Frequency, Amplitude, and Propagation Velocity of Spontaneous Ca++-Dependent Contractile Waves in Intact Adult Rat Cardiac Muscle and Isolated Myocytes

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SUMMARY. Spontaneous contractile waves due to spontaneous calcium cycling by the sarcoplasmic reticulum occur in unstimulated bulk rat papillary muscle and single rat cardiac myocytes with intact sarcolemmal function. We used video analytic techniques to quantify the wave characteristics in both bulk muscle and myocytes; laser-light scattering techniques were also employed in muscle. In muscle bathed in physiological concentrations of calcium, the true periodicity of these waves was a fraction of 1 Hz and increased up to several hertz with increases in cell calcium. This was paralleled by an increase in the frequency of scattered laser light intensity fluctuations. In myocytes, a range of spontaneous contractile wave frequencies similar to that which occurred in the muscle was observed; it could be demonstrated that an increase in superfusate calcium concentrations (2–15 mM at 23°C) increases the oscillation frequency but not amplitude. In both myocytes and muscle, low concentrations of caffeine (0.5 mM) and higher temperature increased the oscillation frequency but diminished their amplitude. However, the scattered light fluctuations did not change with temperature and decreased with caffeine. These results demonstrate that (1) the true frequency of spontaneous sarcoplasmic reticulum oscillations in the unstimulated rat muscle and single myocytes with intact sarcolemmal function is low, i.e., a fraction of a hertz; (2) with cell calcium loading, the oscillation frequency accelerates to those frequencies measured previously in the “calcium overload” state; (3) while scattered light fluctuations which sample myofilament motion are a sensitive, noninvasive method of detecting the oscillations in bulk muscle, they can be insensitive to the divergent changes in oscillation amplitude and frequency. (Circ Res 57: 844–855, 1985)

ISOLATED rat cardiac myocytes, in which sarcolemmal function has been removed to permit direct control of myoplasmic [Ca++] , exhibit spontaneous Ca++ oscillations when bathed in [Ca++] as low as 100 nM (Fabiato and Fabiato, 1972, 1973, 1975; Dani et al., 1979; Rieser et al., 1979; Chiesi et al., 1981; Fabiato, 1983; Fabiato and Baumgarten, 1984). However, the spontaneous Ca++ release does not occur homogeneously throughout the myoplasm, but, rather, is localized and is manifested grossly as localized myofilament interaction which then longitudinally propagates as a contractile wave (Fabiato and Fabiato, 1972, 1975; Rieser et al., 1979; Chiesi et al., 1981). Apparently, the mechanism whereby this propagation occurs is a combination of Ca++ diffusing down its intracellular gradient and a resulting regenerative Ca++-induced Ca++ release induced from the adjacent sarcoplasmic reticulum (SR) (Stern et al., 1984). The frequency at which these waves occur increases with the extent of cell Ca++ loading (Fabiato and Fabiato, 1972, 1973, 1975; Dani et al., 1979; Chiesi et al., 1981; Fabiato, 1983; Fabiato and Baumgarten, 1984), and perturbations that interfere with SR Ca++ pumping and/or release act to inhibit them (Fabiato and Fabiato, 1972, 1973, 1975; Dani et al., 1979; Chiesi et al., 1981). Single isolated rat cardiac myocytes with intact sarcolemmal function also exhibit spontaneous contractile waves in the unstimulated state (Capogrossi et al., 1984; Capogrossi and Lakatta, 1985a; Lakatta et al., 1985).

Studies in the intact bulk muscle strongly suggest that the spontaneous [Ca++] oscillations observed in isolated myocytes (either with or without intact sarcolemmal function) are not an artifact of the methods used to produce those cells or of the experimental conditions in which those studies were implemented. In the unstimulated bulk rat cardiac muscle with intact sarcolemmal function, direct microscopic observation has demonstrated the presence of a spontaneous Ca++-dependent microscopic myofilament contraction that propagates longitudinally in a wave-like manner (Stern et al., 1983). Since these contractile waves are abolished when myoplasmic [Ca++] is buffered to a constant level in detergent-skinned preparations, it has been inferred that, as
in single myocytes, these waves were likewise due to a spontaneous localized increase in myoplasmic [Ca^{2+}] that spreads across the cells in waves (Stern et al., 1983). That spontaneous oscillatory Ca^{2+} release underlies the wave was confirmed by the demonstration of periodic oscillations in the light emitted from aequorin injected into the cells comprising intact muscle tissue (Orchard et al., 1983; Wier et al., 1983). The induced wave-like myofilament motion caused by this propagating Ca^{2+} release modulates a laser beam passed through the tissue to produce scattered light intensity fluctuations (SLIF) (Lappe and Lakatta, 1980; Lakatta and Lappe, 1981; Stern et al., 1983; Kort and Lakatta, 1984). It has been shown that those perturbations which block sarcolemmal ionic conductances do not abolish SLIF, but those that interfere with SR uptake or release, do [i.e., high concentrations of caffeine, ryanodine, or replacement of Ca^{2+} with strontium (Lakatta and Lappe, 1981; Kort and Lakatta, 1983, 1984; Stern et al., 1983, 1984)]. Thus, it has been concluded (Kort and Lakatta, 1983, 1984; Stern et al., 1983, 1984) that SLIF are caused by the very same spontaneous oscillatory SR Ca^{2+} release described earlier in single cardiac cells (Fabiato and Fabiato, 1972, 1973, 1975; Dani et al., 1979; Chiesi et al., 1981; Fabiato, 1983; Fabiato and Baumgarten, 1984). That the differences in the extent of cell Ca^{2+} loading among species required to produce SLIF paralleled those in single cells, further supported this conclusion (Kort and Lakatta, 1984).

Whereas SLIF measurements are noninvasive and have been shown to be a sensitive tool for quantifying the overall magnitude of tissue motion caused by spontaneous Ca^{2+} oscillations, they provide no direct information on either the actual frequency of the spontaneous Ca^{2+} release or the amplitude of the resulting myofilament displacement. Quantitative studies of the frequency and amplitude of spontaneous contractile waves would, under specific experimental conditions, provide unique data on the function of the SR as it occurs in preparations with intact sarcolemmal function. The present study, quantified the frequency, amplitude, and propagation velocity of these waves, both in myocytes with intact sarcolemmal function (Capogrossi et al., 1984; Capogrossi and Lakatta, 1985a; Lakatta et al., 1985) by video imaging, and in the bulk muscle by video imaging and spectral analysis. In addition, in the bulk muscle, changes in these contractile wave parameters were compared to the changes in SLIF that occur in response to perturbations that affect cell Ca^{2+} loading and thus affect Ca^{2+} cycling by the SR.

Methods

Single Myocyte Preparation

Single myocardial cells from the left ventricle of 7-month-old rats were isolated as previously described (Silver et al., 1983; Capogrossi and Lakatta, 1985a). Briefly, the hearts were retrogradely perfused through the aorta with a low Ca^{2+}-collagenase bicarbonate buffer at 37°C (pH 7.4), then minced and mechanically dispersed by gentle pipetting. The single cells then were resuspended and equilibrated for 1 hour in Dulbecco's modified Eagle's medium (DMEM, GIBCO) of the following composition (in mM): NaCl, 109.6; NaHCO3, 44.0; NaH2PO4·H2O, 0.9; KCl, 5.4; MgSO4·7H2O, 0.7; d-glucose, 25; and CaCl2, 1.8; at 37°C and pH 7.4 ± 0.05 maintained by a 5% CO2 humidified atmosphere. Subsequently, cells were washed and resuspended in (N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid) (HEPES) buffer of the following composition (in mM): NaCl, 137; KCl, 5; MgSO4·7H2O, 1.2-HEPES, 20; and dextrose, 15; with variable concentrations of Ca^{2+}. Myocytes used for the study were attached at the cell center (approximately 10% of their length) to the floor of a second chamber with a volume of 1.5 ml and were continuously perfused at the rate of 3 ml/min. In response to threshold stimuli (2–5 msec in duration) delivered by a Grass stimulator (SD9) through two platinum electrodes in the bathing fluid, these cells exhibited a synchronous contraction (twitch). In an unselected subset of these cells (n = 16) resting membrane potential averaged 84.1 ± 4.4 mV (mean ± SEM) (Suarez-Isla et al., 1984). In the absence of stimulation, most of these rat myocytes exhibited infrequent periodic spontaneous contractile waves (Capogrossi et al., 1984; Capogrossi and Lakatta, 1985b).

Measurement of Wave Characteristics in Myocytes

Myocytes were examined by phase-contrast microscopy (Leitz, Diavert) with a 25X objective (see Fig. 1A). The image of a single cell was projected on a video monitor (Panasonic, model WV-52) through a Panasonic TV camera adapted to the microscope. Myocytes selected for study were rod-shaped with clear A-I striations, sharp edges, and no blebs or granulations (Fig. 2a).

Whereas spontaneous contractile waves were easily observed on the video monitor by eye, single frame images of these waves do not adequately demonstrate this phenomenon. Thus, the propagation of such a wave through a series of sarcomeres within an individual myocyte has been depicted in Figure 2b by computer simulation (Fig. 3).
shown in Figure ID. Wave amplitude was measured as the distance from the baseline to the plateau of the video dimension analyzer output (arrow in Fig. 1D); wave frequency was determined simply by monitoring the number of waves that occurred per unit time.

Wave velocity in the myocytes was determined by placing two photoresistors on the video image (Fig. 1) at a known distance from each other (i.e., an equivalent spacing of 50 μm) and measuring the time difference of changes in light intensity sensed at each resistor (Fig. 1C). These changes in light intensity with wave passage, which are demonstrated clearly in the computer simulation in Figure 2b, result from an increase transparency of the tissue as an approaching wave stretched the relatively compliant region of relaxed myofilaments, followed by a decrease in transparency that results from a shortening of the myofilaments in the wave proper, and, finally, an increased transparency as the receding wave stretched the again relaxed myofilaments in the wake of the wave (Fig. 2b). Wave velocity was then simply calculated as:

$$\text{velocity} = \frac{\Delta \text{distance between photoresistors (μm)}}{\Delta \text{time between photoresistor peak (sec)}}$$

**Bulk Muscle Preparation**

Wistar rats weighing 300–500 g were killed by cervical dislocation and the hearts were placed in oxygenated HEPES-buffered solution (Lakatta and Lappe, 1981) containing [Na+] of 140 mM, a [Ca++] of 1 mM, a [K+] of 4.2 mM, a [Mg++] of 1.0 mM, and a glucose concentration of 10 mM; pH was 7.4. Right ventricular papillary muscles (100–400 μm diameter) were dissected, mounted between stainless steel clips, one of which was attached to a force transducer (UC-1; Statham Inc.), and were perfused with buffer solution in either a 3-ml chamber for light-scattering studies (Stern et al., 1983) or in a 5-ml plastic petri dish mounted on the stage of the inverted microscope for both video analysis of displacement and light-scattering measurements. During the equilibration period (typically 3 hours), muscles were field stimulated (SD9; Grass Instrument Co.) at 15/min with platinum wire electrodes.

**Measurements of Wave Characteristics in Bulk Muscle**

The mechanical displacement caused by contractile waves in the bulk muscle preparation was quantified by examining the incoherently illuminated muscle by phase contrast microscopy. The nature of this wave preceded in the bulk muscle appeared similar to that in the myocyte. When a wave occurred, it could easily be observed as a propagating band of increased absorbance. These waves were detected from the video image of the muscle (magnified such that one to two cells lengths occupied the width of the video screen) by a photoresistor sensitive to the light intensity from a region corresponding to a circular area, equivalent to approximately 20 sq μm, placed against the video screen. The output of the photoresistors (after four-pole low-pass filtering at 10 Hz to remove 60-Hz noise) fluctuated with wave passage. Estimates of the magnitude of total motion and of wave frequency were obtained by subjecting this raw signal from one photoresistor to Fourier transform analysis on a Vax 11/780 (Wier et al., 1983).

A similar approach was employed to determine wave velocity in the bulk muscle as in myocytes, except the time difference between wave peaks was determined by performing the cross-correlation between the signals from
the two photoresistors. Two difficulties, however, were initially encountered in this technique: first, there was a slowly varying "noise" superimposed on this signal resulting from a slow translational and gyrational motion of the bulk tissue occurring in response to the continual wave passage through areas remote from the observation site. Second, the raw signal consisted of multidirectional changes in light intensity, and this produced cross-correlation functions with multiple peaks which were uninterpretable. Therefore, before cross-correlation, the raw signals from each of the photoresistors were processed with a pair of analog optimizer circuits that consisted of a signal differentiator circuit (to minimize slow baseline changes), followed by an absolute value circuit (to convert the voltages occurring with wave passage to unidirectional changes). This processing resulted in the multiple humped signals which closely resembled the absolute value of the signals from single myocytes and which were visually observed to correspond to a single wave transversing the tissue between the photoresistors. Records of 10–30 minutes of real time were cross-correlated, the time delay to peak correlation was measured, and then the velocity of wave propagation was calculated as in myocytes.

**SLIF Measurements**

Two methods of measuring SLIF were utilized in the present study. In a series of formal light-scattering studies (method 1) muscles were perfused in a 3-ml chamber with sides made from glass microscope slides, and SLIF were quantified from the half-decay time of the normalized autocorrelation function of scattered light as described previously (Lakatta and Lappe, 1981; Stern et al., 1983). SLIF measurements are presented as $f_{\text{m}}$, which is $1/2\tau_{\text{aut}}$, where $\tau_{\text{aut}}$ is the time for the autocorrelation function to decay 50% of the distance to baseline (Stern et al., 1983). In the muscles studied in the microscopic chamber, since only directional changes in SLIF were required for comparison with directional changes in the wave-displacement measurements, the method to measure SLIF was simplified. The bulk preparation was illuminated by the laser, and the scattered light, collected through the low-power microscopic objective, was projected through a 20-μm pinhole that covered the photomultiplier tube. SLIF were characterized from the resulting autocorrelation functions as described above.

**Results**

Figure 3 illustrates the power spectrum of fluctuations in optical transparency that result from the localized myofilament contraction due to spontaneous contractile waves in a thin unstimulated bulk muscle, and the effect of an increase in cell $Ca^{++}$ loading due to an increase in the superfusate $[Ca^{++}]_{0}$, $(C_{a_{0}})$, on that spectrum. The presence of clear spectral peaks indicates that the motion that causes the optical fluctuations is roughly periodic, just as that described for the spontaneous oscillations in aequorin luminescence in bulk tissue (Orchard et al., 1983; Wier et al., 1983). Detection of clear peaks in the power spectrum required that measurements be made from areas within the preparation sufficiently thin such that striations were visible. In these regions, all the waves appeared to be traveling in the same direction (like waves striking a beach). A monotonic increase in the peak frequency and an increase integrated peak area occurs with increasing $C_{a_{0}}$.

The SLIF frequency in bulk muscle increased with increasing $C_{a_{0}}$ over the range studied in Figure 3 (Table 1A). That an increase in cell $C_{a_{0}}$ loading should increase both the integrated area of the power spectrum (Fig. 3), and SLIF (Table 1) was not unexpected, since both these measurements are related to the quantity of tissue motion, SLIF being a function of the root mean square velocity of motion of the independent light-scattering structures within the illuminated tissue (Stern et al., 1983). This result supports the previous qualitative observations (Stern et al., 1983) that the myofilament-generated motion is responsible for producing SLIF.

The wave frequency and the amplitude of cell displacement caused by an individual wave in cardiac myocytes is listed in Table 2 and illustrated in Figure 4. The marked similarity of the wave frequency in the different $C_{a_{0}}$ in the myocyte and that occurring in the intact muscles (Fig. 3A) is particularly noteworthy: in both single myocytes and intact muscles, in the non-$Ca^{++}$ overload state, e.g., $C_{a_{0}} =

![Figure 3. Power spectra of optical transparency changes induced by the spontaneous propagating contractile waves in representative ultra-thin unstimulated rat papillary muscles. Temperature was 23°C. In a, b, and c, the muscle was bathed in $Ca_{0}$ of 2, 5, and 15 mM, respectively. Note that clear peaks are visible in the power spectrum.](http://circres.ahajournals.org/content/847/1/847/F3)

| Table 1 Effect of $Ca_{0}$, Temperature, or Caffeine on SLIF ($f_{\text{m}}$) in the Bulk Rat Papillary Muscle Preparation |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                  | $f_{\text{m}}$ (Hz) | $f_{\text{m}}$ (Hz) | $f_{\text{m}}$ (Hz) | $f_{\text{m}}$ (Hz) |
| A. $Ca_{0}$ (mM) |                  |                  |                  |                  |
| (23°C; $n = 4$)  |                  |                  |                  |                  |
| 2               | 2.50 ± 0.10      | 2.50 ± 0.10      | 2.50 ± 0.10      | 2.50 ± 0.10      |
| 5               | 4.00 ± 0.30      | 4.00 ± 0.30      | 4.00 ± 0.30      | 4.00 ± 0.30      |
| 15              | 6.30 ± 0.31      | 6.30 ± 0.31      | 6.30 ± 0.31      | 6.30 ± 0.31      |
| B. Temperature  |                  |                  |                  |                  |
| (C$Ca_{0}$ = 3 mM; $n = 3$) |                  |                  |                  |                  |
| 23°C            | 3.0 ± 0.50       | 3.0 ± 0.50       | 3.0 ± 0.50       | 3.0 ± 0.50       |
| 30°C            | 3.4 ± 0.50       | 3.4 ± 0.50       | 3.4 ± 0.50       | 3.4 ± 0.50       |
| C. Caffeine     |                  |                  |                  |                  |
| (23°C, $Ca_{0}$ = 3 mM; $n = 5$) |                  |                  |                  |                  |
| Control         | 3.3 ± 0.30       | 2.1 ± 0.30       | 3.3 ± 0.30       | 2.1 ± 0.30       |
| Caffeine (2 mM) |                  |                  |                  |                  |
| (10 mM)         | 0               | 0               | 0               | 0               |

Results are expressed as mean ± SEM.
TABLE 2

The Effect of Ca, on Spontaneous Contractile Wave Frequency and Amplitude in Single Cardiac Myocytes

<table>
<thead>
<tr>
<th>Ca, (mM)</th>
<th>Wave frequency (min^-1)</th>
<th>Wave amplitude (% resting cell length)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2.45 ± 1.07</td>
<td>3.51 ± 0.33</td>
</tr>
<tr>
<td>5</td>
<td>6.07 ± 2.03</td>
<td>3.63 ± 0.70</td>
</tr>
<tr>
<td>15</td>
<td>23.00 ± 7.58</td>
<td>3.66 ± 0.49</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM.

* 23°C; n = 5.

2.0 mM, the oscillation frequency is rather low, i.e., about 0.1 wave/sec or less (Figs. 3 and 4A). The roughly periodic nature of the wave occurrence in the myocyte is also evident in Figure 4. This strongly suggests that the waves occurring in these unstimulated myocytes with intact sarcolemmal function have a very close correlate in the unstimulated bulk muscle.

Unlike the average wave frequency, the average amplitude of wave motion, measured as in Figure 1B, did not vary with Ca, over the Ca, range studied (Table 2; Fig. 4B). Thus, the increase in the integrated peak areas of the bulk muscle with increased Ca, in Figure 3 apparently are due primarily only to an increase in average wave frequency. Therefore, it can be concluded that an increase in cell Ca++ loading by an increase in Ca, over the range studied in Figures 3 and 4 and Tables 1 and 2, causes the periodic spontaneous Ca++ release to occur more frequently, but does not increase the displacement amplitude per wave. It follows, then, that the increase in SLIF with an increase in Ca, over this range (Table 1A) probably is due entirely to an increase in wave frequency, and not to a marked change in displacement amplitude per wave.

The amplitude of the contractile wave was found to vary in response to other perturbations, however. When temperature was decreased from 37°C to 23°C in myocytes (Fig. 5), a monotonic increase in wave amplitude accompanied the monotonic decrease in wave frequency. At constant temperature and Ca, the addition of a low concentration of caffeine to the bathing fluid caused a marked increase in wave frequency and a diminution in amplitude (Fig. 6A). The complete concentration-effect curve for caffeine in a representative myocyte is illustrated in Figure 6B.

In the bulk muscle, a change in temperature or low concentrations of caffeine produced changes in the power spectrum of wave displacement that were consistent with the observations in the myocytes: an increase in temperature shifted the spectral peak to higher frequencies while reducing the integrated peak area (Fig. 7A). Low concentrations of caffeine also increased wave frequency and decreased the integrated peak area (Fig. 7B). Although, as noted above, the integrated spectral area is a function of both wave frequency and amplitude; a decrease in integrated area accompanying an increase in frequency...
frequency is unambiguously interpretable as a decrease in amplitude.

The effects on SLIF produced by a change in temperature or by low concentrations of caffeine in the bulk muscle are listed in Table 1. With an increase in temperature, SLIF remained unchanged. In four unstimulated muscles, control SLIF averaged 3.6 ± 0.41 Hz at 17°C; at 29°C SLIF was 98 ± 10% control; at 37°C SLIF was 80 ± 20% control. Since SLIF is roughly the product of wave amplitude and frequency, it appears that over this range of temperatures the decrease in wave amplitude as measured directly in the myocyte (Fig. 5) or approximated by the decreased integrated area of spectral peak power in the presence of an increase in frequency (Fig. 7A), was fairly evenly matched by a corresponding increase in wave frequency. Caffeine depressed SLIF (Table 1, and Stern et al., 1983) even though the spectral analysis (Fig. 7B) showed that wave frequency had increased. Thus, decline in wave amplitude with caffeine as shown directly in myocytes (Fig. 6) and indirectly in bulk muscle (Fig. 7B) was of sufficient magnitude to offset the increase in wave frequency. Therefore, when the amplitude of wave displacement decreases, changes in SLIF do not directly track changes in the frequency of the oscillations as they do when the average amplitude remains fairly constant, e.g., when Ca_o is varied. Rather, a decrease in the amplitude of displacement due to the wave causes SLIF to be disproportionately low for the measured wave frequency.

The propagation velocity of the contractile wave increased with an increase in Ca_o or an increase in temperature, but to a lesser extent than did wave frequency. The effects of these perturbations on wave propagation velocity in the bulk muscle are listed in Table 3 and in myocytes are illustrated in Figure 8.

In the results presented thus far, the spontaneous contractile waves in intact bulk muscle and single myocyte studied under approximately similar conditions exhibited a similar periodicity and an ap-
TABLE 3
Effect of Ca, and Temperature on Contractile Wave Propagation Velocity in the Bulk Rat Papillary Muscle Preparation

<table>
<thead>
<tr>
<th>Propagation velocity (µm/sec)</th>
<th>A. Ca (mM) (23°C; n = 5)</th>
<th>B. Temperature (Ca = 3 mM; n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>33 ± 4</td>
<td>23°C 33 ± 5</td>
</tr>
<tr>
<td>5</td>
<td>39 ± 8</td>
<td>30°C 74 ± 19</td>
</tr>
<tr>
<td>15</td>
<td>87 ± 17</td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM.

approximately similar propagation velocity. Also, similar changes in frequency, amplitude, and wave propagation velocities occurred in both preparations in response to the experimental perturbations employed. The absolute oscillation frequencies measured varied from less than 0.1 Hz, when cell or bulk muscle preparations were bathed in physiological Ca, up to several tenths of a hertz when Ca, was increased. In preparations with intact sarcolemma, marked increases in Ca, above those caused by increasing Ca, over this range result from Na-K pump inhibition or from a reduction of Na,. Under these conditions, spontaneous Ca++ fluctuations measured directly by fluctuations in aequorin luminescence (Orchard et al., 1983; Wier et al., 1983) or indirectly by the cellular manifestations of the oscillation, i.e., voltage or current oscillations that result from oscillatory Ca++ modulation of sarcolemmal conductances (Kass et al., 1978a, 1978b; Kass and Tsien, 1982; Matsuda et al., 1982) or the oscillations of resting force (Glitsch and Pott, 1975), exhibited frequencies up to several hertz (see Lakatta et al., 1985, for review). In the present study, Ca++ overload by Na-K blockade or a reduction in Na, was effected to determine whether the frequency of contractile waves could increase to these levels. Ouabain at a temperature of 30°C (Fig. 9A) or a reduction in perfusate [Na+] at 23°C (Fig. 9B) shifted the frequencies of spectral peak of optical fluctuations in muscles to values greater than 1 Hz. Given that the Q10 for wave frequency is at least 2.5 [Figs. 3 and 4, and Fabiato and Fabiato (1972, 1975), Turk et al. (1984)], the results in Figure 9, A and B, suggest that these same experiments performed at 37°C would achieve even higher frequencies. This is demonstrated directly in the muscle in Figure 9C, in which a reduction in Na, at 37°C produced contractile wave frequencies as high as those observed directly as Ca++ oscillations (Orchard et al., 1983; Wier et al., 1983) or their electrical or contractile manifestations (Glitsch and Pott, 1975; Kass et al., 1978a; Matsuda et al., 1982; Kass and Tsien, 1982) in other studies. That ouabain and reduced Na, caused marked increase in SLIF frequency has been demonstrated previously (Lakatta and Lappe, 1981; Walford et al., 1984).

Previous observations indicate that oscillations in aequorin light correlated in frequency with oscillations in resting tension (Orchard et al., 1983), and also that a component of resting force varied directly with SLIF in bulk muscles when Ca, was increased (Lappe and Lakatta, 1980; Lakatta and Lappe, 1981). It has been hypothesized that the summation of asynchronously occurring wave-like contractions within the bulk muscle, damped by the compliant inter-wave regions (and by artifactual compliance at the muscle ends), are the cause of what appears to
be a steady Ca++-dependent resting tone within the bulk preparation (Stern et al., 1983; Kort et al., 1985). Direct evidence that the ensemble average of the spontaneous contractile wave motion is experienced as a component of resting force measured at the ends of a muscle preparation is illustrated in Figure 10 in which the power spectrum from both the video-analyzed wave motion (panel A) and that from the simultaneous force transducer output (panel B) are displayed. Note that both spectra contain peaks at about the same frequency. From this result, and the observation that simultaneous recordings of aequorin luminescence and force exhibit oscillations with the same frequency (Orchard et al., 1983), it can be inferred that spontaneous Ca++ oscillations can cause a Ca++-dependent component of "resting" tension in unstimulated cardiac muscle.

Although oscillations have been observed directly in the tension record in unstimulated bulk muscle during extreme Ca++ overload (Glitsch and Pott, 1975), they are not routinely observed under conditions as in Figure 10, i.e., in the absence of drugs in a Ca of 3.0 mM or less and normal Na. Nonetheless, that these oscillations occur within the preparation is amply demonstrated by spectral analysis and by SLIF measurements. However, whether injury to the ends of the preparation during in vitro mounting causes or contributes to the spontaneous Ca++ oscillations is unclear. Therefore, we measured SLIF in the apparatus designed for formal light-scattering studies (Stern et al., 1983) in the middle portion and at a clamped end of thin and extremely long (>4.5-mm) rat papillary muscles, both initially after mounting, and following equilibration (typically of 3 hours or more). In addition, after equilibration, the effect of an increase in Ca in both regions was measured (Table 4). Immediately after mounting, SLIF measured at a "damaged end," i.e., adjacent to a clamp used to mount the muscle was increased by about 2-fold over that in the midportion of the muscle. During equilibration, SLIF decayed in both areas in a manner similar to that shown previously when only the midportion was examined (Kort and Lakatta, 1984). However, the extent of the decay was greater at the damaged end, so that by the end of the equilibration period, the differences in SLIF between these two regions were abolished. This result suggests that equilibration may involve the death of some initially Ca++-overloaded cells within the damaged region and "healing over" in the others, and this confirms the previous observations that in the equilibrated unstimulated muscle SLIF is uniform throughout the tissue (Lakatta and Lappe, 1981). An increase in cell Ca++ loading in equilibrated tissue produced by an increase in Ca increased the SLIF proportionally in both regions (Table 4). Thus, in an equilibrated muscle, although damaged ends may substantially alter muscle compliance in the unstimulated state, the nature of the spontaneous oscillatory areas in the "damaged ends" does not appear to be different from that of the midportion of bulk muscle.

**Discussion**

The present results demonstrate for the first time that spontaneous contractile waves in unstimulated
Figure 10. The power spectrum of optical fluctuations caused by contractile wave displacement (panel A) and that of the simultaneously measured tension fluctuations (panel B) in an unstimulated rat papillary muscle bathed in Ca\textsubscript{o} of 3.0 mM at 23°C. The similarity in frequency for both peaks implies that a component of resting tension is generated by the contractile waves.

Intact rat cardiac muscle are periodic, and that when bathed in a physiological Ca\textsubscript{o}, the frequency of these oscillatory waves is substantially less than 0.5 Hz. Increases in cell Ca\textsuperscript{++} loading by an increase in Ca\textsubscript{o}, Na\textsuperscript{+}-K\textsuperscript{+} blockade, or a reduction in superfusate [Na\textsuperscript{+}], can increase the oscillation frequency up to several hertz. The propagation velocity of these waves also varies with Ca\textsuperscript{++} loading, but less than does the wave frequency.

The results also show that unstimulated Ca\textsuperscript{++}-tolerant myocytes with intact sarcolemmal function prepared from rat myocardium retain the same properties observed in the intact unstimulated muscle. In a given Ca\textsubscript{o}, the values for wave frequency are roughly the same as those of the intact muscle, and that with increased Ca\textsubscript{o}, the wave frequency in myocytes, as in muscle, increases more than does propagation velocity. The studies in myocytes also show that only a minimal change in the average wave amplitude occurs with an increase in Ca\textsubscript{o}.

Finally, the results show that a change in temperature or the addition of caffeine to the superfusate causes similar changes in wave amplitude and frequency in both the myocytes and bulk preparations.

Similar results for the effects of caffeine and temperature on contractile wave frequency and amplitude have also been observed previously in myocytes without intact sarcolemmal function (Fabiato and Fabiato, 1971, 1975; Chiesi et al., 1981; Fabiato, 1985). In these preparations, it is possible to correct for the slight variations in pH which are due to temperature, and to maintain other ion concentrations constant at each temperature. However, in preparations with intact sarcolemma, it would be difficult to mimic these conditions because a change in temperature likely causes not only a change in cell pH, but also changes in other ionic gradients, e.g., Na\textsuperscript{+}, each of which could change Ca\textsubscript{o}. Since in intact preparations direct and specific control of these ions is not possible, the precise mechanisms for the temperature effect on the oscillation characteristics remain obscure. In these hyperpermeable or "mechanically skinned" rat cardiac myocytes, spontaneous oscillations occur when the preparation is bathed in [Ca\textsuperscript{++}] as low as 100 nM, (Fabiato and Fabiato, 1975; Dani et al., 1979; Chiesi et al., 1981; Fabiato, 1983; Fabiato and Baumgarten, 1984). Although Donnan effects would be expected to increase the [Ca\textsuperscript{++}] in the myofilament space to 135 nM in these preparations, this is still well below the resting myoplasmic levels reported in intact tissue from measurements with either Ca\textsuperscript{++}-selective electrodes, or aequorin luminescence (Blinks et al., 1982), or more recently estimated from Quin 2 fluorescence or aequorin luminescence in rat cardiac cells (Cobbold and Bourne, 1984; Powell et al., 1984; Sheu et al., 1984). Should these estimates of myoplasmic [Ca\textsuperscript{++}] in intact cell be genuine, the occurrence of spontaneous low frequency oscillations in both unstimulated bulk muscle and single rat cardiac myocytes with intact sarcolemmal function could have been predicted from the results of those previous studies in myocytes devoid of sarcolemmal function.

When bathed in physiological Ca\textsubscript{o}, these oscillations are not exhibited in the unstimulated single rabbit myocytes with intact sarcolemmal function prepared in a manner identical to that used for the rat cells in the present study (Capogrossi et al., 1984; Capogrossi and Lakatta, 1985), or in hyperpermeable rabbit single myocytes bathed in 100 nM Ca\textsuperscript{++} (Chiesi et al., 1981). However, in each of these rabbit preparations, and in all other species of mammalian cardiac tissues examined (Kort and Lakatta, 1984), the de novo appearance of spontaneous oscillations

<table>
<thead>
<tr>
<th>Time post-mounting (min)</th>
<th>Cao (mM)</th>
<th>f\textsubscript{o} (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>180</td>
<td>1.8</td>
<td>1.37 ± 0.49</td>
</tr>
<tr>
<td>190</td>
<td>2.5</td>
<td>3.06 ± 0.40</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM.

*30°C; n = 3.
can be produced by an increase in Ca, in the unstimulated state mediated by ionic manipulation or drugs, or by a combination of these and regular electrical stimulation in preparations with intact sarcolemmal function (Lakatta and Lappe, 1981; Capogrossi and Lakatta, 1985). Thus, in either unstimulated myocytes or intact bulk muscle, determining the relative extent of cell Ca** loading from the oscillation frequency requires the knowledge of both the species from which the preparation is derived and the given temperature and Ca,0. For example, unstimulated rat myocytes that are bathed in 1.0 mM Ca, and that exhibit oscillations a frequency greater than 0.5 Hz at 23°C in the absence of drugs, clearly might be described as "Ca** overload" or "Ca** intolerant," whereas unstimulated cat or rabbit myocytes exhibiting spontaneous oscillations at any frequency in physiological Ca, and the absence of drugs might be considered as such. It is also noteworthy that about 10% of rat myocytes prepared by the technique utilized in the present study fail to exhibit oscillations (Capogrossi and Lakatta, 1985b); however, these myocytes do not mimic the functional (twitch) characteristics of the bulk muscle as closely as do those which exhibit oscillations (Capogrossi et al., 1985).

The present results demonstrated that the frequency of propagating contractile waves (when corrected for temperature) in bulk muscle can reach frequencies of oscillation observed in aequorin luminescence (Orchard et al., 1983; Wier et al., 1983), sarcolemmal voltage or current (Kass et al., 1978a, 1978b; Kass and Tsien, 1982; Matsuda et al., 1982), or resting force (Glitsch and Pott, 1975) measured in Ca++ overload states. Glycose-induced acceleration of wave frequency to these levels has also been observed in Ca++-tolerant single rat myocytes (Capogrossi and Lakatta, 1985; Lakatta et al., 1985). These results, coupled with the observation that all mammalian cardiac tissue can exhibit contractile waves (Kort and Lakatta, 1984), strongly suggest that the Ca** oscillations that underlie the aequorin, current, voltage, or force oscillations also arise secondary to the continuous series of regenerative Ca** waves passing through a given point within the tissue. These waves presumably represent a localized increase in myoplasmic Ca** which, by diffusion, produces a Ca**-induced Ca** release from the neighboring SR that reproduces the original level of regional myoplasmic Ca** concentration shifted, or, in effect, propagated down the cell (Stern et al., 1984). However, the precise molecular mechanism(s) effecting this type of release may differ from those in which Ca** release is induced or triggered by application of a small amount of Ca** to preparations which do not exhibit spontaneous release (Fabiato, 1985). It is possible that certain cardiac arrhythmias associated with high cellular Ca** loading may occur due to the wave-induced depolarization resulting from the Ca** modulation of sarcolemmal conductances (Kass et al., 1978a, 1978b; Colquhoun et al., 1981; Kass and Tsien, 1982; Matsuda et al., 1982; Clusin, 1983). As Ca, increased from 2–15 mM, the wave frequency increases approximately an order of magnitude (Fig. 4), but the extent of increase in wave velocity was much smaller (Fig. 8). This implies that increased Ca, results in an increase in the density of waves within the preparation. Since the net depolarization of the tissue from the wave-induced changes in sarcolemmal conductances should increase with an increase in wave density, this provides an explanation as to why high cellular Ca** loading can produce depolarization of sufficient magnitude to induce an arrhythmia (Tsien et al., 1979).

The present results strongly suggest that SLIF are caused by three Ca**-driven contractile waves, and, thus, support previous qualitative observations (Stern et al., 1983). However, the results indicate that the SLIF frequency is not to be interpreted as indicative of the actual wave frequency. The substantial difference in the frequencies of the power spectral peaks in Figure 3 and the SLIF frequency measured under similar conditions in Table 1A is expected, because these measure different parameters. Although characterized in hertz, SLIF actually measures the velocity of tissue motion in units of wavelengths of light/sec, for the case of single-order scattering, and multiples thereof with multiple scattering. Thus, when the mechanical displacement exceeds a wavelength of light, SLIF is proportional to the frequency of wave passage times the root mean square of the ensemble average of the relative distance each light scatter is displaced with the wave passage, i.e., displacement amplitude. (Displacement that is significantly less than a quarter of a wavelength of light, i.e., less than 158 nm, is undetectable by this technique.) Thus, whereas peaks in the power spectrum (Fig. 3) do accurately assess the oscillation frequency, SLIF measurements provide neither the specific frequency nor the amplitude of displacement, but rather, their product. Quantification of oscillation displacement frequency and amplitude are vital for determining the potential impact of the oscillations on cell function or for accessing information about factors that determine Ca** uptake and release from the SR in preparations with intact sarcolemma. In this regard, it is important to consider that, whereas the oscillation frequency simply represents the periodicity of Ca** release into the myofilament space, the oscillation amplitude is determined by the extent of the resultant Ca**-myofilament interaction. This, in turn, is determined by the magnitude of Ca** release, the rate of the SR Ca** uptake, and the Ca** affinity of the myofilaments. Perturbations that cause a decrease in Ca** affinity of the myofilaments [e.g., decrease in sarcomere length (Hibberd and Jervell, 1982), acidosis (Fabiato and Fabiato, 1978), or cyclic adenosine monophosphate-dependent troponin phosphorylation (Holroyde et al., 1979)] or perturbations that might cause a selective increase in the rate of SR
Ca++ uptake without affecting Ca++ release, will cause the wave amplitude for a given Ca++ release to decrease. Should these perturbations also cause an increase in wave frequency or magnitude of Ca++ release (e.g., by Ca++ loading the cell), their combined effects on the lumped parameter SLIF may cancel (Lakatta and Lappe, 1981). The present results demonstrate that when the wave frequency is increased without a concomitant increase in contractile wave amplitude, e.g., with an increase in Ca, (Figs. 3 and 4), a change in SLIF (Table 1A) reflected the proportional change in wave frequency. When the wave amplitude declined, however, e.g., due to an increase in temperature or to relatively low concentration of caffeine (Figs. 3, 5, and 6), SLIF (Table 1, B and C) failed to distinguish the specific changes in oscillation frequency and displacement amplitude.

In spite of these limitations, SLIF measurements remain a reliable, relatively simple, noninvasive, and perhaps the most sensitive (Stern et al., 1983; Kort and Lakatta, 1984) method to determine the presence of subtle wavelike mechanical motion caused by subtle spontaneous Ca++ release within bulk preparations. Furthermore, SLIF measurements are also useful to monitor directional changes in cell Ca++ loading without requiring a test depolarization, e.g., those due to certain inotropic perturbations such as a change in Cao, or addition of cardiac glycosides, or a reduction in Na+, (Lakatta and Lappe, 1981). An increase in SLIF in response to these perturbations in rat muscle (or the de novo appearance of SLIF in other species) not only reflects an increase in average cell Ca++ loading, but because displacement amplitude remains constant with these perturbations, an increase in SLIF also reflects the proportional increase in the frequency of Ca++ release into the myoplasmic space. Thus, SLIF measurements not only provide an accurate detection of the presence of SR Ca++ cycling, but under certain experimental conditions, changes in SLIF may directly reflect changes in the frequency of this SR Ca++ cycling, and thereby parallel the actual measurements of oscillation frequency performed in myocytes (Fabiato and Fabiato, 1972, 1973, 1975; Dani et al., 1979; Chiesi et al., 1981). However, detection of the true oscillation frequency or changes in it caused by other perturbations in intact muscle requires the more tedious video measurements of wave displacement and analysis of its power spectrum as performed in the present study. This can be accomplished only in relatively few muscles that have ultra thin areas (Fig. 2). Even then, the integrated area of the spectral analysis performed on the bulk muscle is not always easy to interpret in terms of the displacement amplitude when the wave frequency also changes (Fig. 3). Given these limitations in the bulk tissue, the Ca++-tolerant isolated cardiac myocyte, which, in the present study, has been shown to exhibit similar contractile wave frequences and propagation velocities as in the intact muscle, looms as the best model for direct measurements of the frequency and amplitude characteristics of the contractile waves. It also is an appropriate model in which to study the functional, i.e., the electrophysiological and contractile, implications (Suarez-Isla et al., 1984; Capogrossi and Lakatta, 1985a; Lakatta et al., 1985) of the spontaneous SR-generated Ca++ oscillations that underlie these contractile waves.

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Lakatta EG, Capogrossi MC, Kort AA, Stern MD (in press) Spontaneous myocardial Ca** oscillations: An overview with emphasis on ryanodine and caffeine. Fed Proc


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