The Interaction of Electrically Stimulated Twitches and Spontaneous Contractile Waves in Single Cardiac Myocytes

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ABSTRACT Spontaneous myofilament motion that propagates within cells as a contractile wave is a manifestation of localized Ca^{2+} release from sarcoplasmic reticulum (SR). At $37°C$, when bathing $[Ca^{2+}]$ (Ca_o) is 1.0 mM, rat myocytes exhibit contractile waves at rest and the interwave interval averages 9.1 ± 1.5 s $(n = 6)$. We determined whether there was an interaction between this type of SR Ca^{2+} release and that induced by electrical stimulation to cause a twitch, and whether such an interaction had functional significance. Progressive decreases in SR Ca^{2+} loading effected by graded concentrations of caffeine produced proportional decreases in the mechanical amplitude of the twitch and of the spontaneous contractile wave. Regular electrical stimulation in physiologic Ca_o abolished the waves and, after stimulation, waves did not reappear for a period of time (delay interval). Over a range of stimulation frequencies $(6-72 \text{ min}^{-1})$, the delay interval ranged from 11.4 ± 3.6 to 12.4 ± 1.7 s and was usually greater than the interwave interval at rest. The delay interval for a wave to occur after a twitch was reduced in the presence of increased Ca_o, glycosides, or catecholamines. When the interstimulus interval exceeded the delay interval, waves could appear between twitches and had a marked effect of shortening the duration of the action potential and decreasing the amplitude of the subsequent twitch. The magnitude of this effect varied inversely with time (up to 2 s) between the onset of the spontaneous diastolic wave and the subsequent stimulated twitch. A reduction of the interstimulus interval to less than the delay interval prevented the occurrence of diastolic waves. These results demonstrate the presence of an interaction between spontaneous and action potential–mediated Ca^{2+} release, which can be interpreted on the basis of a common Ca^{2+} pool and perhaps common release mechanisms. This interaction can explain many of the known effects of electrical stimulation on phenomena that are thought to result from spontaneous Ca^{2+} oscillations in intact tissue.

INTRODUCTION

Spontaneous contractile waves occur in single ventricular cardiac myocytes in which the sarcolemma is either hyperpermeable to Ca^{2+} (Bloom, 1970; Fabiato and Fabiato, 1973; Dani et al., 1979; Rieser et al., 1979; Chiesi et al., 1981) or mechanically removed (Fabiato and Fabiato, 1975). These waves are due to spontaneous localized release and reuptake of Ca^{2+} by the sarcoplasmic reticulum (SR). They occur in roughly periodic manner at a low frequency, i.e., <0.1 Hz, in "skinned" rat myocytes bathed in a $[Ca^{2+}]$ of 100 nM (Fabiato and Fabiato, 1975; Chiesi et al., 1981; Fabiato, 1983), which is at or below most estimates of the resting myoplasmic $[Ca^{2+}]$ (Ca_i) in cardiac muscle preparations with intact sarcolemmal function. Their frequency increases as the bathing $[Ca^{2+}]$ (Ca_o) and SR Ca^{2+} loading increases. However, sarcolemmal function is absent in "skinned" isolated myocytes in which these fundamental properties of spontaneous Ca^{2+} release have been determined. This precludes an assessment of the interactions of the extent of cell Ca^{2+} loading as determined by the sarcolemmal function, the spontaneous SR Ca^{2+} release, and the Ca^{2+} release triggered by an action potential.

Ca^{2+}-tolerant adult rat and rabbit cardiac myocytes, which have intact sarcolemmal function, respond to electrical stimulation with a twitch and can also exhibit spontaneous contractile waves (Capogrossi et al., 1986a). In the absence of stimulation, in 1 mM Ca_o, the waves in these myocytes occur at a frequency similar to those in skinned myocytes bathed in a $[Ca^{2+}]$ of 100 nM (Kort et al., 1985a). If the spontaneous periodic Ca^{2+} release that underlies the spontaneous mechanical waves has the same source and perhaps occurs via the same release mechanisms as that released by an action potential to cause a twitch, then the periodicity or likelihood of the spontaneous contractile wave occurring should be transiently modified by electrical stimulation, which elicits action potential–triggered SR Ca^{2+} release. Specifically, it has been suggested that SR Ca^{2+} loading in rat cardiac muscle is decreased during stimulation after a rest (Bers, 1985). Since the occurrence of spontaneous contractile waves depends on SR Ca^{2+} loading, electrical stimulation might even abolish these waves. If this were indeed found to be the case, it would indicate that the presence of such waves is not "physiologic," insofar as it is "physiologic" for cells to contract continuously in response to repetitive action potentials. Alternatively, the presence of spontaneous contractile waves between stimulated twitches should affect subsequent action potential–mediated SR Ca^{2+} release.

We used Ca^{2+}-tolerant adult myocytes to study the impact of stimulation on characteristics of the individual contractile waves and the effect of their presence during stimulation on the action potential–mediated twitch. Some aspects of these studies are an extension of a prior brief report (Capogrossi and Lakatta, 1985), or have been presented in abstract form (Suarez-Isla et al., 1984; Capogrossi et al., 1984, 1985a, b).

METHODS

Single cardiac myocytes were isolated and bathed in HEPES buffer as described in the preceding article (Capogrossi et al., 1986a). All experiments were performed at 37°C. The methods for measuring the amplitude of cell shortening during a twitch and for

measuring membrane potential were also as described in the preceding article (Capogrossi et al., 1986a). Cell shortening during a contractile wave was measured at the time the wave occupied the central part of the myocyte. Action potentials were elicited by 0.5-ms depolarizing current steps through the impaling microelectrode. Action potential parameters were analyzed from digitized voltage traces (100 μs/point) obtained with a digital oscilloscope (4094, Nicolet Instrument Corp., Madison, WI).

RESULTS

Rat Myocytes

We hypothesized that interactions between spontaneous and action potential–mediated Ca^{2+} release occur in cardiac myocytes and that these interactions are of functional importance. The basis for this hypothesis is the assumption that both types of release originate from the same source, i.e., the SR. To provide experimental support for this assumption, we exposed myocytes to graded concentrations of caffeine in order to produce graded levels of SR Ca^{2+} loading (Fabiato and Fabiato, 1975). We then compared the relative reduction of twitch amplitude during regular stimulation at 72 min^{-1} and contractile wave amplitude in the unstimulated state (see Fig. 6A of the preceding article) at each caffeine concentration. Fig. 1 illustrates that the curve defining the graded reduction in twitch amplitude does not differ from that defining the graded reduction in wave amplitude. We interpreted the proportionate reductions in twitch and wave to indicate that the Ca^{2+} release that underlies the electrically stimulated twitch has the same source or shares release mechanisms with the twitch that underlies the spontaneous contractile wave. Because the wave periodicity depends on SR Ca^{2+} loading (Chiesi et al., 1981; Fabiato, 1985c), a bout of electrical stimulation in triggering Ca^{2+} release from the SR should transiently alter the spontaneous wave occurrence expected on the basis of their periodicity measured before stimulation. Fig. 2A shows a representative example of the effect of stimulation at 48 min^{-1} of a rat cardiac myocyte that exhibited spontaneous contractile waves at rest in a Ca_o of 1.0 mM. Under these conditions, stimulation produced a negative twitch staircase. Contractile waves were not present between twitches, and, after stimulation, there was a period during which no waves occurred. This interval from the last stimulated twitch to the first spontaneous contractile wave is defined as the delay interval. Note that it exceeded the average spontaneous interwave interval at rest. Fig. 2B shows the average delay interval in six cells stimulated over a wide range of frequencies. Waves did not occur between twitches at any frequency of stimulation. After lower stimulation frequencies (6–72 min^{-1}), the delay interval was transiently prolonged to values greater than the interwave interval at rest. Over this frequency range, rat myocytes exhibited a negative twitch amplitude–stimulation frequency relationship (see Fig. 4, control curve). After higher rates of stimulation, the prolongation of the delay interval decreased.

Because the interwave interval at rest decreases as SR Ca^{2+} loading increases, perturbations that cause an increase in SR Ca^{2+} loading during stimulation ought to reduce the delay interval. An increase in cell and SR Ca^{2+} loading at rest can be achieved by a reduction in the [Na] gradient produced by the addition of cardiac glycosides to the bathing fluid (Lee et al., 1980; Sheu and Fozzard, 1982;

Walford et al., 1984). Fig. 3 illustrates that ouabain, which decreases the interwave interval at rest, also decreased the delay interval for the first spontaneous wave to occur after stimulation. Note also that the average extent of cell shortening during the stimulated twitch in ouabain was greater than in control,
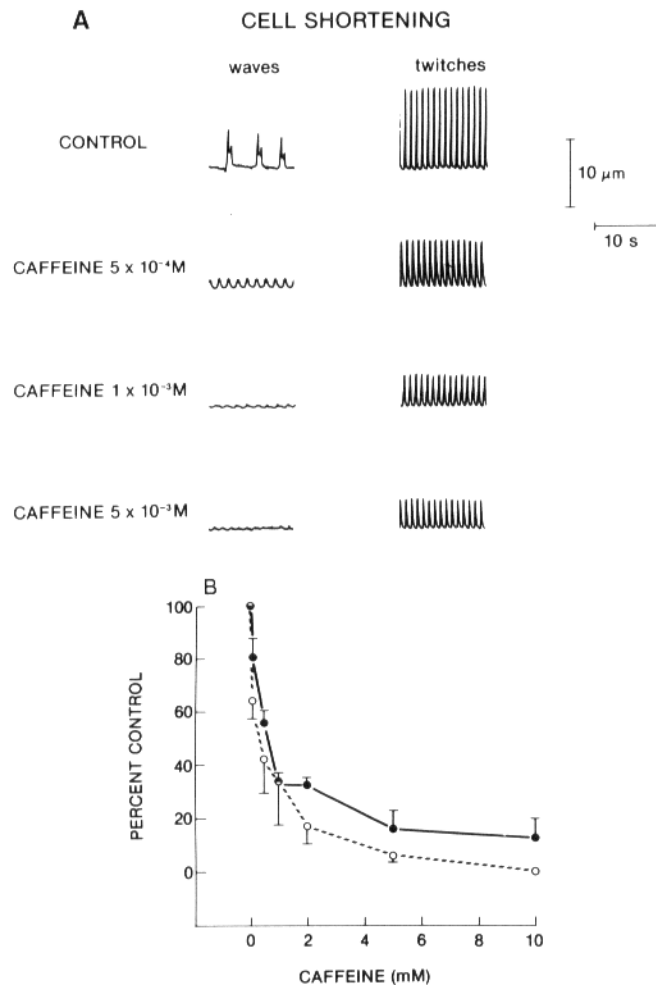
A   **CELL SHORTENING**

FIGURE 1.   The effect of increasing caffeine on twitch and contractile wave amplitude in single rat myocytes bathed in 1 mM Ca_o. Field stimulation was at 72 min^{-1}. (*A*) Tracings from a representative myocyte. (*B*) Average effect of caffeine (*n* = 4). In control, the amplitudes of twitch and wave were 11.4 ± 1.7 and 4.2 ± 0.77 μm, respectively ($\bar{x}$ ± SEM).

and the negative staircase of twitch amplitude upon stimulation that occurred in control was abolished. While the average twitch amplitude increased, the variation in the amplitude of the twitches gave a "contractile alternans" appearance to the tracing. Note the presence of spontaneous contractile waves in the interval between some stimulated twitches. Stimulation in the presence of ouabain also
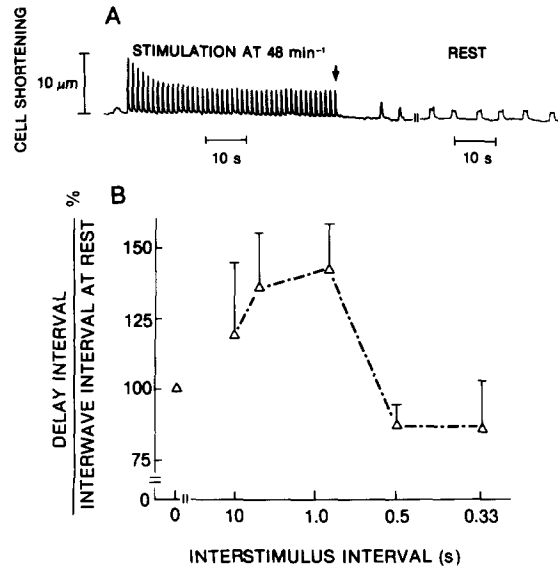
FIGURE 2. (A) The effect of regular field stimulation (48 min^{-1}) of a rat myocyte bathed in 1 mM Ca_o. After stimulation, a delay of interval is required for a spontaneous contractile wave to occur. The duration of the delay interval, i.e., the interval from the arrow (last stimulated twitch) to the first spontaneous oscillation (contractile wave), is longer than the average interwave interval after several minutes of rest. (B) The average effect of the prior rate of stimulation on the delay interval in six rat myocytes bathed in 1.0 mM Ca_o. The cells were stimulated for 1 min at each frequency. Data are expressed as the percent of the resting interwave interval (interstimulus interval of zero), which was 9.1 ± 1.5 s ($\bar{x} \pm$ SEM).

abolished the negative twitch amplitude–stimulation frequency relationship in rat myocytes (Fig. 4). The average effect of ouabain of reducing the interwave interval at rest and the delay interval after stimulation of varying frequency in four cells is illustrated in Fig. 5.
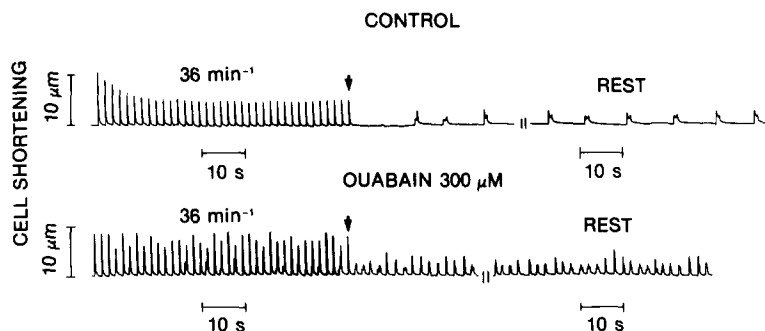

FIGURE 3. (*Left*) Electrical stimulation at 36 min^{-1} for 1 min of a representative rat myocyte bathed in 2.0 mM Ca_o, before (top) and after (bottom) 60 min in ouabain (300 μM). Stimulation was terminated at the arrow. (*Right*) Spontaneous contractile waves at rest in the absence (top) and presence (bottom) of ouabain.
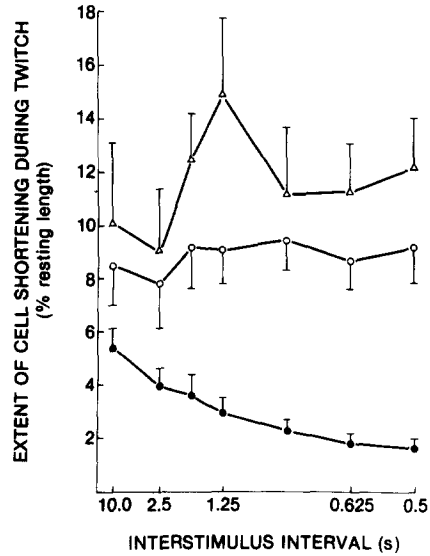
FIGURE 4. The average extent of myocyte shortening during the twitch in the steady state as a function of the rate of stimulation in rat myocytes in the absence and presence of drugs that enhance cell Ca^{2+} loading ($\triangle$, 300 μM ouabain, $n = 4$; $\bigcirc$, 1 μm isoproterenol, $n = 4$). The control curve ($\bullet$) is for all eight cells. Ca_o was 2 mM.
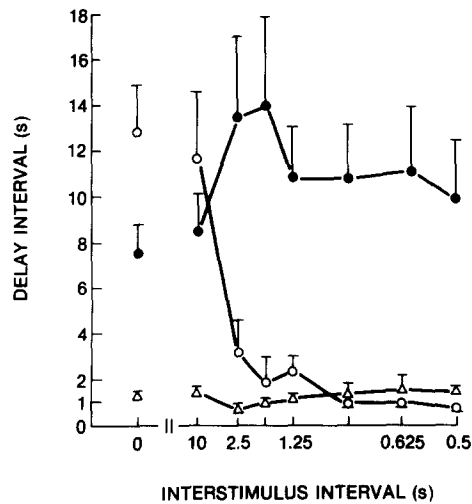

FIGURE 5. The average effect ($\bar{x} \pm$ SEM) of ouabain (300 μM) or isoproterenol (1 μM) on the interwave interval at rest (interstimulus interval of zero) and on the delay interval after 1 min of electrical stimulation of varying frequency. Same myocytes and symbols as in Fig. 4. Ca_o was 2 mM. The magnitude of the variance about the control curve is due to differences among the cells in the stimulation rate at which the maximum delay interval occurred. In the presence of drug, if the delay interval was smaller than the interstimulus interval, waves appeared between twitches (diastolic period). See Fig. 3, left panel, bottom tracing.

Beta-adrenergic agonists enhance cell Ca^{2+} during stimulation. Fig. 4 shows that isoproterenol, like glycosides, also abolished the negative twitch amplitude–stimulation frequency relationship in rat myocytes. However, in contrast to ouabain, there is no evidence of enhanced cell Ca^{2+} loading in the presence of catecholamines in the resting state. This would not be expected, since the only known mechanism through which cAMP-dependent phosphorylation can increase cell Ca^{2+} loading is via enhancement of the slow inward Ca^{2+} current (Watanabe and Besch, 1974; Tsien, 1977), which is inoperative at rest (Bean et al., 1984). Fig. 5 shows the effect of isoproterenol to markedly reduce the delay interval, and this effect increased as the stimulation frequency increased. However, with time after the cessation of stimulation, this effect disappeared and the interwave interval at rest was not reduced but prolonged. This finding can be interpreted as a reduction in "cell Ca^{2+} load" caused by isoproterenol enhancement of the ATP-dependent sarcolemmal Ca^{2+} pump (Caroni and Carafoli, 1981) or activation of the Na^{+}-K^{+} pump (Wasserstrom et al., 1982).

During electrical stimulation, when the delay interval for spontaneous Ca^{2+} release after an action potential–triggered release was smaller than the interstimulus interval, the contractile waves occurred between stimulated twitches, i.e., in the diastolic interval (lower tracing of Fig. 3A). The localized Ca_i achieved within the propagating contractile wave has been estimated to be of the same order of magnitude as that which results from the twitch (Fabiato, 1981, 1985a; Cobbold and Bourne, 1984). When this extent of Ca^{2+} release from the SR is triggered by an action potential to cause a twitch, a restitution period is required for optimal repetitive releases. This is evidenced by the negative staircases with stimulation from rest (Figs. 2A and 3, control) and the negative twitch amplitude–stimulation frequency relationship in the steady state (control curve in Fig. 4) in rat preparations. In physiologic Ca_o, the duration of this restitution period can be as long as 10 s since a reduction in the interstimulus interval from 10 causes a pronounced decrease in twitch amplitude (Fig. 4, control curve). A spontaneous diastolic Ca^{2+} release from the SR might be expected to compromise the Ca^{2+} release by a subsequent action potential if this action potential occurs before full restitution of SR Ca^{2+} loading after the spontaneous release. Thus, spontaneous diastolic Ca^{2+} release may have an effect on a subsequent twitch shortening and action potential duration because of the dependence of cell shortening and the action potential on Ca_i achieved during the twitch (Mitchell et al., 1983; Lab et al., 1984). Figs. 6 and 7 depict the results obtained in a representative microelectrode-impaled rat myocyte in which spontaneous contractile waves occurred in the diastolic period when the cell was stimulated at 24 min^{-1}. The time between a spontaneous contractile wave and a subsequent twitch varied over the range 300–1,800 ms; note that the extent and rate of cell shortening during the ensuing twitch increased as the duration of the interval increased (Fig. 6, A and B). Additionally, it is important to recall that the wave propagation velocity is on the order of milliseconds and that ~1 s elapses between the wave onset and termination. Thus, the presence of spontaneous waves in the period between stimuli compromised twitch displacement and the magnitude of this effect varied directly (up to twofold over the interval examined) with the time elapsed between the onset of the spontaneous contractile wave and the ensuing stimulated twitch.

Fig. 7A shows that after a spontaneous contractile wave, the duration of the transmembrane action potential was abbreviated, and the magnitude of this effect varied directly with the time from the prior spontaneous contractile wave (Fig. 7B). The time-dependent effect on the action potential was related to that in the twitch (Fig. 7C).

The results in Figs. 2, 3, and 5 show that a delay interval must elapse before a spontaneous contractile wave can occur after a twitch. An action potential delivered during the delay interval, i.e., the restitution period for spontaneous release to occur after a twitch ought to discharge the SR, cause a twitch, and thus prevent the occurrence of a spontaneous contractile wave. In other words, the spontaneous diastolic contractile waves ought to be overdriven by regular action potentials that occur at intervals that are shorter than the delay interval
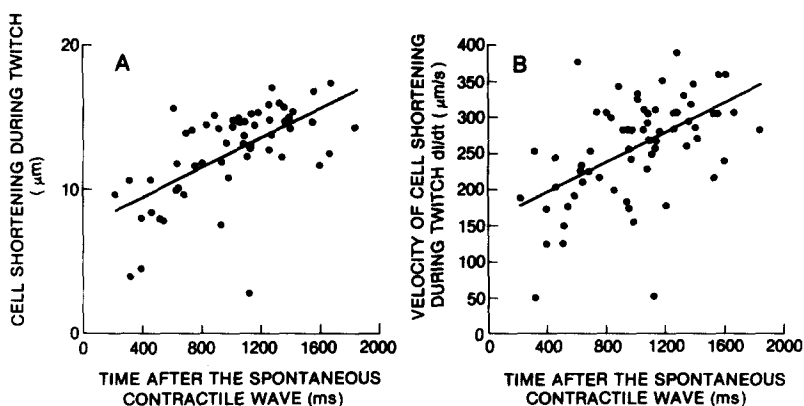

FIGURE 6.   A representative example of the extent (A) and maximum rate (B) of cell shortening of the twitch of a single rat myocyte stimulated at 24 min^{-1} in 1.8 mM Ca$_o$. Both parameters vary with the time elapsed between the preceding spontaneous diastolic contractile wave and the twitch. The least-squares linear fits of the points to the line were $r = 0.61, P < 0.001$ (A) and $r = 0.55, P < 0.001$ (B).

required for spontaneous release after a previous action potential. Furthermore, since this delay interval becomes smaller with increasing cell Ca^{2+} loading (Fig. 5), the interstimulus interval required for such suppression ought to decrease with increasing cell Ca^{2+} loading. This was found to be the case (Fig. 8). The delay interval during stimulation was varied across a wide range by changing Ca$_o$ or by the addition of either ouabain or isoproterenol. Note that the relationship between the interstimulus interval required for the suppression of diastolic waves and the delay interval for the spontaneous wave after the last stimulated twitch form a line of identity.

Rabbit Myocytes

The studies on spontaneous contractile waves described thus far pertain to the rat myocytes in which low-frequency waves occur in the unstimulated state even in a Ca$_o$ as low as 1.0 mM. However, the observations regarding the effect of

stimulation on wave characteristics also pertain to rabbit myocytes under conditions in which spontaneous contractile waves are induced in these cells, e.g., in the presence of high concentrations of ouabain, as shown previously (Capogrossi and Lakatta, 1985).

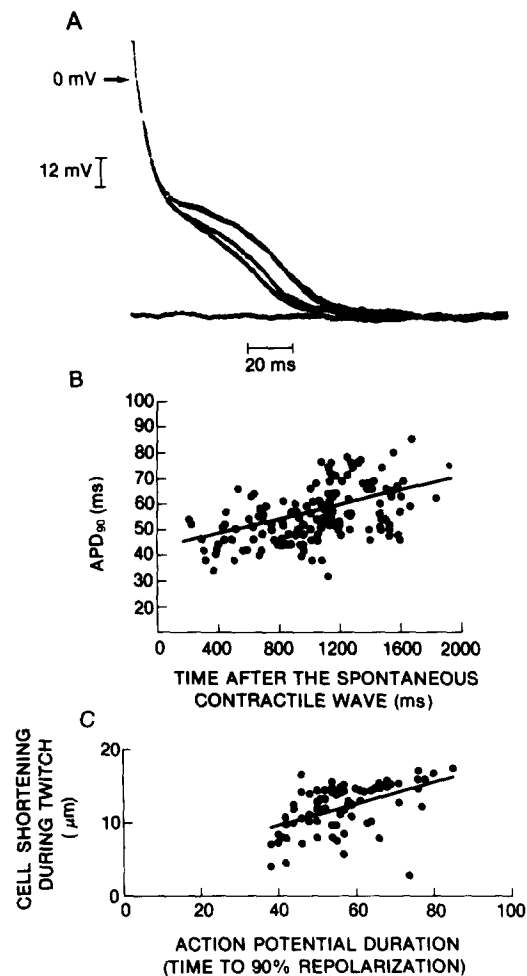Fig. 9 depicts a rabbit myocyte that exhibits all the major findings of the

FIGURE 7. The effect of a spontaneous contractile wave occurring in the interval between stimulated twitches (diastolic period) on the time to 90% repolarization (APD_{90}) of the transmembrane action potential that initiates the following twitch. Same cell as that depicted in Fig. 10. (A) Example of action potentials that followed a spontaneous contractile wave at three different intervals (see text). (B) The time to APD_{90} vs. the interval that had elapsed between the action potential and the beginning of the previous diastolic wave. $r = 0.47$, $P < 0.001$. (C) The effect of diastolic waves on the extent of the subsequent twitch shortening (from Fig. 10A) is related to that on APD_{90} (from Fig. 11B). $r = 0.50, P < 0.001$.
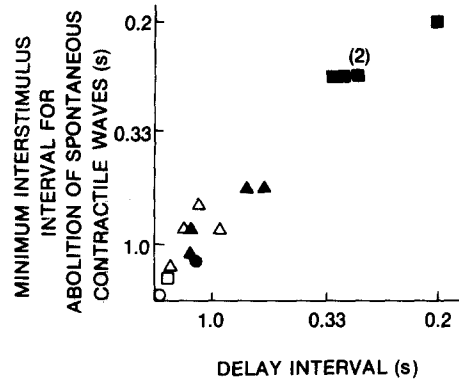
FIGURE 8. The minimum interstimulus interval required to suppress spontaneous waves in rat myocytes varies directly with the delay interval for a wave to occur after a preceding stimulus. Ca_o was 3 (O), 5 (□), or 7 (●) mM in the same cell. There was 2 mM Ca_o and 100 μM ouabain in four other cells (△), 300 μM ouabain in the same four cells (▲), and 1 μM isoproterenol in five other cells (■).

present study. In this cell, which was superfused with buffer containing 1 μM isoproterenol, the following observations were made. (a) Waves were not present in the absence of stimulation, because isoproterenol, although it causes spontaneous contractile waves during or shortly after stimulation at adequate rates ($\geq$48 min^{-1} in this cell), does not cause them at rest. (b) No spontaneous contractile waves were observed during or after stimulation in the presence of isoproterenol at the low frequency of 6 min^{-1}. (c) A spontaneous wave occurred after stimulation at higher rates (48 min^{-1}) but the delay interval was longer than the interstimulus interval; thus, no waves occurred in the diastolic period and each twitch was of constant amplitude. (d) The delay interval decreased as the frequency of stimulation increased. At 96 min^{-1}, it was smaller than the intersti-
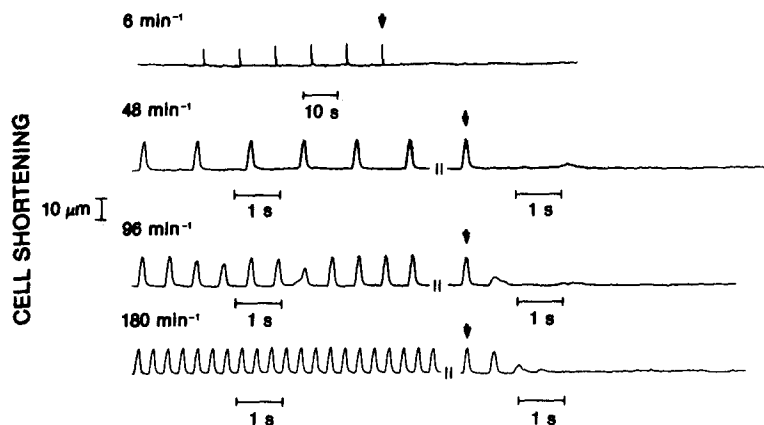

FIGURE 9. The effect of 1 min of stimulation at various frequencies on a representative rabbit myocyte bathed in 3 mM Ca_o in the presence of 1 μM isoproterenol. The arrow indicates the last stimulated twitch. See text for details.

mulus interval; thus, at this frequency of stimulation, spontaneous contractile waves occurred between stimulated twitches. When a wave preceded a twitch, the amplitude of that stimulated twitch decreased and this produced an "alternans" in twitch amplitude, which was well evident in the seventh twitch and, upon closer observation, also in the third and fourth twitches. (*e*) During stimulation at 180 min^{-1}, the interstimulus interval was smaller than the delay interval for spontaneous release, the waves were suppressed, and the twitch amplitude became uniform once again. Additionally, the initial waves after stimulation exhibited various degrees of synchronization before a time-dependent reduction in their frequency and subsequent abolition at rest, as demonstrated previously also in rat cells (Capogrossi and Lakatta, 1985).

DISCUSSION

The present results put to rest the controversy about whether spontaneous contractile waves are a "physiologic" phenomenon in rat cardiac myocytes. Specifically, the results demonstrate that these waves are absent during electrical stimulation of rat myocytes at rates >0.5 Hz in the absence of experimentally induced Ca^{2+} overload. Even during Ca^{2+} overload states, the spontaneous contractile waves are absent if the stimulation rate is increased to the physiologic level for rat myocytes, i.e., ≥ 5 Hz (Fig. 8). Although the absence of contractile waves during electrical stimulation indicates that the underlying spontaneous Ca^{2+} release is not a "physiologic phenomenon," the present results show that when such release does occur between electrically stimulated twitches, its presence is deleterious to myocyte function. In this regard, spontaneous SR Ca^{2+} release in the "diastolic interval" may be considered to be "pathologic." In the unstimulated state, however, as shown in the preceding article (Capogrossi et al., 1986a), their presence cannot be interpreted as pathologic. Neither can their presence in the unstimulated state be described as physiologic, since by definition the absence of stimulation is an unphysiologic state.

After electrical stimulation in the absence of Ca^{2+} overload, the waves reappear only after a delay interval that exceeds the average spontaneous interwave interval at rest. After electrical stimulation in the presence of drugs that increase Ca_i, e.g., cardiac glycosides or isoproterenol, this delay interval was reduced. If this delay interval is smaller than the interstimulus interval, the waves occur in the diastolic period. When they do so, the ensuing twitch amplitude and action potential duration are reduced. Decreasing the interstimulus interval to just less than the delay interval abolishes the spontaneous diastolic contractile waves during stimulation.

It has recently been suggested (Fabiato, 1985d) that in mechanically skinned single myocytes, Ca^{2+}-induced release of Ca^{2+} triggered by a rapid and transient increase in the free $[Ca^{2+}]$ at the outer surface of the SR, caffeine-induced release of Ca^{2+}, and spontaneous cyclic release of Ca^{2+} occur through the same channel across the SR membrane, although the gating mechanism may differ among these types of release. The fact that rat myocytes in the present study exhibit a parallel suppression in the amplitude of the contractile response to both spontaneous and action potential–triggered Ca^{2+} release after incremental SR Ca^{2+} depletion by caffeine (Fig. 1) supports the assumption that both types of Ca^{2+}

release are from the same releasable compartment (SR in Fig. 10) and perhaps have common mechanisms. However, the scheme in Fig. 10 does not imply an identical molecular mechanism for both types of Ca^{2+} release, as this is presently unknown for both cases. In fact, in the case of spontaneous Ca^{2+} release, it appears that some combination of SR Ca^{2+} loading and Ca_i will lead to a spontaneous contractile wave, but neither the present nor previous studies (Bloom, 1970; Fabiato and Fabiato, 1973, 1975; Dani et al., 1979; Rieser et al., 1979; Chiesi et al., 1981; Fabiato, 1985a) have independently controlled these two variables, since both change as the Ca_o bathing the preparations is varied. Thus, the discussion of the present results can consider them a lumped variable, termed the "cell Ca^{2+} load."

Previous studies in rat and other mammalian species have been interpreted to indicate that optimal release of Ca^{2+} by repetitive action potentials requires a restitution period after a previous action potential (Posner and Berman, 1969; Wood et al., 1969; Lee et al., 1970; Wohlfart, 1982; Kurihara and Allen, 1982; Orchard and Lakatta, 1985). Similarly, the delay interval indicates that a restitution period, although it is longer than that for action potential–triggered release, is also required for a spontaneous release after a prior action potential–triggered release. This is because twitches of substantial size can be elicited at intervals after a previous twitch or wave that are substantially smaller than many of the delay intervals measured in the present study. More detailed studies of the restitution of both types of release after a twitch have also indicated that this is the case (Kort and Lakatta, 1985b; Capogrossi et al., 1986b). This may indicate that the two types of release, though sharing certain features, may also differ in some other mechanisms.

Continuous electrical stimulation of rat cardiac preparations under physiologic conditions, at rates that do not allow enough time for repriming of SR, could lead to its Ca^{2+} depletion (via mechanisms for Ca^{2+} loss in Fig. 10), just as repetitive Ca^{2+} triggers deplete SR Ca^{2+} and reduce Ca^{2+} release in the skinned cardiac cell (Fabiato, 1985c). A manifestation of this in the rat myocytes in the present study is the markedly negative staircase in twitch amplitude during repetitive electrical stimulation (Figs. 2A and 3, upper tracing) and a negative twitch amplitude–stimulation frequency relationship (Fig. 4, control curve). Under these conditions, stimulation caused a transient prolongation of the delay interval for spontaneous waves. Other studies in intact muscles stimulated in physiologic Ca_o have demonstrated a post-stimulation transient suppression of scattered light intensity fluctuations, which are caused by the tissue motion generated by spontaneous contractile waves (Kort and Lakatta, 1985a).

That "cell Ca^{2+} load" modulates the restitution time for a subsequent spontaneous Ca^{2+} release follows from the previous observations that the average spontaneous interwave interval in the absence of stimulation varies with cell Ca^{2+} loading (Kort et al., 1985a; Capogrossi and Lakatta, 1985), just as it varies with SR Ca^{2+} loading in "skinned" myocytes (Chiesi et al., 1981; Fabiato, 1985a). If stimulation under certain conditions could enhance cell Ca^{2+} loading to a greater extent than it does under others, the delay interval would decrease. In conjunction with such an effect, the restitution time required for optimal action poten-
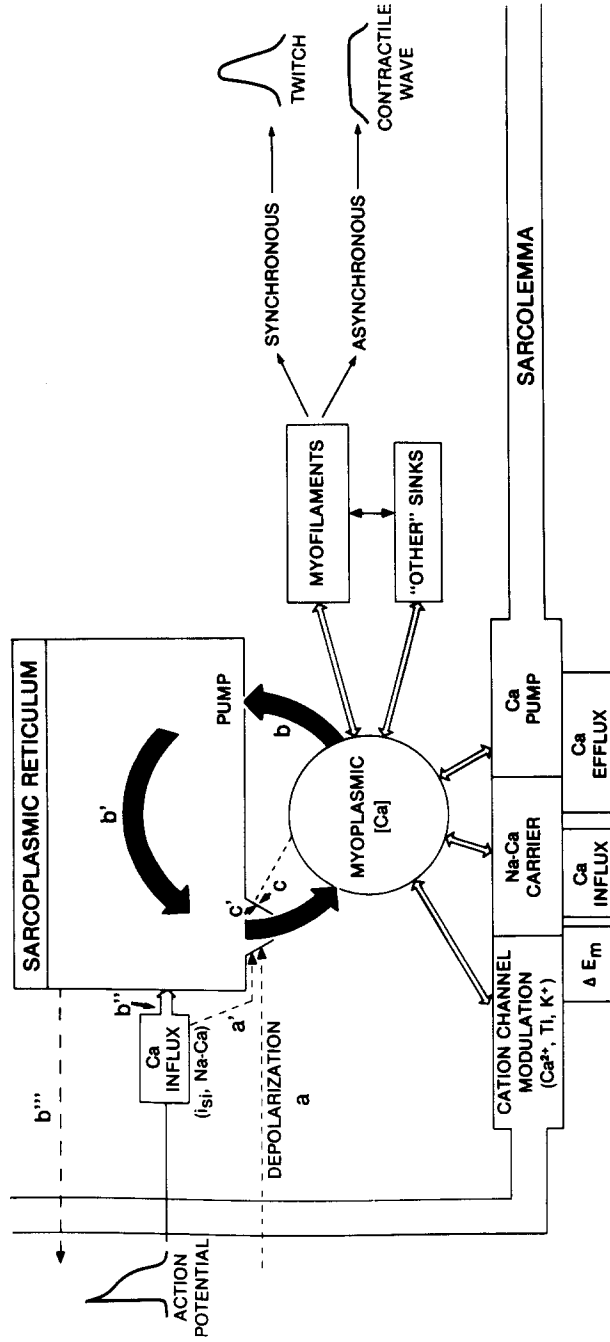
FIGURE 10. A schematic model depicting some mechanisms that are thought to modulate cell Ca^{2+} and SR Ca^{2+} release in the cardiac cell. The action potential causes the release of Ca^{2+} via SR Ca^{2+} channel depolarization (*a*) or by a Ca^{2+} trigger (*a'*). (*b*) SR Ca^{2+} pump. (*b'*) Transfer function relating Ca^{2+} uptake to Ca^{2+} release. (*b''*) SR Ca^{2+} loading from transsarcolemmal influx. (*b'''*) SR Ca^{2+} lost directly into the extracellular space. (*c*) Ca^{2+} modulation of SR Ca^{2+} channel from the myoplasmic Ca^{2+}. (*c'*) Ca^{2+} modulation of SR Ca^{2+} channel from the Ca^{2+} inside the SR.

tial–triggered Ca^{2+} release should also decrease, and thus the reduction in twitch amplitude observed at higher rates of stimulation ought to be less prominent or absent. The present results show that glycosides or isoproterenol (Fig. 5) reduced the delay interval for spontaneous waves to occur. That the restitution time for action potential–mediated release was concomitantly reduced is suggested by the flattening of the negative twitch staircase in rat cells upon stimulation from rest (Fig. 3, lower tracing), with a preservation of the amplitude of the first twitch and the abolition of the negative twitch amplitude–stimulation frequency relationship during steady state stimulation (Fig. 4). The abolition of the steady state negative stimulation interval twitch strength has also been observed in the intact rat cardiac muscle in high Ca_o and is associated with an augmented transient in aequorin luminescence that precedes the contraction (Kurihara and Allen, 1982; Orchard and Lakatta, 1985).

Although the apparent effect of the "cell Ca^{2+} load" on the delay interval can be interpreted as being due to an effect of SR loading, it is not implied that the change in Ca_i that may occur with a change in the "cell Ca^{2+} load" during stimulation is not involved in the modulation of the spontaneous release of Ca^{2+} from SR. It may in fact initiate or inhibit Ca^{2+} release via the SR channel (Fabiato, 1985b), and these two effects cannot be differentiated on the basis of the present results.

Functional Implications of Spontaneous Oscillatory Diastolic Ca^{2+} Release

Variable Ca^{2+}-dependent diastolic tonus. The presence of a diastolic contractile wave causes a change in cell length, and this implies that spontaneous Ca^{2+} release would affect the diastolic stiffness. The variable "diastolic compliance" reported previously in intact cardiac preparations (Scherlag et al., 1966; Hoffman et al., 1967) might be attributed in part to spontaneous Ca^{2+} release. The modulation of the delay interval by the cell Ca^{2+} load could explain the Ca^{2+}-dependent changes in the timing of the aftercontraction in multicellular preparations (Feigl, 1967; Stern et al., 1983; Nieman and Eisner, 1985). Thus, in multicellular myocardial tissue, the observed Ca^{2+}-dependent "diastolic tone" (Lakatta and Lappe, 1981; Walford et al., 1984; Kort and Lakatta, 1985a) could be explained not only by a greater tendency for spontaneous multifocal waves to occur within cells (Capogrossi and Lakatta, 1985), or by an increase in wave propagation velocity (Kort et al., 1985a) as the cell Ca^{2+} load increases, but also by an increase in contractile wave density within the tissue during diastole caused by a shortening of the delay interval (Kort et al., 1985b).

Systolic dysfunction. In the model in Fig. 10, in which an optimal SR Ca^{2+} release after a prior release is time dependent, a critically timed spontaneous release from the SR ought to have a deleterious effect on the subsequent action potential–induced Ca^{2+} release, i.e., a decrease in the Ca^{2+} released by that action potential and therefore a decrease in the strength of the associated twitch. Such an effect does indeed occur and is manifest in single myocytes as "electrical and mechanical alternans," depending on the time elapsed between spontaneous Ca^{2+} release and the action potential (Figs. 6 and 7). A similar dependence of twitch

strength and transmembrane action potential duration on time after an aftercontraction has been observed in multicellular preparations (Ferrier, 1976; Bogdanov et al., 1981; Allen et al., 1985), and a more recent study has demonstrated that twitch strength is enhanced as diastolic Ca^{2+} oscillations dissipate after the reduction in Ca_0 (Lakatta and Lappe, 1981). The addition of ryanodine to the bathing fluid of experimentally Ca^{2+}-overloaded preparations also transiently increases twitch strength (Valdeolmillos and Eisner, 1985).

Membrane depolarization leading to action potentials. Spontaneous release of Ca^{2+} into the myoplasm during diastole with subsequent sarcolemmal depolarization (Fig. 9 of this article and Fig. 16 of the preceding article) is probably an initiating step in some forms of so-called "Ca^{2+}-dependent arrhythmias" (Kass and Tsien, 1982). The present study demonstrates that stimulation in a milieu that produces high cell Ca^{2+} loading leads to an enhanced likelihood of the spontaneous Ca^{2+} release occurring. Previous studies have also shown that stimulation under these conditions results in an enhanced likelihood for Ca^{2+} release to be multifocal (Capogrossi and Lakatta, 1985). These features of spontaneous Ca^{2+} release may underlie triggered automaticity (Ferrier et al., 1973; Ferrier, 1976). Additionally, the depolarization resulting from spontaneous multifocal contractile waves can reach sufficient magnitude to produce a spontaneous action potential (Bahinski et al., 1986; Houser et al., 1986). The present results show that the same spontaneous Ca^{2+} oscillations that are transiently accelerated by stimulation in high Ca^{2+}-loading states can be transiently suppressed by stimulation in normal states of Ca^{2+} loading. Additionally, even in high Ca^{2+}-loading states in vitro, an increase in the stimulation frequency to a level that just exceeds the spontaneous release frequency is able to prevent, or "overdrive," the spontaneous Ca^{2+} release (Figs. 8 and 9, lower tracing). These characteristics of spontaneous diastolic Ca^{2+} release may underlie "abnormal automaticity." Finally, it has been demonstrated that impulse conduction among cells is markedly slowed when Na_i and therefore Ca_i are elevated (De Mello, 1975; Weingart, 1977). The localized level of high Ca_i achieved during a spontaneous contractile wave can be as high as several micromolar (Fabiato, 1981, 1985a; Orchard et al., 1983; Cobbold and Bourne, 1984; Kort et al., 1985b) and may be sufficient to have a role in this slowing of conduction, which is an essential feature of re-entrant arrhythmias.

Enhanced diastolic tone, the occurrence of arrhythmias, and compromised systolic function are cardinal signs of many myocardial disease states. The conceptualization of spontaneous SR Ca^{2+} release as interpreted and discussed in the context of the present findings in cardiac myocytes, though speculative in some regard, provides a logical framework in which to structure testable hypotheses about its potential role in the pathophysiology of some forms of heart disease.

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