Simulation of Calcium Sparks in Cut Skeletal Muscle Fibers of the Frog

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ABSTRACT Spark mass, the volume integral of ΔF/F, was investigated theoretically and with simulations. These studies show that the amount of Ca²⁺ bound to fluo-3 is proportional to mass times the total concentration of fluo-3 ([fluor-3]) ; the proportionality constant depends on resting Ca²⁺ concentration ([Ca²⁺]₀). In the simulation of a Ca²⁺ spark in an intact frog fiber with [fluor-3] = 100 μM, fluo-3 captures approximately one-fourth of the Ca²⁺ released from the sarcoplasmic reticulum (SR). Since mass in cut fibers is several times that in intact fibers, both with similar values of [fluor-3] and [Ca²⁺]₀, it seems likely that SR Ca²⁺ release is larger in cut fiber sparks or that fluo-3 is able to capture a larger fraction of the released Ca²⁺ in cut fibers, perhaps because of reduced intrinsic Ca²⁺ buffering. Computer simulations were used to identify these and other factors that may underlie the differences in mass and other properties of sparks in intact and cut fibers. Our spark model, which successfully simulates calcium sparks in intact fibers, was modified to reflect the conditions of cut fiber measurements. The results show that, if the protein Ca²⁺-buffering power of myoplasm is the same as that in intact fibers, the Ca²⁺ source flux underlying a spark in cut fibers is 5–10 times that in intact fibers. Smaller source fluxes are required for less buffer. In the extreme case in which Ca²⁺ binding to troponin is zero, the source flux needs to be 3–5 times that in intact fibers. An increased Ca²⁺ source flux could arise from an increase in Ca²⁺ flux through one ryanodine receptor (RVR) or an increase in the number of active RYRs per spark, or both. These results indicate that the gating of RYRs, or their apparent single channel Ca²⁺ flux, is different in frog cut fibers—and, perhaps, in other disrupted preparations—than in intact fibers.

Key words: spark mass • ryanodine receptors • excitation-contraction coupling • frog muscle

INTRODUCTION

Ca²⁺ sparks are brief, localized increases in fluorescence that can be detected in confocal images of muscle fibers that contain a Ca²⁺ indicator such as fluo-3 (Cheng et al., 1993; Tsugorka et al., 1995; Klein et al., 1996). These fluorescence signals are driven by local increases in the concentration of myoplasmic free calcium ([Ca²⁺]) that result from the flux of Ca²⁺ from the SR into the myoplasm through one or more RYRs, the Ca²⁺ release channels of the SR.

In frog skeletal muscle, voltage-activated Ca²⁺ sparks differ substantially in intact and cut fibers. For example, the average values of decay time constant, full duration at half maximum (FDHM),* full width at half maximum (FWHM), and spark mass are 1.5- to threefold larger in cut fibers than in intact fibers (Table VII of Hollingworth et al., 2001; see also Table II below). The largest difference is for mass.

The first part of this article describes some of the properties of spark mass, which is defined as the volume integral of ΔF/F. These studies show that the amount of Ca²⁺ bound to fluo-3 is proportional to mass times the total concentration of fluo-3 ([fluor-3]), with a proportionality constant that depends on [Ca²⁺]₀ in an intact fiber simulation with [fluor-3] = 100 μM and [Ca²⁺]₀ = 50 nM (the values that apply to intact fibers; Hollingworth et al., 2001), fluo-3 captures approximately one-fourth of the Ca²⁺ released during a spark. Since mass in cut fibers is several times that in intact fibers, whereas [fluor-3] and [Ca²⁺]₀ are similar, it seems likely that SR Ca²⁺ release is larger in cut fiber sparks or that fluo-3 is able to capture a larger fraction of the released Ca²⁺, perhaps because of reduced intrinsic Ca²⁺ buffering in cut fibers. Other factors, however, may contribute to the differences in spark properties, including the microscope point-spread function (PSF), the ionic composition of the myoplasmic solution, and the procedures used for spark analysis.

The second part of this article describes computer modeling that helps identify the factors that underlie the differences between intact and cut fiber sparks. The spark model of Baylor et al. (2002), which successfully simulates sparks in intact fibers, was modified to mimic the conditions encountered in the cut fiber experiments. The new simulations show that the source flux required for sparks in cut fibers is 3–10 times that in intact fibers; the exact factor depends on the concentrations of [Ca²⁺]₀ and the myoplasmic Ca²⁺ buffering.
proteins such as troponin. Such an increase in Ca$^{2+}$ source flux could arise from an increase in Ca$^{2+}$ flux through one RYR or an increase in the number of active RYRs per spark, or both. In either case, it seems clear that the gating of RYRs, or their apparent single channel Ca$^{2+}$ flux, is different in frog cut fibers—and, perhaps, in other disrupted preparations—than in frog intact fibers.

Some of the results have appeared in abstract form (Baylor et al., 2003; Chandler et al., 2003).

**MATERIALS AND METHODS**

**Measurement of Sparks in Intact Fibers**

Intact single fibers were dissected from leg muscles of *R. pipiens*, microinjected with the membrane-impermeant form of fluo-3, and studied at 18 ± 1°C with a laser-scanning confocal microscope. Fluorescence x-y images were obtained with pixel separations of 0.20 μm in x and 2.048 μm in y. The average [fluoro-3] at the optical site was 0.1 mM. This and other information are given in Hollingworth et al. (2001).

**Simulation of Sparks in Intact Fibers**

Calculations were made with spark model 2 of Baylor et al. (2002). In brief, the myoplasm is assumed to be isotropic, with its constituents distributed homogeneously in the resting state. For computational purposes, the myoplasmic volume is divided into 101 spherically symmetric compartments that are centered at the source of Ca$^{2+}$ release and extend to 5 μm from the source. A spark occurs when a brief flux of Ca$^{2+}$ enters the innermost compartment, a sphere of radius 25 nm. The model is used to calculate, for different times and radial distances from the source, the concentration of myoplasmic-free Ca$^{2+}$, the concentrations of the Ca$^{2+}$-free and Ca$^{2+}$-bound forms of the major intrinsic myoplasmic Ca$^{2+}$ buffers (troponin, ATP, parvalbumin, and the SR Ca$^{2+}$ pump), and the concentrations of the Ca$^{2+}$-free and Ca$^{2+}$-bound forms of fluo-3.

The model considers four different forms of fluo-3: Fluo (Ca$^{2+}$-free, protein-free fluo-3), PrFluo (Ca$^{2+}$-free, protein-bound fluo-3), CaFluo (Ca$^{2+}$-bound, protein-free fluo-3), and CaPrFluo (Ca$^{2+}$-bound, protein-bound fluo-3). The total concentration of Ca$^{2+}$-bound fluo-3, denoted by [Cafluoro-3], is given by

\[
[\text{Cafluoro-3}] = [\text{Fluo}] + [\text{PrFluo}].
\]

CaFluo and CaPrFluo are strongly fluorescent with the same relative intensity (Harkins et al., 1993), denoted by $F_{\text{max}}$, whereas Fluo and PrFluo are weakly fluorescent. To allow for the fluorescence of Ca$^{2+}$-free indicator, it is useful to introduce a derived fluo-3 concentration variable, [FFluo], defined by

\[
[\text{FFluo}] = [\text{Fluo}] + (F_{\text{min}}/F_{\text{max}}) \cdot [\text{PrFluo}].
\]

The model described above for intact fibers was modified to simulate Ca$^{2+}$ sparks in cut fibers. Table 1 lists the differences between the intact and cut fiber simulation conditions (columns 2 and 3, respectively). The information for intact fibers was taken from Hollingworth et al. (2001). The information for cut fibers was taken from experiments in the Schneider laboratory. These experiments were selected for comparison because sparks in the Schneider laboratory and ours were analyzed with similar functions in space and time (Klein et al., 1997; Lacampagne et al., 1999; see below).

**Simulation of Noisy Sparks**

Noisy sparks were simulated with the aid of a random number generator to mimic the known sources of noise and variability in the measurements (Baylor et al., 2002). These include photon counts, the finite size of the confocal PSF, and the finite size of the laser spot. The model considered for the simulation of Ca$^{2+}$ sparks in cut fibers is given by

\[
\Delta F/F = \text{convolving } \Delta [\text{FFluo}]/[\text{FFluo}]_R \text{ with the microscope PSF.}
\]

For the cut fiber simulations, model 2 of Baylor et al. (2002) was modified to incorporate the differences between columns 2 and 3. For adjustment to temperature, the diffusion constants and reaction rate constants in the intact fiber model were scaled by the factors 1.11 and 1.32, respectively (corresponding to Q10s of 1.3 and 2.0, respectively). To adjust for sarcomere length, the concentration of the troponin regulatory sites in the intact fiber model was scaled by the factor 1.2 (the ratio of the sarcomere lengths); this scaling reflects the expected constancy of fiber volume with changes in sarcomere length and the close proximity of the troponin molecules to the SR Ca$^{2+}$ release sites. The association and dissociation rate constants for Ca$^{2+}$'s reaction with EGTA were 3.79 × 10$^{-1}$ M$^{-1}$ s$^{-1}$ and 1.42 s$^{-1}$, respectively (22°C, pH 7.0; Pape et al., 1995). The value of resting free [Mg$^{2+}$] in cut fibers is based on Lacampagne et al. (1998). The other information for cut fibers is based on Lacampagne et al. (1996, 1999) and a personal communication with Dr. M.E. Schneider.
and instrumentation noise as well as variability that arises from random displacements of the scan line relative to the spark source and random offsets in the time of data sampling relative to the time of spark onset.

**Procedures for Spark Analysis in Intact Fibers**

The analysis of an intact fiber spark, both experimental and simulated, followed procedures described in Hollingworth et al. (2001). Briefly, a $3 \times 3$ smoothed $x$-$t$ image was formed from the original $\Delta F/F \times t$ image and an autodetection program was used to tentatively identify a spark as a contiguous region with peak $\Delta F/F \geq 0.3$. The unsmoothed $\Delta F/F$ image was then used to form a $\Delta F/F$ vs. $t$ waveform as the average of the three time lines at $x_0 = -0.2 \mu m$, $x_0 + 0.2 \mu m$, and $x_0$ denotes the spatial center of the spark determined by the autodetection program. This waveform was least-squares fitted with Eq. 1 of Hollingworth et al. (2001). This equation assumes that $\Delta F/F$ starts abruptly, rises exponentially toward a maximum value, then terminates abruptly and decays exponentially to a baseline offset.

The fit determines the 0–100% rise time, time of peak (denoted $t_2$), peak amplitude, decay time constant, and FWHM. Analyzed was fitted with a Gaussian function (Eq. 2 of Hollingworth et al., 1999) and the other morphological parameters satisfied the broad acceptance criteria described in Hollingworth et al. (2001).

**Spark Mass and its Equivalence to the Volume Integral of $\Delta [F/F]_R$**

$\Delta F/F$ is given by the convolution of $\Delta [F/F]_R$ with the microscope PSF,

$$
\frac{\Delta F(x,y,z,t)}{F} = \int \int \Delta [F/F]_R(x',y',z',t) \cdot \frac{1}{[F/F]_R} dx' dy' dz'.
$$

PSF($x' - x', y' - y', z' - z'$) $dx' dy' dz'$,

and mass ($M$) is defined as the volume integral of $\Delta F/F$,

$$
M(t) = \int \int \int \Delta [F/F]_R(x,y,z,t) dx \, dy \, dz.
$$

By changing the order of integration with respect to $x'$, $y'$, $z'$ and $x$, $y$, $z$, and using the fact that the volume integral of PSF equals 1, $M$ can be written

$$
M(t) = \int \int \int \Delta [F/F]_R(x,y,z,t) \cdot \frac{1}{[F/F]_R} \cdot dx \, dy \, dz.
$$

Eq. 6 shows that $M$ is equal to the increase in the total normalized amount of $F/F$ and that this equality does not depend on the spatial resolution of the confocal microscope. The equality holds for any PSF that is continuous in $x$, $y$, and $z$. Because the absolute value of $\Delta [Ca^{2+}]$ is much greater than $\Delta [F/F]_R$, $\Delta [F/F]$ is approximately equal to $\Delta [Ca^{2+}]$, and

$$
M(t) = \int \int \int \Delta [Ca^{2+}] (x,y,z,t) \cdot \frac{1}{[F/F]_R} \cdot dx \, dy \, dz.
$$

Eq. 7 shows that the total amount of $Ca^{2+}$ captured by fluo-3 is approximately equal to $M(t) \cdot [Ca^{2+}]_R$.

**Statistics**

For each set of noisy-spark simulations in Tables IV, V, and VII, sufficient sparks were generated to give 3,176 sparks for inclusion in the analysis. This number is the same as that in the measurements of Hollingworth et al. (2001) and in the simulations of Baylor et al. (2002). Values of the morphological parameters are reported as mean ± SEM. The statistical significance of a difference between means was evaluated with Student’s two-tailed $t$ test at $P < 0.05$.

**Results**

The first part of this article describes simulations and measurements of spark mass in intact muscle fibers of frog. The most accurate estimates of mass are made when the scan line intersects the source of $Ca^{2+}$ release. Experimentally, such “in focus” sparks, if elicited by depolarization, have the following average morphological properties: 0–100% rise time, $\sim 3.9$ ms; peak $\Delta F/F$, $\sim 1.9$; decay time constant, $\sim 4.4$ ms; FWHM, $\sim 5.5$ ms; FWHM (measured at the time of peak $\Delta F/F$), $\sim 1.0 \mu m$ (18°C, Table VII of Baylor et al., 2002; see also Fig. 3, B and D, described below). These and other...
properties of measured sparks in intact fibers are well simulated with spark model 2 of Baylor et al. (2002) with a Ca\(^{2+}\) source flux of 2.5 pA for 4.6 ms and [fluo-3] = 100 μM. Except where noted, these conditions were used for the calculations.

**Spark Mass Equals the Volume Integral of \(\Delta [\text{FFluo}] / [\text{FFluo}]_{R}\)**

Fig. 1 A shows the time course of \(\Delta F / F\) at the Ca\(^{2+}\) source for a standard noise-free simulated spark. The peak amplitude is 2.14 and the time of peak is 4.6 ms, the same as the flux duration. Fig. 1 B shows two nearly identical curves. One is the time course of “true” mass, \(M(t)\), calculated from its definition (Eq. 5). The other is the time course of the volume integral of \(\Delta [\text{FFluo}] / [\text{FFluo}]_{R}\), which is equal to spark mass (Eq. 6); this equality does not depend on the spatial distribution of \(\Delta [\text{FFluo}] / [\text{FFluo}]_{R}\) or on the microscope PSF (see materials and methods). As expected from the theory, the two curves in Fig. 1 B are indistinguishable. At the time of peak \(\Delta F / F\) (4.6 ms), the value of mass is 2.64 μm\(^3\). Although the Ca\(^{2+}\) source flux ceases at 4.6 ms, \(M(t)\) continues to increase; it reaches its peak value, 3.63 μm\(^3\), at 10.8 ms, 6.2 ms after the peak \(\Delta F / F\). The lag between cessation of Ca\(^{2+}\) release and the peak of mass arises from kinetic delays in the reactions between Ca\(^{2+}\) and fluo-3 in the myoplasmic environment (Harkins et al., 1993; Baylor and Hollingworth, 1998; Hollingworth et al., 2000). After 10.8 ms, mass decreases as Ca\(^{2+}\) dissociates from fluo-3 and is captured by parvalbumin and the SR Ca\(^{2+}\) pump.

**The Volume Integral of \(\Delta [\text{Cafluo-3}] / [\text{FFluo}]_{R}\) \(\approx\) Spark Mass**

The continuous curve in Fig. 1 C shows the volume integral of \(\Delta [\text{Cafluo-3}] / [\text{FFluo}]_{R}\). This is proportional to the amount of Ca\(^{2+}\) that is captured by fluo-3, which provides a lower limit of the amount of Ca\(^{2+}\) released during a spark. The peak value of the continuous curve in Fig. 1 C (3.66 μm\(^3\)) times the value of \([\text{FFluo}]_{R}\) (4.22 μM at \([\text{Ca}^{2+}]_{R} = 50 \text{ nM};\) see materials and methods) indicates that 9,312 Ca\(^{2+}\) ions are captured by fluo-3 (3.66 μm\(^3\) \times 4.22 μM = 1.546 × 10\(^{-10}\) moles). This represents ~26% of the 35,888 Ca\(^{2+}\) ions that are released into the myoplasm by the 2.5 pA \times 4.6 ms Ca\(^{2+}\) flux. The capture of about one-fourth of the released Ca\(^{2+}\) by fluo-3 indicates that the buffering action of 100 μM fluo-3 is not negligible during a spark.

The dashed curve in Fig. 1 C shows \(M(t)\). According to Eq. 7, which is illustrated by the similarity of the dashed and continuous curves in Fig. 1 C, the volume integral of \(\Delta [\text{Cafluo-3}]\) is expected to be approximately equal to \(M(t)\) times \([\text{FFluo}]_{R}\). The peak value of \(M(t)\) (3.63 μm\(^3\)) times \([\text{FFluo}]_{R}\) (4.22 μM) gives 9,236 for the number of Ca\(^{2+}\) ions captured by fluo-3, which is 0.99 times the actual value.

**Use of Eq. 3 to Estimate Spark Mass**

Although spark mass depends on the spatial spread of \(\Delta F / F\) in three dimensions, its value can be estimated with Eq. 3 from the spatial spread in the x direction only. Fig. 2 shows noise-free calculations that illustrate the estimation of spark mass (\(M_{x}\)). Fig. 2, A and B, shows the time courses of \(\Delta F / F\) and FWHM, respectively, at the source; these were obtained from fits of a gaussian function to the waveform of \(\Delta F / F\) vs. x at different times t. The curve in Fig. 2 A differs slightly from
that in Fig. 1 A, which is the actual temporal waveform of $\Delta F/F$ at the source. This difference arises because $\Delta F/F$ vs. $x$ is not an exact gaussian function, either in the simulations or in the measurements (Fig. 9, B and E, of Baylor et al., 2002). In spite of this, the $\Delta F/F$ vs. t waveforms in Figs. 1 A and 2 A have similar peak amplitudes, the same time of peak (4.6 ms, which is the time at which the Ca$^{2+}$ source flux terminates), and very similar overall time courses.

The continuous curve in Fig. 2 C shows $M_e(t)$, which was calculated with Eq. 3 from the curves in Fig. 2, A and B. The dashed curve is $M(t)$ from Fig. 1 B, scaled by the factor 0.85 (the ratio of the peak amplitude of $M_e(t)$ to that of $M(t)$). This factor is different from unity because $\Delta F/F$ vs. $x$ is not an exact gaussian function and because the microscope PSF, and consequently $\Delta F/F$, is not symmetrical in $x$, $y$, and $z$ (Table I B, column 2). As shown in Fig. 2 C, $M(t)$ and $M_e(t)$ have identical times of peak (10.8 ms) but somewhat different overall time courses. This comparison shows that Eq. 3 is expected to give reasonable approximations of peak $M(t)$ and of the time of peak $M(t)$ for an in-focus spark. The approximation is less good, however, at the time of peak $\Delta F/F$, where estimates of mass are frequently made.

**Time Course of Mass in Simulated Noisy Sparks and in Sparks in Intact Fibers**

Fig. 3 shows simulated data (asterisks) and measured data (open squares); 0 ms denotes the estimated time of peak $\Delta F/F$. Each set of data was obtained from an average of 179 in-focus sparks, defined as the largest 10% of sparks with peak amplitude $\Delta F/F \approx 0.7$. Noise and variability were included in the simulated data to mimic the measurements (see MATERIALS AND METHODS). Fig. 3 also shows the continuous curves from Fig. 2 time-shifted by $\sim 4.6$ ms so that 0 ms corresponds to the time of peak $\Delta F/F$. Fig. 3, A and B, show the time course of $\Delta F/F$ and Fig. 3, C and D, show FWHM. Both the simulated and measured values of FWHM become noisy after 12 ms; this occurs because $\Delta F/F$ becomes small and the noise in $\Delta F/F$ vs. $x$ makes the gaussian fits less reliable. The simulated data in these panels are in reasonable agreement with the measured data, and, within the noise, both sets of data lie close to the curves, at least out to $\sim 40$ ms.

Fig. 3, E and F, show $M_e(t)$. In both datasets, the time of peak mass is similar to that of the curve, 6.2 ms, consistent with the idea that, within the noise in the data, the kinetic delays in the reactions between Ca$^{2+}$ and fluo-3 in myoplasm are adequately simulated with the model. After 12 ms, the values of mass become less reliable because of the noise in FWHM.

The simulations and measurements in Figs. 1–3 indicate that Eq. 3 provides reasonable estimates of the peak mass and time of peak mass of an in-focus spark.

**Dependence of Simulated Spark Mass on the Amount of Ca$^{2+}$ Released**

Noise-free simulations of sparks at the source of Ca$^{2+}$ flux were also used to study the dependence of $M$ and $M_e$ on the total amount of SR Ca$^{2+}$ released during a spark. Ca$^{2+}$ release was varied by changing either the amplitude or the duration of the source flux.
shows mass at the time of peak $\Delta F/F$ (A) and at the time of peak true mass (B) plotted against the amount of Ca$^{2+}$ released. For releases up to $\sim 30 fC$, both true mass (filled symbols) and estimated mass (open symbols) vary approximately linearly with the amount of Ca$^{2+}$ released. In both panels, the slope of the line fitted to estimated mass (dashed line) is smaller than that fitted to true mass (continuous line). The ratio of the slopes (dashed divided by continuous) is 0.545 in A and 0.832 in B. These simulations show that, for the range of Ca$^{2+}$ releases considered, both the true and estimated mass of an in-focus spark are approximately proportional to the amount of SR Ca$^{2+}$ released, with a proportionality constant that is smaller at the time of peak $\Delta F/F$ than at the time of peak mass. The proportionality constant for peak true mass (slope of the continuous line in Fig. 4 B) corresponds to the capture of 24.9% of the Ca$^{2+}$ released from the SR by fluo-3 ($[\text{fluo-3}] = 100 \mu M$).

Simulations of Sparks in Cut Fibers

Table II gives the average values of spark morphological parameters in intact fibers studied by us and in cut fibers studied in the Schneider laboratory; both laboratories use essentially identical functions to analyze sparks in space and time (Klein et al., 1997; Lacampagne et al., 1999; see MATERIALS AND METHODS). Since the mean values of spark amplitude are similar in intact and cut fibers (0.99 and 1.05, respectively), the underlying Ca$^{2+}$ source fluxes might also be expected to be
similar in the two preparations. This turns out not to be the case, however, as is suggested by the larger value of spark mass in cut fibers and the association of spark mass with the amount of Ca\textsuperscript{2+} captured by fluo-3 that is described above. According to Eq. 7, at the time of peak \( \Delta F/F \), the amount of Ca\textsuperscript{2+} bound to fluo-3 during a cut fiber spark would be expected to be 4–5 times that in intact fibers (threelfold increase in mass times 0.0608/0.0422, the ratio of the values of [Ca\textsubscript{2+}]\textsubscript{R} for a cut fiber spark would be expected to be 4–5 times that in intact fibers (threefold increase in mass times 0.0608/0.0422, the ratio of the values of [Ca\textsubscript{2+}]\textsubscript{R} and [fluor-3T] given in Table I).

The simulations in the following sections elucidate the dependence of mean spark amplitude on Ca\textsuperscript{2+} source strength and other parameters.

**Effects of Fiber Conditions, Microscope PSF, and Analysis Procedures on Properties of Noise-free Sparks at the Ca\textsuperscript{2+} Source**

Fig. 5 A shows the temporal waveforms of five sparks simulated with a Ca\textsuperscript{2+} source flux of 2.5 pA for 4.6 ms and with the scan line through the Ca\textsuperscript{2+} source. Trace a shows \( \Delta F/F \) at \( x = 0 \) for the standard simulation conditions used for intact fiber sparks (Table I, column 2). Trace b shows \( \Delta F/F \) from this same simulation but averaged at three spatial locations (\( x = -0.2, 0, \) and 0.2 \( \mu \)m), as is done in the analysis of sparks in intact fibers. Its amplitude is smaller than that of a because the values of \( \Delta F/F \) at \( x = \pm 0.2 \) \( \mu \)m are smaller than that at \( x = 0 \). Both a and b were calculated with the PSF used for the experiments on intact fibers.

Trace c is similar to b except that the broader PSF from the cut fiber experiments was used (Table I B, column 3). This decreased the peak value of \( \Delta F/F \) from 1.808 in b to 1.040 in c. This shows that the difference in spatial resolution of the confocal microscopes used for the intact and cut fiber experiments is expected to make an almost twofold difference in the peak value of \( \Delta F/F \) near the scan line. Trace d was calculated with the same cut fiber PSF used for c but with the cut fiber analysis procedures described in MATERIALS AND METHODS. The difference between traces c and d is caused by the different number of spatial locations used for averaging the temporal waveforms: three in c (as used for intact fiber sparks) and seven in d (as used for cut fiber sparks).

Trace e was obtained in the same manner as trace d except that cut fiber conditions were used for the simulations (Table I A, column 3). The smaller amplitude of trace e is due mainly to the increase in \([\text{Ca}_{2+}]_{R}\) from 50 to 80 nM. This increases the resting concentration of Ca\textsuperscript{2+}-bound fluo-3 and hence resting fluorescence; as a result, a smaller \( \Delta F/F \) signal is produced for a given Ca\textsuperscript{2+} flux (e.g., Jiang et al., 1999; Baylor et al., 2002).

![Figure 4](https://example.com/fig4.png)

**TABLE II**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Intact fibers</th>
<th>Cut fibers</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–100% rise time (ms)</td>
<td>4.4 ± 0.1</td>
<td>4.7 ± 0.1</td>
</tr>
<tr>
<td>Peak amplitude (( \Delta F/F ))</td>
<td>0.99 ± 0.01</td>
<td>1.05 ± 0.08</td>
</tr>
<tr>
<td>Decay time constant (ms)</td>
<td>4.9 ± 0.1</td>
<td>8.5 ± 0.4</td>
</tr>
<tr>
<td>FWHM (ms)</td>
<td>6.5 ± 0.1</td>
<td>14.8 ± 0.3</td>
</tr>
<tr>
<td>FWHM (( \mu )m)</td>
<td>1.05 ± 0.01</td>
<td>1.51 ± 0.10</td>
</tr>
<tr>
<td>Spark mass (( \mu )m\textsuperscript{3})</td>
<td>1.38</td>
<td>4.36</td>
</tr>
</tbody>
</table>

Mean ± SEM values for intact fibers were measured at 18°C (Table VII of Hollingsworth et al., 2001; amplitude criterion for spark acceptance, \( \Delta F/F \geq 0.5 \)). Cut fiber values were measured at 22°C (Lacampagne et al., 1996, 1999; amplitude criterion, \( \Delta F/F \geq 0.4–0.5 \)). Mass was calculated from the mean values of peak amplitude and FWHM with Eq. 3.
The peak ∆F/F amplitudes in Fig. 5 A progressively decrease from a to e. Trace b, with a peak value of 1.808, represents the temporal waveform of a noise-free simulated intact fiber spark with the scan line through the Ca²⁺ source. Trace e, with a peak value of 0.522, is the comparable waveform for a cut fiber spark. These simulations show that, with a Ca²⁺ source flux of 2.5 pA for 4.6 ms and with the line scan through the Ca²⁺ source, a spark measured in a cut fiber is expected to have an amplitude that is 0.3 times that in an intact fiber.

Fig. 5 B shows the spatial waveforms of ∆F/F that accompany the traces in A. All waveforms in B have been scaled to a peak amplitude of unity to facilitate the comparison of the spatial spread of the sparks. The FWHMs of the waveforms progressively increase from 0.740 μm in a to 1.177 μm in d; waveforms in d and e are indistinguishable.

Additional information about the simulations in Fig. 5 is given in Table III, columns 2–6. From the intact fiber simulation of column 3 to the cut fiber simulation of column 6, there is a 71% reduction in peak amplitude, an 18% increase in F FHDM, a 39% increase in FWHM, and a 23% reduction in spark mass.

**Simulation of Noisy Sparks in Cut Fibers**

(Ca²⁺ Source Flux = 2.5 pA × 4.6 ms)

To further compare our spark simulations with the cut fiber measurements, noisy sparks were simulated and then were analyzed with the cut fiber procedures described in MATERIALS AND METHODS. Table IV, column 2, shows the mean values of the morphological parame-
Thus, even without Ca\(^{2+}\) buffering would be expected to produce a larger spark amplitude for a given Ca\(^{2+}\) flux. Although the value of [Ca\(^{2+}\)]\(_R\) in cut fibers appears to be larger than that in intact fibers (Hollingworth et al., 2001; see also Discussion), it was nonetheless of interest to determine the effect of reducing [Ca\(^{2+}\)]\(_R\) from 80 to 50 nM, the standard value used for spark simulations in intact fibers (Table I). This reduction is expected to reduce resting F and therefore increase ΔF/F for a given Ca\(^{2+}\) flux. Table IV, columns 5–7, are similar to columns 2–4 except that [Ca\(^{2+}\)]\(_R\) = 50 nM. Even without troponin (column 7), the mean values of spark amplitude and mass (0.630 and 2.368 \(\mu\)m\(^3\), respectively) are substantially smaller than those of the measurements (1.05 and 4.36 \(\mu\)m\(^3\), respectively).

Our conclusion from the results in Table IV is that a Ca\(^{2+}\) flux of 2.5 pA is too small to account for the amplitude and some of the other properties of sparks in cut fibers.

**Simulation of Noisy Sparks in Cut Fibers with Mean \(\Delta F/F \approx 1.05\) (Ca\(^{2+}\) Source Flux > 2.5 pA for 4.6 ms)**

Table V shows results similar to those in Table IV except that, for each simulation condition, the Ca\(^{2+}\) flux amplitude was increased in units of 1 pA until average \(\Delta F/F\) was \(\approx 1.05\), similar to that of the cut fiber measurements. In these simulations, sparks that satisfy the criterion \(\Delta F/F \geq 0.4\) can be detected farther from the source so that the average values of D in Table V are substantially larger than those in Table IV. The values of the

<table>
<thead>
<tr>
<th>Variables</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Troponin regulatory sites] (μM)</td>
<td>432</td>
<td>216</td>
<td>0</td>
<td>432</td>
<td>216</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>[Ca(^{2+})](_R) (nM)</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Distance D (μm)</td>
<td>0.522</td>
<td>0.508</td>
<td>0.681</td>
<td>0.741</td>
<td>0.832</td>
<td>0.948</td>
<td></td>
</tr>
<tr>
<td>0–100% rise time (ms)</td>
<td>4.06 ± 0.028</td>
<td>4.25 ± 0.027</td>
<td>4.21 ± 0.026</td>
<td>4.35 ± 0.027</td>
<td>4.34 ± 0.026</td>
<td>4.35 ± 0.025</td>
<td></td>
</tr>
<tr>
<td>Peak amplitude (ΔF/F)</td>
<td>0.493 ± 0.001</td>
<td>0.516 ± 0.002</td>
<td>0.547 ± 0.002</td>
<td>0.560 ± 0.002</td>
<td>0.589 ± 0.002</td>
<td>0.630 ± 0.003</td>
<td></td>
</tr>
<tr>
<td>Decay time constant (ms)</td>
<td>5.17 ± 0.045</td>
<td>5.203 ± 0.041</td>
<td>5.086 ± 0.038</td>
<td>5.851 ± 0.042</td>
<td>5.670 ± 0.039</td>
<td>5.461 ± 0.036</td>
<td></td>
</tr>
<tr>
<td>FWHM (μm)</td>
<td>1.317 ± 0.006</td>
<td>1.343 ± 0.006</td>
<td>1.383 ± 0.006</td>
<td>1.345 ± 0.005</td>
<td>1.378 ± 0.006</td>
<td>1.419 ± 0.005</td>
<td></td>
</tr>
<tr>
<td>Spark mass (μm(^3))</td>
<td>1.649 ± 0.029</td>
<td>1.774 ± 0.028</td>
<td>2.046 ± 0.036</td>
<td>1.877 ± 0.030</td>
<td>2.094 ± 0.032</td>
<td>2.368 ± 0.029</td>
<td></td>
</tr>
</tbody>
</table>

Mean ± SEM values are for 3,176 noisy sparks. The PSF and analysis procedures for cut fibers were used (Table I, column 5). Column 2 was simulated with the standard conditions for cut fibers; in columns 3–7, the concentration of the troponin regulatory sites and [Ca\(^{2+}\)]\(_R\) were varied as indicated. The amplitude criterion for spark acceptance was \(\Delta F/F \geq 0.4\). Here and in Tables V and VII, spark mass was evaluated in each individual simulation. Consequently, the mean value of mass listed in the bottom row is somewhat different from the value obtained with Eq. 3 from the mean values of ΔF/F and FWHM.
other parameters in Table V, columns 2–7, are broadly consistent with the experimental results in Table II, column 3. Consequently, none of the six combinations of [troponin] and [Ca\(^{2+}\)]\(_{R}\) can be definitely ruled out. As expected, the largest Ca\(^{2+}\) flux (23 pA, column 2) occurs with the standard values of [troponin] and [Ca\(^{2+}\)]\(_{R}\), and the smallest flux (8 pA, column 7) occurs with [troponin] = 0 and [Ca\(^{2+}\)]\(_{R}\) = 50 nM. Even the 8 pA value is more than three times that required for the simulation of sparks in intact fibers, 2.5 pA.

Tables IV and V give the values of Ca\(^{2+}\) source flux and peak \(\Delta F/F\) at the source for the six simulation conditions, columns 2–7. In each case, the relative increase in \(\Delta F/F\) is smaller than the relative increase in the source flux. This indicates that the relation between \(\Delta F/F\) at the source and source flux is convex (has a slope that decreases with increasing flux), perhaps due to factors such as the saturation of fluo-3 by Ca\(^{2+}\) near the source. The relation between mean \(\Delta F/F\) and \(\Delta F/F\) at the source is also convex. This occurs, as mentioned above, because, as source flux is increased, sparks that satisfy a fixed detection criterion such as \(\Delta F/F \geq 0.4\) are detected farther from the source, as evidenced by an increase in the value of \(D\). These distant sparks, of small amplitude, make a progressively larger contribution to the mean value of \(\Delta F/F\) as the source flux is increased. As a result, the relation between mean \(\Delta F/F\) and \(\Delta F/F\) at the source is convex.

Simulations with Increased Myoplasmic Diffusion Constants and Increased Myoplasmic Water Volume

Table VI shows the apparent diffusion constants of six indicator dyes studied in cut fibers in the Chandler laboratory and in intact fibers in the Baylor laboratory. On average, apparent diffusion constants in cut fibers are \(\sim 1.3\) times those in intact fibers (Table VI, column 4). A possible explanation, which is supported by the measurements of Irving et al. (1987), is that the myoplasmic water volume is increased in cut fibers compared with intact fibers. These authors measured intrinsic birefringence (optical retardation per unit path length, which primarily reflects the birefringence of myosin) in both intact and cut fibers and found that cut fibers, on average, have values that are \(\sim 0.85\) times those in intact fibers. This suggests that the optical path length in cut fibers is 1/0.85 times that in intact fibers, and that myoplasmic water volume is increased according to the factor 1.4 (\(\sim 1/0.85\)). An increase in water volume would be expected to reduce the viscosity of myoplasm and, thus, to increase the actual diffusion constants of all diffusible myoplasmic constituents (including the indicator dyes). An increase in water volume would also be expected to dilute the concentrations of poorly diffusible myoplasmic constituents of high molecular weight, such as soluble and structural proteins, to which indicator molecules readily bind (e.g., Konishi et al., 1988; Kurebayashi et al., 1993). This reduction in concentration of binding sites would be expected to further increase the apparent diffusion constants of the indicators.

To investigate these possibilities, simulations similar to those in Table V, columns 2–7, were performed with two modifications: the diffusion constants in the model were multiplied by 1.3 and the concentrations of binding sites on myoplasmic proteins were divided by 1.4; these sites are the Ca\(^{2+}\) regulatory sites on troponin, the Ca\(^{2+}\) transport sites on the SR Ca\(^{2+}\) pump, the Ca\(^{2+}\)/Mg\(^{2+}\) sites on parvalbumin, and the binding sites for fluo-3 on (unspecified) protein molecules (Baylor et al., 2002). Table VII, columns 2–7, give the results.
The apparent diffusion constants (columns 2 and 3) have been referred to 16°C based on the temperature of the original measurements (16–17°C for intact fibers; 13–18°C for cut fibers) and a Q10 of 1.3. Column 4 is the ratio of column 2 to column 3.

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The Ca²⁺ source fluxes in Table VII are all very similar to the corresponding fluxes in Table V (ranges, 9–22 pA and 8–25 pA, respectively). One noticeable difference in the morphological parameters is that the values of FWHM and spark mass in Table VII are 15–18% and 51–61% larger, respectively, than those in Table V and those in Table II, column 3. Since the values in Table V and Table II, column 3, are in good agreement, the assumptions underlying the simulations of Table VII may be less accurate than those of Table V.

Each of the two modifications used for Table VII was also tested separately. With the concentrations of the protein binding sites left unchanged but with the diffusion constants of the myoplasmic constituents increased by the factor 1.3, the Ca²⁺ fluxes required for these simulations ranged from 10 to 26 pA (not shown). When the diffusion constants were left unchanged and the concentrations of the protein binding sites were divided by the factor 1.4, the Ca²⁺ fluxes ranged from 7 to 18 pA (not shown). In both types of simulations, the increases in FWHM and spark mass were somewhat less marked than those in Table VII. For the first type of simulation, the increases in FWHM and mass were, respectively, 8–10% and 25–31% larger than the corresponding values in Table V; for the second type, the increases were 5–7% and 14–24%, respectively. These simulations, and those in Table VII, do not change the conclusion that Ca²⁺ sparks in cut fibers require a Ca²⁺ source flux that is 3–10-fold larger than that required in intact fibers.

**DISCUSSION**

**General Properties of Spark Mass**

This article shows that spark mass, defined as the volume integral of ΔF/F, is equal to the volume integral of Δ[FFluo]/[FFluo]₀ and that this equality does not depend on the PSF of the confocal microscope or on the spatial distribution of Δ[FFluo]. Furthermore, the amount of Ca²⁺ captured by fluo-3 is expected to be approximately equal to the product of mass and [FFluo]₀. Simulations with the intact fiber model described by Baylor et al. (2002) show that fluo-3 captures about two orders of magnitude less Ca²⁺ than in the intact fiber.
one-fourth of the Ca\(^{2+}\) released during a spark ([fluo-
3\(_{2+}\)] = 100 \(\mu\)M). The time of maximal capture occurs
6.2 ms after that of peak \(\Delta F/F\), owing to kinetic delays
in the reactions between Ca\(^{2+}\) and fluo-3 in the myo-
plasmic environment. Although spark mass depends
on the values of \(\Delta F/F\) in \(x\), \(y\), and \(z\), the simulations
show that, with the laser scan line positioned near the
source of Ca\(^{2+}\) release, a reasonable estimate of spark
mass can be obtained with Eq. 3 from the values of
\(\Delta F/F\) and FWHM obtained from the \(\Delta F/F\) vs. \(x\) wave-
form. This method is believed to be more reliable
than those used previously to estimate mass (next sec-
tions).

**Signal Mass in Nonmuscle Cells**

The concept of signal mass was introduced by Sun et al.
(1998), who studied Ca\(^{2+}\) signaling events ("blips" and
"puffs") mediated by inositol-tris-phosphate in oocytes
injected with the fluorescent Ca\(^{2+}\) indicator Oregon
green 488 Bapta-1. Signal mass (the volume integral of
\(\Delta F/F\)) was estimated from \(\Delta F/F\) vs. \(x\) with a method
that is different from that used in this article. During
blips (the smallest resolved events, \(\Delta F/F = 0.25\)), sig-
nal mass increased at about the same time as \(\Delta F/F\)
or shortly thereafter, and peak mass (~5 \(\mu\)m\(^3\)) was
reached \(\approx 15\) ms after peak \(\Delta F/F\) (Fig. 4 C of Sun et al.,
1998). During puffs (larger events, \(\Delta F/F = 1–2\)), the
peak value of mass was an order of magnitude larger
(~80 \(\mu\)m\(^3\)) and it occurred \(\approx 100\) ms after the peak of
\(\Delta F/F\) (Fig. 4 D of Sun et al., 1998). The delay from
peak \(\Delta F/F\) to peak mass was attributed to a continued
but diminished flux of Ca\(^{2+}\) into the cytoplasm.

Thus, the peak values of mass in oocytes are either
comparable to, or many times larger than, that esti-
mated for an averaged in-focus spark in frog intact mus-
cle fibers (5–100 \(\mu\)m\(^3\) compared with ~3.8 \(\mu\)m\(^3\)) and
the lag between peak \(\Delta F/F\) and peak mass in oocytes is
at least several times larger than that in intact muscle
fibers (\(\approx 15\) ms and \(\approx 100\) ms compared with ~6 ms).
Some of the lag in oocytes is likely due to kinetic delays
in the reactions between Ca\(^{2+}\) and the indicator in the
cytoplasmic environment, similar to the situation with
sparks in frog intact muscle fibers.

**Signal Mass in Cut Fibers**

As far as we are aware, the only estimate of mass in cut
muscle fibers was reported by Gonzalez et al. (2000).
With Eq. 3, however, mass can be calculated from other
articles if the values of \(\Delta F/F\) and FWHM are given. Ta-
ble VII in Hollingworth et al. (2001) tabulates such val-
ues at the time of peak \(\Delta F/F\): 3.7–5.2 \(\mu\)m\(^3\) for voltage-
activated sparks in cut fibers and 4.4–22.5 \(\mu\)m\(^3\) for
spontaneous sparks in permeabilized cut fibers (ampli-
tude threshold for spark acceptance, \(\Delta F/F \approx 0.5\) to
1.0). These values of mass are 2.5–15 times those ob-
tained for voltage-activated sparks in intact fibers at the
time of peak \(\Delta F/F\), 1.4–1.5 \(\mu\)m\(^3\).

In the paper by Gonzalez et al. (2000), frog fibers
were permeabilized by saponin and exposed to Impera-
 toxin A, an agent that, in bilayer experiments, binds to
open RyRs and induces a long-lived substrate that has
about one-third the normal conductance (Tripathy et
al., 1998). The toxin-related events usually had an ini-
itial \(\Delta F/F\) that was similar to a spark followed by a small
maintained \(\Delta F/F\) that lasted ~1 s (Gonzalez et al.,
2000). The spark-like event in their Fig. 2, A–D, had a
peak \(\Delta F/F \approx 3\), a FWHM at time of peak \(\Delta F/F \approx 1.9\)
\(\mu\)m, and a peak mass \(\approx 50 \mu m^3\). An average of nine
such events was simulated with a Ca\(^{2+}\) source flux of
peak amplitude of ~11 pA and half-duration of ~9 ms
(Fig. 2 F of Gonzalez et al., 2000). Both the peak mass
of the single event, 50 \(\mu m^3\), and the amount of Ca\(^{2+}\)
release estimated for the averaged event, ~99 fC, are
an order of magnitude larger than the values estimated
for in-focus sparks activated by voltage in frog intact fi-
bers, 3–4 \(\mu m^3\) (Fig. 3, E and F) and 11.5 fC, respectively.

**Simulation of Sparks in Cut Fibers**

The main conclusion of this article is that the simulation
of Ca\(^{2+}\) sparks in cut fibers requires a Ca\(^{2+}\) source flux
that is substantially larger than the 2.5 pA required to
simulate sparks in intact fibers. The required source flux
is also substantially larger than the 1.4 pA used in the
spark simulations by the Schneider laboratory (Jiang et
al., 1999). With the standard concentrations of troponin
and resting Ca\(^{2+}\) in the cut fiber model, a Ca\(^{2+}\) source
flux of 23 pA is required (Table V, column 2). Even un-
der the extreme conditions that [Ca\(^{2+}\)]\(_R\) = 50 nM and
the troponin regulatory sites bind no Ca\(^{2+}\) at all, a
source flux of 8 pA is required (Table V, column 7), which
is three times that required in intact fibers. Be-
cause the values of the morphological parameters in
each row of Table V, columns 2–7, are close to one an-
other and to those in the cut fiber experiments (Table II,
column 3), all six model conditions in Table V produce a
reasonable simulation of sparks in cut fibers. Thus, these
simulations do not establish the likely value of [Ca\(^{2+}\)]\(_R\) or
the concentration of the troponin sites available for Ca\(^{2+}\)
binding. Similar conclusions apply to the simulations in
Table VII, which include increases in myoplasmic diffu-
sion constants and myoplasmic water volume. These sim-
ulations, which are in less satisfactory agreement with
the measurements than those in Table V, also required
large Ca\(^{2+}\) source fluxes (9–22 pA).

**Comparisons with the Measurements and Simulations
by the Ríos Laboratory**

Voltage-activated Ca\(^{2+}\) sparks in cut fibers appear to be
different in the Ríos and Schneider laboratories (Table
VII of Hollingworth et al., 2001). For example, spark
amplitude is substantially larger in the Rios laboratory (1.85 ± 0.12; amplitude acceptance criterion, ΔF/F ≥ 0.6; 18 ± 1°C), even though the values of FWHM for the microscope PSF in the Rios laboratory (0.47 µm in x and y and 1.44 µm in z; Rios et al., 1999) are similar to or larger than those in the Schneider laboratory (0.5 µm in x and y and 1.0 µm in z, respectively; Table I, column 3). Since FWHM is slightly smaller in the Rios laboratory (1.33 vs. 1.5 µm; Table VII of Hollingworth et al., 2001), spark mass is only slightly larger (5.2 vs. 4.36 µm³). The larger spark amplitude and slightly larger value of mass in the Rios laboratory make it likely that the underlying Ca²⁺ source flux is at least as large as the 8–23 pA required for the simulation of sparks from the Schneider laboratory (row 3 of Tables V and VII). This expectation is in agreement with spark simulations by the Rios laboratory, which required Ca²⁺ source fluxes of 8 to 27 pA, depending on conditions (Table IV of Rios et al., 1999).

Significance of a Larger Amplitude Ca²⁺ Source Flux in Cut Fibers

A larger Ca²⁺ source flux in cut fibers could be caused by an increase in RYR single channel Ca²⁺ flux, an increase in mean open probability, an increase in the number of active RYRs per spark, or a combination of these possibilities. There are several differences between cut and intact fibers that might explain such an increase(s). First, as considered in the last section of results, cut fibers appear to be more hydrated than intact fibers and this swelling might alter RYR function, perhaps by changing the physical interactions between adjacent RYRs or between the RYRs and other proteins at the triadic junction. Second, the relative amplitude of fluo-3’s resting fluorescence at the z- and m-lines differs between intact fibers (Hollingworth et al., 2001) and cut fibers (Tsugorka et al., 1995; Klein et al., 1996; Lacampagne et al., 1996; Shirokova and Rios, 1997). The cut fiber pattern can be mimicked in intact fibers by increasing the concentration of K⁺ in the bathing solution from 2.5 to 7.5–30 mM. Since an increase in [Ca²⁺]r accompanies an elevation in [K⁺], it seems likely that the pattern of fluo-3’s resting fluorescence is a rough indicator of [Ca²⁺]r. By this criterion, [Ca²⁺]r is larger in cut fibers than in intact fibers (Hollingworth et al., 2001). Third, the duration of an action-potential–stimulated Ca²⁺ transient progressively increases with time during a 2 h experiment in a cut, but not an intact, fiber (Maylie et al., 1987b,c). This increase, which occurs in the absence of changes in indicator concentration, suggests that Ca²⁺ uptake is progressively slowed during the 2 h period, perhaps because of a progressive loss of intrinsic myoplasmic Ca²⁺ buffers or of modulators that maintain the normal activity of the SR Ca²⁺ pump; a decrease in the concentration of parvalbumin does not appear to occur during this period (Irving et al., 1989). In addition to these three documented differences between cut and intact fibers, small mobile molecules such as monovalent and divalent ions, ATP, phosphocreatine, and peptides would be expected to diffuse out of a fiber after cutting so that the composition of myoplasm in cut fibers would be expected to become progressively different from that in intact fibers (even though additions are usually made to the cut fiber end-pool solution to keep the concentrations of some of these constituents near the normal range). For example, [Mg²⁺]r and [Ca²⁺]r, which strongly affect RYR function, are probably 0.5–0.7 mM and 0.08–0.1 µM, respectively, in cut fibers and ~1 mM and ~0.05 µM, respectively, in intact fibers (Table I).

The differences between cut and intact fibers listed above might account for some, perhaps all, of the increased Ca²⁺ source flux in cut fiber sparks. For example, an increase in [Ca²⁺]r in cut fibers would be expected to increase the activity of the SR Ca²⁺ pump, which, in turn, should increase free [Ca²⁺] inside the SR and thereby increase RYR single channel Ca²⁺ flux. The diffusive loss of small molecules from the myoplasm of cut fibers could, in theory, increase the Ca²⁺ flux through an RYR if channel blockers or modulators that decrease the mean open probability were removed. The smaller value of [Mg²⁺]r and the larger value of [Ca²⁺]r (and the possible associated increase in SR Ca²⁺ content) in cut fibers could increase the number of RYRs per spark by augmenting Ca²⁺-induced Ca²⁺ release, a process that has been described in cut fibers (Jacquemond et al., 1991; Stern et al., 1997; Gonzalez et al., 2000; see also Rios and Pizarro, 1988). Although the cause(s) of the increased Ca²⁺ source flux in sparks in cut fibers is poorly understood at this time, the presence of this difference between RYR function in intact and cut fibers suggests that intact fibers contain structural or regulatory factors that are altered or missing in cut fibers—and, perhaps, in other disrupted preparations.

We thank Dr. Martin F. Schneider for helpful discussions regarding the measurement and interpretation of Ca²⁺ sparks in cut fibers.

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