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^{2+}) 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^{2+} 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^{2+} entry by this mechanism then "primes" the diadic cleft so that subsequent Ca^{2+} entry through Ca^{2+} channels can more efficiently trigger Ca^{2+} release from the sarcoplasmic reticulum. In NCX knockout mice, this mechanism is inoperative (Na^{+} current has no effect on the Ca^{2+} transient), and excitation-contraction coupling relies upon the elevated diadic cleft Ca^{2+} that arises from the slow extrusion of cytoplasmic Ca^{2+} by the ATP-dependent sarcolemmal Ca^{2+} 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.

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L. Annunziato (ed.), *Sodium Calcium Exchange: A Growing Spectrum of Pathophysiological Implications*,
Advances in Experimental Medicine and Biology 961, DOI 10.1007/978-1-4614-4756-6_30,

Keywords
Sodium-calcium exchange • Excitation-contraction coupling • Heart failure • Calcium channels • Sodium current • Contractility

30.1 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; 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 US 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 one million myocardial infarctions annually in the United States. Fifty percent 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.

30.2 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^{2+} content (Studer et al. 1994; Flesch et al. 1996; Hobai and O'Rourke 2000; Hasenfuss and 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^{2+} 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^{2+} loading to adrenergic signals (Koch et al. 2000). Other factors contribute to contractile dysfunction: these include defective SR Ca^{2+}-ATPase activity, leading to reduced SR Ca^{2+} content (Schmidt et al. 1998); myofilament dysfunction, which decreases the contractile response to released Ca^{2+} (Hajjar and Gwathmey 1990); mitochondrial dysfunction, which leads to energy starvation; and fibrosis, which replaces myocytes with noncontracting cells (Ingwall and Weiss 2004).

30.3 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^{2+} influx via Ca^{2+} current (I_{Ca}), (2) increased SR Ca^{2+} uptake rate (via phospholamban (PLB) phosphorylation),

and (3) increased myofilament Ca^{2+} 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^{2+} load leading to SR Ca^{2+} overload, which has a variety of deleterious consequences including arrhythmia and cell death.

30.4 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^{2+}-induced Ca^{2+} release (CICR) mechanism controls EC coupling in cardiac cells (Fabiato 1983). Ca^{2+} entering through sarcolemmal L-type Ca^{2+} channels (LCCs) triggers release of Ca^{2+} by RyRs on the SR surface (London and 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^{2+} into a restricted junctional region (the diadic cleft), leading to a significant rise in Ca^{2+} concentration. This Ca^{2+} gates a cluster of RyRs on the apposing membrane of the junctional SR, allowing Ca^{2+} release from the SR to generate a Ca^{2+} 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^{2+} transients. However, we now know that action potentials in healthy cells trigger each couplon simultaneously in a coordinated and synchronous manner (Inoue and Bridge 2003). This synchronous activity appears to be critical for optimum contractility.

Failing cardiac muscle is characterized by the loss of synchronized Ca^{2+} release upon depolarization, as exemplified by postinfarct remodeling in the rabbit (Litwin et al. 2000). We have found similar loss of synchronization of Ca^{2+} release in rabbit cells exposed to metabolic inhibitors (Fig. 30.1), 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^{2+} spark probability and distribution of spark latencies are predicted by LCC latency, open time, and opening probability (P_o). Primary changes in RyR behavior (Meissner 1994) and cellular structure (Gomez et al. 2001) may also contribute to loss of synchronization.

30.5 Excitation-Contraction Coupling in NCX KO Mice

We wondered whether NCX might alter EC coupling independent of changes in SR Ca^{2+} stores and Ca^{2+} 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^{2+}, preserved SR Ca^{2+} stores, and normal Ca^{2+} transients in response to electrical stimulation (Henderson et al. 2004). Because NCX is absent and no other Ca^{2+} efflux mechanism increases to compensate, Ca^{2+} removal in response to caffeine-induced SR Ca^{2+} release is dramatically reduced. A major adaptation in this model appears to be a reduction in Ca^{2+} influx through LCCs and an associated increase in EC coupling gain (Pott et al. 2005). The reduced Ca^{2+} current

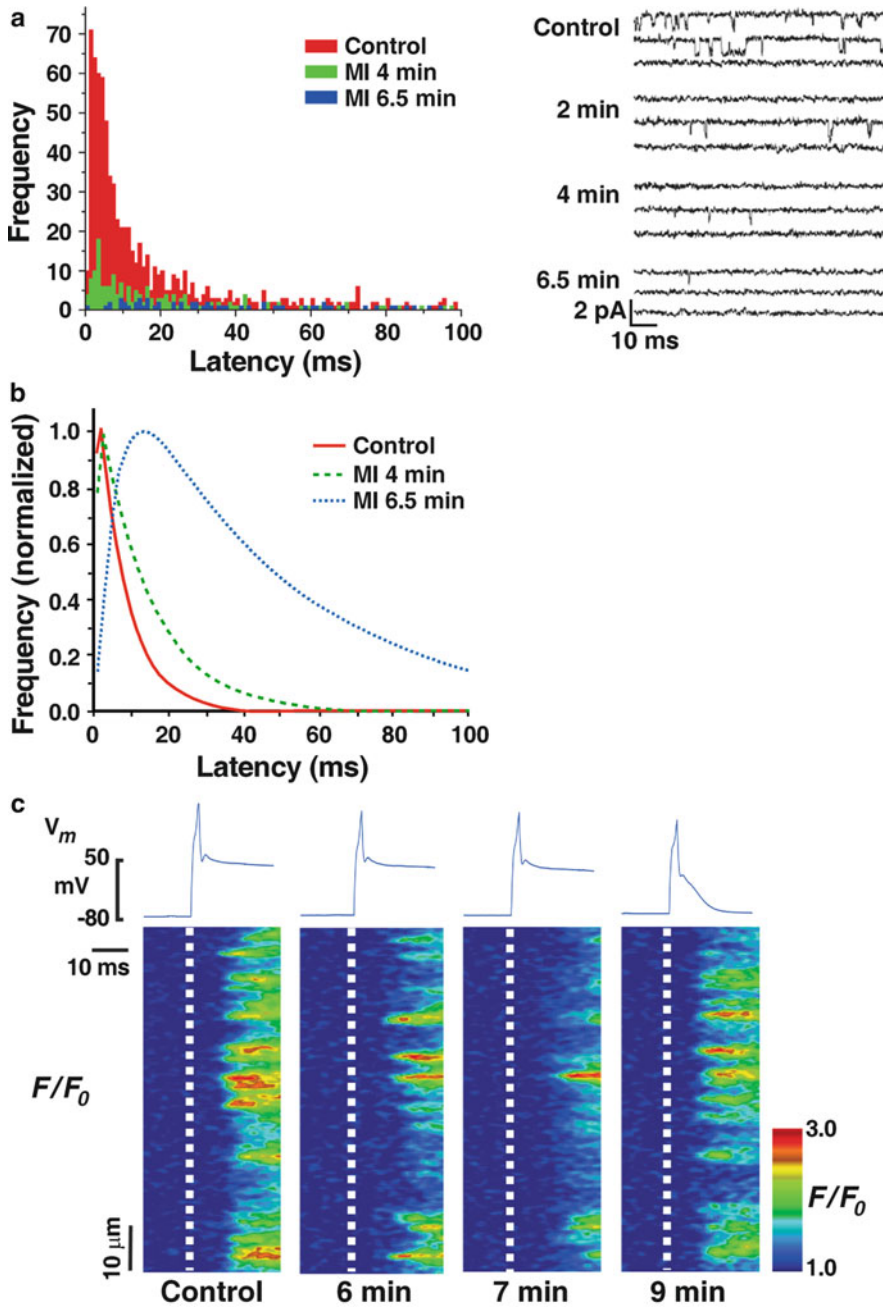

Fig. 30.1 Effect of metabolic inhibition on LCCs and triggered Ca^{2+} sparks in isolated adult rabbit ventricular myocytes. (**a**) Frequency distribution of single L-type Ca^{2+} channel latency under control conditions (*red*), and after 4 (*green*) and 6.5 (*blue*) min of oxidative and glycolytic 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. (**c**) Shows high-speed line-scan images of Ca^{2+} 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^{2+} 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^{2+} influx caused by rapid early repolarization. The ability to trigger Ca^{2+} release with a short action potential indicates that RyRs can still respond to Ca^{2+} even during advanced MI and that changes in Ca^{2+} channel behavior are the primary reason for reduced spark probability and increased latency (From Chantawansri et al. 2008 with permission)

(I_{Ca}) is caused by an increase in subsarcolemmal/diadic cleft Ca^{2+} concentration and the resulting Ca^{2+}-dependent inactivation (Pott et al. 2007a). Action potential shortening caused by upregulation of the transient outward current (I_{TO}) also limits Ca^{2+} entry during depolarization (Pott et al. 2007b). Resting Ca^{2+} sparks, the elementary events of EC coupling that reflect CICR activity at the single-couplon level, are reduced in frequency compared to wild-type 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^{2+} removal from the diadic cleft in KO cells. Spark activity and size equalize when cells from WT and KO mice are permeabilized to eliminate the influence of NCX, I_{Ca}, and differences in cleft Ca^{2+} (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.

30.6 Reverse NCX and SR Ca Release Triggering

How then does NCX affect cleft Ca^{2+} and microscopic EC coupling during depolarization? In the cardiac-specific NCX knockout (KO) mouse, effective EC coupling is dependent upon elevated diadic cleft Ca^{2+} throughout the cardiac cycle. This is made clear by experiments buffering Ca^{2+} in the cytoplasm using EGTA. Under strong Ca^{2+} buffering conditions, KO mice exhibit reduced coupling efficiency (exemplified by decreased spark number and increased spark latency), whereas wild-type (WT) mice display normal coupling (Fig. 30.2, 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^{2+} 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^{2+} 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^{2+} release (LeBlanc and 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 and Goknur 1991; Nuss and Houser 1992; Kohmoto et al. 1994; Wasserstrom and Vites 1996; Lines et al. 2006), others refuted NCX ability to trigger SR Ca^{2+} 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 and Niggli 1994; Lopez-Lopez et al. 1995; Sipido et al. 1995, 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^{2+} channels by voltage protocols, instability in SR Ca^{2+} content, incomplete blockade of I_{Ca} by voltage-dependent blockers, and nonphysiologic 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^{2+} 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 tetrodotoxin (TTX) and without introducing voltage errors or unplanned changes in Ca^{2+} channel activity. Using this protocol, we found that eliminating I_{Na} selectively decreases (but does not eliminate) Ca^{2+} release in WT but has no effect in NCX KO (Fig. 30.3). 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}

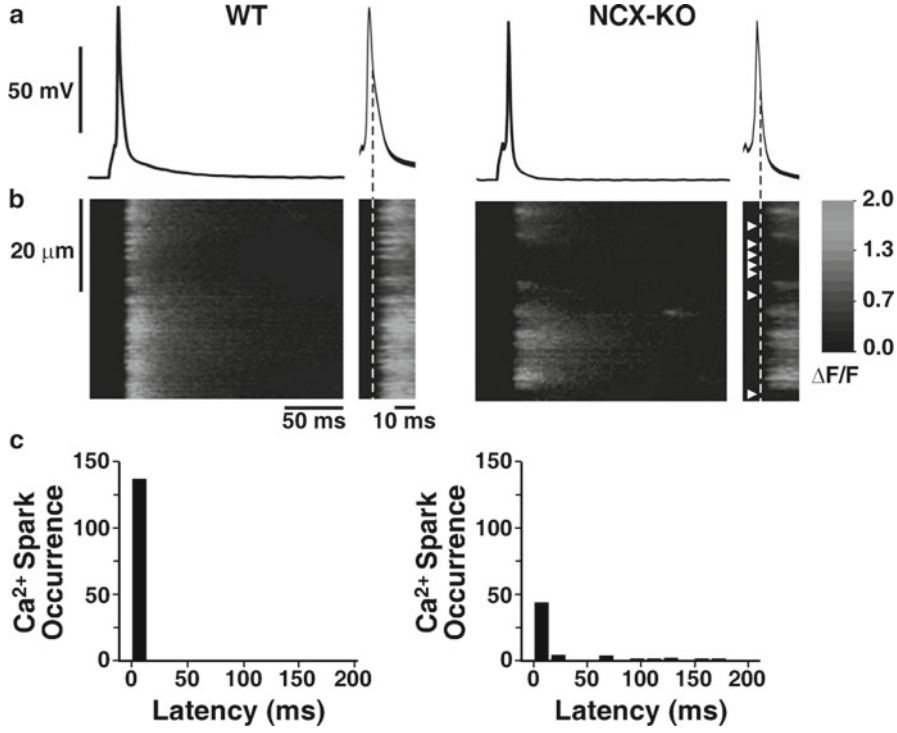

Fig. 30.2 Buffering Ca^{2+} in the diadic cleft reduces spark probability in NCX knockouts, but not in wild type. (**a**) Representative action potentials stimulated by current commands, and (**b**) corresponding high-speed (0.24 ms/line) line-scan images recorded simultaneously in representative WT and NCX KO myocytes. Cells were loaded with 1 mM fluo-3 and 3 mM EGTA via the patch pipette to buffer Ca^{2+} 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 □F/F magnitude as indicated in the adjoining palette. (**c**) Ca^{2+} 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^{2+} via reverse NCX (From Neco et al. 2010, with permission).

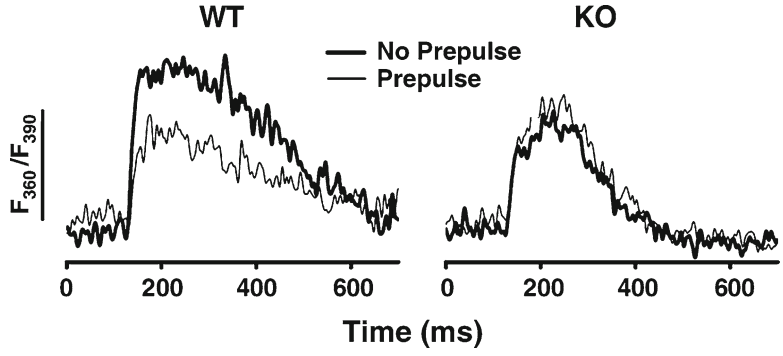

Fig. 30.3 Reverse NCX is an essential component of the Ca^{2+}-induced Ca^{2+} release mechanism of cardiac EC coupling. We used a ramp prepulse to inactivate I_{Na} immediately prior to application of an action potential voltage clamp waveform in WT (*left*) and NCX KO (*right*) myocytes. In this representative example of Ca transients recorded with Fura-2 in patch clamped myocytes, we found that in WT the prepulse markedly reduced the Ca^{2+} 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^{2+} release from the SR (From Larbig et al. 2010, with permission)

makes an important contribution to the triggering process. To confirm this finding using a different approach, we applied the Na^+ channel blocker TTX (5 μM) using a rapid solution exchange device 1 s prior to depolarization by the action potential voltage clamp. TTX rapidly and reversibly reduced the Ca^{2+} transient without reducing SR Ca^{2+} load, confirming the effect of Na-induced reverse NCX on CICR.

30.7 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) (Gershome et al. 2011). Blocking these channels in rats apparently has no effect on EC coupling (Brette and 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^{2+} release. We tested this hypothesis in rabbit, a species which is more dependent on Ca^{2+} 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^{2+} release similar to the reduction in Ca^{2+} release observed during a slow ramp prepulse and similar to that described above for mouse (Torres et al. 2010).

30.8 Essential Role of NCX in Priming the Diadic Cleft

Our results suggest that NCX plays an essential role in the process of Ca^{2+}-induced Ca^{2+} release, not simply by direct triggering of RyRs (which seems unlikely based on the relative inefficiency of NCX as demonstrated by Sham et al. (1992) and 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^{2+}. We know that the relationship between RyR P_o and activating Ca^{2+} is sigmoid (Copello et al. 1997). The NCX-mediated priming of cleft Ca^{2+} moves Ca^{2+} concentration along the flat part of this sigmoid curve without increasing RyR P_o appreciably. However, the Ca^{2+} 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^{2+} entry upon activation of I_{Ca} will further raise Ca^{2+} 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 nonlinear fashion (Torres et al. 2010). Without this priming effect, the entry of Ca^{2+} 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, the cleft Ca^{2+} is elevated throughout the cardiac cycle, so further priming by I_{Na} and NCX is not required (Larbig et al. 2010).

30.9 Conclusion

These findings raise the intriguing possibility of manipulating NCX as a therapeutic tool in HF, not simply to alter Ca^{2+} efflux and SR Ca^{2+} 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^{2+} overload and the consequent arrhythmias and cellular damage. The increase in Ca^{2+} 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^{2+} 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^{2+}-dependent inactivation, independent of SR Ca^{2+} load and global cytoplasmic Ca^{2+} levels, which are unchanged. The reduction in LCC activity also reduces the frequency of resting Ca^{2+} sparks. Nevertheless, the size of Ca^{2+} 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^{2+}. Conversely, 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^{2+} and increase 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^{2+} by facilitating Ca^{2+} efflux. NCX is therefore a potentially major therapeutic target with a higher safety margin than current agents.

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