Enhanced Ca\(^{2+}\)-Activated Na\(^{+}\)-Ca\(^{2+}\) Exchange Activity in Canine Pacing-Induced Heart Failure

Ion A. Hobai, Brian O’Rourke

Abstract—Defective excitation-contraction coupling in heart failure is generally associated with both a reduction in sarcoplasmic reticulum (SR) Ca\(^{2+}\) uptake and a greater dependence on transsarcolemmal Na\(^{+}\)-Ca\(^{2+}\) exchange (NCX) for Ca\(^{2+}\) removal. Although a relative increase in NCX is expected when SR function is impaired, few and contradictory studies have addressed whether there is an absolute increase in NCX activity. The present study examines in detail NCX density and function in left ventricular midmyocardial myocytes isolated from normal or tachycardic pacing–induced failing canine hearts. No change of NCX current density was evident in myocytes from failing hearts when intracellular Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_i\)) was buffered to 200 nmol/L. However, when [Ca\(^{2+}\)]\(_i\) was minimally buffered with 50 μmol/L indo-1, Ca\(^{2+}\) extrusion via NCX during caffeine application was doubled in failing versus normal cells. In other voltage-clamp experiments in which SR uptake was blocked with thapsigargin, both reverse-mode and forward-mode NCX currents and Ca\(^{2+}\) transport were increased >2-fold in failing cells. These results suggest that, in addition to a relative increase in NCX function as a consequence of defective SR Ca\(^{2+}\) uptake, there is an absolute increase in NCX function that depends on [Ca\(^{2+}\)]\(_i\), in the failing heart. (Circ Res. 2000;87:690-698.)

Key Words: Na\(^{+}\)-Ca\(^{2+}\) exchange ■ heart failure ■ tachycardia

Defective excitation-contraction coupling lies at the core of heart failure pathophysiology,\(^1,2\) having an impact on both contractile function and action potential shape and duration.\(^3\) Cardiac cells isolated from failing human hearts typically exhibit intracellular Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_i\)) transients with decreased amplitudes and slowed rates of relaxation,\(^1\) consistent with decreased contractility in vivo. In a previous study from our laboratory, we have reported\(^2\) similar changes in cellular Ca\(^{2+}\) transients in canine tachycardia-induced heart failure. In this model, as well as in human heart failure,\(^4,5\) the protein level of the sarcoplasmic reticulum (SR) Ca\(^{2+}\) ATPase (SERCA) is reduced and that of Na\(^{+}\)-Ca\(^{2+}\) exchange (NCX) is increased.\(^6\) In accord with these changes, the primary Ca\(^{2+}\) handling defect is diminished SR Ca\(^{2+}\) uptake and a shift toward transsarcolemmal Ca\(^{2+}\) extrusion as a major Ca\(^{2+}\) removal pathway.\(^2\)

A relative increase in NCX function is expected in the face of decreased SR Ca\(^{2+}\) uptake, and we did not previously determine whether an absolute increase in NCX function occurred in proportion to the increased NCX protein levels. Therefore, the present study specifically examines NCX currents and transsarcolemmal Ca\(^{2+}\) transport using several techniques. The findings indicate a significant enhancement of NCX activity in heart failure but only when measured with minimal cytosolic Ca\(^{2+}\) buffering. In light of contradictory reports indicating a reduced,\(^6\) unchanged,\(^7\) or increased\(^8,9\) NCX density in other animal models of heart failure, this is an important issue to address, because NCX upregulation has been correlated with improved diastolic function during the development of heart failure\(^10\) and may also contribute to systolic function in the failing human heart.\(^11\)

Materials and Methods

Canine Pacing-Induced Tachycardia Heart Failure Model

Induction of heart failure was carried out as described previously\(^12\) using protocols approved by the Institution’s Animal Care and Use Committee. In brief, a ventricular inhibited pacemaker (Medtronic) was implanted in mongrel dogs of either sex. Pacing at 240 bpm was maintained for 3 to 4 weeks, during which time the animals developed typical symptoms of heart failure including lethargy, loss of appetite, ascites, etc. Hemodynamic decompensation was confirmed by recording left ventricular pressure waveforms (under anesthesia with 25 mg/kg thiopental) before euthanization, using a micromanometer-tipped left ventricular catheter inserted through the right femoral artery. An increased end-diastolic pressure (normal hearts, 4.7±1.0 mm Hg, versus failing hearts, 29.0±3.9 mm Hg \([P<0.001, n=10 \text{ normal and } 7 \text{ failing hearts for this and subsequent experiments}])\), slowed rate of pressure rise (dP/dt: normal hearts, 2738.1±170.9 mm Hg/s, versus failing hearts, 1216.6±89.5 mm Hg/s), and slowed relaxation rate (dP/dt: normal hearts, −3591.7±238.4 mm Hg/s, versus failing hearts, −1260.1±85.3 mm Hg/s; Figure 1) were evident in failing hearts.

Isolation of midmyocardial cardiomyocytes.\(^2,13\) Single-cell electrophysiological studies,\(^2\) and fluorometric Ca\(^{2+}\) recording\(^2\) were

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performed using published methods and are detailed in the online-only Materials and Methods (data supplement available at http://www.circresaha.org).

Myocytes were whole-cell patch-clamped at 37°C. Internal and external solution compositions for each experiment are shown in the Table. Consistent with previous studies,2,12 cell capacitance was similar in cells isolated from failing and normal hearts (normal cells, 162 ± 7 pF [n=110 cells from 11 dogs; number of cells/number of dogs appears throughout the text in the form 110/11]; failing cells, 171 ± 7 pF; n=90/9; P=NS). After whole-cell configuration was established, the external solution was changed using a custom-built heated rapid-switching device (see online-only Materials and Methods [data supplement available at http://www.circresaha.org]).

For determination of NCX current with [Ca2+]i buffered to 200 nmol/L (compare Figure 2), the external solution (Table) was K1-free (to block the inward rectifier K1 current, IK1, and also the Na1/K1-pump) and also contained (in mmol/L) niflumic acid 100 (to block Ca2+-activated Cl currents), strophanthidin 10 (Na1/K1-pump inhibitor), and nitrendipine 10 (dihydropyridine antagonist). The internal solution (pipette solution 1) is shown in the Table. Cesium and tetraethylammonium inhibited outward K1 currents. The mixture of 5 mmol/L BAPTA and 1.75 mmol/L Ca2+ in the pipette solution gave a free [Ca2+]i of 200 nmol/L (calculated using the Maxchelator program, D. Bers, Loyola University, Chicago, Ill). The pipette-to-bath liquid junction potential was found to be –2.7 mV and was not corrected.

For determination of NCX activity during caffeine applications (compare Figure 3), the external solution (Table) contained 100 mmol/L niflumic acid, and pipette solution 2 was used (Table). For the experiments shown in Figures 3 through 6, we used an external solution (Table), with added quantities (in mmol/L) of niflumic acid 100, nitrendipine 10, and thapsigargin 1 (to inhibit SERCA), and pipette solution 3 (Table). For both pipette solutions 2 and 3 the pipette-to-bath liquid junction potential was –20 mV and was corrected.

[Ca2+]i measurement was performed as described previously2 using the K1 salt form of indo-1. Cellular autofluorescence was recorded before rupturing the cell-attached patch and subtracted before determining R (ratio of 405 nm emission/495 nm emission). [Ca2+]i was calculated according to the equation [Ca2+]i = Kd × R× ([R−Rmin]/[Rmax−R]),14 using a Kd of 844 nmol/L,15 and experimentally determined Rmin=1, Rmax=4, and β=2.

An expanded Materials and Methods section can be found in an online data supplement available at http://www.circresaha.org.

Results

In a previous study,2 we reported that diastolic [Ca2+]i removal was slowed to a lesser extent in failing than in normal cells when SERCA was inhibited with cyclopiazonic acid, indicating an increased contribution of NCX. In addition, data also suggested a trend toward a faster absolute rate of NCX-mediated Ca2+ removal (the time constant of Ca2+ decay [τCa] with cyclopiazonic acid was 599 ± 48 ms in failing cells versus 813 ± 269 ms in normal cells, not statistically significant), but the difficulty of fitting the exponential decay of the small Ca2+ transients and the absence of selective measurements of NCX currents precluded a definitive conclusion on this point. Thus, multiple methods were used in the present study to determine whether there is an absolute increase in NCX function under various conditions (eg, freely rising [Ca2+]i versus buffered conditions) and modes of activity (forward- and reverse-mode currents and Ca2+ fluxes).

Figure 1. In vivo hemodynamic parameters. Shown are in vivo left ventricular pressure and dP/dt cycles in dogs from normal (N) group (A and B) and failing (F) group (C and D). On average, failing hearts exhibited increased end-diastolic pressure (E) and slowed dP/dt for both systole (F) and diastole (G). *Statistical significance (P<0.05).
NCX Current With \([\text{Ca}^{2+}]_i\) Buffered to 200 mmol/L

With all of the other major ion channels and transporters inhibited (see Materials and Methods), repeated families of pulses between \(-100\) and \(+100\) mV from a holding potential of \(-40\) mV induced a time-independent current that was partially blocked by 10 mmol/L Ni\(^{2+}\) (Figure 2A). Under these conditions, the Ni\(^{2+}\)-sensitive current represents NCX current.\(^{16,17}\) The voltage dependence of membrane currents in control solution and in Ni\(^{2+}\) is shown in Figure 2B. Similar Ni\(^{2+}\)-sensitive currents were recorded in failing cells (Figures 2C and 2D). Importantly, the average NCX current density was similar in both experimental groups at all membrane potentials (Figure 2E). NCX current had an apparent reversal potential \((E_{\text{rev}})\) close to the holding potential \((-40\) mV). This differed from the calculated reversal potential of \(-90\) mV but was consistent with previous studies that attributed this result to local ionic gradients near the sarcolemma.\(^{17,18}\) A small but statistically significant hyperpolarizing shift in the 0 current potential was evident in failing cells when \(E_{\text{rev}}\) was calculated individually for each cell \((-41.24\pm1.27\) mV in normal cells and \(-47.7\pm2.34\) mV in failing cells; \(P=0.018\)). To correct for this shift in estimating NCX density, the voltage dependence of the whole-cell conductance \((G_{\text{NCX}}=I/[V_{M}-E_{\text{rev}}])\) was determined for individual cells. This parameter was also not significantly different between groups under these conditions (Figure 2F).

Components (in mmol/L) of Solutions Used

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TEA indicates tetraethylammonium.

**Figure 2.** Measurement of NCX current with \([\text{Ca}^{2+}]_i\) buffered. A, In a normal cell (N), membrane current recordings in control solution (left), 10 mmol/L Ni\(^{2+}\) (middle), and Ni\(^{2+}\)-sensitive current (right). Shown are depolarizations between \(-80\) and \(+80\) in 20-mV steps for 0.3 second every 4 seconds. B, Current-voltage relation (I-V) of average membrane current (measured 100 ms after depolarization) in control solution (C) and Ni\(^{2+}\) (N), \(n=25/4\). C and D, Results of the same experiment in a failing cell (F). E, Current-voltage relation of Ni\(^{2+}\)-sensitive current for normal \((n=25/4)\) compared with failing cells \((n=19/3)\). No significant difference was observed at any membrane potential. In this and subsequent figures, normal and failing data are shown with open and closed symbols, respectively. F, Voltage dependence of NCX conductance \((G_{\text{NCX}})\) in failing and normal cells. No significant difference was observed at any membrane potential.
NCX Activity During Caffeine Application

SR Ca\(^{2+}\) release was induced by rapid caffeine application after a conditioning train of depolarizations (not shown) in 2 mmol/L external Ca\(^{2+}\) for normal cells and 5 mmol/L Ca\(^{2+}\) for failing cells, to compensate for diminished steady-state SR Ca\(^{2+}\) loading in failing cells. After the conditioning train was stopped, the holding potential was set to \(-80\) mV (and, in failing cells, external solution was rapidly changed back to 2 mmol/L Ca\(^{2+}\)), and caffeine was applied. Figures 3A and 3B show representative caffeine-induced [Ca\(^{2+}\)]\(_i\) signals during a caffeine-induced Ca\(^{2+}\) release. B, Same experiment in a failing cell (F). C, Caffeine-induced [Ca\(^{2+}\)]\(_i\) transients were normalized and superimposed. It can be seen that [Ca\(^{2+}\)]\(_i\) decay was faster in failing than in normal cells. D, [Ca\(^{2+}\)]\(_i\) decay was fitted with a monoeponential, the average time constant of which was significantly reduced in failing vs normal cells (n=15/5 for normal and 15/3 for failing cells, P<0.0001). "Statistical significance (P<0.05). E, NCX current density plotted (as positive value) against [Ca\(^{2+}\)]\(_i\) for a normal and a failing cell. The relation was fitted with a linear function, the slope of which was not significantly different in failing cells (as shown in panel F). G, In a normal cell, [Ca\(^{2+}\)]\(_i\) signals and membrane current during application of caffeine with NCX blocked by a Na\(^+\) - and Ca\(^{2+}\)-free external solution. For comparison, the thin, interrupted trace shows [Ca\(^{2+}\)]\(_i\) decay during application of caffeine (in standard external solution) in the same cell, normalized for amplitude (and thus not corresponding to the same y axis). H, Similar experiment in a failing cell. I, Time constant of [Ca\(^{2+}\)]\(_i\) decay in the absence of NCX was similar in normal and failing cells (n=9/3 for normal and 8/3 for failing cells, P=NS).

Cells, which was \(\approx2.2\)-fold faster than in normal cells (\(\tau_0=761 \pm 64\) ms in normal versus 347 \(\pm 52\) ms in failing cells, P<0.0001).

The doubling of the NCX-mediated Ca\(^{2+}\) efflux rate with minimal Ca\(^{2+}\) buffering, together with the similar Ni\(^{2+}\)-sensitive currents in the presence of buffer (Figure 2), suggested that NCX may be differentially modulated by [Ca\(^{2+}\)]\(_i\) in the systolic range in failing cells. To investigate this possibility directly, we plotted NCX current density against [Ca\(^{2+}\)]\(_i\) for a normal and a failing cell. The slope of the relation between NCX current density and [Ca\(^{2+}\)]\(_i\) was not significantly different between groups (Figure 3F).

To examine the possibility that [Ca\(^{2+}\)]\(_i\) removal mechanisms other than NCX (eg, the sarcotremal Ca\(^{2+}\) pump and the mitochondrial unipporter) could have contributed to the differences observed, we applied caffeine in the absence of external

Figure 3. NCX activity during caffeine release. A, In a normal cell (N), membrane currents and [Ca\(^{2+}\)]\(_i\) signals during a caffeine-induced Ca\(^{2+}\) release. B, Same experiment in a failing cell (F). C, Caffeine-induced [Ca\(^{2+}\)]\(_i\) transients were normalized and superimposed. It can be seen that [Ca\(^{2+}\)]\(_i\) decay was faster in failing than in normal cells. D, [Ca\(^{2+}\)]\(_i\) decay was fitted with a monoeponential, the average time constant of which was significantly reduced in failing vs normal cells (n=15/5 for normal and 15/3 for failing cells, P<0.0001). "Statistical significance (P<0.05). E, NCX current density plotted (as positive value) against [Ca\(^{2+}\)]\(_i\) for a normal and a failing cell. The relation was fitted with a linear function, the slope of which was not significantly different in failing cells (as shown in panel F). G, In a normal cell, [Ca\(^{2+}\)]\(_i\) signals and membrane current during application of caffeine with NCX blocked by a Na\(^+\) - and Ca\(^{2+}\)-free external solution. For comparison, the thin, interrupted trace shows [Ca\(^{2+}\)]\(_i\) decay during application of caffeine (in standard external solution) in the same cell, normalized for amplitude (and thus not corresponding to the same y axis). H, Similar experiment in a failing cell. I, Time constant of [Ca\(^{2+}\)]\(_i\) decay in the absence of NCX was similar in normal and failing cells (n=9/3 for normal and 8/3 for failing cells, P=NS).
Na\(^+\) and Ca\(^{2+}\) (replaced with Li\(^+\) and Ni\(^{2+}\), respectively) to block both forward- and reverse-mode NCX (Figures 3G and 3I). Under these conditions, Ca\(^{2+}\) decay was substantially slowed in both groups, but there was no difference in non-NCX Ca\(^{2+}\) extrusion time constants (3367.6\pm63 ms in normal versus 3236.3\pm648 ms in failing cells, NS; Figure 3I).

### NCX Activity Under Thapsigargin

In a third series of experiments, also under physiological Ca\(^{2+}\) buffering conditions, we selectively measured both reverse- and forward-mode NCX-mediated Ca\(^{2+}\) transport and currents when SR Ca\(^{2+}\) uptake was inhibited with 1 \(\mu\)mol/L thapsigargin.

From a holding potential of –100 mV, 0.5-second depolarizations from 0 to +120 mV were applied every 4 seconds. Reverse-mode NCX elicited an outward current and an almost linear rise in [Ca\(^{2+}\)], (Figure 4A, left). Repolarization initiated [Ca\(^{2+}\)] decay and an inward tail current, generated by forward-mode NCX. Ni\(^{2+}\) (10 mmol/L) partially blocked the outward current during depolarization and virtually eliminated the rise in [Ca\(^{2+}\)], and the tail current (Figure 4A, middle). In failing cells, the same voltage protocol elicited a substantially larger Ni\(^{2+}\)-sensitive outward current, [Ca\(^{2+}\)]\(_r\), rise, and tail current as compared with normal cells (Figure 4B).

To quantify NCX function, we measured 4 different parameters (Figure 5), as follows:

1. The Ni\(^{2+}\)-sensitive outward current at both 100 and 485 ms after depolarization (Figures 5A and 5B). NCX current density was larger in failing compared with normal cells over the whole range of membrane potentials, with the largest differences (2- to 4.5-fold) evident between +60 and +120 mV.

2. The amplitude of the tail current on repolarization to –100 mV (Figure 5C). This parameter was increased 2- to 3.3-fold in failing compared with normal cells (similar to the increase in peak [Ca\(^{2+}\)], mentioned below).

3. The [Ca\(^{2+}\)]\(_r\) rise during depolarization at 100 and 485 ms (Figures 5D and 5E). This was also increased 2- to 3-fold in failing cells between +60 and +120 mV.

4. The time constant of [Ca\(^{2+}\)]\(_r\) decay mediated by forward-mode NCX. [Ca\(^{2+}\)]\(_r\) decline (after repolarization to –100 mV following a depolarization to +100 mV) was fitted with a single-exponential function. The average time constant was faster in failing versus normal cells (Figure 5F), reflecting a \(\approx\) 2.26-fold acceleration of Ca\(^{2+}\) removal via forward-mode NCX activity, similar to the results obtained with caffeine.

It was important to ascertain that the Ni\(^{2+}\)-sensitive currents, and particularly the tail currents, were exclusively due to NCX. Although all of the other known Ca\(^{2+}\)-activated currents (such as those carried by K\(^+\) or Cl\(^-\)) were blocked in our protocol, the possibility remained that part of the Ni\(^{2+}\)-sensitive currents were due to some unidentified Ca\(^{2+}\)-activated ion channel. If this were the case, such a channel would continue to conduct current on repolarization as long as [Ca\(^{2+}\)]\(_r\) remained elevated.

To test this, after a series of control pulses to +100 mV, during one depolarization we effected a rapid exchange to Na\(^+\)-free solutions (Na\(^+\) replaced by Li\(^+\), which is not transported by NCX, Figure 6A). The change in the electrochemical gradient for Na\(^+\) quickly increased the outward current, consistent with an increased driving force for reverse-mode NCX. The [Ca\(^{2+}\)]\(_r\) rise was also enhanced by the solution change (the somewhat smaller increase in [Ca\(^{2+}\)]\(_r\)), than expected from the increase in NCX current is probably due to the Ca\(^{2+}\) indicator approaching saturation). With the repolarization, still in Na\(^+\)-free solution, [Ca\(^{2+}\)]\(_r\) remained

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**Figure 4.** Measurement of NCX with thapsigargin. A, In a normal cell (N), membrane currents and [Ca\(^{2+}\)] recordings during depolarizations from –100 mV to +40, +60, and +80 mV in control condition (left) and in Ni\(^{2+}\) (middle). Right panel shows Ni\(^{2+}\)-sensitive current. B, Similar experiment in a failing cell (F) elicited a much larger [Ca\(^{2+}\)], reverse-mode NCX-mediated Ca\(^{2+}\) uptake was inhibited with 1 \(\mu\)mol/L thapsigargin.
high, but the inward current tail was completely eliminated. Restoration of Na⁺-containing solution initiated Ca²⁺ efflux once again, and this was accompanied by the development of an inward current. The integral of the inward current activated by Na⁺ reapplication was close to that of the tail current in control solution (118.6 ± 24.3% of control tail current; \( P = \text{NS}; n = 8 \) experiments).

As the tail current was completely blocked in Na⁺-free solution (even if [Ca²⁺] remained high), this suggested strongly that it was generated only by NCX and not by some other Ca²⁺-activated conductance.

We then analyzed the relation between tail NCX current and [Ca²⁺], from the experiments of Figures 4 and 5. Figure 6B shows representative plots from normal and failing myocytes, which were not markedly different. The NCX current versus Ca²⁺ relations were close to linear, and their slopes were not significantly increased in failing cells compared with normal cells (Figure 6C).

Therefore, the measurements of forward- and reverse-mode NCX driven by voltage-clamp depolarizations in the presence of thapsigargin generally confirmed the findings of the caffeine experiments; whole-cell NCX activity (Ca²⁺ transport and current density) was significantly increased in failing cells, but this difference disappeared when NCX current density was normalized for [Ca²⁺⁺].

Estimation of Cellular Ca²⁺ Buffering Capacity

For the interpretation of all [Ca²⁺] measurements, we needed to ascertain that the buffering capacity of cardiac cells is not changed in heart failure. We used the method of Trafford et al. Briefly, during caffeine application, the backward integral of NCX current, representing the charge moved by NCX efflux during repolarization, was measured (with respect to holding current) both in control solution and in Ni²⁺ and then subtracted. Membrane current was measured 100 ms after depolarization (A) and at the end of the test pulse (B). The Ni²⁺-sensitive (NCX) current was larger in failing (F) than in normal (N) cells for depolarizations between +60 and +120 mV \( (P < 0.05, n = 10/4 \text{ for normal and } 7/3 \text{ for failing cells}). \) C, Magnitude of tail inward current at different membrane potentials. Tail current was measured as peak inward current 30 ms after repolarization to −100 mV (and thus after the capacitative spike) minus steady-state holding current (which was usually negligible). The tail current was larger in failing than in normal cells between +60 and +120 mV \( (n = 10/4 \text{ for normal and } 7/3 \text{ for failing cells}). \) D and E, Voltage dependence of the rise in [Ca²⁺⁺], via reverse-mode NCX in normal and failing cells. Shown are measurements taken 100 ms after depolarization (D) and at the end of the test depolarization (E). Reverse NCX induced a larger rise in [Ca²⁺] in failing vs normal cells at all potentials between +40 and +120 mV \( (n = 10/4 \text{ for normal and } 7/3 \text{ for failing cells}). \) F, Average time constants for [Ca²⁺⁺] decay after repolarization from +100 mV \( (P = 0.016, n = 12/4 \text{ for normal and } 7/3 \text{ for failing cells}). \)

![Figure 5. Parameters of NCX function under thapsigargin. A and B, Amplitude of the Ni²⁺-sensitive current. Outward membrane current during depolarization was measured (with respect to holding current) both in control solution and in Ni²⁺ and then subtracted. Membrane current was measured 100 ms after depolarization (A) and at the end of the test pulse (B). The Ni²⁺-sensitive (NCX) current was larger in failing (F) than in normal (N) cells for depolarizations between +60 and +120 mV \( (P < 0.05, n = 10/4 \text{ for normal and } 7/3 \text{ for failing cells}). \) C, Magnitude of tail inward current at different membrane potentials. Tail current was measured as peak inward current 30 ms after repolarization to −100 mV (and thus after the capacitative spike) minus steady-state holding current (which was usually negligible). The tail current was larger in failing than in normal cells between +60 and +120 mV \( (n = 10/4 \text{ for normal and } 7/3 \text{ for failing cells}). \) D and E, Voltage dependence of the rise in [Ca²⁺⁺], via reverse-mode NCX in normal and failing cells. Shown are measurements taken 100 ms after depolarization (D) and at the end of the test depolarization (E). Reverse NCX induced a larger rise in [Ca²⁺] in failing vs normal cells at all potentials between +40 and +120 mV \( (n = 10/4 \text{ for normal and } 7/3 \text{ for failing cells}). \) F, Average time constants for [Ca²⁺⁺] decay after repolarization from +100 mV \( (P = 0.016, n = 12/4 \text{ for normal and } 7/3 \text{ for failing cells}). \)]
but only when \([\text{Ca}^{2+}]\) is permitted to rise freely in the cytoplasm. When all of the findings are considered together, we can offer two potential mechanistic explanations for the results.

First, allosteric modulation of NCX by \([\text{Ca}^{2+}]\) may be enhanced in the failing heart. It is well known that \([\text{Ca}^{2+}]\) stimulates NCX, binding to a site on the intracellular loop distinct from the transport site. \(19\) \([\text{Ca}^{2+}]\) stimulation is thought to be a direct process, with a half-maximal \([\text{Ca}^{2+}] (K_d)\) close to 0.3 \(\mu\text{mol/L}\) and a Hill coefficient close to 1 (in giant patches\(^{19}\)).

On the other hand, because the 2-fold increase in NCX activity in failing cells (with minimal \([\text{Ca}^{2+}]\) buffering) is similar to the 2-fold increase in NCX protein expression in this model,\(^{2}\) it is tempting to speculate that the mechanism involves an increased number of exchangers in the membrane. To rationalize the lack of a difference in NCX current density under highly buffered conditions, it is possible that NCX in failing cells is differentially regulated by \([\text{Ca}^{2+}]\),

Figure 6. A, Experiment illustrating that the inward current tail was generated by forward-mode NCX. Membrane currents and \([\text{Ca}^{2+}]\) signals in control solution (unmarked trace) and during a rapid switch to \(\text{Na}^{+}\)-free solution (bar) during depolarization and extending into the repolarizing pulse (arrows). The tail current was blocked in \(\text{Na}^{+}\)-free solution, consistent with an exclusive contribution by forward-mode NCX. This experiment was performed in 8 cells from both normal and heart failure dogs with similar results. B, Tail NCX current density plotted against \([\text{Ca}^{2+}]\) in a normal and a failing cell. C, NCX current vs \([\text{Ca}^{2+}]\) relations were fitted in each cell with a linear function, the slope of which was not significantly different in normal and failing cells (n=14/5 normal and 10/3 failing cells, \(P=\text{NS}\)).

Figure 7. Estimation of cellular \([\text{Ca}^{2+}]\) buffering properties. A, Membrane currents and \([\text{Ca}^{2+}]\) signals during a caffeine application. B, Time course of the calculated backward integral of NCX current (\(\text{Ca}_t\), aligned for time with panel A). C, Two representative examples from a normal (N) and a failing (F) heart showing that the relation between total \([\text{Ca}^{2+}] (\text{Ca}_t)\) and free \([\text{Ca}^{2+}] ([\text{Ca}^{2+}])\) was largely linear and similar in the 2 groups. D, Mean ratio of free-to-total \([\text{Ca}^{2+}]\) was not different in normal and failing cells \((P=0.81, n=8/3\) normal and 8/3 failing cells). Right bars show that a similar analysis using the data obtained with thapsigargin yielded identical results \((P=0.85, n=9/4\) normal and 10/3 failing cells).
through either a change in cooperativity, a difference in the effective $K_0$ for activation, or the presence of a nonfunctional pool of exchangers recruitable by a prolonged Ca$^{2+}$ stimulus (a “Ca$^{2+}$-switch” mechanism, separate from Ca$^{2+}$ allosteric activation). Such a mechanism could represent an intriguing adaptive mechanism that would enable the myocyte to remove Ca$^{2+}$ from the cell only when cytoplasmic [Ca$^{2+}$], overload threatens (perhaps exacerbated by depressed SERCA function).

Both an increase in NCX number and stimulation by [Ca$^{2+}$], should be evident as an increase in the slope of the relation between NCX current and [Ca$^{2+}$], but we did not observe any significant difference in these plots in separate series of experiments (Figures 3E and 6B). We considered two possible explanations for this apparent inconsistency.

First, there may be limitations in the methodology. We made every effort to exclude some potential experimental artifacts by testing the specificity of the current measured (Figure 6A), the intrinsic buffer capacity of the cells (Figure 7), and the contribution of non–NCX-mediated Ca$^{2+}$ transport (Figure 3). These factors, while providing useful new information on the basic cellular properties of this model, could not explain the differences between groups. Other problems related to the non–steady-state conditions, such as differences in subsarcolemmal ion homeostasis or kinetic limitations, could not be ruled out and could confound the interpretations based on bulk [Ca$^{2+}$], recordings.

A second hypothesis is that the stoichiometry of NCX in the failing heart is changed. If, for example, the stoichiometry was 5Na$^+:2$Ca$^{2+}$ instead of the usual 3Na$^+:1$Ca$^{2+}$, only half the charge would move for the same amount of Ca$^{2+}$ transport. Thus, NCX could show an increased whole-cell Ca$^{2+}$ transport without a change in the NCX current density–versus-Ca$^{2+}$ relation. The fact that we did see increased NCX tail currents in failing cells in the experiments shown in Figures 4 and 5 could be due to a larger influx of Ca$^{2+}$ through reverse-mode exchange during the depolarization. Interestingly, a double-sized NCX transcript (14 kb mRNA, as opposed to normal 7 kb) has been reported in rabbit failing heart (and in other tissues; see, eg, Reference 9 and references within), but whether it codes for a different protein isoform has not been determined. Expression of an exchanger having a different stoichiometry in heart failure is therefore a possibility. Two recent reports also suggest that the stoichiometry of the exchanger may be dynamically modulated. Fujioka et al. found that the stoichiometry of the exchanger conform better to a 4Na$^+:1$Ca$^{2+}$ exchange ratio and that it increased at higher internal Na$^+$ concentration and decreased at high [Ca$^{2+}$]. Additionally, Egger and Niggli reported that external acidification decreased NCX current without a change in Ca$^{2+}$ transport rate. Thus, NCX stoichiometry may be variable and modulated by local factors, some of them possibly changed in heart failure.

Relevance for Cell Contractility

Although the precise molecular mechanism for the increase in NCX remains to be determined, the consistent doubling of NCX-mediated whole-cell Ca$^{2+}$ transport shown in this study may be expected to influence profoundly cell contractility. As NCX is largely responsible for Ca$^{2+}$ extrusion and only partially for Ca$^{2+}$ entry (together with the L-type Ca$^{2+}$ current), its increase might be expected to decrease SR load and contractility. Combined with decreased SR Ca$^{2+}$ uptake, this is the result obtained in computer simulations of the Ca$^{2+}$ transient in the canine cardiac cell model and has been confirmed by experiments in which NCX was increased (by adenovirus infection) in cultured adult rabbit cells. Nevertheless, reverse NCX may activate the myofilaments directly, and an increased NCX has been recently correlated with increased contractility in a canine model of cardiac hypertrophy. The physiological effects will depend on the membrane potential during the plateau of the action potential; the amplitude of the [Ca$^{2+}$] transient; and, in light of the present results, the average level of [Ca$^{2+}$]. It also remains to be determined whether an increase in NCX may be pro- or antiarrhythmic, given that NCX current will influence both the action potential plateau and spontaneous depolarizations during diastole.

Comparison With Other Heart Failure Models

NCX activity has been shown to be increased in 2 heart disease models not characterized by reduced contractility and recently in a rabbit aortic insufficiency/pressure overload failure model. Contrary to the present finding, NCX has been shown to be decreased or unchanged in a similar tachy- cardiac pacing model of heart failure in rabbit, with either decreased or elevated NCX protein levels, respectively. Thus, both the depression of SERCA activity and compensatory upregulation of NCX apparently vary depending on the model and/or the etiology of the disease. With regard to NCX upregulation, the present findings suggest that some of the discrepancies may also depend on how the NCX function was determined. It is also relevant to add that Pogwizd et al. identified a largely parallel increase in NCX mRNA, protein, whole-cell current, and Ca$^{2+}$ extrusion, so the apparent inconsistency reported here may not be generally applicable.

Relevance for Human Disease

In human heart failure, NCX expression (as mRNA and protein levels) has been reported to be increased and was positively correlated with preserved diastolic function. Measurements in sarcolemmal vesicles have also suggested an increased NCX. One of the few physiological studies reported indirect evidence for an “increased functional relevance” of NCX in failing human myocardium; Flesch et al. showed that BDF (a Na$^+$ channel activator) increased contractility of papillary muscle strips more in failing than in normal cells, which suggested that NCX may be upregulated (although involvement of changes in the action potential shape and other Na$^+$ transport mechanisms could not be ruled out). A prominent role for reverse-mode NCX in supporting contraction of myocytes from failing human cells has also been suggested by Dripa et al., who demonstrated that a tonic component of action potential–evoked Ca$^{2+}$ transients and contractions was insensitive to SR inhibition but sensitive to a NCX inhibitor compound. This suggestion is consistent with our experimental results and model simulations of the behavior of NCX in the failing canine cardiomyocytes.
In summary, the present experiments identified a 2-fold increase in NCX activity in cells isolated from failing canine cells. This increase was only evident when \([\text{Ca}^{2+}]\) was allowed to rise, suggesting that \([\text{Ca}^{2+}]\)-dependent modulation of the exchanger may be involved. Such an increase is likely to play an important role in the contractility and electrophysiology of the failing heart.

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Enhanced Ca\(^{2+}\)-Activated Na\(^{+}\)-Ca\(^{2+}\) Exchange Activity in Canine Pacing-Induced Heart Failure

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