Effects of Electrical Shocks on Ca$_{i}^{2+}$ and V$_{m}$ in Myocyte Cultures

Vladimir G. Fast, Eric R. Cheek, Andrew E. Pollard, Raymond E. Ideker

Abstract—Changes in intracellular calcium concentration (ΔCa$_{i}^{2+}$) induced by electrical shocks may play an important role in defibrillation, but high-resolution ΔCa$_{i}^{2+}$ measurements in a multicellular cardiac tissue and their relationship to corresponding V$_{m}$ changes (ΔV$_{m}$) are lacking. Here, we measured shock-induced ΔCa$_{i}^{2+}$ and ΔV$_{m}$ 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 Ca$_{i}^{2+}$-sensitive dye Fluo-4FF. Shocks (E=5 to 40 V/cm) were applied during the action potential plateau. Shocks caused transient Ca$_{i}^{2+}$ decrease at sites of both negative and positive ΔV$_{m}$. Similar Ca$_{i}^{2+}$ changes were observed in an ionic model of adult rat myocytes. Simulations showed that the Ca$_{i}^{2+}$ decrease at sites of ΔV$_{m}$ was caused by the outward flow of I$_{CaL}$ and troponin binding; at sites of ΔV$_{m}$ it was caused by inactivation of I$_{CaL}$ combined with extrusion by Na–Ca exchanger and troponin binding. The important role of I$_{CaL}$ was supported by experiments in which application of nifedipine eliminated Ca$_{i}^{2+}$ decrease at ΔV$_{m}$ sites. Largest ΔCa$_{i}^{2+}$ were observed during shocks of ~10 V/cm causing simple monophasic ΔV$_{m}$. Shocks stronger than ~20 V/cm caused smaller ΔCa$_{i}^{2+}$ and postshock elevation of diastolic Ca$_{i}^{2+}$. This was paralleled with occurrence of biphasic negative ΔV$_{m}$ that indicated membrane electroporation. Thus, these data indicate that shocks transiently decrease Ca$_{i}^{2+}$ at sites of both ΔV$_{m}$ and ΔV$_{m}$. Outward flow of I$_{CaL}$ plays an important role in Ca$_{i}^{2+}$ decrease in the ΔV$_{m}$ areas. Very strong shocks caused smaller negative ΔCa$_{i}^{2+}$ and postshock elevation of diastolic 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 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 Ca$_{i}^{2+}$ concentration caused by a direct effect of the shock on myocytes.

The direct assessment of the mechanisms of shock-Ca$_{i}^{2+}$ interaction and its role in defibrillation requires measurements of shock-induced Ca$_{i}^{2+}$ and V$_{m}$ changes with high spatial and temporal resolution. Previously, shock-induced Ca$_{i}^{2+}$ changes were measured in single myocytes and at a single point in whole hearts. No spatially resolved data on shock-induced 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 ΔV$_{m}$ in the heart. It is well established that shocks produce highly nonuniform patterns of ΔV$_{m}$ with areas of positive, negative, or negligible polarizations present in different parts of cardiac tissue, suggesting that 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 ΔV$_{m}$ of 2 main types. Shocks of moderate strength induce asymmetric ΔV$_{m}$, with negative ΔV$_{m}$ being much larger than positive ΔV$_{m}$. Stronger shocks can induce ΔV$_{m}$ of another type, which is characterized by a nonmonotonic behavior of negative ΔV$_{m}$ in which strong hyperpolarization is followed by a return of V$_{m}$ to more positive levels. In cell cultures, the transition from one nonlinear ΔV$_{m}$ type to another with increasing shock...
strength was paralleled by the occurrence of postshock arrhythmias originating from the area of shock-induced negative polarization\(^1\) as well as with membrane electroporation in the same areas.\(^{13}\) 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,\(^{11,16}\) 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.\(^{17}\) Neonatal rat myocytes were obtained from 1- to 2-day-old Wistar rats and cultured according to the previously published procedures.\(^{11}\) Cell cultures were incubated in UltraCulture medium (BioWhittaker) supplemented with 20 \(\mu\)g/mL vitamin B\(_{12}\), antibiotics, 0.1 mmol/L of bromodeoxyuridine, and 0.5 mmol/L of epinephrine at 37°C in a humidified atmosphere containing 4% CO\(_2\). Culture medium was exchanged on the next day before incubating with a dye solution for 30 to 45 minutes. After that, cells were transfected into experimental chamber and superfused with Hanks solution (Life Technologies) having a pH of 7.4 and a temperature of 36°C.

Previous \(\text{Ca}^{2+}\) measurements using dye Fluo-3 demonstrated very long durations of \(\text{Ca}^{2+}\) transients (CaD).\(^{18}\) We hypothesized that such long CaD were caused by the high affinity of Fluo-3 to \(\text{Ca}^{2+}\) ions. Because this can affect measurements of shock-induced \(\Delta \text{Ca}^{2+}\), we examined dyes with different affinities to \(\text{Ca}^{2+}\) ions from 2 major groups. The first group included Fluo-3 analogs: Fluo-4, Fluo-5F, Fluo-4FF, and Fluo-5N (Molecular Probes) that have nominal excitation wavelength of 480/40 nm and emitted light was measured at \(>560\) 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\(^{18}\) 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\(^{17}\) 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 were applied to the values of \(\sim 5, 10, 20, 30,\) and \(40 \text{ V/cm}\) 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.\(^{1}\) 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.\(^{16}\) 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 (\% ACa).

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 \(V_m-\text{Ca}^{2+}\) 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.\(^{19}\) The Ca-handling system included \(I_{\text{NCX}}\), Na-Ca exchange (NCX), sarcoplasmic reticulum (SR), sarcollemmal pump current (\(I_{\text{CaP}}\)), and buffering by troponin (Tpn). SR and Tpn buffering were slightly modified according to a previous publication.\(^{20}\) Full description of the 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.\(^{21}\) 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 \(\text{CaD}_{\text{sto}}\) (Figure 1B) measured with Fluo-4 and Fluo-5F (243±29 ms, \(n=6\), and 220±15 ms, \(n=7\), number of strands) was \(\sim 80\%\) 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 \(\text{CaD}_{\text{sto}}\) (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...
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.

**Shock-Induced Ca\textsuperscript{2+} Changes and Monophasic Asymmetric ΔV\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\textsuperscript{−}\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.
Similar to the effect of the weaker shock, Ca\textsuperscript{2+} was transiently reduced at sites of both negative and positive \( \Delta V_m \) (Figure 3B), but these changes were smaller than \( \Delta \text{Ca}_{\text{a}}^{2+} \) during the weaker shock. At sites of maximal \( \Delta V_m^+ \) and \( \Delta V_m^- \), the decreases of Ca\textsuperscript{2+} were 16\% and 5\% ACa, respectively. Another difference was that the duration of Ca\textsuperscript{2+} transients was somewhat prolonged in the areas of both positive and negative \( V_m \). A small elevation of the diastolic Ca\textsuperscript{2+} level was observed, more prominent at the anodal edge of the strand where it was \( \approx 12\% \) ACa 300 ms after the rising phase (Figure 3C, trace 1). In addition, the shock induced an extra beat, with a coupling interval of \( \approx 360 \) ms.

Shock-induced \( V_m \) and Ca\textsuperscript{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 Ca\textsuperscript{2+} responses of the 2 types described were observed. Thus, all shocks caused Ca\textsuperscript{2+} decreases at sites of both \( \Delta V_m^+ \) and \( \Delta V_m^- \) (Figure 4B). The \( \Delta \text{Ca}_{\text{a}}^{2+} \) magnitude was always larger at \( \Delta V_m^- \) sites than at \( \Delta V_m^+ \) sites. The largest \( \Delta \text{Ca}_{\text{a}}^{2+} \) (\( \approx 16\% \) ACa) 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 Ca\textsuperscript{2+} transients was increased in a strength-dependent fashion with mean \( \Delta \text{CaD}_{\text{a}} \) at the strand edges reaching 25\% to 30\% at 40-V/cm shocks (Figure 4D). The diastolic Ca\textsuperscript{2+} measured 300 ms after the Ca\textsuperscript{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 Ca\textsuperscript{2+}, but the magnitude of this elevation was very small (\( \approx 3\% \) to 4\% ACa). Measurements of diastolic Ca\textsuperscript{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 Ca\textsuperscript{2+} level was registered, with \( \Delta \text{CaD}_{\text{a}, \text{an}} \), measuring 12\% and 7\% ACa at the anodal and cathodal edges, respectively.
Cai
c
tively inhibited during the shock. As shown in Figure 5B, CaD50 was measured at the 50% level of Ca i

outward (trace 2), causing removal of Ca2 + that were qualitatively similar to those observed in experiments. Thus, shock-induced po-

tude. B, C, and D, Plots of mean ΔCa2+ end, ΔCa2+ dia, and ΔCaD50 as functions of shock strength, respectively. The number of

measurements (number of cell strands) for each shock strength was 7 to 10. *P<0.05 from zero.

Computer Modeling

Figure 4. Dependence of ΔCa2+ on shock strength. A, Definition of end-shock (ΔCa2+ end), diastolic (ΔCa2+ dia) Ca2+ changes as well as changes of Ca2+ transient duration (ΔCaD). ΔCa2+ end was measured 9 ms after the shock onset; ΔCa2+ dia was measured 300 ms after the Ca2+ transient rising phase; ΔCaD was measured at the 50% level of Ca2+ transient amplitude. B, C, and D, Plots of mean ΔCa2+ end, ΔCa2+ dia and ΔCaD50 as functions of shock strength, respectively. The number of measurements (number of cell strands) for each shock strength was 7 to 10. *P<0.05 from zero.

Figure 5 illustrates effects of shocks in a computer model. A shock caused ΔV m and ΔCa2+ that were qualitatively similar to those observed in experiments. Thus, shock-induced po-
larizations were asymmetric with ΔV m< ΔV + m and Ca2+ was decreased at sites of both ΔV m and ΔV + m (Figure 5A). I CaL recordings showed that shocks caused rapid inactivation of this current in the ΔV m area (trace 1), which contributed to Ca2+ decrease at this location. In the ΔV + m region, I CaL remained active, but it changed its direction from inward to outward (trace 2), causing removal of Ca2+ ions from the intracellular space.

To examine the roles of various Ca2+ handling pathways in shock-induced Ca2+ decrease, these pathways were selec-
tively inhibited during the shock. As shown in Figure 5B, Ca2+ decrease in ΔV 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 CaL, or I CaP did not reduce ΔCa2+. In the area of ΔV + m (site 2), Ca2+ decrease became smaller after disabling of I CaL or Tpn. Blocking other pathways (NCX, SR, I CaP) produced no or minor effects.

The effect of I CaL inhibition on ΔCa2+ 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 Ca2+ in the ΔV m area (Figure 5C).

In these conditions, ΔV m + and ΔV m became equal. Thus, block of I CaL resulted in elimination of ΔV m asymmetry.

With increasing shock strength, Ca2+ decrease became larger in the ΔV m area (Figure 5D). In the ΔV + m area, ΔCa2+ dependence was nonmonotonic, with ΔCa2+ becoming positive at stronger shocks. This ΔCa2+ reversal was eliminated by blocking NCX (not shown), indicating that positive ΔCa2+ was caused by inflow of Ca2+ via reversed NCX at large ΔV + m. Within the range of ΔV m observed experimentally (<≈100 mV), however, shock-induced ΔCa2+ were negative (Figure 5E).

Effects of Nifedipine, Caffeine, and Thapsigargin on Shock-Induced ΔCa2+

The roles of I CaL and SR in ΔCa2+ were investigated in cell cultures using drug application. Figure 6 illustrates the effects of 1 μmol/L nifedipine on ΔCa2+. Nifedipine completely eliminated the shock-induced Ca2+ 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 ΔCa2+ (P<0.05 from control), which became not different from zero (NS); the average anodal ΔCa2+ was reduced by ≈37% (P<0.05). In 2 monolayers, ΔV m were measured together with ΔCa2+. In accordance with a previous publication,15 nifedipine reduced ratio of ΔV m+/ ΔV m caused by an increase of ΔV m (Figure 6C).

Figure 7 demonstrates the effects of caffeine (10 mmol/L) and thapsigargin (1 μmol/L) on ΔCa2+. Although both drugs caused slowing of rising and recovery phases of Ca2+ transients, they did not eliminate shock-induced Ca2+ decrease (Figure 7A and 7B). The average ΔCa2+ was not strongly affected by both drugs (Figure 7C and 7D).

Measurements of Shock-Induced ΔCa2+ With High-Affinity Dyes

As shown in Figure 1, dye affinity affects measurements of Ca2+ transient duration. To examine whether it also affects measurements of shock-induced ΔCa2+, 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 ΔCa2+ than those measured with Fluo-4FF. A 9-V/cm shock caused only ≈5% decrease of Ca2+ at the anodal strand edge (trace 1) and no Ca2+ change at the cathodal edge (trace 2). In addition, Fluo-4 resulted in measurements of larger postshock Ca2+ changes. Thus, the 29-V/cm shock caused elevation of the anodal diastolic Ca2+ level by ≈65% ACa (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+}/H\textsubscript{11001} 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+}/H\textsubscript{11001} in areas of both positive and negative polarizations; computer simulations and experiments with channel blockers indicate that ICaL played an important role in Ca\textsuperscript{2+}/H\textsubscript{11001} decrease; (2) the magnitude of Ca\textsuperscript{2+}/H\textsubscript{11001} 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+}/H\textsubscript{11001} changes were strongly dependent on the affinity of the Ca\textsuperscript{2+}-sensitive dye.

Role of Dye Affinity in Ca\textsuperscript{2+}/H\textsubscript{11001} Measurements

It is well-recognized that dye affinity plays an important role in Ca\textsuperscript{2+}/H\textsubscript{11001} 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+}/H\textsubscript{11001} imaging.\textsuperscript{22} High-affinity dyes may misrepresent Ca\textsuperscript{2+}/H\textsubscript{11001} 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+}/H\textsubscript{11001} transient durations (see online supplement for detailed explanation). This mechanism may also exaggerate small Ca\textsuperscript{2+}/H\textsubscript{11001} changes near the resting level, which can explain the large elevation of diastolic Ca\textsuperscript{2+}/H\textsubscript{11001} level measured with high-affinity dyes. The fact that these dyes may underestimate Ca\textsuperscript{2+}/H\textsubscript{11001} changes near the peak of the Ca\textsuperscript{2+}/H\textsubscript{11001} transient contributes to the apparent small shock-induced ΔCa\textsuperscript{2+}/H\textsubscript{11001}.

Shock-Induced ΔCa\textsuperscript{2+}/H\textsubscript{11001} and Relationship With ΔV\textsubscript{m}

This is the first study in which spatio-temporal Ca\textsuperscript{2+}/H\textsubscript{11001} 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+}/H\textsubscript{11001} 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+}/H\textsubscript{11001} 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+}/H\textsubscript{11001}-handling pathways in shock-induced ΔCa\textsuperscript{2+}/H\textsubscript{11001}. Simulations showed that in ΔV\textsuperscript{m} areas, Ca\textsuperscript{2+}/H\textsubscript{11001} reduction was caused by inactivation of ICaL, combined with extrusion of Ca\textsuperscript{2+} ions by NCX and troponin binding. Unexpectedly, SR played no role...
in Ca\textsuperscript{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\textsuperscript{2+} transient amplitude.

In regions of ΔV\textsubscript{m}, I\textsubscript{CaL} remained active but it changed its direction from inward to outward when V\textsubscript{m} exceeded the I\textsubscript{CaL} reversal potential causing a reduction of Ca\textsuperscript{2+} at these locations. This mechanism accounted for a significant portion of shock-induced Ca\textsuperscript{2+} decrease with the remaining portion being caused by troponin binding. NCX did not play a role in Ca\textsuperscript{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\textsubscript{CaL} in ΔCa\textsuperscript{2+}. Thus, inhibition of I\textsubscript{CaL} by nifedipine caused elimination of Ca\textsuperscript{2+} decrease at the cathodal strand side indicating that the reversed flow of I\textsubscript{CaL} contributed to Ca\textsuperscript{2+} decrease in this area in control conditions. In accordance with the model, disabling of SR by caffeine and thapsigargin did not affect ΔCa\textsuperscript{2+}. This may suggest that, similar to the model,
SR played a negligible role in $\Delta \text{Ca}^{2+}$ in cell cultures, which is consistent with the relatively low level of SR development in neonatal rat myocytes,$^{23,24}$ Alternatively, the lack of drug effects may be explained by their inhibition of both $\text{Ca}^{2+}$ release and uptake. This may affect amplitudes of $\text{Ca}^{2+}$ transients and $\Delta \text{Ca}^{2+}$ to the same extent, leaving relative $\Delta \text{Ca}^{2+}$ unchanged.

The fact that $\text{Ca}^{2+}$ was decreased during shocks at the majority of locations is in apparent contradiction with previous studies in whole hearts reporting an increase of peak $\text{Ca}^{2+}$ in response to shocks applied during the absolute refractory period.$^6$ Also, no $\text{Ca}^{2+}$ decrease during shocks was reported in a study in isolated cells.$^7$ 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 $\Delta \text{Ca}^{2+}$ near peak of $\text{Ca}^{2+}$ transient. Second, these studies did not assess regional differences in $\text{Ca}^{2+}$ changes; according to the present work, $\Delta \text{Ca}^{2+}$ are nonuniform and may remain unchanged during shocks in areas where $\Delta V_m$ are small. Therefore, the definitive resolution of the discrepancy between this and the previous studies requires spatially and temporally resolved measurements of shock-induced $\Delta \text{Ca}^{2+}$ and $\Delta V_m$ in whole hearts or whole tissue preparations using low-affinity $\text{Ca}^{2+}$ 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.”$^{3-5}$ Two alternative mechanisms were proposed to explain this effect. First, shocks could have a direct effect on myocytes by increasing their peak $\text{Ca}^{2+}$. 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 $\approx 5$ V/cm.$^{25}$ According to the present study, shocks of such strength should not increase $\text{Ca}^{2+}$ directly. Instead, the main effect of 5 V/cm shocks was a decrease of $\text{Ca}^{2+}$ during the shocks at sites of both positive and negative $\Delta V_m$ (Figure 4). There was also a small increase of the $\text{Ca}^{2+}$ transient duration (by $\approx 10\%$), 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 $\text{Ca}^{2+}$. More likely, it is caused by parasympathetic nerve stimulation.

Strong shocks (E $\approx 20$ V/cm) that caused biphasic $\Delta V_m$ produced smaller $\text{Ca}^{2+}$ decreases than the weaker shocks that caused monophasic $\Delta V_m$. The stronger shocks also often caused a small but statistically significant postshock elevation of $\text{Ca}^{2+}$, which was more prominent in the areas of negative $\Delta V_m$ (Figure 4). Shocks of such strength were shown to cause membrane electroporation.$^{15}$ Therefore, both $\text{Ca}^{2+}$ changes can be explained by shock-induced electroporation causing entry of $\text{Ca}^{2+}$ 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 $\Delta \text{Ca}^{2+}$ at sites of $\Delta V_m$. At sites of $\Delta V_m$, $\Delta \text{Ca}^{2+}$ dependence was nonmonotonic, with negative $\Delta \text{Ca}^{2+}$ first increasing and then decreasing because of $\text{Ca}^{2+}$ entry via reverse NCX. Because this effect was associated with relatively large positive $\Delta V_m$ which were not observed experimentally, it is unlikely that this mechanism played a large role in $\Delta \text{Ca}^{2+}$ in cell cultures.

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

Postshock $\text{Ca}^{2+}$ elevation was previously described in a study on isolated myocytes.$^7$ In that work, $\text{Ca}^{2+}$ elevation comparable to the amplitude of $\text{Ca}^{2+}$ transients ($\approx 100\%\Delta \text{Ca}$) was observed after shocks with strengths of 30 V/cm and higher. Such $\text{Ca}^{2+}$ changes are much larger than the diastolic $\text{Ca}^{2+}$ 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 $\text{Ca}^{2+}$ 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 $\text{Ca}^{2+}$.

![Figure 8. Shock-induced $\Delta \text{Ca}^{2+}$ measured with high-affinity dye Fluo-4. A. $\text{Ca}^{2+}$ transients with shocks of different strength in comparison to control recordings (black traces) at anodal (site 1) and cathodal (site 2) strand edges. B. Corresponding longer $\text{Ca}^{2+}$ recordings. The arrow indicates apparent elevation of the diastolic $\text{Ca}^{2+}$ level.](http://circres.ahajournals.org/lookup/fig/0/4/5/8/0/1)
Limitations
Ca$_{2+}$ changes were measured in strands of only one width. Because Ca$_{2+}$ 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. There are species- and age-dependent differences in ionic currents and handling of intracellular calcium that may affect shock-induced Ca$_{2+}$ 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$_{2+}$ should be qualitatively similar as well.

Acknowledgments
This work was supported by NIH Grants HL67748 and HL67961 and AHA Grant 0255025B. We thank Reuben Collins for help with preparation of cell cultures.

References
Effects of Electrical Shocks on $\text{Ca}^{2+}_{i}$ and $V_m$ in Myocyte Cultures
Vladimir G. Fast, Eric R. Cheek, Andrew E. Pollard and Raymond E. Ideker

_Circ Res._ 2004;94:1589-1597; originally published online May 20, 2004;
doi: 10.1161/01.RES.0000132746.94360.8b

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circres.ahajournals.org/content/94/12/1589

Data Supplement (unedited) at:
http://circres.ahajournals.org/content/suppl/2004/06/14/94.12.1589.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation Research_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the _Permissions and Rights Question and Answer_ document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation Research_ is online at:
http://circres.ahajournals.org/subscriptions/
Cable Model

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

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

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 \( \text{Ca}^{2+} \) 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, $I_i$

\[ I_i = I_{Na} + I_{Ca} + I_1 + I_{Na} + I_{K} + I_{Ca} - I_{Na} + I_{NaCa} + I_{CaP} \]

**Na⁺ current, $I_{Na}$**

\[ I_{Na} = g_{Na} m^3 h j (V - E_{Na}) \]

\[ \dot{m} = \frac{1}{1 + e^{(V + 45.0)/6.5}} \]

\[ \dot{h} = \frac{1}{1 + e^{(V + 78.13)/6.07}} \]

\[ E_{Na} = \frac{RT}{F} \ln \frac{[Na^+]_o}{[Na^+]} \]

\[ \frac{dm}{dt} = \frac{\dot{m} - m}{\tau_m} \]

\[ \frac{dh}{dt} = \frac{\dot{h} - h}{\tau_h} \]

\[ \frac{dj}{dt} = \frac{\dot{j} - j}{\tau_j} \]

\[ \tau_m = \frac{0.00136}{0.32(V + 47.13) + 0.08e^{V/11}} \]

if $V \geq -40$ mV

\[ \tau_h = 0.0004537(1.0 + e^{(V + 10.0)/11.1}) \]

\[ \tau_j = 0.01163(1.0 + e^{-0.1(V + 32.0)}) \]

else if $V \leq -40$ mV

\[ \tau_h = 0.0349 \]

\[ \tau_j = 0.00349 \]

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

\[ I_{Ca} = g_{Ca} d \left[ \left( 0.9 + \frac{Ca_{inact}}{10.0} \right) f_{11} + \left( 0.1 - \frac{Ca_{inact}}{10.0} \right) f_{12} \right] (V - E_{Ca}) \]

\[ \dot{d} = \frac{1}{1 + e^{(V + 15.0)/5.0}} \]

\[ \dot{f}_{11} = \frac{1}{1 + e^{(V + 26.9)/5.4}} \]

$E_{Ca} = 65.0$

$K^+$ current, $I_K$

\[ I_K = 0.00305e^{-0.0045(V + 7.0)^2} + 0.00105e^{-0.002(V - 18.0)} + 0.00025 \]

\[ \tau_{f1} = 0.105e^{-((V + 45.0)/12.0)^3} \frac{0.04}{(1.0 + e^{(V + 25.0)/25.0}) + 0.017} \]

\[ \tau_{f2} = 0.041e^{-((V + 47.0)/12.0)^3} \frac{0.08}{(1.0 + e^{(V + 55.0)/25.0}) + 0.017} \]

\[ \tau_{Ca_{inact}} = \frac{1.0}{(1.0 + [Ca^{2+}]_o/0.01)} \]

\[ \tau_{Ca_{inact}} = 0.009 \]

\[ \frac{dd}{dt} = \frac{\dot{d} - d}{\tau_d} \]

\[ \frac{df_{11}}{dt} = \frac{\dot{f}_{11} - f_{11}}{\tau_{f1}} \]

\[ \frac{df_{12}}{dt} = \frac{\dot{f}_{12} - f_{12}}{\tau_{f2}} \]

\[ \frac{dCa_{inact}}{dt} = \frac{Ca_{inact} - Ca_{inact}}{\tau_{Ca_{inact}}} \]

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

\[ I_r = g_r (a_{s} + b_{slow}) (V - E_K) \]

\[ \tau_r = \frac{1}{1 + e^{(V + 10.0)/11.42}} \]

\[ \tau_{slow} = \frac{1}{1 + e^{(V + 45.35)/8.884}} \]

\[ \tau_{slow} = 1.0 \]

\[ \tau_{inact} = 0.55e^{-((V + 70.0)/25.0)^2} + 0.049 \]

\[ \tau_{slow} = 3.3e^{-((V + 70.0)/25.0)^2} + 0.049 \]

\[ \frac{dr}{dt} = \frac{\dot{r} - r}{\tau_r} \]

\[ \frac{ds}{dt} = \frac{\dot{s} - s}{\tau_s} \]

\[ \frac{ds_{slow}}{dt} = \frac{\dot{s}_{slow} - s_{slow}}{\tau_{slow}} \]

\[ E_K = \frac{RT}{F} \ln \frac{[K^+]_o}{[K^+]_i} \]

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

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

$$\tilde{r}_s = \frac{1}{1 + e^{(V+11.5)/-18.2}}$$

$$\tilde{w}_s = \frac{1}{1 + e^{(V+87.5)/10.3}}$$

$$10.0$$

$$\tau_{v_s} = 45.16e^{-0.03757(V+93.0)} + 98.9e^{0.1(V+38.0)}$$

$$\tau_{w_s} = 2.1$$

$$\frac{dr_{ss}}{dt} = \frac{\tilde{r}_s - r_{ss}}{\tau_{v_s}}$$

$$\frac{dw_{ss}}{dt} = \frac{\tilde{w}_s - w_{ss}}{\tau_{w_s}}$$

Inward rectifier, $I_{K1}$

$$I_{K1} = \left[ \frac{48}{(e^{(V+37.25)} + e^{(V+37.25-25)}) + 10} \right]$$

$$\cdot \left[ \frac{0.0001}{1 + e^{(V-E_K-17.3) / 19}} \right]$$

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

$$+ \frac{g_{K1}(V - E_K - 1.73)(1 + e^{(V+138.6)/10.48})}{(1 + e^{-1.6138(V+138.6)/1.73)}) \cdot (1 + e^{(V-E_K-0.9888)/0.124})$$

Hyperpolarization-activated current, $I_r$

$$I_r = g_r[y_{Na}(V - E_{Na}) + f_K(V - E_K)]$$

$$y_r = \frac{1}{1 + e^{(V+11.5)/10.48}}$$

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

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

$$\tau_y = \frac{1}{0.11885e^{(V+80.05)/28.37} + 0.56236e^{(V+80.05)/14.19}}$$

Background currents

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

$$I_{BK} = g_{BK}(V - E_K)$$

$$I_{BCa} = g_{BCa}(V - E_{Ca})$$

$$I_B = I_{BNa} + I_{BCa} + I_{BK}$$

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

$$I_{NaK} = I_{NaK}\left( \frac{1}{1.0 + 0.1245e^{0.1V_{RT}} + 0.0365\sigma e^{0.1V_{RT}}} \right)$$

$$\cdot \left( \frac{[K^+]_{o}^n + k_{n,K}}{[K^+]_{o}} \right)$$

$$\sigma = e^{(V_{Na}-107.3) / 7.0} - 1.0$$

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

$$I_{CaP} = I_{CaP}\left( \frac{[Ca^{2+}]_{o}^n}{[Ca^{2+}]_{o} + k_{o,Ca}} + 0.00004 \right)$$

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

$$I_{NaCa} = k_{NaCa}\left( \frac{[Ca^{2+}]_{o}^n [Ca^{2+}]_{o}^{0.03743(y_{NaCa})}}{[Na^+]_{o} [Na^+]_{o}^{0.03743(y_{NaCa})+1}}} \right)$$

$$+ (1 + d_{NaCa} [Na^+]_{o}^{[Ca^{2+}]_{o}^n} + [Na^+]_{o} [Ca^{2+}]_{o}^{n})$$

$Ca^{2+}$ handling mechanisms

Calcium release channel in sarcoplasmic reticulum

$$\frac{dP_{C1}}{dt} = -k_1^{*} [Ca^{2+}]^n P_{C1} + k_2 P_{o1}$$

$$\frac{dP_{o1}}{dt} = k_1^{*} [Ca^{2+}]^n P_{C1} - k_1 P_{o1} - k_2 P_{o1} + k_2 P_{o2}$$

$$\frac{dP_{o2}}{dt} = k_1^{*} [Ca^{2+}]^n P_{o1} - k_2 P_{o2}$$

$$\frac{dP_{C2}}{dt} = k_2 P_{o1} - k_2 P_{C2}$$

$$J_{rel} = v_r (P_{o1} + P_{o2}) ([Ca^{2+}]_{SR} - [Ca^{2+}]_{o})$$

SERCA2a $Ca^{2+}$ pump

$$f_b = \frac{([Ca^{2+}]_{o}/K_{fb})^n_{NSR}}{r_b = ([Ca^{2+}]_{NSR}/K_{rb})^n_{NSR}}$$

$$J_{up} = K_{SR} - f_b - r_b$$

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

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

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

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

$$\frac{d[HTRPNCa]}{dt} = k_{up}[Ca^{2+}] ([HTRPNCa]_{tot}$$

$$- [HTRPNCa] - k_{down}[HTRPNCa]$$

$$\frac{d[LTRPNCa]}{dt} = k_{up}[Ca^{2+}] ([LTRPNCa]_{tot}$$

$$- [LTRPNCa] - k_{down}[LTRPNCa]$$
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>
</tr>
</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 (\mu S)</td>
</tr>
<tr>
<td>(g_{CaL})</td>
<td>Maximum conductance for (I_{CaL})</td>
<td>0.031 (\mu S)</td>
</tr>
<tr>
<td>(g_t)</td>
<td>Maximum conductance for (I_t)</td>
<td>0.0163 (\mu S)</td>
</tr>
<tr>
<td>(g_{ss})</td>
<td>Maximum conductance for (I_{ss})</td>
<td>0.007 (\mu S)</td>
</tr>
<tr>
<td>(g_{KL})</td>
<td>Maximum conductance for (I_{KL})</td>
<td>0.024 (\mu S)</td>
</tr>
<tr>
<td>(g_{BNa})</td>
<td>Maximum conductance for (I_{BNa})</td>
<td>8.015e-05 (\mu S)</td>
</tr>
<tr>
<td>(g_{BCa})</td>
<td>Maximum conductance for (I_{BCa})</td>
<td>3.24e-05 (\mu S)</td>
</tr>
<tr>
<td>(g_{BK})</td>
<td>Maximum conductance for (I_{BK})</td>
<td>13.8e-05 (\mu S)</td>
</tr>
<tr>
<td>(g_f)</td>
<td>Maximum conductance for (I_f)</td>
<td>0.00145 (\mu S)</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_{m,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>
\( k_{NaCa} \) Scaling factor for \( I_{NaCa} \) 0.9984e-05 (mM)^{-4}

\( d_{NaCa} \) Denominator constant for \( I_{NaCa} \) 0.0001 (mM)^{-4}

\( Y_{NaCa} \) 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_{c+} )</td>
<td>RyR ( P_{c1} - P_{o1} ) rate constant</td>
<td>12.15e12 mM(^{-4})s(^{-1})</td>
</tr>
<tr>
<td>( K_{c-} )</td>
<td>RyR ( P_{o1} - P_{c1} ) rate constant</td>
<td>0.576e03 s(^{-1})</td>
</tr>
<tr>
<td>( K_{o+} )</td>
<td>RyR ( P_{o1} - P_{o2} ) rate constant</td>
<td>4.05e09 mM(^{-3})s(^{-1})</td>
</tr>
<tr>
<td>( K_{o-} )</td>
<td>RyR ( P_{o2} - P_{o1} ) rate constant</td>
<td>1.930e03 s(^{-1})</td>
</tr>
<tr>
<td>( K_{r+} )</td>
<td>RyR ( P_{o1} - P_{o2} ) rate constant</td>
<td>0.1e03 s(^{-1})</td>
</tr>
<tr>
<td>( K_{r-} )</td>
<td>RyR ( P_{c2} - 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^{+}_\text{trpnm} )</td>
<td>Ca(^{2+}) on rate for troponin high-affinity sites</td>
<td>20e03 mM(^{-1})s(^{-1})</td>
</tr>
<tr>
<td>( K^{-}_\text{trpnm} )</td>
<td>Ca(^{2+}) off rate for troponin high-affinity sites</td>
<td>66.0e-03 s(^{-1})</td>
</tr>
<tr>
<td>( K^{+}_\text{trpm} )</td>
<td>Ca(^{2+}) on rate for troponin low-affinity sites</td>
<td>40e03 mM(^{-1})s(^{-1})</td>
</tr>
<tr>
<td>( K^{-}_\text{trpm} )</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_{n})</td>
<td>Ca(^{2+}) half-saturation constant for calmodulin</td>
<td>2.38e-03 mM</td>
</tr>
<tr>
<td>( K_{m})</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_{1i} )</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>( C_{a\text{inact}} )</td>
<td>Ca(^{2+})-inactivation gating variable</td>
<td>9.913102e-01</td>
</tr>
<tr>
<td>( r )</td>
<td>( I_{i} ) activation gating variable</td>
<td>2.191519e-03</td>
</tr>
<tr>
<td>( s )</td>
<td>( I_{i} ) fast inactivation gating variable</td>
<td>9.842542e-01</td>
</tr>
<tr>
<td>( s_{slow} )</td>
<td>( I_{i} ) 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>
</tr>
<tr>
<td>( s_{ss} )</td>
<td>( I_{ss} ) inactivation gating variable</td>
<td>3.142767e-01</td>
</tr>
<tr>
<td>( y )</td>
<td>( I_{y} ) inactivation gating variable</td>
<td>3.578708e-03</td>
</tr>
<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>6.60742e-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>$PC_1$</td>
<td>Fraction of channels in state $PC_1$</td>
<td>6.348229e-01</td>
</tr>
<tr>
<td>$Po_1$</td>
<td>Fraction of channels in state $Po_1$</td>
<td>4.327548e-04</td>
</tr>
<tr>
<td>$Po_2$</td>
<td>Fraction of channels in state $Po_2$</td>
<td>6.062540e-10</td>
</tr>
<tr>
<td>$PC_2$</td>
<td>Fraction of channels in state $PC_2$</td>
<td>3.647471e-01</td>
</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\textsuperscript{2+} Measurements

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

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

where \( \text{Ca} \) – free calcium ion concentration; \( \text{D} \) – free dye concentration; \( \text{CaD} \) – bound dye concentration. Optical signals are proportional to \( \text{CaD} \). For steady-state conditions:

\[ \text{CaD} = \text{Kd} \times \text{D} \]

where \( \text{Kd} \) - dissociation constant. If \( \text{Dt} \) – total dye concentration (constant), then:

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

and

\[ \text{CaD} = \frac{\text{Ca} \times \text{Dt}}{\text{Kd} + \text{Ca}} \]

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

![Figure S1. Binding curves for high- (A) and low-affinity (B) dyes.](image)

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

Figure S2 illustrates how dye affinity may affect measurements of \( \text{Ca} \) transient duration. Applying binding curves to a test \( \text{Ca} \) transient (Panel A) results in two different \( \text{CaD} \) 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\(_{2+}\) 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 ΔCa.

In this case, the high-affinity dye reports ΔCa ~3-times smaller than the actual value (blue trace) whereas the low-affinity dye reports ΔCa₂⁺ correctly (red trace).

References


