Effects of Electrical Shocks on $\text{Ca}_{i}^{2+}$ and $V_m$ in Myocyte Cultures

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Abstract—Changes in intracellular calcium concentration ($\Delta \text{Ca}_{i}^{2+}$) induced by electrical shocks may play an important role in defibrillation, but high-resolution $\Delta \text{Ca}_{i}^{2+}$ measurements in a multicellular cardiac tissue and their relationship to corresponding $V_m$ changes ($\Delta V_m$) are lacking. Here, we measured shock-induced $\Delta \text{Ca}_{i}^{2+}$ and $\Delta V_m$ in geometrically defined myocyte cultures. Cell strands (width $=0.8$ mm) were double-stained with $V_m$-sensitive dye RH-237 and a low-affinity $\text{Ca}_{i}^{2+}$-sensitive dye Fluo-4FF. Shocks ($E\approx5$ to 40 V/cm) were applied during the action potential plateau. Shocks caused transient $\text{Ca}_{i}^{2+}$ decrease at sites of both negative and positive $\Delta V_m$. Similar $\Delta \text{Ca}_{i}^{2+}$ changes were observed in an ionic model of adult rat myocytes. Simulations showed that the $\text{Ca}_{i}^{2+}$ decrease at sites of $\Delta V_m$ was caused by the outward flow of $I_{\text{CaL}}$ and troponin binding; at sites of $\Delta V_m$ it was caused by inactivation of $I_{\text{CaL}}$ combined with extrusion by Na–Ca exchanger and troponin binding. The important role of $I_{\text{CaL}}$ was supported by experiments in which application of nifedipine eliminated $\text{Ca}_{i}^{2+}$ decrease at $\Delta V_m$ sites. Largest $\Delta \text{Ca}_{i}^{2+}$ were observed during shocks of $\approx10$ V/cm causing simple monophasic $\Delta V_m$. Shocks stronger than $\approx20$ V/cm caused smaller $\Delta \text{Ca}_{i}^{2+}$ and postshock elevation of diastolic $\text{Ca}_{i}^{2+}$. This was paralleled with occurrence of biphasic negative $\Delta V_m$ that indicated membrane electroporation. Thus, these data indicate that shocks transiently decrease $\text{Ca}_{i}^{2+}$ at sites of both $\Delta V_m$ and $\Delta V_m$. Outward flow of $I_{\text{CaL}}$ plays an important role in $\text{Ca}_{i}^{2+}$ decrease in the $\Delta V_m$ areas. Very strong shocks caused smaller negative $\Delta \text{Ca}_{i}^{2+}$ and postshock elevation of diastolic $\text{Ca}_{i}^{2+}$, likely caused by membrane electroporation. (Circ Res. 2004; 94:1589-1597.)

Key Words: defibrillation ■ fluorescent imaging ■ membrane potential ■ intracellular calcium

Calcium ions play crucial roles in regulation of cardiac excitation and contractility, and they may be an important determinant of the tissue response to defibrillation shocks. The interaction between electrical field and $\text{Ca}_{i}^{2+}$ may affect the outcome of a defibrillation attempt in several ways. First, it was suggested that very strong shocks cause calcium overload, which can lead to abnormal impulse generation, re-induction of rapid arrhythmias, and defibrillation failure. Second, it was reported that relatively weak shocks with an energy below the defibrillation threshold applied during fibrillation can prevent the loss of cardiac contractility often observed after successful defibrillation, so-called pulseless electrical activity syndrome. In a related study, it was reported that cardiac contractility is enhanced when shocks are applied during the absolute refractory period. Two alternative mechanisms were proposed to explain these effects: stimulation of intracardiac sympathetic nerves by the shock or an increase of peak $\text{Ca}_{i}^{2+}$ concentration caused by a direct effect of the shock on myocytes.

The direct assessment of the mechanisms of shock-$\text{Ca}_{i}^{2+}$ interaction and its role in defibrillation requires measurements of shock-induced $\text{Ca}_{i}^{2+}$ and $V_m$ changes with high spatial and temporal resolution. Previously, shock-induced $\text{Ca}_{i}^{2+}$ changes were measured in single myocytes and at a single point in whole hearts. No spatially resolved data on shock-induced $\text{Ca}_{i}^{2+}$ changes and colocalized $V_m$ changes in multicellular cardiac tissue are currently available. Such data are especially important because of the known complexity of shock-induced $\Delta V_m$ in the heart. It is well established that shocks produce highly nonuniform patterns of $\Delta V_m$ with areas of positive, negative, or negligible polarizations present in different parts of cardiac tissue, suggesting that $\text{Ca}_{i}^{2+}$ changes may also be nonuniform. In addition, shocks produce different types of $V_m$ responses that depend on the shock strength and the tissue geometry. With the exception of very weak shocks, shocks applied during the action potential (AP) plateau produce nonlinear $\Delta V_m$ of 2 main types. Shocks of moderate strength induce asymmetric $\Delta V_m$ with negative $\Delta V_m$ being much larger than positive $\Delta V_m$. Stronger shocks can induce $\Delta V_m$ of another type, which is characterized by a nonmonotonic behavior of negative $\Delta V_m$ in which strong hyperpolarization is followed by a return of $V_m$ to more positive levels. In cell cultures, the transition from one nonlinear $\Delta V_m$ type to another with increasing shock

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strength was paralleled by the occurrence of postshock arrhythmias originating from the area of shock-induced negative polarization as well as with membrane electroporation in the same areas. It can be hypothesized that these 2 types of nonlinear \( \Delta V_m \) are associated with different \( \text{Ca}^{2+} \) changes as well.

The purpose of the present study was to use high-resolution optical mapping of \( V_m \) and \( \text{Ca}^{2+} \) to determine spatio-temporal \( \text{Ca}^{2+} \) changes during and after shocks, as well as the relationship between these \( \Delta \text{Ca}^{2+} \) and different types of \( \Delta V_m \) in multicellular cardiac tissue. Because shock-induced \( \Delta V_m \) strongly depend on the tissue geometry, experiments were performed in geometrically defined cultured cell monolayers. To elucidate the ionic mechanisms of \( \text{Ca}^{2+} \) changes, shock effects were also investigated in a computer model of rat ventricular myocardium.

### Methods

#### Cell Cultures

Growth substrates containing linear strands (width = 0.8 mm) were prepared as described previously. Neonatal rat myocytes were obtained from 1- to 2-day-old Wistar rats and cultured according to the previously published procedures. Cell cultures were incubated in UltraCulture medium (BioWhittaker) supplemented with 20 \( \mu \)g/mL vitamin B12, antibiotics, 0.1 mmol/L of bromoethoxyuridine, and 0.5 mmol/L of epinephrine at 37°C in a humidified atmosphere containing 4% CO2. Culture medium was exchanged on the next day after preparation and every second day thereafter. Experiments were performed after 4 to 6 days in culture.

#### Optical Mapping of \( V_m \) and \( \text{Ca}^{2+} \)

\( V_m \) and \( \text{Ca}^{2+} \) changes were measured using a previously described optical mapping technique with some modifications. Cells were first stained with 5 mmol/L of a \( \text{Ca}^{2+} \)-sensitive dye (see later) by incubating with a dye solution for 30 to 45 minutes. After that, cultures were transferred into experimental chamber and superfused with Hanks solution (Life Technologies) having a pH of 7.4 and a buffer capacity of 10 mmol/L, respectively. The second group included Rhod-2 and Rhod-FF, with \( \text{K}_d \) of 0.57 and 17 mmol/L, respectively. The second group included Rhod-2 and Rhod-FF, with \( \text{K}_d \) of 0.57 and 17 mmol/L, respectively. Based on these measurements (see Results), the dye Fluo-4FF was selected for measurement of shock-induced \( \Delta \text{Ca}^{2+} \) and \( \Delta V_m \) in double-stained preparations. \( \Delta \text{Ca}^{2+} \) and \( \Delta V_m \) were measured sequentially using different sets of optical filters. For \( V_m \) measurements, fluorescence was excited at 500/55 nm and emitted light was measured at >650 nm. For \( \text{Ca}^{2+} \) measurements using Fluo dyes, the respective wavelength ranges were 480/40 nm and 530/50 nm. With Rhod dyes, they were 530/40 nm and 580/40 nm. Tests described previously showed that there was no optical cross-talk between RH-237 and Fluo dyes. Fluorescence was measured using a 16×16 photodiode array (Hamamatsu) and a microscopic mapping system at a sampling rate of 2 to 5 kHz/channel and spatial resolution of 110 or 55 \( \mu \)m/diode.

Cells were paced at a cycle length of 500 ms. Rectangular uniform-field shocks with a duration of 10 ms were delivered via 2 platinum plate electrodes. The field strength (\( E \)) was measured using a bipolar electrode. Delivery of shocks was synchronized with stimulation pulses. The delay between AP upstrokes and shocks was 10 to 20 ms. At each mapping location, 3 to 4 shocks of different strengths selected from the values of ~5, 10, 20, 30, and 40 V/cm were applied. A shock of the same strength was applied twice to measure \( \Delta V_m \) and \( \Delta \text{Ca}^{2+} \). For shocks of 20 V/cm or stronger, a 3-minute interval was allowed after shocks for tissue recovery. Before shock application, control (no shocks) \( V_m \) and \( \text{Ca}^{2+} \) recordings were made. To check for data reproducibility, at the end of each series, measurements with the initial shock strength were repeated. In some cases, shocks of the same strength were applied repeatedly. Both \( \Delta V_m \) and \( \Delta \text{Ca}^{2+} \) measurements were highly reproducible. Changing the shock polarity resulted in symmetric reversal of \( V_m \) and \( \text{Ca}^{2+} \) responses.

A shock-induced \( \Delta V_m \) was measured as the difference between a linear fit of the plateau phase and the magnitude of the shock response 9 ms after the shock onset and normalized by the action potential amplitude. To measure a shock-induced change in \( \text{Ca}^{2+} \), 2 recordings of \( \text{Ca}^{2+} \) transients taken with and without a shock were normalized according to their levels at the moment preceding the shock. This normalization compensated for changes in optical signals caused by dye washout and photobleaching. The \( \Delta \text{Ca}^{2+} \) was calculated at the shock end (\( t = 9 \) ms) as the difference between the 2 signals and expressed as a percentage of the amplitude of the control \( \text{Ca}^{2+} \) transient (% \( \Delta \text{Ca}^{2+} \)).

An activation time was measured at the level of 50% change of the AP upstroke amplitude. The onset of \( \text{Ca}^{2+} \) transients was measured at the level of 30% change. The difference between the 2 times was taken as the \( \text{V} - \text{Ca}^{2+} \) delay. The durations of AP and \( \text{Ca}^{2+} \) transients were measured as time intervals between their respective onsets and as 50% or 80% levels of signal recovery.

Data were expressed as mean±SD. Differences were compared using the 2-tailed t-test or the 1-way ANOVA test. Results were considered statistically significant if \( P < 0.05 \).

#### Computer Modeling of Shock Effects

Computer simulations were performed in a 1-dimensional cable with an ionic model of adult endocardial rat ventricular myocytes. The Ca-handling system included L_{Ca}, Na-Ca exchange (NCX), sarco-plasmic reticulum (SR), sarcoplasmic pump current (I_{CaP}), and buffering by troponin (Tpn). SR and Tpn buffering were slightly modified according to a previous publication. Full description of ionic model and cable parameters is presented in the online data supplement at http://circres.ahajournals.org. Cable equations were solved numerically using previously described procedures. The cable was stimulated at all nodes simultaneously. After 15 ms of the stimulus, a current was injected at one cable end and the same current was withdrawn from the other end. Such current injection is equivalent to application of extracellular uniform-field shocks.

#### Results

##### Selection of \( \text{Ca}^{2+} \)-Sensitive Dye

Figure 1 illustrates the effects of dye affinity on optical measurements of \( \text{Ca}^{2+} \) transients. Calcium transients (Figure 1A) measured with high-affinity dyes Fluo-4 and Fluo-5F were much longer than those measured with low-affinity Fluo-4FF. The average \( \text{Cao} \) (Figure 1B) measured with Fluo-4 and Fluo-5F (243±29 ms, \( n = 6 \), and 220±15 ms, \( n = 7 \), number of strands) was ~80% longer than with Fluo-4FF (132±10 ms, \( n = 6 \); \( P < 0.001 \)). A further reduction of dye affinity by using Fluo-5N did not reduce \( \text{Cao} \) (141±11 ms, \( n = 7 \)) in comparison to Fluo-4FF. In all series of measurements, AP durations were not significantly different (Figure 1B, right panel). Decreasing the dye affinity caused reduction of the signal-to-noise ratio (Figure 1A).

Contrary to the recovery phase of \( \text{Ca}^{2+} \) transients, the parameters of the rising phase were not dependent on the dye
Optical recordings of Ca\textsuperscript{2+} transients. A, Optical recordings of Ca\textsuperscript{2+} and V\textsubscript{m} in double-stained cell monolayers using analogs of Fluo-4. B, Dependence of Ca\textsuperscript{2+} transient duration (CaD\textsubscript{80}) on dye affinity (left panel). Action potential duration (APD\textsubscript{80}) was similar in these series of measurements (right panel). C, Dependence of Ca\textsuperscript{2+} rise time (left) and V\textsubscript{m}−Ca\textsuperscript{2+} delay (right) on dye affinity. D and E, Ca\textsuperscript{2+} transients (D) and CaD\textsubscript{80} (E) measured with dyes Rhod-2 and Rhod-FF. *P<0.05.

Shock-Induced Changes of Ca\textsuperscript{2+} and V\textsubscript{m}

It was previously shown that depending on the field strength, shocks applied during AP plateau produce nonlinear ΔV\textsubscript{m} of 2 main types, monophasic asymmetric ΔV\textsubscript{m} and biphasic ΔV\textsubscript{m}.

Figure 2 illustrates ΔV\textsubscript{m} of the first type and accompanying Ca\textsuperscript{2+} changes induced by a 10-V/cm shock. The shock caused negative and positive ΔV\textsubscript{m} on the opposite strand edges with a gradual transition of ΔV\textsubscript{m} amplitude in between (Figure 2A). The ΔV\textsubscript{m} distribution was strongly asymmetric with maximal ΔV\textsuperscript{−}\textsubscript{m} (trace 1) being ≈2.5-times larger than maximal ΔV\textsuperscript{+}\textsubscript{m} (trace 7). At the strand edges and at most other locations, polarizations had simple monophasic shapes. A more complex ΔV\textsubscript{m} shape was observed at the boundary between areas of positive and negative polarizations (trace 5), reflecting electrotonic interactions with areas of ΔV\textsuperscript{+}\textsubscript{m} and ΔV\textsuperscript{−}\textsubscript{m}. After the shock, V\textsubscript{m} returned to levels similar to those in the control.

As shown in Figure 2B, a shock of the same strength reduced Ca\textsuperscript{2+} at sites of both negative (traces 1 to 3) and positive polarizations (traces 6, 7). At the intermediate location where ΔV\textsubscript{m} was small (trace 5), ΔCa\textsuperscript{2+} was negligible. Ca\textsuperscript{2+} reduction was larger at sites of negative, rather than at positive, ΔV\textsubscript{m}, with ΔCa\textsuperscript{2+} magnitude being −25% and −11% ACa at sites of maximal ΔV\textsubscript{m} (trace 1) and ΔV\textsuperscript{+}\textsubscript{m} (trace 7), respectively.

After the shock, Ca\textsuperscript{2+} continued to decrease for ≈4 ms at sites of negative ΔV\textsubscript{m}, where ΔCa\textsuperscript{2+} could reach −40%ACa (trace 1). After that, Ca\textsuperscript{2+} began to rise and, at most locations, Ca\textsuperscript{2+} returned to the control level soon after the shock (Figure 2C). A slight postshock increase of peak Ca\textsuperscript{2+} amplitude was observed at some locations (trace 6). Similarly, the duration of Ca\textsuperscript{2+} transients could be slightly prolonged (traces 6, 7). The diastolic level of Ca\textsuperscript{2+} was not changed.

Shock-Induced ΔCa\textsuperscript{2+} and Biphasic ΔV\textsuperscript{−}

Figure 3 illustrates Ca\textsuperscript{2+} changes during a shock (E=29 V/cm) that produced another nonlinear ΔV\textsubscript{m} type. In this case, the negative ΔV\textsubscript{m} waveforms (Figure 3A, traces 1 to 3) were biphasic, exhibiting a strong positive shift after the initial large hyperpolarization. At the same time, the positive ΔV\textsubscript{m} at the cathodal strand edge (trace 7) was not different from the weaker shock.

choice. As shown in Figure 1C, the rise-times of Ca\textsuperscript{2+} transients measured using high-affinity and low-affinity dyes Fluo-4 and Fluo-4FF dyes were 16.3±1.0 ms and 17.5±1.3 ms (n=6, NS), respectively. The respective delays between AP and Ca\textsuperscript{2+} upstrokes were 3.1±0.1 and 3.1±0.3 (n=6, NS).

Similar data were obtained using Rhod dyes. The low-affinity Rhod-FF resulted in markedly shorter Ca\textsuperscript{2+} transients than the high-affinity Rhod-2 (Figure 1D). The CaD\textsubscript{80} values measured by these 2 dyes (Figure 1E) were similar to those measured by Fluo-4FF and Fluo-4, respectively.

These data indicate that the dye affinity plays an important role in optical measurements of Ca\textsuperscript{2+}. Long Ca\textsuperscript{2+} transients measured with high-affinity dyes can be explained by dye saturation (see Discussion). Therefore, in conditions in which in situ dye calibration compensating for this saturation is not available, low-affinity dyes are more appropriate for the faithful reproduction of Ca\textsuperscript{2+} time course. Because Fluo-4FF exhibited a much higher signal-to-noise ratio than Fluo-5N, it was selected for measurements of shock-induced ΔCa\textsuperscript{2+} and ΔV\textsubscript{m} in double-stained preparations described later.
Similar to the effect of the weaker shock, $\Delta \text{Ca}^{2+}$ was transiently reduced at sites of both negative and positive $\Delta V_m$ (Figure 3B), but these changes were smaller than $\Delta \text{Ca}^{2+}$ during the weaker shock. At sites of maximal $\Delta V_m$ and $\Delta V_m^-$, the decreases of $\text{Ca}^{2+}$ were 16% and 5% $\Delta \text{Ca}$, respectively. Another difference was that the duration of $\Delta \text{Ca}^{2+}$ transients was somewhat prolonged in the areas of both positive and negative $\Delta V_m$. A small elevation of the diastolic $\text{Ca}^{2+}$ level was observed, more prominent at the anodal edge of the strand where it was $\sim 12\% \Delta \text{Ca} 300$ ms after the rising phase (Figure 3C, trace 1). In addition, the shock induced an extra beat, with a coupling interval of $\sim 360$ ms.

Shock-induced $V_m$ and $\Delta \text{Ca}^{2+}$ changes were measured in a total of 19 cell strands from 15 cell monolayers. Each shock strength was examined in 7 to 10 strands. In all strands, $V_m$ and $\Delta \text{Ca}^{2+}$ responses of the 2 types described were observed. Thus, all shocks caused $\Delta \text{Ca}^{2+}$ decreases at sites of both $\Delta V_m$ and $\Delta V_m^-$ (Figure 4B). The $\Delta \text{Ca}^{2+}$ magnitude was always larger at $\Delta V_m^-$ sites than at $\Delta V_m^+$ sites. The largest $\sim \Delta \text{Ca}^{2+}$ ($\sim 16\% \Delta \text{Ca}$) were observed at a shock strength of 10 V/cm, and they decreased with increasing shock strength ($P<0.05$, 40-V/cm group versus 10-V/cm group).

The average duration of $\Delta \text{Ca}^{2+}$ transients was increased in a strength-dependent fashion with mean $\Delta \text{CaD}_{\text{Ca}}$ at the strand edges reaching 25% to 30% at 40-V/cm shocks (Figure 4D). The diastolic $\text{Ca}^{2+}$ measured 300 ms after the $\text{Ca}^{2+}$ rising phase was not significantly affected by shocks with strength of 10 V/cm or less (Figure 4C). Increasing the shock strength to 20 V/cm caused elevation of diastolic $\text{Ca}^{2+}$, but the magnitude of this elevation was very small ($\sim 3\%$ to $4\% \Delta \text{Ca}$). Measurements of diastolic $\text{Ca}^{2+}$ changes by shocks stronger than 20 V/cm were precluded in the majority of cases because of occurrences of extra beats with relatively short coupling intervals. In 1 case, when a 30-V/cm shock induced an extra beat with a coupling interval longer than 300 ms, a more substantial elevation of diastolic $\text{Ca}^{2+}$ level was registered, with $\Delta \text{Ca}^{2+}_{\text{dia}}$ measuring 12% and 7% $\Delta \text{Ca}$ at the anodal and cathodal edges, respectively.
Computer Modeling

Figure 5 illustrates effects of shocks in a computer model. A shock caused \( \Delta V_m \) and \( \Delta C_{ai}^{2+} \) that were qualitatively similar to those observed in experiments. Thus, shock-induced polarizations were asymmetric with \( \Delta V_m^+ > \Delta V_m^- \) and \( C_{ai}^{2+} \) was decreased at sites of both \( \Delta V_m^+ \) and \( \Delta V_m^- \) (Figure 5A). \( I_{cal} \) recordings showed that shocks caused rapid inactivation of this current in the \( \Delta V_m^- \) (trace 1), which contributed to \( C_{ai}^{2+} \) decrease at this location. In the \( \Delta V_m^+ \) region, \( I_{cal} \) remained active, but it changed its direction from inward to outward (trace 2), causing removal of \( C_{ai}^{2+} \) ions from the intracellular space.

To examine the roles of various \( C_{ai}^{2+} \) handling pathways in shock-induced \( C_{ai}^{2+} \) decrease, these pathways were selectively inhibited during the shock. As shown in Figure 5B, \( C_{ai}^{2+} \) decrease in \( \Delta V_m^- \) area (site 1) became smaller when NCX or Tpn buffering was disabled (green and blue traces). Inhibition of SR (both release and uptake), \( I_{cald} \) or \( I_{cad} \) did not reduce \( \Delta C_{ai}^{2+} \). In the area of \( \Delta V_m^+ \) (site 2), \( C_{ai}^{2+} \) decrease became smaller after disabling of \( I_{cal} \) or Tpn. Blocking other pathways (NCX, SR, \( I_{soc} \)) produced no or minor effects.

The effects of \( I_{cal} \) inhibition on \( \Delta C_{ai}^{2+} \) was more pronounced when \( I_{cal} \) was partially inhibited during the whole simulation. With \( I_{cal} \) reduced by 60%, a shock caused practically no change in \( C_{ai}^{2+} \) in the \( \Delta V_m^- \) area (Figure 5C).

In these conditions, \( \Delta V_m^+ \) and \( \Delta V_m^- \) became equal. Thus, block of \( I_{cal} \) resulted in elimination of \( \Delta V_m^- \) asymmetry.

With increasing shock strength, \( C_{ai}^{2+} \) decrease became larger in the \( \Delta V_m^- \) area (Figure 5D). In the \( \Delta V_m^- \) area, \( \Delta C_{ai}^{2+} \) dependence was nonmonotonic, with \( \Delta C_{ai}^{2+} \) becoming positive at stronger shocks. This \( \Delta C_{ai}^{2+} \) reversal was eliminated by blocking NCX (not shown), indicating that positive \( \Delta C_{ai}^{2+} \) was caused by inflow of \( C_{ai}^{2+} \) via reversed NCX at large \( \Delta V_m^- \). Within the range of \( \Delta V_m^- \) observed experimentally (<\( 100 \) mV), however, shock-induced \( \Delta C_{ai}^{2+} \) were negative (Figure 5E).

Effects of Nifedipine, Caffeine, and Thapsigargin on Shock-Induced \( \Delta C_{ai}^{2+} \)

The roles of \( I_{cal} \) and SR in \( \Delta C_{ai}^{2+} \) were investigated in cell cultures using drug application. Figure 6 illustrates the effects of 1 \( \mu \)mol/L nifedipine on \( \Delta C_{ai}^{2+} \). Nifedipine completely eliminated the shock-induced \( C_{ai}^{2+} \) decrease at the cathodal side (Figure 6A, traces 1). This effect was reversible (not shown). Similar results were obtained in 7 monolayers. As shown in Figure 6B, nifedipine caused radical reduction of the average cathodal \( \Delta C_{ai}^{2+} \) (\( P<0.05 \) from control), which became not different from zero (NS); the average anodal \( \Delta C_{ai}^{2+} \) was reduced by \( \approx \)37% (\( P<0.05 \)). In 2 monolayers, \( \Delta V_m \) were measured together with \( \Delta C_{ai}^{2+} \). In accordance with a previous publication,15 nifedipine reduced ratio of \( \Delta V_m^- / \Delta V_m^+ \) caused by an increase of \( \Delta V_m^- \) (Figure 6C).

Figure 7 demonstrates the effects of caffeine (10 mmol/L) and thapsigargin (1 \( \mu \)mol/L) on \( \Delta C_{ai}^{2+} \). Although both drugs caused slowing of rising and recovery phases of \( C_{ai}^{2+} \) transients, they did not eliminate shock-induced \( C_{ai}^{2+} \) decrease (Figure 7A and 7B). The average \( \Delta C_{ai}^{2+} \) was not strongly affected by both drugs (Figure 7C and 7D).

Measurements of Shock-Induced \( \Delta C_{ai}^{2+} \) With High-Affinity Dyes

As shown in Figure 1, dye affinity affects measurements of \( C_{ai}^{2+} \) transient duration. To examine whether it also affects measurements of shock-induced \( \Delta C_{ai}^{2+} \), these measurements were repeated using a high-affinity dye Fluo-4. As shown in Figure 8A, use of this dye resulted in measurements of much smaller negative \( \Delta C_{ai}^{2+} \) than those measured with Fluo-4FF. A 9-V/cm shock caused only \( \approx \)5% decrease of \( C_{ai}^{2+} \) at the anodal strand edge (trace 1) and no \( C_{ai}^{2+} \) change at the cathodal edge (trace 2). In addition, Fluo-4 resulted in measurements of larger postshock \( C_{ai}^{2+} \) changes. Thus, the 29-V/cm shock caused elevation of the anodal diastolic \( C_{ai}^{2+} \) level by \( \approx \)65% \( \Delta C_{ai} \) (Figure 8B). Qualitatively similar results were observed in a total of 4 cell monolayers stained with Fluo-4.
Discussion
This study presents high-resolution measurements of Ca\textsuperscript{2+} and V\textsubscript{m} changes caused by electrical shocks in cultured cell monolayers. The main findings are: (1) shocks induced transient decreases of Ca\textsuperscript{2+} in areas of both negative and positive polarizations; (2) the magnitude of the Ca\textsuperscript{2+} decrease had a nonmonotonic dependence on shock strength, decreasing at stronger shocks; this was paralleled with the occurrence of biphasic negative V\textsubscript{m}; and (3) optical measurements of Ca\textsuperscript{2+} changes were strongly dependent on the affinity of the Ca\textsuperscript{2+}-sensitive dye.

Role of Dye Affinity in Ca\textsuperscript{2+} Measurements
It is well-recognized that dye affinity plays an important role in Ca\textsuperscript{2+} measurements.\textsuperscript{22} Although there is no “gold” standard for validation of fluorescent dyes, it is generally agreed that low-affinity dyes are more appropriate for dynamic Ca\textsuperscript{2+} imaging.\textsuperscript{22} High-affinity dyes may misrepresent Ca\textsuperscript{2+} transients because of several factors, including (1) saturation at high Ca\textsuperscript{2+} concentrations generated by cardiac cells and (2) slow dye dissociation from Ca\textsuperscript{2+} ions. Without in situ calibration compensating for dye saturation, using high-affinity dyes may result in “clipping” of the upper portion of Ca\textsuperscript{2+} transients, which can explain long measured Ca\textsuperscript{2+} transient durations (see online supplement for detailed explanation). This mechanism may also exaggerate small Ca\textsuperscript{2+} changes near the resting level, which can explain the large elevation of diastolic Ca\textsuperscript{2+} level measured with high-affinity dyes. The fact that these dyes may underestimate Ca\textsuperscript{2+} changes near the peak of the Ca\textsuperscript{2+} transient contributes to the apparent small shock-induced ΔCa\textsuperscript{2+}.

Shock-Induced ΔCa\textsuperscript{2+} and Relationship With ΔV\textsubscript{m}
This is the first study in which spatio-temporal Ca\textsuperscript{2+} changes were measured and directly related with colocalized V\textsubscript{m} changes during electrical shocks in multicellular cardiac tissue. It was found that shocks of all strengths caused transient decreases of Ca\textsuperscript{2+} in the areas of both negative and positive polarizations. Qualitatively similar results were obtained in a computer model of rat myocytes in which shocks induced asymmetric ΔV\textsubscript{m} and Ca\textsuperscript{2+} decrease at sites of ΔV\textsubscript{m} and ΔV\textsubscript{m} within the range of ΔV\textsubscript{m} observed experimentally. The model allowed evaluating roles of different Ca\textsuperscript{2+}-handling pathways in shock-induced ΔCa\textsuperscript{2+}. Simulations showed that in ΔV\textsubscript{m} areas, Ca\textsuperscript{2+} reduction was caused by inactivation of I\textsubscript{CaL} combined with extrusion of Ca\textsuperscript{2+} ions by NCX and troponin binding. Unexpectedly, SR played no role.
in Ca\(^{2+}\) decrease. This could be because of the fact that SR activity is relatively low in this model; it is responsible for only \(\approx 30\%\) of Ca\(^{2+}\) transient amplitude.

In regions of \(\Delta V_{m}\), I\(_{\text{CaL}}\) remained active but it changed its direction from inward to outward when \(V_{m}\) exceeded the I\(_{\text{CaL}}\) reversal potential causing a reduction of Ca\(^{2+}\) at these locations. This mechanism accounted for a significant portion of shock-induced Ca\(^{2+}\) decrease with the remaining portion being caused by troponin binding. NCX did not play a role in Ca\(^{2+}\) decrease in this area because NCX in this model reverses at potentials above \(\approx 0\) mV.

Experiments in cell cultures supported the important role of I\(_{\text{CaL}}\) in \(\Delta V_{m}\). Thus, inhibition of I\(_{\text{CaL}}\) by nifedipine caused elimination of Ca\(^{2+}\) decrease at the cathodal strand side indicating that the reversed flow of I\(_{\text{CaL}}\) contributed to Ca\(^{2+}\) decrease in this area in control conditions. In accordance with the model, disabling of SR by caffeine and thapsigargin did not affect \(\Delta V_{m}\). This may suggest that, similar to the model,
SR played a negligible role in ΔCa^{2+} in cell cultures, which is consistent with the relatively low level of SR development in neonatal rat myocytes.\textsuperscript{23,24} Alternatively, the lack of drug effects may be explained by their inhibition of both Ca^{2+} release and uptake. This may affect amplitudes of Ca^{2+} transients and ΔCa^{2+} to the same extent, leaving relative ΔCa^{2+} unchanged.

The fact that Ca^{2+} was decreased during shocks at the majority of locations is in apparent contradiction with previous studies in whole hearts reporting an increase of peak Ca^{2+} in response to shocks applied during the absolute refractory period.\textsuperscript{8} Also, no Ca^{2+} decrease during shocks was reported in a study in isolated cells.\textsuperscript{7} These discrepancies may be caused by a combination of several factors. First, previous studies used high-affinity dyes that, because of their saturation and slow dissociation, may underestimate negative ΔCa^{2+} near peak of Ca^{2+} transient. Second, these studies did not assess regional differences in Ca^{2+} changes; according to the present work, ΔCa^{2+} are nonuniform and may remain unchanged during shocks in areas where ΔVm are small. Therefore, the definitive resolution of the discrepancy between this and the previous studies requires spatially and temporally resolved measurements of shock-induced ΔCa^{2+} and ΔVm in whole hearts or whole tissue preparations using low-affinity Ca^{2+} dyes.

It was previously reported that shocks with a strength below the defibrillation threshold applied during the absolute refractory period or during fibrillation can cause a positive inotropic effect, alleviating postshock “pulseless electrical activity.”\textsuperscript{3–5} Two alternative mechanisms were proposed to explain this effect. First, shocks could have a direct effect on myocytes by increasing their peak Ca^{2+}. Second, shocks could affect myocytes indirectly via stimulation of sympathetic intracardiac nerves. The shock strength used in these studies was below the defibrillation threshold, which is estimated at \textasciitilde 5 V/cm.\textsuperscript{25} According to the present study, shocks of such strength should not increase Ca^{2+} directly. Instead, the main effect of 5-V/cm shocks was a decrease of Ca^{2+} during the shocks at sites of both positive and negative ΔVm (Figure 4). There was also a small increase of the Ca^{2+} transient duration (by \textasciitilde 10%), but such an effect is unlikely to cause an increase in peak force. Therefore, these data do not support the hypothesis relating inotropic shock effect with the direct increase of Ca^{2+}. More likely, it is caused by parasympathetic nerve stimulation.

Strong shocks (E \textasciitilde 20 V/cm) that caused biphasic ΔVm produced smaller Ca^{2+} decreases than the weaker shocks that caused monophasic ΔVm. The stronger shocks also often caused a small but statistically significant postshock elevation of Ca^{2+}, which was more prominent in the areas of negative ΔVm (Figure 4). Shocks of such strength were shown to cause membrane electroporation.\textsuperscript{15} Therefore, both Ca^{2+} changes can be explained by shock-induced electroporation causing entry of Ca^{2+} ions via membrane pores. This interpretation is also supported by simulations in a model without membrane electroporation in which increasing shock strength caused larger negative ΔCa^{2+} at sites of ΔV_m. At sites of ΔV_m, ΔCa^{2+} dependence was nonmonotonic, with negative ΔCa^{2+} first increasing and then decreasing because of Ca^{2+} entry via reverse NCX. Because this effect was associated with relatively large positive ΔVm, which were not observed experimentally, it is unlikely that this mechanism played a large role in ΔCa^{2+} in cell cultures.

Shock-induced Ca^{2+} overload has been implicated in the generation of postshock focal arrhythmias\textsuperscript{1} via activation of calcium-dependent inward currents.\textsuperscript{26} The threshold for these arrhythmias in 0.8-mm-wide cultured cell strands is \textasciitilde 20 V/cm.\textsuperscript{1} Although such shocks caused a statistically significant increase of diastolic Ca^{2+}, the change was too small (\textasciitilde 4%) to make a definitive conclusion about possible contribution of such Ca^{2+} changes to arrhythmia generation. It is more likely that Ca^{2+} elevation plays a role in arrhythmias induced by stronger shocks.

Postshock Ca^{2+} elevation was previously described in a study on isolated myocytes.\textsuperscript{7} In that work, Ca^{2+} elevation comparable to the amplitude of Ca^{2+} transients (\textasciitilde 100%ACa) was observed after shocks with strengths of 30 V/cm and higher. Such Ca^{2+} changes are much larger than the diastolic Ca^{2+} changes observed here at comparable shock strengths (Figure 4C). This difference may be partially attributed to the high affinity of Fura-2 used in these experiments. When dye Fluo-4 with a similar affinity was used here, significant postshock elevation of Ca^{2+} was observed after 30-V/cm shocks, also (Figure 7). This and the arguments presented indicate that previous measurements might have substantially overestimated shock-induced elevation of diastolic Ca^{2+}.
Limitations
Ca\textsuperscript{2+} changes were measured in strands of only one width. Because Ca\textsuperscript{2+} changes are mediated by \( V_m \) changes, shock-induced \( \Delta Ca^{2+} \) in strands of other widths can be estimated using the dependence of \( \Delta V_m \) magnitude on the strand width published previously.\textsuperscript{11} There are species- and age-dependent differences in ionic currents and handling of intracellular calcium\textsuperscript{23,24,27} that may affect shock-induced Ca\textsuperscript{2+} changes. On the qualitative level, however, calcium and other ionic currents have similar voltage dependence and kinetic properties in different cell types, indicating that the effects of shocks on Ca\textsuperscript{2+} should be qualitatively similar as well.

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References
Effects of Electrical Shocks on $\text{Ca}^{2+}$ and $V_m$ in Myocyte Cultures

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Cable Model

Computer simulations were performed in a cable model described by the following equation:

\[
C_m \frac{\partial V_m}{\partial t} = \frac{1}{S_v R_i} \frac{\partial^2 V_m}{\partial \lambda^2} - I_i + I_{ex}
\]

where \( V_m \) – membrane potential (mV), \( C_m \) – the specific membrane capacitance (1 \( \mu F/cm^2 \)), \( S_v \) - surface-to-volume ratio (6250 cm\(^{-1} \)), \( R_i \) – the specific intracellular resistivity (500 \( \Omega cm \)), \( I_i \) - ionic current density (\( \mu A/cm^2 \)), \( I_{ex} \) - external current density (\( \mu A/cm^2 \)). Application of uniform-field shocks was modeled by injection of external current, \( I_{ex} \), at one end of the cable and withdrawal of the same current at the other end. The \( I_{ex} \) strength was chosen to generate \( V_m \) changes with magnitudes similar to those observed experimentally.

The cable length was 0.96 mm. The cable was divided into 32 elements with a length of 30 \( \mu m \) and the cable equation was solved numerically\(^1\) using an explicit integration algorithm with temporal step of 0.1 \( \mu s \).

Formulation for ionic current, \( I_i \), was based on the whole-cell ionic model of adult rat endocardial ventricular myocytes\(^2\). Full description of ionic model is presented below. Units: mV (membrane potential), nA (current), \( \mu S \) (conductance), mM (concentration), mM*\( s^{-1} \) (ion flux), s (time constants), \( s^{-1} \) (rate constants).

The Ca-handling system in this model included L-type Ca current (\( I_{CaL} \)) with Ca-dependent inactivation, Na-Ca exchanger (NCX), release and uptake from sarcoplasmic reticulum (SR), sarcolemmal pump current (\( I_{CaP} \)) and intracellular Ca\(^{2+} \) buffering by troponin (Tpn) and calmodulin. Parameters of SR and Tpn buffering were slightly modified according to the previous publication\(^3\).

For use in the cable equation, the whole-cell current was divided by the cell surface area (\( 10^{-4} \) cm\(^2 \)). Specific membrane resistivity obtained by dividing the cell input resistance by the cell surface area was 7132 \( \Omega cm^2 \). Electrotonic space constant calculated from membrane resistance, \( R_i \) and \( S_v \) was 478 \( \mu m \). Thus, the ratio between the cable length and the space constant was approximately 2, which is similar to the ratio between the strand width used in experiments in this work and the space constant value reported previously for cultured cell monolayers (~350 \( \mu m \)).\(^4\)
Ionic current, $I_i$

$$I_i = I_{Na} + I_{CaL} + I_L + I_{Na} + I_I + I_K + I_{Na} + I_{NaCa} + I_{Cap}$$

**Na**$^+$ current, $I_{Na}$

$$I_{Na} = g_{Na}m^3hj(V - E_{Na})$$

$$m = \frac{1}{1 + e^{V + 45.09}/6.5}$$

$$h = j = \frac{1}{1 + e^{V + 70.13}/13.07}$$

$$E_{Na} = \frac{RT}{F} \ln \left(\frac{[Na^+]_o}{[Na^+]}\right)$$

$$\frac{dm}{dt} = \tau_m \left(0.00136 \frac{0.32(V + 47.13)}{1.0 - e^{-0.1(V + 47.13)}} + 0.08e^{V/11}\right)$$

if $V \geq -40$ mV

$$\tau_m = 0.0004537(1.0 + e^{(V+10.60)/11.1})$$

$$\tau_j = 0.01163(1.0 + e^{-0.1(V+32.0)}) \left(e^{-2.335 \times 10^{-17}V}\right)$$

else if $V \leq -40$ mV

$$0.00349 \frac{0.135e^{V+80.00}+3.56e^{0.09V}+3.1 \times 10^4e^{0.35V}}{1.0 + e^0.31(V+79.93)} \left(-1.27140e^{0.2444V}ight.$$  

$$-3.47 \times 10^{-5}e^{0.0491V} + \frac{0.1212e^{-0.00025V}}{1.0 + e^{-0.00025V}(V+40.14)}$$

$L$-type Ca$^{2+}$ current, $I_{CaL}$

$$I_{CaL} = g_{CaL}d\left[0.9 + \frac{C_{inact}}{10.0}\right]f_{t1} + \left(0.1 - \frac{C_{inact}}{10.0}\right)f_{t2}(V - E_{CaL})$$

$$d = \frac{1}{1 + e^{(V+15.3)}/5.0}$$

$$f_{t1} = f_{t2} = \frac{1}{1 + e^{(V+26.7)/5.4}}$$

$$E_{CaL} = 65.0$$

$$\tau_{d} = 0.00305e^{-0.0045(V+7.0)^2} + 0.00105e^{-0.002(V+18.0)} + 0.00025$$

$$\tau_{t1} = 0.105e^{-(V+45.0)/12.0} + \frac{0.04}{(1.0 + e^{(V+55.0)/25.0})}$$

$$+ 0.015 \left(1.0 + e^{(V+75.0)/25.0}\right) + 0.017$$

$$\tau_{t2} = 0.041e^{-(V+47.0)/12.0} + \frac{0.08}{(1.0 + e^{(V+55.0)/25.0})}$$

$$+ 0.015 \left(1.0 + e^{(V+75.0)/25.0}\right) + 0.017$$

$$C_{Ca} = \frac{1.0}{(1.0 + [Ca^{2+}]/0.01)}$$

$$\tau_{Ca} = 0.009$$

$$d\frac{d}{dt} = \frac{d - d}{\tau_{d}}$$

$$d\frac{f_{t1}}{dt} = \frac{f_{t1} - f_{t1}}{\tau_{t1}}$$

$$d\frac{f_{t2}}{dt} = \frac{f_{t2} - f_{t2}}{\tau_{t2}}$$

$$dC_{inact}/dt = \frac{C_{inact} - C_{inact}}{\tau_{Ca}}$$

Ca$^{2+}$-independent transient outward K$^+$ current, $I_i$

$$I_i = g_{K}(as + bs_{slow})(V - E_K)$$

$$\tilde{r} = \frac{1}{1 + e^{-(V+10.60)/-11.42}}$$

$$\tilde{s} = \tilde{s}_{slow} = \frac{1}{1 + e^{(V+45.30)/8.841}}$$

$$\tau_{r} = 45.16e^{0.0337(V+50.0)} + 98.9e^{-0.1(V+38.0)}$$

$$\tau_{s} = 0.55e^{-(V+70.0)/25.0} + 0.049$$

$$\tau_{slow} = 3.3e^{-(V+70.0)/25.0} + 0.049$$

$$\frac{dr}{dt} = \frac{\tilde{r} - r}{\tau_{r}}$$

$$\frac{ds}{dt} = \frac{\tilde{s} - s}{\tau_{s}}$$

$$\frac{ds_{slow}}{dt} = \frac{\tilde{s}_{slow} - s_{slow}}{\tau_{slow}}$$

$$E_K = \frac{RT}{F} \ln \left[\frac{[K^+]_o}{[K^+]_s}\right]$$

$$a = 0.583; \quad b = 0.417$$
Steady-state outward $K^+$ current, $I_{ss}$

$$I_{ss} = g_{Na}(V - E_K)V$$

$$\tau_{ss} = \frac{1}{1 + e^{(V+11.5)/-18.2}}$$

$$\delta_{ss} = \frac{1}{1 + e^{(V+17.5)/10.3}}$$

$$\tau_{ss} = 45.16e^{0.0377(V+50.0)} + 98.9e^{-0.1(V+88.0)}$$

$$\tau_{ss} = 2.1$$

$$\frac{dr_{ss}}{dt} = \frac{r_{ss} - r_{ss}}{\tau_{ss}}$$

$$\frac{ds_{ss}}{dt} = \frac{\delta_{ss} - \delta_{ss}}{\tau_{ss}}$$

Inward rectifier, $I_{K1}$

$$I_{K1} = 48 \left( \frac{1}{(e^{V+37.25} + e^{V+37.25})} + 10 \right) \cdot \frac{0.0001}{1 + e^{(V-E_{K1})-17}}$$

$$g_{K1}(V - E_K - 1.73)$$

$$+ (1 + e^{1.613(V-E_{K1})-17}) \cdot (1 + e^{(K_{1})-0.9885-0.124})$$

Hyperpolarization-activated current, $I_p$

$$I_p = g_{Na}(V - E_{Na}) + g_{K}(V - E_{K})$$

$$y_p = \frac{1}{1 + e^{(V+138.6)/10.48}}$$

$$f_{Na} = 0.2, f_K = 1 - f_{Na}$$

$$\frac{dy}{dt} = \frac{y_s - y}{\tau_y}$$

$$\tau_y = \frac{1}{(0.11885e^{(V+80.00)/28.37} + 0.56236e^{(V+80.00)/14.19})}$$

Background currents

$$I_{BNa} = g_{BNa}(V - E_{Na})$$

$$I_{BK} = g_{BK}(V - E_{K})$$

$$I_{B} = I_{BNa} + I_{B} + I_{BK}$$

$Na^+-K^+$ pump current, $I_{NaK}$

$$I_{NaK} = I_{max} \left( \frac{1.0}{1.0 + 0.1245e^{0.11V_{FR/ST}} + 0.0365e^{0.365V_{FR/ST}}} \right)$$

$$\sigma = e^{[Na^+]/67.3} - 1.0$$

Sarcolemmal $Ca^{2+}$ pump current, $I_{cap}$

$$I_{cap} = I_{max} \left( \frac{[Ca^{2+}]}{[Ca^{2+}]} + 0.00004 \right)$$

$Na^+-Ca^{2+}$ ion exchanger current, $I_{NaCa}$

$$I_{NaCa} = k_{NaCa} \left( \frac{[Na^+][Ca^{2+}]e^{0.03743V_{NaC}}}{[Na^+][Ca^{2+}]e^{0.03743V_{NaC}} + [Na^+][Ca^{2+}]e^{0.03743V_{NaC}}} \right)$$

$Ca^{2+}$ handling mechanisms

Calcium release channel in sarcoplasmic reticulum

$$\frac{dP_{Cl}}{dt} = -k_{p} [Ca^{2+}]P_{cl} + k_{p} P_{o1}$$

$$\frac{dP_{o1}}{dt} = k_{p} [Ca^{2+}]P_{cl} - k_{p} P_{o1} - k_{p} [Ca^{2+}]P_{o1}$$

$$+ k_{p} P_{o2} - k_{p} P_{o1} + k_{p} P_{c2}$$

$$\frac{dP_{o2}}{dt} = k_{p} [Ca^{2+}]P_{o1} - k_{p} P_{o2}$$

$$\frac{dP_{c2}}{dt} = k_{p} P_{o1} - k_{p} P_{c2}$$

$$J_{n} = \nu_{1} (P_{o1} + P_{c2})([Ca^{2+}]_{SR} - [Ca^{2+}]_{o})$$

SERCA2a $Ca^{2+}$ pump

$$f_{b} = ([Ca^{2+}]_{b}/K_{b})^{N_{b}}$$

$$r_{b} = ([Ca^{2+}]_{b}/K_{b})^{N_{b}}$$

$$J_{n} = \nu_{max} f_{b} - \nu_{max} r_{b}$$

$$1 + f_{b} + r_{b}$$

Intracellular and sarcoplasmic reticulum $Ca^{2+}$ fluxes

$$J_{u} = \frac{[Ca^{2+}]_{SR} - [Ca^{2+}]_{SR}}{\tau_{u}}$$

$$J_{a} = \frac{[Ca^{2+}]_{SR} - [Ca^{2+}]_{SR}}{\tau_{x}}$$

$$J_{n} = \frac{d[HTRPNCa]}{dt} + \frac{d[LTRPNCa]}{dt}$$

$$\frac{d[HTRPNCa]}{dt} = k_{max} [Ca^{2+}] ([HTRPNCa]) - k_{max} [HTRPNCa]$$

$$\frac{d[LTRPNCa]}{dt} = k_{max} [Ca^{2+}] ([LTRPNCa]) - k_{max} [LTRPNCa]$$
### Extracellular ion concentrations

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$[K^+]_o$</td>
<td>Extracellular K$^+$ concentration</td>
<td>5.4</td>
</tr>
<tr>
<td>$[Na^+]_o$</td>
<td>Extracellular Na$^+$ concentration</td>
<td>140.0</td>
</tr>
<tr>
<td>$[Ca^{2+}]_o$</td>
<td>Extracellular Ca$^{2+}$ concentration</td>
<td>1.2</td>
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</tbody>
</table>

### Membrane current parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T$</td>
<td>Absolute temperature</td>
<td>295 K</td>
</tr>
<tr>
<td>$g_{Na}$</td>
<td>Maximum conductance for $I_{Na}$</td>
<td>1.064 µS</td>
</tr>
<tr>
<td>$g_{CaL}$</td>
<td>Maximum conductance for $I_{CaL}$</td>
<td>0.031 µS</td>
</tr>
<tr>
<td>$g_{t}$</td>
<td>Maximum conductance for $I_{t}$</td>
<td>0.0163 µS</td>
</tr>
<tr>
<td>$g_{ss}$</td>
<td>Maximum conductance for $I_{ss}$</td>
<td>0.007 µS</td>
</tr>
<tr>
<td>$g_{KL}$</td>
<td>Maximum conductance for $I_{KL}$</td>
<td>0.024 µS</td>
</tr>
<tr>
<td>$g_{BNa}$</td>
<td>Maximum conductance for $I_{BNa}$</td>
<td>8.015e-05 µS</td>
</tr>
<tr>
<td>$g_{BCa}$</td>
<td>Maximum conductance for $I_{BCa}$</td>
<td>3.24e-05 µS</td>
</tr>
<tr>
<td>$g_{BK}$</td>
<td>Maximum conductance for $I_{BK}$</td>
<td>13.8e-05 µS</td>
</tr>
<tr>
<td>$g_{f}$</td>
<td>Maximum conductance for $I_{f}$</td>
<td>0.00145 µS</td>
</tr>
<tr>
<td>$I_{NaK}$</td>
<td>Maximum $I_{NaK}$ current</td>
<td>0.08 nA</td>
</tr>
<tr>
<td>$K_{m,Na}$</td>
<td>Half-maximum Na$^+$ binding constant for $I_{NaK}$</td>
<td>10.0 mM</td>
</tr>
<tr>
<td>$K_{am,K}$</td>
<td>Half-maximum K$^+$ binding constant for $I_{NaK}$</td>
<td>1.5 mM</td>
</tr>
<tr>
<td>$I_{CaP}$</td>
<td>Maximum $I_{CaP}$ current</td>
<td>0.004 nA</td>
</tr>
</tbody>
</table>
Scaling factor for $I_{NaCa}$ & 0.9984e-05 (mM)$^{-4}$
Denominator constant for $I_{NaCa}$ & 0.0001 (mM)$^{-4}$
Position of energy barrier controlling voltage dependence for $I_{NaCa}$ & 0.5

### Parameters of SR and Ca$^{2+}$ buffers

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$v_t$</td>
<td>Maximum RyR (ryanodine) channel Ca$^{2+}$ flux</td>
<td>1.8e03 s$^{-1}$</td>
</tr>
<tr>
<td>$K_{fb}$</td>
<td>Forward half-saturation constant for Ca$^{2+}$-ATPase</td>
<td>0.168e-03 mM</td>
</tr>
<tr>
<td>$K_{rb}$</td>
<td>Backward half-saturation constant for Ca$^{2+}$-ATPase</td>
<td>3.29 mM</td>
</tr>
<tr>
<td>$K_{SR}$</td>
<td>Scaling factor for Ca$^{2+}$-ATPase</td>
<td>1.0</td>
</tr>
<tr>
<td>$N_{fb}$</td>
<td>Forward cooperativity constant for Ca$^{2+}$-ATPase</td>
<td>1.2</td>
</tr>
<tr>
<td>$N_{rb}$</td>
<td>Reverse cooperativity constant for Ca$^{2+}$-ATPase</td>
<td>1.0</td>
</tr>
<tr>
<td>$v_{maxf}$</td>
<td>Ca$^{2+}$-ATP-ase forward rate parameter</td>
<td>0.8131 mM s$^{-1}$</td>
</tr>
<tr>
<td>$v_{maxr}$</td>
<td>Ca$^{2+}$-ATP-ase reverse rate parameter</td>
<td>0.318 s$^{-1}$</td>
</tr>
<tr>
<td>$\tau_{fer}$</td>
<td>Time constant for transfer from NSR to JSR</td>
<td>0.5747e-03 s</td>
</tr>
<tr>
<td>$K_+$</td>
<td>RyR $P_{c1} - P_{o1}$ rate constant</td>
<td>12.15e12 mM$^{-4}$s$^{-1}$</td>
</tr>
<tr>
<td>$K_-$</td>
<td>RyR $P_{o1} - P_{c1}$ rate constant</td>
<td>0.576e03 s$^{-1}$</td>
</tr>
<tr>
<td>$K_+$</td>
<td>RyR $P_{o1} - P_{o2}$ rate constant</td>
<td>4.05e09 mM$^{-3}$s$^{-1}$</td>
</tr>
<tr>
<td>$K_-$</td>
<td>RyR $P_{o2} - P_{o1}$ rate constant</td>
<td>1.930e03 s$^{-1}$</td>
</tr>
<tr>
<td>$K_+$</td>
<td>RyR $P_{o1} - P_{o2}$ rate constant</td>
<td>0.1e03 s$^{-1}$</td>
</tr>
<tr>
<td>$K_-$</td>
<td>RyR $P_{o2} - P_{o1}$ rate constant</td>
<td>0.0008e03 s$^{-1}$</td>
</tr>
<tr>
<td>$n$</td>
<td>RyR Ca$^{2+}$ cooperativity parameter $P_{c1} - P_{o1}$</td>
<td>4</td>
</tr>
<tr>
<td>$m$</td>
<td>RyR Ca$^{2+}$ cooperativity parameter $P_{o1} - P_{o2}$</td>
<td>3</td>
</tr>
<tr>
<td>$[LTRPN]_{tot}$</td>
<td>Total troponin low-affinity site concentration</td>
<td>70e-03 mM</td>
</tr>
<tr>
<td>$[HTRPN]_{tot}$</td>
<td>Total troponin high-affinity site concentration</td>
<td>140e-03 mM</td>
</tr>
<tr>
<td>$K_{trpnm}$</td>
<td>Ca$^{2+}$ on rate for troponin high-affinity sites</td>
<td>20e03 mM$^{-1}$s$^{-1}$</td>
</tr>
<tr>
<td>$K_{trpn}$</td>
<td>Ca$^{2+}$ off rate for troponin high-affinity sites</td>
<td>66.0e-03 s$^{-1}$</td>
</tr>
<tr>
<td>$K_{trpm}$</td>
<td>Ca$^{2+}$ on rate for troponin low-affinity sites</td>
<td>40e03 mM$^{-1}$s$^{-1}$</td>
</tr>
<tr>
<td>$K_{trpn}$</td>
<td>Ca$^{2+}$ off rate for troponin low-affinity sites</td>
<td>0.04e03 s$^{-1}$</td>
</tr>
<tr>
<td>$[CMDN]_{tot}$</td>
<td>Total myoplasm calmodulin concentration</td>
<td>50.0e-03 mM</td>
</tr>
<tr>
<td>$[CSQN]_{tot}$</td>
<td>Total myoplasm calsequestrin concentration</td>
<td>15.0 mM</td>
</tr>
<tr>
<td>$K_{htrpn}$</td>
<td>Ca$^{2+}$ half-saturation constant for calmodulin</td>
<td>2.38e-03 mM</td>
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<tr>
<td>$K_{CSQN}$</td>
<td>Ca$^{2+}$ half-saturation constant for calsequestrin</td>
<td>0.8 mM</td>
</tr>
</tbody>
</table>

### Initial values for state variables

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_m$</td>
<td>Membrane potential</td>
<td>-80.5 mV</td>
</tr>
<tr>
<td>$m$</td>
<td>$I_{Na}$ activation gating variable</td>
<td>4.164108e-03</td>
</tr>
<tr>
<td>$h$</td>
<td>$I_{Na}$ fast inactivation gating variable</td>
<td>6.735613e-01</td>
</tr>
<tr>
<td>$j$</td>
<td>$I_{Na}$ slow inactivation gating variable</td>
<td>6.729362e-01</td>
</tr>
<tr>
<td>$d$</td>
<td>$I_{Ca}$ activation gating variable</td>
<td>2.171081e-06</td>
</tr>
<tr>
<td>$f_{11}$</td>
<td>$I_{Ca}$ fast inactivation gating variable</td>
<td>9.999529e-01</td>
</tr>
<tr>
<td>$f_{12}$</td>
<td>$I_{Ca}$ slow inactivation gating variable</td>
<td>9.999529e-01</td>
</tr>
<tr>
<td>$Ca_{inact}$</td>
<td>Ca$^{2+}$-inactivation gating variable</td>
<td>9.913102e-01</td>
</tr>
<tr>
<td>$r$</td>
<td>$I_{r}$ activation gating variable</td>
<td>2.191519e-03</td>
</tr>
<tr>
<td>$s$</td>
<td>$I_{r}$ fast inactivation gating variable</td>
<td>9.842542e-01</td>
</tr>
<tr>
<td>$s_{slow}$</td>
<td>$I_{r}$ slow inactivation gating variable</td>
<td>6.421196e-01</td>
</tr>
<tr>
<td>$r_{ss}$</td>
<td>$I_{ss}$ activation gating variable</td>
<td>2.907171e-03</td>
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<tr>
<td>$s_{ss}$</td>
<td>$I_{ss}$ inactivation gating variable</td>
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</tr>
<tr>
<td>$y$</td>
<td>$I_{y}$ inactivation gating variable</td>
<td>3.578708e-03</td>
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<tr>
<td>$[Na^+]_i$</td>
<td>Intracellular Na$^+$ concentration</td>
<td>1.073519e+01 mM</td>
</tr>
<tr>
<td>$[K^+]_i$</td>
<td>Intracellular K$^+$ concentration</td>
<td>1.392751e+02 mM</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
<td>Value</td>
</tr>
<tr>
<td>--------</td>
<td>--------------------------------------------------</td>
<td>------------------------------------</td>
</tr>
<tr>
<td>$[Ca^{2+}]_i$</td>
<td>Intracellular Ca$^{2+}$ concentration</td>
<td>7.418e-05 mM</td>
</tr>
<tr>
<td>$[Ca^{2+}]_{NSR}$</td>
<td>NSR Ca$^{2+}$ concentration</td>
<td>6.600742e-02 mM</td>
</tr>
<tr>
<td>$[Ca^{2+}]_{SS}$</td>
<td>Restricted subspace Ca$^{2+}$ concentration</td>
<td>8.737212e-05 mM</td>
</tr>
<tr>
<td>$[Ca^{2+}]_{JSR}$</td>
<td>JSR Ca$^{2+}$ concentration</td>
<td>6.607948e-02 mM</td>
</tr>
<tr>
<td>$P_{C1}$</td>
<td>Fraction of channels in state $P_{C1}$</td>
<td>6.348229e-01</td>
</tr>
<tr>
<td>$P_{o1}$</td>
<td>Fraction of channels in state $P_{o1}$</td>
<td>4.327548e-04</td>
</tr>
<tr>
<td>$P_{o2}$</td>
<td>Fraction of channels in state $P_{o2}$</td>
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<tr>
<td>$P_{C2}$</td>
<td>Fraction of channels in state $P_{C2}$</td>
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</tr>
<tr>
<td>$ltrpn$</td>
<td>Concentration of Ca$^{2+}$-bound low-affinity troponin sites</td>
<td>5.161900e-03 mM</td>
</tr>
<tr>
<td>$htrpn$</td>
<td>Concentration of Ca$^{2+}$-bound high-affinity troponin sites</td>
<td>1.394301e-01 mM</td>
</tr>
</tbody>
</table>
**Role of Dye Affinity in Ca\(^{2+}\) Measurements**

Binding between calcium ions and dye molecules is described by the following equation:

\[
\text{Ca} + \text{D} \leftrightarrow \text{CaD}
\]

where Ca – free calcium ion concentration; D – free dye concentration; CaD – bound dye concentration.

Optical signals are proportional to CaD. For steady-state conditions:

\[
\text{Ca} \times \text{D} = K_d \times \text{CaD}
\]

where \(K_d\) - dissociation constant. If \(D_t\) – total dye concentration (constant), then:

\[
D_t = \text{D} + \text{CaD}
\]

and

\[
\text{CaD} = \frac{\text{Ca} \times D_t}{K_d + \text{Ca}}
\]

Let’s assume that Ca is varied in the range between 0 and 1 and consider two dyes with relatively high and low affinities, for instance, with \(K_d\)=0.3 and 10. The equation given above results in binding curves for these two dyes shown in Figure S1.

![Figure S1](image)

**Figure S1.** Binding curves for high- (A) and low-affinity (B) dyes.

Whereas the low-affinity dye is linear within the specified Ca range, the high-affinity dye has a non-linear response. Without exact dye calibration curves, which are difficult to obtain in a multicellular preparation *in situ*, the high-affinity dye may misrepresent Ca changes.

Figure S2 illustrates how dye affinity may affect measurements of Ca transient duration. Applying binding curves to a test Ca transient (Panel A) results in two different CaD traces for low- and high-affinity dyes (Panel B).
Figure S2. Effect of dye affinity on measurements of Ca transient duration.

Because of its non-linearity, the high-affinity dye (Kd=0.3) reports an abnormally long Ca transient (blue trace). In contrast, the low-affinity dye reproduces the shape of Ca transient correctly. For the same reason, the high-affinity dye can overestimate diastolic Ca elevation. Figure S3 shows a test Ca signal (Panel A) with the post-transient diastolic level elevated by 10%ACa and normalized CaD traces for high- and low-affinity dyes (Panel B).

Figure S3. Effect of dye affinity on measurements of diastolic Ca elevation.

The high-affinity dye reports a diastolic Ca level of ~35%ACa, 3.5-times higher than the actual level (blue trace). At the same time, the low-affinity dye reports it correctly (red trace).

The saturation effect may also contribute to the underestimation of shock-induced ∆Ca\textsuperscript{2+} measured by high-affinity dyes. Figure S4 shows a test Ca signal with simulated ∆Ca of ~27%ACa (Panel A) and normalized CaD traces for high and low affinity dyes (Panel B).
**Figure S4.** Effect of dye affinity on measurements of shock-induced $\Delta Ca$.  

In this case, the high-affinity dye reports $\Delta Ca \sim$3-times smaller than the actual value (blue trace) whereas the low-affinity dye reports $\Delta Ca^{2+}_i$ correctly (red trace).

**References**


