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Masterclass

Functional implications of spontaneous sarcoplasmic reticulum Ca^{2+} release in the heart

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A fair degree of order among cells is required for normal heart function, ie, effective pumping of blood and efficient filling between cycles require that the cystolic [Ca^{2+}], Ca_i, oscillation that underlies each heart beat occurs uniformly within each cell and relatively synchronously among cells comprising the myocardium. The Ca^{2+} oscillations triggered by an action potential to cause a normal heart beat (fig 1) are due to the rapid release of Ca^{2+} from an intracellular storage site, the sarcoplasmic reticulum. The sarcoplasmic reticulum subsequently pumps back into itself a large fraction of the Ca^{2+} it releases into the cytosol, resulting in a fall in Ca_i to the diastolic level (fig 1, upper panel). Thus, at closer inspection, the heartbeat is in essence an organised cycling of Ca^{2+} from the sarcoplasmic reticulum into the cytosol and back again. In a machine of this design, the potential for *spontaneous* sarcoplasmic reticulum Ca^{2+} recycling, ie, spontaneous Ca^{2+} oscillations (S-CaOs) to occur between action potential initiated heart beats is ever present (fig 1, lower panel). A substantial body of evidence gleaned from a variety of mammalian cardiac preparations has indeed documented the occurrence of S-CaOs and has, in part, defined their characteristics and functional sequelae.

S-CaOs in individual cardiac ventricular cells

The probability of S-CaOs occurrence is determined by the extent of Ca^{2+} loading of the cardiac cell and of its sarcoplasmic reticulum, and by the Ca^{2+} pumping release characteristics of the latter.[2-4] The Ca_i determines the Ca^{2+} available for pumping by the sarcoplasmic reticulum; thus regulation of Ca_i by sarcolemmal ion pumps and carriers is a major determinant of S-CaOs occurrence. Increasing the bathing [Ca^{2+}], addition of catecholamines or other inotropic drugs, eg, inhibition of the Na-K pump,[5-18] or situations like acidosis, metabolic inhibition,[19 20] reoxygenation following anoxia,[21 22] reflow following ischaemia,[23 24] free radical exposure,[25-29] or the Ca^{2+} paradox,[30] each of which leads to an increase in Ca_i, enhance the probability of S-CaOs occurrence. Other factors that directly modulate the sarcoplasmic reticulum Ca^{2+} pump or release channel, eg, Mg^{2+}, ATP, ADP, cAMP, and IP_3, caffeine, ryanodine, and temperature, also determine the likelihood of S-CaOs occurrence.[2-4 9-13 31-35] "Skinned" rat cells bathed in a [Ca^{2+}] of as low as 100 nM exhibit S-CaOs.[36 37] In fact about 80% of all rat cells exhibit S-CaOs under this condition.[2] In contrast, for skinned rabbit cells to exhibit S-CaOs an ambient bathing [Ca^{2+}] of twice the value in rat is required.[2] In the absence of regularly occurring action potentials in intact cardiac cells, S-CaOs occurrence is roughly periodic; the periodicity is Ca^{2+} dependent and ranges from less than 0.1 to a few Hz (see [32] for review). When bathed in physiological [Ca^{2+}] in the absence of drugs, 50% of healthy *unstimulated* rat myocardial cells with intact sarcolemmal function exibit S-CaOs; however, the S-CaOs frequency under these conditions is low, ie, 2-3 per minute.[38 39] In

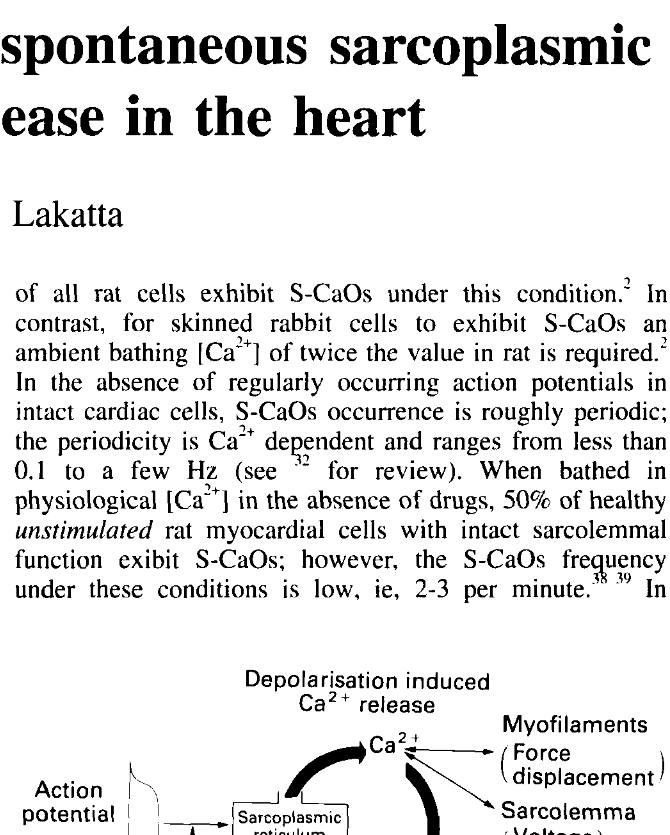

Figure 1 *Top: Action potential (AP) induces Ca^{2+} release from the sarcoplasmic reticulum (SR) via Ca^{2+}-induced Ca^{2+} release. Ca^{2+} interacts with myofilament binding sites to cause a contraction and with sarcolemmal binding sites to produce inward current, which affects the repolarisation of the AP. SR is both a source and sink for the Ca_i, ie, it is a Ca^{2+} oscillator. In addition to triggering the release of Ca^{2+} from SR, Ca^{2+} current via L type sarcolemmal Ca^{2+} channels activated during the AP place a Ca^{2+} load on the cytosol and SR. In the steady state, this Ca^{2+} loading is balanced by other sarcolemmal Ca^{2+} extrusion mechanisms (not shown). Bottom: Spontaneous Ca^{2+} oscillations (S-CaOs) can occur in the absence of an AP. The increase in Ca_i from S-CaOs has the same sequelae as those induced by an AP. In myocardial tissue these sequelae occur heterogeneously within and among cells during the diastolic interval. From Lakatta.[1]*

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contrast, rabbit cells, prepared by the same method as rat cells, do not exhibit S-CaOs under these conditions, but require an increase in the bathing [Ca^{2+}] to approximately 10 mM for induction of low frequency S-CaOs.[39]

While the Ca^{2+} dependence of S-CaOs has clearly been established, the mechanisms involved in the *initiation* of this Ca^{2+} release have not been defined. S-CaOs, unlike an action potential triggered sarcoplasmic reticulum Ca^{2+} release, occurs *locally* within cardiac cells.[4,18,20,23,38,40–48] At a given instant, a single focus or multiple foci of higher Ca_i due to S-CaOs can be present with a cardiac cell.[40,42–44,49] The local Ca_i achieved during S-CaOs is as high as that triggered by an action potential to cause systole.[20,40,43,50] Ca^{2+}-induced Ca^{2+} release from sarcoplasmic reticulum has been shown to occur in chemically or mechanically "skinned" cardiac cells under conditions in which these preparations behave as sarcoplasmic reticulum *in situ*,[2–4,35] and in sarcoplasmic reticulum vesicles reconstituted into lipid bilayers.[33,34,51–53] Recent advances in single cardiac cells with an intact sarcolemma have clearly shown that during normal excitation-contraction coupling Ca^{2+} entering the cell during the action potential via L type sarcolemmal Ca^{2+} channels triggers the release of Ca^{2+} from within the sarcoplasmic reticulum (see [54] for review). This Ca^{2+}-induced Ca^{2+} release from the sarcoplasmic reticulum had been predicted from prior experiments in mechanically skinned cardiac cells.[31,36,37] S-CaOs initiation may also be initiated by Ca^{2+}-induced Ca^{2+} release from the sarcoplasmic reticulum.[54] However, without a detailed kinetic knowledge of the Ca^{2+} release channel, it is impossible to model Ca^{2+} induced S-CaOs initiation mechanisms quantitatively. But this mechanism must be subject to the constraints that there exists a stable resting equilibrium with low Ca_i and that the cell must return to this equilibrium following a release. By mathematical modelling it was found that models satisfying these constraints give rise to S-CaOs when provided with a sufficient source of Ca^{2+}.[37,50,55] As the Ca^{2+} in the sarcoplasmic reticulum rises, a point is reached at which the loop gain of the positive feedback process exceeds unity. Traces of Ca^{2+} released will then trigger the release of further Ca^{2+} in an explosive process, resulting in a rapid spontaneous release of large amounts of Ca^{2+}.[37,55] Following release, the sarcoplasmic reticulum reloads with Ca^{2+} and the process will repeat itself with a frequency that depends on the availability of Ca^{2+} to the cell. The regeneration of Ca^{2+}-induced Ca^{2+} release thus provides an attractive mechanism for the spontaneous *initiation* of S-CaOs.

During electrical stimulation of cardiac cells (fig 2), the occurrence of S-CaOs in diastole is separated from the preceding action potential triggered sarcoplasmic reticulum Ca^{2+} release by a "delay" interval (time from the prior electrically stimulated beat to the onset of S-CaOs, arrows in fig 2) which becomes shorter as the Ca^{2+} load available to the sarcoplasmic reticulum increases (fig 3). The delay interval may result from a net cell Ca^{2+} loss that causes Ca^{2+} depletion of some or all cellular compartments; evidence for a substantial Ca^{2+} efflux during electrical stimulation has recently been provided from measurements of extracellular [Ca^{2+}] with Ca^{2+} sensitive dyes or microelectrodes.[56–61] In rat ventricular preparations, in contrast to those of most other species, S-CaOs occurs in the absence of Ca^{2+} overload in the unstimulated state.[11,12,45,46] Recent studies indicate that Ca^{2+} *influx* occurs immediately following cessation of stimulation in rat cardiac muscle.[61] The observed gradual recovery of S-CaOs with rest following stimulation in rat tissue in the absence of experimental Ca^{2+} overload (fig 14A) may be due to slow "diastolic" reloading of cells, which may relate to the higher Na_i measured in rat preparations in some[61–63] but not in all[64] studies. It is also noteworthy that suspensions of rat cardiac myocytes have a higher apparent resting Ca_i than myocytes of other species[65]; this may indicate that in rat myocardium the Na-Ca exchanger operates closer to equilibrium than in other species.[61,66]

A transient removal of Ca^{2+} from the sarcoplasmic reticulum "releasable pool" but not from the cell is a second mechanism that might explain the delay interval in non-Ca^{2+}-overloaded rat cardiac muscle. The idea of a transient inaccessibility of Ca^{2+} for release has traditionally been seen in relation to a two compartment sarcoplasmic reticulum model with pumping occurring into a non-releasable compartment followed by subsequent transfer to the release compartment.[67,68] Some anatomical evidence for this has been provided from autoradiographic and electron microscope probe studies that suggest a time dependent recycling of Ca^{2+} after contractile activity from the longitudinal sarcoplasmic reticulum to the terminal cisternae in various muscle types.[69,70] Alternatively, a delay in the restitution of Ca^{2+} loading of the sarcoplasmic reticulum release site could occur if Ca^{2+} released by an action potential were taken up by or bound to other cell buffers (ie, myofilaments, mitochondria, calmodulin, or phospholipids within the sarcolemma) from which it was only slowly released, or if complete Ca^{2+} filling of the sarcoplasmic reticulum involved a relatively slow pumping rate at the relatively low Ca_i subsequent to the contraction itself.[71]

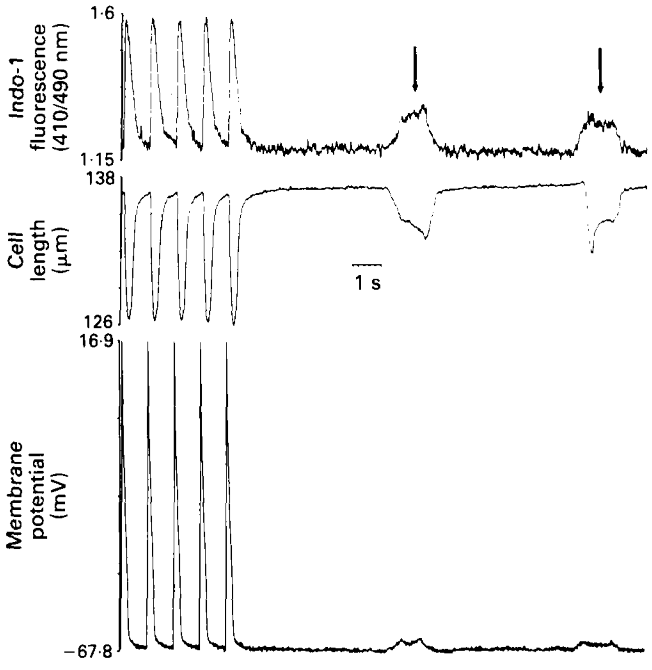

***Figure 2** The normal heart beat at the cellular level. Simultaneous recordings of Ca_i measured by Indo-1 fluorescence 410/490 nm (top trace), cell length measured via a photodiode array (middle trace), and transmembrane action potential (AP), measured via a patch pipette (lower trace) in a single isolated rat ventricular myocyte in response to field stimulation. Following a cessation of electrical stimulation spontaneous Ca^{2+} oscillations (S-CaOs) occur (arrow) producing a transient shortening of the cell and a transient membrane depolarisation. Note that the Indo fluorescence, which provides an index of cell Ca^{2+}, is measured from the entire cell and that the integral of the increase in Indo fluorescence during the S-CaOs is roughly equal to that caused by the AP. The reason for this will become clear later. From Talo* et al.[79]

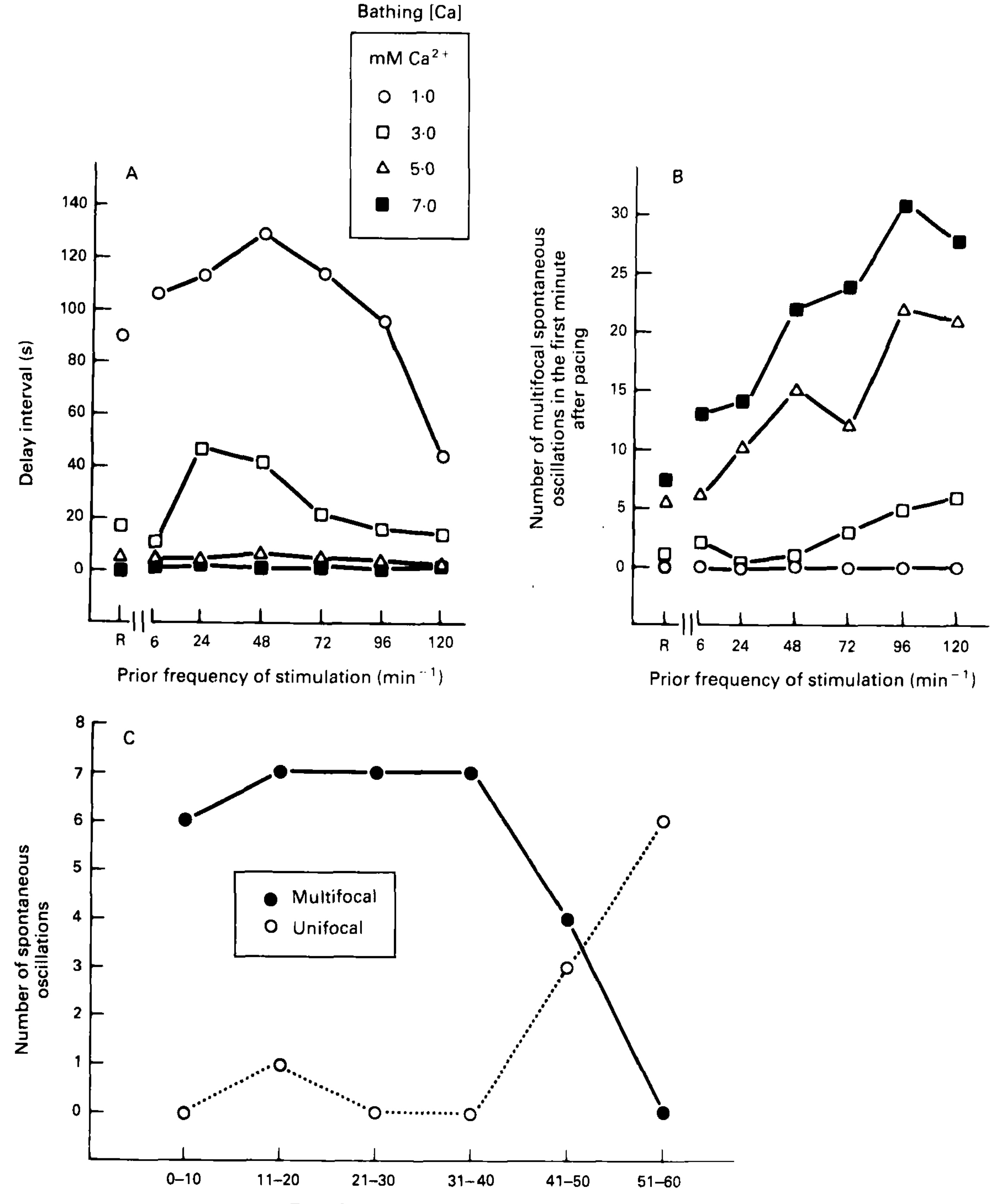

Figure 3 *(A): Effect of electrical stimulation and bathing [Ca^{2+}] on the delay interval between onset of the last stimulated contraction and the first contractile oscillation driven by spontaneous Ca^{2+} oscillations (S-CaOs) following cessation of stimulation in a rat ventricular myocyte. (B) and (C): The effect of electrical stimulation on the likelihood for multifocal S-CaOs occurring within a rat cell. Prior stimulation rate in C was 96·min^{-1} and bathing [Ca^{2+}] was 7 mM. See text for details. From Capogrossi and Lakatta.*[38]

A relative refractoriness of the sarcoplasmic reticulum release channel is a third possible mechanism involved in the delay interval, particularly during states of high Ca^{2+} loading when the delay interval is short. At the present time, however, no data are available to support this hypothesis. Ca^{2+} inhibition of sarcoplasmic reticulum release, not necessarily related to Ca^{2+} loading and resulting from the increase in Ca_i elicited by a prior action potential, is a fourth possible explanation for the delay interval after stimulation. However at the moment this hypothesis has no direct evidence in intact preparations or in reconstituted cardiac sarcoplasmic reticulum Ca^{2+} release channels[34] in its favour. In fragments of single cells, the time constant for removal of the hypothesised Ca^{2+} inactivation of sarcoplasmic reticulum Ca^{2+} release mechanisms[72] is very short compared to the prolonged suppression of S-CaOs during the delay interval following stimulation.

The interval between successive action potentials is a determinant of S-Ca^{2+} occurrence.[6,38,45] In some situations in which sarcoplasmic reticulum Ca^{2+} loading at rest is moderate, diastolic S-CaOs occurrence can be "overdriven" by a modest increase in the stimulation frequency,[45,46,73] ie, successive action potentials fall within the delay interval. However, the action potential, in addition to discharging Ca^{2+}

from the sarcoplasmic reticulum, also serves to load the cardiac cell with Ca^{2+}. Under conditions in which cell and sarcoplasmic reticulum Ca^{2+} loading is already high in diastole, or at rest, electrical stimulation or an increase in the stimulation frequency increases S-CaOs frequency and thus decreases the delay interval and exacerbates diastolic S-CaOs.[6 38 45 73] Figure 3A illustrates the effect of stimulation and bathing [Ca^{2+}] on the delay interval in a representative rat myocyte. As noted above, in the unstimulated state rat myocytes show spontaneous oscillations in the absence of experimental Ca^{2+} overload. During electrical stimulation at rates of 6-72·min^{-1} in 1 mM bathing [Ca^{2+}] the delay interval (top curve in fig 3A) is longer than the average spontaneous interval at rest (designated as R in the figure). One explanation for the prolongation of the delay interval at the lower rates of stimulation is that the time averaged membrane potential is less than the reversal potential for the Na-Ca exchanger and that rat cell Ca^{2+} unloads during stimulation at these rates. Additional mechanisms for the suppression of S-CaOs during stimulation in rat cells bathed in normal [Ca^{2+}] are discussed below. Figure 3A also shows that after stimulation at 120·min^{-1} in this bathing [Ca^{2+}] the delay interval becomes shorter than the average spontaneous interval at rest. Over the entire range of stimulation rates in fig 3A (in 1 mM bathing [Ca^{2+}]), the interstimulus interval never exceeds the delay interval, and therefore S-CaOs do not occur between stimulated contractions under these conditions. However, as bathing [Ca^{2+}] is progressively increased, the S-CaOs frequency at rest increases and the stimulation effect to prolong the delay interval becomes progressively blunted. In bathing [Ca^{2+}] of 5 and 7 mM prolongation of the delay above the S-CaOs interval at rest does not occur; rather there is a reduction in the period of the S-CaOs at rest and a reduced delay interval during stimulation, permitting the S-CaOs to occur in the diastolic interval.

β Adrenergic receptor agonists, by increasing Ca_i during stimulation and thus increasing sarcoplasmic reticulum Ca^{2+} loading, and possibly also by an effect of cAMP on the sarcoplasmic reticulum Ca^{2+} channel release, also reduce the delay interval and therefore increase the likelihood that S-CaOs will occur during stimulation in diastole. This is exemplified in a rabbit ventricular myocyte in fig 4. Unlike rat myocytes, rabbit ventricular myocytes do not exhibit S-CaOs in the absence of electrical stimulation in physiologic bathing [Ca^{2+}].[6 11] In the presence of the β adrenergic agonist isoprenaline S-CaOs do not occur in the absence of electrical stimulation (because, unlike an increase in bathing [Ca^{2+}], β adrenergic receptor stimulation does not increase Ca_i at rest).[73] During or after stimulation in the presence of isoprenaline at the low frequency of 6·min^{-1} no S-CaOs are observed. After cessation of stimulation at a higher rate (48·min^{-1}) a single S-CaOs occurs, but the delay interval is longer than was the interstimulus interval during stimulation; thus no S-CaOs occurred in the diastolic period during the stimulation. As the frequency of stimulation increases the delay interval decreases: following stimulation at 96·min^{-1}, the delay interval becomes shorter than the interstimulus interval and thus S-CaOs occur in diastole; following cessation of stimulation multiple S-CaOs occur that differ in shape (see below). Note that during stimulation at 96·min^{-1} when a diastolic S-CaOs directly precedes an electrically stimulated twitch, the amplitude of that twitch decreases and a contractile "alternans" occurs. This is clearly evident in the seventh twitch during stimulation at 96·min^{-1} and, upon closer observation, also in the third and fourth twitches. (A mechanism relating diastolic S-CaOs to contractile alternans is discussed below.) During stimulation at 180·min^{-1}, the interstimulus interval again becomes less than the delay interval, the S-CaOs are suppressed, and the twitch amplitude becomes uniform once again.

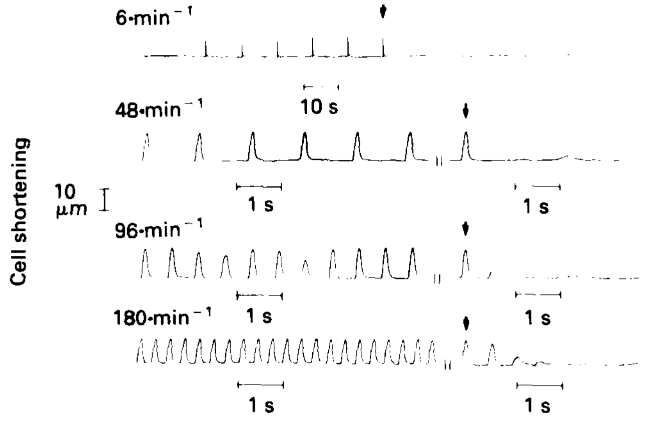

Figure 4 *The effect of electrical stimulation of varying frequency on a representative rabbit myocyte bathed in a* [Ca^{2+}] *of 3 mM in the presence of 1 μM isoprenaline. The arrow indicates the last stimulated twitch. See text for details. From Capogrossi* et al.[45]

Localised origin of S-CaOs within individual cardiac cells

Unlike the sarcoplasmic reticulum Ca^{2+} release caused by an action potential, S-CaOs occur locally within individual cells. Figure 5A (top traces) shows a schematic representation of localised myofilament shortening, ie, the contractile manifestation of localised spontaneous Ca^{2+} release from the sarcoplasmic reticulum in the cytosol. The localised myofilament shortening produces a "contractile band." Diffusion of Ca^{2+} from the initial release locus triggers Ca^{2+} release from the sarcoplasmic reticulum at adjacent sites, resulting in a propagation of Ca^{2+} release as a wave, thus producing a propagating contractile band[55] as shown in fig 5A. Model calculations[55] show that, under conditions in which spontaneous release is initiated, such a wave is always self sustaining. The propagation velocity of the contractile band calculated from such models agrees with the experimentally observed velocity, ie, 80-150 μ·s^{-1}, in individual cells.[12 41 55] Thus theoretical studies conclude that regeneration of Ca^{2+}-induced Ca^{2+} release is a plausible mechanism for both the initiation and propagation of spontaneous Ca^{2+} release. However, it has been noted in experiments employing "caged" Ca^{2+} that the local liberation of Ca^{2+} by flash photolysis produces a local contraction that does not propagate.[74]

S-CaOs originating from a single localised area within the cell may produce only a single band of contracted sarcomeres, as in fig 5A. In fig 5B, the contractile band is initiated in the central region of the myocyte and propagates bidirectionally, giving rise to two discrete contractile bands. Note in the recordings beneath the diagrams in fig 5 that the presence of a contractile band within a cardiac myocyte causes the resting cell length to decrease. When spontaneous sarcoplasmic reticulum Ca^{2+} release originates nearly simultaneously from more than a single focus, the resultant myofilament shortening activity summates to cause a greater reduction in myocyte length (fig 5B versus 5A, lower traces). The likelihood that S-CaOs occurrence will be multifocal, like the S-CaOs frequency, varies directly with the extent of cell and sarcoplasmic reticulum Ca^{2+} loading.[38 45] Figure 3B shows that an increase in bathing [Ca^{2+}] increases the

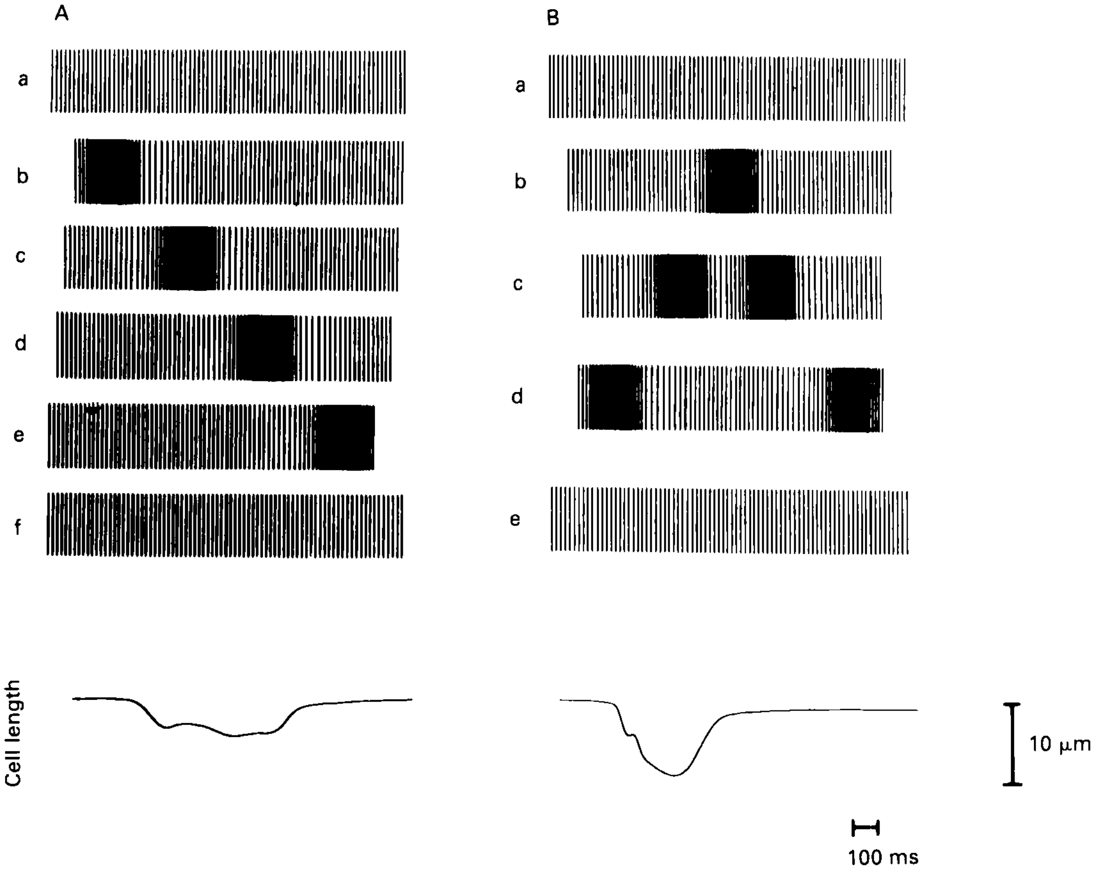

Figure 5 *Upper tracings: schematic diagrams of localised, propagating myofilament activation initiated by spontaneous Ca^{2+} oscillations (S-CaOs). Lower tracings: cell length oscillations driven by propagation of localised S-CaOs. From Capogrossi* et al.[44]

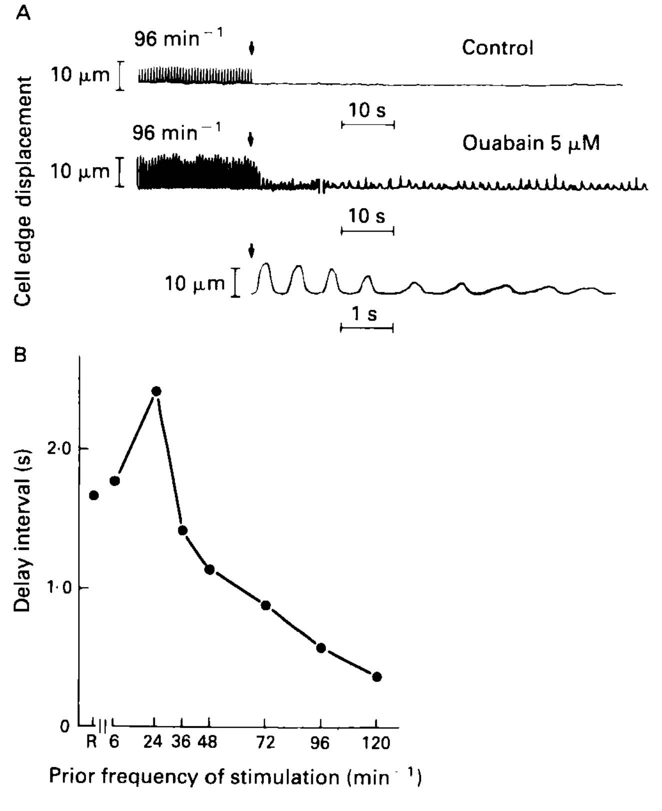

Figure 6 *(A): Stimulation of a single rabbit myocyte in bathing [Ca^{2+}] of 5 mM in the absence (top tracing) and presence of 5 μM ouabain (lower tracings). In the absence of drug no spontaneous Ca^{2+} oscillations (S-CaOs) occurred; ouabain causes contractile oscillations driven by S-CaOs to occur at rest, and stimulation transiently increases the oscillation frequency. Following termination of stimulation (arrow), in the tracing displayed on a more rapid time base (third line), note the progressive increase in time to peak displacement and decrease in peak displacement in each subsequent S-CaOs. This is due to the transition from multifocal to unifocal S-CaOs with time following stimulation. Note also that during stimulation a marked "alternans" of twitch displacement (cf. also fig 3) occurs when S-CaOs are present in the diastolic interval in the presence of drug (middle tracing). Systolic contraction alternans is absent in control (top tracing). (B): Bimodal effect of electrical stimulation on S-CaOs frequency: at 6 and 24·min^{-1} S-CaOs frequency, as reflected in time to first S-CaOs, following cessation of stimulation is less than at rest (R), but during stimulation at rates of 36·min^{-1} or greater S-CaOs becomes enhanced. From Capogrossi and Lakatta.*[48]

likelihood for the S-CaOs to be multifocal at rest in rat myocytes. Note that at a given bathing [Ca^{2+}] the probability that multifocal S-CaOs will occur is enhanced by electrical stimulation, and that the magnitude of this effect is dependent on the rate of stimulation and wanes with time following cessation of stimulation (fig 3C). Note also in fig 4 that S-CaOs occurring immediately after cessation of stimulation of this *rabbit* cell exhibit varying shapes, indicative of multifocal S-CaOs with varying degrees of synchronisation of contractile activity.

Figure 6 shows the transient synchronisation of S-CaOs by electrical stimulation of a rabbit myocyte in the presence of cardiac glycosides. Ouabain causes the de novo appearance of S-CaOs in rabbit myocytes at rest (panel A). Electrical stimulation alters the S-CaOs frequency (panel B): at low

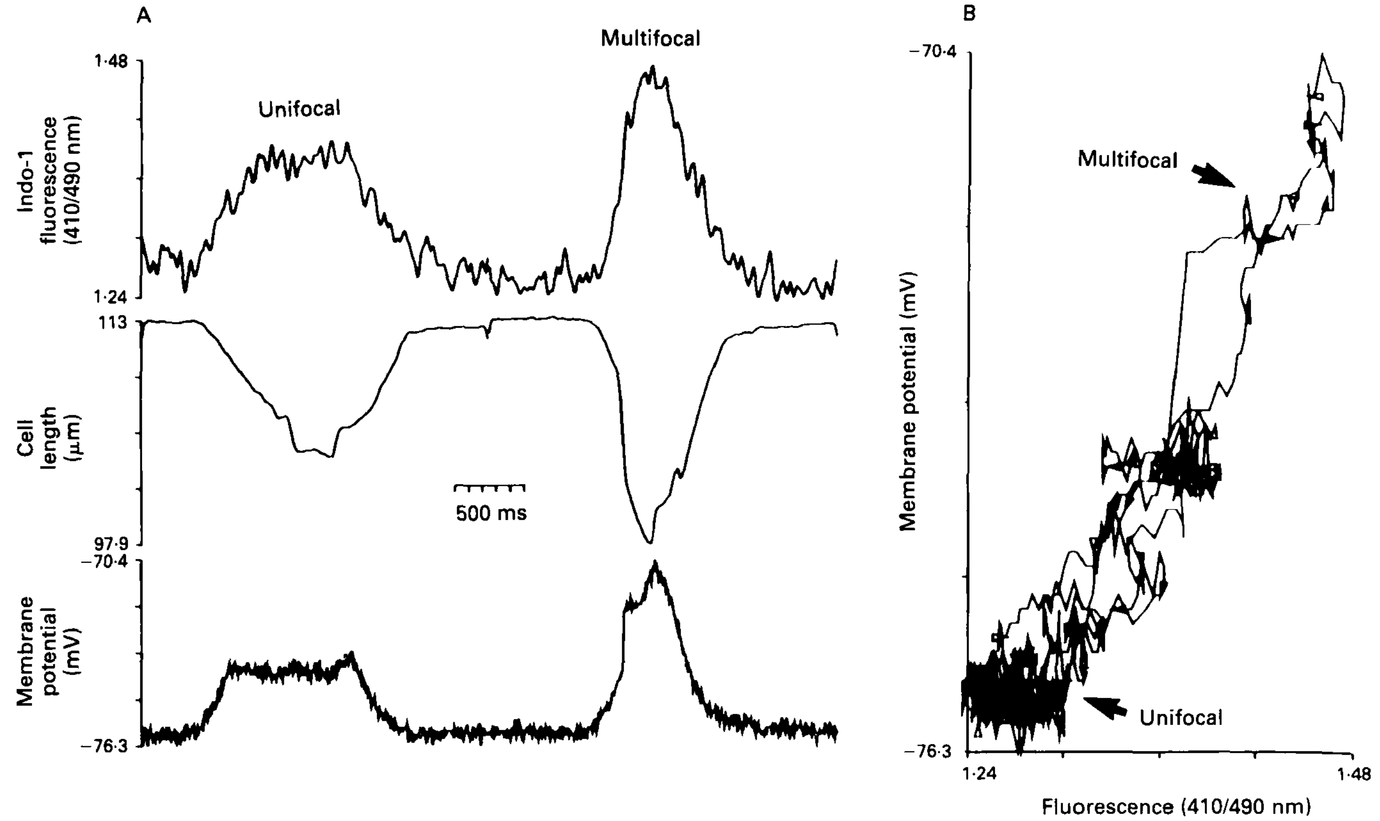

Figure 7 *(A): Spontaneous Ca^{2+} oscillations reported by oscillations in fluorescence of the Ca$_i$ indicator, Indo-1 (top), contraction (middle), and membrane potential (lower) traces in a single rat cardiac myocyte. Note that the increase in Ca$_i$ (measured as the whole cell average of Indo fluorescence) is greater during multifocal S-CaOs (right tracings) than in unifocal S-CaOs (left tracings) and this is associated with a larger membrane deplorisation and contraction. (B): Ca$_i$ and membrane potential phase plane diagrams for events in panel A form a continuum. From Talo* et al.[79]

frequencies of stimulation the S-CaOs period is transiently prolonged from that at resting level (R): at higher rates of stimulation the S-CaOs frequency is enhanced. The lower tracing in panel A shows this on an expanded time scale and also shows that the initial S-CaOs following termination of stimulation are partially synchronised; the extent of synchronisation and the S-CaOs frequency both wane with time following stimulation.

Synchronisation of localised S-CaOs release within single cells

The localised increase in Ca$_i$ caused by S-CaOs not only produces localised Ca^{2+} dependent myofilament interaction and cell contraction but also causes an inward current that is due to the Ca^{2+} activation of cell membrane ionic channels, eg, the non-specific cation conductance, I$_{Ti}$, or of the Na-Ca exchanger.[25 75-78] This produces sarcolemmal depolarisation (fig 2). Thus, in contrast to the normal heartbeat in which a sarcoplasmic reticulum Ca^{2+} release is triggered by events that result from a depolarisation, CaOs *causes* a depolarisation.

The area of sarcolemmal membrane surface exposed to a given increase in Ca$_i$ at any instant determines the magnitude of the resultant Ca^{2+} modulation of the ionic mechanisms that produce inward current, and thus determines the magnitude of the resultant depolarisation. Since a greater sarcolemmal area is exposed to incqeased Ca$_i$ at any instant during multifocal versus unifocal S-CaOs, the former causes a larger sarcolemmal depolarisation (and contraction) than does unifocal S-CaOs (fig 7). It may also be possible that the increase in Ca$_i$ in a given locus during conditions of multifocal release may be greater than that during unifocal release. This is unlikely, however, as the frequency of S-CaOs increases during conditions of multifocal occurrence; increases in S-CaOs frequency are associated with decreases in amplitude of Ca^{2+} release, as measured from the amplitude of local contraction.[12] When of sufficient magnitude, the depolarisation caused by multifocal of S-CaOs can activate Na channels and trigger an action potential (fig 8). Thus a synchronisation of localised increased in Ca$_i$ due to the occurrence of multifocal S-CaOs within single cells of ventricular myocardium is a mechanism of "abnormal automaticity." Note that the rates of depolarisation, cell edge displacement (contraction), or Ca$_i$ at the initiation of the spontaneous action potential and contraction in fig 8 differ from the electrically stimulated events: the former are preceded by slower depolarisation, Ca$_i$ increase, or cell edge displacement; this results from the occurrence of S-CaOs which initiate these events. The frequency of the S-CaOs in cell in fig 8B is indicative of a relatively high degree of Ca^{2+} loading (see fig 3). In such cells action potentials triggered by S-CaOs by external stimulation often hang up at the plateau potential. The specific mechanisms for this are numerous and beyond scope of this discussion. Acidosis which also is known to be associated with an enhanced likelihood of arrhythmias occurring, enhances the occurrence of multifocal S-CaOs.[19]

While the discussion thus far has focused on diastolic S-CaOs, the occurrence of S-CaOs during the plateau of a "long" action potential, due to the resultant inward current and deplorisation, would be expected to modulate the removal of I$_{Ca}$ inactivation. Thus S-CaOs may have a role in determining the likelihood that early afterdepolarisations will occur.[27 29 79-82]

Figure 8 *(A): Left panel, externally stimulated action potential (AP) (lower tracing) tracing* Ca_i *indexed as Indo-1 fluorescence (upper tracing) and contraction (middle tracing) in a single rat ventricular myocyte. Middle panel – spontaneous* Ca^{2+} *oscillation (S-CaOs) triggers an AP and contraction. Right panel – tracings in left and middle panels are superimposed. Note that the spontaneous AP is preceded by a "phase 4 like," spontaneous slow depolarisation that occurs in phase with the spontaneous diastolic rise in* Ca_i *due to spontaneous sarcoplasmic reticulum* Ca^{2+} *release. From Talo* et al.[79] *(B): Unifocal and multifocal S-CaOs and membrane potential oscillations in a single rat cardiac myocyte. The multifocal S-CaOs (indicated by an arrow) depolarises the membrane sufficiently to trigger a sponaneous AP that hangs up in the plateau phase. This is shown in an expanded time scale in panel C. From Talo* et al.[79] ▶

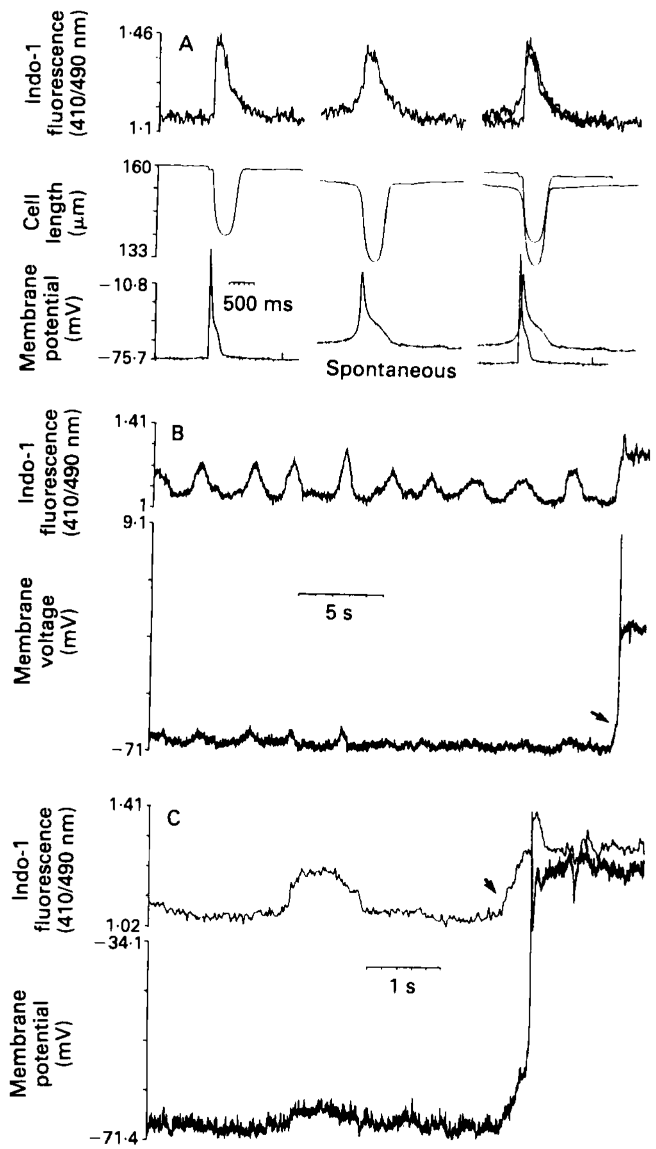

Figure 9 *(A): Left tracings: simultaneously recorded* Ca_i *transient (top), twitch contractions (middle) and action potential (AP) (bottom) from a nine beat stimulus train at 0.5 Hz in a rat ventricular myocyte. Right tracings: superimposed* Ca_i *transients (top), twitch contractions (middle), and AP (bottom) from beats 1 and 9 of the stimulus train shown at left. (B): Left tracings: the effect of ryanodine (0·1 μM) on the stimulation dependence of the* Ca_i *transient and AP,* Ca_i *transients (top), and AP (bottom) in response to 0·5 Hz stimulation after a 2 min rest from the same cell shown in panel A after 10 min exposure to 0.1 μM ryanodine. (B): Right tracings: superimposed* Ca_i *transients (top) and AP (bottom) from beats 1 and 9 of left panel. From duBell* et al.[83]
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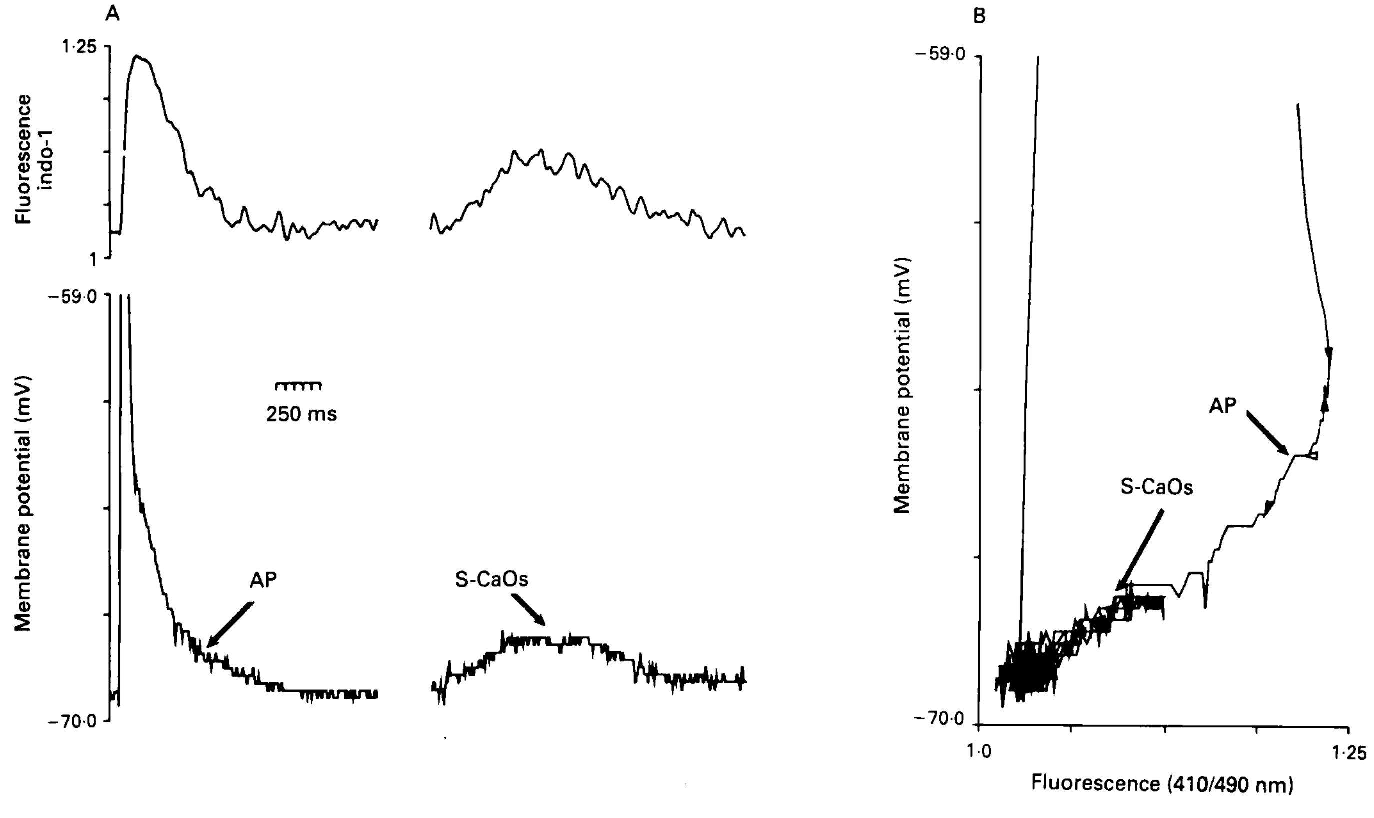


Diastolic S-CaOs modulate excitation-contraction coupling in cardiac cells

Action potential — The same mechanisms that govern the Ca^{2+} dependent modulation of membrane potential during S-CaOs release also cause Ca^{2+} dependent modulation of the membrane potential during the action potential of the normal heart beat[83–87] (fig 9). In the left tracings of fig 9A, note that the action potential of this rat cell narrows as the Ca$_i$ transient decreases in amplitude with successive beats following a period of rest. The right tracings in panel A show the first and ninth beat on an expanded scale. Figure 9B, right tracings, shows that in the absence of a Ca$_i$ transient (due to prior ryanodine induced depletion of the sarcoplasmic reticulum Ca^{2+} store) the action potential has a briefer duration than when a Ca$_i$ transient is present and no beat dependence of the action potential duration occurs. As expected from Ca$_i$ modulation of the membrane potential, the relationships between depolarisation and Ca$_i$ resulting from diastolic S-CaOs and the relationships occurring during a normal heart beat as the resting membrane potential is approached during action potential repolarisation are superimposable (fig 10). It is noteworthy that in other

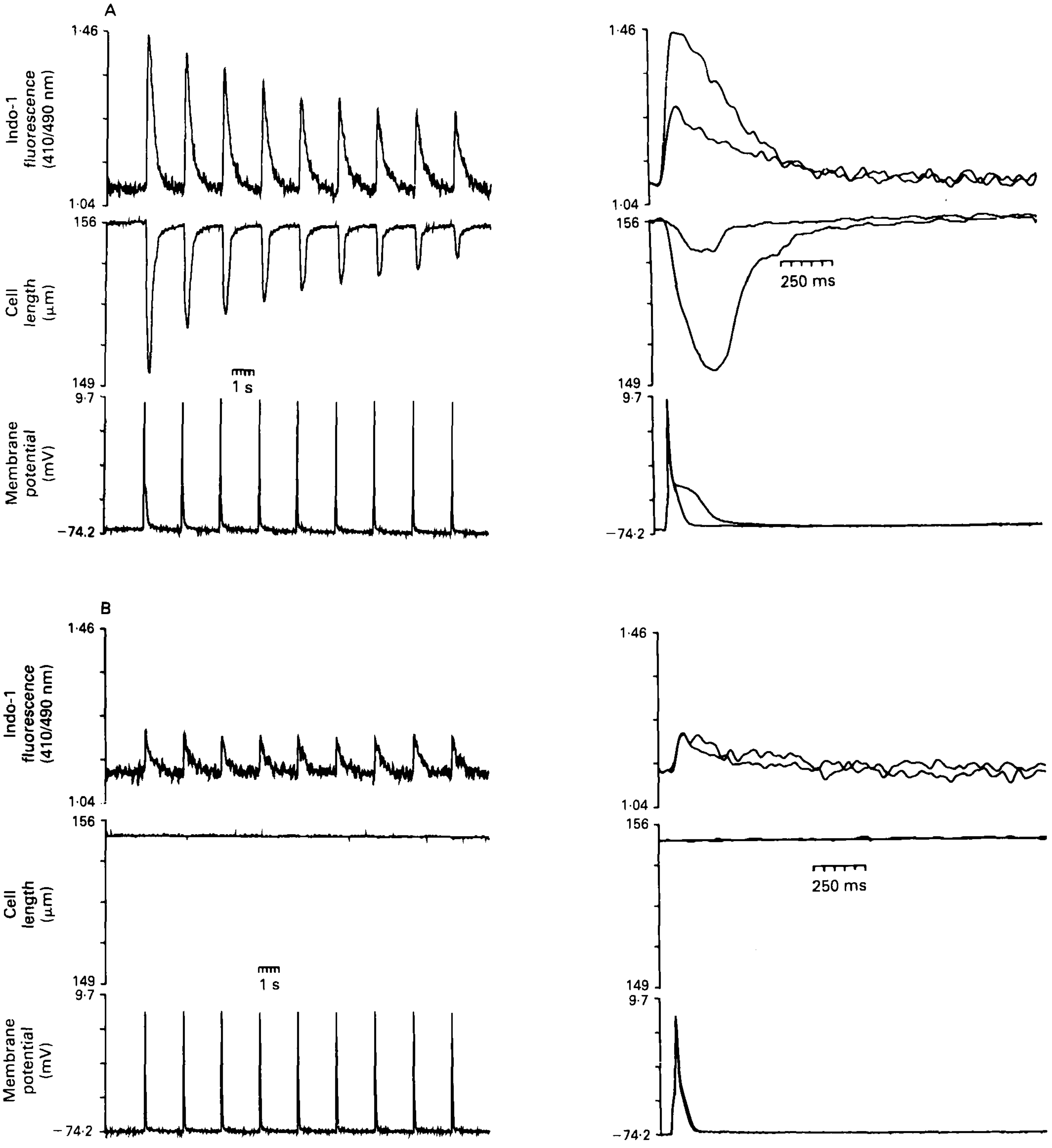


Figure 10 *(A): Simultaneously recorded Ca^{2+} transients (top) and membrane potential (bottom) from a rat cell in which diastolic spontaneous Ca^{2+} oscillations (S-CaOs) occurred just prior to a stimulated action potential. (B): High gain plot of the phase-plane diagrams of the stimulated beat and the S-CaOs shows common trajectory between the transient and the transmembrane AP and the spontaneous membrane potential oscillation. From duBell* et al.[83]

species, eg, guinea pig, abolition of the Ca^{2+} transient by ryanodine prolongs the action potential.[86] The species difference may be due to the presence of Ca^{2+} dependent K channels in the guinea pig (eg, the delayed rectifier channel) that are not present in rat cells. An additional explanation for the species difference of the Ca_i effect on action potential duration may relate to the level of the plateau potential, and thus to directional fluxes of Ca^{2+} and Na^+ via the Na-Ca exchanger.

During contraction alternans caused by S-CaOs in diastole, eg, as in figs 4 and 6, the contractions that differ in amplitude differ in action potential duration (fig 11). Thus diastolic S-CaOs can produce not only contractile alternans, but also produce substantial "alternans" in action potential repolarisation time. In the intact myocardium the asynchronous occurrence of S-CaOs (see below) might be expected to "increase the dispersion of refractory periods" among cells comprising the tissue. The reduction in action potential repolarisation time by S-CaOs occurrence in the preceding diastole occurs via at least two mechanisms: (a) reduction in the amplitude of the Ca_i transient due to a reduction in the extent of sarcoplasmic reticulum Ca^{2+} release (fig 12A) due to partial sarcoplasmic reticulum Ca^{2+} depletion by the S-CaOs during the preceding diastole, and (b) reduction of the whole cell I_{Ca} (fig 12C) due to localised inactivation of Ca^{2+} channels by the localised increase in Ca_i induced by S-CaOs.[88-90]

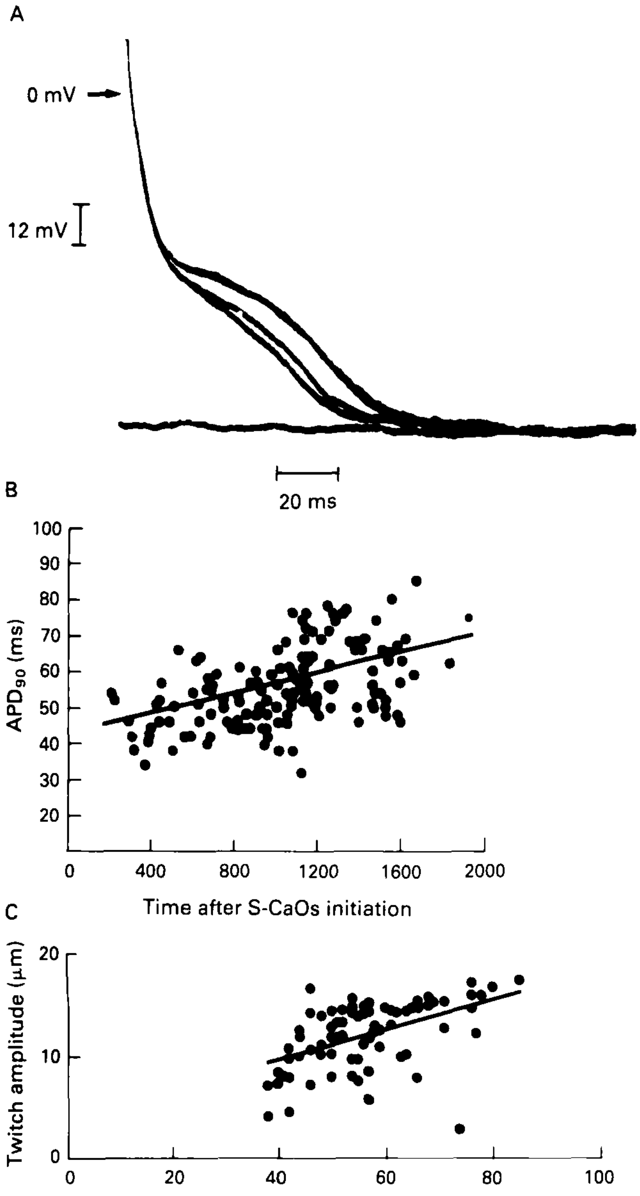


Figure 11 *The effect of spontaneous Ca^{2+} oscillations (S-CaOs) occurring in the interval between stimulated twitches (diastolic period) on the time to 90% repolarisation (APD_{90}) of the action potential (AP) that initiates the following twitch in a rat myocyte. (A): Example of AP that followed the initiation of S-CaOs at three different intervals (see text). (B): The time to APD_{90} versus the interval that had elapsed between the AP and the beginning of the S-CaOs in the preceding diastole ($r = 0.47$, $p<0.001$). (C): The effect of diastolic S-CaOs on twitch amplitude initiated by the subsequent AP is correlated with the APD_{90} of the transmural action potential ($r = 0.50$, $p<0.001$). From Capogrossi* et al.[45]

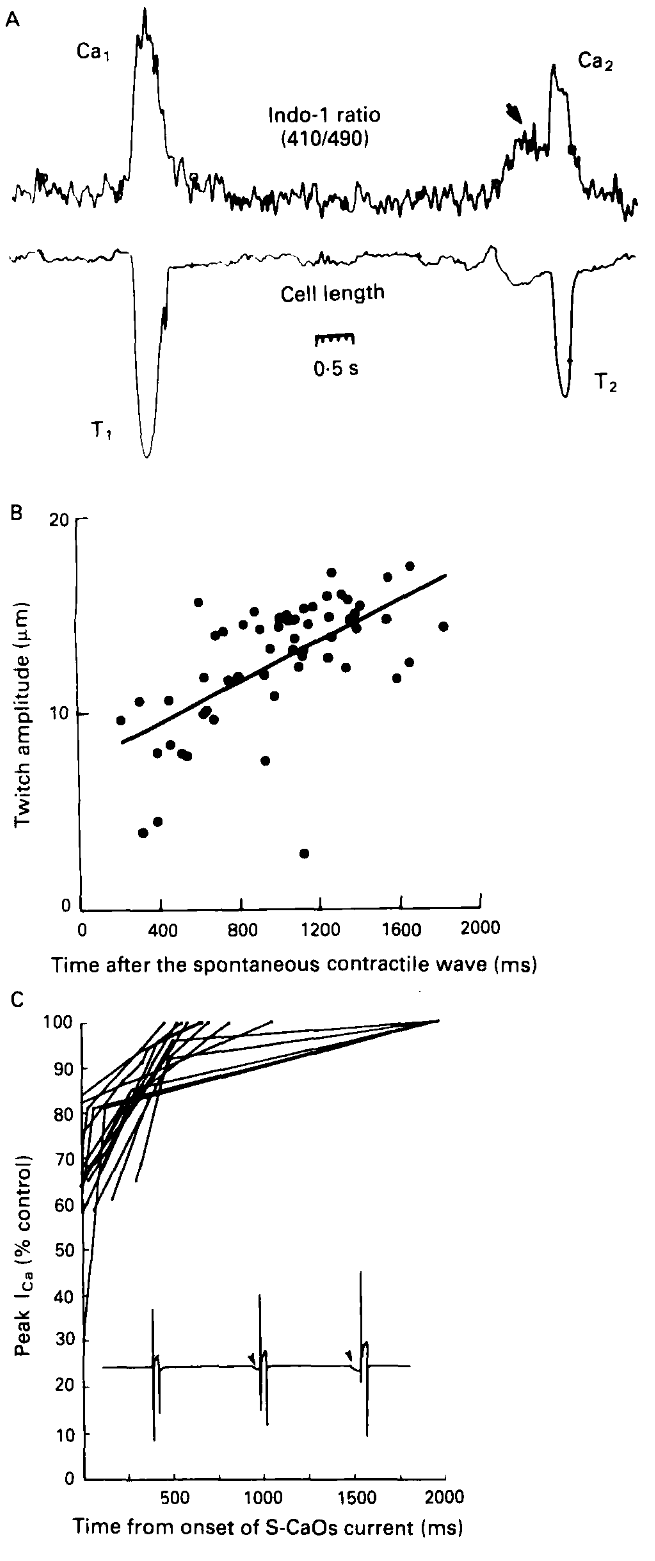
Twitch amplitude — Based upon the foregoing discussion, the reduction in the Ca_i transient elicited by an action potential following a diastole in which S-CaOs occurs can thus be attributed both to Ca^{2+} depletion from the sarcoplasmic reticulum and to a reduced trigger (I_{Ca}) for Ca^{2+} release. The reduced Ca_i transient leads to a reduction in twitch amplitude (figs 11C, 12A and 12B). It has been hypothesised that the occurrence of spontaneous Ca^{2+} release in the cardiac diastole might be a mechanism for the saturation of systolic contractile function upon exposure to intropic disturbances that *increase* cell Ca^{2+} loading.[46] To establish the validity of this hypothesis, the time when spontaneous sarcoplasmic reticulum Ca^{2+} release first occurs and its relationship to twitch saturation was established (fig 13). In individual myocytes, regardless of whether twitch amplitude is enhanced by an increase in bathing $[Ca^{2+}]$ (fig 13A), by addition of isoprenaline to the bathing fluid, or by permitting a period of rest following stimulation (which in rat myocytes leads to sarcoplasmic reticulum Ca^{2+} loading), *saturation* of the twitch amplitude elicited by electrical excitation in a given myocyte occurs simultaneously with the onset of spontaneous Ca^{2+} release from the sarcoplasmic reticulum (fig 13B).

S-CaOs within intact myocardial tissue

Detection of S-CaOs in cardiac tissue

When intact isolated muscles are examined at a magnification of greater than 30 times under incoherent illumination shortly after mounting in a bathing $[Ca^{2+}]$ of 1 mM, a continuous chaotic "squirming" motion is observed which gradually diminishes in amplitude with time, becoming quite subtle after several hours.[10,11] This corresponds to a low frequency S-CaOs observed in unstimulated healthy single rat cells in the absence of Ca^{2+} overload (fig 3). When the muscle is Ca^{2+} loaded by replacing Na^+ in the medium with Li^{3+}, the squirming motions become larger, somewhat faster, and possibly more disordered. The overall impression then is of a "washing machine" with prominent periodic oscillation of small domains of the muscle, completely asynchronous from one region to another even within the 200 µm length of the microscope field.[11] Thus in intact cardiac muscle contractile waves driven by S-CaOs occur asynchronously *among* cells.[10,11]

Without the use of microscopy, the subtle motion caused by contractile waves due to S-CaOs release in isolated unstimulated intact myocardial tissue can be measured non-invasively by various methods. One strategy employs a photoresistor to sense light absorption changes due to subtle motion generated by the contractile sequelae of S-CaOs within the tissue.[12] Fourier analyses of the raw photoresistor



◀ **Figure 12** *(A): The appearance of spontaneous Ca^{2+} release in the diastolic interval between stimulated twitches has a negative effect on the ensuing stimulated twitches: two twitches (T_1 and T_2) in a single rat ventricular myocyte loaded with Indo-1 AM, bathed in [Ca^{2+}] of 3 mM, and stimulated at 0.2 Hz at 23°C. The upper tracing shows Indo-1 fluorescence and the lower tracing depicts cell length, both measured as in fig 2. The appearance of spontaneous sarcoplasmic reticulum Ca^{2+} release in the diastolic interval (arrow) leads to a diminution in the ensuing Ca_i transient and decrease in the amplitude of the following twitch (T_2) (from Lakatta* et al.[50]*) (B): The time dependence of the effect of diastolic spontaneous Ca^{2+} oscillation (S-CaOs) on the twitch amplitude following the ensuing action potential in a rat myocyte (from Capogrossi* et al.[45]*) (C): The effect of S-CaOs, manifest as the occurrence of the spontaneous inward current it causes (arrows) to modulate the I_{Ca} amplitude during a subsequent externally driven voltage clamp step. The figure shows that S-CaOs occurrence can decrease the amplitude of I_{Ca} and that the magnitude of this varies with the interval between S-CaOs occurrence and of I_{Ca} by activation by voltage clamp step. From Walker* et al.[88]

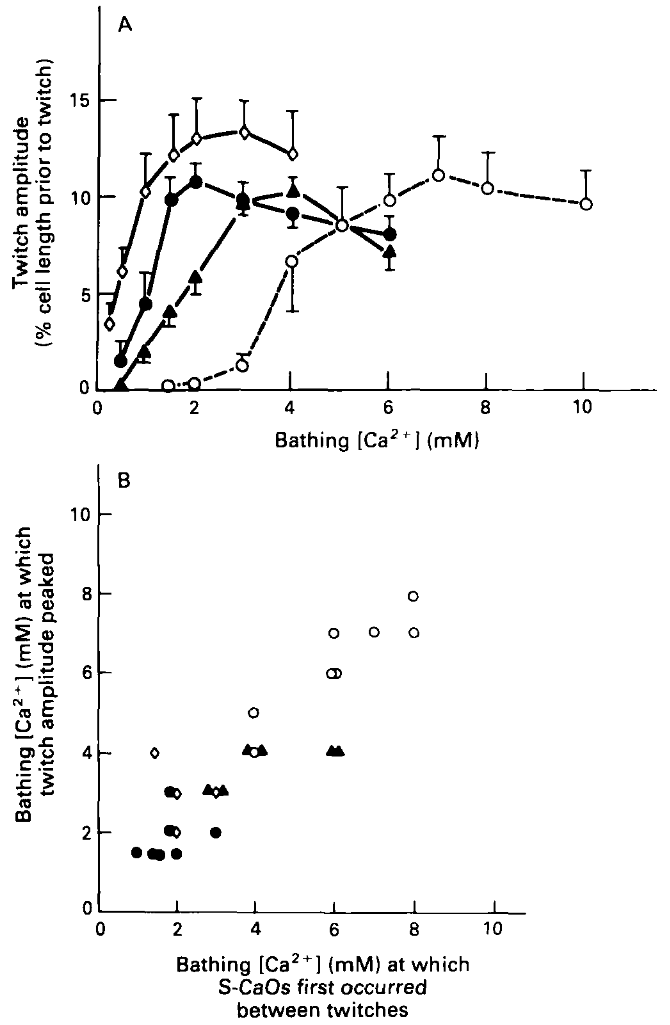


Figure 13 *(A): Twitch amplitude at varying bathing [Ca^{2+}] in rat myocytes continuously stimulated at 0.2 Hz (▲), at 1 Hz (●; n = 5), in the presence of isoprenaline at 1 Hz (◇; n = 5), or following a brief exposure to 1 μM ryanodine (○; n = 5). The maximum twitch amplitude is similar in the four groups, although it occurs at varying bathing [Ca^{2+}]. (B): A unique relationship between bathing [Ca^{2+}] at which the twitch amplitude peaks and spontaneous Ca^{2+} oscillation (S-CaOs), manifest as spontaneous contractile waves, first appeared for all cells studied in four protocols for all myocytes from panel A. Regardless of the drug or stimulation frequency, the bathing [Ca^{2+}] at which the twitch saturated varied linearly with the bathing [Ca^{2+}] at which S-CaOs first became manifest: $y = 0.70 + 8.83x$; $r = 0.90$, $p = <0.001$, $n = 25$. From Capogrossi* et al.[46]

output signal (fig 15B) indicates that the contractile motion in intact bulk muscle and contractile waves in single myocytes studied under approximately similar conditions show similar periodicity and an approximately similar propagation velocity, ie, 50-150 $\mu m \cdot s^{-1}$.[12] More rapid apparent cell to cell propagation velocities of S-CaOs-induced contractions observed in some studies in intact cardiac muscle under extreme conditions of Ca^{2+} overload[91 92] may relate to electrical propagation due to the effect of partially synchronised S-CaOs, allowing the membrane to be sufficiently depolarised to elicit slow action potentials. Similar changes in frequency, amplitude, and wave propagation velocities also occur in both isolated myocytes and intact muscle in response to various experimental disturbances that vary cell Ca^{2+} loading.[12] During marked Ca^{2+} overload S-CaOs can also be detected in intact myocardial tissue by noise analyses of Ca^{2+} indicator signals, eg, aequorin luminescence,[13, 93] or of apparent tonic force.[12] When marked Ca^{2+} overload occurs S-CaOs can also often be detected by the presence of gross oscillations in the force[94] or aequorin signal records.[5, 93] Because, as noted, sarcolemmal ionic conductances are modulated by Ca_i, the oscillatory currents or resultant membrane depolarisation caused by S-CaOs can also be detected as spontaneous voltage or current fluctuations within the tissue.[25, 95–103]

Laser spectroscopy has also been employed in intact preparations as a non-invasive tool for the detection of the mechanical sequelae of S-CaOs and changes in their magnitude: the inhomogeous contractile motion caused by the asynchronous oscillations phase modulates a laser beam as it passes through the tissue, producing intensity fluctuations in the scattered light (SLIF). Of all these methods currently employed to detect S-CaOs or their manifestations in intact tissue, laser spectroscopy is the most sensitive; it can detect the presence of subtle oscillations in otherwise apparently quiescent tissue.[6, 10–12, 14, 39, 73, 95] Unstimulated rat and canine tissues exhibit S-CaOs as detected by SLIF, even when the bathing $[Ca^{2+}]$ is as low as 1-2 mM; in unstimulated rabbit muscle the bathing $[Ca^{2+}]$ must be considerably higher (10 mM) for S-CaOs to occur;[11] drugs or other manoeuvres that increase Ca_i in the unstimulated state, eg, Na-K pump inhibition, cause de novo SLIF in unstimulated rabbit muscle and augment the SLIF magnitude in unstimulated rat muscles.[6, 1, 73] Frog cardiac tissues (which have a sparse sarcoplasmic reticulum) do not exhibit S-CaOs or SLIF even under high Ca^{2+} loading conditions.[11] SLIF are abolished by high caffeine concentrations or by ryanodine.[10, 11, 73] These species, tissue, pharmacological, and bathing $[Ca^{2+}]$ profiles for S-CaOs detected as SLIF bear a striking similarity to that for Ca^{2+}-induced Ca^{2+} release from sarcoplasmic reticulum in mechanically skinned cardiac cells.[104] Additionally, the $[Ca^{2+}]$ dependence of SLIF in intact isolated cardiac tissue within a species is the same as that for the periodicity of the contractile waves measured directly in single myocytes.[12, 39] However, the SLIF "frequency," as measured from the half decay time of the autocorrelation function of the scattered light in published studies, is the *product* of the amplitude and frequency of the oscillatory motion caused by S-CaOs within the tissue. Thus SLIF "frequency," as discussed in subsequent sections, is greater than the actual S-CaOs frequency that occurs within individual cells, and is also not identical to the frequency of S-CaOs determined from noise analyses of tension or of Ca_i in intact myocardial tissue.[12]

Predictions of S-CaOs functional sequelae in intact muscle from observations made in individual isolated cardiac cells

Interaction of S-CaOs and electrically stimulated sarcoplasmic reticulum Ca^{2+} release — Studies in single rat cardiac cells show that S-CaOs occurs in the absence of stimulation and that following stimulation their occurrence is suppressed or exaggerated depending on the Ca^{2+} loading of the cell at rest (figs 2 and 3). Figure 14 shows that rat cardiac muscle bathed in physiological $[Ca^{2+}]$ (1.5 mM) and bathing $[K^+]$ (4.2 mM) exhibits a steady level of SLIF (dotted line in fig 14A) in the unstimulated or resting state; resting SLIF appear 5 s after termination of stimulation and reach their steady level 15-20 s after prior stimulation at 60 min.

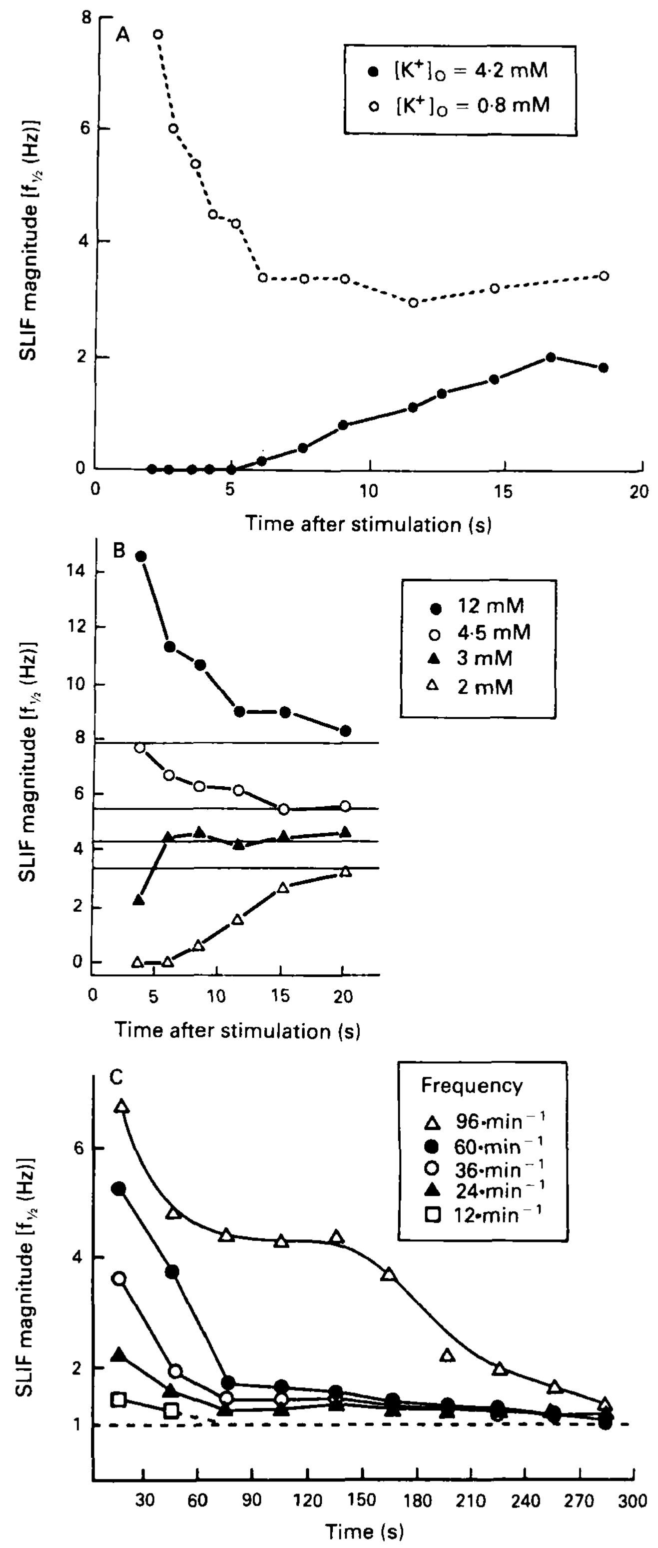


Figure 14 *(A) A representative rat papillary muscle showing the time course of scattered light intensity fluctuation (SLIF) restitution following regular electrical stimulation at 60·min^{-1} in a rat papillary muscle. Bathing $[Ca^{2+}]$ 1.5 mM; bathing K^+ either 4.2 mM or 0.8 mM (to inhibit the Na-K pump and Ca^{2+} load the cell). Data points are placed at the midpoint of the actual time windows of SLIF measurements. (B) Plot of effects of bathing $[Ca^{2+}]$ on the time course of SLIF restitution following stimulation at 60·min^{-1} in a rat muscle. The horizonatal lines indicate the value of SLIF prior to stimulation in each bathing $[Ca^{2+}]$. From Kort and Lakatta.[73] (C) A typical example of the effect of the rate of prior stimulation at different rates on the decay in SLIF magnitude ($f_{1/2}$) that follows termination of that stimulation in a cat papillary muscle. Bathing $[Ca^{2+}]$ was 0.4 mM. From Lakatta and Lappe.[14]*

However, for a 5 s interval following electrical stimulation, SLIF are transiently abolished (this interval corresponds to the delay interval observed in single cells and discussed above). Because the 5 s interval before the onset of SLIF recovery is longer than the interstimulus interval during prior stimulation of this muscle, S-CaOs, manifest as SLIF, did not occur (by inference) during the diastolic period during prior stimulation. In other words, S-CaOs was "overdriven" by electrically stimulated sarcoplasmic reticulum Ca^{2+} release.

Figure 14A also shows that in reduced bathing [K^+] (0.8 mM), resting SLIF are increased (due to Ca^{2+} loading of the cell by Na-K pump inhibition). Stimulation of the muscle during this Na-K pump inhibition transiently enhances, rather than suppresses, SLIF in the early poststimulation period. Specifically, SLIF are now not only present during the same (delay) interval following the last stimulation in which they were transiently abolished in normal bathing [K^+], but they are also augmented by 2·5-fold over the already increased resting level in the reduced bathing [K^+]. This augmentation of SLIF during stimulation in reduced bathing [K^+] is due to the relatively greater net Ca^{2+} loading of cell than in a normal bathing [K^+]. The greater Ca^{2+} gain is likely to result from a decrease in Ca^{2+} efflux via the Na-Ca exchanger during stimulation, which is due to the collapse of the Na^+ electromechanical gradient caused by the disabled Na-K pump when bathing [K^+] is reduced.

Figure 14B shows the effect of stimulation on SLIF restitution in a representative muscle bathed in varying [Ca^{2+}]. Note the increase in resting SLIF with increasing [Ca^{2+}]. Differences in the pattern and time course of SLIF recovery following stimulation in the different bathing [Ca^{2+}] are also clearly defined. In the earliest measurement window, SLIF are abolished in bathing [Ca^{2+}] of 2.0 mM but increase with progressive increases in [Ca^{2+}]; in the two highest bathing [Ca^{2+}] studied, SLIF are greater than at rest, that is, a transient overshoot in SLIF occurred. At longer times following stimulation, SLIF proceed to increase or decrease so as to equilibrate back to the resting level characteristic of that bathing [Ca^{2+}], and thus to a given resting cell [Ca^{2+}] load. The interactive effects of electrical stimulation and bathing [Ca^{2+}] on SLIF in muscle as shown in fig 16 are thus predicted from the observations made in single rat myocytes. (cf figs. 3A, 4, and 6B). In non-rat ventricular muscle, electrical stimulation at varying rates increases cell Ca^{2+} loading. Figure 14C shows that the SLIF measured shortly after stimulation increases with the prior stimulation rate; as

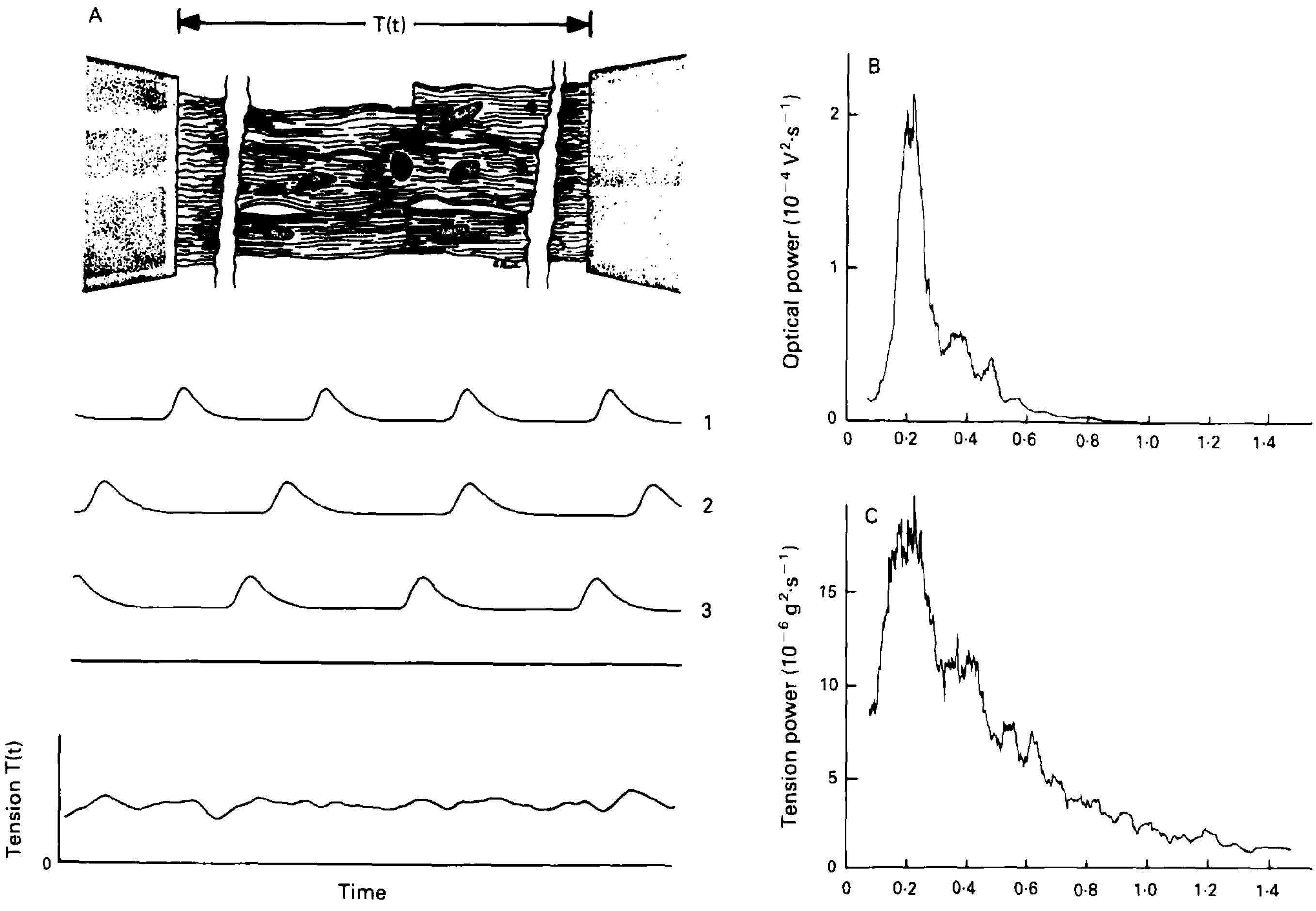


Figure 15 *(A) A schematic of the model of independent spontaneous Ca^{2+} oscillations (S-CaOs) within cells or intact cardiac tissue at rest, in the absence of stimulation. The sum of independent periodic asynchronous CaOs in cells 1-3 summates to cause a fluctuating tension T(t), measured at the ends of the preparation. The curve shown as T(t) in the lower part of the panel was computer-stimulated by adding up 100 curves similar to curves in 1-3 but with arbitrary phases and periods normally distributed about an average value. Note that this average Ca^{2+} dependent "tone" is non-zero and thus contributes to the overall diastolic force. An increase in CaOs frequency due to enchanced cell Ca^{2+} loading augments this Ca^{2+} dependent tonus via partial synchronisation of the individual oscillations. From Stern* et al.[10] *The power spectrum of optical fluctuations caused by contractile wave displacement (panel B) and that of the simultaneously measured tension fluctuations (panel C) in an unstimulated rat muscle bathed in [Ca^{2+}] 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. From Kort* et al.[12]

the time following stimulation increases, SLIF decays to its resting level.

Partial synchronisation of S-CaOs: effects on diastolic tone — As noted earlier, the occurrence of a contractile wave generated by S-CaOs within a cardiac cell causes a reduction in cell length (figs 2 and 5). Recall that in intact unstimulated muscle the occurrence of these contractile waves is asynchronous among cells.[10] It has been hypothesised[10,46] that the summation of asynchronously occurring wave like contractions within the bulk muscle, damped by the compliant interwave regions (and by artifactual compliance at the muscle ends), is the cause of what appears to be a steady Ca^{2+} dependent portion of resting tone within the bulk preparation (fig 15A). Direct evidence that the overall 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 fig 15B, in which the power spectrum from both the wave motion within the tissue detected by a photoresistor (panel B) and from the simultaneous force transducer output (panel C) 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 show oscillations with the same frequency,[93] it can be inferred that S-CaOs can cause a Ca^{2+} dependent component of "resting" tension in unstimulated cardiac muscle. A Ca^{2+} dependent component of resting force in intact muscles[14,105] varies directly with SLIF when the bathing $[Ca^{2+}]$ is increased (fig 16). Note that the magnitude of this Ca^{2+} dependent resting tonus is small relative to the passive resting tension induced by stretching isolated cardiac ventricular musle to a length at which maximum twitch force is observed.

The effect of S-CaOs on diastolic tonus in intact cardiac muscle depends on the distribution of frequencies and phases of spontaneous Ca^{2+} release cycles throughout the muscle at any instant of stimulation.[10,13,46] This distribution depends, in turn, on the interval since the last stimulus, ie, the summation of "delay intervals" of individual cardiac cells (figs 2-4) which determine the "quiet interval" during which SLIF are absent following stimulation in intact muscle (fig 14A and B). Recall that myocardial cells stimulated during high Ca^{2+} loading states, eg, in the presence of glycosides, enhanced bathing $[Ca^{2+}]$, or catecholamines (figs 3, 4, and 6), show not only a decrease in the delay interval for spontaneous Ca^{2+} release to occur following the prior twitch, leading to the appearance of S-CaOs in the diastolic period, but also an enhanced probability of S-CaOs occurring in more than a single focus (figs 3, 4, and 6). Both Ca^{2+} dependent phenomena, ie, an increase in the probability of S-CaOs occurrence (due to an increase in their frequency) and an increase in the probability for S-CaOs to be multifocal or partially synchronised, have a summation effect on the resultant S-CaOs dependent diastolic contractile amplitude.[10,13] Periodic aftercontractions occur during high Ca^{2+} loading, especially in the rat and the dog, species that are prone to S-CaOs at rest in physiological $[Ca^{2+}]$.[97,105-108] The high SLIF magnitude following stimulation in the presence of Na-K blockade or high bathing $[Ca^{2+}]$ in fig 14 is accompanied by aftercontractions (see fig 17D). With increased time following the cessation of stimulation, SLIF decays and the aftercontractions become damped and cease. The decay of SLIF magnitude with time reflects the decay in the extent of synchronisation of S-CaOs among myocardial cells due to a reduction in the frequency of S-CaOs occurrence within individual cells. The reduction in the extent of synchronisation is attributed to a reduction in the cell Ca^{2+} load which is facilitated by enchanced Ca^{2+} extrusion via the Na-Ca exchanger at the resting potential, ie, in the absence of regular cyclic depolarisation during action potentials. Partial synchronisation of S-CaOs following a prior action potential mediated sarcoplasmic reticulum

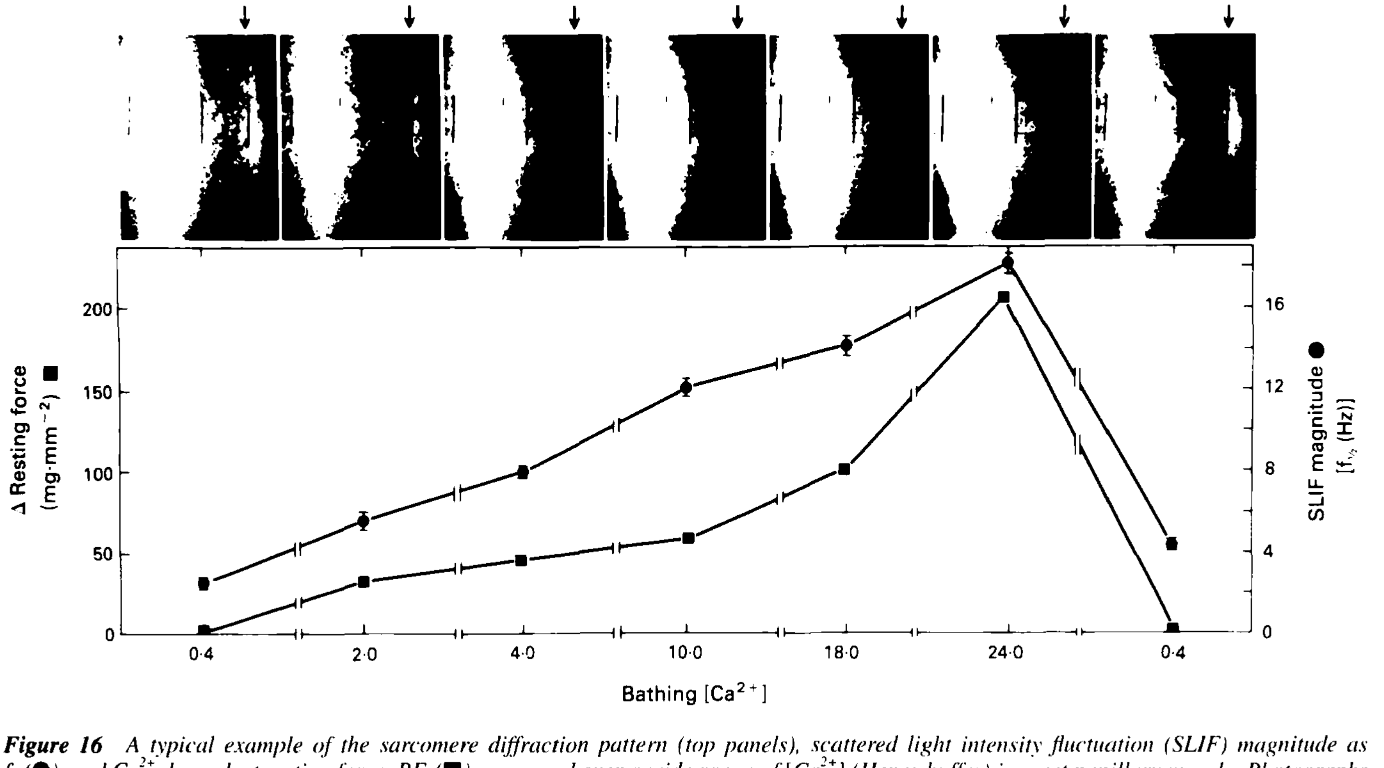


Figure 16 *A typical example of the sarcomere diffraction pattern (top panels), scattered light intensity fluctuation (SLIF) magnitude as $f_{½}$ (●), and Ca^{2+} dependent resting force, RF, (■), measured over a wide range of $[Ca^{2+}]$ (Hepes buffer) in a rat papillary muscle. Photographs of the diffraction pattern were made in the same area of the muscle at the same camera setting in each $[Ca^{2+}]$. Arrow indicates the first order of diffraction. RF is the difference between RF in a given $[Ca^{2+}]$ and that in a reference control in $[Ca^{2+}]$ of 0.4 mM. Cross sectional area was 0.24 mm.^{2} From Lakatta and Lappe.*[14]

release during states of high cell Ca^{2+} loading in a simple quantitative model (fig 17A) predicts the development of hyperrelaxation, aftercontractions, and oscillatory restitution of diastolic tension in states of high Ca^{2+} loading.[10 46] The model in fig 17A interprets the aftercontractions as a "storm" of asynchronous spontaneous Ca^{2+} release events, clustered in time because of the synchronising influence of the previous stimulus. If this interpretation is correct, then the SLIF magnitude, which is a manifestation of the microscopic motion owing to these release events, should vary in phase with the aftercontractions. Panels B-D in fig 17 show that this is indeed the case. Thus hyperrelaxation and oscillatory recovery of diastolic tension phenomena, which are amply documented to occur in intact muscle[97 108-110] but are not observed in single cells, are best understood as a statistical effect of the partially synchronised occurrence throughout the muscle of S-CaOs in individual cells.[10 46]

Diastolic S-CaOs effects on systolic function — It has been long recognised that optimal Ca^{2+} loading of intact muscle first causes an increase in twitch amplitude; further increases in cell Ca^{2+} lead to a decline in twitch amplitude and an increase in resting tension. This "supraoptimal" Ca^{2+} loading

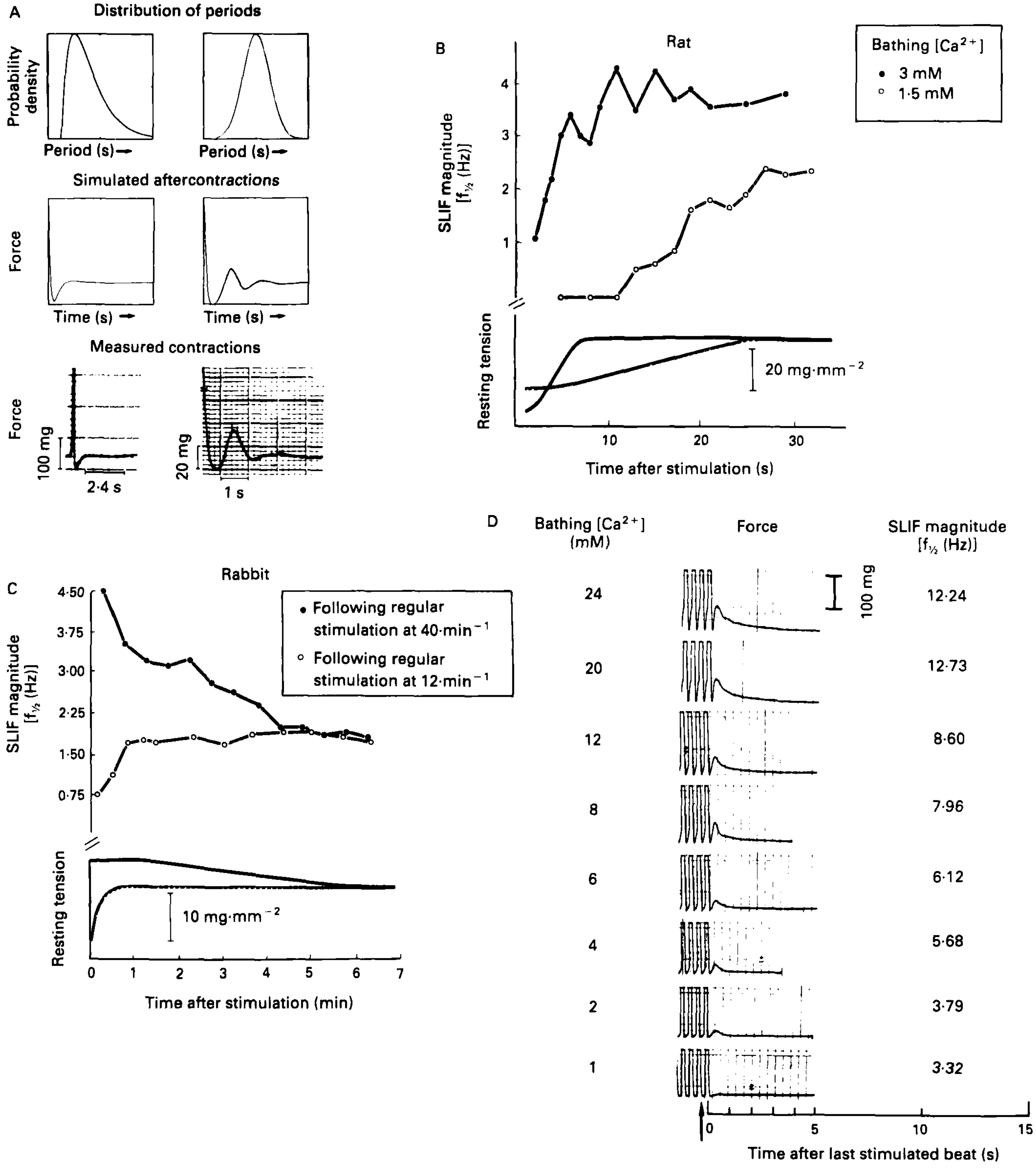


Figure 17 The legend to figure 17 is on the opposite page

is associated with increases in the magnitude of S-CaOs as reflected by direct measurements of Ca_i,[93,111,112] afterdepolarisations,[113] or by changes in SLIF magnitude.[5,14] Figure 18A depicts this pattern of events during progressive Ca^{2+} loading of a rat muscle by ouabain. A concept that follows from observations of S-CaOs in single cardiac cells, as described above, is that in multicellular tissues in which Ca^{2+} release occurs asynchronously among cells, contractility never reaches the highest value of which the tissue is capable because some myocytes within the tissue begin to exhibit diastolic S-CaOs before others have achieved their maximum inotropic state (fig 18B). The average twitch amplitude in several single myocytes (the ensemble average stimulates a tissue) measured across a range of bathing [Ca^{2+}] is depicted in fig 18B. The figure shows that the average twitch amplitude of an ensemble of cells can continue to increase, even though some cells begin to exhibit spontaneous Ca^{2+} release. Thus it may be expected that in intact muscle, excessive Ca^{2+} loading must be defined not by the presence of spontaneous Ca^{2+} release in some cells but as the state when the average magnitude of spontaneous Ca^{2+} release is sufficient to limit the average systolic function among the cells, ie, to limit twitch amplitude. A mathematical model has been constructed in order to predict the effect of spontaneous Ca^{2+} release measured in individual cells on the systolic function of the intact muscle and to relate it to the behaviour of intact muscles.[46] In this model the "contractility" of the "muscle" is represented by the average releasable Ca^{2+} of an ensemble of cells, subject to spontaneous Ca^{2+} release. A schematic diagram of this concept is shown in fig 18C. The same simple synchronisation model that predicts the limitation of the steady state level of twitch force by S-CaOs also predicts a steady Ca^{2+} dependent tonus due to S-CaOs (figs 15A), oscillatory patterns in the recovery of resting tension (eg, as in fig 17), and an out of phase relationship in the oscillatory restitution of twitch and resting tension with rest following stimulation.[6,10,46,106,107]

Evidence linking S-CaOs to arrhythmias in intact cardiac tissue — As shown in figs 2 and 7-10, membrane potential of individual cardiac cells is modulated by Ca_i: a steady increase in Ca_i produces a steady depolarising influence; oscillatory increases in Ca_i produce oscillatory depolarisations. Aftercontractions in cardiac tissue are accompanied by diastolic afterdepolarisations.[16,17,95-98,101-103,107,108,110,113] It has long been inferred that S-CaOs cause diastolic afterdepolarisations.[95-97] In intact cardiac tissue these have been implicated in "triggered arrhythmias".[96,98,102,103,107] The model of the aftercontraction based upon synchronisation of S-CaOs discussed above implies that the afterpotential that accompanies the diastolic afterdepolarisations is generated by partial synchronisation of the depolarisations of individual cells caused by spontaneous Ca^2 release. A similar conclusion has been reached by analysis of electrical noise within cardiac Purkinje tissue.[114] The link between S-CaOs, delayed afterdepolarisations, and aftercontractions and arrhythmias in intact tissue has been further solidified in recent experiments.[115] After the addition of ouabain (1 μM), afterpotentials, aftercontractions, and spontaneous oscillations of the membrane potential and of resting tension amplitude of guinea pig muscle become significantly increased. The power spectra of spontaneous oscillations of the membrane potential and of resting tension under these conditions have similar resonance harmonics with the frequency of about 5 Hz. Three to five minutes after the addition of ryanodine (0.1-0.5 μM), which selectively abolishes S-CaOs, the afterpotentials, aftercontractions, and spontaneous oscillations of the membrane potential and resting tension are abolished, thus linking these oscillations with S-CaOs. In experiments performed in vivo, ouabain induced (75-115 $\mu g \cdot kg^{-1}$) ventricular arrhythmias could be also terminated with intravenous injection of ryanodine (15 $\mu g \cdot kg^{-1}$) and sinus rhythm was completely restored.[115]

Spontaneous action potentials in intact tissue have been referred to as a manifestation of abnormal automaticity.[99] As indicated earlier, synchronisation of S-CaOs in the absence of electrical stimulation in individual cardiac cells causes spontaneous action potentials. A role of synchronisation of S-CaOs in intact cardiac muscle has also been linked to spontaneous action potentials in unstimulated intact cardiac muscle.[115,116]

S-CaOs effects in the intact heart

Effects of Ca^{2+} overload

It has recently been shown that, with suitable precautions to minimise bulk motion, it was possible to detect SLIF from the epicardial surface of isolated intact hearts.[24] These

***Figure 17** (On previous page)* *(A) Simulated force transients generated by numerical integration of an infinite number of oscillations with intrinsic periods distributed with a probability density P(t). If f(t) describes the periodic force produced by one oscillator with unit period, then the predicted total force at time t after synchronisation by an action potential, ie, a twitch, will be F(f) = P(t)(t/T)dT. Syncronisation of oscillators with a skewed distribution (panel A, upper tracing) gives rise to a simulated force transient (twitch) with a prominent hyperrelaxation and a subtle non-periodic "aftercontraction" (middle tracing), resembling a measured transient (lower tracing) from a rat papillary muscle in bathing [Ca^{2+}] of 1 mM. A symmetrical (Gaussian) distribution of periods (panel B, upper tracing) gives rise to a periodic series of aftercontractions (middle tracing) resembling the measured aftercontractions (lower tracing) from a rat papillary muscle in bathing [Ca^{2+}] of 2.5 mM and caffeine concentration of 2.5 mM. From Stern* et al.[10] *(B) Scattered light intensity fluctuation (SLIF) and resting tension restitution after stimulation at 20·min^{-1} in a representative rat muscle (cross sectional area 0.21 mm_2) bathed in [Ca^{2+}] of 1.5 mM or 3.0 mM. The recovery of SLIF and resting tension is accelerated in the higher bathing [Ca^{2+}], but in both cases SLIF recovery occurs in phase with resting tension. The detection of the resting tension transient following a prior twitch in most muscles under this experimental condition requires recording at high sensitivity because the magnitude of the resting tension transient is relatively small. In this muscle the change in resting tension over the restitution interval was only about 20 mg, that is 2.5 orders of magnitude less than the steady state twitch. Normalistion of the two resting tension curves is necessary because the absolute resting tension decreases over the relatively long period required to implement this time gating protocol.[14,72] From Kort and Lakatta.[6] (C) The effect of different stimulation rates on SLIF and resting tension in a rabbit muscle (cross sectional area 0.30 mm^2) bathed in [Ca^{2+}] of 20 mM. The restitution of SLIF varies from an undershoot (after stimulation at the low rate) to an overshoot after stimulation at the higher rate. Except for the early period following stimulation at the higher rate, these undershoots and overshoots are mirrored in the restitutions of resting tension. From Kort and Lakatta.[6] (D) Transients in resting force (RF) (aftercontractions) and SLIF magnitude ($f_{1/2}$) following periods of regular stimulation in a typical cat muscle. Each panel represents an actual record of the force of high sensitivity of the force recording apparatus. Arrow indicates termination of regular stimulation at 60·min^{-1} in a given bathing [Ca^{2+}]. The number to right of each panel is SLIF magnitude measured over the 20 s period following the transient in RF. From Lakatta and Lappe.[14]*

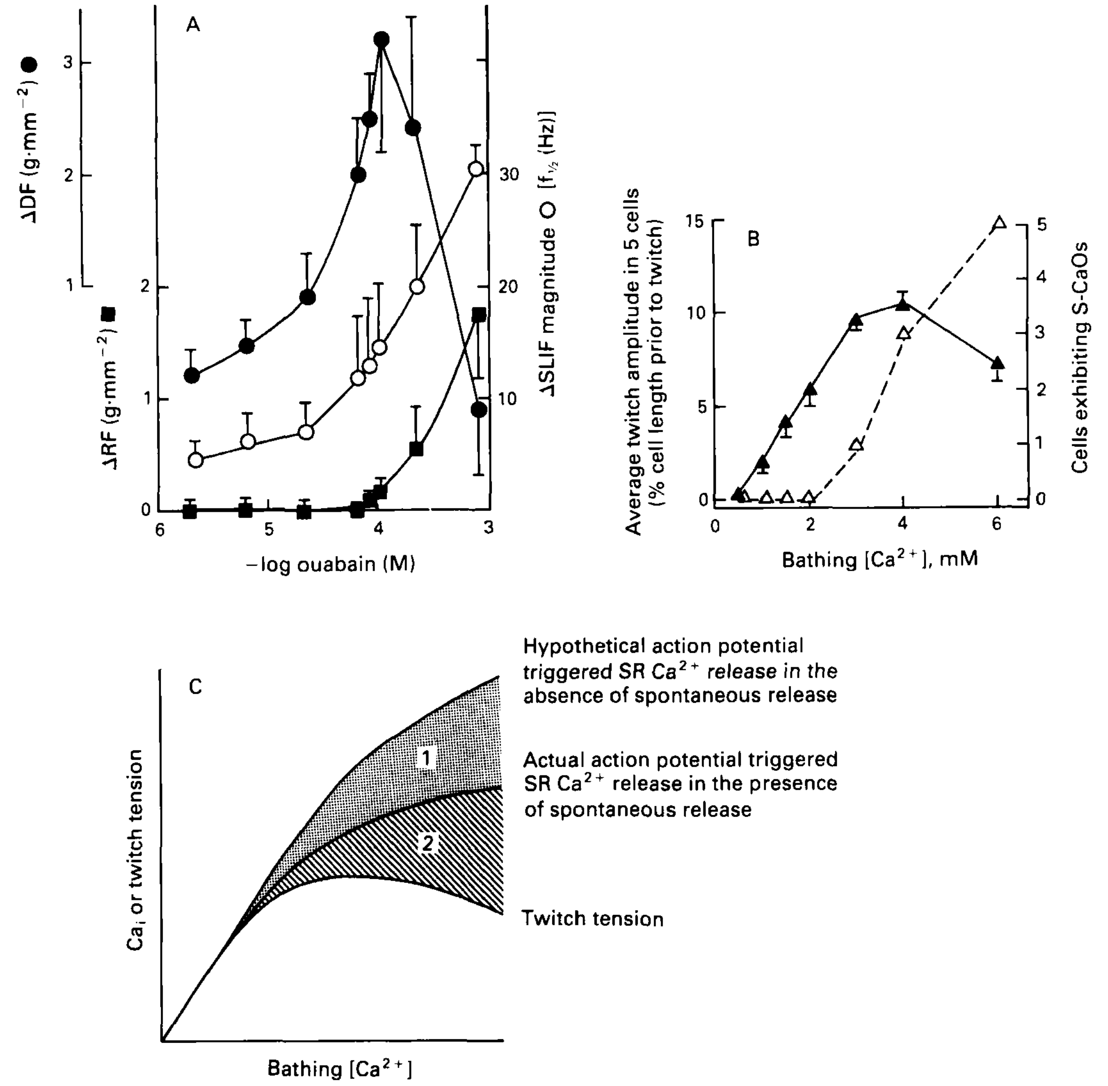


Figure 18 *(A) The effect of ouabain on scattered light intensity fluctuation (SLIF) magnitude ($f_{1/2}$), developed force (DF), and resting force (RF) in rat papillary muscles; $\Delta f_{1/2}$ (○), ΔRF (■), and ΔDF (●) represent the change from control level in 0.2 mM [Ca^{2+}]. The points represent the mean three muscles, bars = SEM. At control, DF was 0.67 (SEM 0.31) g·mm^{-2}, $f_{1/2}$ was 3.61(0.92) Hz, and RF was 712.0(118.0) mg·mm^{-2}. Cross sectional area was 0.40(0.10) mm.^{2} From Lakatta and Lappe.[14] (B) Average twitch amplitude of five individual rat myocytes in response to an increase in bathing [Ca^{2+}] during 1 Hz stimulation. The dotted line indicates how many of these cells exhibited spontaneous Ca^{2+} oscillations (S-CaOs) in each bathing [Ca^{2+}]. From Capogrossi* et al.[11] *(C) A schematic explanation for the effects of S-CaOs on twitch tension. The upper curve represents an estimation of the level of sarcoplasmic reticulum (SR) Ca^{2+} loading in the absence of S-CaOs, that is, in the absence of SLIF. This curve assumes that the K_m for SR Ca^{2+} pumping is sufficiently high and that only a mild plateauing occurs over the range of bathing [Ca^{2+}]. In the presence of S-CaOs (middle curve), the SR Ca^{2+} load is reduced. Area 1 is related to the SLIF magnitude and corresponds to the decrement in action potential (AP) triggered SR Ca^{2+} release due to the presence of diastolic S-CaOs, reflecting a functional SR Ca^{2+} saturation of some cells. (No inference as to whether the "actual SR Ca^{2+} loading" continues to increase or decrease at higher bathing [Ca^{2+}] is intended.) The lowest curve corresponds to the actual twitch tension in response to an AP. Area 2 corresponds to the difference between the spatially averaged SR Ca^{2+} release by an AP and the resultant twitch tension. The difference is due to the inhomogeneity of tissue compliance associated with the regions of spontaneous Ca^{2+} release. Note that both areas 1 and 2 increase with increasing bathing [Ca^{2+}] (and thus with increasing cell and SR Ca^{2+} loading). From Kort and Lakatta.[6]*

fluctuations occur reliably in the presence of physiological levels of perfusate Ca^{2+} in the rat (fig 19B). SLIF from whole hearts display the species dependence and pharmacological signature characteristic of intracellular S-CaOs seen in cells and muscles.[11] Figure 19B shows the effect of transient depolarisation of the heart by a pulse of KCl on SLIF and resting pressure in a representative heart. The transient in resting pressure is accompanied by an in phase transient increase in SLIF magnitude.

An example of the manifestations of systolic and diastolic dysfunction and ventricular tachycardia produced by Ca^{2+} overload and accompanied by S-CaOs in an *intact* heart is illustrated in fig 20. When the perfusate [Ca^{2+}] is increased (to Ca^{2+} load the myocytes), systolic pressure first increases dramatically, while small increases in resting pressure and SLIF occur. With further Ca^{2+} loading, diastolic pressure and SLIF monotonically increase, but systolic pressure plateaus and then decreases from its optimum level. Still further increases in Ca^{2+} loading (fig 20, lower tracings) lead to visible oscillations in diastolic pressure and ventricular tachycardia. (While this functional profile might be considered to resemble gross heart failure, a biopsy would prove normal!) This pattern of Ca^{2+} overload on systolic and diastolic function and on average tissue S-CaOs magnitude

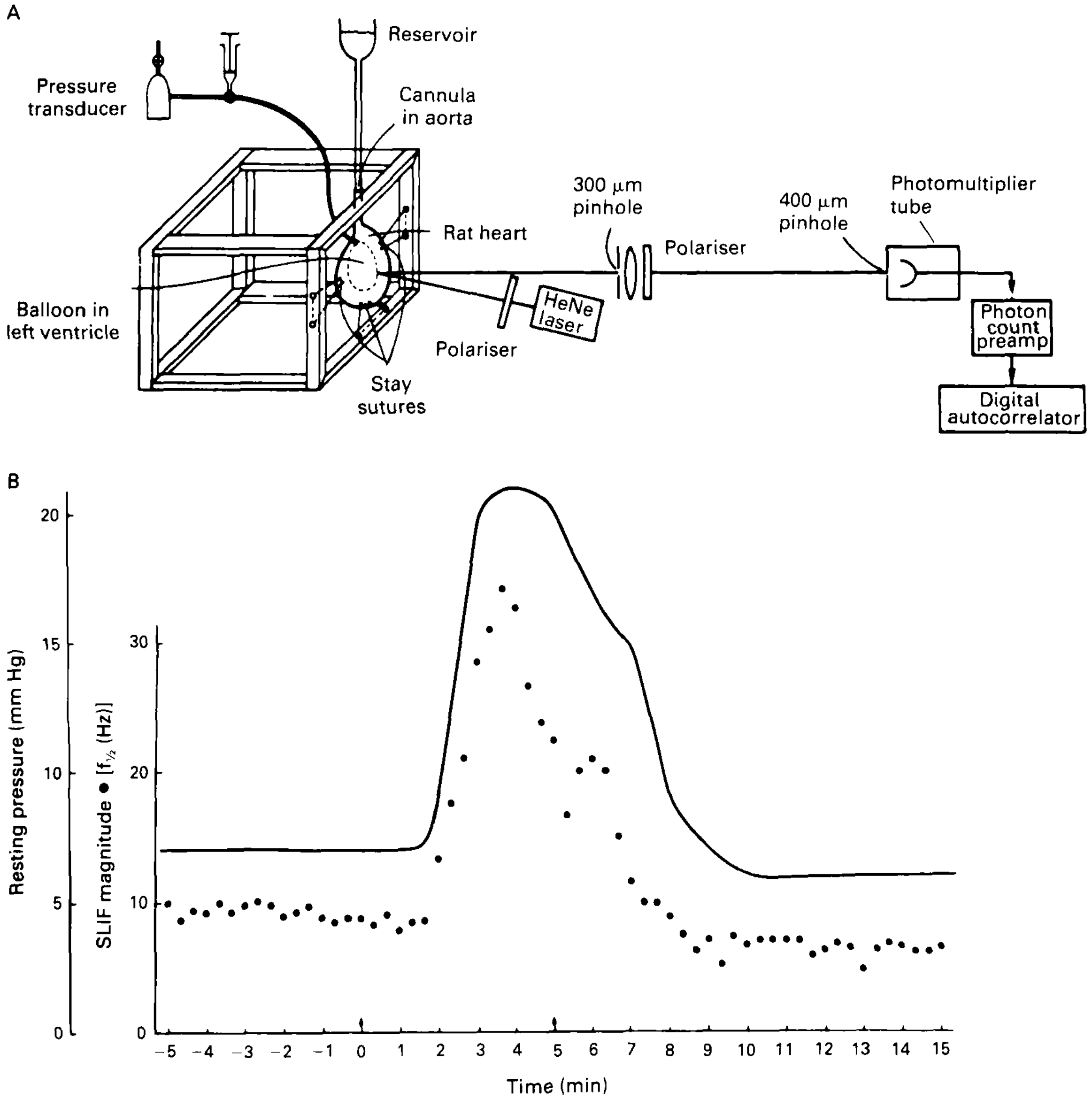


Figure 19 *(A) Schematic of apparatus for measurement of scattered light intensity fluctuation (SLIF) by backscatter of helium-neon laser light from the epicardial surface of intact perfused hearts. From Stern* et al.[24] *(B) Increase of SLIF frequency in an isolated rat heart during replacement of perfusate Na (144 mM including that used to titrate Hepes) quantitatively by K^+. Increase of SLIF is consistent with expected increase in intracellular Ca^{2+} due to Na-Ca exchange, despite complete depolarisation of sarcolemma, which would abolish any conduction electrical arrhythmias that might be an artifactual source of SLIF. From Stern* et al.[24]

as monitored SLIF in the intact heart is identical to that produced by ouabain in the isolated muscle in fig 18A. The occurrence and characteristics of ventricular fibrillation occurring in isolated hearts during Ca^{2+} overload by increasing the bathing [Ca^{2+}] or by cardiac glycosides are modulated by ryanodine.[118,117]

Phenotypic and genotypic adaptations of the chronic pressure loaded myocardium include a marked increase in the action potential and Ca_i transient durations and a reduced rate of Ca^{2+} pumping by the sarcoplasmic reticulum, apparently due in part to a transcriptionally regulated decrease in its pump site density (see [119] for review). This pattern of adaptation is associated with a reduced cell Ca^{2+} tolerance and a reduced threshold for the occurrence of S-CaOs.[119] Recent observations indicated that cardiomyopathic hamster hearts, even in the pre-hypertrophic stage, show a reduced threshold for Ca^{2+} loading and the occurrence of S-CaOs.[120] The myocardium of normotensive aged rat heart exhibits biophysical and molecular changes that are strikingly similar to the hypertensive younger rat heart[121,122]; these changes are also associated with a decreased Ca^{2+} tolerance and a reduced threshold for aftercontractions and afterdepolarisations and ventricular fibrillation.[123]

Ischaemia and reflow

One of the earliest clues to the effect of ischaemia-like states on S-CaOs was the observation that the frequency of SLIF declined when muscles were deprived of oxygen and glucose, even while the resting tension of these muscles was rising.[14] The decline in SLIF frequency during this "ischaemic" contracture was in striking contrast to the increase in SLIF associated with most "contractures" produced by Ca^{2+} overload. It is also of note that anoxia reduces oscillatory potentials and aftercontractions,[100] and inhibits reperfusion and digitalis induced arrhythmias.[100,124] That anoxia decreases or abolishes the S-CaOs frequency in single cardiac cells[125] and that metabolic inhibition abolishes current oscillations due to S-CaOs in single cells[126] suggests that energy depletion and its associated changes in cytosolic pH and inorganic phosphate in the intact heart (perhaps in part a cause of sarcoplasmic reticulum Ca^{2+} depletion due to a diminution in pump function) reduce SLIF. The reduction in SLIF may also be related to an unloading of the Ca^{2+} store in sarcoplasmic reticulum due to an effect of the above factors on its Ca^{2+} release channel during ischaemia. If such unloading of sarcoplasmic reticulum Ca^{2+} stores occurred heterogeneously among myocardial cells it might be a factor in arrhythmogenesis during this time, eg, ventricular fibrillation during ischaemia. This may be an explanation for why ryanodine can prevent ventricular fibrillation not only during reflow following ischaemia (see below) but also during ischaemia.[127]

After 30 minutes of global ischaemia at 30°C, SLIF become undetectable in the majority of rat hearts (fig 21). If ischaemia is further prolonged, isovolumetric resting pressure rises after about 45-60 minutes of ischaemia ("ischaemic contracture"). This rise is never associated with the redevelopment of SLIF (fig 21). When the heart is then reperfused, however, SLIF frequency rises rapidly to 3-5 times preischaemic control within 3-5 minutes, and this is paralleled by a further rise in resting pressure ("reflow contracture"). The bulk of the Ca^{2+} overloading of the heart during reflow occurs during this period; the fact that this contracture is a dynamic contracture, ie, associated with an increase in S-CaOs manifest as a marked SLIF overshoot, suggests that at least some of this Ca^{2+} overload is occurring in cells which are still potentially viable. In single myocytes reoxygenation following anoxia also leads to a marked increase in S-CaOs frequency.[125]

The SLIF overshoot during reflow is inversely correlated with the recovery of contractile function (fig 22C). The SLIF overshoot during the reflow period occurs at a time when ^{31}phosphorus nuclear magnetic resonance spectra show that pH, ATP, phosphocreatine, and inorganic phosphate have already recovered to a steady value (fig 22B). Reperfusion for the initial 10 minutes with 0.08 mM [Ca^{2+}] prior to a perfusate with 1.5 mM [Ca^{2+}] has significant beneficial effects on functional recovery and Ca^{2+} overload during reperfusion. SLIF overshoot at 5-10 min is prevented (fig 23A), the recovery of systolic function is enhanced, and intracellular Ca^{2+} overload is decreased (fig 23B). These results are compatible with the hypothesis that Ca^{2+} overload and asynchronous CaOs may contribute, in part, to the depression of muscle function, ie, myocardial "stunning" during reflow.

S-CaOs may also be implicated in reflow arrhythmias, such as ventricular fibrillation. Recall that S-CaOs is a mechanism whereby Ca_i is transiently amplified *locally* to systolic levels. Thus this phenomenon can transiently produce levels of Ca^{2+} that may affect impulse

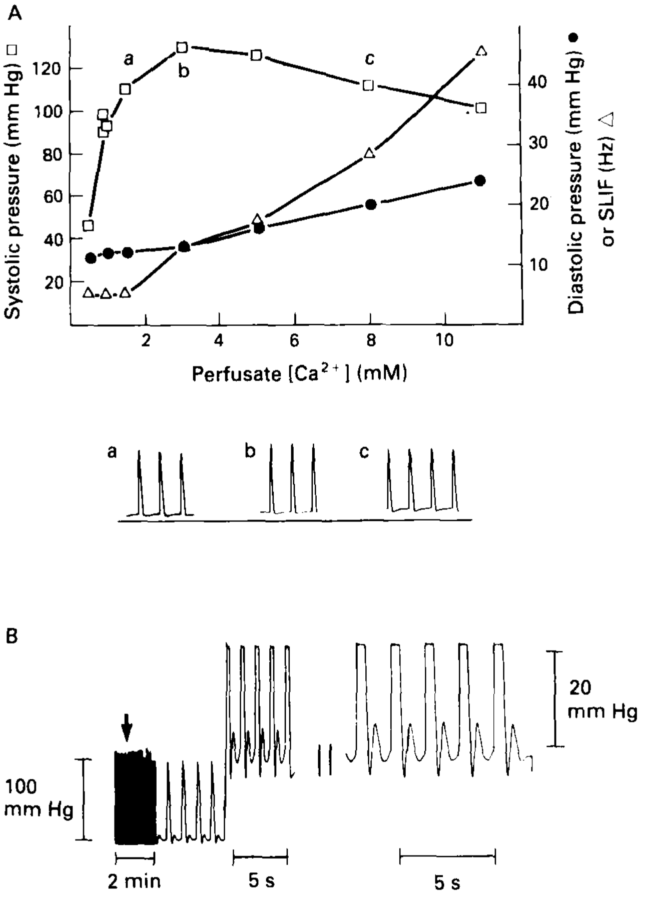


Figure 20 *Response of isolated rat heart to increasing Ca^{2+} loading. (A) The Ca^{2+} dependence of systolic and diastolic pressure and scattered light intensity fluctuation (SLIF), a manifestation of diastolic intracellular spontaneous Ca^{2} oscillations (S-CaOs). The preparation was perfused retrogradely from the aorta and rendered quiescent by an atrioventricular block and the addition of 1 μg·ml^{-1} propranolol to the perfusate. The left atrium was removed, and a balloon was inserted into the left ventricle to maintain constant end diastolic volume. Stimulation as 20·min^{-1} at 37°C produced isovolumetric systoles. Actual records, measured at a, b, and c, are shown below (see text for further details). (B) When the heart in (A) was perfused with a bathing [Ca^{2+}] of 15 mM, it began to beat spontaneously (arrow) and the oscillation in diastolic pressure become more pronounced. From Lakatta.[131]*

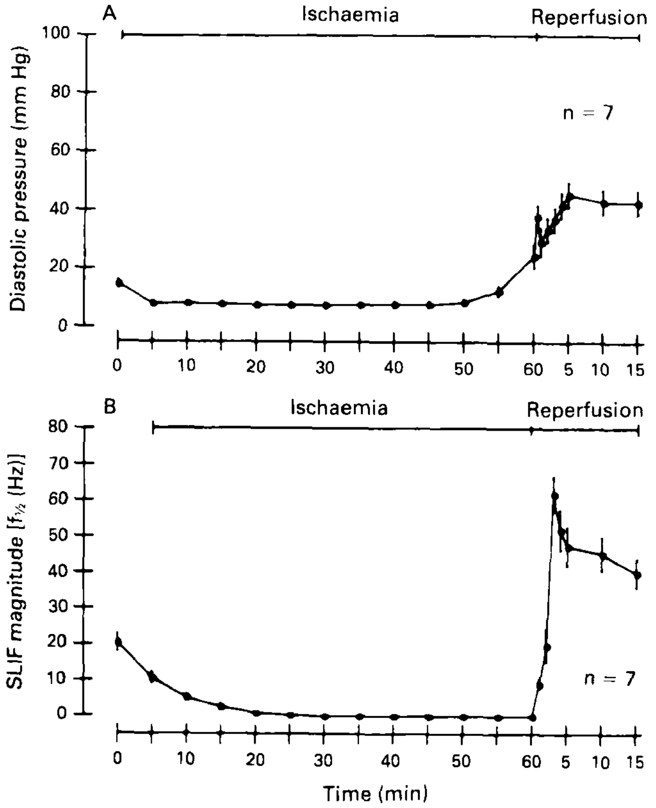


Figure 21 *Average resting pressure (top) and scattered light intensity fluctuation (SLIF) (bottom) from seven beats subjected to global ischeamia at 30°C, followed by reperfusion. From Stern* et al.[24]

conduction,[128-130] due to effects on cell-cell coupling and on cell excitability (resulting from an effect of the Ca_i dependent depolarisation to inactivate Na channels).

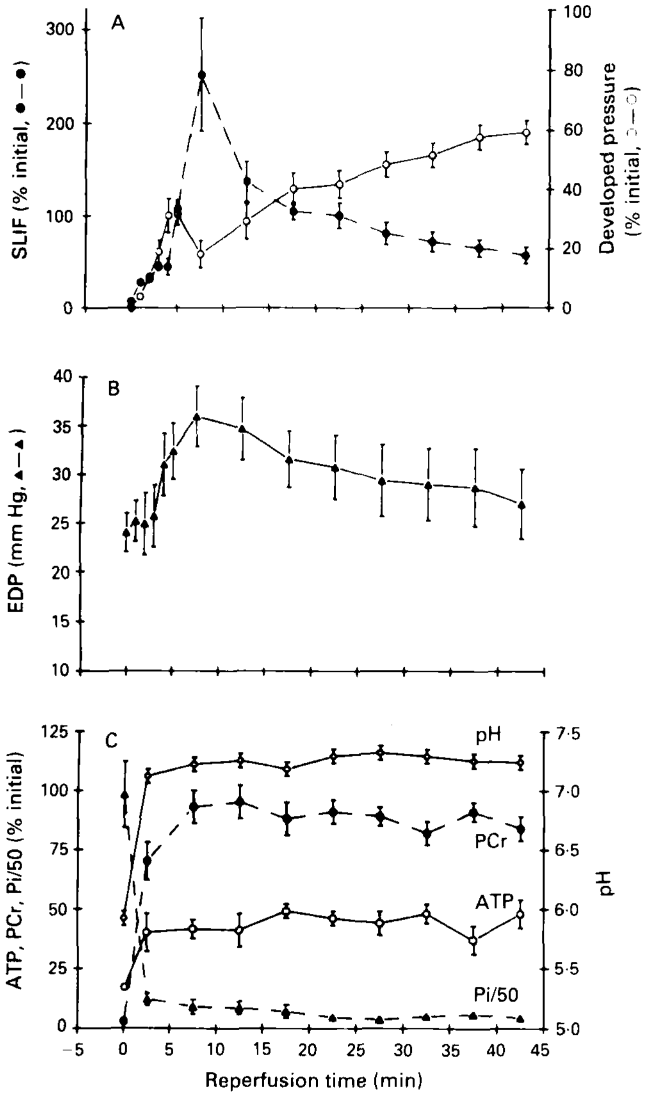


Figure 22 (A) Contractile and metabolic recovery and diastolic spontaneous Ca^{2+} oscillations (S-CaOs) detected by scattered light intensity fluctuation (SLIF) measurements during reperfusion of rat hearts following ischaemia (heart rate = 20 beats·min^{-1}). Mean values of developed pressure (as percent initial, $n = 10$), end diastolic pressure (EDP) (mm Hg, $n = 10$), and SLIF (Hz, $n=5$) are plotted in (A) v reperfusion time (min). Developed pressure recovers during the first 5 min of reperfusion but then abruptly falls, reaching a nadir at 7.5 min. Thereafter, developed pressure increases slowly, attaining 60% of its initial value after 45 min of reperfusion. (B) EDP climbs rapidly in the first min of reperfusion possibly because of the "garden hose" effect. It reaches a maximum at 7.5 min of reperfusion, which is significantly higher than that at 2 min of reperfusion ($p<0.01$) and which occurs as developed pressure is falling. Phosphocreatine (PCr), ATP, and inorganic phosphate (P_i) are presented as percentage of initial, preischaemic values. The mean preischaemic values of PCr/ATP and P_i/ATP were 2.02 (SEM 0.10) and 0.10(0.01), respectively. (C) Metabolic variables ($n = 5$) recover to their full extent within 5-7.5 min of reperfusion and then plateau at a time when contractile function is falling. Diastolic S-CaOs, indexed by SLIF, increase from the end ischaemic value of zero and peak at a mean value of three times baseline at 7.5 min of reperfusion. This SLIF peak coincides with the transient fall in developed pressure and the peak in EDP. Subsequently, SLIF decrease monotonically over the next 35 min of reperfusion while developed pressure recovers. From Weiss et al.[23]

Accordingly S-CaOs may have a role in "re-entrant" type arrhythmias in the intact heart, eg, ventricular fibrillation. Recent studies have indeed implicated S-CaOs in the initiation of ventricular fibrillation during postischaemic reflow.[117,127]

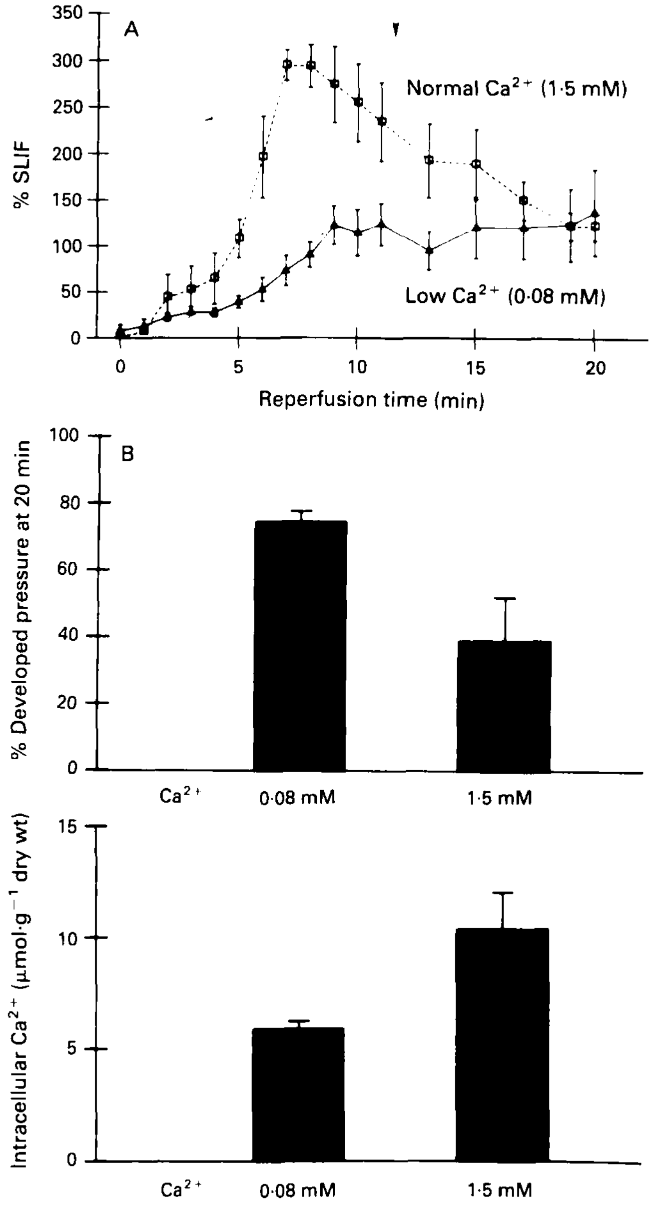


Figure 23 (A) Scattered light intensity fluctuations (SLIF) during reperfusion of rat hearts with low (0.08 mM) and normal (1.5 mM) Ca^{2+} containing solutions. In hearts reperfused with low Ca^{2+} perfusate ($n = 4$) after 65 min of no flow ischaemia, SLIF increase slowly and do not "overshoot" to three times the baseline values as seen in hearts reperfused with normal Ca^{2+} containing solution ($n = 4$). SLIF levels are similar at 15 min of reperfusion in both groups when perfusate Ca^{2+} is 1.5 mM in all hearts. The arrow indicates when perfusate Ca^{2+} of 1.5 mM was restored in the low Ca^{2+} (0.08 mM) group. (B) Developed pressure and cellular Ca^{2+} following reperfusion with normal and low Ca^{2+} containing perfusate. Hearts reperfused initially with 1.5 mM Ca^{2+} have significantly higher cell contents of Ca^{2+} and lower developed pressure at 20 min than hearts perfused initially (0-10 min of reperfusion) with low (0.08 mM) Ca^{2+} containing solution. From Weiss et al.[23]

Summary

The Ca_i oscillation generated by the sarcoplasmic reticulum in response to an action potential occurs relatively synchronously within and among cells. The sarcoplasmic reticulum can also generate spontaneous Ca_i oscillations (S-CaOs), ie, not triggered by sarcolemmal depolarisation. The local increase in Ca_i due to S-CaOs is equivalent to that induced by an action potential. Heterogeneity of diastolic Ca_i among cells within myocardial tissue caused by asynchronous S-CaOs leads to heterogeneous myofilament activation, the summation of which produces a Ca^{2+} dependent component to diastolic tone. The local increases in Ca_i due to S-CaOs also cause oscillatory sarcolemmal depolarisations due to Ca^{2+} modulation of the Na-Ca exchanger and of non-specific cation channels. When local S-CaOs within a myocardial cell is sufficiently synchronised the resultant depolarisation summates and can be sufficient to trigger a spontaneous action potential. Inhomogeneous levels of diastolic Ca_i among cells may lead to heterogeneity in cell coupling and thus may also affect the impulse conduction in myocardial tissue. The magnitude of the S-CaOs induced diastolic tonus and depolarisation varies with the extent to which S-CaOs are synchronised; partially synchronised S-CaOs following an action potential induced Ca^{2+} release produce an aftercontraction and afterdepolarisation.

Inhomogeneity of *diastolic* sarcoplasmic reticulum Ca^{2+} loading and sarcomere lengths within individual cardiac cells due to S-CaOs leads to inhomogeneous *systolic* Ca_i levels and sarcomere inhomogeneities in response to a subsequent action potential, which compromise the systolic contraction amplitude. Heterogeneity of systolic Ca_i among cells due to diastolic S-CaOs also leads to heterogeneity of action potential repolarisation times, due to heterogeneous Ca_i modulation of the Na-Ca exchanger, the non-specific cation channel, the L type Ca^{2+} channel and, depending upon species, Ca^{2+} activated K^+ channels. S-CaOs occurrence during a long action potential plateau may also modulate the removal of voltage inactivation of L type Ca^{2+} channels, and affects the likelihood of the occurrence of "early afterdepolarisations." Thus, as a single entity, S-CaOs may be implicated in diverse manifestation of heart failure – impaired systolic performance, increased diastolic tonus, and an increased probability for the occurrence of arrhythmias.

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Key terms: cardiac cells; calcium; sarcoplasmic reticulum; spontaneous Ca^{2+} oscillations; diastolic function; arrhythmias.

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