Synchronous Occurrence of Spontaneous Localized Calcium Release From the Sarcoplasmic Reticulum Generates Action Potentials in Rat Cardiac Ventricular Myocytes at Normal Resting Membrane Potential

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Under certain conditions, spontaneous release of Ca\(^{2+}\) from the sarcoplasmic reticulum occurs in resting mammalian myocardium. In single rat ventricular myocytes, such spontaneous Ca\(^{2+}\) release appears localized rather than homogeneous. When the increase in cytosolic Ca\(^{2+}\) is present in a single locus within a cell, it causes a small depolarization, which, at the normal resting potential, is subthreshold for generating an action potential. However, when spontaneous Ca\(^{2+}\) release occurs simultaneously at more than a single discrete locus, the resultant sarcolemmal depolarization is augmented to levels that can induce an action potential, even when this depolarization begins at the normal resting membrane potential. Thus, the synchronous occurrence of multifocal localized increases in cytosolic Ca\(^{2+}\) due to spontaneous Ca\(^{2+}\) release from the sarcoplasmic reticulum within ventricular myocytes is a mechanism for “abnormal automaticity.” (Circulation Research 1987;61:498-503)

Previous studies in cardiac tissue have attributed spontaneous action potentials following depolarizing voltage clamp steps or bouts of stimulation to spontaneous Ca\(^{2+}\) release from the sarcoplasmic reticulum (SR) that had been “triggered” by the prior depolarization. Hence, this type of arrhythmogenesis has been termed “triggered activity.” In contrast, spontaneous action potentials arising in unstimulated ventricular myocardial tissue are presently referred to as “abnormal automaticity,” and its mechanism, while unknown, is thought to be distinctly different from “triggered activity.” We hypothesized that spontaneous SR Ca\(^{2+}\) release, which is considered to be the basis of triggered activity, can also be a cause of “abnormal automaticity.” In this report, we demonstrate a subcellular mechanism by which this can occur in single cardiac myocytes at the normal resting membrane potential.

Materials and Methods

Cell Isolation

Single cardiac myocytes were isolated as previously described. Briefly, hearts from 6-8-month-old Wistar rats were retrogradely perfused with a low-Ca\(^{2+}\) collagenase bicarbonate buffer at 37°±0.5° C (pH 7.4). Following approximately 30 minutes of perfusion, the heart was flaccid. The left ventricle was dissected free, minced, and the fragments were gently pipetted in the dissociation medium. The solution was filtered through gauze and allowed to form a pellet by gravity. Finally, the supernatant was removed, and the pellet was resuspended in HEPES-buffered Tyrode’s solution containing 1 mM [Ca\(^{2+}\)]. All experiments were at 37° C and pH 7.4. All solutions were made fresh on the day of the experiment.

Changes in cell length were monitored with a video analytical technique as previously described.

Electrophysiologic Techniques

Transmembrane potential was recorded simultaneously with changes in cell length using standard microelectrodes (20-50 MΩ) filled with 3 M KCl. Microelectrodes were connected to an amplifier (Axoclamp II) with a high input impedance. Action potentials were evoked by brief current pulses through the recording microelectrode. These techniques are described in greater detail in previous publications.

Results and Discussion

Localized spontaneous SR Ca\(^{2+}\) release in ventricular cardiac myocytes results in a localized area of increased cytosolic Ca\(^{2+}\) concentration, [Ca\(^{2+}\)]. This causes localized Ca\(^{2+}\)-myofilament interaction, manifest as a discrete band of sarcomere shortening (Figure 1A). As Ca\(^{2+}\) diffuses from the localized area of high concentration, it induces Ca\(^{2+}\) release from neighboring SR, resulting in a propagation of SR Ca\(^{2+}\) release and, thus, a propagation of the contractile band at approximately 100 μm/sec. While the localized increase in [Ca\(^{2+}\)], within the contractile band and its propagation has been observed using fluorescent Ca\(^{2+}\) indicators, the localized increase in [Ca\(^{2+}\)], has not been
FIGURE 1. Diagrammatic representation of spontaneous contractile waves in Ca²⁺-tolerant isolated rat ventricular myocytes, prepared as described in Silver et al.¹ A: Unifocal contractile wave: events a–f occur sequentially for approximately 1 second. (a), The cell is at rest with uniform sarcomere length throughout; (b), a localized band of contracted sarcomeres arises at the left end of the cell. This propagates through the cell (c, d, and e) as a "contractile wave" and is followed (f) by the return to mechanical quiescence. Note that when the contractile band is present in the cell its length shortens. c' is an actual myocyte photograph (Zeiss planapochromat 63 ×, 1.4 NA, oil immersion DIC objective and DIC condenser with 0.63 NA. The picture is part of a sequence on a Kodak technical pan film 2415 obtained with a Photo-Sonics 16 mm-IVN camera at the rate of one picture every 20 msec) at the time corresponding to c on the schematic. Arrow indicates the band of contracted sarcomeres which produces a bulge at the myocyte edge. B: Multifocal contractile wave. (a), Cell is quiescent. (b), Localized band of contracted sarcomeres arises in the center of the cell and propagates in both directions (c, d) to the cell ends. (e), The cell reaches the quiescent state in less time than in unifocal. Another variant of multifocal waves (not shown) results from the simultaneous origin at both cell ends of contractile bands that propagate to the cell center. Note that in b, c, and d more than one discrete band of contracted sarcomeres is present and that the shortening of the cell ends exceeds that in Panel A. c'' is a photograph of a myocyte at the time corresponding to c on the schematic. C: Records of the cell edge mechanical displacement (lower) and membrane depolarization (upper) that accompany unifocal (left) and multifocal (right) spontaneous contractile waves in a representative unstimulated rat ventricular myocyte. D: Relations between the magnitude of depolarization (ΔEᵢ) and contractile magnitude measured as cell edge displacement for unifocal and multifocal spontaneous contractile waves in a representative unstimulated myocyte.
Figure 2. A: Recording of spontaneous action potential and twitch resulting from multifocal spontaneous Ca\(^{2+}\) release. Arrow indicates cell edge displacement due to the spontaneous contractile wave. B: Recording of an electrically driven action potential and the resultant contraction shown for comparison. C and D: Comparison of the action potentials shown in A and B at higher resolution. Note that while the threshold potential in the spontaneous versus the driven action potentials are similar, the upstroke velocity of the spontaneous action potential (dV/dt = 45 V/sec) is less than that of the driven one (dV/dt = 120 V/sec). In D, the driven beat has a faster upstroke and overshoots zero potential. In C, the large arrow points to the beginning of the spontaneous depolarization that accompanies the contractile wave, and in both C and D the small arrow indicates the stimulus artifact of the driven beat. It is well known that with regular stimulation of rat myocytes the twitch amplitude markedly decreases and the action potential duration shortens compared with those following a long pause. The relatively large twitch and transmembrane action potential duration of the spontaneous events relative to the stimulated events in the figure arise because the latter were measured during steady-state stimulation at 1 Hz, while the former occurred in the absence of regular stimulation. Thus, the design of this particular experiment precludes comparison of amplitudes of spontaneous versus stimulated twitch amplitudes or of the duration of the transmembrane action potentials. Rather, the point of the figure is to contrast the take off potential and the foot of the mechanical record in both types of events.

Quantitated. However, studies employing the chemiluminescent indicator aequorin have estimated the localized [Ca\(^{2+}\)], within the contractile band to be greater than micromolar. The band of contracted sarcomeres and its propagation can be readily tracked with microscopic videoanalytic techniques, and these studies have shown that the contractile band width remains fairly constant during its propagation, presumably because of SR Ca\(^{2+}\) pumping in the wake of the wave.
The example in Figure 1A depicts only a single such contractile band occurring within the cell at any instant and, thus, only a single localized area of increased $[\text{Ca}^{2+}]$, within the cell. This unifocal, spontaneous SR Ca$^{2+}$ release is the most common type observed in unstimulated rat myocytes at normal resting membrane potential in non-Ca$^{2+}$ overloaded states.$^5,11,14$ It produces a small displacement of the cell edge (Figure 1C) and a small inward current, i.e., $<10$ pA at $23^\circ$ C.$^5$ This current, which is thought to be due to Ca$^{2+}$ modulation of a sarcolemmal nonspecific cation conductance (T, channel) or a Na–Ca exchange carrier,$^6–19$ produces a small depolarization (Figure 1C). As myocyte Ca$^{2+}$ loading increases, the likelihood for more than a single contractile band to be present within a cell at a given instant increases.$^8$ In Figure 1B, the contractile band is initiated in the central region of the myocyte and propagates bidirectionally, giving rise to two discrete contractile bands. Because more than one contractile band is now present within the myocyte at a given instant, this is referred to as multifocal spontaneous SR Ca$^{2+}$ release. This causes a greater dis-
placement of the cell edge than the unifocal type (cf., Figures 1A, 1B, 1C, lower tracings).

The area of sarcolemmal membrane surface exposed to high [Ca\(^{2+}\)], at any instant might be expected to determine the magnitude of the resultant Ca\(^{2+}\) modulation of inward conductances and thus the magnitude of the resultant depolarization. Since during multifocal spontaneous SR Ca\(^{2+}\) release a greater sarcolemmal area is exposed to increased [Ca\(^{2+}\)], at any instant, this type of spontaneous calcium release might be expected to cause a larger sarcolemmal depolarization than unifocal release. Figure 1C (upper tracings) and 1D show (in a representative cell with a resting potential of \(-80 \text{ mV}\)) that this is indeed the case. In this myocyte, the average magnitude of the depolarization associated with the occurrence of a unifocal contractile wave was \(3.05 \pm 0.09 \text{ mV} (\pm \text{SEM}; n = 82)\) and never generated action potentials at the normal resting membrane potential. If the membrane is depolarized by passing a constant subthreshold current (not shown), the occurrence of a unifocal spontaneous contractile wave at any later point in time can produce a spontaneous action potential. The average magnitude of the depolarization associated with multifocal contractile waves that did not elicit an action potential at the normal resting potential was \(10.4 \pm 0.18 \text{ mV} (n = 29)\). In other multifocal waves (not depicted in Figure 1D) depolarization averaged \(4.0 \pm 1.1 \text{ mV} (n = 11)\) and reached threshold for sodium channel activation (i.e., about \(-67 \text{ mV}\)), to produce spontaneous action potentials that elicited twitches. Figure 2A illustrates a representative example of this. For comparison, an action potential elicited by electrical stimulation and the resultant twitch are shown in Figure 2B. The rates of depolarization and cell edge displacement at the initiation of the action potential (Figures 2C and 2D) and of the contraction (Figure 2A) differ between the spontaneous action potential and twitch and the electrically stimulated ones: the spontaneous events are preceded by slower depolarization and cell edge displacement because of the multifocal localized spontaneous Ca\(^{2+}\) release.

The preceding results show that when myofilament interaction due to spontaneous Ca\(^{2+}\) release occurs simultaneously at more than a single focus, the resultant depolarization is greater and can be sufficient to elicit an action potential, even when the depolarization is initiated at the normal resting membrane potential. Could experimentally induced synchronization of SR Ca\(^{2+}\) release elicit an action potential? We hypothesized that the rapid application of a high concentration of caffeine ought to be sufficient to trigger an action potential. This idea is based on studies in which caffeine was used in mechanically skinned myocardial preparations to cause a synchronous release of Ca\(^{2+}\) from the SR to initiate contraction\(^{21}\) as well as to produce inward currents in embryonic cell clusters.\(^{19,21}\) Figure 3 shows representative examples of the electrical and mechanical responses of single myocytes to the rapid addition of caffeine to the bathing milieu. In some instances, this caused repetitive spontaneous action potentials (Figure 3A), and in others, it caused a single action potential with a long (4 seconds) plateau (Figure 3B); with time after the addition of caffeine, all contractile and electrical evidence of spontaneous SR Ca\(^{2+}\) release was abolished. In contrast, the slower application of caffeine to the bathing medium produced smaller depolarizations that were not sufficient to cause action potentials (Figure 3C); rather, it produced a marked increase in the spontaneous contractile wave frequency and a progressive decrease in amplitude with ultimate disappearance of the waves as observed previously,\(^{14}\) presumably due to SR Ca\(^{2+}\) depletion. The wave associated depolarization behaved similarly (Figure 3C).

We conclude that spontaneous localized increases in [Ca\(^{2+}\)], due to spontaneous SR Ca\(^{2+}\) release can induce a transmembrane action potential at normal resting membrane potentials only if such release occurs in more than a single locus within the cell, i.e., when these loci of high [Ca\(^{2+}\)], occur synchronously. This can be mimicked by caffeine-induced Ca\(^{2+}\) release if a high concentration of caffeine is rapidly delivered to the cell. Thus, a synchronization of localized increases in [Ca\(^{2+}\)], within single cells of ventricular myocardium is a mechanism of "abnormal automaticity."

KEY WORDS • cardiac myocytes • spontaneous calcium oscillations • abnormal automaticity • arrhythmias • multifocal SR Ca\(^{2+}\) release
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