Altered Na/Ca exchange distribution in ventricular myocytes from failing hearts

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Na/Ca exchange (NCX) is normally located predominantly in the T tubules of cardiac ventricular myocytes. However, redistribution of NCX occurs in myocytes from failing hearts, resulting in more uniform distribution between T tubule and surface sarcolemma; this alters access of NCX to Ca released from sarcoplasmic reticulum and thus cellular Ca handling.

CONTRACTION of CARDIAC VENTRICULAR myocytes is initiated by Ca influx across the cell membrane via the L-type Ca current (I_CaL), which activates ryanodine receptors (RyRs) in adjacent sarcoplasmic reticulum (SR) membrane, triggering Ca-induced Ca release (CICR) from the SR. Relaxation occurs as a result of Ca removal from the cytoplasm into the SR, via a Ca ATPase (SERCA), and across the cell membrane, predominantly via Na/Ca exchange (NCX) (2).

In mammalian ventricular myocytes, I_CaL, CICR, and Ca efflux via NCX occur predominantly at invaginations of the surface membrane, called T tubules (5). Presumably because of this colocation, Ca close to the site of CICR appears to be more effective than bulk cytoplasmic Ca at stimulating Ca efflux via NCX, and thus—since NCX carries three Na for each Ca—the associated inward (depolarizing) NCX current (I_{NCX}) (35). This is important not only for normal Ca efflux but also because under conditions of Ca overload, spontaneous SR Ca release occurs, activating inward I_{NCX} and causing delayed afterdepolarisations (DADs), which can trigger action potentials and arrhythmias (18). Because of the proximity of RyRs and NCX, it seems likely that such activity will arise predominantly at T tubules.

Heart failure (HF) is associated with disruption of the t-tubular network (21, 22) and redistribution of I_CaL from the T tubules to the surface membrane, although RyR distribution appears unaltered (7). However, the effect of HF on the distribution of I_{NCX} is unknown but is important because changes may alter the proximity of NCX to the site of CICR, and thus I_{NCX} and Ca efflux. In the present study we investigated the distribution of I_{NCX} between the t-tubular and surface membranes, and its functional consequences, in myocytes from normal and HF rats.

MATERIALS AND METHODS

Surgical model of HF. Ligation of the left anterior descending coronary artery of adult male Wistar rats (CAL) was performed as previously described (7). The corresponding sham operation (Sham) was identical except that no tie was placed around the coronary artery. All procedures were performed in accordance with UK legislation and approved by the University of Bristol Ethics Committee. As reported in another study using cells from these animals, CAL had no significant effect on body weight or tibia length, but significantly increased heart and lung weights relative to body weight and tibia length, decreased ejection fraction, and increased left ventricular diastolic and systolic volumes, indicative of early stage HF (7).

Myocyte isolation and detubulation. Myocytes were isolated from the left ventricular free wall and septum of Sham and CAL animals 18.6 ± 0.3 and 18.5 ± 0.3 wk after surgery, respectively, as previously described (7) and stored for 2–8 h before use on the day of isolation. Myocyte detubulation (DT), physical and functional uncoupling of the T tubules from the surface membrane, was achieved using formamide-induced osmotic shock, as previously described (4, 6, 7).

Solutions. All reagents were obtained from Sigma-Aldrich (Poole, UK) unless otherwise specified. Cells were superfused with solution containing (in mmol/l) 133 NaCl, 1 MgSO_4, 1 CaCl_2, 1 Na_2HPO_4, 10 D-glucose, and 10 HEPES (pH 7.4, NaOH); 5 CsCl was added to inhibit K currents. The pipette solution contained (in mmol/l) 110 CsCl, 20 tetrabutylammonium chloride, 0.5 MgCl_2, 5 MgATP, 10 HEPES, 0.4 GTP-Tris (pH 7.2, CsOH), and 0.1 pentapotassium salt of the fluorescent Ca indicator fluo-4 (Life Technologies, Paisley, UK).

Measurement of I_CaL and I_{NCX}. Myocytes were placed in a chamber mounted on a Diaphot inverted microscope (Nikon UK, Kingston-upon-Thames, UK). Membrane currents and cell capacitance were recorded with the whole cell patch-clamp technique using an Axopatch 200B patch clamp amplifier, a Digidata 1440A analog-to-digital

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from Sample sizes (pipette solution. the appropriate (Sham or CAL) buffering power (calculated by plot-
ments). Student’s 
P
hoc test were used as appropriate. Statistical significance was taken as

\[
C_{\text{Ca}} = \frac{K_d F/F_0}{[C_{\text{Ca}}]_{\text{rest}} - F/F_0 + 1}
\]

where resting Ca ([C_{\text{Ca}}]_{\text{rest}}) was assumed to be 0.1 \(\mu\)mol/l and the dissociation constant \(K_d\) for fluo-4 in situ was 1.1 \(\mu\)mol/l (8, 20).

The rate of decay of Ca transients was obtained by fitting single exponential functions to the declining phase of the \(I_{C_{\text{Ca}}}\) and caffeine-induced Ca transients. The fitted rate constants \(k\) were multiplied by the appropriate (Sham or CAL) buffering power (calculated by plotting \([C_{\text{Ca}}]\) against \([C_{\text{Ca,at}}]\) as previously described) (12, 33) to correct for the effect of buffering power and thus allow comparison of the rate of Ca extrusion in Sham and CAL myocytes. This corrected rate constant \(k\) during application of caffeine \((k_{\text{caf}})\) was used as an index of the rate of total sarcolemmal Ca efflux, and that in the presence of caffeine plus Ni \((k_{\text{Ca,Ni}})\) as the slow (non-SR, non-CX) Ca extrusion pathways. The rate of Ca removal via NCX \((k_{\text{NCX}})\) was calculated as \(k_{\text{Ca,Ni}}/k_{\text{Ca,F}}\), and the rate of Ca uptake by SR \((k_{\text{SR}})\) was calculated as the difference between the rate of decline of the \(I_{C_{\text{Ca}}}\) induced Ca transient \(k_{\text{Ca,F}}\). These rate constants were also used to calculate the percent contribution of these pathways to Ca removal from the cell cytoplasm, as previously described (25, 36).

**Statistical analysis.** Data are expressed as means \(\pm\) SE. The errors of derived variables and the subsequent statistical analysis were calculated using propagation of errors from the constituent measurements. Student’s \(t\)-tests and 2-way ANOVA with the Bonferroni post hoc test were used as appropriate. Statistical significance was taken as \(P < 0.05\). All statistical tests were performed on the number of cells. Sample sizes \((n)\) are given as \(c/h\), where \(c\) is the number of cells used from \(h\) hearts.

**RESULTS**

The effect of CAL on NCX distribution. Cell capacitance (a function of membrane area) was significantly larger in CAL myocytes \(240.2 \pm 20.8\) pF (Sham) vs. 375.0 \(\pm\) 63.0 pF (CAL); \(n = 12/6\) and 8/4, respectively; \(P = 0.004\); this was accompanied by a nonsignificant increase in cell volume \(33.5 \pm 5.1\) pl (Sham) vs. 49.0 \(\pm\) 6.1 pl (CAL), resulting in no significant difference in cell surface area-to-volume ratio, as previously reported during cellular hypertrophy (15, 29).

Figure 1A shows representative caffeine-induced Ca transients (top) and accompanying membrane currents (bottom) recorded from Sham and CAL myocytes. Caffeine-induced Ca transient amplitude was significantly smaller in CAL compared with Sham myocytes \(2.42 \pm 0.63\) \(\mu\)M (Sham) vs. 0.78 \(\pm\) 0.44 \(\mu\)M (CAL); \(P < 0.05\) and buffering power, assessed as described in the MATERIALS AND METHODS, was significantly larger in CAL myocytes \(134 \pm 66\) (Sham) vs. 294 \(\pm\) 46 (CAL); \(P < 0.01\). Representative caffeine-induced Ca transients and \(I_{\text{NCX}}\) in DT Sham and CAL cells are shown in Fig. 1B: DT did not significantly alter peak \([C_{\text{Ca}}]\), in Sham cells \(2.06 \pm 0.69\) \(\mu\)M but increased peak \([C_{\text{Ca}}]\) in CAL cells to \(2.30 \pm 1.09\) \(\mu\)M.

In Sham myocytes, the decline of the caffeine-induced Ca transient, corrected for buffering power (see MATERIALS AND METHODS), was significantly slowed by DT \(k_{\text{Ca,F}}\) (106.8 \(\pm\) 9.5 (Sham) vs. 46.3 \(\pm\) 5.0 s\(^{-1}\) (Sham DT); \(P < 0.001\); Fig. 1C), consistent with loss of t-tubular Ca efflux pathways. In CAL myocytes, the decline of the caffeine-induced Ca transient was not significantly different from Sham myocytes and not significantly altered by DT \(k_{\text{Ca,F}}\) (120.4 \(\pm\) 15.2 (CAL) vs. 141.8 \(\pm\) 7.7 s\(^{-1}\) (CAL DT); Fig. 1C), consistent with reduced t-tubular sarcolemmal Ca efflux in CAL myocytes.

To investigate the role of NCX in these changes, exposure to caffeine was repeated in the presence of Ni to inhibit NCX. Figure 1D shows the rate of Ca extrusion via NCX \((k_{\text{NCX}})\); DT significantly decreased \(k_{\text{NCX}}\) in Sham cells \(86.8 \pm 7.8\) (Sham) vs. 34.3 \(\pm\) 4.8 s\(^{-1}\) (Sham DT); \(P < 0.0001\), compatible with loss of t-tubular NCX. In CAL myocytes, \(k_{\text{NCX}}\) was not significantly different from Sham myocytes and not significantly altered by DT \(102.5 \pm 14.5\) (CAL) vs. 102.2 \(\pm\) 8.0 s\(^{-1}\) (CAL DT), implying that although the rate of Ca extrusion via NCX is similar in Sham and CAL myocytes, there is little Ca extrusion via t-tubular NCX in these cells.

We used \(I_{\text{NCX}}\) in intact and DT myocytes to quantify its distribution between the surface and t-tubular membranes. Since NCX activity depends on Ca adjacent to the exchanger, we measured \(I_{\text{NCX}}\) at a \([C_{\text{Ca}}]\) of 400 \(\mu\)mol/l during the descending phase of the caffeine transient, when Ca has been shown to be uniformly distributed throughout the cytoplasm in both intact and DT cells (3). Figure 1E shows that whole cell \(I_{\text{NCX}}\) density determined in this way was not significantly different in Sham and CAL myocytes and that in Sham myocytes, \(I_{\text{NCX}}\) density is significantly greater in the t-tubular membrane than at the cell surface, as previously reported (14), resulting in a T tubule-to-surface sarcolemma \(I_{\text{NCX}}\) ratio of 25:1. However, the distribution of \(I_{\text{NCX}}\) is different in CAL myocytes, decreasing at the T tubules by \(\approx 50\%\) and increasing at the surface membrane by \(\approx 300\%\), resulting in no significant difference in \(I_{\text{NCX}}\) density between the two membranes in these cells and an \(I_{\text{NCX}}\) T tubule-to-surface sarcolemma ratio of 3:1. This suggests that the slower Ca extrusion via NCX following DT of Sham cells is due to loss of t-tubular NCX and that the lack of effect of DT on the rate of Ca extrusion via NCX in CAL cells is due to its relocation away from T tubules.

The effect of CAL on cellular Ca handling. The preceding data show redistribution of \(I_{\text{NCX}}\) in CAL myocytes. NCX is one of the major Ca efflux pathways that compete for cytoplasmic Ca (1, 25), so that a decrease of NCX activity at the T tubules
(the site of CICR and SERCA) (24, 32) might alter the balance of Ca removal via NCX and SR (30), thereby altering SR Ca content and thus Ca release and the associated inward current (K\textsubscript{NCX}) during the application of 10 nM caffeine (white bar) in detubulated (DT) Sham and CAL cells. Scale bars represent 2 s.

**Fig. 1.** A: representative records of intracellular free Ca concentration ([Ca\textsubscript{i}]) and the associated inward Na/Ca exchange (NCX) current (I\textsubscript{NCX}) during the application of 10 nM caffeine (white bar) in sham operation (Sham) and coronary artery ligation (CAL) cells. Scale bars represent 2 s. B: representative records of [Ca\textsubscript{i}] and the associated inward current (K\textsubscript{NCX}) during the application of 10 nM caffeine (white bar) in detubulated (DT) Sham and CAL cells. Scale bars represent 2 s. C: mean rate constants for the decline of the caffeine-induced Ca transient (k\textsubscript{caff}); n = 12/6, 11/5, 8/4, 7/4 for Sham, Sham DT, CAL, and CAL DT, respectively. D: mean K\textsubscript{NCX}; n = 12/6, 11/5, 8/4, 5/3 for Sham, Sham DT, CAL, and CAL DT, respectively. E: I\textsubscript{NCX} density in the whole cell and at the surface and t-tubular membranes, determined at 400 nM [Ca\textsubscript{i}], during the declining phase of the caffeine transient. ***P < 0.001 with Bonferroni posttest.
between bulk cytoplasmic Ca and colocation of NCX and RyRs. NCX activity by altering release. However, efflux. The consequent increase in SR Ca content will alter suggests that SR Ca load might be increased following loss of the SR, thereby increasing fractional SR Ca uptake; they also CAL myocytes decreases access of NCX to Ca released from the cytoplasm of Sham, DT Sham, CAL, and DT CAL cells; n = 12/6, 11/5, 8/4, 5/3, respectively. B: distribution of Ca current (I\textsubscript{NCX}) density between the surface and t-tubular membranes; n = 12/6 and 8/4 for Sham and CAL, respectively; only statistical comparisons between t-tubular and surface membranes are shown. *P < 0.05, **P < 0.01, and ***P < 0.001 with Bonferroni posttest.

The effect of CAL on the relationship between [Ca\textsubscript{i}] and I\textsubscript{NCX}. The data above suggest that decreased t-tubular NCX in CAL myocytes decreases access of NCX to Ca released from the SR, thereby increasing fractional SR Ca uptake; they also suggest that SR Ca load might be increased following loss of T tubules, as a result of the relative changes in Ca influx and efflux. The consequent increase in SR Ca content will alter NCX activity by altering release. However, I\textsubscript{NCX} activity may also be altered directly as a result of the change in the colocalization of NCX and RyRs.

The mechanism of redistribution is unclear, although reminiscent of the redistribution of \( \beta_2 \)-adrenoceptors and I\textsubscript{Ca} from their normal t-tubular location to a more uniform distribution in cells from failing hearts (6, 7, 27). It has been suggested that localization of NCX activity at the T tubules is due, in part, to local protein kinase A activity (10); however, protein kinase A activity appears to increase at the T tubules in CAL myocytes (7), making it unlikely that this can explain the decrease in t-tubular I\textsubscript{NCX} observed in these cells. These changes may reflect reversion in HF toward a more neonatal phenotype, in which cell activation is dependent on Ca influx and efflux across the surface, rather than t-tubular, membrane (11, 31) and a general loss of t-tubular protein localization.

Functional consequences of redistribution. Computer modeling suggests that the relative location of NCX, SERCA, and sarcoplasmic Ca ATPase alters their ability to compete for cytoplasmic Ca and thus the amount of Ca removed by each pathway (30). The present work shows that DT of Sham myocytes decreases Ca efflux via NCX, as a result of loss of t-tubular NCX, and increases SR Ca uptake. A similar decrease...
in Ca efflux via NCX and increased Ca uptake via SERCA occurred in CAL myocytes, compared with Sham cells, even though total NCX density at a given [Ca]i is the same in CAL and Sham myocytes. This can be explained by redistribution of NCX away from the T tubules in CAL cells, so that it no longer has “privileged” access to Ca released from the SR as a result of its proximity to RyRs; this will reduce Ca extrusion via NCX and allow a greater fraction of the cytoplasmic Ca to be removed by SERCA, much of which also appears to be located at T tubules (24) where Ca release occurs. Although a large fractional decrease in Ca extrusion via NCX results in a relatively small fractional increase in SR Ca uptake, this reflects the relatively small fraction of Ca removed by NCX compared with the SR. Altered NCX location in CAL myocytes, which was measured when cytoplasmic Ca was relatively uniformly distributed, is likely to be important during the systolic Ca transient, since NCX activity close to the site of CICR at the T tubules, where the majority of Ca efflux normally occurs (Fig. 1D), will be reduced, although this may be offset by reduced Ca release at the T tubules due to decreased t-tubular Ica.

It is notable that although fractional SR Ca uptake was greater in CAL than in Sham myocytes (Fig. 2A), SR Ca content was not significantly different and the caffeine-induced rise of cytoplasmic Ca was smaller (Fig. 1A). However, calculated peak [Ca]i depends on [Ca]rest, which was taken as 0.1 μmol/l in the present study; increasing [Ca]rest would increase peak [Ca]i, but previous reports of [Ca]rest in CAL have been inconsistent, showing an increase, decrease, or no change (9, 17, 19, 37). Nevertheless, the present observations may be reconciled by increased fractional SR Ca uptake being offset by decreased local Ica, which would tend to decrease SR Ca content and the increased Ca buffering observed in CAL myocytes, which would decrease free Ca for a given release. This may also account for the greater slope of the relationship between Ca and I_{NCX} in CAL myocytes (Fig. 3), since a given Ca extrusion would result in a smaller change in free Ca. However, DT sufficiently increased SR Ca content to cause a larger caffeine-induced rise of cytoplasmic Ca with hysteresis evident in I_{NCX} between the rising and falling phases of Ca release. An alternative explanation for the increased SR Ca uptake is increased SERCA activity in CAL myocytes, although this seems unlikely since previous work has shown decreased SERCA activity in HF (16) and this alone would not explain the lack of effect of DT on the contribution of different efflux pathways to Ca removal.

It is also notable that despite the decreased percent contribution of NCX to Ca removal in CAL myocytes, k_{NCX} was not significantly different from that in Sham cells. Thus it appears that NCX can rapidly remove Ca from the cytoplasm in the absence of a functional SR (k_{NCX}), but its fractional contribution is decreased, presumably because its ability to compete with SERCA is decreased as a result of its relocation. This redistribution of I_{NCX} is also likely to be important because HF is associated with disorganization and loss of T tubules (13, 21–23) and redistribution of Ica (and thus Ca release) from the T tubules to the surface membrane. The present work shows that t-tubular I_{NCX}/Ica density is higher in CAL myocytes than in Sham, suggesting that loss of T tubules will lead to greater loss of Ca efflux, compared with influx, in CAL myocytes, and thus greater Ca accumulation consistent with the observed effect of DT on SR Ca content in these cells. Thus loss of T tubules in HF may result in increased SR Ca content, which will increase both systolic Ca release and the probability of spontaneous SR Ca release and thus of DADs.

The proximity of the majority of NCX adjacent to RyRs at the T tubules may also be important in the genesis of arrhythmias due to activation of NCX by spontaneous SR Ca release in conditions of Ca

**Fig. 3.** Averaged hysteresis loops for I_{NCX} density vs. [Ca], during application of caffeine in Sham, DT Sham, CAL, and DT CAL myocytes; n = 11/5, 9/5, 7/4, 7/4, respectively. Inset: loop on expanded scales: x and y-scale bars represent 0.1 μM and 0.2 pA/pF, respectively.

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This image contains graphs illustrating the hysteresis loops for NCX density vs. free calcium ([Ca]free) in different conditions (Sham, DT Sham, CAL, DT CAL) during application of caffeine. The loops are shown on expanded scales, with x and y-scale bars representing 0.1 μM and 0.2 pA/pF, respectively. The loops demonstrate the relationship between NCX activity and cytoplasmic calcium levels under different conditions.
overload (28). The hysteresis between bulk cytoplasmic Ca and \( I_{\text{NCX}} \) observed during application of caffeine or during spontaneous Ca release (35; Fig. 3) is consistent with Ca released from SR having privileged access to NCX. The observation that DT of Sham myocytes decreased this hysteresis suggests that it arises at the T tubules as a result of the proximity of the majority of NCX to the site of Ca release in the T tubules. However, this hysteresis was evident in CAL and DT CAL myocytes, so that it appears to be occurring at the surface of these cells. The hysteresis in CAL cells cannot be explained by a change in \([\text{Ca}]_{\text{in}}\) altering the calibration of \([\text{Ca}]_{\text{i}}\), which would alter the \(x\)-axis gain of the hysteresis loops, but the hysteresis would remain. It seems likely, therefore, that the hysteresis in CAL myocytes is due to the redistribution of \( I_{\text{NCX}} \) to the surface membrane, resulting in enhanced \( I_{\text{NCX}} \) in response to Ca at the cell surface, which itself may be increased by the observed redistribution of \( I_{\text{Ca}} \), even in the apparent absence of changes in RyR distribution (7). Thus it appears that privileged access occurs at the cell surface in CAL myocytes, so that loss of T tubules in HF may not protect against DADs, which may be generated at the surface of these cells. The hysteresis in CAL cells cannot be explained by a change in \([\text{Ca}]_{\text{in}}\) altering the calibration of \([\text{Ca}]_{\text{i}}\), which would alter the \(x\)-axis gain of the hysteresis loops, but the hysteresis would remain. It seems likely, therefore, that the hysteresis in CAL myocytes is due to the redistribution of \( I_{\text{NCX}} \) to the surface membrane, resulting in enhanced \( I_{\text{NCX}} \) in response to Ca at the cell surface, which itself may be increased by the observed redistribution of \( I_{\text{Ca}} \), even in the apparent absence of changes in RyR distribution (7). Thus it appears that privileged access occurs at the cell surface in CAL myocytes, so that loss of T tubules in HF may not protect against DADs, which may be generated at the surface of these cells.

**Conclusions.** These data suggest that the cellular distribution of NCX is altered in CAL myocytes and that this will alter NCX activity both directly, by altering the proximity of NCX to the site of SR Ca release, and indirectly, by increasing SR Ca uptake, both in intact myocytes, by decreasing the ability of NCX to compete with SERCA, and following loss of T tubules, which will result in greater loss of NCX than \( I_{\text{Ca}} \). These changes will alter \( I_{\text{NCX}} \) and thus action potential configuration, Ca balance, and the probability, magnitude and site of DAD generation in HF.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**


**REFERENCES**


