Calcium sparks in mouse ventricular myocytes at physiological temperature

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Submitted 10 September 2002; accepted in final form 9 June 2003

Ferrier, Gregory R., Robin H. Smith, and Susan E. Howlett. Calcium sparks in mouse ventricular myocytes at physiological temperature. *Am J Physiol Heart Circ Physiol* 285: H1495–H1505, 2003. First published June 12, 2003; 10.1152/ajpheart.00802.2002.—In cardiac muscle, Ca$^{2+}$ is released from the sarcoplasmic reticulum (SR) in units called Ca$^{2+}$ sparks. Ca$^{2+}$ spark characteristics have been studied almost entirely at room temperature. This study compares characteristics of spontaneous sparks detected with fluo 3 in resting mouse ventricular myocytes at 22 and 37°C. The incidence and frequency of Ca$^{2+}$ sparks decreased dramatically at 37°C compared with 22°C. Also, spark amplitudes and times to peak were significantly reduced at 37°C. In contrast, spatial width and decay times were unchanged. During field stimulation, peak spatially averaged transients were similar at 22 and 37°C, and experiments with fura 2 demonstrated that diastolic and systolic Ca$^{2+}$ concentrations were unchanged. However, SR Ca$^{2+}$ content decreased significantly at 37°C. Restoration of SR Ca$^{2+}$ by superfusion with 5 mM Ca$^{2+}$ increased spark frequency but did not reverse the effects of temperature on spark parameters. Thus effects of temperature on spark frequency may reflect changes in SR stores, whereas changes in spark amplitude and rise time may reflect known effects of temperature on ryanodine receptor function.

CONTRACTION OF CARDIAC MUSCLE is initiated by a rapid rise in intracellular Ca$^{2+}$, called the Ca$^{2+}$ transient. Most of this Ca$^{2+}$ originates from release of intracellular stores of Ca$^{2+}$ through ryanodine receptors in the sarcoplasmic reticulum (SR) (1). Cheng et al. (5) first demonstrated that Ca$^{2+}$ release from the SR occurs as discrete quanta. They named these quanta Ca$^{2+}$ sparks because of the brief localized light emission they induced in Ca$^{2+}$-sensitive fluorescent dyes. Sparks originate near specialized Ca$^{2+}$ release regions at junctions between the SR and t-tubules or sarcolemma in cardiac muscle (2, 6, 21, 26) and are believed to represent concerted opening of groups of ryanodine receptors (1).

The spatial and temporal dimensions of sparks are stochastic properties, which vary as probability distributions independent of the initiating stimulus. Sparks typically have a width of ~2 μm, a rise time of ~10 ms, and a time constant of decay of ~20 ms (1, 4, 8, 28). At the peak, Ca$^{2+}$ rises to >1.5 times resting levels (4, 12, 29). Decay of spark intensity is believed to represent termination of release flux by ryanodine receptor inactivation (19) combined with diffusion of released Ca$^{2+}$ away from the site of release and to a lesser extent by uptake into the SR (8). Spontaneous Ca$^{2+}$ sparks occur at a low rate in quiescent myocytes. However, when a cell is depolarized, it is believed that many sparks are activated in near unison and the combined Ca$^{2+}$ release constitutes the Ca$^{2+}$ transient (3, 15, 16, 23).

It is not clear whether the characteristics and behavior of Ca$^{2+}$ sparks determined in earlier studies apply to mammalian cardiac myocytes at physiological temperature. One study (30) has demonstrated sparks in quiescent rat trabeculae at 33°C. Therefore, sparks can occur above room temperature and are not restricted to dissociated myocytes. However, virtually all other studies that have described and characterized Ca$^{2+}$ sparks have been conducted in myocytes at room temperature, typically 22–24°C. Changes in temperature can impact on many factors related to cellular Ca$^{2+}$ dynamics, including SR Ca$^{2+}$ load, L-type Ca$^{2+}$ current, duration of contraction, and time course of contraction (1). Interestingly, Sitgesas et al. (27) have reported that the open probability of the single ryanodine receptor in lipid bilayers is markedly temperature sensitive, with open probability being substantially reduced at higher temperatures. In addition, ryanodine receptors exhibited long open times at low temperature and brief openings at higher temperatures (27). One might speculate that brief open times at physiological temperature might be less likely to result in spontaneous sparks in cardiac myocytes. It is also possible that the characteristics of Ca$^{2+}$ sparks might be altered by changes in temperature. Therefore, we initiated studies to determine the impact of temperature on the occurrence and characteristics of Ca$^{2+}$ sparks. The specific objectives of this study were as follows: 1) to determine and compare the incidence and frequency of spontaneous Ca$^{2+}$ sparks in quiescent mouse ventricular myocytes at 22 and 37°C; 2) to compare the amplitudes, widths, and time courses of sparks at 22 and 37°C; 3) to determine whether changes in sparks with temperature reflect changes in...
SR Ca\(^{2+}\) stores; and 4) to document changes in Ca\(^{2+}\) transients and contractions occurring with the same changes in temperature in mouse myocytes.

**METHODS**

Experiments were conducted in accordance with the guidelines published by the Canadian Council on Animal Care and were approved by the Dalhousie University Committee on Laboratory Animals. Ventricular myocytes were isolated from male or female adult mice purchased from Charles River (CD-1) or from wild-type mice originally purchased from Jackson Laboratories (Bar Harbor, ME) and raised in house. Mice were anesthetized with pentobarbital sodium (200 mg/kg ip) co-injected with heparin (100 units) to prevent coagulation. Hearts were cannulated in situ and then perfused at 2.2 ml/min with 37°C nominally Ca\(^{2+}\)-free buffer of the following composition (in mM): 130 NaCl, 5 KCl, 1 MgCl\(_2\), 0.33 Na\(_2\)H\(_4\)PO\(_4\), 25 HEPES, 20 glucose, 3.0 Na pyruvate, and 1.0 Na lactate (pH 7.4 with NaOH). After 10 min, perfusion was switched to the Ca\(^{2+}\) stock to 800 μM CaCl\(_2\) plus dispase II (10 mg/50 ml, Boehringer Mannheim); collagenase (24 mg/30 ml, Worthington type I), and trypsin (1 mg/30 ml, Sigma). After perfusion for an additional 10 min, hearts were removed from the cannula and cut into small pieces in high-potassium substrate-enriched solution of the following composition (in mM): 30 KCl, 75 KOH, 30 KH\(_2\)PO\(_4\), 3 MgSO\(_4\), 50 glutamic acid, 20 taurine, 0.5 EGTA, 10 glucose, and 10 HEPES (pH 7.4 with KOH).

Myocytes released from the pieces of ventricular muscle by gentle swirling were filtered through 225-μm polyethylene mesh (Spectrum). Ca\(^{2+}\) sparks were recorded from myocytes loaded with fluo 3 by incubation in 20 μM fluo 3-AM (Molecular Probes) for 20–25 min at room temperature in the dark. Cells were loaded by dye with adding 200 μl of a fluo 3-AM stock to 800 μl of cell suspension. Fluo 3-AM stock was prepared by dissolving 1 mg of fluo 3-AM in 0.86 ml of anhydrous DMSO (Sigma). To this was added 9 ml of a solution containing 300 μl of pluronic F-127 (Molecular Probes) dissolved and sonicated in 12 ml of fetal calf serum. Myocytes loaded with fluo 3 were transferred to an experimental chamber (volume: ~1.5 ml) mounted on the stage of a Zeiss LSM 510 laser scanning microscope (Axiovert 100). Cells were allowed to settle (~5–10 min) and adhere to the glass bottom of the chamber, which was formed by a glass coverslip (24 × 50 mm, 0.08–0.13 mm in thickness, VWR Scientific). Coverslips were coated with poly-L-lysine (Sigma) at a concentration of 5–10 μg/cm\(^2\). Surfaces treated with poly-L-lysine were air dried and washed with distilled water before use. After myocytes had adhered, they were superfused at 1.3 ml/min at either room temperature (22–24°C) or 37°C with a solution of the following composition (in mM): 145 NaCl, 4 KCl, 1 CaCl\(_2\), 1 MgCl\(_2\), 10 HEPES, and 10 glucose (pH 7.4 with NaOH). The temperature of the superfusate was controlled by a water-jacketed heat exchanger, in which the physiological buffer passes through stainless steel tubing to maximize heat exchange. The heat exchanger was positioned immediately before the bath inlet. The temperature gradient across the bath was <1°C. Effects of the temperature gradient were minimized by always selecting cells from the middle of the bath.

Experiments were conducted on quiescent myocytes or on myocytes stimulated through bipolar silver wire electrodes positioned for field stimulation. Stimuli were rectangular pulses, 5 ms in duration, delivered at 1 Hz (model SD9, Grass Instruments). The stimulus voltage was adjusted to ~25% higher than the value required to initiate visible contractions.

Changes in free Ca\(^{2+}\) were measured in line scan mode. Fluo 3 was excited at 488 nm, and emission intensity was measured at 525 nm (Zeiss oil immersion objective, ×40/1.3 numerical aperture). Myocytes were repetitively scanned along the entire length of the cell at 1.5 ms intervals, for a maximum of 6 s. The confocal pinhole was adjusted to 54 μm to provide a maximum x-y-z resolution of 0.26 × 0.26 × 0.75 μm, and each line was composed of 512 pixels (voxels). The laser intensity was reduced to ≤5% maximum to decrease cell damage and dye bleaching. Line scan diagrams were constructed by stacking emission lines, corresponding to excitation scans, in temporal order.

Images were analyzed with Scion Image (Scion), ImageJ (NIH), and SigmaPlot (version 5.0, Jandel Scientific). Ca\(^{2+}\) sparks were identified as local peak elevations of fluorescent intensity (F) that were ≥1.5 times the surrounding background levels (F\(_0\)). The parameters that were measured included amplitude (F/F\(_0\)), spatial width [full width at half-maximum intensity (FWHM)], time to peak (TTP), amplitude, and the incidence (%) of myocytes exhibiting sparks during the 6-s recording period were measured. In field-stimulated myocytes, Ca\(^{2+}\) transients were determined by averaging the intensity of each sequential scan line and plotting the mean intensity as a function of time. These Ca\(^{2+}\) transients represent the average change in Ca\(^{2+}\) along the length of the cell but are restricted to the volume excited by the laser scan. TTP, T\(_{1/2}\), and F/F\(_0\) were measured for Ca\(^{2+}\) transients recorded at both temperatures. Fluorescence emission was not corrected for temperature, because spark amplitude was measured as the ratio of the peak fluorescence to adjacent background diastolic levels. Because changes to peak and background fluorescence are expected to be proportional, the ratio should be unaffected. This was confirmed in vitro for the same optical path and laser intensity by determining the ratio of emission recorded for 300 and 150 nM free Ca\(^{2+}\) at both 22 and 37°C. The emission ratio was identical at both temperatures. Because the ratio of F to F\(_0\) was not affected by temperature, the remaining spark parameters as well as our criterion for spark detection should not be affected by temperature.

In additional experiments, Ca\(^{2+}\) concentrations were measured ratiometrically with fura 2 with a Photon Technology International DeltaRAM system and Felix software (PTI; Brunswick, Md) as described previously (17, 31). Fura 2-AM stock solution (2.5 mM) was prepared in anhydrous DMSO. Cells were incubated in 5 μM fura 2-AM for 20–30 min at room temperature in the dark. Myocytes were transferred to an experimental chamber mounted on the stage of a Nikon inverted microscope (Eclipse TE200) and field stimulated at 1 Hz. Cells loaded with fura 2 were excited alternately at 340 and 380 nm, and emission was recorded at 510 nm. Light collection was restricted to the cell by an adjustable aperture. Background fluorescence was subtracted for each excitation wavelength, and the ratio of emission during excitation at 340 and 380 nm was converted to Ca\(^{2+}\) concentration with calibration curves determined in vitro with the same optical path. To compensate for changes in fluorescence with temperature, calibration curves were determined at both 22 and 37°C. Free Ca\(^{2+}\) concentrations were buffered by EGTA in the calibration solution (1 μM fura 2, 10 mM EGTA, 100 mM KCl, and 10 mM K-MOPS). pH was adjusted to neutrality with KOH (~30 mM) at both temperatures, and free Ca\(^{2+}\) concentrations in the calibration solutions were calculated.
for each temperature with Maxchelator (version 2.40, WinMAXC). Unloaded cell shortening was measured with a video edge detector (Crescent Electronics; Sandy, UT) coupled to a closed circuit television system (31).

In some experiments, SR Ca$^{2+}$ stores were assessed by rapid application of 10 mM caffeine with a temperature-controlled rapid solution switcher (11). For this purpose, caffeine was dissolved in solution with 0 mM Na$^+$ and 0 mM Ca$^{2+}$ to prevent efflux of Ca$^{2+}$ through Na$^+/Ca^{2+}$ exchange (13). The composition of this solution was (in mM) 140 LiCl, 4 KCl, 4 MgCl$_2$, 5 HEPES, 10 glucose, 0.3 lidocaine, and 4 4-aminopyridine (pH 7.4 with LiOH). Released Ca$^{2+}$ was measured by the 340-to-380-nm fura 2 ratio as described above. Cells in these experiments were activated regularly with 200-ms voltage-clamp pulses from a holding potential of −80 to 0 mV. Caffeine was applied for 1 s after a train of conditioning pulses.

Statistical analyses were conducted with SigmaStat (Jandel Scientific). Differences between means were tested for significance with either a t-test or a rank sum test. Differences in incidence were subjected to a $\chi^2$-test. Differences were considered statistically significant for $P < 0.05$.

**RESULTS**

Figure 1, A and B, shows representative line scan diagrams recorded from quiescent ventricular myocytes at room temperature (22°C) and 37°C, respectively. The line scan diagram recorded at 22°C shows multiple Ca$^{2+}$ sparks occurring spontaneously. The changes in intensity with time at selected sites a and b in Fig. 1A are shown below the line scan diagram. These intensity-time profiles show that the sparks had a rapid onset and more gradual decline. Line a shows two sparks occurring at or near the same site. Figure 1B shows the same size line scan diagram recorded from a different cell at 37°C, which showed only a single Ca$^{2+}$ spark. The intensity-time profile of the location corresponding to this spark is shown below the line scan diagram.

The line scan diagrams in Fig. 1, A and B, show a difference in the number of sparks occurring in resting cells at different temperatures. We evaluated the effect of temperature on the number of cells exhibiting sparks during a 6-s line scan. Figure 1C shows that close to 90% of cells exhibited Ca$^{2+}$ sparks at 22°C, whereas only 20% of myocytes generated Ca$^{2+}$ sparks when the temperature was 37°C. The percentage of cells exhibiting sparks was significantly higher at 22°C compared with 37°C. We also determined the overall frequency of sparks occurring at the two different temperatures. Figure 1D shows that the frequency of Ca$^{2+}$ sparks was markedly and significantly decreased at 37°C compared with 22°C.

We next determined and compared the characteristics of Ca$^{2+}$ sparks recorded at both temperatures. Figure 2, A and B, shows line scan diagrams of representative Ca$^{2+}$ sparks recorded at 22 and 37°C, respectively. The same sparks are shown rotated and with intensity axes added in the vertical plane in Fig. 2. C and D. The example recorded at 22°C appears somewhat slower in time course than the example recorded at 37°C. Intensity-time profiles for the same sparks are

![Figure 1](http://ajpheart.physiology.org/)

**Fig. 1.** The incidence and frequency of Ca$^{2+}$ sparks are significantly decreased at physiological temperature. A: representative line scan diagram showing multiple Ca$^{2+}$ sparks recorded at 22°C. Intensity-time profiles illustrating representative sparks are indicated as a and b. The intensity-time profiles represent the average intensity collected for four contiguous pixels at each of the points indicated on the distance axis. B: line scan diagram showing a single Ca$^{2+}$ spark recorded at 37°C. The intensity-time profile for point c is shown below the line scan. C: percentage of cells in which Ca$^{2+}$ sparks occurred during a 6-s line scan recording was significantly lower at 37°C ($\chi^2$-test). D: mean frequency of Ca$^{2+}$ sparks, measured as the number of sparks per 100 μm per second, was significantly decreased at 37°C. F/F0, fluorescent intensity/surrounding background levels. Data are from line scans recorded from 25 cells at each temperature. *P < 0.05.
presented in Fig. 2. Here, the relative intensity is presented as the ratio of the fluorescence intensity at any given point (F) to the background intensity preceding the spark (F₀). The spatial contours of the same sparks are shown by the relative intensity-distance profiles in Fig. 2, G and H. The widths of these representative Ca²⁺ sparks appeared to be similar at the two temperatures.

Quantitative data for Ca²⁺ spark characteristics were derived through measurements illustrated in Fig. 3A. Figure 3A shows a surface plot of fluorescence intensity during a Ca²⁺ spark. Time and distance are plotted on the x- and y-axes, and intensity is plotted on the z-axis. The amplitudes of sparks were determined as F/F₀ at the peak of the spark. Mean data for amplitudes of sparks at 22 and 37°C are shown in Fig. 3B. Spark amplitudes were significantly lower at 37°C compared with amplitudes measured at 22°C. Spatial widths of sparks were measured as FWHM, as indicated in Fig. 3A. Figure 3C shows that FWHM was virtually identical at the two temperatures tested. Time courses of Ca²⁺ sparks were divided into rising and decaying phases. The rising phase was measured as TTP, and the decay phase was measured as T₁/₂, as indicated in Fig. 3A. Figure 3D shows that TTP was significantly shorter for Ca²⁺ sparks recorded at 37°C. In contrast, there were no significant differences between T₁/₂ for sparks at 22 and 37°C.

The frequency distributions of spark characteristics at both temperatures are plotted in Fig. 4. Figure 4A shows the distribution of spark amplitudes. The amplitude with the greatest number of events was the same at both temperatures. However, there was a greater number of large sparks at 22°C. Thus the
frequency distribution for amplitudes was broader at 22°C compared with 37°C. The frequency distributions for FWHM were similar at both temperatures (Fig. 4B). However, the frequency distributions for TTP indicate that the distribution of rise times was shifted to shorter times at 37°C (Fig. 4C). The TTP with the greatest number of events also was shorter at 37°C. The distribution frequencies for $T_{1/2}$ were similar for both temperatures (Fig. 4D).

Because Ca$^{2+}$ sparks are believed to represent the sum of numerous Ca$^{2+}$ sparks, we determined whether changes in temperature also resulted in changes in Ca$^{2+}$ transients in field-stimulated myocytes. Figure 5A shows a representative line scan diagram recorded at 22°C. Three stimulated Ca$^{2+}$ transients were triggered during this line scan. To visualize the time courses of the large Ca$^{2+}$ transients, fluorescent intensity was averaged for each line in the diagram and plotted as a function of time below the line scan diagram to obtain a spatially averaged transient. A representative line scan diagram recorded at 37°C plus the corresponding spatially averaged Ca$^{2+}$ transients below are shown in Fig. 5B. The time courses of the Ca$^{2+}$ transients recorded at the two temperatures were similar. In both line scans shown in Fig. 5, the scan lines extended slightly beyond both ends of the cells. Therefore, the contractions of the cells are visible as scalloping of the edges of the fluorescent signal. The contractions at 37°C appeared to be more rapid than the contractions at 22°C. In addition to the large Ca$^{2+}$ transients, several Ca$^{2+}$ sparks were observed between transients at both temperatures.

Mean data for amplitudes and time courses of Ca$^{2+}$ transients recorded at both temperatures are summarized in Fig. 5, C–E. Figure 5C shows that there was no difference between the mean amplitudes of Ca$^{2+}$ transients expressed as F/F$_0$. Mean TTP is shown in Fig. 5D. Although there was a trend toward shorter TTP at 37°C, this difference was not statistically significant. Figure 5E shows that $T_{1/2}$ was similar at both temperatures. There also was no significant difference in the number of cells exhibiting Ca$^{2+}$ sparks between stimulated transients. At 22°C, 42% of 31 cells exhibited Ca$^{2+}$ sparks, and at 37°C, 47% of 19 cells exhibited sparks.

Spatially averaged transients determined with fluo 3 provide a measure of changes in free intracellular Ca$^{2+}$ relative to resting levels (F$_0$) but do not provide a measure of actual Ca$^{2+}$ concentrations. To determine whether Ca$^{2+}$ concentrations achieved during transient...
transients were different at 22 and 37°C, we conducted experiments with similar field stimulation protocols in cells loaded with the ratiometric dye fura 2. Unloaded cell shortening was measured simultaneously. Figure 6, A and B, shows representative recordings of transients and contractions measured at 22 and 37°C, respectively. Mean data for amplitudes and time courses of contractions were shown in Fig. 6, C–F. Figure 6C presents mean concentrations of Ca2+ for transients recorded at both temperatures. The diastolic Ca2+ concentration, measured immediately before the beginning of transients, was not significantly different at 37°C compared with 22°C. The peak systolic Ca2+ concentration and the amplitudes of Ca2+ transients also were not significantly different at 37°C compared with 22°C. The mean amplitudes of contractions were slightly larger at 37°C. The mean amplitudes of contractions were significantly larger at 37°C, although this was not significant. We next investigated whether changes in Ca2+ sparks observed with increased temperature were caused by decreased SR Ca2+. To do this, we determined the Ca2+ spark frequency at 37°C in myocytes in which SR Ca2+ stores were increased by superfusion with extracellular solution containing 5 mM Ca2+. SR Ca2+ stores were assessed in cells loaded with fura 2 by rapid application of 10 mM caffeine in 0 mM Na+ and
Fig. 6. Amplitudes and time courses of Ca\(^{2+}\) transients and contractions at 22 and 37°C in field-stimulated mouse myocytes loaded with fura 2. A: representative examples of fura 2 Ca\(^{2+}\) transients (top) and contractions (bottom) in a myocyte at 22°C. B: representative examples of fura 2 Ca\(^{2+}\) transients (top) and contractions (bottom) in a myocyte at 37°C. C: mean Ca\(^{2+}\) concentrations recorded during field stimulation at 22 and 37°C. Diastolic and systolic Ca\(^{2+}\) concentrations, as well as Ca\(^{2+}\) transient amplitudes, were not significantly different at 22 and 37°C. D: mean amplitude of contractions was slightly but not significantly greater at 37°C. E: mean time courses of Ca\(^{2+}\) transients. TTP and \(T_{1/2}\) were significantly shorter at 37°C. F: mean time courses of contractions. TTP and \(T_{1/2}\) of contractions also were significantly shorter at 37°C. [Ca\(^{2+}\)]\(_i\), intracellular Ca\(^{2+}\) concentration. \(n = 8\) at 22°C and 10 at 37°C. *\(P < 0.05\).

Fig. 7. Mean sarcoplasmic reticulum (SR) Ca\(^{2+}\) content was significantly decreased at 37°C in mouse ventricular myocytes. A: representative example of changes in Ca\(^{2+}\) concentration in a ventricular myocyte at 22°C. Caffeine (10 mM) was applied with a rapid solution switcher for 1 s after a train of 5 conditioning pulses. Caffeine was applied in solution with 0 mM Na\(^+\) and 0 mM Ca\(^{2+}\) to prevent loss of released Ca\(^{2+}\) through Na\(^+\)/Ca\(^{2+}\) exchange. B: recording from a different cell showing representative changes in Ca\(^{2+}\) concentration in response to conditioning pulses and caffeine at 37°C. C: comparison of mean resting free Ca\(^{2+}\) concentrations and mean peak Ca\(^{2+}\) concentrations in response to caffeine in myocytes at 22 and 37°C. Although resting Ca\(^{2+}\) concentration was unaffected, peak caffeine-induced Ca\(^{2+}\) transients were significantly decreased at 37°C in mouse myocytes. \(n = 5\) at both temperatures. *\(P < 0.05\).
approximated the relative decrease caused by increasing temperature. We then used the same increase in extracellular Ca\(^{2+}\) concentration to assess whether increasing SR Ca\(^{2+}\) would counteract the effects of temperature on spark frequency and parameters in cells loaded with fluo 3. Figure 8 compares the frequency of Ca\(^{2+}\) sparks measured at 22°C and 37°C in cells loaded with fluo 3. Control spark frequency was determined at 22°C in 1 mM Ca\(^{2+}\). Increasing the temperature to 37°C significantly reduced the spark frequency in 1 mM Ca\(^{2+}\) but not in 5 mM Ca\(^{2+}\). n = 12 myocytes/group. *P < 0.05; ns, not significant.

DISCUSSION

This study was designed to determine and compare the incidence, frequency, and characteristics of spontaneous Ca\(^{2+}\) sparks in quiescent mouse ventricular myocytes at 22 and 37°C. In addition, we investigated whether effects of temperature on sparks reflected changes in SR Ca\(^{2+}\) stores and characterized changes in Ca\(^{2+}\) transients and contractions occurring with the same changes in temperature in mouse myocytes. Our results show that the incidence and frequency of Ca\(^{2+}\) sparks decrease dramatically at 37°C compared with 22°C. In addition, both spark amplitude and TTP were significantly reduced at 37°C. However, there was no change in the spatial width or T\(_{50}\) of sparks with

Fig. 8. Elevation of SR Ca\(^{2+}\) stores attenuates the reduction in spark frequency observed at 37°C. A: representative caffeine-induced fura 2 transients recorded from myocytes superfused solution containing 1 or 5 mM Ca\(^{2+}\). B: comparison of mean resting Ca\(^{2+}\) concentration and peak amplitudes of caffeine-induced transients in the presence of 1 and 5 mM Ca\(^{2+}\). Amplitudes of caffeine-induced transients were significantly increased in 5 mM Ca\(^{2+}\). C: elevation of extracellular Ca\(^{2+}\) prevents significant reduction of spark frequency at 37°C in cells loaded with fluo 3. Control spark frequency was determined at 22°C in 1 mM Ca\(^{2+}\). Increasing the temperature to 37°C significantly reduced the spark frequency in 1 mM Ca\(^{2+}\) but not in 5 mM Ca\(^{2+}\). n = 12 myocytes/group. *P < 0.05; ns, not significant.
temperature. These changes in occurrence and characteristics of sparks were accompanied by a decrease in SR Ca$^{2+}$ stores at 37°C. When SR Ca$^{2+}$ stores were increased to compensate for this effect of temperature, spark frequency was no longer decreased, but changes in spark parameters were not eliminated. Changes in Ca$^{2+}$ sparks with increased temperature were not accompanied by changes in the amplitudes of Ca$^{2+}$ transients measured with fluo 3 or fura 2 in field-stimulated cells. However, TTP and half-relaxation of fura 2 transients and contractions were significantly faster at 37°C, although fluo 3 spatially averaged transients did not exhibit changes in time course.

One of the key observations in this study is that the occurrence of spontaneous Ca$^{2+}$ sparks is greatly decreased at 37°C in mammalian ventricular myocytes. Thus observations of spark frequency and incidence at room temperature are not a good measure of their occurrence at physiological temperature. Several possible mechanisms that might explain why the occurrence of sparks decreases at physiological temperature should be considered. First, it is unlikely that the temperature dependence of Ca$^{2+}$ sparks reflects changes in open probability of L-type Ca$^{2+}$ channels, as spontaneous sparks are not believed to be triggered by opening of L-type Ca$^{2+}$ channels (25, 29). Second, it has been reported that Ca$^{2+}$ spark frequency increases with increases in cytosolic Ca$^{2+}$ concentration (1). However, in the present study, we did not observe significant changes in diastolic Ca$^{2+}$ when temperature was increased to 37°C. Third, SR Ca$^{2+}$ stores might play an important role in determining Ca$^{2+}$ spark frequency. Several studies (18, 24, 25) have reported an increase in spark frequency when SR Ca$^{2+}$ increases. In some mammalian species, such as the rabbit and ferret, SR Ca$^{2+}$ stores are greater at low temperatures compared with higher temperatures (22). However, in other species, such as the cat, SR Ca$^{2+}$ stores do not change significantly between 25 and 35°C (22). When we measured SR Ca$^{2+}$ stores in mouse ventricular myocytes with caffeine applications and fura 2, we found that there was a significant decrease at 37°C compared with 22°C. Interestingly, when SR Ca$^{2+}$ stores were increased at 37°C, a significant decrease in spark frequency was no longer observed. This suggests that changes in SR Ca$^{2+}$ are likely to contribute to the decrease in spark incidence and frequency observed at 37°C in mouse myocytes.

It also is possible that temperature alters ryanodine receptor function. Increased temperature decreases the open probability and open times of single sheep ryanodine receptors in lipid bilayers (27). If similar changes occur in vivo in mouse myocytes, this may provide another mechanism that contributes to the decreased incidence and frequency of Ca$^{2+}$ sparks at physiological temperature. Decreased open probability of ryanodine receptors at 37°C compared with 22°C might decrease the likelihood that any given opening of a ryanodine receptor would recruit neighboring ryanodine receptors and initiate a spark. The decreased open times reported by Sitsapesan et al. (27) also might explain the reduced TTP and decreased amplitude of Ca$^{2+}$ sparks observed at 37°C in the present study. If ryanodine receptors close sooner at physiological temperature and thereby curtail Ca$^{2+}$ release, both a decreased TTP and decreased spark amplitude would be expected. Interestingly, the effects of temperature on TTP and amplitude of sparks were not reversed by increasing SR Ca$^{2+}$ stores. This suggests that these effects of temperature are not mediated by changes in SR stores and therefore may reflect changes in ryanodine receptor kinetics. This interpretation is supported by observations by Lukyanenko et al. (18), who investigated the effects of changing SR stores at room temperature. They found that Ca$^{2+}$ spark frequency paralleled SR stores; however, spark amplitudes were unchanged.

We also found that neither spatial width nor $T_{1/2}$ of sparks changed with temperature. The absence of a
change in spark width suggests that the number of ryanodine receptors comprising a spark unit was the same at both 22 and 37°C. The absence of a significant change in T\text{\textsubscript{vs}} between 22 and 37°C in the present study indicates that decay of sparks occurs by a mechanism that is not strongly affected by temperature. Indeed, T\text{\textsubscript{vs}} is believed to be determined primarily by diffusion of Ca\textsuperscript{2+} away from the release sites, whereas reuptake of Ca\textsuperscript{2+} by the SR plays a lesser role (8). Our results are in keeping with diffusion being the main determinant of spark decay, as the Q\textsubscript{10} for aqueous diffusion is only 1.3 (10).

The changes observed in spontaneous Ca\textsuperscript{2+} spark amplitude did not correlate with changes in the amplitudes of whole cell transients determined with either fura 2 or fluo 3. Spark amplitudes decreased, whereas the amplitudes of Ca\textsuperscript{2+} transients were unchanged. Thus the change in unitary release of SR Ca\textsuperscript{2+} observed with spontaneous sparks did not impact on the amplitudes of Ca\textsuperscript{2+} transients. However, Ca\textsuperscript{2+} transients are not caused by spontaneous sparks, and it is possible that depolarization-induced sparks respond differently to temperature than spontaneous sparks. Alternatively, an increase in number of spark units recruited by depolarization at 37°C might compensate for reduced spark amplitudes.

The TTP for whole cell Ca\textsuperscript{2+} transients measured with fura 2 was shorter at 37°C compared with 22°C. A trend toward shorter TTP was also observed with spatially averaged fluo 3 transients, although this was not statistically significant. It is not clear whether abbreviation of rise times of Ca\textsuperscript{2+} transients reflects the stochastic properties of Ca\textsuperscript{2+} sparks. Other factors such as synchrony of spark recruitment and the length of the latent period between depolarization and initiation of sparks may play a significant role in determining the TTP. For example, slowed rise times of Ca\textsuperscript{2+} transients observed in myocytes from failed hearts have been associated with a greater dispersion in latency for spark initiation (14). Furthermore, synchronization of sparks and shortening of latent periods with isoproterenol resulted in shorter rise times for transients (14). It is unknown whether increasing temperature causes a similar synchronization and decreased latency in depolarization-induced Ca\textsuperscript{2+} sparks. However, if such a change occurs, it might explain why the peak amplitude of Ca\textsuperscript{2+} transients was preserved despite a reduction of SR Ca\textsuperscript{2+} stores at 37°C. In addition, the magnitude of trigger Ca\textsuperscript{2+} current is increased substantially at 37°C. L-type Ca\textsuperscript{2+} current exhibits a Q\textsubscript{10} of ~3, which is believed to reflect in part an increase in phosphorylation levels and therefore open probability (20). L-type Ca\textsuperscript{2+} current also may make a larger contribution to the transient and thereby help preserve the amplitudes of the transients despite the reduction in SR Ca\textsuperscript{2+} stores.

The T\text{\textsubscript{vs}} of Ca\textsuperscript{2+} transients measured with fura 2 decreased significantly at 37°C compared with 22°C. In contrast, there was no significant change in T\text{\textsubscript{vs}} of spatially averaged Ca\textsuperscript{2+} transients detected with fluo 3. The rate of dissociation of Ca\textsuperscript{2+} from fura 2 is much slower than that from fluo 3 (7). Thus the decay observed in the fura 2 transients may largely represent the slow kinetics of Ca\textsuperscript{2+} dissociation from fura 2. Because of this, the marked change in decay time of Ca\textsuperscript{2+} transients detected with fura 2 may reflect the substantial temperature dependence of the fura 2 dissociation constant (9). The dissociation constants for fluo 3 are much higher than those for fura 2 at both room and physiological temperatures (7), and therefore the time courses of fluo 3 transients more likely reflect changes in free Ca\textsuperscript{2+}. Therefore, our data suggest that there is little change in T\text{\textsubscript{vs}} of Ca\textsuperscript{2+} transients at 37°C compared with 22°C in mouse ventricular myocytes.

Although the time course of decay of fluo 3 Ca\textsuperscript{2+} transients changed little with temperature, times to half-relaxation of contractions decreased at 37°C compared with 22°C. It is not clear what causes this disparity in time courses. It is possible that additional temperature-sensitive events involved in contraction and relaxation may be affected differently from events related to SR Ca\textsuperscript{2+} release. For example, rates of Ca\textsuperscript{2+} dissociation from myofilaments as well as steps in hydrolysis of ATP and myofilament movement may vary substantially at different temperatures. Thus the relationship between declining Ca\textsuperscript{2+} concentrations and relaxation might differ at 22 and 37°C.

The present study demonstrates that the stochastic properties as well as the frequency and incidence of spontaneous Ca\textsuperscript{2+} sparks recorded in isolated mouse myocytes at room temperature do not accurately reflect spark characteristics at physiological temperature. This finding has important implications regarding our understanding of the dynamic exchange of Ca\textsuperscript{2+} between intracellular and extracellular compartments. The level of SR Ca\textsuperscript{2+} reflects a balance between uptake and release. Spontaneous Ca\textsuperscript{2+} sparks provide a mechanism for spontaneous SR Ca\textsuperscript{2+} leak. The present study suggests that SR Ca\textsuperscript{2+} leak in the form of sparks is much less at physiological temperature than at room temperature, as frequency, rise times, and amplitudes of sparks are decreased. Changes in SR Ca\textsuperscript{2+} stores may account in part for the effect of temperature on spark frequency, although other factors such as changes in the open probability of ryanodine receptors also may contribute.

The authors thank Peter Nicholl, Dr. Jiequan Zhu, and Steve Foster for excellent laboratory technical support and assistance in preparation of the figures and Steven Whitefield for outstanding technical support of confocal microscopy.

DISCLOSURES

This work was supported in part by grants from the Heart and Stroke Foundation of Nova Scotia and from The Canadian Institutes for Health Research. During this study, R. Smith was supported by graduate studentships from the Nova Scotia Health Research Foundation and the Heart and Stroke Foundation of Canada.
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