Calcium entry via Na/Ca exchange during the action potential directly contributes to contraction of failing human ventricular myocytes

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Abstract

Prolongation of the Ca2+ transient and action potential (AP) durations are two characteristic changes in myocyte physiology in the failing human heart. The hypothesis of this study is that Ca2+ influx via reverse mode Na+/Ca2+ exchanger (NCX) or via L-type Ca2+ channels directly activates contraction in failing human myocytes while in normal myocytes this Ca2+ is transported into the sarcoplasmic reticulum (SR) to regulate SR Ca2+ stores. Methods: Myocytes were isolated from failing human (n=6), nonfailing human (n=3) and normal feline hearts (n=9) and whole cell current and voltage clamp techniques were used to evoke and increase the duration of APs (0.5 Hz, 37 °C). Cyclopiazonic acid (CPA 10−6 M), nifedipine (NIF;10−6 M) and KB-R 7943 (KB-R; 3×10−6 M) were used to reduce SR Ca2+ uptake, Ca2+ influx via the L-type Ca2+ current and reverse mode NCX, respectively. [Na+]i was changed by dialyzing myocytes with 0, 10 and 20 mM Na+ pipette solutions. Results: Prolongation of the AP duration caused an immediate prolongation of contraction and Ca2+ transient durations in failing myocytes. The first beat after the prolonged AP was potentiated by 21±6 and 27±6% in nonfailing human and normal feline myocytes, respectively (P<0.05), but there was no significant effect in failing human myocytes (±5±4% vs. steady state). CPA blunted the potentiation of the first beat after AP prolongation in normal feline and nonfailing human myocytes, mimicking the failing phenotype. NIF reduced steady state contractility and abolished the potentiation of the first beat after AP prolongation (2±6±1% vs. steady state). Increasing [Na+]i shortened AP, Ca2+ transient and contraction durations and increased steady state and post AP prolongation contractions. Dialysis with 0 Na+ eliminated these effects. Conclusions: Ca2+ enters both normal and failing cardiac myocytes during the late portion of the AP plateau via reverse mode NCX. In (normal) myocytes with good SR function, this Ca2+ influx helps maintain and regulate SR Ca2+ load. In (failing) human myocytes with poor SR function this Ca2+ influx directly contributes to contraction. These studies suggest that the Ca2+ transient of the failing human ventricular myocytes has a higher than normal reliance on Ca2+ influx via the reverse mode of the NCX during the terminal phases of the AP.

Keywords: Ca-channel; Calcium (cellular); Heart failure; Membrane potential; Myocytes; Na/Ca-exchanger; SR (function)

1. Introduction

Depressed contractility and prolongation of systole are characteristic features of the failing human heart [1–3]. Changes in myocyte Ca2+ regulation are thought to be centrally involved in these heart failure related contractile abnormalities [4]. Depressed Ca2+ transport by the sarcoplasmic reticulum (SR) Ca2+ ATPase (SERCA) and increased activity of the sarcolemmal Na+/Ca2+ exchanger (NCX) are thought to be responsible for this abnormal Ca2+ regulation (for review see Refs. [5,6]). Recent studies suggest that [Na+]i, is elevated in the failing heart and this is also likely to contribute to altered Ca2+ regulation via effects on the thermodynamics of NCX.

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[7,8]. The purpose of present study was to define the contributions of NCX mediated Ca\(^{2+}\) fluxes during the action potential (AP) to the alterations in contractility of the failing human ventricular myocyte.

During each action potential (AP), Ca\(^{2+}\) enters the cell via voltage-gated L-type Ca\(^{2+}\) channels and induces SR Ca\(^{2+}\) release by activating the SR Ca\(^{2+}\) release channels (ryanodine receptors) [9,10]. The resulting Ca\(^{2+}\) transient is the sum of Ca\(^{2+}\) influx and SR Ca\(^{2+}\) release [11]. In the steady state, the decay of the Ca\(^{2+}\) transient occurs as Ca\(^{2+}\) is transported back into the SR by SERCA and the amount of Ca\(^{2+}\) that enters during the AP is transported from the cell via forward mode NCX [12,13]. The relative contribution of Ca\(^{2+}\) influx and SR Ca\(^{2+}\) release to the Ca\(^{2+}\) transient varies in different species [14–16] and these differences appear to be dependent on AP duration and the activity of SERCA and NCX. In most normal cardiac myocytes there is thought to be very little Ca\(^{2+}\) influx via reverse mode NCX and this is thought to occur during the upstroke of the AP [14].

Reduced SERCA protein abundance and function are thought to be major causes of altered Ca\(^{2+}\) cycling in the failing human heart [17–20]. Depressed Ca\(^{2+}\) transport by the SR is thought to reduce SR Ca\(^{2+}\) stores, reduce SR Ca\(^{2+}\) release, and slow the decay of the Ca\(^{2+}\) transient in heart failure [1,21–23]. The abundance and activity of the NCX appears to be increased in the failing human heart. Since the NCX is the principal Ca\(^{2+}\) efflux mechanism in cardiac myocytes [13] it has been suggested that it makes a larger than normal contribution to the decay of the Ca\(^{2+}\) transient in the failing heart [20,24–28]. However, recent studies suggest that in failing human myocytes Ca\(^{2+}\) efflux via the NCX only occurs upon AP repolarization and that during the AP plateau there may be Ca\(^{2+}\) entry via the NCX [23,29,30].

Reverse mode (Ca\(^{2+}\) entry) NCX is promoted by depolarization, low intracellular [Ca\(^{2+}\)]\(_i\) and high [Na\(^{+}\)]\(_i\) [8,31], all of which are features of the failing human ventricular myocyte. The hypothesis of this study is that Ca\(^{2+}\) influx via the NCX during late portions of the AP plateaux in failing human myocytes with poor SR function slows the decay of the Ca\(^{2+}\) transient and prolongs contractile duration. Our results suggest that Ca\(^{2+}\) can enter both normal and failing human myocytes during the late portions of the AP plateau via the NCX. In normal myocytes this Ca\(^{2+}\) entry maintains SR Ca\(^{2+}\) stores while in failing myocytes with depressed SR function the predominant effect is to prolong the duration of contraction via direct elevation of cytosolic Ca\(^{2+}\).

2. Methods

2.1. Human myocardium and myocyte isolation

Failing human myocardium (n=6) was obtained at the time of transplantation. Nonfailing human hearts (n=3 hearts) were obtained in cases when the hearts could not be used for transplantation for technical reasons. Myocytes were isolated as reported previously [30]. After isolation, myocytes were kept in Krebs–Henseleit buffer containing 200 \(\mu\)M Ca\(^{2+}\) and 2% bovine albumin.

2.2. Feline cell isolation

Myocytes were isolated from normal feline hearts (n=9) as described in detail previously [21,22]. The care of the animals used in this study as well as the anesthesia were carried out in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.3. Physiological experiments

Cells were placed in a heated chamber mounted on an inverted microscope (Zeiss, Germany) and superfused with a modified Tyrode’s solution (37°C, pH 7.4, containing in mM/l: Ca\(^{2+}\) 1.0, glucose 10, Hepes 5, KCl 5.4, MgCl\(_2\) 6H\(_2\)O 1.2, NaCl 152, Na-pyruvate 2). For subsets of experiments, cells were loaded with fluo-3 AM (15 \(\mu\)g/ml for 10 min at room temperature). Borosilicate glass pipettes (3–5 m\(\Omega\)) used for whole cell patch clamp experiments were filled with a solution containing (in mM): l-aspartate-K 120, KCl 20, MgCl\(_2\) 1, Hepes 10, GTP 0.1, NaCl 10, pH 7.2 with KOH. APs were evoked in current clamp mode at 0.5 Hz using an Axoclamp 2B (Axon Industries) amplifier and voltage signals were recorded as described in detail previously [16,25]. To vary the AP duration, small depolarizing currents (<5 nA) were injected through the patch electrode for one beat. This caused the resting potential to depolarize from \(-71 \pm 1\) to 69±1 mV in failing and from 71±1 to 69±1 mV in nonfailing myocytes. Contraction and Ca\(^{2+}\) transients (fluor-3 AM) were measured as described in detail previously [21,32].

The duration of depolarization was also changed using voltage clamp (VC) techniques that have been described previously [29,32]. Membrane potential was held at -70 mV so that Na\(^{+}\) currents would be present.

2.4. Pharmacological interventions

SR function was reduced with Cyclopiazonic acid (CPA 10^-6 M), Nifedipine (NIF 10^-6 M) was used to reduce L-type Ca\(^{2+}\) currents and KB-R 7943 (KB-R: 3×10^-6 M, Kanebo, Japan) was used to reduce reverse mode NCX activity. [Na\(^{+}\)], was varied using 0 or 20 mM Na\(^{+}\) pipette solution (2–3 m\(\Omega\) pipettes).
2.5. Statistics

To determine the significance of the effects of prolongation of AP duration on test step and post test step beats, the dependent variables, APD50%, contraction magnitude and duration (at 50% recovery), fluorescence transient duration (at 50% recovery), and fractional shortening %, were treated as continuous measurements. Means and standard error of the means are presented for the variables for each group at each test period (Steady state, test step, 1st post test step). The experimental design was a two-factor (group and test period) design with repeated measures on test period. The null hypothesis was that there was no difference in the dependent variables among the different groups and periods. The data for the dependent variables were significantly non-normal. In order to apply ANOVA methods, a ‘normalized-rank’ transformation was applied to the data. The rank-transformed data were analyzed using a mixed-model ANOVA for repeated measures followed by multiple comparisons to detect significant individual mean differences between groups and periods. Multiple pair-wise comparisons used the Bonferroni adjustment to maintain an experiment-wise type I error of 0.05 or less. Differences between group means (rejection of the null hypothesis) were considered significant if the probability of chance occurrence was ≤0.05 using two-tailed tests.

3. Results

Steady state shortening magnitude at 0.5 Hz was significantly smaller in failing versus nonfailing human myocytes (3.6±0.7 vs. 4.6±1.1 μm). Fractional shortening was even more depressed because the diastolic cell length of failing myocytes was greater than nonfailing myocytes (148±10 vs. 134±13 μm). In the steady state, the duration of contractions and Ca²⁺ transients (time to 50% recovery; T50%) were significantly prolonged in failing versus nonfailing cells (531±103 vs. 363±37 ms and 615±88 vs. 402±38 ms), respectively. Likewise, the steady state APD 50% was significantly longer in failing versus nonfailing human myocytes (654±36 vs. 512±27 ms, Table 1). The effects of AP prolongation on contraction were similar in nonfailing human and normal feline ventricular myocytes (Table 1).

Lengthening the duration of a single AP with current injection caused a greater prolongation of the corresponding contraction (Fig. 1A) and Ca²⁺ transient (Table 1) in failing versus nonfailing human myocytes. The Ca²⁺ transient during the prolonged AP was significantly prolonged compared to steady state only in the failing myocytes (759±142 vs. 615±88 ms in the steady state; P<0.05). However, the first beat after the prolonged AP was significantly potentiated (increased contractile amplitude versus steady state contraction) only in the nonfailing myocytes (Fig. 1, Table 1).

The current injection technique used to prolong the duration of the AP also caused depolarization of the resting potential and changed the membrane potential during the AP plateau. Therefore, to verify that the changes in the Ca²⁺ transients and contractions we observed were primarily caused by prolongation in the AP duration, we also used square wave voltage clamp (VC) steps to change depolarization duration in the same myocytes. In these experiments (n=5), prolongation of a single VC test step after steady state stimulation caused effects on contraction that were almost identical to those in current clamp experiments (Fig. 2A).

Next we tested the idea that potentiation of contraction by AP prolongation requires normal SR function. Reducing SR Ca²⁺ reuptake with CPA in nonfailing human myocytes reduced the steady state contraction amplitude and abolished the potentiation of the contraction of the first beat after AP prolongation (Fig. 2A,C). Identical experiments were carried out in normal feline myocytes. As in nonfailing human myocytes, prolongation of the AP

| Contraction magnitude (μM) | 1st post TS beat | 5.5±1* | 11.1±1.2* |
| APD 50% (ms) | Test step 628±145** | 417±37 | 349±43* |
| Ca²⁺ T 50% (ms) | Test step 613±40* | 464±24 | 333±28 |

*P<0.05 versus steady state within group, **P<0.05 failing versus nonfailing human myocytes.

Table 1

| Twitch contraction, action potential and Ca²⁺ transient parameters in human and feline myocytes |
|-------------------------------------|------------------|------------------|------------------|
| Contraction | Steady state 3.6±0.7* | 4.6±1 | 9.5±1.1 |
| n=7 | | | |
| Test step (TS) | 3.6±0.7 | 4.5±1 | 8.9±1.1 |
| (μM) | | | |
| 1st post TS beat | 3.7±0.8* | 5.5±1* | 11.1±1.2* |
| Contraction | Steady state 531±103* | 363±37 | 297±22 |
| n=10 | | | |
| Test step | 628±145** | 417±37 | 349±43* |
| (ms) | | | |
| 1st post TS beat | 526±101* | 323±25 | 275±19 |
| Base | 654±36* | 512±27 | 380±33 |
| | 921±70** | 867±67* | 614±63* |
| | 1st post TS beat | 613±40* | 464±24 | 333±28 |
| | | | |
| Ca²⁺ T 50% | Steady state 615±88* | 402±38 | – |
| n=17 | | | |
| Test step | 759±142** | 441±51 | – |
| | 1st post TS beat | 613±67* | 392±39 | – |
Fig. 1. (A) Representative examples of the effects of AP prolongation in nonfailing and failing human myocytes on contraction. Upper trace: action potentials, lower trace: contraction. The first AP is the steady state (SS, control) AP, the middle AP is the prolonged AP (test step) and the third AP is the first post test step AP after the prolonged AP. The dashed lines under the AP traces are the time during which current was passed into the cell to prolong the AP duration. AP prolongation prolonged contraction in the failing myocyte and potentiated the post prolongation beat in the nonfailing myocyte. The first beat after the prolonged AP was only significantly potentiated in the nonfailing myocytes. (B) Mean values±S.E.M. of changes in contractile amplitude (AP experiments) compared to steady state. *P<0.05 versus steady state (basal value), †P<0.05 versus failing myocytes.

Caused potentiation of the next contraction and this potentiation was reduced by CPA (Fig. 2B,C).

We next examined the roles of Ca\(^{2+}\) influx via the L-type Ca\(^{2+}\) current versus reverse mode NCX in the potentiation of contractility caused by AP prolongation. Test steps from \(-70\) to \(+10\) mV were used to maximally activate the L-type Ca\(^{2+}\) channels. Steps to \(+30\) mV were used to cause smaller Ca\(^{2+}\) currents and larger amounts of Ca\(^{2+}\) influx via NCX. The contraction caused by the first post test step was potentiated when depolarized to \(+30\) mV but not to \(+10\) mV (Fig. 3). To further investigate this issue, myocytes were exposed to NIF to reduce the L-type Ca\(^{2+}\) current. NIF reduced the magnitude of steady state contraction and shortened the AP duration (Fig. 4A), but
Fig. 2. (A) Representative example of the effects of prolonging the duration of depolarization with voltage clamp techniques in a nonfailing human myocyte. A single prolonged voltage clamp step caused potentiation of the next contraction. In the same cell, the SR inhibitor cyclopiazonic acid (CPA) reduced basal contractility, shifted the peak of contraction to the right and abolished the potentiation of the first beat after the prolonged beat. (B) Representative example of the effects of AP prolongation for a single beat in a normal feline myocyte before and after CPA treatment. Results were similar to those in nonfailing human myocytes. (C) Mean values±S.E.M. of changes in contractile amplitude compared to steady state (AP experiments). Upper panel: Nonfailing human myocytes and feline myocytes show a comparable potentiation of the first beat after the prolonged test step. Lower panel: SR inhibition with CPA abolishes the potentiation of the first post test step beat and brings out a positive inotropic effect of AP prolongation in nonfailing myocytes. *P<0.05 versus steady state (within group).

The relative potentiation of the contraction after the prolonged test step was similar to that observed under control conditions. However, since the AP was significantly shorter in duration after NIF we repeated these experiments using voltage clamp techniques, in the same cells. Again, NIF led to a significant reduction of the steady state contraction and the potentiation of the post test step beat was maintained (Fig. 4B).

To study the idea that Ca\(^{2+}\) influx via reverse mode NCX during the prolonged test steps contributes to the slow decay of contraction and Ca\(^{2+}\) transients in failing myocytes and is the source of Ca\(^{2+}\) that loads the SR and potentiates the subsequent beat in normal myocytes, we exposed myocytes to KB-R to block reverse mode NCX [33]. KB-R reduced the size of the typical second component of the contraction associated with the prolonged voltage clamp step and also reduced the potentiation of the next contraction (Fig. 5A,B). The average effects in normal feline myocytes are summarized in Fig. 5C. Due to the small number of available nonfailing human myocytes we were only able to test the effects of KB-R on failing human myocytes. In these experiments, KB-R eliminated the second component of contraction associated with prolonged AP duration and abolished the modest potentiation of the first beat after the prolonged AP (Fig. 5B).

If Ca\(^{2+}\) entry via the NCX during the AP plateau is involved in the regulation of contraction and in SR Ca\(^{2+}\) loading, then the effects we observed with prolongation of AP duration should be sensitive to [Na\(^+\)]. Therefore, we tested the effects of dialysis with 0 or 20 mM Na\(^+\) on contraction and Ca\(^{2+}\) transients in failing human myocytes (n=8). Representative data are shown in Fig. 6. The steady...
state contractions and Ca\(^{2+}\) transient amplitudes were greater in the 20 mM versus 0 mM [Na\(^{+}\)]\(_{\text{pip}}\) myocytes (6.4\(±\)1.6 vs. 3.3\(±\)0.7 \(\mu\)m and \(F/F_{0}\): 2.2\(±\)0.2 vs. 1.7\(±\)0.2, \(P<0.05\), respectively). Failing myocytes dialyzed with 20 mM [Na\(^{+}\)]\(_{\text{pip}}\) had shorter duration APs, contractions, and Ca\(^{2+}\) transients than failing myocytes dialysed with 0 mM [Na\(^{+}\)]\(_{\text{pip}}\). It is noteworthy that there was a significant increase in intracellular Ca\(^{2+}\) and contraction (18\(±\)4\%) during the AP plateau in the 20 mM [Na\(^{+}\)]\(_{\text{pip}}\) cells, but not in the 0 mM [Na\(^{+}\)]\(_{\text{pip}}\) cells. AP prolongation for one beat caused an immediate, additional increase in contraction and Ca\(^{2+}\) transients in cells dialysed with 20 mM [Na\(^{+}\)]\(_{\text{pip}}\) but not in cells dialyzed with 0 mM [Na\(^{+}\)]\(_{\text{pip}}\). These experiments show that intracellular [Na\(^{+}\)] influences AP wave shape and the size and duration of Ca\(^{2+}\) transients and contractions. These results suggest that sodium-dependent Ca\(^{2+}\) influx during the plateau phase of the AP directly contribute to Ca\(^{2+}\) transients and contraction in failing human ventricular myocytes.

4. Discussion

The main findings of this study are that prolongation of the AP duration causes an immediate prolongation of contractions and Ca\(^{2+}\) transients in failing myocytes and potentiates the contractility of the next beat in nonfailing myocytes. The SERCA2a antagonist CPA made the effects of AP prolongation on normal myocytes similar to those seen in failing myocytes in the absence of CPA. The Ca\(^{2+}\) channel antagonist NIF reduced steady state contractions but did not significantly change the effects of AP prolongation on contractility. The reverse mode NCX antagonist KB-R reduced steady state contractions and significantly reduced the effects of AP prolongation on contractility. Increasing intracellular Na\(^{+}\) in failing human myocytes increased basal contractility and was associated with APD-induced potentiation of contraction. Prolongation of depolarization duration under conditions that reduce Ca\(^{2+}\) influx via reverse mode NCX activity (KB-R, 0 mM
[Na$^+$]$_{pip}$ were not associated with an increased amplitude of contraction. These results are consistent with the hypothesis that in (normal) myocytes with normal SR function, Ca$^{2+}$ influx during the AP plateau is immediately taken up by the SR and potentiates the next beat. In (failing) myocytes with depressed SR function, Ca$^{2+}$ influx during the AP plateau is not fully transported into the SR and directly contributes to contraction. These data suggest that contraction of failing human myocytes is more reliant on transsarcolemmal Ca$^{2+}$ influx during the AP plateau on a beat-to-beat basis than in nonfailing myocytes.

4.1. **Ca$^{2+}$ homeostasis is altered in heart failure**

The cellular and molecular bases of abnormal intracellular Ca$^{2+}$ homeostasis in failing human myocytes still have not been clearly identified. Investigations of the protein or mRNA levels of SERCA, the SR regulatory protein phospholamban, the L-type Ca$^{2+}$ channels and the sarcolemmal NCX have not produced consistent results. Most [17,18,34,35] but not all [36,37] previous studies suggest reduced Ca$^{2+}$ transport capacity of the SR is the primary alteration that causes changes in the size and shape of the Ca$^{2+}$ transient in heart failure (for review see Refs. [5,6]). Some [20,24,25,27] but not all [32,37] studies suggest that the abundance and activity of the NCX are increased in the failing human heart. The NCX is the sarcolemmal protein that is primarily responsible for Ca$^{2+}$ efflux in the heart and which mainly adjusts intracellular Ca$^{2+}$ levels by removing Ca$^{2+}$ from the cytosol during diastole [13]. It has been suggested that an increase in forward mode NCX activity partially compensates for the reduced SR function in heart failure (for review see Refs. [5,6,38]). The present results suggest that this concept is incomplete because the SR and NCX do not work in concert to lower cytosolic Ca$^{2+}$ during the AP plateau. Our results suggest that instead of removing Ca$^{2+}$ from the cell, Ca$^{2+}$ actually enters via reverse mode NCX during the AP plateau, slowing the rate of decay of the Ca$^{2+}$ transient and prolonging contraction. These effects are likely to be
dependent on small Ca\(^{2+}\) transients resulting from depressed SR function.

4.2. Ca\(^{2+}\) currents in the late portion of the AP plateau

It is well established that Ca\(^{2+}\) can enter cardiac myocytes during the late portions of the AP plateau via the L-type Ca channel ([39–41]; for review, see Ref. [42]). Our results suggest that this process occurs in both normal and failing myocytes when the AP plateau is sufficiently long. While the L-type Ca\(^{2+}\) channel inactivates via a [Ca\(^{2+}\)]-dependent mechanism [39], in myocytes with long duration APs, the Ca\(^{2+}\) channel can reactivate during the decaying phase of the Ca\(^{2+}\) transient and produce additional Ca\(^{2+}\) influx. This Ca\(^{2+}\) channel reactivation is known to be responsible for early afterdepolarizations [39,40]. This process should be enhanced in failing myocytes because the smaller size of the Ca\(^{2+}\) transient should induce less Ca\(^{2+}\) mediated inactivation. The present studies suggest that in addition to Ca\(^{2+}\) entry via the L-type Ca channel, Ca\(^{2+}\) can enter the myocyte during the late portion of the action potential via reverse mode NCX. This idea is controversial [31] but is supported by recent studies in failing human myocytes [23,30].

Fig. 4. Effects of the Ca\(^{2+}\) channel antagonist nifedipine (NIF) in feline myocytes. (A) Contractility was reduced after NIF application, but the relative potentiation of contraction after the prolonged test step was still present. The AP duration was significantly shortened by NIF. (B) To study the effects of NIF in the absence of AP shortening a similar series of experiments was carried out using voltage clamp techniques. Under these conditions, prolongation of the duration of depolarization caused a prolongation of contraction and potentiation of the first contraction after the prolonged VC step.
Fig. 5. Effects of the reverse-mode NCX inhibitor KB-R 7943. (A) The effects of KB-R on contraction were studied in a feline myocyte under voltage clamp conditions. KB-R reduced basal contractility, reduced the NCX related second component of contraction during the prolonged step and abolished the potentiation of contraction after the prolonged voltage step. (B) In a human myocyte under current clamp conditions KB-R shortened the AP duration. The potentiation of contraction, which is present to a smaller extent in some failing myocytes (see mean data in Fig. 1B) following the prolonged AP was abolished. (C) Mean data (AP experiments) of changes in contraction in feline myocytes before (control) and after exposure to nifedipine or KB-R 7943 at steady state (SS), the prolonged beat (test step) and the first beat after the prolonged beat. The potentiation (% of steady state) of the contraction was maintained when cells were exposed to the NIF and was eliminated by KB-R. *P<0.05 versus steady state (within group), †P<0.05 versus control.

4.3. How does NCX contribute to the contraction of the myocyte?

The direction of Ca^{2+} transport by the electrogenic NCX is determined by the membrane potential, the subsarcolemmal [Ca^{2+}]_i and [Na^+]_o and the [Na^+]_o and [Ca^{2+}]_o [31]. Forward mode NCX (Ca^{2+} efflux) is promoted by negative membrane potentials (diastole), high [Ca^{2+}]_i (early systole), and low [Na^+]_o. Reverse mode NCX (Ca^{2+} influx) is promoted by positive membrane potentials
Fig. 5. (continued)

Fig. 6. Representative examples of the effects of dialysis with 0 and 20 mM [Na\textsuperscript{+}]\textsubscript{pip} on APs, Ca\textsuperscript{2+} transients and contractions. AP duration was shortened in 20 mM [Na\textsuperscript{+}]\textsubscript{pip}, but Ca\textsuperscript{2+} transient and contractile amplitude were larger. During the AP plateau, an increase in [Ca\textsuperscript{2+}], was observed in the 20 mM [Na\textsuperscript{+}]\textsubscript{pip} cell, whereas the Ca\textsuperscript{2+} transient decays in the 0 mM [Na\textsuperscript{+}]\textsubscript{pip} cell (see arrows). AP prolongation (middle APs) immediately increased the contraction and the Ca\textsuperscript{2+} transient in the 20 mM [Na\textsuperscript{+}]\textsubscript{pip} cell and potentiated the contraction of the next beat. There was no noticeable effect of AP prolongation in the cells dialyzed with 0 mM [Na\textsuperscript{+}]\textsubscript{pip} (n=8 cells).

4.4. NCX activity depends on Na\textsuperscript{+} and Ca\textsuperscript{2+} concentrations and therefore on SERCA function

As discussed earlier, the direction of Ca\textsuperscript{2+} transport by the NCX activity is determined by the concentrations of Na\textsuperscript{+} and Ca\textsuperscript{2+} inside and outside the cell and on the membrane potential. In human heart failure, there are changes in all of these parameters. The smaller Ca\textsuperscript{2+} transients appear to be largely caused by depressed SR function leading to smaller than normal SR Ca\textsuperscript{2+} stores [44]. This smaller peak Ca\textsuperscript{2+} will reduce inactivation of the L-type Ca\textsuperscript{2+} channel [41] and bias the NCX toward reverse mode activity. In addition, the lengthening of the AP duration (long QT) in heart failure lengthens the time available for reverse mode NCX. These increases in Ca\textsuperscript{2+} influx during the late phases of the AP further slow the decay of the Ca\textsuperscript{2+} transient in the failing human heart cell.

We conclude that Ca\textsuperscript{2+} enters failing human myocytes via reverse mode NCX because of changes in the thermodynamic determinants of Ca\textsuperscript{2+} fluxes through this transporter. This additional Ca\textsuperscript{2+} influx supports contractility but slows the decay of the Ca\textsuperscript{2+} transient and predisposes the failing heart to diastolic dysfunction.
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