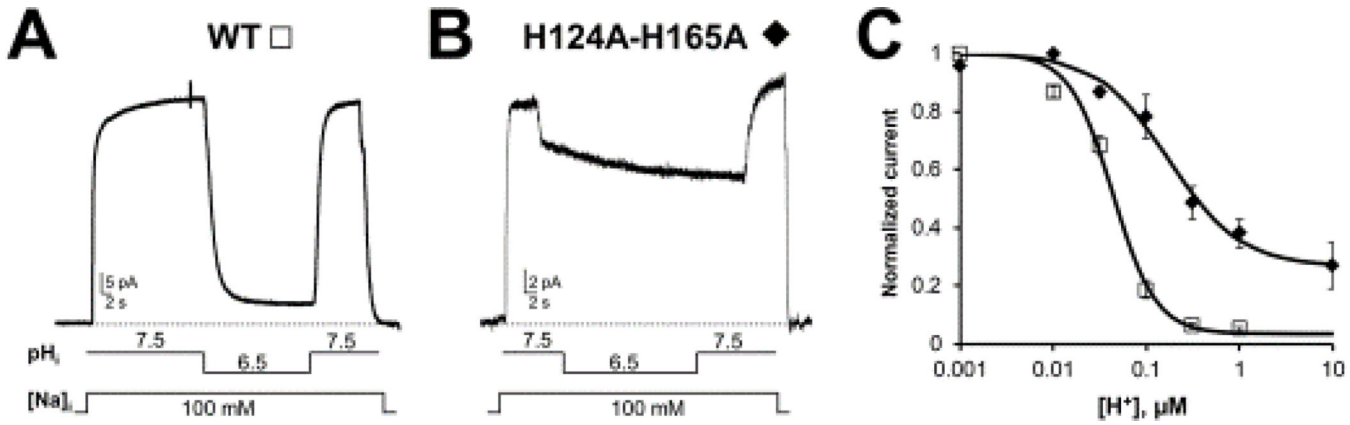

Figure 3: Two histidines are important for NCX pH regulation

Exchanger currents were recorded using the giant excised patch technique in the inside out configuration from *Xenopus laevis* oocytes expressing the indicated constructs (modified from [9]). A. The WT cardiac exchanger is highly sensitive to changes in intracellular pH and its activity is almost completely abolished in the presence of cytoplasmic pH 6.5. B. Mutation of histidine 124 and 165 to alanine drastically decreased the exchanger pH sensitivity, underlying the role of these two residues in the regulation of NCX by protons. C. Dose response curves for cytoplasmic H^+ for WT (cardiac isoform NCX1.1) and H124A-H165A mutant exchangers. Replacement of histidine 124 and 165 drastically decreased the sensitivity of NCX to cytoplasmic protons and significantly changed the cooperativity of binding.