Is Modulation of Sodium-Calcium Exchange a Therapeutic Option in Heart Failure?

Gerd Hasenfuss, Wolfgang Schillinger

Besides the sarcoplasmic reticulum (SR) Ca\textsuperscript{2+}-ATPase (SERCA), the sarcolemmal Na\textsuperscript{+}–Ca\textsuperscript{2+} exchanger (NCX) is the most important Ca\textsuperscript{2+} transport protein responsible for maintaining the Ca\textsuperscript{2+} balance of the myocyte. It catalyzes the transport of Ca\textsuperscript{2+} across the membrane in exchange for Na\textsuperscript{+} in a reversible manner. Its activity is called “forward” when Na\textsuperscript{+} is transported inward and Ca\textsuperscript{2+} outward and “reversed” when ions are transported in the opposite directions. The driving force of NCX depends on Na\textsuperscript{+} and Ca\textsuperscript{2+} concentrations at either side of the plasma membrane and on the membrane potential. NCX is electrogenic and carries inward (depolarizing) current in forward mode and outward (repolarizing) current in reversed mode.\textsuperscript{1} NCX consists of 9 transmembrane helices and a large cytoplasmic loop. This loop has been shown to contain Ca\textsuperscript{2+}- and Na\textsuperscript{+}-binding regulatory sites, which are distinct from the transport sites. Thus, Na\textsuperscript{+} and Ca\textsuperscript{2+} ions are both transport substrates and modulators of activity. At the N-terminal end of the cytoplasmic loop near the membrane–lipid interface, there is a 20-amino acid segment, designated the endogenous XIP region. This region is considered to function as an autoinhibitory domain that plays a central role in NCX regulation. In addition, PIP\textsubscript{2} protons, ATP, and PKC-dependent effects regulate NCX activity.\textsuperscript{1-3} More recently, it was shown that the Ca\textsuperscript{2+} binding protein sorcin exerts stimulatory actions on NCX.\textsuperscript{4}

Altered expression and activity of the sarcolemmal NCX may play a key role for disturbed contractile function and arrhythmogenesis in hypertrophy and heart failure. It has become clear that disturbed excitation–contraction coupling attributable to altered SR Ca\textsuperscript{2+} accumulation significantly contributes to heart failure pathophysiology.\textsuperscript{3} Three major factors seem to contribute to disturbed SR Ca\textsuperscript{2+} accumulation in human heart failure: (1) increased leak of Ca\textsuperscript{2+} through ryanodine receptors, (2) reduced SERCA activity, and (3) increased transsarcolemmal elimination of Ca\textsuperscript{2+} by NCX. SR Ca\textsuperscript{2+} accumulation depends on the activity of SERCA relative to transsarcolemmal Ca\textsuperscript{2+} elimination by NCX. When protein levels of NCX were measured relative to SERCA, it was observed that this ratio was increased by a factor of 3 in endstage failing myocardium, indicating a relative dominance of NCX over SERCA Ca\textsuperscript{2+} transport.\textsuperscript{5} Interestingly, two different phenotypes were identified: (1) endstage failing hearts with a predominant increase in protein levels of NCX, and (2) endstage failing hearts with a predominant decrease in SERCA protein levels. In the former subgroup, diastolic function was preserved because overall cellular capacity to eliminate cytosolic Ca\textsuperscript{2+} is high. However, systolic function was impaired because Ca\textsuperscript{2+} is eliminated across the sarcolemmal membrane and therefore SR Ca\textsuperscript{2+} accumulation is decreased. In the latter group, both SR Ca\textsuperscript{2+} uptake and global cytosolic Ca\textsuperscript{2+} elimination are reduced and therefore systolic as well as diastolic function was severely compromised. Enhanced transsarcolemmal relative to SR Ca\textsuperscript{2+} cycling is most pronounced at high heart rates.\textsuperscript{5,6} As was indicated in clinical and experimental studies, increased forward mode exchange is arrhythmogenic because of delayed afterdepolarizations after inward current generation.\textsuperscript{1,3} Furthermore, it was shown that increased expression of NCX increases sensitivity to digitalis and predisposes to free radical induced myocyte dysfunction.\textsuperscript{5,7}

From these considerations, modulation of NCX function in heart failure may be a therapeutic option. Stimulation of forward mode NCX activity would reduce cytosolic Ca\textsuperscript{2+}, impair systolic, and improve diastolic function. Vice versa, stimulation of reversed mode NCX would increase intracellular Ca\textsuperscript{2+} and contractility with the risk of diastolic impairment. Inhibition of NCX function should have opposite effects. Apparently, the consequence of nonselective stimulation or inhibition of NCX function is quite complex and unpredictable. Several inhibitors of NCX function have been developed:\textsuperscript{2} KB-R7943 is a well-characterized inhibitor. Intriguingly, KB-R7943 was suggested to exert a preferential effect on reverse-mode NCX activity. First studies also reported high selectivity of the drug; this was yet questioned by other authors. Synthetic peptides are considered as potent and highly selective NCX inhibitors. The NCX inhibiting peptide (XIP) is derived from the primary sequence of cardiac NCX1, binds at the large cytoplasmic loop, and decreases the V\textsubscript{max} of NCX activity. However, it does not appear to permeate through the cell membrane, which limits its use for therapeutic interventions in heart failure. In addition, other peptides, such as the cyclic hexapeptide FRCCRFa and its cell-permeant, N-myristylated derivative Myr-FRCCRFa, which are much smaller than XIP, have been reported to effectively inhibit NCX activity. Recently, new compounds with particularly high selectivity for NCX such as SEA0400 were synthesized.\textsuperscript{2,8}
In this issue of *Circulation Research*, Hobai *et al.* present an interesting study suggesting that inhibition of NCX may be a therapeutic option in heart failure by normalizing Ca²⁺ cycling and improving contractile function. They studied isolated myocytes from dog hearts with pacing-induced heart failure using single cell electrophysiology techniques with XIP added directly to the intracellular solution. The canine pacing tachycardia model was previously shown to present with decreased SERCA and increased NCX protein levels. The main findings are that NCX inhibition by XIP increases SR Ca²⁺ load and Ca²⁺ transients; an estimated 27% inhibition of NCX induced an 80% increase in the amplitude of the Ca²⁺ transient in nonfailing myocytes at 0.5 Hz. In failing myocytes with doubling of NCX function, a 27% inhibition of NCX induced a 3.86-fold increase in the Ca²⁺ transient amplitude. Quite surprisingly, inhibition of NCX was not associated with increased diastolic Ca²⁺ and rather accelerated relaxation kinetics. The finding of increased SR Ca²⁺ load with reduced NCX activity is consistent with findings using the opposite approach in a previous study. When protein levels of NCX were increased by adenosine mediated gene transfer in isolated rabbit myocytes, this resulted in decreased SR Ca²⁺ content, decreased myocyte shortening, and blunted forced frequency relation, i.e., findings similar to those observed in the failing human heart. However, it should be mentioned that in other models and experimental conditions, increased NCX expression and function was shown to upregulate SR Ca²⁺ levels. In these studies, high Na⁺ levels may have been the main cause promoting reversed mode NCX activity.

What are the subcellular mechanisms coupling SR Ca²⁺ load to NCX function? Most likely, NCX inhibition transiently increases intracellular Ca²⁺, which subsequently results in stimulation of SERCA. It has been recognized that with increasing stimulation rates, a marked “frequency-dependent acceleration of relaxation” (FDAR) can be found in mammalian ventricular muscle. Apparently, FDAR is linked to the frequency-dependent increases in [Ca²⁺], and is independent from β-adrenergic activation. There are several mechanisms to explain SERCA stimulation by Ca²⁺. Involvement of phosphorylation of phospholamban (PLN) by Ca²⁺/calmodulin-dependent protein kinase (CaMKII) relieving the inhibitory action of PLN on SERCA has first been suggested. Accordingly, FDAR was abolished after inhibition of CaMKII with KN-62 or KN-93. However, it was also present in PLN knockout mice, implying that PLN is not essential. Interestingly, direct phosphorylation of SERCA by CaMKII resulting in stimulation of the Vₘₐₓ of Ca²⁺ uptake has been described as an alternative pathway. However, there is still controversy and the appropriate target of CaMKII involved in accelerating Ca²⁺ transport during FDAR is not yet identified unequivocally. After SERCA stimulation, intracellular Ca²⁺ declines and a new steady state with higher SR Ca²⁺ levels and higher SR Ca²⁺ release and uptake develops. Of course, under steady state conditions, NCX Ca²⁺ elimination matches with L-type Ca²⁺ current influx.

Although the finding of increased SR load and Ca²⁺ transients after NCX inhibition is plausible for nonfailing as well as failing myocardium, the finding of improved rate of relaxation may depend on the experimental model. In the failing human heart, diastolic Ca²⁺ was shown to be increased. Furthermore, in muscle strip preparations from failing human hearts, diastolic function varied considerably between patients. Diastolic function was related to expression levels of SERCA and NCX. It was normal in patients with increased NCX and preserved SERCA levels. In those patients, NCX inhibition may improve function by increasing reduced SR Ca²⁺ load. In patients with reduced SERCA, diastolic function was impaired. In those patients, NCX inhibition may further deteriorate diastolic performance. This may be most pronounced at high heart rates with reduced time for SR Ca²⁺ transport. On the same line, intracellular Na⁺ is high in human heart failure. Because increased Na⁺ promotes reversed mode NCX activity, the consequences of XIP induced NCX inhibition may critically depend on intracellular Na⁺.

Another aspect needs to be considered. In heart failure, Ca²⁺ leak from the SR is increased due to enhanced ryanodine receptor open probability. Because leak as measured by Ca²⁺ sparks is increased with higher SR Ca²⁺ load any intervention to increase SR Ca²⁺ load without reducing the leak decreases efficiency of excitation–contraction coupling with increased energy consumption and arrhythmias as potential side effects. Finally, XIP acts at the intracellular surface of the NCX, which was suggested to be involved in Na⁺-dependent inactivation of the transporter. This together with the finding that XIP did not inhibit NCX function during caffeine induced Ca²⁺ release in the study by Hobai *et al.* may suggest that the observed functional changes are specific for XIP and may not be generally transferable to other nonspecific NCX inhibitors.

In summary, because NCX is a complex molecule, the regulation of which is incompletely understood, the effects of NCX modulation on intracellular Ca²⁺ and contractile function is rather unpredictable and may deeply depend on experimental conditions and models. The study by Hobai *et al.* stimulates to consider inhibitors of NCX for improving excitation–contraction coupling in heart failure. However, more studies are needed to understand the effects of XIP and analogues under different conditions such as high stimulation rates, increased intracellular Na⁺, increased SR Ca²⁺ leak, and various SERCA and NCX expression levels.

**References**


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