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Cardiac Sodium-Calcium Exchange and Efficient Excitation-Contraction Coupling: Implications for Heart Disease

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Abstract

Cardiovascular disease is a leading cause of death worldwide, with ischemic heart disease alone accounting for >12% of all deaths; more than HIV/AIDS, tuberculosis, lung and breast cancer combined. Heart disease has been the leading cause of death in the United States for the past 85 years, and is a major cause of disability and health-care expenditures. The cardiac conditions most likely to result in death include heart failure and arrhythmias, both a consequence of ischemic coronary disease and myocardial infarction, though chronic hypertension and valvular diseases are also important causes of heart failure. Sodium-calcium exchange (NCX) is the dominant calcium (Ca) efflux mechanism in cardiac cells. Using ventricular-specific NCX knockout mice, we have found that NCX is also an essential regulator of cardiac contractility independent of sarcoplasmic reticulum Ca load. During the upstroke of the action potential, sodium (Na) ions enter the diadic cleft space between the sarcolemma and the sarcoplasmic reticulum. The rise in cleft Na, in conjunction with depolarization, causes NCX to transiently reverse. Ca entry by this mechanism then “primes” the diadic cleft so that subsequent Ca entry through Ca channels can more efficiently trigger Ca release from the sarcoplasmic reticulum. In NCX knockout mice, this mechanism is inoperative (Na current has no effect on the Ca transient), and excitation-contraction coupling relies upon the elevated diadic cleft Ca that arises from the slow extrusion of cytoplasmic Ca by the ATP-dependent sarcolemmal Ca pump. Thus our data support the conclusion that NCX is an important regulator of cardiac contractility. These findings suggest that manipulation of NCX may be beneficial in the treatment of heart failure.

Keywords

Sodium-calcium exchange; Excitation-contraction coupling; Heart Failure; Calcium channels; Sodium current; Contractility

INTRODUCTION

Heart disease, including heart failure (HF), myocardial infarction (MI) and their complications, is a global problem accounting for more than 12% of all deaths worldwide in 2011 according to the World Health Organization (2011). In the United States, 5.8 million people carry a diagnosis of heart failure (HF). 1.1 million are hospitalized with HF each year as a primary diagnosis, and 3.39 million patients visit an outpatient clinic annually because of HF (Roger *et al.*, 2011). The CDC estimates the U.S. cost of HF in 2010 to be \$39.2 billion (2011). This is an enormous financial expenditure as well as disease burden. There are also 1 million myocardial infarctions annually in the U.S. 50% of patients with MI will die of arrhythmia before hospitalization. Another 5% develop cardiogenic shock, and half of

these patients die as well (Roger *et al.*, 2011). Thus the severity and the prevalence of heart disease in the world are astounding. In this chapter, we will briefly review the pathogenesis of HF, and then discuss how new insights into the role of NCX in excitation-contraction (EC) coupling may offer opportunities to improve the treatment of this debilitating disease.

Pathogenesis of Heart Failure

The pathogenesis of HF has been an intense area of investigation. Although several lines of evidence suggest that NCX activity is increased in HF and contributes to contractile dysfunction by depleting sarcoplasmic reticulum (SR) Ca content (Studer *et al.*, 1994; Flesch *et al.*, 1996; Hobai & O'Rourke, 2000; Hasenfuss & Pieske, 2002; Armoundas *et al.*, 2007), recent clinical advances have ignored the exchanger and instead target abnormal activation of neuroendocrine signals. Neuroendocrine activation has multiple deleterious effects but with respect to EC coupling, it is thought to lead to hyperphosphorylation of ryanodine receptors (RyRs) by kinases (PKA and/or CaMKII), leading to SR Ca leak (Marks, 2000). Neuroendocrine activation also promotes beta-adrenergic receptor downregulation and associated abnormal G protein signaling, which likewise blunts the response of LCCs and SR Ca loading to adrenergic signals (Koch *et al.*, 2000). Other factors contribute to contractile dysfunction: these include defective SR CaATPase activity, leading to reduced SR Ca content (Schmidt *et al.*, 1998); myofilament dysfunction, which decreases the contractile response to released Ca (Hajjar & Gwathmey, 1990); mitochondrial dysfunction, which leads to energy starvation; and fibrosis, which replaces myocytes with non-contracting cells (Ingwall & Weiss, 2004).

Manipulating Contractility in Heart Failure

Although targeting the neuroendocrine system through the use of beta blockers, angiotensin converting enzyme inhibitors, and aldosterone antagonists has been a relatively effective strategy to manage HF (Fonarow *et al.*, 2011), patients still complain of fatigue, shortness of breath and limited exercise tolerance. Ultimately, their disease progresses and hospitalizations for decompensation become more frequent as resting blood flow to vital organs decreases. Thus another approach is necessary. One such approach is to employ inotropic agents to directly stimulate contractile function. These agents most commonly operate by further stimulating beta-adrenergic receptors, which in turn trigger a signaling cascade that results in: 1) increased Ca influx via Ca current (I_{Ca}), 2) increased SR Ca uptake rate (via PLB phosphorylation), and 3) increased myofilament Ca responsiveness. However, several seminal studies have established that inotropes increase mortality and morbidity in the HF population (Felker *et al.*, 2003) despite improved pump function. For example, the ADHERE registry of >10,000 patients showed significantly higher in-hospital mortality (adjusted by propensity score) for HF patients treated with the beta agonist dobutamine or the phosphodiesterase inhibitor milrinone instead of vasodilators (Abraham *et al.*, 2005). The ESCAPE trial of severe HF patients undergoing evaluation for heart transplantation found that those who were "electively" treated with inotropes had a 1.8-fold increase in 6-month mortality (Elkayam *et al.*, 2007). Thus inotropes, while sometimes unavoidable in the short run, are dangerous in the long run. The problem appears to be the very thing that improves contractility: increased cellular Ca load leading to SR Ca overload, which has a variety of deleterious consequences including arrhythmia and cell death.

A Modern View of Excitation-Contraction Coupling in Health and Disease

Recent developments in understanding of the role of NCX in EC coupling may help reveal new and safer strategies to improve contractility than the current generation of inotropes. We have long known that a Ca-induced Ca-release (CICR) mechanism controls EC coupling

in cardiac cells (Fabiato, 1983). Ca entering through sarcolemmal L-type Ca channels (LCCs) triggers release of Ca by RyRs on the SR surface (London & Krueger, 1986). This reaction occurs throughout the ventricular cell within functional units known as couplons (Stern *et al.*, 1997; Franzini-Armstrong *et al.*, 1999). These units, which are located primarily along transverse (t)-tubules, permit sarcolemmal LCCs to admit Ca into a restricted junctional region (the diadic cleft), leading to a significant rise in Ca concentration. This Ca gates a cluster of RyRs on the apposing membrane of the junctional SR, allowing Ca release from the SR to generate a Ca spark (Cheng *et al.*, 1993). The spatial separation between couplons is sufficient to permit their local control (Stern, 1992), which explains the voltage-dependence of Ca transients. However, we now know that action potentials in healthy cells trigger each couplon simultaneously in a coordinated and synchronous manner (Inoue & Bridge, 2003). This synchronous activity appears to be critical for optimum contractility.

Failing cardiac muscle is characterized by the loss of synchronized Ca release upon depolarization, as exemplified by post-infarct remodeling in the rabbit (Litwin *et al.*, 2000). We have found similar loss of synchronization of Ca release in rabbit cells exposed to metabolic inhibitors (Fig. 1), an experimental condition that recapitulates the metabolic stress of HF (Chantawansri *et al.*, 2008). The loss of synchronization can in large part be explained by changes in the single channel characteristics of LCCs. For example, the Ca spark probability and distribution of spark latencies are predicted by LCC latency, open time, and P_o . Primary changes in RyR behavior (Meissner, 1994) and cellular structure (Gomez *et al.*, 2001) may also contribute to loss of synchronization.

Excitation-Contraction Coupling in NCX KO Mice

We wondered whether NCX might alter EC coupling independent of changes in SR Ca stores and Ca channel activity. To explore this possibility, we took advantage of our ventricular-specific NCX knockout mice. These mice live into adulthood with normal cardiac function. Isolated cells from these mice exhibit normal resting Ca, preserved SR Ca stores and normal Ca transients in response to electrical stimulation (Henderson *et al.*, 2004). Because NCX is absent and no other Ca efflux mechanism increases to compensate, Ca removal in response to caffeine-induced SR Ca release is dramatically reduced. Major adaptations in this model appears to be a reduction in Ca influx through LCCs and an associated increase in EC coupling gain (Pott *et al.*, 2005). The reduced I_{Ca} is caused by an increase in subsarcolemmal/diadic cleft Ca concentration and the resulting Ca-dependent inactivation (Pott *et al.*, 2007b). Action potential shortening caused by upregulation of the transient outward current (I_{TO}) also limits Ca entry during depolarization (Pott *et al.*, 2007a). Resting Ca sparks, the elementary events of EC coupling that reflect CICR activity at the single couplon level, are reduced in frequency compared to wildtype cells. However, the sparks that do occur are larger and last longer (Neco *et al.*, 2010). The frequency reduction is consistent with reduced diastolic triggering of sparks by the smaller KO I_{Ca} , and the difference in spark size is caused by the lack of NCX-mediated Ca removal from the diadic cleft in KO cells. Spark activity and size equalizes when cells from WT and KO mice are permeabilized to eliminate the influence of NCX, I_{Ca} , and differences in cleft Ca (Neco *et al.*, 2010). This indicates that RyR function is not responsible for differences in spark frequency, and directly implicates I_{Ca} and NCX as the responsible elements.

Reverse NCX and SR Ca Release Triggering

How then does NCX affect cleft Ca and microscopic EC coupling during depolarization? In the cardiac-specific NCX KO Mouse, effective EC coupling is dependent upon elevated diadic cleft Ca throughout the cardiac cycle. This is made clear by experiments buffering Ca

in the cytoplasm using EGTA. Under strong Ca buffering conditions, KO mice exhibit reduced coupling efficiency (exemplified by decreased spark number and increased spark latency), whereas WT mice display normal coupling (Fig. 2, from Neco *et al.*, 2010). Keep in mind that under these highly buffered conditions we expect I_{Ca} to be as large in the KO as it is in the WT (Pott *et al.*, 2007b). The best explanation for preserved EC coupling in buffered WT cells is that NCX helps maintain coupling during depolarization. We have hypothesized that reverse NCX primes the diadic cleft with a subthreshold amount of Ca during the initial upstroke of the action potential in response to Na entry via I_{Na} into the subsarcolemmal space. Only a small amount of additional Ca brought in by LCCs is needed to trigger release in all couplons. A similar argument was proposed by LeBlanc and Hume in 1990 when they showed that blocking I_{Na} reduced Ca release (LeBlanc & Hume, 1990). However, these authors argued that reverse NCX was a *direct* trigger. Although subsequent reports from several other groups supported LeBlanc and Hume's findings (Haworth & Goknur, 1991; Nuss & Houser, 1992; Kohmoto *et al.*, 1994; Wasserstrom & Vites, 1996; Lines *et al.*, 2006), others refuted NCX's ability to trigger SR Ca release in any fashion that was remotely close to what could be triggered by I_{Ca} (Bers *et al.*, 1990; Sham *et al.*, 1992; Lipp & Niggli, 1994; Lopez-Lopez *et al.*, 1995; Sipido *et al.*, 1995; Sipido *et al.*, 1997). Furthermore, many of the experiments supportive of Leblanc and Hume were criticized on technical grounds: poor voltage control, inadvertent activation or inactivation of Ca channels by voltage protocols, instability in SR Ca content, incomplete blockade of I_{Ca} by voltage-dependent blockers, and non-physiologic intracellular Na concentrations.

To address these criticisms, we once again took advantage of the NCX KO mouse and also carefully constructed voltage clamp protocols and waveforms in the shape of an action potential so as to minimize voltage errors and inactivation of I_{Ca} that might confound interpretation. In order to trigger Ca release in the absence of I_{Na} the action potential clamp was preceded by a linear ramp depolarization from -70 to -40 mV over a period of 1.3 s. This prepulse strategy was designed to inactivate I_{Na} without first generating the large Na influx that typifies square-wave prepulses. It also prevented unwanted activation of LCCs by voltage errors produced by saturating Na currents (I_{Na}) activated during the prepulse. This was verified in control experiments. Thus we were able to expeditiously eliminate I_{Na} without the use of TTX and without introducing voltage errors or unplanned changes in Ca channel activity. Using this protocol, we found that eliminating I_{Na} selectively decreases (but does not eliminate) calcium release in WT, but has no effect in NCX KO (Fig. 3). The absence of an effect of inactivating I_{Na} in NCX KO confirms that reverse NCX in response to rapid influx of Na via I_{Na} makes an important contribution to the triggering process. To confirm this finding using a different approach, we applied the Na channel blocker TTX (5 μ M) using a rapid solution exchange device 1 second prior to depolarization by the action potential voltage clamp. TTX rapidly and reversibly reduced the Ca transient without reducing SR Ca load in WT but had no effect in NCX KO. The TTX results confirm the effect of Na-induced reverse NCX on CICR.

Importance of Na-channel Isoforms Concentrated in Transverse Tubules

Ventricular myocytes contain numerous isoforms of Na channels in addition to the cardiac isoform $Na_v 1.5$. One group of isoforms ($Na_v 1.1$, 1.2 , 1.3 and 1.6), often referred to collectively as "neuronal Na channels", appears to be concentrated in transverse-tubules (t-tubules) (Gershon *et al.*, 2011). Blocking these channels in rats apparently has no effect on EC coupling (Brette & Orchard, 2006). However, some other groups have suggested that these channels do have an effect on contractility (Maier *et al.*, 2002). We reasoned that since the process of EC coupling in ventricular myocytes is mainly concentrated in couplons located in t-tubules, then selective inhibition of "neuronal" Na channels should be sufficient to eliminate the contribution of reverse NCX to the trigger for SR Ca release. We tested this

hypothesis in rabbit, a species which is more dependent on Ca influx from LCCs for triggering than mouse (i.e. less EC coupling gain). When we exposed rabbit cells to 100 nM TTX, a low concentration that specifically inhibits “neuronal” Na channels (Goldin, 2001; Catterall *et al.*, 2005), we found reduced SR Ca release similar to the reduction in Ca release observed during a slow ramp prepulse and similar to that described above for mouse.

Essential Role of NCX in Priming the Diadic Cleft

Our results suggest that NCX plays an essential role in the process of Ca-induced Ca release, not simply by direct triggering of RyRs (which seems unlikely based on the relative inefficiency of NCX as demonstrated by Sham *et al.* (Sham *et al.*, 1992) and Sipido *et al.* (Sipido *et al.*, 1997), but through the following sequence of events: in response to t-tubular “neuronal” Na channel activation upon depolarization, the rise in junctional Na concentration activates reverse NCX which *primes* the diadic cleft with Ca. We know that the relationship between RyR P_o and activating Ca is sigmoid (Copello *et al.*, 1997). The NCX-mediated priming of cleft Ca moves Ca concentration along the flat part of this sigmoid curve without increasing RyR P_o appreciably. However, the Ca concentration reaches all the way to the inflection point for the steep portion of the sigmoid curve. We propose that this priming takes place during the 4 ms of the action potential that precedes activation of I_{Ca} . Subsequent Ca entry upon activation of I_{Ca} will further raise Ca in a concentration range where it is related steeply to RyR P_o , so that the NCX and I_{Ca} effectively sum their activities in a non-linear fashion (Torres *et al.*, 2010). Without this priming effect, the entry of Ca via I_{Ca} may still be sufficient to trigger, but with less efficiency than when the system is first primed by NCX. Thus it seems that NCX is necessary to increase the coupling efficiency (Polakova *et al.*, 2008) of CICR. In NCX KO myocytes in the absence of Ca buffering, the cleft Ca is elevated throughout the cardiac cycle, so further priming by I_{Na} and NCX is not required (Larbig *et al.*, 2010).

Conclusion

These findings raise the intriguing possibility of manipulating NCX as a therapeutic tool in HF, not simply to alter Ca efflux and SR Ca load like a cardiac glycoside (e.g. digitalis), but rather as a way to prime the diadic cleft and maximize coupling efficiency. The goal is to provide maximum inotropic support without provoking SR Ca overload and the consequent arrhythmias and cellular damage. The increase in Ca entry via reverse NCX required to accomplish this increase in coupling efficiency is unknown, but should be minimal (Torres *et al.*, 2010). On the other hand, we have shown evidence that ablation of NCX substantially reduces ischemia/reperfusion injury (Imahashi *et al.*, 2005) and may also reduce triggered arrhythmias (Nagy *et al.*, 2004). Thus we are faced with two opposing strategies for involving NCX in the protection and improvement of cardiac function: enhancing reverse NCX to optimize CICR, and blocking NCX during acute ischemia/reperfusion to prevent Ca overload. Unfortunately, pharmacological agonists and antagonists of the exchanger lack the specificity for these purposes and will require further development. Hopefully new work involving structure/function of NCX (John *et al.*, 2011) will soon lead to a new family of pharmacological agents.

In summary, we have found that knocking out NCX in the ventricle reduces LCC activity through Ca-dependent inactivation, independent of SR Ca load and global cytoplasmic Ca levels, which are unchanged. The reduction in LCC activity also reduces the frequency of resting Ca sparks. Nevertheless the size of Ca sparks is increased, supporting the concept that NCX resides within or at least very near couplons and thereby locally regulates the removal of diadic cleft Ca. Additionally, we have found that effective EC coupling in mouse and rabbit requires activation of TTX-sensitive Na channels in order to promote reverse

NCX, which primes the diadic cleft with Ca and increases coupling fidelity. We conclude that cardiac NCX is a key transporter responsible for normal contractility in addition to its classic function as a regulator of cellular Ca by facilitating Ca efflux. NCX is therefore a potentially major therapeutic target with a higher safety margin than current agents.

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Figure 1. EFFECT OF METABOLIC INHIBITION ON L-TYPE CA CHANNELS AND TRIGGERED CA SPARKS IN ISOLATED ADULT RABBIT VENTRICULAR MYOCYTES

A: Frequency distribution of single L-type Ca channel latency under control conditions (red), and after 4 (green) and 6.5 (blue) minutes of oxidative and glycolytic metabolic inhibition (MI) with FCCP (50 nM) and 2-deoxyglucose (10 mM). Normalized frequency distribution is shown in Panel **B**. Note the increase in the proportion of delayed latencies during MI. Panel **C** shows high speed line scan images of Ca sparks in response to depolarization by action potentials (shown above each image) under control conditions and during MI. Spark latency increases during MI while spark probability declines, in parallel with the increased latency and reduced open probability of LCCs as shown in **A** and in the inset of original single Ca channel records. In this example, spark probability and latency

returned to normal as the action potential duration became very short, the result of increased Ca influx caused by rapid early repolarization. The ability to trigger Ca release with a short action potential indicates that RyRs can still respond to Ca even during advanced MI, and that changes in Ca channel behavior are the primary reason for reduced spark probability and increased latency (from Chantawansri *et al*, 2008 with permission).

Figure 2. BUFFERING CALCIUM IN THE DIADIC CLEFT REDUCES SPARK PROBABILITY IN NCX KNOCKOUTS, BUT NOT IN WILDTYPE

A: Representative action potentials stimulated by current commands and **B**, corresponding high speed (0.24 ms/line) line-scan images recorded simultaneously in representative WT and NCX KO myocytes. Cells were loaded with 1 mM fluo-3 and 3 mM EGTA via the patch pipette to buffer Ca in the diadic cleft. Action potentials and images are also shown on a higher-resolution temporal scale (scale bar, 10 ms). Dashed line indicates the time when the earliest Ca spark was activated. Arrowheads mark the positions where couplons failed to activate. Fluorescence intensities are reported in self-ratioed $\Delta F/F$ magnitude as indicated in the adjoining palette. **C:** Ca spark latency histograms (15-ms bins) constructed from line-scan images recorded in WT (left, $n=8$ cells from four mice) and NCX KO (right, $n=8$ cells from four mice) myocytes. Note the increased spark latency in the KO compared to WT, a consequence of buffering the diadic cleft with EGTA. These data show that KO mice require elevated cleft Ca for efficient EC coupling, whereas WT mice are able to prime the diadic cleft with Ca via reverse NCX (from Neco *et al.*, 2010, with permission).

Figure 3. REVERSE NCX IS AN ESSENTIAL COMPONENT OF THE CALCIUM-INDUCED CALCIUM RELEASE MECHANISM OF CARDIAC EXCITATION-CONTRACTION COUPLING

We used a ramp prepulse to inactivate I_{Na} immediately prior to application of an action potential voltage clamp waveform in WT (left) and NCX KO (right) myocytes. In this representative example of Ca transients recorded with Fura-2 in patch clamped myocytes, we found that in WT the prepulse markedly reduced the Ca transient, whereas in KO there was no effect. This shows that reverse NCX driven by Na entry during the upstroke of the action potential increases coupling fidelity, i.e. the probability of triggering Ca release from the SR (from Larbig *et al.*, 2010, with permission).