Increased Exchange Current but Normal \( \text{Ca}^{2+} \) Transport via \( \text{Na}^+-\text{Ca}^{2+} \) Exchange During Cardiac Hypertrophy After Myocardial Infarction

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Abstract—Hypertrophied and failing cardiac myocytes generally show alterations in intracellular \( \text{Ca}^{2+} \) handling associated with changes in the contractile function and arrhythmogenicity. The cardiac \( \text{Na}^+-\text{Ca}^{2+} \) exchange (NCX) is an important mechanism for \( \text{Ca}^{2+} \) extrusion and cell relaxation. Its possible involvement in changes of excitation-contraction coupling (EC-coupling) with disease remains uncertain. We analyzed the NCX function in rat ventricular myocytes 5 to 6 months after experimental myocardial infarction (PMI) produced by left coronary artery ligation and from sham-operated (SO) hearts. Caged \( \text{Ca}^{2+} \) was dialyzed into the cytoplasm via a patch-clamp pipette and \( \text{Ca}^{2+} \) was released by flash photolysis to activate NCX and measure the associated currents (\( I_{\text{NaCa}} \)), whereas [\( \text{Ca}^{2+} \)], changes were simultaneously recorded with a confocal microscope. \( I_{\text{NaCa}} \) density normalized to the [\( \text{Ca}^{2+} \)] jumps was 2.6-fold higher in myocytes from PMI rats. The level of total NCX protein expression in PMI myocytes was also increased. Interestingly, although the \( I_{\text{NaCa}} \) density in PMI cells was larger, PMI and SO myocytes presented virtually identical \( \text{Ca}^{2+} \) transport via the NCX. This discrepancy was explained by a reduced surface/volume ratio (34.8\%) observed in PMI cells. We conclude that the increase in NCX density may be a mechanism to maintain the required \( \text{Ca}^{2+} \) extrusion from a larger cell to allow adequate relaxation. (Circ Res. 2002;91:323-330.)

Key Words: sodium-calcium exchange ▪ cardiac myocyte ▪ hypertrophy ▪ intracellular calcium ▪ calcium transport

Cardiac hypertrophy and heart failure are associated with alterations in \( \text{Ca}^{2+} \) handling that are involved in contraction-relaxation abnormalities.\(^1\)\(^-\)\(^4\) The \( \text{Na}^+-\text{Ca}^{2+} \) exchanger (NCX) contributes to heart muscle relaxation by \( \text{Ca}^{2+} \) extrusion and may be involved in excitation-contraction coupling.\(^5\)\(^-\)\(^7\) The 3:1 stoichiometry for \( \text{Na}^+ \) and \( \text{Ca}^{2+} \) and a consecutive process for the ion exchange are widely accepted (but see Fujioka et al\(^9\)). Nevertheless, details of the molecular mechanism and regulation of the \( \text{Na}^+-\text{Ca}^{2+} \) exchange are not well understood and subject of intense investigations\(^10\)\(^-\)\(^12\) (see Egger and Niggli\(^2\) for a recent review). Beside the role of the NCX in excitation-contraction coupling, alterations in its function during cardiac hypertrophy could be involved in other important aspects of cardiac remodeling. In fact, [\( \text{Ca}^{2+} \)], whose level is in part maintained by the NCX, is a key factor for the cellular regulation of protein expression. Cardiac remodeling involves the selective synthesis of some proteins.\(^1\) In this regard, “long-term” changes in the \( \text{Ca}^{2+} \) homeostasis could affect the up- or downregulation of proteins by the so-called “excitation-transcription coupling.”\(^12\) Some of the proteins, whose expression has been shown to be altered in hypertrophy and failure, are (1) ionic channels, which shape the action potential, such as \( \text{K}^+ \) channels,\(^13\) whose expression has been shown to depend on [\( \text{Ca}^{2+} \)];\(^14\) (2) contractile proteins\(^1\); and (3) proteins that are involved in excitation-contraction coupling (eg, L-type \( \text{Ca}^{2+} \) channels, NCX, sarcoplasmic reticulum [\( \text{SR} \) \( \text{Ca}^{2+} \)-ATPase]). Although it is generally accepted that the \( \text{SR} \) \( \text{Ca}^{2+} \)-ATPase (SERCA) function is decreased in hypertrophy, data regarding the NCX are controversial. An increase in the NCX function has been reported\(^15\)\(^-\)\(^16\) but modifications of the NCX activity during hypertrophy seem to depend on the experimental conditions,\(^17\) the animal model, and the disease progression.\(^18\) Moreover, the precise cellular mechanisms and consequences underlying possible NCX alterations in pathophysiological conditions are not well understood, and it is difficult to discern whether the observed modifications in hypertrophied and failing myocytes constitute a primary cause of the dysfunction, or a secondary adaptation to the new cardiac situation after the insult.
The goal of the present study was to examine whether and how heart remodeling after myocardial infarction induces functional changes in the sarcolemmal NCX activity at the cellular level. We produced myocardial infarctions in rats by left coronary artery ligation. This method acutely induces infarction and the survivor animals develop heart remodeling, characterized by chamber dilatation, cellular hypertrophy, and finally, heart failure.1,4 We activated the NCX via flash photolysis and due to Ca$^{2+}$ transport by the NCX. We found that myocytes isolated from infarcted rat hearts exhibited increased functional NCX protein expression and $I_{\text{NaCa}}$ density and surprisingly normal NCX Ca$^{2+}$ transport capacity. Preliminary results have been presented to the Biophysical Society in abstract form.20

### Materials and Methods

#### Cell Isolation and Solutions

The experiments were performed according to the guidelines of the Swiss Animal Protection Law and with the permission of The State Veterinary Office, Bern, Switzerland. Myocardial infarction was produced in young Wistar male rats (120 to 220 g, Centre d’Elevage Janvier, Le Genest Saint Isle, France) by left coronary artery ligation as previously described.21 Ventricular myocytes were isolated from postmyocardial infarcted (PMI) and sham-operated (SO) rat hearts with enzymatic methods.1 For this study, cells from the left ventricular free wall were used and treated with 10 mmol/L ryanodine and 0.1 mmol/L thapsigargin (both from Alomone Labs, Israel) at room temperature (20 to 24°C) for at least 60 minutes to exclude the SR-function.

Changes of the extracellular solutions were performed using a gravity-driven superfusion system ($t_{1/2}$ ~400 ms, 1 mL/min). The superfusion solution contained (in mmol/L) NaCl 140, KCl 5, MgCl$_2$ 1, CaCl$_2$ 1.8, CsCl 1, BaCl$_2$ 0.5, HEPES 10, glucose 10, pH 7.4 (NaOH). The pipette filling solution contained (in mmol/L) Cs-aspartate 120, tetraethylammonium-Cl 20 (Sigma), Na$_2$-DM-nitrophen 2 (Calbiochem, USA), fluo-3 0.1 (TEFLABS, USA), CaCl$_2$, 0.5, reduced glutathione 2, K$_2$-ATP 5, HEPES 20, pH 7.2 (CsOH). Cells were loaded for at least 3 minutes by dialysis or with moderate pressure injection. All experiments were performed at room temperature (20 to 23°C).

#### Flash Photolysis

Light from a xenon short-arc flash lamp (Strobex 238/278, 230 Ws, pulse duration ~1 ms) was used to photolyze intracellular DM-nitrophen in an epi-illumination arrangement on the confocal microscope. Part of the produced ultraviolet spectrum (<390 nm) was passed through the microscope objective (Zeiss Neofluor lens, 63× Oil/NA = 1.25, Carl Zeiss) generating a homogeneous illumination of the entire visible field.

#### Voltage Clamp and Data Analysis

The membrane capacitance ($C_m$) was determined at the beginning of each experiment by measuring capacity transients induced by 10-ms hyper and depolarizing voltage-clamp steps. The obtained capacity currents were integrated and divided by the voltage step.

In some cells, the myocyte volumes were obtained together with $C_m$. Stacks of confocal fluorescence images were recorded and cell volume calculated by the method described by Satoh et al.23 Spherical aberration was calculated and corrected from the 3-D ellipsoidal image obtained from a microsphere of known ratio.23

The $I_{\text{NaCa}}$ was recorded in the whole-cell configuration of the patch-clamp technique as previously described.11

All values were expressed as mean±SEM. Student’s unpaired $t$ tests were used to test for significance ($P<0.05$ or $P<0.001$, as indicated).

#### Confocal Microscopy

The setup for fluorescence measurements was based on a confocal laser-scanning microscope (MRC 1000, Bio-Rad or LSM 510 Zeiss). Fluo-3 was excited at 488 nm with 50 μW intensity and the fluorescence was detected at wavelengths >515 nm. Amplitude and time course of Ca$^{2+}$ concentrations were computed off-line from line-scan images using a customized version of the NIH Image software and IDL software (Research Systems). The [Ca$^{2+}$]$_i$ was derived using a self-ratio method assuming a resting [Ca$^{2+}$]$_i$ of 100 mmol/L and a dissociation constant of the fluo-3:Ca$^{2+}$ complex (K_D) of 400 mmol/L.23

#### Protein Binding Assay and mRNA Quantification by RT-PCR

Binding experiments were performed with the H-labeled high-affinity antibody R3F1 in order to determine the amounts of the NCX present in whole-cell lysates as previously described.24 The protein concentrations in the lysates were quantified using the Micro BCA Protein Assay (Pierce) according to the manufacturer supplied protocol. For semiquantitative PCR, total RNA was extracted from isolated cardiac myocytes by the guanidinium thiocyanate-phenol-chloroform method.25

Images were acquired with a CCD camera and quantitatively analyzed using the BioRad software. For each sample, the ratio NCX/GAPDH was calculated.

For calibration of RT-PCR reactions from different RNA samples, GAPDH was used as an internal standard. Each sample was analyzed twice independently (RT and PCR reactions). For the PCR reactions (NCX and GAPDH), the optimal number of cycles (logarithmic phase) was determined and was 27, and either 21 or 24, respectively. Two different cycle numbers for GAPDH were used in order to exclude a bias of the PCR results due to the selection of the reference point. No systematic differences between the results obtained with 21 or 24 cycles were observed, and thus, results were combined.

### Results

#### Morphological and Cellular Characteristics

Some of the operated animals were used for hemodynamic measurements. These results have been published recently and showed that the PMI animals were suffering from heart failure.4 Other animals were used to analyze NCX function and these results are presented here. At the time of experiments, PMI animals showed very dilated hearts and large infarction sizes between 3 to 5 (number of parts occupied by the scar of a total of 6 by visual division of the left ventricular free wall). The heart-to-body weight ratio was significantly higher in PMI animals than in sham-operated controls (see Table).

Myocytes isolated from the surviving left ventricular wall showed hypertrophy. The morphological parameters and the cell surface, indirectly measured by membrane capacitance, were significantly increased in PMI myocytes ($\approx$2.3-fold, see Table). The average myocyte volume was found to be $\approx$3.5-fold larger in PMI rats (see Materials and Methods and Table).

#### Increased $I_{\text{NaCa}}$ in PMI Cardiomyocytes

The quantification of the NCX function is not straightforward because of competing Ca$^{2+}$ transport mechanisms (eg, SERCA2 and the sarcolemmal Ca$^{2+}$-ATPase). SR function was blocked (see Materials and Methods) to eliminate it as a...
sink or source for Ca\(^{2+}\). \(I_{\text{NaCa}}\) was induced via UV-flash photolysis of caged Ca\(^{2+}\) (DM-nitrophen) at −40 mV, whereas L-type Ca\(^{2+}\) currents \(I_{\text{Ca,L}}\) were activated with a voltage step (200 ms) from −40 mV to 0 mV. The \(I_{\text{NaCa}}\) activation via photorelease of “caged” Ca\(^{2+}\) was chosen because this methodology offers the advantage that the substrates for the exchange process can be controlled nearly perfectly.\(^{26}\) The [Ca\(^{2+}\)] transient amplitude can be adjusted with the UV-flash intensity, whereas the [Ca\(^{2+}\)] and [Na\(^{+}\)] are controlled by the superfusion solution composition. In addition, no voltage steps are required to activate \(I_{\text{NaCa}}\). In previous studies, it has been shown that currents activated by [Ca\(^{2+}\)], jumps induced by flash photolysis were sensitive to 5 mmol/L extracellular Ni\(^{2+}\) and removal of extracellular Na\(^{+}\), suggesting that the observed currents are only mediated by the NCX (data not shown).\(^{26}\) Figure 1 shows the experimental protocol and \(I_{\text{NaCa}}\) together with the corresponding [Ca\(^{2+}\)] transients. Interestingly, although the [Ca\(^{2+}\)] transients induced by UV-flash photolysis were of comparable amplitude, PMI myocytes showed larger currents and more charge was moved. Because in our study \(I_{\text{NaCa}}\) was evoked by [Ca\(^{2+}\)], jumps, \(I_{\text{NaCa}}\) density (ie, current per membrane capacitance) was normalized to the [Ca\(^{2+}\)], transient amplitude, accounting for variability of the [Ca\(^{2+}\)] jumps induced by flash photolysis. In this way, the \(I_{\text{NaCa}}\) density per activating [Ca\(^{2+}\)], at −40 mV was still markedly increased in PMI myocytes (see Table 1; Figure 1C), whereas the \(I_{\text{Ca,L}}\) density was similar in SO and PMI myocytes (Table 1). Thus, \(I_{\text{NaCa}}\) was significantly larger in PMI myocytes even when activated by [Ca\(^{2+}\)], jumps of comparable amplitude (Figures 1A and 1B).

### Ca\(^{2+}\) Transport Mediated by the NCX

The simultaneous measurements of \(I_{\text{NaCa}}\) and the changes of [Ca\(^{2+}\)], open a way to correlate the \(I_{\text{NaCa}}\) (or net charge transport) with the Ca\(^{2+}\) removal (Ca\(^{2+}\) transport) mediated by the NCX. In previous experiments, a linear relationship between \(I_{\text{NaCa}}\) and [Ca\(^{2+}\)] has been found under conditions of [Ca\(^{2+}\)] jumps evoked by flash photolysis of caged Ca\(^{2+}\).\(^{11}\) Recently, it has been proposed that the stoichiometry of the NCX may vary depending on [Na\(^{+}\)], and/or [Ca\(^{2+}\)]\(^{5}\). Therefore, we compared matched pairs with similar [Ca\(^{2+}\)], jumps in PMI and SO myocytes. Figure 2 shows surface plots calculated from the line scan data given in Figure 1. In this example, a pair of matched [Ca\(^{2+}\)] jumps and the corresponding Ca\(^{2+}\) transport were analyzed. Interestingly, Figure 2B shows no difference in the activation of Ca\(^{2+}\) transport by [Ca\(^{2+}\)]. The finding of comparable Ca\(^{2+}\) transport in SO cells and PMI cells is summarized in Figure 2C and contrasts with increased \(I_{\text{NaCa}}\) in PMI myocytes (Figure 1C; see Table). In other words, the ratio of the rate of [Ca\(^{2+}\)] decay and \(I_{\text{NaCa}}\) density was increased in PMI myocytes, suggesting that PMI cells generate more \(I_{\text{NaCa}}\) per change of [Ca\(^{2+}\)] (see Table). How can we explain the discrepancy between the \(I_{\text{NaCa}}\) density and the Ca\(^{2+}\) transport mediated by the NCX in hypertrophied myocytes? In principle, this apparent discrepancy could result from (1) a change in the stoichiometry of the NCX exchange cycle; (2) changes of the intracellular Ca\(^{2+}\) buffer capacity; and (3) changed cellular morphometric characteristics.

### NCX Stoichiometry

Important information about changes in the stoichiometry and/or electrogenicity of the NCX can be derived from the voltage dependence of \(I_{\text{NaCa}}\). The \(I_{\text{NaCa}}\) was larger at more negative potentials and exhibited no reversal potential, as expected (because \(I_{\text{NaCa}}\) represents a Ca\(^{2+}\)-activated difference current and not the total current).\(^{26}\) To compare the voltage-dependence between 2 cell groups, we normalized to the \(I_{\text{NaCa}}\) density at −40 mV. The \(I_{\text{NaCa}}\)/voltage relationship was indistinguishable in SO and PMI myocytes, suggesting that the stoichiometry and the electrogenicity of the NCX was most likely not affected (Figure 3A).

### Buffer Capacity

A second possibility to explain the difference between \(I_{\text{NaCa}}\) density and the maximal rate of [Ca\(^{2+}\)] decay in SO and PMI myocytes could be an elevated cytosolic Ca\(^{2+}\) buffer capacity in PMI myocytes. By simultaneously recording \(I_{\text{Ca,L}}\) and [Ca\(^{2+}\)], in myocytes with blocked SR function and blocked NCX function (Na\(^{+}\)-free pipette solution), we obtained quantitative information on Ca\(^{2+}\) buffer capacity. For this purpose, we integrated the amount of charge entering the cell during the Ca\(^{2+}\) current normalized to the cell volume (see Materials and Methods) to obtain the predicted total Ca\(^{2+}\) concentration.
change, if all Ca\(^{2+}\) entered through the \(I_{\text{Ca,L}}\) remained free. Simultaneously, we determined the resulting change of [Ca\(^{2+}\)\], (Figures 3B through 3D). The recorded increase of [Ca\(^{2+}\)] due to \(I_{\text{Ca,L}}\) was divided by the calculated total Ca\(^{2+}\) concentration change to determine the buffer capacity.\(^{27}\) The buffer capacity was not significantly changed during hypertrophy (Figure 3D; see Table 1). Thus, the slower decrease in [Ca\(^{2+}\)], induced by a given \(I_{\text{NaCa}}\) in PMI myocytes did not result from a change in buffer capacity.

Figure 1. Enhanced NCX function in myocytes from PMI rats. Data recorded in a SO myocyte (A) and in a PMI myocyte (B), both after SR inhibition. Traces shown from top to bottom: applied voltage protocols, the NCX current (\(I_{\text{NaCa}}\)) followed by the L-type calcium current (\(I_{\text{Ca,L}}\)) recorded in the whole-cell configuration of the patch-clamp technique. Expanded from the inset box: the [Ca\(^{2+}\)] transients, the line scan images of fluo-3 fluorescence, the corresponding \(I_{\text{NaCa}}\), and the transported charge (\(\int I_{\text{NaCa}}\,dt\)), \(I_{\text{NaCa}}\) was induced by UV-flash photolysis of caged Ca\(^{2+}\) (DM-nitrophen) at –40 mV, whereas \(I_{\text{Ca,L}}\) were activated with a voltage step (200 ms) from –40 mV to 0 mV. 

NCX Function Normalized to the Myocyte Volume

Another possibility for a discrepancy between \(I_{\text{NaCa}}\) density and Ca\(^{2+}\) transport could be due to surface/volume changes, because \(I_{\text{NaCa}}\) is measured per surface, but the changes in [Ca\(^{2+}\)], depend on cell volume. In fact, comparison of the membrane surface (\(C_m\)) with the cell volume leads to a smaller \(C_m/volume\) ratio in hypertrophied PMI myocytes (4.62 pF/pL versus 3.01 pF/pL, see Table), or in other words, the cell volume increased more than the surface in PMI myocytes. The change of the surface/volume relationship might explain the apparent discrepancy between current density and Ca\(^{2+}\) transport. To test this hypothesis, instead of normalizing to \(C_m\), the \(I_{\text{NaCa}}\) were normalized to the cellular volume. As a result, the differences in \(I_{\text{NaCa}}\) observed in myocytes from PMI and SO rats were no longer evident (Figures 4A and 4B). In addition, the corresponding charge transport (Figure 4C) was now similar under both conditions. Although the \(I_{\text{Ca,L}}\) density exhibited no significant change, normalization for the cell volume revealed a significant reduction in PMI myocytes (to \(\approx 50\%\); Figure 4E). That is, a similar \(I_{\text{Ca,L}}\) will induce a smaller increase in global [Ca\(^{2+}\)], in PMI myocytes.

Quantitative Changes in NCX Protein Expression During Myocardial Remodeling

The NCX protein expression was measured in isolated myocyte lysates from the left ventricles using a well-established binding assay\(^{24}\) (expressed as the number of counts per microgram of protein of whole-cell homogenate representing the specifically bound radiolabeled NCX antibody R3F1; SO, 2149 specific counts \(\mu g^{-1}\) total protein; PMI, 3884 specific counts \(\mu g^{-1}\) total protein; \(n=3; P<0.001\)). The amount of NCX in PMI rats was increased to 180±11% of SO rats. We then examined whether this protein increase was also reflected at the mRNA level and thus, semiquantitative RT-PCR was performed. The amounts of specific PCR products for NCX were normalized using GAPDH as internal standard for each PCR reaction. Compared with SO rats, the NCX PCR product was slightly elevated in PMI myocytes (109±13%), but the differences were not statistically significant.

Two possible explanations can be envisaged, either the RT-PCR method is not sensitive enough to detect small differences in mRNA levels or the NCX protein expression is regulated at the translation or even at the posttranscriptional level. It is likely that the first point needs to be considered, because differences in mRNA levels have to be about 50% to obtain significant differences. However, changes of the mRNA levels do not always reflect the amount of the functionally expressed protein, and up- or downregulation of protein expression does not necessarily go in parallel with changes of the global protein function. Additionally, regulatory or modulatory factors (eg, protein phosphorylation) may compensate for changes in the total amount of the expressed protein. Regarding the unchanged NCX function per cell volume and the increased NCX protein in PMI cells, it is possible that changes in protein expression may occur without changes in the NCX function.\(^{28}\)
Discussion

Our results obtained in a rat postmyocardial infarction model showed significant alterations of NCX function and expression during remodeling. In addition, our approach using a combination of biophysical methods also revealed some apparent discrepancies between the electrical charge and the Ca\(^{2+}\) transport mediated by this protein. We have used these discrepancies to derive a new conclusion regarding a possible role of NCX remodeling in the progression of and adaptation to the disease.

How Can We Explain the Discrepancy Between \(I_{\text{NaCa}}\) and \(\text{Ca}^{2+}\) Transport Mediated by the NCX?

To our initial surprise, the time course of the decline of \([\text{Ca}^{2+}]_i\), due to the NCX activity was very similar in both PMI and SO myocytes, despite the fact that \(I_{\text{NaCa}}\) density was clearly larger in PMI myocytes. What could be possible reasons for this apparent discrepancy?

Generally, an increase of \(I_{\text{NaCa}}\) could result from a larger number of active NCX molecules or a faster turnover of the ion exchange. In this regard, catalytic effects of \([\text{Ca}^{2+}]_i\), and, according to some studies, phosphorylation of the NCX may be important for the regulation of NCX turnover. However, during our UV-flash experiments phosphorylation or catalytic modulation of NCX function in PMI cells would increase the \(I_{\text{NaCa}}\) amplitude and accelerate the decline of \([\text{Ca}^{2+}]_i\), to the same extent. Thus, the \(I_{\text{NaCa}}/d[\text{Ca}^{2+}]_i dt^{-1}\) ratio would remain unchanged; however, we observed such a discrepancy. Taken together, allosteric regulation or phosphorylation of NCX cannot explain the paradox found in our experiments.

Other possibilities to explain the discrepancy between \(I_{\text{NaCa}}\) and \(\text{Ca}^{2+}\) transport include the following: (1) a change in the electrogenicity of the NCX that does not affect \(\text{Ca}^{2+}\) transport; (2) a modification of the \(\text{Ca}^{2+}\) buffer capacity of the cell; and (3) a shift in the surface-volume relationship of the cells.

The unchanged \(I_{\text{NaCa}}-V\) relationship (Figure 3A) indicates that the charge moved during the rate-limiting step of the NCX cycle, and thus the stoichiometry, was probably not affected. This makes a change in the stoichiometry an unlikely mechanism for resolving the observed paradox.

The decay time course of the \([\text{Ca}^{2+}]_i\) jump depends on the \([\text{Ca}^{2+}]_i\) transient amplitude as well as on cytosolic \(\text{Ca}^{2+}\) buffering.\(^{29}\) However, our estimates of the cytosolic \(\text{Ca}^{2+}\) buffer capacity in SO and PMI cells showed no significant differences (Figure 3C) and, therefore, also fail to explain the discrepancy between \(I_{\text{NaCa}}\) and \(\text{Ca}^{2+}\) transport observed in PMI cells.

Cardiac hypertrophy is a gradual process that also includes changes of the cell geometry. Whereas \(I_{\text{NaCa}}\) depends on the surface of the cell, the amount of \(\text{Ca}^{2+}\) transport correlates with the cell volume. Thus, \(\text{Ca}^{2+}\) transport and membrane currents can only be compared directly if the ratio of membrane surface and cellular volume remains unchanged. At the stage of hypertrophy used in our experiments, this was clearly not the case (see Table). The surface-volume ratio had decreased by 34.8%. Consistent with volume increase, we found that for a similar free \([\text{Ca}^{2+}]_i\), jump, a larger amount of total charge was moved by the NCX in PMI cells (Figure 1).

The conversion of \(I_{\text{NaCa}}\) into “volume current densities” (Figure 4B) represents a method for a direct comparison of \(I_{\text{NaCa}}\) with intracellular \([\text{Ca}^{2+}]_i\) changes. Surprisingly, performing this normalization resolved the apparent discrepancy and led to an alternative interpretation of our data. Although hypertrophied myocytes generated more \(I_{\text{NaCa}}\) per cell volume remained essentially constant (Figure 4B).

From this analysis, we conclude that the \(\text{Ca}^{2+}\) transport capacity via the NCX remains constant in PMI myocytes despite extensive remodeling of NCX expression and a marked increase in \(I_{\text{NaCa}}\) density. The observation that the cells maintain a constant \(\text{Ca}^{2+}\) transport also indicates that the upregulation of the \(I_{\text{NaCa}}\) density more likely represents a secondary change to account for the increased cell volume. Although our findings on \(I_{\text{NaCa}}\) confirm the results of several other studies,\(^{15,17,30,31}\) in combination with the \(\text{Ca}^{2+}\) measure-

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**Figure 2.** \(\text{Ca}^{2+}\) transport by the NCX. A, Surface plots of \([\text{Ca}^{2+}]_o\) transients of comparable amplitude elicited via flash photolysis in SO and PMI myocytes. B, Rate of free \([\text{Ca}^{2+}]_i\) decay (d\([\text{Ca}^{2+}]_i/dt\), \(\text{Ca}^{2+}\) transport) obtained from the experimental data shown in Figure 1 plotted as a function of \([\text{Ca}^{2+}]_i\). (white dots indicate SO; red dots, PMI). C, Rate of decay of free \([\text{Ca}^{2+}]_i\) obtained from myocytes with comparable \([\text{Ca}^{2+}]_i\) transients (n=5).
and CICR. In hypertrophied cells, an increase in the NCX activity, even if a secondary event, would have subsequent consequences. From the importance of the NCX for the SR Ca$^{2+}$ load, an increased $I_{\text{NaCa}}$ has been implied to be responsible for both an increase$^{32}$ and a decrease$^{30}$ in contractility. In the steady state, Ca$^{2+}$ removal via NCX has to balance Ca$^{2+}$ influx via L-type Ca$^{2+}$ current. When the amplitude of the Ca$^{2+}$ transient is reduced in hypertrophy and heart failure, as has been reported by others$^{4,33,34}$ the NCX would tend to remove less Ca$^{2+}$ and an upregulation would be required. The increased NCX expression and maintained Ca$^{2+}$ transport observed here is consistent with the reduced SERCA expression and with the hyperphosphorylation of RyRs$^{35}$ reported from other cardiac disease models. Both mechanisms would tend to decrease the amplitude of the Ca$^{2+}$ transient by lowering the SR Ca$^{2+}$ content. Similarly, the [Ca$^{2+}$]$_i$ transients were reported to be reduced because of an altered spark trigger probability.$^4$ However, this picture is still very incomplete and needs to be elaborated in more detail and with different experimental approaches.

**Comparison With Other Disease Model Studies**

So far, several studies have reported changes in the NCX function or expression during various cardiac diseases. However, the results regarding remodeling of NCX function and expression are sometimes not consistent. An increase in mRNA and protein levels has been reported in human$^{18,36,37}$ and experimental rabbit heart failure,$^{31}$ although changes in NCX activity were controversial.$^{36}$ However, most investigators found an increased $I_{\text{NaCa}}$ both in hypertrophy and heart failure$^{16,17,32}$; eg, Litwin and Bridge$^{15}$ found 32% increased $I_{\text{NaCa}}$ in the infarcted rabbit model and Pogwizd et al$^{31}$ described a parallel 2-fold increase in NCX mRNA. NCX expression, rate of [Ca$^{2+}$]$_i$ decline, and $I_{\text{NaCa}}$ in an arrhythmogenic rabbit model of heart failure. Recently, it was proposed that NCX upregulation may be the critical link between contractile dysfunction and arrhythmogenesis,$^{32,34}$ Interestingly, Hobai and O’Rourke$^{17}$ also identified a 2-fold increase in the NCX activity from the decline of caffeine-induced [Ca$^{2+}$]$_i$ transients from dog hearts. They found that the Ca$^{2+}$ transport and the outward $I_{\text{NaCa}}$ were increased, but not the inward $I_{\text{NaCa}}$. This was taken as evidence to suggest that allosteric modulation of NCX by [Ca$^{2+}$]$_i$ may be enhanced in failing myocytes and responsible for this asymmetry.$^{17}$

The reports about the L-type Ca$^{2+}$ current density in hypertrophied myocytes are also controversial as far as increases, decreases, and no changes have been observed (for review see Tomasiell and Marbán$^{39}$ and Bénitah et al$^{40}$). Regarding $I_{\text{Ca,L}}$ in our study, the maintained current density would induce a smaller “global” [Ca$^{2+}$]$_i$ increase, as shown in the volume normalization (Figure 4E). However, L-type Ca$^{2+}$ current is modulated in intact animals, thus the findings obtained in isolated cells need to be interpreted very carefully. What could be the consequences for CICR? The amount of Ca$^{2+}$ released during the CICR is determined by both, the $I_{\text{Ca,L}}$ and the SR Ca$^{2+}$ load. The $I_{\text{Ca,L}}$ serves two main functions: (1) it provides the trigger Ca$^{2+}$ for CICR and (2) it reloads the SR with Ca$^{2+}$. The relevant volume for the

![Image](http://circres.ahajournals.org/)

**Figure 3.** Buffer capacities of PMI and SO cells are comparable. A, Voltage dependence of $I_{\text{NaCa}}$, normalized to the peak $I_{\text{NaCa}}$ at $-40$ mV in SO myocytes (red) and in PMI myocytes (black). Experimental data can be fitted with a monoexponential function ($n$=3). B, Data observed in a SO myocyte. C, Data recorded in a PMI myocyte. Traces shown from top to bottom: applied voltage protocols, [Ca$^{2+}$]$_i$ transients, line-scan images of fluo-3 fluorescence, and $I_{\text{NaCa}}$. D, Calculated buffer capacity in 4 SO myocytes (white bar) and 6 PMI cells (black bar) both with inhibited SR function.

**Consequences for EC-Coupling and Ca$^{2+}$-Induced Ca$^{2+}$ Release (CICR)**

In our experiments, we examined the NCX function in complete isolation by experimentally eliminating all other Ca$^{2+}$ transport systems. Therefore, we can only speculate about possible consequences of our findings for EC-coupling

ments, we reach a slightly different conclusion. We note that the Ca$^{2+}$ transport rate via the NCX is maintained at a constant level by the cardiomyocytes at this stage of hypertrophy; therefore, they need to increase the functional NCX protein density in the membrane.
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The trigger function is determined by the volume of the dyadic cleft. However, this volume might not rise in parallel with the cell volume but rather with the surface of the T-tubules. In the hypertrophied myocytes examined in this study, I$_{\text{Ca,L}}$ densities were not affected, in other words, there should be no consequences for triggering CICR. Nevertheless, it has been reported that the "gain" of the CICR triggered by I$_{\text{Ca,L}}$ was depressed in failing myocytes, also in the same animal model studied here and at the same stage of disease. Accordingly the shift of the "gain" was attributed to structural/geometrical changes in the dyadic cleft and this may also depend on the stage of the disease.

Taken together, some of the reported discrepancies in NCX function and expression could be explained by different species, disease models, the state of the disease, the measured functional parameter of the NCX, or the methodology used. This seriously limits direct comparisons of different studies. The present work also suggests that the procedure for normalization used in each study could be very important.

**Outlook and Relevance for Human Disease**

A common problem in all studies of cardiac Ca$^{2+}$/H$^{+}$ signaling during diseases is to distinguish between causality and adaptation for a given alteration. For the NCX, this problem is even more difficult because of the two modes of the exchange process (ie, the Ca$^{2+}$ efflux and Ca$^{2+}$/H$^{+}$ influx mode). A strategy for future studies could be to monitor the development of the hypertrophy from the initial to the final state (eg, collect geometrical parameters, membrane currents, Ca$^{2+}$ signaling, and protein and/or mRNA data). The second open question is whether findings of one particular study can be generalized to other disease models. Changes in the expression of proteins involved in the excitation-contraction coupling are expected to occur continuously during the progress and development of the disease. However, consequences and alterations of NCX function are not necessarily identical in various disease models, and species-dependent differences most likely exist. Moreover, its is very difficult to extrapolate from single cell data to the situation on the tissue and organ level. Therefore, additional studies will be required to determine whether a maintained Ca$^{2+}$/H$^{+}$ transport via the NCX is a general phenomenon in cardiomyocytes during hypertrophy.

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**References**


Increased Exchange Current but Normal Ca$^{2+}$ Transport via Na$^{+}$-Ca$^{2+}$ Exchange During Cardiac Hypertrophy After Myocardial Infarction

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