Artifactual contractions triggered by field stimulation of cardiomyocytes

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Bøkenes, Janny, Ivar Sjaastad, and Ole M. Sejersted. Artifactual contractions triggered by field stimulation of cardiomyocytes. J Appl Physiol 98: 1712–1719, 2005. First published January 7, 2005; doi:10.1152/japplphysiol.00630.2004.—Although cell shortening in patch-clamped cells (current-clamp mode) is triggered by an ordinary action potential, the trigger mechanism in field-stimulated cells is not so obvious. The contraction characteristics of the two methods differ, and we, therefore, examined the triggering sequence in field-stimulated cells. Isolated rat cardiomyocytes were plated on laminin-coated coverslips that were mounted on an inverted light microscope and superfused with HEPES-Tyrode buffer (pH 7.4; 37°C). The cells were stimulated to contract either by a 0.5-ms current injection (CC cells) through high-resistance electrodes or a 5-ms biphasic field-stimulation pulse (FS cells), and drugs were added to block sarcoplasmin proteins involved in excitation-contraction coupling. Time to peak contraction (TTP) was significantly longer in FS cells and was not affected by the polarity or the length of the stimulus pulse. Tetrodotoxin (TTX; 20 μM) blocked cell shortening in CC cells but not in FS cells. Ni2+ (5 mM) blocked cell shortening in FS cells, whereas KB-R7943 (KB; 5 μM) had no effect either on cell shortening or TTP. In FS cells, nifedipine (Nif; 100 μM) and Ca2+ (300 μM) reduced fractional shortening by 34 and 63%, respectively, but only Cd2+ are fundamentally different. A current injection triggers a uniform depolarization, whereas an electric field will expose each part of the membrane to different potentials during stimulation (8, 22, 31, 41, 47, 48). We observed that contraction characteristics depend on the stimulation method used. Because cell shortening might be triggered differently in field-stimulated (FS) and current-clamped (CC) cells, this difference might be reflected in contractile characteristics. In this work, we wanted to compare the two methods of stimulation, in particular we wanted to examine the trigger sequence of FS cells and we hypothesize that it is different from the sequence seen in CC cells.

MATERIALS AND METHODS

Animals were cared for according to the Norwegian Animal Welfare Act, which conforms to the National Institutes of Health guidelines (NIH publication No. 85–23, revised 1996). Two animals were kept in each cage and housed in a temperature-regulated room with a 12:12-h day-night cycle and given access to food and water ad libitum.

Animals and Cell Isolation

Male Wistar rats (Møllegaard Breeding and Research Centre, Skensved, Denmark) weighing ~400 g were intubated and ventilated on a Zoovent ventilator (Triumph Technical Services, Milton Keynes, UK) with 68% N2O, 29% O2, and 2–3% isoflurane (Abbott Laboratories, North Chicago, IL). After an intravenous injection of heparin, the hearts were excised and immediately put in ice-cold saline for cell isolation.

Left ventricular myocytes were isolated using a modified Langendorff setup. From the ice-cold buffer, the hearts were immediately mounted by the aorta and retrograded perfused with medium A. After 5 min of perfusion with medium A at ~10 ml/min, the hearts were perfused for 30 min with medium B at ~7 ml/min. The right ventricle and the atria were removed before the heart was cut down, put in a petri dish with medium B containing 0.02 mg/ml DNase (Worthington, NJ), and minced. This suspension was transferred to a glass flask and gently shaken in a 37°C water bath for 15 min. The cells were then centrifuged twice at 500 rpm (48 g) for 30 s. After the first centrifugation, the cells were suspended in the proper volume of CM and stored on ice. The supernatant was removed and the sediment was allowed to sediment for 10 min. The supernatant was removed and the cells resuspended in cultivation medium (CM) and allowed to sediment for another 10 min. The supernatant was removed and the cells were suspended in the proper volume of CM and stored on laminin-coated (Natural Mouse Laminin, Gibco) coverslips placed in 6-well trays and kept at 37°C until used (within 10 h). The diastolic cell length was 125.2 ± 1.4 μm (n = 74).

All solutions were continuously gassed with 5% CO2–95% O2, and solutions and cells were kept at 37°C throughout the isolation procedure.

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**Solutions**

*Medium A.* Medium A was composed of Joklik’s medium (GIBCO) added to 23.8 mM NaHCO₃, 1 mM MgSO₄, and 1 mM dl-carnitine, equilibrated with 5% CO₂ and 95% O₂ for 30 min at 37°C, and pH adjusted to 7.4 with NaOH.

*Medium B.* Medium B was a medium A added to ~200 U/ml collagenase (Type II, Worthington) and 0.1% BSA.

*CM.* CM was composed of medium 199 (Sigma) mixed with 2 mg/ml BSA, 2 mM dl-carnitine, 5 mM creatine, 5 mM taurine, 0.1 µM insulin, 0.1 mM triiodothyronine, 100 IU/ml penicillin, and 100 µg/ml streptomycin.

*HT buffer.* HT buffer was comprised of (in mM): 140 NaCl, 1.8 CaCl₂, 0.5 MgCl₂, 5.0 HEPES, 5.5 glucose, 0.4 Na₂HPO₄, and 5.4 KCl, adjusted to pH 7.4 with NaOH.

*Tetrodotoxin.* Tetrodotoxin (TTX; Tocris, Bristol, UK) was first dissolved in HT buffer, pH 4.8, before being diluted in HT buffer, pH 7.4. Diluted TTX was used within 1 wk. Final concentration of TTX was 21 µM.

*KB-R7943.* KB-R7943 (KB, Tocris) was dissolved in DMSO as a 20 mM stock solution and stored at −20°C in proper portions to avoid repeatedly thawing.

*Ni²⁺, nifedipine,* and *Cd²⁺* were dissolved in double-distilled H₂O as stock solutions.

**Experimental Procedures**

The coverslips were used as the floor of the experimental chamber on the stage of a Nikon Diaphoto-TMD inverted microscope. The chamber (~200 µl) was superfused with prewarmed solution at a rate of ~4 ml/min. A custom-made rapid switcher allowed us to change the solution around the cell within 500 ms. All experiments were performed at 37°C, and only single, rod-shaped myocytes with preserved striation and no blebs were used.

**Field Stimulation**

Two platinum electrodes were placed on each side of a myocyte. The distance between the two platinum wires was ~4 mm. The orientation of the cells was random in relation to the electrodes. The isolated myocytes were field stimulated at 1 Hz and, if not otherwise specified, with a biphasic pulse of 5 ms total length. Amplitude and duration of the pulse was controlled by a Pulsar 6bp-as stimulator (FHC) and monitored with an oscilloscope (Gould, Essex, UK). The stimulus amplitudes were set to ~20% above threshold. The effect of the different drugs was monitored by first recording 20 control contractions and then the first 20 contractions after switching the solution. An acute effect was defined as contractions 4–6.

**Current-Clamp Stimulation**

Isolated myocytes were impaled with high-resistance microelectrodes (20–35 MΩ filled with 2.7 M KCl) to minimize cell dialysis and buffering of intracellular Ca²⁺. After impalement and correction of series resistance, the cells were given a 0.5-ms current at 1 Hz. The strength of the pulse was increased stepwise until a stable action potential with an explicit peak was established (~20% above threshold). Recordings were made with an Axoclamp-2A amplifier (Axon Instruments, Foster City, CA) in bridge mode for current-clamp experiments. Membrane potentials and cell shortening were recorded and analyzed.

**Field Stimulation Measured in Current-Clamp Mode**

The cells were first impaled with high-resistance electrodes as described above, after which the platinum electrodes were placed on each side of the cell. The cells were then field stimulated as described while membrane potential and cell shortening were measured simultaneously.

**Data Sampling and Analysis**

Protocols were written in pClamp software (Axon Instruments). The data were digitized with a Digidata 1200B and sampled and stored on a computer. Unloaded cell shortening was measured using a charge-coupled device camera and a video edge detector (Crescent Electronics, Sandy, UT). Sampled data were analyzed with pClamp8 or pClamp9 software.

**Statistics**

All data are presented as means ± SE. Number of cells is given as n. When groups are compared statistically, paired or unpaired t-tests with proper tailing and Bonferroni correction are used.

**RESULTS**

**Size and Duration of Cell Shortening in FS and CC Cells**

Time to peak contraction (TTP) in FS cells was 19 ms longer than in CC cells, and cell shortening was also larger by 28% (Fig. 1A). In both groups, TTP increased with increasing cell shortening (Fig. 1B). The relationship was not very steep, and the r values for FS and CC cells were 0.58 (not significant) and 0.69 (P < 0.05), respectively. The regression lines were statistically different, but the slopes were not different between the two groups. From the regression lines it can be calculated that only 3 ms of the difference in TTP between the FS and CC
cells can be accounted for by the slope of the regression relationship, which means that 16 ms of the difference (vertical distance between the regression lines) is due to mode of stimulation.

Relevant contraction data are summarized in Table 1. Time to 10% contraction and the time from 10 to 90% contraction are longer in FS cells than in CC cells, although the maximum rate of contraction (+dL/dtmax) was not different. However, +dL/dtmax normalized to fractional shortening, R+dL/dtmax, was slower in FS cells than in CC cells. The total contraction-relaxation cycle [from 10% contraction to 90% relaxation (CRC)] was ~10% shorter in FS cells than in CC cells, which was due to a more rapid relaxation of FS cells. Maximum rate of relaxation was equal for the two stimulation methods, but was due to a more rapid relaxation of FS cells. Maximum rate of contraction was not different. However, R10-90, time to 10% relaxation and from 10 to 90% relaxation were 0.6 and 0.4 times the values of CC cells. As visualized in Fig. 1A, this was due to a pronounced "tail" on the cell length record of CC cells during the last one-third of the relaxation phase. Thus the longer TTP in FS cells reflects that FS cells were slower than CC cells throughout the whole CRC cycle, except for the very last phase.

Shape and Duration of Stimulus During Field Stimulation

Because we used a standard 5-ms biphasic pulse for the FS cells and a 0.5-ms current injection for the CC cells, it could be questioned whether TTP in FS cells would be shorter with a shorter stimulation pulse. Thus we compared cell shortening triggered by a 5-ms and a 0.5-ms biphasic pulse. In addition, we also tested whether mono- and biphasic pulses resulted in different TTP. There was no dependence of TTP on the pulse duration and no difference in TTP between cell shortening induced by mono- or biphasic pulses (Table 2).

Blocking of Sodium Current

Cell shortening is normally triggered by an action potential. The different shape of the cell shortening record in FS cells compared with CC cells could be caused by a longer action potential or that cell shortening was triggered by another mechanism. We therefore tested the effect of TTX (21 μM), which blocks the I\textsubscript{Na}.

TTX did not block cell shortening in FS cells (Fig. 2A). In some cells, the cell shortening was slightly reduced by the drug and in others the cell shortening became slightly enlarged and TTP was prolonged. Pulse duration did not affect the lack of response to TTX, nor was it necessary to increase the field strength to maintain cell shortening.

TTX almost abolished the cell shortening in CC cells (Fig. 2B). The effect was detectable from the second beat after application of the drug and completed by the fourth beat. The corresponding action potential shows that the peak depolarization is delayed and diminished and thus no longer represent the fast I\textsubscript{Na}. This confirms that the cell shortening in CC cells is induced by action potentials with a fast upstroke, whereas the initiation of contraction in the FS cells is different.

Depolarization During Field Stimulation

Although cell shortening in FS cells is not likely to be initiated by the I\textsubscript{Na}, a depolarization of the cell membrane is most probable. To monitor the depolarization pattern in FS cells, the membrane potential was simultaneously measured in current-clamp mode during field stimulation.

The diastolic membrane potential was −78.9 ± 2.3 (n = 10) and −78.8 ± 4.4 mV (n = 3) for CC and FS cells, respectively. Membrane potentials from a CC cell and an FS cell are superimposed for comparison in Fig. 3. For both types of stimulation, a large artifact reflects the stimulation pulse followed by a depolarization and a subsequent cell shortening (not shown). Compared with the action potential of CC cells, the peak in FS cells was delayed (times to peak 1.4 ± 0.1 and 5.5 ± 1.6 ms for CC and FS cells, respectively; P < 0.05) and lower (21.8 ± 2.9 and 20.5 ± 19.5 mV for CC and FS cells, respectively; P < 0.05). This supports the theory that this depolarization is not a result of the opening of Na\textsuperscript{+} channels.

Effect of Ni\textsuperscript{2+} on Cell Shortening

Taken together that cell shortening in FS cells did not seem to be triggered by the I\textsubscript{Na} and that the cell membrane depolarizes, it is possible that the electric field directly activates the L-type Ca\textsuperscript{2+} channels or Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange. To test this we used Ni\textsuperscript{2+} (5 mM), which blocks both of them (14, 29).

Ni\textsuperscript{2+} reversibly blocked cell shortening from one beat to another in FS cells (Fig. 4). The experiment was repeated in three cells.

Blocking of I\textsubscript{Ca,L}

We then tested whether the effect of Ni\textsuperscript{2+} was due to a blocking of the L-type Ca\textsuperscript{2+} channels, the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger (NCX), or both in combination. Nif (100 μM) reduced

<table>
<thead>
<tr>
<th>Pulse Duration, ms</th>
<th>Monophasic</th>
<th>Biphasic</th>
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<tbody>
<tr>
<td>0.5</td>
<td>64.3±2.4</td>
<td>63.9±2.8</td>
</tr>
<tr>
<td>5.0</td>
<td>61.4±2.4</td>
<td>63.5±2.3</td>
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Values are mean ± SE, n = 8.
fractional shortening by 34% in FS cells without any significant effect on TTP (Fig. 5). Cd$_{2+}$ (300 μM) reduced fractional shortening by 63% and TTP by 48%. To test the degree of block of the $I_{Ca,L}$ obtained by these doses of Nif and Cd$_{2+}$, we performed voltage-clamp experiments. Nif completely blocked the $I_{Ca,L}$, whereas Cd$_{2+}$ only blocked about two-thirds of the current (results not shown). These results suggest that the $I_{Ca,L}$ may not be the only trigger of cell shortening in FS cells.

### Blocking the NCX

The NCX current is able to trigger cell shortening when it operates in “reverse-mode” (Na$^+$/Ca$^{2+}$ in) (44). At low concentrations, the drug KB (12, 20) selectively blocks reverse-mode Na$^+$/Ca$^{2+}$ exchange. However, fractional shortening and TTP in FS cells were not affected by 5 μM of KB (Fig. 4). We therefore conclude that the effect of Ni$^{2+}$/ and reverse-mode Na$^+$/Ca$^{2+}$ exchange are not simply additive but probably interact synergistically to trigger contraction in FS cells as indicated by the effect of Ni$^{2+}$.

### Blocking Both the $I_{Ca,L}$ and Reverse-Mode Na$^+$/Ca$^{2+}$ Exchange

To verify that the effect of Ni$^{2+}$ was due to simultaneous block of the $I_{Ca,L}$ and reverse-mode Na$^+$/Ca$^{2+}$ exchange and to further substantiate the idea that the channel and the transporter interact, we applied a combination of L-type Ca$^{2+}$ channel blockers with KB.

The combination of Nif (100 μM) and KB (5 μM) reduced cell shortening by 50% and shortened the TTP significantly compared with control. This effect was not significantly different from the effect of Nif alone. On the other hand, the combination of Cd$_{2+}$ and KB almost abolished cell shortening. In only one of 13 cells, a small contraction remained after addition of the two blockers in combination.
concealed within the stimulus artifact, but we can conclude that it is not required for opening of the L-type Ca\textsuperscript{2+} channels.

**Pulse Waveform**

As described by others (2, 49), we used two platinum electrodes placed on each side of the cell, stimulating with biphasic pulses of alternating direction of the pulse to avoid electrolysis. Alternating polarity of the pulse will result in different patterns of depolarization of a cell (32, 41, 47, 48), and Tung and Borderies (41) predicted mathematically that the time for the membrane to reach the threshold potential for triggering an action potential depended on the total length of the pulse. However, they also predicted that the excitability would be independent of the waveform (mono- or biphasic). We used a 5-ms biphasic pulse as a standard field-stimulation pulse, which triggered myocyte contractions with a stable and reproducible TTP (6). Switching from biphasic to monophasic pulses or altering the duration of the pulse did not affect fractional shortening or TTP; the lack of alteration is in accordance with the predictions of Tung and Borderies (41).

**Depolarization During Field Stimulation**

The action potential starts with the opening of Na\textsuperscript{+} channels, resulting in a fast and large inward \( I_{Na} \) that is selectively and completely blocked by TTX (21, 35). TTX caused the disappearance of the fast upstroke of the action potential and abolished cell shortening in CC cells but was without effect on cell shortening in FS cells, although there is no reason to believe that TTX should be less effective in FS cells. Because cell shortening in our FS cells was independent of \( I_{Na} \), we

**Field Stimulation**

Numerous investigators have used field stimulation to study contraction of isolated cardiomyocytes. The method is so well accepted that information about the nature of the pulse is either insufficiently described (2, 3, 37, 38) or not present at all (11, 18, 30), and the method is regarded as a means of triggering a physiological contraction. Field stimulation is claimed to elicit an action potential (8, 31, 33, 38). However, although the membrane depolarizes, in our hands, field stimulation does not seem to trigger the normal sequence of channel activation. What can the reason be for this? When a cell is stimulated by a uniform electric field and aligned parallel to it, the two ends are nonuniformly (and oppositely) polarized, and different regions of the cell experience very different transmembrane voltages (8, 22, 31, 33, 41, 47, 48). Spatial differences have also been observed in the intracellular Ca\textsuperscript{2+} ([Ca\textsuperscript{2+}]\textsubscript{i}) transient (7, 32). How these spatial inhomogeneities of membrane depolarization and [Ca\textsuperscript{2+}]\textsubscript{i} transients affect the cell shortening has not been investigated. In our experiments the field strength was set to \( \sim 20\% \) above the voltage threshold that caused cell shortening without concern of the alignment of the cell with respect to the electric field. Increasing the voltage more did not increase cell shortening. It seems reasonable to conclude that field stimulation causes a nonhomogenous depolarization of the cell that directly activates the L-type Ca\textsuperscript{2+} channels. We cannot tell whether an \( I_{Na} \) is present or not, because it may be

**DISCUSSION**

In this study we show that field stimulation of cardiomyocytes triggers contraction independent of the \( I_{Na} \) and that cell shortening was slower compared with CC cells. In the CC cells, the \( I_{Na} \) is triggered by a small current injection resulting in a normal action potential, and cell shortening follows subsequent to an inward Ca\textsuperscript{2+} current through L-type Ca\textsuperscript{2+} channels. We previously showed that under these circumstances cell shortening disappears in the presence of Ca\textsuperscript{2+} channel blockers (36). In the FS cells on the other hand, cell shortening was only partially depressed by Nif or Cd\textsuperscript{2+} and complete depression of contraction required the simultaneous inhibition of the NCX.

**Fractional Shortening**

![Fractional Shortening](image1)

**Time to Peak**

![Time to Peak](image2)
assume that the electric field created during the field stimulation directly activated other electrogenic channels and transporters like L-type Ca\(^{2+}\) channels or the NCX. The depolarization observed in our field-stimulated current-clamped cells peaked later than in CC cells and did not reach a positive potential. Thus it probably does not reflect opening of Na\(^{+}\) channels. Normally $I_{\text{Na}}$ peaks within 1–2 ms. By using voltagesensitive dyes, the $I_{\text{Na}}$ might be possible to detect, but there are few relevant reports in the literature. The recordings leave the impression that rate of depolarization is slower than the true $I_{\text{Na}}$ under conditions that are comparable to the present experiments (8, 22, 33, 48). Thus the $I_{\text{Na}}$ might be absent.

$I_{\text{Ca,L}}$

$I_{\text{Ca,L}}$ exhibits a bell-shaped voltage relationship (36) and represents by far the largest source of trigger Ca\(^{2+}\) into the cytosol. In our voltage-clamp experiments, Nif effectively blocked this current (unpublished data). Shen et al. (34) showed that Nif blocks the $I_{\text{Ca,L}}$, with only minor influence on other currents (14, 29, 50), whereas other investigators showed that it does not completely abolish the [Ca\(^{2+}\)]\(_i\), transients (13, 32). In our FS cells, Nif only reduced cell shortening by ~35%. This could be due to an insufficient dose or Nif not fully blocking $I_{\text{Ca,L}}$ when cells are stimulated to contract by an electric field in contrast to current injection during current clamp. We therefore tried another blocker, Cd\(^{2+}\), which is frequently used to block L-type Ca\(^{2+}\) channels (1, 14). The minimum concentration of Cd\(^{2+}\) that completely blocks $I_{\text{Ca,L}}$ is reported to be 20 (17) to 100 $\mu$M (16, 34). Surprisingly, when we used 300 $\mu$M of Cd\(^{2+}\), cell shortening in FS cells was only reduced by 60%. $I_{\text{Ca,L}}$ was blocked to the same extent (unpublished data). Others have found that Cd\(^{2+}\) does not totally block the [Ca\(^{2+}\)]\(_i\), transients (35), and, in addition, it can affect the $I_{\text{Na}}$ (35) and the $I_{\text{NCX}}$ (16, 19) as well. Because neither Cd\(^{2+}\) nor Nif abolished all contractions, the remaining cell shortening could be caused by the NCX operating in reverse mode (43).

$\text{Na}^{+}/\text{Ca}^{2+}$ Exchange Current

The NCX operates both in forward ($\text{Na}^{+}$ in/ Ca\(^{2+}\) out) or reverse ($\text{Na}^{+}$ out/ Ca\(^{2+}\) in) mode (5) depending on the membrane potential and the intra- and extracellular Na\(^{+}\) and Ca\(^{2+}\) concentrations. Although reverse-mode Na\(^{+}/\text{Ca}^{2+}\) exchange can trigger cell shortening (23, 34, 43), the significance of this mechanism under physiological conditions is controversial (35, 42, 46).

During the normal excitation-contraction cycle, the reverse-mode $I_{\text{NCX}}$ is caused by the combination of a transient increase in subsarcolemmal Na\(^{+}\) concentration and depolarization during the upstroke of the action potential. The isothiourea derivative KB (19) is a selective inhibitor of reverse-mode NCX (12, 20, 45). On our FS cells, KB had no effect on the cell shortening, although the concentration we used selectively inhibits Ca\(^{2+}\) influx via the NCX (30) and is twice the IC\(_{50}\) (12, 20). A lack of effect on cell shortening, however, does not necessarily mean that the reverse-mode NCX is not blocked (30). Others have found that KB does not affect spontaneously beating cardiomyocytes (20). The reason for a lack of effect could be that the duration of reverse-mode $I_{\text{NCX}}$ is too short to give a substantial increase in [Ca\(^{2+}\)]\(_i\) (46) and that its role is to finely adjust [Ca\(^{2+}\)] in the subsarcolemmal space. Therefore, it seems that reverse-mode $I_{\text{NCX}}$ might not be required to trigger contractions under normal physiological conditions (35, 42). Blocking $I_{\text{Na}}$ with TTX would also block the reverse-mode $I_{\text{NCX}}$. Because neither TTX nor KB had any blocking effect on our FS cells, the NCX could not be the only source to trigger Ca\(^{2+}\).

$I_{\text{Ca,L}}$ and NCX in Combination

Confocal microscopy showed that Ca\(^{2+}\) release from the SR followed different patterns whether it was triggered by Ca\(^{2+}\) influx through L-type Ca\(^{2+}\) channels or reverse-mode Na\(^{+}/\text{Ca}^{2+}\) exchange (24). Furthermore, the relation between the two triggers is not simple and linear (15, 49) but they may act synergistically in the modulation of SR release (25). Although the effects on contraction obtained by blocking L-type Ca\(^{2+}\) channels and NCX separately do not predict that the two drugs can act additively, it is possible that $I_{\text{NCX}}$ can take over as trigger of Ca\(^{2+}\) release when the $I_{\text{Ca,L}}$ is blocked. When both the L-type Ca\(^{2+}\) channels (29) and the NCX were blocked with Ni\(^{2+}\) (14), all cell shortening in the FS cells was effectively abolished. A combination of KB and Nif reduced cell shortening more than Nif alone, and a combination of KB and Cd\(^{2+}\) almost abolished the cell shortening. This strongly suggests that the cell shortening in the FS cells is primarily triggered by $I_{\text{Ca,L}}$, but that reverse-mode Na\(^{+}/\text{Ca}^{2+}\) exchange can add to activation of Ca\(^{2+}\) release provided that the $I_{\text{Ca,L}}$ is reduced.

Possible Explanations for the Slower Contraction in FS Cells

The explanation for the 15–20 ms longer TTP in FS compared with CC cells is not obvious. The nonuniformity of the polarization of the FS cells is reflected in nonuniform and delayed [Ca\(^{2+}\)]\(_i\), transients (32) measured by multisite optical mapping. Confocal microscopy in line-scan mode has also shown nonuniformity of the [Ca\(^{2+}\)]\(_i\), transient with a variation of up to 10 ms (7, 40). If the Ca\(^{2+}\) wave propagates at ~110 $\mu$m/s (40), it would take ~20 ms to reach the core from the activated surface in an average rat cardiomyocyte. A dysynchronous start and upstroke of the [Ca\(^{2+}\)]\(_i\), transient could well delay the onset of a contraction. Others again have not detected any dysynchrony of importance in their cellular line scans (4, 9, 10, 39), unless the myocytes were harvested from infarcted hearts (26) or cell cultures (27, 28). This dys synchrony of the [Ca\(^{2+}\)]\(_i\), transient signal is thought to be a result of partial detubulation. Again, the models and experimental conditions vary so that the results are difficult to compare. Normally, only global, and thus the cell average, [Ca\(^{2+}\)]\(_i\), transient have been studied. We are not aware of any studies where the timing, shape, and characteristics, or possible dys synchrony, of the [Ca\(^{2+}\)]\(_i\), transients are matched up with contraction characteristics in isolated cardiomyocytes.

An alternative explanation to dysynchronous release of Ca\(^{2+}\) could be later onset of Ca\(^{2+}\) reuptake so that Ca\(^{2+}\) transients were longer. In that case, one would also expect a larger transient and a more pronounced cell shortening. On average, cell shortening was larger in FS compared with CC cells, but as shown in Fig. 1B there was considerable overlap and TTP was longer in the FS cells even at cell shortening similar to that seen in CC cells. It is unlikely that field stimulation would affect the relationship between [Ca\(^{2+}\)]\(_i\) and the rate of reuptake by the SR.
It is obvious from our results that the prolongation of TTP must be a consequence of field stimulation per se and not a result of the pattern or length of the stimulus pulse. It is necessary with further experiments to reveal the nature of this effect.

In summary, the sequence of currents and their relation to myocyte contraction is evident under current-clamp conditions when action potentials are the obvious initial triggers of the excitation-contraction cycle. The origin of cell shortening in FS cells is more complex, because TTX, Cd$^{2+}$, Nif, and KB have different effects in these cells compared with CC cells. The relative contribution of the currents involved may be different from CC cells. The polarization patterns created by different stimuli may also influence the interplay of the Ca$^{2+}$ channel, the NCY, and the Ca$^{2+}$ release channel of SR. From our results we conclude that the $I_{Na}$ is not necessary for triggering a contraction in FS cells. The $I_{Ca,L}$ seems to be directly triggered by the electric field and can induce cell shortening without influence of the $I_{Ncy}$. However, when the $I_{Ca,L}$ is reduced or abolished, the $I_{NCX}$ contributes as a trigger of cell shortening.

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