Role of Sodium-Calcium Exchanger in Modulating the Action Potential of Ventricular Myocytes From Normal and Failing Hearts

Antonis A. Armoundas, Ion A. Hobai, Gordon F. Tomaselli, Raimond L. Winslow, Brian O’Rourke

Abstract—Increased Na\(^+\)-Ca\(^{2+}\) exchange (NCX) activity in heart failure and hypertrophy may compensate for depressed sarcoplasmic reticular Ca\(^{2+}\) uptake, provide inotropic support through reverse-mode Ca\(^{2+}\) entry, and/or deplete intracellular Ca\(^{2+}\) stores. NCX is electrogenic and depends on Na\(^+\) and Ca\(^{2+}\) transmembrane gradients, making it difficult to predict its effect on the action potential (AP). Here, we examine the effect of [Na\(^+\)] on the AP in myocytes from normal and pacing-induced failing canine hearts and estimate the direction of the NCX driving force using simultaneously recorded APs and Ca\(^{2+}\) transients. AP duration shortened with increasing [Na\(^+\)], and was correlated with a shift in the reversal point of the NCX driving force. At [Na\(^+\)] \(\approx\) 10 mmol/L, outward NCX current during the plateau facilitated repolarization, whereas at 5 mmol/L [Na\(^+\)], NCX had a depolarizing effect, confirmed by partially inhibiting NCX with exchange inhibitory peptide. Exchange inhibitory peptide shortened the AP duration at 5 mmol/L [Na\(^+\)], and prolonged it at [Na\(^+\)] \(\approx\) 10 mmol/L. With K\(^+\) currents blocked, total membrane current was outward during the late plateau of an AP clamp at 10 mmol/L [Na\(^+\)], and became inward close to the predicted reversal point for the NCX driving force. The results were reproduced using a computer model. These results indicate that NCX plays an important role in shaping the AP of the canine myocyte, helping it to repolarize at high [Na\(^+\)], especially in the failing heart, but contributing a depolarizing, potentially arrhythmogenic, influence at low [Na\(^+\)]. (Circ Res. 2003;93:46-53.)

Key Words: heart failure ■ Na\(^+\)-Ca\(^{2+}\) exchanger ■ reversal potential ■ Ca\(^{2+}\) transients

The Na\(^+\)-Ca\(^{2+}\) exchanger (NCX) catalyzes the electrogenic exchange of Na\(^+\) for Ca\(^{2+}\) across the cardiac sarcolemma and is reversible, operating in either forward (Ca\(^{2+}\)-efflux) or reverse (Ca\(^{2+}\)-influx) modes, depending on the prevailing electrochemical driving forces for Ca\(^{2+}\) and Na\(^+\). This complex dependence on transmembrane ion and voltage gradients, which are both rapidly changing during the cellular action potential (AP), makes predictions about the overall influence of NCX current (I\(_{\text{NCX}}\)) on excitation-contraction coupling challenging. NCX is the primary Ca\(^{2+}\) extrusion mechanism in the heart and is required to remove the increment of Ca\(^{2+}\) entering the myocyte via Ca\(^{2+}\) channels on each beat, but the timing of the transition from reverse-mode exchange (outward I\(_{\text{NCX}}\)) to forward-mode exchange (inward I\(_{\text{NCX}}\)) has been difficult to determine. Because the membrane impedance during the AP plateau of large mammals (including humans) is high, the net direction of current flow through NCX is likely to be a fundamental determinant of AP duration (APD).

Early investigations into the net effect of I\(_{\text{NCX}}\) on the AP demonstrated that it contributed net inward current and prolonged the AP in the rat. This is expected in this species, because of the rapid AP repolarization brought about by large transient outward K\(^+\) currents. In guinea pig myocytes, experimental data supported predominantly outward I\(_{\text{NCX}}\) during the AP plateau, but in that study, I\(_{\text{NCX}}\) was measured with sarcoplasmic reticular (SR) Ca\(^{2+}\) release minimized with a Ca\(^{2+}\) channel antagonist. This unphysiological condition would tend to exaggerate the magnitude of reverse-mode NCX. More recently, Weber et al. have argued that I\(_{\text{NCX}}\) may be inward during most of the AP at physiological [Na\(^+\)], with NCX sensing a higher submembrane concentration of Ca\(^{2+}\) than that reported by intracellular Ca\(^{2+}\) dyes.

In the failing heart, where SR function is impaired, a greater dependence on NCX for Ca\(^{2+}\) removal is expected. Moreover, a prominent increase in NCX function, in both a relative and absolute sense, has been demonstrated in animal models and in human heart failure, in accord with an increase in NCX mRNA and/or protein. There are multiple consequences of increased NCX. More forward-mode exchange compensates for defective SR Ca\(^{2+}\) removal, but it is at the expense of depleting the releasable pool of Ca\(^{2+}\) and contributing more depolarizing current, increasing the propensity for arrhythmias triggered by delayed afterdepolarization.
were acquired simultaneously and analyzed offline. Indo 1 transients (Molecular Probes). Electrophysiological and fluorescence signals were carried out as described previously 8,9,21 using protocols approved by the institution’s Animal Care and Use Committee (please see the online data supplement).

Materials and Methods

Induction of heart failure and ventricular cardiomyocyte isolation were carried out as described previously8,9,21 using protocols approved by the institution’s Animal Care and Use Committee (please see the online data supplement, available at http://www.circresaha.org).

Isolated midmyocardial ventricular myocytes were superfused (at 37°C) with a physiological salt solution containing (mmol/L) NaCl 140, KCl 5, MgCl2 0.5, MgATP 5, and HEPES 10 (pH 7.4 with NaOH). The intracellular solution contained (mmol/L) potassium glutamate 130, KCl 9, NaCl 5 to 15, MgCl2 0.5, MgATP 5, and HEPES 10 (pH 7.2 with KOH), along with 50 μmol/L indo 1 (Molecular Probes). Electrophysiological and fluorescence signals were acquired simultaneously and analyzed offline. Indo 1 transients were calibrated in terms of [Ca2+], as described previously8,9,21 (also see the online data supplement).

Exchange-inhibitory peptide (XIP) was synthesized and purified by the protein synthesis core facility of The Johns Hopkins University. A small concentration-dependent shift in the pipette-to-bath junction potential was introduced by intracellular XIP, as evidenced by a 3- to 5-mV right shift in the zero current potential for the inward rectifier K+ current (data not shown). This offset was corrected in the AP records.

APs and [Ca2+], were measured in current-clamp mode pacing at 1 Hz to steady state (after at least 20 stimuli). The driving-force reversal point (RP) of the NCX was measured as the membrane potential (Em) at which the NCX driving force (Em – ENCX) equaled zero, with ENCX defined as 3Em – 2ECa. Em and ECa are the equilibrium potentials for Na+ and Ca2+, respectively, with ECa = (RT/zF)log([X]/[X]i), where R is the ideal gas constant; T, absolute temperature; z, valence; and F, Faraday constant, and all ions except [Ca2+], were assumed to be equivalent to their bulk concentrations (see Discussion). The time to RP (TTRP) was defined as the time from the AP upstroke to the NCX RP and was normalized to the duration of the AP for determination of the fraction of the duty cycle the exchanger was in reverse versus forward mode.

Effect of Intraacellular Na+ on NCX Driving Force and APD

Figure 1 illustrates the approach used to estimate the driving force for fNCX at 5, 10, or 15 mmol/L [Na+], and shows representative APs and Ca2+ transients for myocytes from normal and failing hearts. ENCX (Figure 1, blue traces) was calculated as 3Em – 2ECa (3:1 stoichiometry) from the Ca2+ transient, assuming a constant extracellular Ca2+ and transmembrane Na+ gradient. The difference between Em and ENCX...
was determined and is plotted on the same voltage scale for comparison (Figure 1, magenta traces). The NCX RP was taken as the membrane voltage corresponding to the point at which the NCX driving force reversed the sign (E_m = E_{NCX}). It is clear from the figure that this point shifted in the hyperpolarized direction at higher [Na^+]i, favoring more outward current during the AP plateau, suggesting that repolarization should be facilitated at higher [Na^+]i. Notably, the NCX driving force was more outward in myocytes from failing hearts at any given [Na^+]i, as a consequence of both a more depolarized early plateau potential and a smaller cytosolic Ca^{2+} transient.

There were no statistically significant differences of the resting potential either between groups or as a function of [Na^+]i. (Table). Consistent with the shift in NCX driving force, APD at 90% repolarization (APD_{90}) was inversely correlated with [Na^+]i (Figure 2A). APD_{90} decreased on elevating [Na^+]i from 5 to 15 mmol/L in myocytes from normal (by ∼175 ms) and failing (by ∼125 ms) hearts (Table). Hyperpolarization of the NCX RP was associated with AP shortening (Figure 2B), and the fraction of the APD the NCX spent in the reverse mode (ie, the TTRP normalized to the APD [TTRP/APD_{90}]) was increased at high [Na^+]i (Figure 2C).

The qualitative observation that the net balance of NCX driving force was shifted toward more reverse-mode exchange in myocytes from failing hearts was confirmed by calculating the ratio of reverse-mode to forward-mode exchange driving force during the AP. This was determined by integrating the area bounded by the NCX driving-force curve from the upstroke of the AP to the RP (Int_{rev}) divided by the forward-mode integral from the RP to phase 3 repolarization of the AP (Int_{fwd}) (Figure 4A). Int_{rev}/Int_{fwd} was higher in the failing group at all three [Na^+]i levels, reaching statistical significance at 15 mmol/L [Na^+]i (Figure 4B). Because this parameter reflects a change in the balance of outward versus inward NCX driving force, it underestimates the difference of the NCX contribution in shaping the AP between groups, inasmuch as the total I_{NCX} in the failing heart would also take into account the increase in I_{NCX} density in this model, which is approximately twice that of the normal myocyte.\(^8\) At 10 and 15 [Na^+]i, Int_{rev}/Int_{fwd} was >1, suggesting a net repolarizing influence of NCX at these levels of [Na^+]i. Inspection of the running integral of NCX shows that the NCX driving force for reverse-mode exchange rises more rapidly and is larger in the failing group at all levels of [Na^+]i (Figure 4C). Furthermore, in the context of increased resting [Na^+]i, in heart failure,\(^9\) Int_{rev}/Int_{fwd} significantly differed between cells from failing hearts at 10 mmol/L [Na^+]i, and cells from normal hearts at 5 mmol/L [Na^+]i, (P=0.01).

### Effect of NCX Inhibition on APD

To test the validity of the assumption that NCX driving force calculated from the Ca^{2+} transient was a suitable method for estimating the RP and net effect of NCX on the AP wave-
To avoid the confounding secondary effect of XIP to increase 

Figure 3. [Na\(^+\)]\(_i\) dependence of APD using paired pipette tech-

tique. [Na\(^+\)]\(_i\) was varied within individual myocytes using 

sequential pipettes containing different levels of [Na\(^+\)]. The 

direction and amplitude of the change in [Na\(^+\)]\(_i\) are indicated by 

the arrows. Black and red arrows denote myocytes from normal 

and failing hearts, respectively. In one cell from a normal heart 

and four cells from failing hearts, a third pipette was used to 

sequentially patch the same cell with a different [Na\(^+\)]. 

also confirmed in separate unpaired experiments in myocytes 

paced at 0.25 Hz (Figure 5C).

Model Simulations

We developed an AP-clamp variation of the canine car-

diomyocyte computer model described by Winslow, 

Greenstein, and colleagues\(^{20,23}\) that allowed the use of an 

experimentally recorded AP as an input function to simul-

ate the Ca\(^{2+}\) transient and underlying membrane cur-

rents.\(^{24}\) By comparing experimental and model results, we 

reasoned that if a given AP waveform produces a simu-

lated Ca\(^{2+}\) transient and NCX driving force that reproduce 

experimental estimates, then the assumptions of the model 

do not grossly misrepresent the behavior of the myocyte. 

Model-derived Ca\(^{2+}\) transients in the AP clamp at 5, 10, or 

15 mmol/L [Na\(^+\)]\(_i\), were compared with experimental trans-

ients recorded in current-clamp mode (Figure 6A). The 

model-derived NCX driving-force trajectories and RPs ro-

bustly reproduced the experimental data, and model RPs were 

correlated over the range of [Na\(^+\)]. \(R=0.99,\) Figure 

6B). Importantly, the slope of this relationship was 1.0, 

indicating that the experimental RP did not deviate signifi-

cantly from that determined by the model. This would not 

have been the case if the driving force for NCX was primarily 

governed by Ca\(^{2+}\) in a microdomain that differs from the bulk 

Ca\(^{2+}\) reported by indo 1. Thus, when NCX in the model was 

reformatted to respond to Ca\(^{2+}\) in the subsarcolemmal 

junctional subspace (by modifying equations A.39 and A.91 

of Winslow et al\(^{20}\); see online supplement), a marked reduc-

tion in Ca\(^{2+}\) transient amplitude was observed that was due to 

enhanced forward-mode exchange and Ca\(^{2+}\) extrusion from 

the myocyte (Figure 6C). If these conditions existed, the 

result would be a shift in the NCX RP; consequently, the 

experimentally obtained RP values would have been shifted 

to more negative values and the slope of the relation in Figure 

6B would not have been 1. From a different perspective, 

when NCX is formulated to respond to Ca\(^{2+}\) within the 

subspace, the shift in \(I_{NCX}\) substantially alters the AP wave-

form, we reasoned that the APD\(_{90}\) should be predictably 

influenced by partial inhibition of NCX (Figure 5A), depend-

ing on [Na\(^+\)]. At 5 mmol/L [Na\(^+\)], \(I_{NCX}\) is inward for almost 

the entire APD; therefore, inhibition of this depolarizing 
current should shorten the APD\(_{90}\). In contrast, at 10 or 

15 mmol/L [Na\(^+\)], inhibition of NCX should prolong the AP 

if it is contributing outward current during the mid to late 

plateau. This was tested using the same paired-pipette method 
described above for changing [Na\(^+\)]. (Figure 3), except that in 

this case, [Na\(^+\)] was held constant, and 100 \(\mu\)mol/L XIP was 

included in the second pipette. As predicted, XIP shortened 
the AP at 5 mmol/L [Na\(^+\)], but prolonged the AP at 10 or 

15 mmol/L [Na\(^+\)], in both experimental groups (Figure 5B). 

To avoid the confounding secondary effect of XIP to increase 
SR Ca\(^{2+}\) load, we matched Ca\(^{2+}\) transient recordings that had 

similar rise times and amplitudes by selecting records in the 
presence of XIP during the pulse train before the maximal SR 
Ca\(^{2+}\) load was attained. The significant \(P<0.05\) prolonga-
tion of the AP by XIP (30 \(\mu\)mol/L) at 10 mmol/L [Na\(^+\)] was 

\(P<0.05\) for normal vs failing myocytes; \(\dagger P<0.05\) for myocytes from failing hearts 
at 10 mmol/L [Na\(^+\)]. vs myocytes from normal hearts at 5 mmol/L [Na\(^+\)].

Figure 4. Ratio of integrated reverse-mode to forward-mode NCX driving force during the AP. A, \(I_{rev}\) represents the integral of the \(E_{rev}-E_{NCX}\) curve from the upstroke of the AP to the NCX RP. \(I_{for}\) is the integral from the driving-force RP to the point of AP repolariza-
tion (taken as the minimum of the NCX driving-force curve). B, \(I_{rev}/I_{for}\) increased as a function of [Na\(^+\)] and was significantly higher 
in the failing group. C, Averaged running integrals for all data sets illustrate more rapid reverse-mode NCX activation in myocytes from 
failing (F) than normal (N) hearts at all levels of [Na\(^+\)]. \(\dagger P<0.05\) for normal vs failing myocytes; \(\dagger P<0.05\) for myocytes from failing hearts 
at 10 mmol/L [Na\(^+\)]. vs myocytes from normal hearts at 5 mmol/L [Na\(^+\)].
form when the model is not constrained under AP-clamp conditions (Figure 6D). In this case, the enhanced inward $I_{NCX}$ results in overshoot of the AP dome and prolongation of the APD.

$I_{NCX}$ During AP Clamp

Direct measurement of $I_{NCX}$ during a physiological AP and Ca$^{2+}$ transient would yield the ultimate answer to the central question of the present study; however, selectively measuring $I_{NCX}$ without perturbing other parameters is difficult. For example, inhibiting NCX with Ni$^{2+}$ or KBR-7943$^{25}$ will also block Na$^+$ and Ca$^{2+}$ channels (among others), which will profoundly change the AP profile and the Ca$^{2+}$ transient, as will selective inhibition of L-type Ca$^{2+}$ current ($I_{Ca,L}$).$^{5}$ Therefore, we sought confirmation that our estimation of timing of the reversal of the NCX driving force matches that of the $I_{NCX}$ by performing an AP-clamp experiment (in 10 mmol/L Na$^+$) with K$^+$ currents blocked by Cs$^+$ substitution and Ca$^{2+}$-activated Cl$^-$ currents eliminated using the guinea pig ventricular myocyte. This limited the major currents flowing during the plateau to $I_{Ca,L}$ and $I_{NCX}$ (with a minor contribution of the Na$^+,K^+-$ATPase current; see Discussion). Under these conditions, a net outward current was recorded beginning approx 100 ms after the upstroke of the AP and extending until just before phase 3 repolarization (Figure 7). Consistent with predictions, the kinetics and late RP of the plateau current corresponded well with the RP of the NCX driving force. A notable deviation between the NCX driving-force estimate and the presumed NCX current at the resting potential was evident (see Discussion).

Discussion

The results indicate that the APD is strongly dependent on [Na$^+$], with increased [Na$^+$], resulting in shortening of the...
APD90. This finding is consistent with a shift in the balance of reverse-mode versus forward-mode NCX. Inhibition of NCX with XIP supports the hypothesis that at high [Na+]i (>10 mmol/L), outward I_{NCX} during the plateau contributes to AP repolarization, whereas at low [Na+]i (5 mmol/L), I_{NCX} is predominantly inward. The net direction of the NCX driving force during the AP plateau can be reasonably determined from the cytosolic Ca2+ transient.

APD90 decreased significantly in both groups when [Na+]i increased from 5 to 15 mmol/L [Na+]i, compared with 10 mmol/L [Na+]i. (Figure 2). This relationship was almost perfectly linear over the whole range of [Na+]i in normal myocytes (r=0.998, P<0.05 for the data in Figure 2A), but in the failing group, the [Na+]i-induced shortening of APD90 appeared to reach a limit at 10 mmol/L [Na+]i. This was true despite the fact that the NCX RP shifted to a more hyperpolarized point (Figure 2B), reached later in the AP (see TTRP/APD90; Figure 2C), when [Na+]i was increased from 10 mmol/L to 15 mmol/L in the failing group. Furthermore, the ratio of integrated reverse-mode to forward-mode exchange very strongly favored repolarization at 15 mmol/L [Na+]i, compared with 10 mmol/L [Na+]i. (Figure 4). The diminished effect of increasing [Na+]i, from 10 to 15 mmol/L on APD90 in the failing group suggests that an upper limit of outward I_{NCX} may have been reached at 10 mmol/L [Na+]i, or that the contribution of I_{NCX} to the AP is overwhelmed by other factors in this range of [Na+]i.

In our hands, blocking Na+-K+-ATPase (10 μmol/L strophanthidin) prolonged the APD (data not shown); thus, a limitation of the present study is that the Na+-K+ pump may partially contribute to [Na+]i-mediated AP shortening. However, the extent of its contribution cannot be easilyascertained using inhibitors, because [Na+]i homeostasis is dramatically altered in their presence. Further investigation will be necessary to determine how much of the [Na+]i-mediated AP shortening might be related to Na+-K+-ATPase current, but we estimate the pump current to be less than 1/4 the density of I_{NCX} at plateau potentials. Moreover, the pump will always have a hyperpolarizing influence and therefore could not explain the differential effects of XIP at low versus high [Na+]i.

At all levels of [Na+]i, myocytes from failing hearts, compared with cells from normal hearts, displayed a higher ratio of outward/inward NCX driving force (Intrev/Int for , Figure 4). This difference could be explained by two factors: (1) less early repolarization, presumably due to diminished transient outward K+ current,21 and (2) a reduction in the amplitude of the Ca2+ transient in the failing myocyte.9,26 The result is earlier activation of reverse-mode exchange and more outward I_{NCX} during the AP plateau. It is important to note that this conclusion is based solely on the estimate of NCX driving force and would be true whether or not the current density of NCX9,20,21 or [Na+]i 27 was increased in heart failure, two factors that would further enhance reverse-mode exchange. This could be considered as an adaptation that would counteract excessive AP prolongation resulting from decreased Ca2+-dependent inactivation of I_{Ca,L} in the failing myocyte20 but would result in Ca2+ extrusion occurring primarily on completion of repolarization. Whereas the former effect would be beneficial, the latter could contribute to an enhanced susceptibility to delayed afterdepolarizations, as suggested previously.28

Early afterdepolarizations also could be strongly influenced by the RP of I_{NCX}. Once turning inward (which happens very early at low [Na+]i), I_{NCX} is a depolarizing force that is enhanced during the vulnerable late phase of the AP, when Ca2+ channels may be recovering from inactivation. In the context of the present study, in paired-pipette experiments, we have observed early afterdepolarizations triggered in response to lowering [Na+]i, from 10 to 5 mmol/L (data not shown).

The results obtained using the AP-clamp variation of the canine myocyte model24 were consistent with experimental results with respect to the profile of the Ca2+ transient and the influence of [Na+]i on the AP. Because the only input to the model was the AP waveform, this implies that the model assumptions for describing the Ca2+ transient waveform and the behavior of I_{NCX} were generally correct (Figures 6B through 6D). These include a 3:1 Na+:Ca2+ stoichiometry for NCX, distribution of the exchanger in the nonjunctional compartment, and NCX driving force determined by the bulk cytoplasmic Ca2+ signal.

Recent reports suggest that NCX is exclusively localized in the T tubules29,30 but does not have direct access to the Ca2+ released into the junctional space of the dyad,31 inasmuch as caffeine-evoked SR Ca2+ release does not activate I_{NCX} when [Ca2+]i is buffered to within ~50 nm of the release sites.32 Our XIP and computer simulation results (Figures 6A through 6C) indicate that NCX is unlikely to sense Ca2+ in the dyadic cleft, a conclusion that is in agreement with the results of López-López et al.,33 who have shown that reverse-mode NCX cannot directly trigger Ca2+ sparks in the absence of I_{Ca,L}. However, the possibility still exists that subsarcolemmal [Na+] or [Ca2+] gradients may be present during the AP. Na+ entering the myocyte through the Na+ channel into a hypothetical “fuzzy space”34 has been proposed to account for...
NCX-triggered \( \text{Ca}^{2+} \) release. This idea has been challenged, but the issue has not been definitively resolved. The increase in \([\text{Na}^+]\), due to the \( \text{Na}^+ \) current has been estimated to be on the order of 25 \( \mu \text{mol/L} \), an amount that should not have a major impact on our conclusions, because the changes we made were in the millimolar range.

\( \text{Ca}^{2+} \) released from the SR into the junctional space does greatly exceed that measured by an evenly distributed \( \text{Ca}^{2+} \) indicator in the cytoplasm. NCX in proximity to the release sites could theoretically respond to a local submembrane \( \text{Ca}^{2+} \) microdomain, in effect switching the exchanger to forward mode much earlier in the AP. A recent report by Weber et al, who used NCX tail currents as an index of submembrane \( \text{Ca}^{2+} \) during truncated AP-clamp stimuli, suggested that the driving force for NCX during an AP may be much more inward than had been previously determined (eg, see Grantham and Cannell) or estimated from our experiments. This would imply that the balance of reverse-mode to forward-mode NCX activity could favor depolarization rather than repolarization at higher \([\text{Na}^+]\), a conclusion that was not supported by the XIP experiments or the AP-clamp experiment. We cannot rule out that early in the AP, subsarcolemmal \( \text{Ca}^{2+} \) gradients exist that could cause the exchanger to transiently shift to forward mode during the SR \( \text{Ca}^{2+} \) release phase of the \( \text{Ca}^{2+} \) transient. Late in the plateau, however, the present findings indicate that the measured \( [\text{Ca}^{2+}] \) is a good approximation of that seen by the NCX and is useful in determining the effect of NCX on \( \text{APD}_{90} \). This is in partial agreement with the conclusions of Weber et al, with the only modification being that the subsarcolemmal gradients probably dissipate earlier in the AP (ie, during the notch phase) than they predicted.

In 10 mmol/L \([\text{Na}^+]\), with \( K^+ \) currents blocked, total membrane current during the late plateau in the AP-clamp experiment (Figure 7) was outward and had an RP similar to that of the calculated NCX driving force. These data provide additional support for our conclusions but also highlight a limitation of the method, ie, that the NCX current magnitude (but not the direction of current flow) can deviate substantially from the driving force estimate during different phases of the cardiac cycle. This can be seen at the resting \( E_m \) in 10 mmol/L \([\text{Na}^+]\), where there is substantial driving force for inward \( I_{\text{NCX}} \) but the net inward current diminishes. A reduction in NCX activity at the resting potential, described previously in voltage-clamp experiments, could explain this observation; however, further studies will be required to reconcile this aspect of the results.

In summary, these findings underscore the prominent role of NCX in modulating the APD in myocytes from normal and failing hearts and provide support for the hypothesis that \( I_{\text{NCX}} \) can be a significant repolarizing force in ventricular myocytes from large mammals. In the context of heart failure, in which \([\text{Na}^+]\), may increase >10 mmol/L, this effect could help to prevent excessive AP prolongation and provide inotropic support via reverse-mode exchange. At \([\text{Na}^+]\), <10 mmol/L, this balance shifts toward more forward-mode exchange, so that \( I_{\text{NCX}} \) contributes to depolarization of the AP plateau and AP prolongation, factors that may contribute to arrhythmogenesis.

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Canine tachycardic pacing model of heart failure

Male mongrel dogs were anesthetized and surgically instrumented under sterile conditions for implantation of a VVI pacemaker (Medtronic). Rapid pacing at 240 bpm was initiated 1 to 2 days after surgery and maintained for 3 to 4 weeks, at which time hemodynamic decompensation was confirmed by recording left ventricular pressure waveforms (under anesthesia with 25 mg/kg thiopental), using a micromanometer-tipped left ventricular catheter inserted through the right femoral artery. In this study, an increased end-diastolic pressure (normal hearts, 4.6±0.8 mm Hg, versus failing hearts, 35.2±2.8 mm Hg, p<0.05), slowed rate of pressure rise (dP/dt: normal hearts, 2026±812 mm Hg/s, versus failing hearts, 1122±148 mm Hg/s, p<0.05), and slowed relaxation rate (-dP/dt: normal hearts, -2160±147 mm Hg/s, versus failing hearts, -1211±329 mm Hg/s, p<0.05) were evident in the failing hearts (hemodynamic data are expressed as mean±standard error). Hearts were harvested by left lateral thoracotomy, immersed in ice-cold cardioplegic solution (in mmol/L, KCl 104, NaCl 32, NaHCO₃ 10, Taurine 10, 2,3-butanedione monoxime (BDM) 20; pH 7.4 with KOH), and quickly excised. Normal hearts were similarly obtained from nonpaced dogs. Midmyocardial myocytes were enzymatically isolated as previously described.

Single-Cell Physiological Studies

Isolated ventricular myocytes were placed in a heated (37°C) chamber on the stage of an inverted fluorescence microscope (Diaphot 200; Nikon, Inc).
Data have been corrected for liquid junction potentials between the pipette and bath solutions\(^2\).

*Fluorescence recording and Ca\(^{2+}\) calibration*

A xenon arc lamp was used to excite indo-1 fluorescence at 365 nm (390 nm dichroic mirror), and the emitted fluorescence was recorded using a dual channel photomultiplier tube assembly (ESP associates, Toronto, Ontario) at wavelengths of 405 and 495 nm. Cellular autofluorescence at both emission wavelengths was recorded before rupturing the cell-attached patch. The ratio of indo-1 fluorescence \(R=F_{405\text{ nm}}/F_{495\text{ nm}}\) was determined after subtraction of cellular autofluorescence and was used to calculate free intracellular Ca\(^{2+}\) according to the equation \([\text{Ca}^{2+}]_i=K_d\beta(\frac{R-R_{\text{min}}}{R_{\text{max}}-R})^3\), using a \(K_d\) of 844 nmol/L, as reported for rabbit cardiomyocytes \(^4\). The \(R_{\text{min}}, R_{\text{max}},\) and \(\beta\) for the fluorescence system were determined to be 0.8, 5.8 and 2.0 respectively.

*Model Simulations*

In some simulations the NCX was reformulated such that a fraction of the total NCX current responded to Ca\(^{2+}\) in the subsarcolemmal subspace (SS) by modifying equation A.39 \(^5\) as follows

\[
I_{\text{NaCa}} = I_{\text{NaCa}}^{\text{ss}} + I_{\text{NaCa}}^{\text{SS}}
\]

where

\[
I_{\text{NaCa}}^{\text{ss}} = (1-\lambda)k_{\text{NaCa}}\frac{5000}{K_{m,Na}^3+[Na^+]_o^3}\frac{1}{K_{m,Ca}+[Ca^{2+}]_o} \frac{1}{1+k_{sat}e^{(\eta-1)VF/RT}}
\]

\[
(\lambda e^{\eta V F/RT} x[Na^+]_o^3[Ca^{2+}]_o - e^{(\eta-1)VF/RT}[Na^+]_o^3[Ca^{2+}]_o)
\]

2
and

\[ I_{\text{NaCa}_{\text{SS}}}^{\text{SS}} = \frac{\lambda k_{\text{NaCa}}}{K_{m,\text{Na}}^3 + [\text{Na}^+]_o^3} \frac{1}{K_{m,\text{Ca}}} + [\text{Ca}^{2+}]_o \frac{1}{1 + k_{\text{sat}} e^{(\eta-1)/VF/RT}} \]

\[ (e^{(\eta-1)/VF/RT} [\text{Na}^+]_o [\text{Ca}^{2+}]_o - e^{(\eta-1)/VF/RT} [\text{Na}^+]_o [\text{Ca}^{2+}]_{\text{SS}}) \]

In addition, equation A.91 was modified to allow Ca$^{2+}$ flux through the NCX into the subspace as follows

\[ \frac{d[\text{Ca}^{2+}]_{SS}}{dt} = \lambda \left\{ J_{\text{rel}} \frac{V_{\text{JSR}}}{V_{SS}} - J_{\text{sfc}} \frac{V_{\text{myo}}}{V_{SS}} - (I_{Ca} - \lambda 2 I_{\text{NaCa}}) \frac{A_{\text{cap}} C_{sc}}{2V_{SS} F^2} \right\} \]

where, \( \lambda \) is the percent of the NCX that is located in the subspace and all other symbols are as described by Winslow et al \(^5\).

References


