Published in final edited form as:
J Mol Cell Cardiol. 2013 August ; 61: 28–33. doi:10.1016/j.yjmcc.2013.06.001.

Na/Ca exchange and contraction of the heart

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Abstract

Sodium-calcium exchange (NCX) is the major calcium (Ca) efflux mechanism of ventricular cardiomyocytes. Consequently the exchanger plays a critical role in the regulation of cellular Ca content and hence contractility. Reductions in Ca efflux by the exchanger, such as those produced by elevated intracellular sodium (Na) in response to cardiac glycosides, raise sarcoplasmic reticulum (SR) Ca stores. The result is an increased Ca transient and cardiac contractility. Enhanced Ca efflux activity by the exchanger, for example during heart failure, may reduce diadic cleft Ca and excitation-contraction (EC) coupling gain. This aggravates the impaired contractility associated with SR Ca ATPase dysfunction and reduced SR Ca load in failing heart muscle. Recent data from our laboratories indicate that NCX can also impact the efficiency of EC coupling and contractility independent of SR Ca load through diadic cleft priming with Ca during the upstroke of the action potential.

Keywords

Sodium calcium exchanger; Calcium; Heart contractility; Excitation contraction coupling; Heart failure; Genetically modified mice

Introduction

The sodium-calcium exchanger (NCX) is the dominant Ca efflux mechanism in cardiac myocytes. Because of its essential role in Ca regulation, particularly sarcoplasmic reticulum (SR) Ca content, it has an enormous influence on contractility. In this article, we will discuss how NCX can influence contractility in the heart under physiological and pathophysiological conditions.

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Disclosures: none declared

1. Relationship of NCX Structure to Excitation-Contraction Coupling

The structure of NCX is reviewed in an accompanying manuscript in this issue. For the purposes of the following discussion, we will consider the cardiac isoform of the exchanger, NCX1. In brief, NCX1 (referred to as NCX from this point on) is a plasma membrane protein that consists of 10 transmembrane segments that bind and transport Na and Ca across the plasma membrane [1, 2]. A large cytoplasmic loop between transmembrane segments 5 and 6 contains two regulatory domains for Ca. Thus NCX not only moves Ca across the sarcolemmal membrane but is also regulated allosterically by intracellular Ca [3, 4]. Exchangers appear to assemble as dimers in the plasma membrane, though it is unknown whether this is essential for regulation of transport [5, 6].

Immunolocalization studies have indicated that NCX is distributed throughout the surface membrane including the sarcolemma of the transverse tubular sarcolemma (t-tubules) [7]. There is some indication that NCX density is higher within the t-tubules [8, 9], though NCX localization within myoyctes remains controversial.

NCX transports 3 Na ions in exchange for 1 Ca ion, resulting in electrogenic transport that can be measured as an ionic current. NCX can operate in a bidirectional fashion. In “forward mode” the exchanger extrudes Ca from myocytes in exchange for Na influx. Conversely, during “reverse mode” the exchanger extrudes Na in exchange for Ca influx. The mode in which NCX operates is governed by the Na and Ca gradients across the cell membrane, as well as the membrane potential (E_m), following a driving force equal to $E_m - E_{Na/Ca}$ (where $E_{Na/Ca} = 3E_{Na} - 2E_{Ca}$ with E_{Na} and E_{Ca} the Na and Ca equilibrium potentials, respectively). The most recognized action of NCX is its Ca removal function in forward mode at membrane voltages less than the equilibrium potential, [10]. During the cardiac contraction-relaxation cycle, Ca enters the cell by flowing down its concentration gradient through L-type Ca channels (LCCs) when they open in response to depolarization. This Ca then triggers ryanodine receptors (RyRs) to open allowing Ca release from the SR into the cytoplasm where it binds to myofilaments resulting in contractile force generation. The relationship between SR Ca release flux and the Ca current that triggers it is referred to as the gain of excitation-contraction (EC) coupling and is an expression of its efficiency [11]. Changes in gain do not necessarily correlate with an improvement or decline in SR Ca release. They merely reflect a change in the effectiveness of Ca current at triggering SR Ca release. Myocyte relaxation begins upon Ca removal from the cytoplasm. There are two major routes of removal: one is by the SR Ca ATPase (SERCA), which pumps Ca back into the SR; the other is NCX. Under physiologic conditions, NCX removes the same amount of Ca that entered the cell through LCCs in order to maintain cellular Ca balance. This was first demonstrated by Bridge *et al.* [12], who were able to effectively separate and measure both Ca entry through Ca current (I_{Ca}) as well as corresponding Ca efflux through NCX from a single contraction/relaxation cycle. The plasma membrane Ca ATPase (PMCA) and mitochondria are considered minor players in the balance of cellular Ca, although their contribution should not be dismissed as there is accumulating evidence that they can meaningfully influence cellular Ca stores and modify EC coupling (see [13] for review). A persistent reduction in cellular Ca efflux not matched by a decrease in Ca influx will result in increased cellular Ca. The expectation is that this increased cellular Ca will accumulate in the SR rather than the cytoplasm if SERCA is performing normally. The increased SR store typically results in larger Ca transients and therefore contractility [14, 15]. Conversely, an increase in Ca efflux will deplete SR stores and depress contractility. Among different species, the tendency to deplete or load SR Ca in resting cells appears to depend upon intracellular Na concentration: in species with higher Na concentrations (e.g. mice/rats), SR loading appears to occur at rest whereas those with lower Na concentrations (e.g. rabbits/guinea pigs/humans) are associated with SR Ca depletion at rest. The prevailing hypothesis is that these changes in SR Ca load in response to the levels of intracellular Na are mediated

by NCX [16]. Higher intracellular Na concentration reduces NCX mediated Ca efflux, which allows SERCA to compete more effectively for Ca during the relaxation phase of the Ca transient as described above. Whether the reduced NCX-mediated Ca efflux in the setting of elevated Na raises diastolic Ca levels within the diadic cleft is uncertain. However this could be a mechanism for increased EC coupling gain in species with higher intracellular Na. The general concept of SR loading and unloading in response to intracellular Na concentration has important implications for treatment of contractile dysfunction in heart failure, as discussed below and elsewhere [17, 18].

2. Influence of NCX on SR Ca Stores and Contractility in Heart Failure

SR Ca store depletion and associated contractile dysfunction are characteristics of systolic heart failure (HF) that are thought to be the consequence of depressed SERCA function, leaky RyRs and increased expression of NCX [19–27]. Na-K pump inhibitors such as digitalis have been used for over 200 years to improve contractility in failing myocardium. In the 1970s and 80s the mechanism of action of these cardiac glycosides was attributed to Na accumulation in the cytoplasm producing Ca influx through reverse NCX [28, 29]. Subsequently, Satoh et al [30] demonstrated that actual Ca influx via NCX is not required for Ca to accumulate in the SR. Instead Na accumulation reduces the thermodynamic drive for Ca efflux through NCX, which shifts the burden of Ca removal to SERCA. This results in higher SR Ca loads and the consequent inotropic effect observed with digitalis treatment. Most studies continue to support the hypothesis that NCX is required for the inotropic effect of digoxin [31, 32]. However these studies frequently rely upon relatively non-selective NCX blockers such as SEA-0400 and KB-R7943 [33, 34] to draw their conclusions. Furthermore, a number of alternative mechanisms for the inotropic effect of digitalis (e.g. Sagawa *et al.*, [35]) have been proposed.

Because of the poor selectivity of NCX blockers and inconsistent experimental results, Reuter *et al.* used an NCX knockout (KO) mouse to unequivocally prove that NCX is required for the inotropic effect of digoxin [36]. The global knock-out of NCX ($NCX^{-/-}$) is embryonic lethal. However, $NCX^{-/-}$ heart tubes can be isolated for study at 9.5 days post coitum [36]. When loaded with the Ca indicator fura-2, these heart tubes demonstrate normal Ca transients and regional contractions in response to electrical stimulation, despite a poorly developed SR. Application of the cardiac glycoside ouabain increases Ca transients in wild type (WT) heart tubes, eventually leading to Ca overload (Fig. 1). In response to ouabain, the relaxation rate of the Ca transient declines, indicative of reduced Ca extrusion from the cell. In contrast, ouabain had no effect on Ca transients in NCX KO heart tubes, even when much higher doses were tested [36]. These results indicate that NCX is required for the action of cardiac glycosides in this model.

3. Overexpression of NCX in Non-Failing Heart: Effects on EC Coupling and Contractility

The demonstration that thermodynamic and pharmacologic manipulation of NCX can support SR Ca and contractility in HF (where NCX expression is increased) still provides only indirect evidence that increased NCX activity by necessity depletes SR Ca and depresses contractility. Indeed, Weisser-Thomas et al [37] found that the NCX blocker KB-R7943 actually reduced contractility in failing human myocytes by blocking NCX-mediated Ca entry during the late plateau phase of the prolonged action potential (AP). This would argue that increased NCX activity in HF is essential to maintain SR Ca content rather than to deplete it.

Because of inconsistent conclusions, and the use of drugs such as KB-R7943 that are relatively non-specific, genetic overexpression of NCX has been used to provide additional information about NCX's effect on contractility. Several groups have reported that

heterozygous constitutive overexpression of NCX (2.3-fold increase in NCX activity) had no effect on contractility and did not result in heart failure [38–40]. Induced overexpression of NCX in mice using a tetracycline transactivator (~40% increase in NCX current) and gene transfer of NCX to cultured adult rat ventricular myocytes (2.8-fold increase in protein expression) likewise showed no effect on contractility [41, 42]. However these studies showed that increased expression does result in exaggerated changes in SR Ca content and contractility (increased or decreased) in response to increases and decreases in bath Ca. On balance the results suggest a relatively neutral effect of increasing NCX activity on contractility as long as there is no externally imposed perturbation of ion concentrations or membrane voltage.

To investigate the effects of further increases in NCX activity on contractility and EC coupling we generated a homozygous overexpressing NCX mouse that produces a 3.1-fold increase in NCX activity [43]. Although these animals demonstrated mild hypertrophy at 3.5 months (22% increase in heart weight to tibia length), they had normal baseline cardiac function and no evidence of heart failure unless stressed hemodynamically [44]. We found an insignificant trend for reduction in SR Ca content. However, we did identify a significant decrease in the amplitude of the Ca transient in response to a standard voltage clamp designed to maximize the Ca current (−40 mV to 0 mV). Although the Ca current *increased* by almost 50%, the Ca transient amplitude unexpectedly *decreased* by almost half. This decrease in the extent of Ca release in response to Ca current is an indication of reduced EC coupling gain in overexpressing myocytes (Fig. 2). The cause of the increased Ca current appears to be a reduction in the concentration of Ca in the vicinity of LCCs produced by the overabundance of Ca-removing NCX transporters. Thus excessive NCX activity in this model leads to defective EC coupling and reduced Ca transients, most likely by directly interfering with the efficiency of the trigger mechanism for Ca release. More studies are necessary to further corroborate this hypothesis. Nevertheless these animals survive without evidence of reduced contractility, suggesting that compensatory increases in I_{Ca} and the AP duration help to maintain contractility. Interestingly, defects in EC coupling are typical of many heart failure models [45, 46] where NCX may also be increased. The prevailing hypothesis is that the increase in NCX compensates for the reduction in SERCA activity [46]. Although this helps to prevent cytoplasmic Ca overload, it results in unloading of SR Ca and consequent reduced contractility. The extent to which increased NCX activity versus other heart failure-induced changes (e.g. t-tubular remodeling and loss of couplons [47]) participate in EC coupling defects is unclear.

4. NCX, Diadic Cleft Calcium and EC Coupling Efficiency

The forgoing studies demonstrate that NCX is capable of influencing contractility in two different fashions: the first involves altering SR Ca content through competition with SERCA; the second involves modulating SR Ca triggering by influencing local Ca in the vicinity of LCCs and RyRs. To study this second concept further, we produced a conditional knockout of NCX in mice. As noted above, global knockouts of NCX are embryonic lethal. To address this concern, we generated a conditional knockout of NCX that lives into adulthood [48]. We crossed mice floxed at exon 11 of NCX gene with mice expressing cre-recombinase under the control of the ventricular-specific myosin light chain (MLC2v) promoter. This strategy eliminates NCX in approximately 80% of myocytes in the adult mouse ventricle. The small percentage of cells with normal NCX is expected when using the cre-lox system in the ventricle [49, 50]. Echocardiograms show mildly reduced but adequate systolic function [48] along with mild hypertrophy at 7.5 weeks of age. However as the mice age, they are less tolerant to hemodynamic stress than WT controls [51]. There is no evidence of structural abnormalities by thin-section electron microscopy and no changes in expression of other major myocardial proteins including SERCA, calsequestrin, PMCA and

dihydropyridine receptor (DHPR). No other NCX isoforms are expressed in these hearts. We found no changes in SR Ca load as assessed by caffeine pulses in fura-2 loaded myocytes, and Ca transients in paced cells were indistinguishable from WT. However, there is a ~50% decrease in the amplitude of I_{Ca} in KO cells. Since SR Ca release is normal despite the decrease in I_{Ca}, we conclude that EC coupling gain is increased in KO myocytes [52]. The decrease in I_{Ca} and the increased gain in KO mice are the exact opposite of what we found in NCX *overexpressing* mice (see above), supporting the idea that NCX can influence EC coupling without altering SR Ca load. The question remains how the elimination of NCX can increase gain to maintain EC coupling despite a smaller Ca current. The answer is related to an increase in subsarcolemmal or diadic cleft Ca in the vicinity of the LLCs and RyRs. This was demonstrated by buffering cellular Ca using the fast Ca chelator BAPTA (10 mM in the patch electrode), which restored I_{Ca} to wildtype levels [53]. Increased local Ca in the diadic cleft raises the gain of EC coupling in the KO mouse by reducing the amount of Ca entry (through LCCs) required to trigger RyRs.

The importance of NCX and diadic cleft Ca as a regulator of EC coupling and contractility is illustrated very clearly in the following experiment. In patch clamped ventricular myocytes isolated from NCX KO mice, Ca buffering with 3 mM EGTA and 1 mM fluo-3 in the patch pipette reduces the probability of Ca sparks triggered by the AP. However the same buffering protocol has no deleterious effect on triggering in WT [54]. We proposed that KO myocytes are dependent on elevated Ca in the cleft to increase the coupling fidelity of the reduced I_{Ca}. The mechanism relies upon the sigmoid dependence of RyR open probability on gating Ca concentration [55]. RyR open probability depends upon Ca concentration in the diadic cleft. In WT animals, we assume resting Ca concentration resides at the foot of the sigmoid curve, but in KO animals cleft Ca is already elevated to the point where the slope of the curve has begun to steepen (because the only remaining Ca efflux mechanism, PMCA, is less effective at Ca removal than NCX). Thus any further increase in cleft Ca by LCCs openings produces a marked increase in the open probability of RyRs. Buffering cleft Ca in KO animals lowers the Ca concentration back down to the foot of the curve, and I_{Ca} (even though it increases in the absence of inactivating Ca) is incapable of reliably triggering RyRs without the assistance of NCX. WT cells on the other hand appear to overcome this problem by employing reverse NCX to prime the diadic cleft with Ca just before LCCs open [54]. Further evidence for this is described below.

In 1990, LeBlanc and Hume proposed that reversal of NCX by Na current (I_{Na}) could trigger Ca release by RyRs directly [56]. They argued that I_{Na} activated during the upstroke of the AP increases subsarcolemmal Na very rapidly. As depolarization continues, reverse NCX becomes thermodynamically favorable and Ca entry through NCX triggers RyRs. It is straightforward to demonstrate that reverse NCX can trigger Ca release with increased intracellular Na, for example when high Na is included in the patch pipette solution [57–62]. However, Sipido et al [63] found that NCX produced little triggering or did so very slowly, and Weber *et al.* [64] argued that Ca entry through LCCs would prevent the exchanger from reversing [65] after the first 15 ms (although EC coupling is complete within 3–5 ms). Nevertheless this early Ca entry is sufficient to prime the diadic cleft with Ca, particularly if Na channels and NCX are located together in the cleft. To address these issues, we took a novel approach by using our ventricular-specific conditional NCX KO mice to assess the effect of I_{Na}, and thus reverse NCX, on Ca release in murine ventricular myocytes [66]. We recorded intracellular Ca transients in the presence or absence of I_{Na} in patch clamped cells using voltage waveforms designed to simulate an AP. We eliminated I_{Na} prior to the AP clamp by applying a ramp to −45 mV. We verified in separate cells that this ramp eliminated I_{Na} without compromising I_{Ca}. We found that in WT cells the prepulse reduced the Ca transient amplitude by 51.1 ± 4.6% ($P < 0.001$, $n = 14$), but had no effect on Ca transients in NCX KO cells (Fig. 3). By disabling the SR using thapsigargin and ryanodine, we were able

to measure Ca entering the cell during this AP waveform protocol. We observed a marked reduction of Ca entry when I_{Na} was blocked by the prepulse. We could also reduce the Ca transient in WT cells by applying 10 μM TTX, but this had no effect on KO. Thus reverse NCX in response to I_{Na} is necessary for efficient EC coupling in mice, presumably by increasing coupling fidelity.

Although these experiments clearly describe the contribution of NCX to RyR triggering during the upstroke of the AP, EC coupling in mice does not accurately reflect the process in humans. Most notably, the AP of the mouse is much shorter than in humans, with a higher expression and activity of I_{TO}. To address this concern, Torres *et al.* [67] investigated the contribution of reverse NCX triggering using isolated rabbit ventricular myocytes. Unlike mice, rabbit APs have less I_{TO} activity and a long plateau phase similar to that in humans. EC coupling gain in rabbits is also lower than in mice, and is also representative of human EC coupling. Ca entry from LCCs contributes about 20% of the contractile Ca as opposed to 10% in mice [68]. Torres *et al.* [67] used an AP waveform clamp to minimize loss of voltage control and applied a voltage ramp to inactivate I_{Na}. As in WT mice, the ramp prepulse reduced Ca release. Interestingly, they found that they could similarly reduce Ca release using a low dose of TTX (100 nM, see Fig. 4) to block TTX-sensitive Na channels (Na_v 1.1 and 1.4 in rabbit ventricle) which are more sensitive to TTX than the cardiac isoform (Na_v 1.5) [69–74]. These TTX-sensitive Na channels, which resemble neuronal isoforms, are thought to be concentrated in the t-tubules, in close proximity to LCCs, RyRs, and probably Na channels and NCX [75, 76], potentially priming the diadic cleft with Na [67]. This would in turn reverse NCX prior to I_{Ca} activation, which would prime the diadic cleft with Ca and increase EC coupling gain as described above in mice [54]. It is possible that a different mechanism for control of gain is present in rats [77].

Conclusions

In summary, NCX plays a major role in modulating contractility in cardiac myocytes through multiple mechanisms. Clearly NCX can influence SR Ca load and hence contractility through competition with SERCA during the decline of the Ca transient in diastole. NCX can also impact the efficiency of EC coupling and contractility independent of SR Ca load through effects on diadic cleft Ca. In response to I_{Na}, NCX primes the diadic cleft with Ca, thereby increasing the probability of triggering RyRs within a couplon. Further studies will be necessary to clarify how this mechanism is related to different populations of Na channels located in the t-tubule system. Finally, increased expression of NCX in HF may not only reduce SR Ca by mass action, but also may reduce EC coupling gain by depleting Ca from the diadic cleft.

Acknowledgments

The authors thank Scott Lamp for figure preparation. The work described in this review was supported by the Doroty and E. Phillip Lyan Endowment in Laser Research, AHA Research Grant 12IRG9140020 to JIG and the following NIH grants: R01HL70828 to JIG and R01HL48509 to JIG and KDP.

Abbreviations

NCX	sodium calcium exchanger
KO	NCX knockout mouse
Ca	calcium
AP	action potential

EC	excitation-contraction coupling
RyRs	ryanodine receptor
SR	sarcoplasmic reticulum
LCCs	L-type Ca channels

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Highlights

- NCX transports Ca in and out of the cell in exchange for Na, controlling Ca homeostasis
- Changes in Na concentration alter the driving force for Ca efflux by NCX
- Reduced Ca efflux by NCX increases Ca in the sarcoplasmic reticulum, increasing contractility
- Increased NCX activity or expression in heart failure may reduce contractility
- Genetically modified mice lacking or overexpressing NCX are useful to study NCX role in heart failure

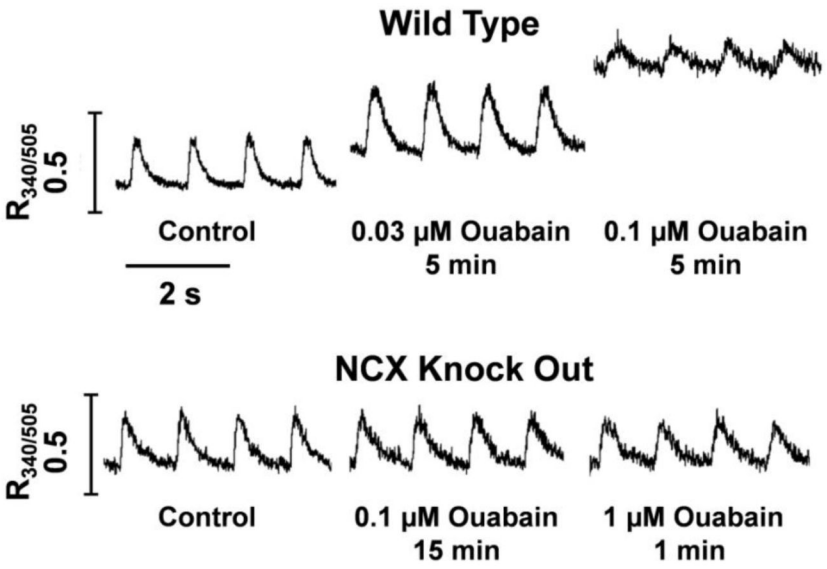

Figure 1. Effects of ouabain on Ca transients in embryonic heart tubes isolated from wild type and NCX$^{-/-}$ mice

In wildtype heart tubes loaded with the Ca indicator fura-2 (*upper panels*), 0.03 μM ouabain moderately increased Ca transients whereas 0.1 μM ouabain caused Ca overload. However in NCX$^{-/-}$ heart tubes (*lower panels*) ouabain at either concentration had no effect, demonstrating that NCX is required for the effect of ouabain on Ca transients (modified from Reuter *et al.*, Circ Res. **90,** 305–308, 2002 and used with permission).

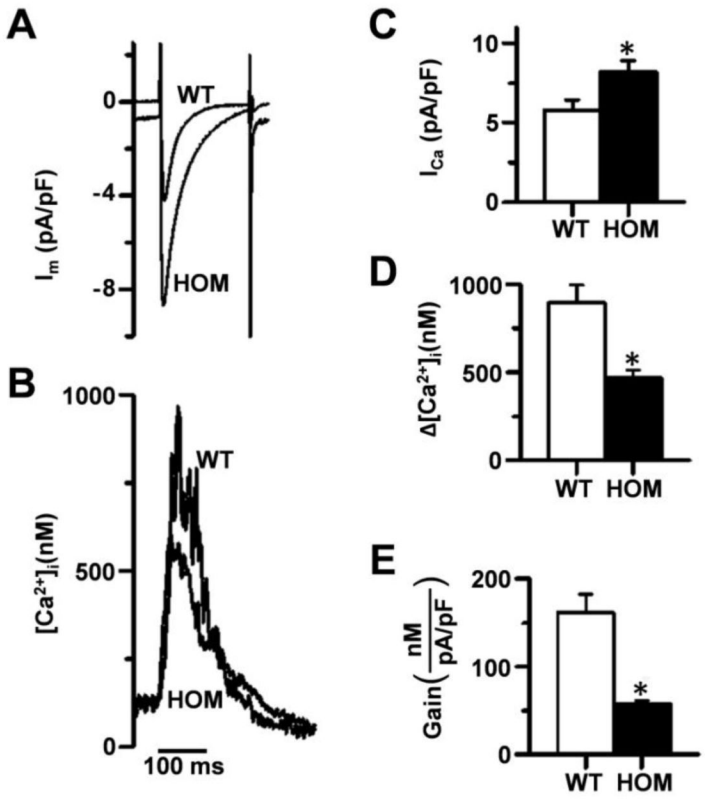

Figure 2. Reduced Excitation-Contraction Coupling Gain in NCX Homozygous Overexpressing Mice

Representative Ca currents (I_m) from wildtype (WT) and NCX homozygous overexpressing mice (HOM) are shown in **A**. Although the HOM Ca current is larger in the WT (summarized in **C**), the corresponding Ca transient ($\Delta[Ca^{2+}]_i$) measured with fura-2 (**B**) is smaller. The decreased Ca transient (summarized in **D**) in response to the increased current indicates a reduction in gain (**E**). (This figure was modified from Reuter *et al.*, J Physiol. **554,** 779–789, 2004, and used with permission [43]).

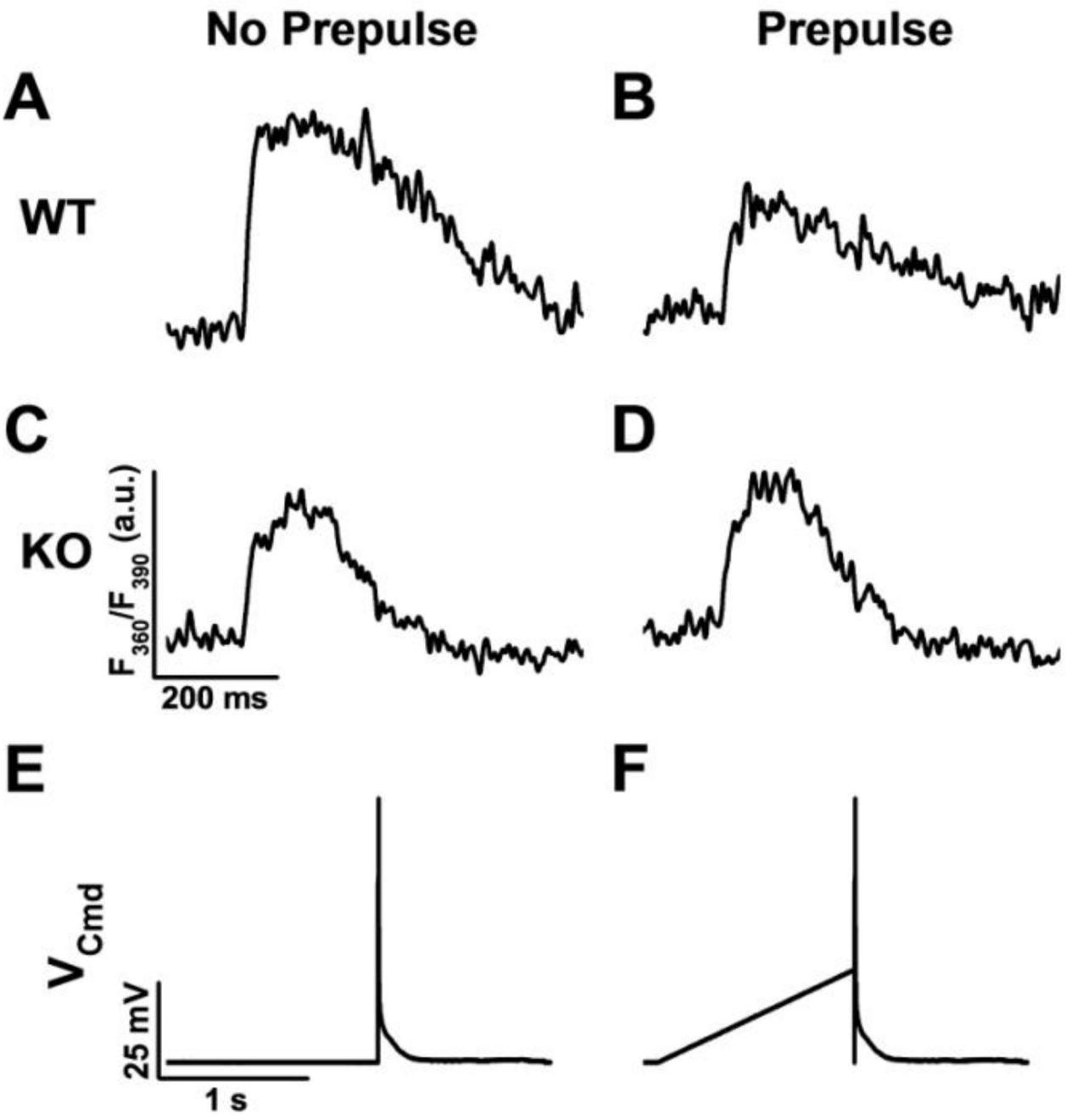

Figure 3. Effects of I_{Na} inactivation on Ca transients in WT and conditional NCX KO myocytes

Ca transients from a patch clamped wildtype (WT) ventricular myocyte before (**A**) and after (**B**) inactivating I_{Na} using a ramp prepulse. Note the decreased Ca transient after applying the inactivating prepulse (**B**). In contrast, the ramp prepulse has no effect in KO (**C**, **D**). Command voltage waveforms are shown in **E** (no prepulse) and **F** (prepulse). (Figure modified from Larbig *et al.* J Physiol. **588,** 3267–3276 with permission [66]).

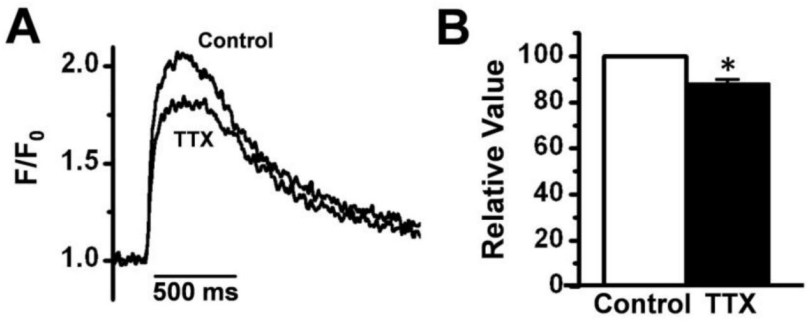

Figure 4. Effect of 100 nM TTX on Ca transient in isolated patch clamped rabbit myocytes

Representative Ca transients with and without 100 nM TTX present in the extracellular solution (**A**). The amplitude of the Ca transient decreased 12±2% (n=6) with TTX (summarized in **B**). *p<0.05. (modified from Torres *et al.* J Physiol. 588:4249–60. 2010 with permission [67]).