Regulation of Cardiac L-Type Ca^{2+} Current in Na^+-Ca^{2+} Exchanger Knockout Mice: Functional Coupling of the Ca^{2+} Channel and the Na^+-Ca^{2+} Exchanger

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ABSTRACT L-type Ca^{2+} current (I_{Ca}) is reduced in myocytes from cardiac-specific Na^+-Ca^{2+} exchanger (NCX) knockout (KO) mice. This is an important adaptation to prevent Ca^{2+} overload in the absence of NCX. However, Ca^{2+} channel expression is unchanged, suggesting that regulatory processes reduce I_{Ca}. We tested the hypothesis that an elevation in local Ca^{2+} reduces I_{Ca} in KO myocytes. In patch-clamped myocytes from NCX KO mice, peak I_{Ca} was reduced by 50%, and inactivation kinetics were accelerated as compared to wild-type (WT) myocytes. To assess the effects of cytosolic Ca^{2+} concentration on I_{Ca}, we used Ba^{2+} instead of Ca^{2+} as the charge carrier and simultaneously depleted sarcoplasmic reticular Ca^{2+} with thapsigargin and ryanodine. Under these conditions, we observed no significant difference in Ba^{2+} current between WT and KO myocytes. Also, dialysis with the fast Ca^{2+} chelator BAPTA eliminated differences in both I_{Ca} amplitude and decay kinetics between KO and WT myocytes. We conclude that, in NCX KO myocytes, Ca^{2+}-dependent inactivation of I_{Ca} reduces I_{Ca} amplitude and accelerates current decay kinetics. We hypothesize that the elevated subsarcolemmal Ca^{2+} that results from the absence of NCX activity inactivates some L-type Ca^{2+} channels. Modulation of subsarcolemmal Ca^{2+} by the Na^+-Ca^{2+} exchanger may be an important regulator of excitation-contraction coupling.

INTRODUCTION

The Na^+-Ca^{2+} exchanger (NCX) is the main Ca^{2+} extrusion mechanism of the cardiac myocyte, maintaining Ca^{2+} homeostasis by removing Ca^{2+} that enters the myocyte during each cardiac cycle (1–3). Although functional excitation-contraction coupling would seem impossible without NCX, we have produced a murine cardiac-specific knockout (KO) of NCX. Strikingly, these animals survive into adulthood and exhibit only minor cardiac dysfunction (4,5). One mechanism that enables KO myocytes to maintain Ca^{2+} homeostasis in the absence of NCX is a reduction of Ca^{2+} entry via the L-type Ca^{2+} current (I_{Ca}) (5), which is accentuated by a shortening of the action potential (6).

The mechanism underlying the reduction in I_{Ca} is unknown. Because dihydropyridine receptor (DHPR) expression is unaltered in KO myocytes (7), regulatory processes must be responsible. We have demonstrated previously that the voltage dependence of I_{Ca} is similar in WT and KO, and I_{Ca} is reduced by a similar amount at all voltages in KO compared to WT (5,7). Thus, it seems unlikely that the mechanisms involved in the reduction of I_{Ca} in KO are primarily voltage dependent.

Intracellular Ca^{2+} itself potently inactivates I_{Ca} by a process known as Ca^{2+}-dependent inactivation (8,9). Changes in local subsarcolemmal Ca^{2+} caused by the absence of normal Ca^{2+} extrusion via NCX could explain the reduction of I_{Ca} in NCX KO mice. Such a functional relationship between NCX and I_{Ca} has indeed been demonstrated in a noncardiac mammalian cellular expression system (10). However, the experimental investigation of this relationship in intact cardiac myocytes remains challenging: Pharmacological manipulation of NCX activity is difficult because of a lack of sufficiently specific inhibitors (11). Furthermore, when NCX is acutely blocked by removal of external Na^+, there is an almost immediate increase in the sarcoplasmic reticular (SR) Ca^{2+} load and the Ca^{2+} transient (12). Thus, it is difficult to directly link changes in I_{Ca} to altered NCX activity and to establish a functional relationship between the two proteins.

Cardiac-specific NCX KO mice exhibit unaltered SR Ca^{2+} load, and their global systolic and diastolic Ca^{2+} concentrations measured with the Ca^{2+}-sensitive indicator fura-2 are indistinguishable from those in wild-type (WT) littermates (5,7). Thus, these animals present a unique tool for investigating the effects of NCX on the behavior of the L-type Ca^{2+} current. An increase in subsarcolemmal Ca^{2+} might increase the degree of I_{Ca} inactivation and reduce current amplitude yet be undetectable by conventional fluorescent indicator techniques. We report here that Ca^{2+}-dependent inactivation is indeed responsible for the reduction of I_{Ca} in NCX KO mice. We attribute this to elevated subsarcolemmal Ca^{2+} in the absence of NCX activity.

Submitted July 2, 2006, and accepted for publication October 26, 2006.

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0006-3495/07/02/1431/07 $2.00

doi: 10.1529/biophysj.106.091538

METHODS

Generation of transgenic mice

NCX cardiac-specific KO mice were generated using cre/lox technology as previously described (7). Animals used in this study were between 8 and 12 weeks of age, did not display any gross pathology, had good cardiac function, and exhibited no evidence of heart failure.

Isolation of ventricular myocytes from adult mouse hearts

Before (20 min) explantation of the hearts, mice were injected with 200 μl heparin (10,000 units/ml) i.p. Animals were then anesthetized with 200 μl nembutal (50 mg/ml) i.p., and hearts were quickly removed via thoracotomy. Isolation of ventricular myocytes by collagenase/protease digestion was performed as reported previously (5,7). All procedures were in accordance with the guidelines of the UCLA Office for Protection of Research Subjects. Isolated cardiomyocytes were stored for up to 6 h at room temperature in modified Tyrode solution containing (in mM) 136 NaCl, 5.4 KCl, 10 Hepes, 1.0 $MgCl_2$, 0.33 NaH_2PO_4, 1.0 $CaCl_2$, 10 glucose, pH 7.4 with NaOH. To prevent Ca^{2+} activated Cl^- current, 0.1 mM DIDS (4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid disodium salt hydrate) was present when SR Ca^{2+} release was not depleted by thapsigargin and ryanodine. DIDS was prepared as a 100 mM stock solution by heating in 0.1 M $KHCO_3$. The above solution was also used, with modifications described below, as the standard bath for electrophysiological recordings.

Electrophysiology

Whole-cell membrane currents were recorded as described previously (5,7). The pipette solution contained (in mM) 120 CsCl, 10 TEA-Cl, 10 NaCl, 20 Hepes, 5 MgATP, 0.05 cAMP, pH 7.2 with CsOH, with modifications described below. We recorded whole-cell membrane current using an Axopatch 200 or 200B patch-clamp amplifier (Axon Instruments, Union City, CA) in voltage-clamp mode and a Digidata 1322A (Axon Instruments) data acquisition system controlled by pCLAMP 9 software (Axon Instruments). We applied series resistance compensation to all recordings. To measure differences in decay kinetics of I_{Ca}, single exponential curves were fitted, and the time constant (τ) was calculated.

Rapid solution exchange

Miniature solenoid valves (The Lee Co., Westbrook, CT) controlled by PCLAMP digital outputs controlled the bath solution flow through a micromanifold (ALA Scientific Instruments, Westbury, NY). This enabled precise timing of solution exchanges in relation to the voltage-clamp protocol. The solution surrounding a patched cell exchanged with a half-time of $<$150 ms. The procedure has been described previously (5,7).

Statistical analysis

Data are expressed as means $\pm$ SE. Student's unpaired *t*-test was used for direct comparisons of WT versus KO.

RESULTS

Determination of cellular phenotype

It has been a common finding that cardiac-specific gene knockout using cre/lox technology does not occur with 100% efficiency (13,14). In NCX KO mice, those cells in which gene excision occurs have complete absence of NCX, and the remaining cells have normal (WT) expression levels. Immunofluorescence and functional data indicate that 80–90% of myocytes from NCX KO mice have no detectable NCX (5).

To identify the 10–20% of myocytes isolated from KO animals that express NCX, each myocyte was tested for NCX inward current (I_{NCX}) before exposure to thapsigargin and ryanodine. Cells were held at a voltage of -40 mV. After six 100-ms conditioning pulses from -40 to 0 mV (at 1 Hz), cells were depolarized from -40 to 0 mV for 50 ms to induce I_{Ca} and SR Ca^{2+} release. In WT myocytes, a slowly inactivating inward current is detected on repolarization to -40 mV, which is generated by the forward mode of NCX to remove the elevated cytosolic Ca^{2+} (15). This ''tail'' NCX current is absent in myocytes with a KO phenotype (Fig. 1). Because NCX KO myocytes have the same SR Ca^{2+} content and Ca^{2+} release on depolarization as WT, the absence of inward current is indicative of the absence of NCX. As expected, only 10–20% of myocytes isolated from NCX KO mice expressed I_{NCX}. This result is consistent with findings from our previous studies (5,7) and the published efficiency of the cre recombinase system (13,14). Cells with detectable I_{NCX} were excluded from the KO group.

I_{Ca} amplitude and kinetics in NCX KO myocytes

We recorded I_{Ca} in WT and KO cells. After six prepulses to 0 mV (1 Hz; 100 ms) to ensure steady-state SR Ca^{2+} load,

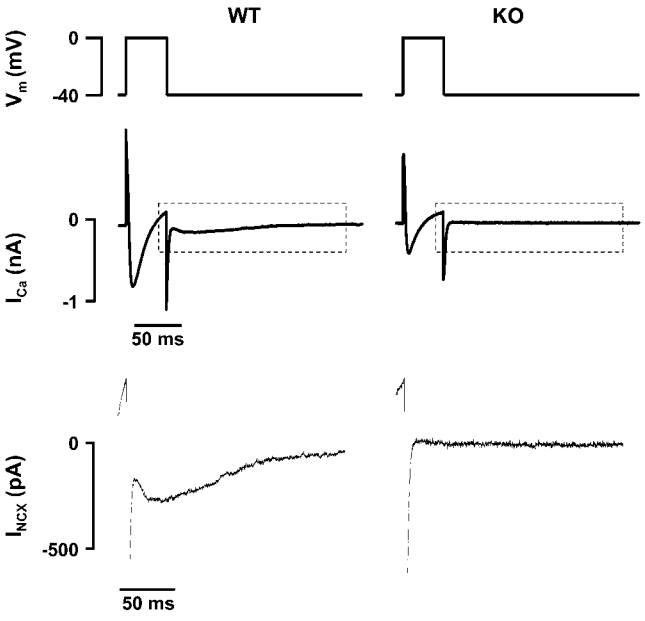

FIGURE 1 Identification of cellular phenotype. (*Top panel*) Cells were held at -40 mV and then depolarized to 0 mV for 50 ms. (*Middle panel*) Depolarization induces I_{Ca}. In cells with a WT phenotype, this led to a second inward current on repolarization generated by NCX. This NCX ''tail current'' is absent in myocytes with a KO phenotype. Insets from the middle panel are magnified in the bottom panel to visualize I_{NCX} on repolarization.

cells were depolarized from −75 to −40 mV for 100 ms to inactivate Na^+ current and then further depolarized to 0 mV (200 ms) to elicit I_{Ca}. Consistent with our previous studies (5,7), we observed a reduced amplitude of I_{Ca} in KO myocytes as compared to WT myocytes (KO, −6.2 ± 0.8 pA/pF, $n = 9$; WT, −12.3 ± 0.8 pA/pF, $n = 15$; $p < 0.01$) (Fig. 2 *A*, Fig. 3). We also found that the decay rate of I_{Ca} was accelerated in KO compared to WT (KO, $\tau = 17.8 \pm 2.5$ ms; WT, $\tau = 27.6 \pm 1.0$ ms; $p < 0.01$) (Fig. 2 *B*, Fig. 3). These changes in amplitude and decay rate are consistent with increased Ca^{2+}-dependent inactivation of the Ca^{2+} current, which we hypothesize is caused by increased subsarcolemmal Ca^{2+} resulting from the elimination of NCX.

Effect of SR Ca^{2+} on I_{Ca} in NCX KO myocytes

To assess the effect of SR Ca^{2+} on I_{Ca} in NCX KO cells, we recorded I_{Ca} as described above after exposing the cells to thapsigargin (Tg; 0.2 μM) and ryanodine (Ry; 10 μM) for 10–15 min to deplete SR Ca^{2+} stores. Tg/Ry eliminated the difference in decay kinetics between KO and WT (KO, $\tau =$ 26.5 ± 2.0 ms; WT, $\tau = 29.1 \pm 0.9$ ms; $p > 0.05$; Fig. 2 *B*, Fig. 3) but did not affect the amplitude of I_{Ca} in either KO or WT (KO, −6.0 ± 0.9 pA/pF, $n = 5$; WT, −12.0 ± 1.5 pA/pF, $n = 4$; $p < 0.01$) (Fig. 2 *B*, Fig. 3). Thus, although SR Ca^{2+} has an important influence on I_{Ca} kinetics in KO cells, this source of Ca^{2+} does not account for all differences in I_{Ca} between WT and KO. Interestingly, elimination of SR Ca^{2+} had a greater effect on I_{Ca} decay kinetics in KO myocytes than in WT. We discuss the significance of this finding in more detail below.

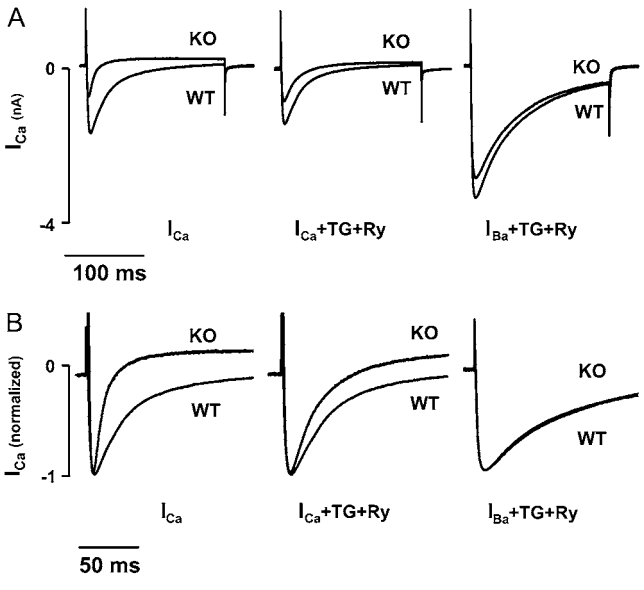

FIGURE 2 Using Ba^{2+} as a charge carrier while depleting SR Ca^{2+} eliminates differences in I_{Ca}. (*A*) Representative traces of I_{Ca} (*left*), I_{Ca} after depletion of SR Ca^{2+} with Tg and Ry (*middle*), and I_{Ba} in the presence of Tg and Ry (*right*) for KO and WT myocytes. (*B*) Recordings shown in *A* were normalized to peak to emphasize the equivalent rates of inactivation after eliminating SR Ca^{2+} release (I_{Ca} in the presence of Tg and Ry, *middle*) and Ca^{2+} entry into the diadic cleft (I_{Ba} in the presence of Tg and Ry, *right*).

Effect of Ca^{2+} entry on I_{Ca} in NCX KO myocytes

We next tested the contribution of extracellular Ca^{2+} entry through L-type Ca^{2+} channels on I_{Ca} kinetics in KO myocytes. To do this, we replaced bath Ca^{2+} (1 mM) with Ba^{2+} (3 mM). These experiments were conducted in the presence of Tg and Ry to deplete SR stores so that no Ca^{2+} would be available to influence I_{Ca}. Ba^{2+} is readily conducted by L-type Ca^{2+} channels. However, unlike Ca^{2+}, Ba^{2+} has no direct effect on I_{Ca} inactivation. Thus, under these conditions, inactivation of I_{Ca} is predominantly voltage dependent (9). When Ba^{2+} was used as the charge carrier, the differences in I_{Ca} amplitude between KO and WT were completely eliminated (KO, −15.9 ± 2.9 pA/pF, $n = 6$; WT, −17.8 ± 3.7 pA/pF, $n = 4$; $p > 0.05$) (Fig. 2 *A*, Fig. 3), and decay kinetics were further slowed (KO, $\tau = 57.8 \pm 4.3$ ms; WT, $\tau = 52.0 \pm 6.9$ ms; $p > 0.05$) (Fig. 2 *B*, Fig. 3). These data indicate that both intact SR Ca^{2+} stores and also Ca^{2+} entry through L-type Ca^{2+} channels are required to produce the differences in I_{Ca} amplitude and kinetics observed between WT and KO cells. Because global cytosolic Ca^{2+} concentration measured with fura-2 is the same in both cell types (5,7), this finding suggests that differences in subsarcolemmal Ca^{2+} are responsible for the smaller and more rapidly inactivating Ca^{2+} current we observe in NCX KO mice.

Effect of intracellular Ca^{2+} buffering on I_{Ca} in NCX KO myocytes

To further investigate the influence of intracellular Ca^{2+} on I_{Ca} in NCX KO myocytes, we dialyzed myocytes with the fast Ca^{2+} chelator BAPTA (10 mM) via the patch pipette to buffer cytosolic Ca^{2+} (Fig. 4). With Ca^{2+} as the charge carrier, BAPTA eliminated differences in I_{Ca} amplitude (KO, −11.8 ± 1.1 pA/pF, $n = 9$; WT, −13.9 ± 1.3 pA/pF, $n = 11$; $p > 0.05$) (Fig. 4, *A* and *B*) and decay kinetics (KO, $\tau = 44.7 \pm 4.1$ ms; WT, $\tau = 44.7 \pm 3.0$ ms; $p > 0.05$) (Fig. 4, *A* and *B*) between KO and WT myocytes. Thus, buffering cytosolic Ca^{2+} with BAPTA negates the effect of knocking out NCX on I_{Ca} amplitude and inactivation.

Abrupt block of NCX

In cardiac myocytes, blockade of NCX leads to an almost immediate increase in SR Ca^{2+} load and the Ca^{2+} transient (12). To evaluate the effects of NCX blockade on I_{Ca} before loading of the SR can occur, we used the following protocol: I_{Ca} was measured during constant pulsing from a holding potential of −40 mV to 0 mV at 1 Hz. After seven pulses, external Na^+ was rapidly replaced with Li^+ using the rapid solution exchanger described in the Methods. To prevent

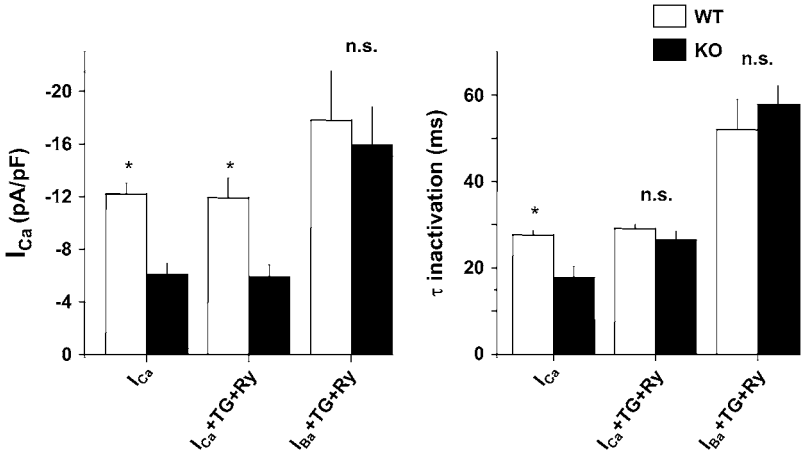

FIGURE 3 Summary data comparing amplitude of I_{Ca} in KO and WT (KO, $n = 9$; WT, $n = 15$) under three conditions: 1), using Ca^{2+} as the charge carrier with intact SR, 2), using Ca^{2+} as the charge carrier with SR Ca^{2+} eliminated by Tg/Ry (KO, $n = 5$; WT, $n = 4$), and 3), using Ba^{2+} as the charge carrier (I_{Ba}) in the presence of Tg/Ry (KO, $n = 6$; WT, $n = 4$). Amplitude is shown on the left, and decay kinetics on the right. *$p < 0.01$ for WT versus KO.

reverse Na^+-Ca^{2+} exchange, pipette Na^+ was replaced with Cs^+ for these experiments. I_{Ca} during the final pulse in control solution was then compared with I_{Ca} elicited during the first pulse in Li^+ solution, i.e., before an increase in SR Ca^{2+} load and the Ca^{2+} transient could occur. An example is shown in Fig. 5. In WT cells, acute blockade of NCX reduced I_{Ca} amplitude to 86.8 ± 1.3% of control ($n = 14$; $p < 0.05$), whereas in KO cells, rapid removal of external Na^+ had no effect on I_{Ca} (98.0 ± 6.5%; $n = 10$; $p > 0.05$).

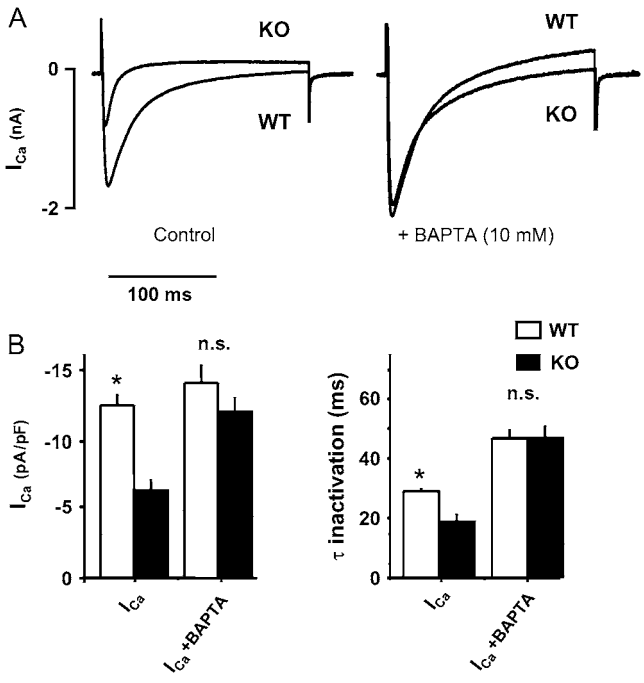

FIGURE 4 Magnitude and decay kinetics of I_{Ca} are equal when cytosolic Ca^{2+} is buffered with BAPTA. (*A*) Representative tracings of a KO and a WT myocyte under control conditions (*left*) and from a separate set of KO and WT myocytes that had been dialyzed with BAPTA (*right*). (*B*) Summary data comparing I_{Ca} in the presence and absence of BAPTA (KO, $n = 9$; WT, $n = 11$). Amplitude is shown on the left-hand graph, and decay kinetics on the right. *$p < 0.01$ for WT versus KO.

These results suggest that abrupt blockade of NCX in WT cells can raise subsarcolemmal Ca^{2+} and thereby inactivate Ca^{2+} channels sufficiently to reduce I_{Ca}. This is consistent with the report by Goldhaber et al. (16), who found that acute blockade of NCX (without reversal) rapidly raises subsarcolemmal Ca^{2+} and activates Ca^{2+} sparks in rat ventricular myocytes. In KO cells, removing Na^+ has no effect on subsarcolemmal Ca^{2+} or I_{Ca} because NCX is already blocked.

Experimental limitations

As mentioned earlier, some myocytes (10–20%) isolated from KO hearts have a WT phenotype. To identify and exclude these cells from the study, we tested for the presence of Na^+-Ca^{2+} exchange current as described above. However, testing for I_{NCX} depends on the presence of cytosolic Ca^{2+}. It was not feasible, then, to test for I_{NCX} when cells were dialyzed with BAPTA. Thus, for this subset of experiments (Fig. 4), we cannot exclude the possibility that some cells in the KO group actually have a WT phenotype. However, no more than 20% of KO cells are likely to be of WT phenotype (5,7), which would not alter our conclusions.

DISCUSSION

The Na^+-Ca^{2+} exchanger is the dominant Ca^{2+} efflux mechanism in cardiac myocytes. Surprisingly, knocking out the exchanger in a cardiac-specific manner is not lethal. NCX KO mice live into adulthood with only modest hemodynamic abnormalities (5,7). NCX KO mice do not up-regulate alternative Ca^{2+} extrusion mechanisms and therefore have severely impaired Ca^{2+} removal capacity (7). To compensate for the absence of NCX, KO mice limit Ca^{2+} entry via I_{Ca}, and the net effect is a decrease in transsarcolemmal Ca^{2+} flux without compromising contractility (5,7). Although I_{Ca} is reduced in NCX KO mice, DHPR expression is unaltered (5). Thus, regulatory mechanisms must underlie the reduction of I_{Ca} in NCX KO mice. How does this occur?

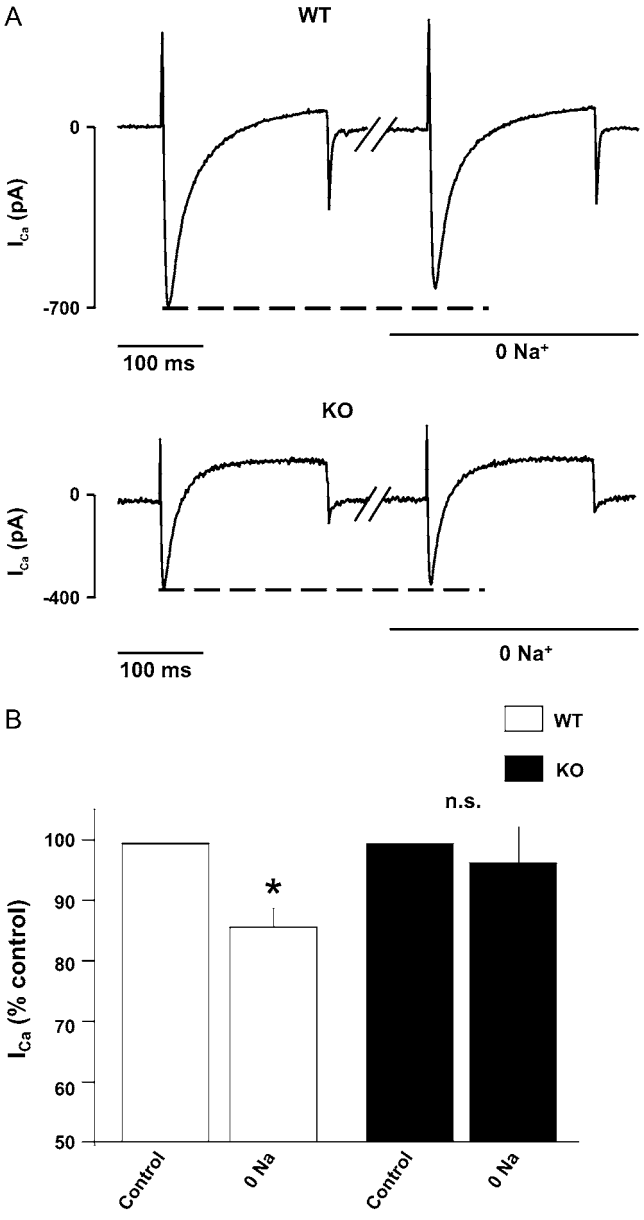

FIGURE 5 Effect of abrupt blockade of NCX on I_{Ca}. (*A*) Representative traces showing effect on I_{Ca} of rapidly replacing external Na^+ with Li^+ before loading of SR Ca^{2+} can occur. The left-hand traces show the seventh and final Ca^{2+} current elicited in Na^+-containing solution, whereas the right hand traces show the first Ca^{2+} current elicited in Li^+-containing solution. Note that Li^+ replacement causes a small but significant reduction in I_{Ca} amplitude in the WT cell (*upper panel*) but no change in the KO cell (*lower panel*) because of the lack of NCX. (*B*) Summary results ($*p < 0.05$, n.s. = difference not significant).

Knockout of NCX promotes Ca^{2+}-dependent inactivation of I_{Ca}

Ca^{2+}-dependent inactivation is one of the strongest modulators of the cardiac L-type Ca^{2+} channel and can regulate Ca^{2+} influx on a beat-to-beat basis. Ca^{2+}-dependent inactivation of I_{Ca} occurs when Ca^{2+} binds to DHPR-bound calmodulin (17). Although the amount of Ca^{2+} released from the SR is severalfold higher than that entering the cell via the L-type Ca^{2+} channel, both sources of Ca^{2+} are major contributors to Ca^{2+}-dependent inhibition of I_{Ca} (18–21), and both sources may thereby influence I_{Ca} amplitude (10).

In this study, we show that either buffering intracellular Ca^{2+} (Fig. 4), or eliminating both SR Ca^{2+} release and Ca^{2+} entry (Fig. 3) eliminates the differences in I_{Ca} between NCX KO and WT mice. Thus, we conclude that the mechanism underlying the reduced I_{Ca} in NCX KO mice is most likely Ca^{2+}-dependent inactivation. Because global cytosolic Ca^{2+} levels are unchanged in NCX KO cells, we conclude that I_{Ca} is inactivated by elevated subsarcolemmal Ca^{2+}.

Regulation of I_{Ca} by subsarcolemmal Ca^{2+}

Suppression of SR Ca^{2+} release is not sufficient to normalize I_{Ca} amplitude in NCX KO myocytes (Figs. 2 and 3). Only the additional inhibition of Ca^{2+} entry (Figs. 2 and 3) or heavily buffering cytosolic Ca^{2+} (Fig. 4) leads to normalization of I_{Ca} amplitude and decay kinetics. These observations suggest that in NCX KO myocytes, the absence of a robust Ca^{2+} efflux mechanism keeps subsarcolemmal Ca^{2+} elevated, even when SR Ca^{2+} is eliminated. We do not completely understand the source of the elevated subsarcolemmal Ca^{2+} in the absence of SR Ca^{2+}. One possibility is that stochastic openings of L-type Ca^{2+} channels are sufficient to raise subsarcolemmal Ca^{2+} (even before depolarization) in the absence of NCX. Also, we cannot exclude the possibility of other Ca^{2+} sources or adaptations of which we are not yet aware. For example, ablation of NCX may lead to an increase in the sensitivity of L-type Ca^{2+} channels to inactivating Ca^{2+}, although we have no direct evidence for this.

I_{Ca} amplitude in WT myocytes is also regulated by subsarcolemmal Ca^{2+}. However, in WT cells, with an active efflux of Ca^{2+} by NCX, subsarcolemmal Ca^{2+} is lower at rest than in KO myocytes. Thus, I_{Ca} amplitude is greater and inactivation slower in WT compared with KO even at baseline. Furthermore, because subsarcolemmal Ca^{2+} is relatively low in WT myocytes to begin with, elimination of SR Ca^{2+} using thapsigargin and ryanodine has a relatively small effect on subsarcolemmal Ca^{2+} (and therefore inactivation of I_{Ca}; see Fig. 3) compared to the effect in KO.

Conventional fluorescent indicator methods are incapable of measuring the local Ca^{2+} concentration in the diadic cleft or the subsarcolemmal space. Indeed, resting and systolic cytoplasmic Ca^{2+} levels measured by fura-2 and fluo-3 are similar in WT and KO myocytes (5,7). Nevertheless, as shown in this study, a Ca^{2+}-dependent mechanism underlies the reduced I_{Ca} in NCX KO mice. The Ca^{2+} current thereby serves as a reporter of subsarcolemmal and diadic cleft Ca^{2+}. Elevated Ca^{2+} in these restricted spaces caused by a lack of normal Ca^{2+} extrusion by the exchanger results in reduced I_{Ca} amplitude and accelerated inactivation kinetics.

Effect of acute NCX blockade on I_{Ca}

Unlike KO of NCX, rapid blockade of NCX by Li^+ substitution in WT myocytes had only a small effect on the I_{Ca} recorded during the first pulse after blockade (Fig. 5). An explanation for the mild effect of acute NCX blockade on I_{Ca} could be that several cardiac cycles in the absence of NCX activity are necessary to sufficiently raise subsarcolemmal Ca^{2+} in WT cells to levels required to inactivate I_{Ca}. This is difficult to investigate in cardiac myocytes because the blockade of NCX leads to a rapid increase of SR Ca^{2+} load and Ca^{2+} transients during continued pulses (12,22). Isaev et al. (10) addressed this problem by investigating the effects of NCX on I_{Ca} in a noncardiac mammalian expression system in which DHPR and NCX were coexpressed. Cytosolic Ca^{2+} was raised to levels expected in the subsarcolemmal space of cardiomyocytes during systole. Under these conditions, they found that suppression of NCX leads to a reduction of I_{Ca} similar to what we have described here in KO myocytes (Fig. 2).

We cannot exclude the possibility that there are longer-term adaptive responses participating in the survival of the NCX KO mice. Adaptations could, for example, sensitize KO myocytes to Ca^{2+}-induced inactivation of I_{Ca}; we do not presently have evidence for such adaptations.

Ca^{2+}-dependent versus voltage-dependent inhibition of I_{Ca}

We have reported previously that there is no difference in the relative voltage dependence of I_{Ca} in WT versus KO myocytes (5,7). In this study, we demonstrate a complete reversal of the reduction in I_{Ca} in KO myocytes when regulatory Ca^{2+} is eliminated. We therefore conclude that the underlying mechanism is primarily Ca^{2+}-dependent rather than voltage-dependent.

However, evidence has been presented recently that subsarcolemmal Ca^{2+} can alter the voltage sensor of the DHPR (10), and thus, voltage-dependent and Ca^{2+}-dependent regulatory mechanisms may be interconnected. Therefore, we cannot exclude the possibility that differences in subsarcolemmal Ca^{2+} may differentially influence the voltage-dependent availability of I_{Ca} in KO versus WT. Future experimental work will be necessary to investigate this issue.

Functional implications

The reduction of I_{Ca} in NCX KO mice via a Ca^{2+}-dependent mechanism suggests a functional coupling between NCX and the L-type Ca^{2+} channel (Fig. 6): Reduced Ca^{2+} extrusion capacity leads to an accumulation of subsarcolemmal Ca^{2+}. This promotes Ca^{2+}-dependent inactivation of I_{Ca}, leading to a reduction of I_{Ca}. The reduction of Ca^{2+} influx balances Ca^{2+} fluxes. There is then an overall reduction in transsarcolemmal Ca^{2+} traffic.

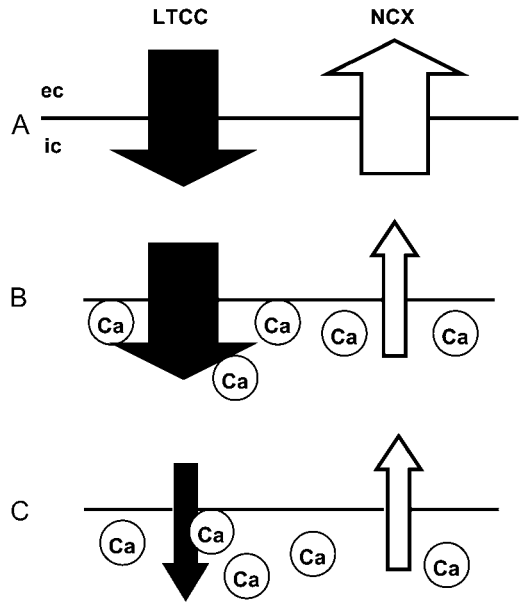

FIGURE 6 Schematic diagram of functional coupling between NCX and the L-type Ca^{2+} channel (LTCC). (*A*) Under physiological conditions, Ca^{2+} influx via I_{Ca} is in balance with Ca^{2+} efflux via NCX. (*B*) In situations of reduced Ca^{2+} extrusion capacity, Ca^{2+} accumulates in the subsarcolemmal space. (*C*) The increased subsarcolemmal Ca^{2+} concentration promotes Ca^{2+}-dependent inactivation of I_{Ca}. This reduces Ca^{2+} influx so that a new balance between influx and efflux is established. ec, extracellular; ic, intracellular.

This feedback mechanism may enable the cardiomyocyte to adapt to impaired Ca^{2+} extrusion and may stabilize the balance of Ca^{2+} fluxes. The interplay between the Na^+-Ca^{2+} exchanger and the L-type Ca^{2+} channel may be essential for maintenance of Ca^{2+} homeostasis in both healthy and diseased heart.

The authors appreciate helpful discussions with Dr. R. Olcese.

This research was supported by Köln Fortune and the German Research Foundation (DFG PO 1004\1-1 and 1-2) (C.P.), National Institutes of Health grants HL70828 (J.I.G.) and HL48509 (K.D.P.), and the Laubisch Foundation.

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