In mammalian cardiac myocytes, contractile activation occurs in response to Ca\(^{2+}\) influx from the extracellular space, triggering Ca\(^{2+}\) release via Ca\(^{2+}\)-sensitive channels located in the membrane of the sarcoplasmic reticulum (SR) (Bers, 1991; Stern & Lakatta, 1992; Eisner et al. 1998). Despite its self-regenerating nature, this process of Ca\(^{2+}\)-induced Ca\(^{2+}\) release (CICR) in myocytes is not normally all-or-none but instead is smoothly graded according to the amounts of trigger Ca\(^{2+}\) entering into the cell (Cannell et al. 1987; Beuckelmann & Wier, 1988). Under certain conditions, such as increased cellular Ca\(^{2+}\) load, CICR waves may be initiated by elevating extracellular Ca\(^{2+}\) concentrations ([Ca\(^{2+}\)]\(_{out}\)) or at normal Ca\(^{2+}\) loads when the sensitivity of the release sites to cytosolic Ca\(^{2+}\) was enhanced by low doses of caffeine. When we prevented the accumulation of extra Ca\(^{2+}\) in the luminal compartment of the sarcoplasmic reticulum (SR) with thapsigargin, focal caffeine pulses failed to trigger self-sustaining Ca\(^{2+}\) waves on elevation of [Ca\(^{2+}\)]\(_{cyt}\). Inhibition of SR Ca\(^{2+}\) uptake by thapsigargin in cells already preloaded with Ca\(^{2+}\) above normal levels did not prevent local Ca\(^{2+}\) elevations from triggering propagating waves. Moreover, wave velocity increased by 20%. Tetracaine (0·75 mM) caused transient complete inhibition of both local and propagating Ca\(^{2+}\) signals, followed by full recovery of the responses due to increased SR Ca\(^{2+}\) accumulation.

Computer simulations using a numerical model with spatially distinct Ca\(^{2+}\) release sites suggested that increased amounts of releasable Ca\(^{2+}\) might not be sufficient to generate self-sustaining Ca\(^{2+}\) waves under conditions of Ca\(^{2+}\) overload unless the threshold of release site Ca\(^{2+}\) activation was set at relatively low levels (\(<\) 1·5 ìÒ).

We conclude that the potentiation of SR Ca\(^{2+}\) release channels by luminal Ca\(^{2+}\) is an important factor in Ca\(^{2+}\) wave generation. Wave propagation does not require the translocation of Ca\(^{2+}\) from the spreading wave front into the SR. Instead, it relies on luminal Ca\(^{2+}\) sensitizing Ca\(^{2+}\) release channels to cytosolic Ca\(^{2+}\).

In mammalian cardiac myocytes, contractile activation occurs in response to Ca\(^{2+}\) influx from the extracellular space, triggering Ca\(^{2+}\) release via Ca\(^{2+}\)-sensitive channels located in the membrane of the sarcoplasmic reticulum (SR) (Bers, 1991; Stern & Lakatta, 1992; Eisner et al. 1998). Despite its self-regenerating nature, this process of Ca\(^{2+}\)-induced Ca\(^{2+}\) release (CICR) in myocytes is not normally all-or-none but instead is smoothly graded according to the amounts of trigger Ca\(^{2+}\) entering into the cell (Cannell et al. 1987; Beuckelmann & Wier, 1988). Under certain conditions, such as increased cellular Ca\(^{2+}\) load, Ca\(^{2+}\) release does show signs of uncontrollable behaviour. Spontaneous discharges of SR Ca\(^{2+}\) begin locally, then propagate as regenerative Ca\(^{2+}\) waves through the cell (Kort et al. 1985; Wier et al. 1987; Takamatsu & Wier, 1990; Lipp & Niggli, 1994; Trafford et al. 1995; Engel et al. 1995; Wussling & Salz, 1996; Cheng et al. 1996; Lukyanenko et al. 1996). This chaotic behaviour has been implicated in certain cardiac abnormalities including after-depolarizations and Ca\(^{2+}\)-dependent arrhythmias (Ishide, 1996). At the present time, the factors responsible for preventing CICR from self regenerating or the alterations responsible for transition from graded release to all-or-none spontaneous release are not precisely understood.

The role of luminal Ca\(^{2+}\) in the generation of Ca\(^{2+}\) waves in rat ventricular myocytes

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1. We used confocal Ca\(^{2+}\) imaging and fluo-3 to investigate the transition of localized Ca\(^{2+}\) releases induced by focal caffeine stimulation into propagating Ca\(^{2+}\) waves in isolated rat ventricular myocytes.

2. Self-sustaining Ca\(^{2+}\) waves could be initiated when the cellular Ca\(^{2+}\) load was increased by elevating the extracellular [Ca\(^{2+}\)] ([Ca\(^{2+}\)]\(_{out}\)) and they could also be initiated at normal Ca\(^{2+}\) loads when the sensitivity of the release sites to cytosolic Ca\(^{2+}\) was enhanced by low doses of caffeine. When we prevented the accumulation of extra Ca\(^{2+}\) in the luminal compartment of the sarcoplasmic reticulum (SR) with thapsigargin, focal caffeine pulses failed to trigger self-sustaining Ca\(^{2+}\) waves on elevation of [Ca\(^{2+}\)]\(_{cyt}\). Inhibition of SR Ca\(^{2+}\) uptake by thapsigargin in cells already preloaded with Ca\(^{2+}\) above normal levels did not prevent local Ca\(^{2+}\) elevations from triggering propagating waves. Moreover, wave velocity increased by 20%. Tetracaine (0·75 mM) caused transient complete inhibition of both local and propagating Ca\(^{2+}\) signals, followed by full recovery of the responses due to increased SR Ca\(^{2+}\) accumulation.

3. Computer simulations using a numerical model with spatially distinct Ca\(^{2+}\) release sites suggested that increased amounts of releasable Ca\(^{2+}\) might not be sufficient to generate self-sustaining Ca\(^{2+}\) waves under conditions of Ca\(^{2+}\) overload unless the threshold of release site Ca\(^{2+}\) activation was set at relatively low levels (\(<\) 1·5 ìÒ).

4. We conclude that the potentiation of SR Ca\(^{2+}\) release channels by luminal Ca\(^{2+}\) is an important factor in Ca\(^{2+}\) wave generation. Wave propagation does not require the translocation of Ca\(^{2+}\) from the spreading wave front into the SR. Instead, it relies on luminal Ca\(^{2+}\) sensitizing Ca\(^{2+}\) release channels to cytosolic Ca\(^{2+}\).
receptors (RyRs) to cytosolic Ca²⁺ is enhanced by increased second mechanisms when the sensitivity of ryanodine

To distinguish between these possibilities we monitored the propagation of the Ca²⁺ wave is cytosolic Ca²⁺. Luminal Ca²⁺ acts at an intraluminal site on the SR Ca²⁺ release channel following its uptake into the SR (Takamatsu & Wier, 1990; Fabiato, 1992). This possibility is consistent with recent evidence showing that increased luminal Ca²⁺ can activate the Ca²⁺ release channels in lipid bilayers (Sitsapesan & Williams, 1994; Lukyanenko et al. 1996). It is also consistent with results indicating that increased SR Ca²⁺ load can potentiate CICR release in intact and patch-clamped myocytes (Han et al. 1994; Janczewski et al. 1994; Bassani et al. 1995; Lukyanenko et al. 1996; Györke et al. 1997; Györke & Györke, 1998). (3) A combination of the first and second mechanisms when the sensitivity of ryanodine receptors (RyRs) to cytosolic Ca²⁺ is enhanced by increased luminal Ca²⁺. In support of this possibility, increased luminal Ca²⁺ has been shown to increase the sensitivity of RyR to cytosolic Ca²⁺ in lipid bilayers (Lukyanenko et al. 1996; Györke & Györke, 1998).

To distinguish between these possibilities we monitored the subcellular properties of SR Ca²⁺ release using confocal Ca²⁺ imaging and fluo-3 in rat ventricular myocytes. We examined the transition of localized Ca²⁺ release into propagating Ca²⁺ waves under various experimental conditions, including inhibition of the SR Ca²⁺ pump by thapsigargin, sensitization of the SR Ca²⁺ release channels to cytosolic Ca²⁺ by caffeine, and inhibition of the SR Ca²⁺ channels by tetracaine. In addition we investigated the factors for successful Ca²⁺ wave propagation using a numerical model of the Ca²⁺ wave. Our results indicate that the primary factor responsible for propagation of the Ca²⁺ wave is cytosolic Ca²⁺. Luminal Ca²⁺ plays a modulatory role by enhancing the sensitivity of the Ca²⁺ release channels to cytoplasmic Ca²⁺.

**METHODS**

**Cell isolation and experimental solutions**

Adult Sprague–Dawley rate (200–300 g) were killed by lethal injection of pentobarbital sodium (Nembutal; 100 mg kg⁻¹ i.p.) as approved by the Animal Care and Use Committee. Single ventricular myocytes were obtained by enzymatic dissociation as described before (Györke et al. 1997). The cells were loaded with fluo-3 by a 20 min incubation with 5 μM fluo-3 AM (acetoxymethyl ester form; Molecular Probes) at 23 °C.

The Tyrode solution contained (mM): 140 NaCl, 2 KCl, 0-5 MgCl₂, 1–5 CaCl₂, 10 HEPES, 25 NaH₂PO₄, 5/6 glucose, pH 7.3. Tetrodotoxin (Sigma) at 10 μg was added to the bathing solution to avoid depolarization-induced Ca²⁺ release due to spontaneous action potentials. Caffeine, thapsigargin (Calbiochem) or tetracaine were used at the concentrations indicated.

**Confocal microscopy**

Experiments were performed using an Olympus laser scanning confocal microscope (LSM-GB200) equipped with an Ar Argon laser (514 nm), 63 Na objective (Lukyanenko et al. 1996). Fluo-3 was excited by light at 488 nm (25 mW argon laser, intensity attenuated to 1–3%), and fluorescence was measured at wavelengths of >515 nm (confocal detector aperture set to 25% of maximum). As determined with 0.1 μm fluorescent beads (Molecular Probes), the system provided an axial resolution of 0.4 μm and a depth of field of about 0.7 μm. Images were acquired in the linescan mode at a rate of 8 or 32 milliseconds per scan. For calibration purposes, the total linescan [Ca²⁺] in 1 mm [Ca²⁺], was assumed to be 100 nM and it served as a reference point for the determination of [Ca²⁺] in the course of an experiment. [Ca²⁺] was calculated from fluo-3 fluorescence using an equation and calibration parameters described previously (Cheng et al. 1993). Image processing and analysis were performed by using NIH Image (NIH, Bethesda, MD, USA) and IDL software (Research Systems Inc., Boulder, CO, USA).

**Lipid bilayer experiments**

Heavy sarcoplasmic reticulum (SR) microsomes were isolated by differential centrifugation from the ventricles of dog heart (Dettbarn et al. 1994). The dogs were killed by lethal injection of Nembutal (70 mg kg⁻¹ i.v.) as approved by the Animal Care and Use Committee. SR microsomes were fused into planar lipid bilayers and single channels were recorded as described previously (Lukyanenko et al. 1996). SR vesicles were added to one side of the bilayer (defined as cis) and the other side was defined as trans (virtual ground). Bilayers contained 80% phosphatidylethanolamine and 20% phosphatidylcholine dissolved in decane at a final concentration of 50 mg ml⁻¹. Standard solutions contained 350 mM cis CaCl₂, 20 mM trans CaCl₂, 20 μM Hepes (pH 7.4). After channel incorporation, the trans CaCl₂ was adjusted to 350 mM. Single channel recording was carried out using an Axopatch 200A (Axon Instruments) patch-clamp amplifier. Data were filtered at 2 kHz and digitized at 5 kHz. Acquisition and analysis of data were performed using pCLAMP 6.01 software (Axon Instruments).

**Model of the Ca²⁺ wave**

To simulate Ca²⁺ waves, we used a numerical model of CICR with spatially discrete release sites. The model includes 50 individual release sites (equally spaced at intervals of 2 μm), two spatially homogeneous removal fluxes (SR Ca²⁺ pump and solute buffers), diffusible dye, the calcium–dye complex (Ca·dye) and calcium bound to endogenous buffers (Ca·B). The spatiotemporal distributions of the free cytoplasmic Ca²⁺, the Ca·dye complex and the free dye, along with the temporal evolution of the bound buffers, are described by the following coupled system of reaction–diffusion equations and ordinary differential equation:

\[
\frac{\partial [Ca^{2+}]}{\partial t} = D_{Ca} \nabla^2 [Ca^{2+}] + g_{rel} - g_{rem} + g_{reso}
\]  
\[
\frac{\partial [Ca·dye]}{\partial t} = D_{dye} \nabla^2 [Ca·dye] + g_{dye} - g_{dyeo}
\]  
\[
\frac{\partial [dye]}{\partial t} = D_{dye} \nabla^2 [dye] - g_{dye}
\]  
\[
\frac{\partial [Ca·B]}{\partial t} = g_v.
\]
Here $D_{Ca}$, $D_{Ca-dye}$ and $D_{dye}$ are the diffusion coefficients of $Ca^{2+}$, Ca-dye and free dye, respectively (the endogenous buffers are assumed to be immobile, see below); $\nabla^2[Ca^{2+}]$, $\nabla^2[Ca-dye]$ and $\nabla^2[dye]$ are the Laplacians; $q_{rel}$ is the release flux and $q_{rem}$ the removal flux. The removal flux consists of three contributions:

$$q_{rem} = q_b + q_{SR} + q_{dye}$$  \hspace{1cm} (5)

The quantity $q_b$ is the removal by binding to endogenous buffers, and $q_{SR}$ is the removal by the $Ca^{2+}$-ATPase of the SR. $q_{dye}$ is the binding flux of free calcium to the indicator dye. The quantity $q_{rel}$ is the release from the SR under basal conditions. Removal by the $Ca^{2+}$-ATPase and Na$^{+}$-$Ca^{2+}$ exchanger of the sarcolemma (taken to be constant throughout the model) is ignored in this study. The $Ca^{2+}$ release flux, $q_{rel}$, of each release site was implemented using the model equations and parameter values in the appendix of Keizer et al. (1998; eqns 7–10).

It is given by the following expression:

$$q_{rel}(r,t) = \left\{ \begin{array}{ll}
(\tau_{rel}^0)([Ca^{2+}])_{SR} - (Ca^{2+})_{SR} & \text{if } ([Ca^{2+}])_{SR} > \sigma^* \text{ and } r = r_i \\
0 & \text{otherwise.} \end{array} \right.$$  \hspace{1cm} (6)

Here the coefficient $\tau_{rel}$ is the conductance of the release site, $f_{rel}([Ca^{2+}])_{r,t}$ is the calcium-dependent fractional activation of each site and $([Ca^{2+}])_{r,t}$ is the local free calcium concentration. The quantity $\sigma^*$ is the threshold value of myoplasmic calcium above which the site is able to release calcium. $r_i$ is the location of release site $i$. $([Ca^{2+}])_{SR}$ is the calcium concentration in the sarcoplasmic reticulum (taken to be constant in a given simulation).

With regard to buffering, the flux was calculated from the following equation, which lump all the endogenous buffers into a single immobile pool:

$$q_b = \dot{k}_{rel}[Ca^{2+}][[B] - [Ca:B]] - \dot{k}_{rel}[Ca:B]$$  \hspace{1cm} (7)

The rate constants for binding and dissociation are given by $k_{rel}$ and $k_{off}$ respectively. $[B]$ is the total concentration of buffers and $[Ca:B]$ is the concentration of the calcium--buffer complexes. Implicit in this expression is the use of the conservation relation:

$$[B] = [B] + [Ca:B].$$  \hspace{1cm} (8)

Then the concentration of the free buffer at any time, $[B]$, can simply be found by difference. The use of this relation is valid when the initial distribution of the buffers is uniform and binding of calcium to the buffer does not change the diffusion coefficient of the buffer. The flux transported by the $Ca^{2+}$-pump was calculated from the expression given by Tang & Othmer (1994):

$$q_{pump} = V_{pump}[Ca^{2+}]_{SR}/[K_{pump}]_{SR} + [Ca^{2+}]_{SR},$$  \hspace{1cm} (9)

where $V_{pump}$ is the maximal capacity and $K_{pump}$ the calcium activity at which the transport is half-maximal for a particular process. The flux of calcium binding to the indicator is given by the following expression:

$$q_{dye} = \dot{k}_{dye}[[Ca^{2+}][dye]] - \dot{k}_{dye}[[Ca:dye]],$$  \hspace{1cm} (10)

As with the endogenous buffers, a conservation relation among the total dye, bound dye and free dye may be employed when the initial distribution of dye is uniform and the diffusion coefficient of the free and bound dye are the same. However, we chose in this case to model the free dye in an equivalent fashion with a reaction diffusion equation instead of the conservation relations because we wished to incorporate the flexibility to relax the assumptions that permit the conservation relation in future studies.

The term $q_{rel}$ is given by:

$$q_{rel} = V_{rel}[Ca^{2+}]_{SR}/[K_{rel}]_{rel} + [Ca^{2+}]_{SR}$$  \hspace{1cm} (11)

The parameters of release, diffusion, buffering and uptake of $Ca^{2+}$ are presented in Table 1.

The governing equations are subject to the following initial and boundary conditions:

$$[Ca^{2+}](r,0) = [Ca^{2+}]_0, [Ca^{2+}](r,t) = [Ca^{2+}]_{SR},$$  \hspace{1cm} (12)

$$[Ca:dye](r,0) = [Ca:dye]_0, [Ca:dye](r,t) = [Ca:dye]_{SR},$$

$$[dye](r,0) = [dye]_0, [dye](r,t) = [dye]_{SR},$$

$$[Ca:B](r) = [Ca:B]_0 = [Ca^{2+}]_{SR}.$$

The value of the basal calcium level was taken to be $[Ca^{2+}]_{SR} = 0·1 \mu M$. The basal values $[Ca:dye]_0$ and $[dye]_0$ were taken to be in equilibrium with $[Ca^{2+}]_{SR}$, ([Ca:dye]$_0$ = 5 $\mu$M, $[dye]_0$ = 45 $\mu$M). The dimension $a = 100 \mu$m, and is the radius of the spherical domain being simulated. The coupled reaction–diffusion equations were solved numerically using an explicit Euler scheme. The time derivatives were made discrete using a second-order, central finite difference approximation. Our time step was $\Delta t = 8·571 \times 10^{-4}$ ms, and our space step was $\Delta x = 0·1 \mu$m. The modelling code was written in VISUAL FORTRAN Standard Edition 5.0.A (Digital) and executed on a Pentium IIID 266 desktop computer.

### RESULTS

#### Generation and measurement of $Ca^{2+}$ waves

To study the mechanisms underlying $Ca^{2+}$ wave propagation, we used the technique of local caffeine application. It has been demonstrated that $Ca^{2+}$ release by caffeine can be confined to small regions of the cell to trigger $Ca^{2+}$ waves propagating away from the area of application (Trafford et al. 1995). Caffeine (10 mM) was applied through a micropipette (~1 $\mu$m diameter) positioned near the surface of fluo-3-loaded cells and the resulting $[Ca^{2+}]$, changes were measured by repeatedly scanning a single line along the longitudinal axis of the cell. The spatial characteristics of our local solution application system were visualized upon...
injection of fluorescein-containing solution into the bath (Fig. 1Aa). As can be seen, the solution application was confined within an area of <20 μm diameter. Local application of caffeine to cells induced Ca²⁺ waves which propagated from the area of initiation giving rise to a characteristic inverted 'V' fluorescence pattern on the linescan image (Fig. 1Ac).

In accordance with previous studies (Trafford et al. 1990, 1995), the potency of the cells to generate propagating responses depended on their Ca²⁺ loading status. With [Ca²⁺]₀ < 2 mM, most responses triggered by caffeine typically decayed in both amplitude and propagation velocity, and died out within 20–30 μm of their site of origin. With [Ca²⁺]₀ > 2 mM, most responses propagated throughout the entire cell at constant amplitude and velocity. Figure 1A shows images from a typical experiment in which abortive waves measured with 1 mM [Ca²⁺]₀ (Fig. 1Ab) became regenerative 1 min after increasing [Ca²⁺]₀ to 3 mM (Fig. 1Ac). Similar results were obtained in eight out of ten cells in which [Ca²⁺] was changed from 1 to between 2 and 5 mM. In the two remaining experiments (at 2 mM [Ca²⁺]₀), local caffeine pulses failed to trigger propagating Ca²⁺ waves. Figure 1B plots time-dependent fluorescence changes measured from the site of caffeine applications before and after elevating [Ca²⁺]₀. In accordance with our previous studies (Lukyanenko et al. 1996), elevating [Ca²⁺]₀ resulted in a slight increase in the resting fluorescence and a larger increase in the amplitude of the peak caffeine-induced fluorescence transients. The position of the Ca²⁺ wave in the x–t plane was defined at half-maximal amplitude of the rising phase of the signal using a computer selection algorithm (Fig. 1Ad). The average velocity of the propagating wave was determined by fitting a linear function to the position of the wave (Fig. 1C). The average wave velocity varied from 32 to 74 μm s⁻¹ (46.0 ± 2.3 μm s⁻¹, n = 25) at [Ca²⁺]₀ of 3 mM. These estimates of the Ca²⁺ wave velocities should not be affected significantly by local shortening of the cell. It has been shown previously that

**Figure 1.** The effects of [Ca²⁺]₀ on [Ca²⁺] responses to focal caffeine applications

Aa, linescan image illustrating diffusion of fluorescein in water following pressure injection (10 p.s.i. for 20 ms) of fluorescein-containing solution (5 μg ml⁻¹) from a micropipette. b and c, linescan images of fluorescence changes induced by focal application of caffeine (10 μM) with injection settings similar to those in a at 1 mM [Ca²⁺]₀ (non-propagating response; b) and 1 min after increasing [Ca²⁺]₀ to 3 mM (propagating Ca²⁺ wave; c). The experimental protocol is presented schematically at the top. d, portion of the image in c marked by arrows; white dots indicate the position of the wave defined at half-maximal amplitude of the local [Ca²⁺]₀. Calibration bars: horizontal, 14 μm; vertical, 0.28 s. B, time-dependent changes in fluorescence at the site of caffeine applications (marked in Aa and c by bars) at 1 mM [Ca²⁺]₀ (black trace) and 3 mM [Ca²⁺]₀ (grey trace). C, linear fit to the position of the wave as defined in Ad; the reciprocal of the slope of the line yields the average wave velocity v = 51 μm s⁻¹. D, space-averaged Ca²⁺ transients elicited by application of 20 μM caffeine to the whole bath before (lefthand trace) and 1 min after increasing [Ca²⁺]₀ from 1 to 3 mM (righthand trace).
contractile waves follow calcium waves with a delay of 50–100 ms (Kort et al. 1985). Thus, as has been argued previously (Wussling & Salz, 1996), although regional shortening can affect initial Ca\(^{2+}\) wave velocity it should have no impact on the velocity of the Ca\(^{2+}\) wave at later times (>100 ms), when the Ca\(^{2+}\) and contractile waves travel at the same speed.

The effects of increasing \([\text{Ca}^{2+}]_o\) on SR Ca\(^{2+}\) content were assessed in a separate series of experiments, in which caffeine (20 mm) was applied to the whole bath and changes in space-averaged \([\text{Ca}^{2+}]\) were measured (Lukyanenko et al. 1996). As indexed by changes in the amplitude of the caffeine-induced Ca\(^{2+}\) transients (0.84 ± 0.05 μM vs. 1.10 ± 0.06 μM, \(n = 8\)), the SR Ca\(^{2+}\) load increased by 26% upon elevating \([\text{Ca}^{2+}]_o\) from 1 to 3 mm (Fig. 1D). In addition, the resting \([\text{Ca}^{2+}]\) tended to increase slightly (from 100 ± 6 to 112 ± 10 nm). These results are in agreement with our previous assessment of changes in SR Ca\(^{2+}\) load caused by elevations in extracellular \([\text{Ca}^{2+}]_o\) (Lukyanenko et al. 1996).

**Effects of thapsigargin**

The transition from an abortive Ca\(^{2+}\) wave to a regenerative Ca\(^{2+}\) wave on elevation of \([\text{Ca}^{2+}]_o\), could be caused by increases in either cytosolic or intra-SR \([\text{Ca}^{2+}]\). To distinguish between these possibilities, we examined the ability of local caffeine application to induce propagating Ca\(^{2+}\) waves under conditions when the SR Ca\(^{2+}\) uptake was inhibited by thapsigargin before elevating bathing \([\text{Ca}^{2+}]\). Thapsigargin selectively inhibits the SR Ca\(^{2+}\) ATPase (Thastrup et al. 1989; Kirby et al. 1992). As illustrated in Fig. 2A, exposure of the cells to thapsigargin prior to elevation of \([\text{Ca}^{2+}]_o\), resulted in a failure of caffeine pulses to trigger regenerative Ca\(^{2+}\) waves. In these experiments, thapsigargin (10 μM) was introduced to the bath 1 min before elevating \([\text{Ca}^{2+}]\) from 1 to 3 mm and the caffeine pulse was applied 2 min after the Ca\(^{2+}\) elevation. At this concentration and exposure time, thapsigargin has been shown to inhibit SR Ca\(^{2+}\) uptake by about 90% without causing a significant change in the SR Ca\(^{2+}\) load (Bassani et al. 1993). At the site of caffeine application the basal fluorescence was again slightly elevated, but the peak amplitude of the caffeine-induced fluorescence signal did not show a significant change in the presence of thapsigargin (Fig. 2B). This result would be consistent with the notion that thapsigargin, while preventing uptake of extra Ca\(^{2+}\) into the SR, did not cause a measurable loss of Ca\(^{2+}\) from the SR during the time period of the measurements. Similar results were obtained in four out of four other experiments.

The effects of thapsigargin on the Ca\(^{2+}\) load and Ca\(^{2+}\) uptake capacity of the SR were further investigated in a separate series of experiments with caffeine application to the whole bath. Figure 2C illustrates caffeine-induced Ca\(^{2+}\) transients measured under control conditions and also after successive introductions to the bath of thapsigargin (10 μM) and elevated Ca\(^{2+}\) (3 mm). Consistent with the results of experiments employing local caffeine applications (Fig. 2B), elevation of \([\text{Ca}^{2+}]_o\) in the presence of thapsigargin, while resulting in a small elevation in the basal \([\text{Ca}^{2+}]\) (from 100 ± 9 to 140 ± 15 μM, \(n = 5\)), did not cause a significant increase in amplitude of the caffeine-induced Ca\(^{2+}\) transients (0.81 ± 0.09 vs. 0.86 ± 0.10 μM). The amplitude of the second caffeine-induced Ca\(^{2+}\) transient measured 2 min later in the continuous presence of thapsigargin was reduced dramatically (by 72 ± 11%), verifying a substantial inhibition in SR Ca\(^{2+}\) uptake. These results confirm that thapsigargin can effectively prevent the accumulation of extra Ca\(^{2+}\) in the SR on increasing \([\text{Ca}^{2+}]_o\) under our experimental conditions. Taken together, these results suggest that the increased accumulation of Ca\(^{2+}\) in the SR is a critical factor in the generation of propagating Ca\(^{2+}\) waves under conditions of increased cellular Ca\(^{2+}\) load. The remainder of this study was therefore concerned with defining the mechanisms through which increased \([\text{Ca}^{2+}]_o\) results in wave propagation.

It has been suggested that propagation of Ca\(^{2+}\) waves is mediated by Ca\(^{2+}\) uptake into a highly loaded SR and subsequent activation of the release mechanism from within the SR (Takamatsu & Wier, 1990; Fabiato, 1992; Lukyanenko et al. 1996). To test this hypothesis directly, we examined the ability of thapsigargin to inhibit propagation of Ca\(^{2+}\) release following focal caffeine applications in cells already preloaded by Ca\(^{2+}\) above normal levels. Figure 3A illustrates the effects of exposure of the cells to thapsigargin (10 μM for 1 min) on the caffeine-induced responses in the presence of 3 mm Ca\(^{2+}\) in the bathing solution. Rather than inhibiting wave propagation, thapsigargin actually enhanced it (Fig. 3B). Again, the peak amplitude of the caffeine-induced responses was without a change, indicating no significant alterations in the SR Ca\(^{2+}\) load (Fig. 3C). Based on the results of six experiments, the velocities of Ca\(^{2+}\) waves before and after application of thapsigargin were 43.0 ± 1.2 and 50.2 ± 1.7 μm s\(^{-1}\), respectively (\(P < 0.05\)). The effects of thapsigargin on space-averaged caffeine-induced Ca\(^{2+}\) transients are shown in Fig. 3D. Again, inhibition of the Ca\(^{2+}\) uptake capacity of the SR is confirmed by the reduced amplitude of the caffeine-induced Ca\(^{2+}\) signal in the presence of the drug. Similar results were obtained in four other experiments.

Since the enhancement of Ca\(^{2+}\) wave propagation by thapsigargin could be also due to a potentiation of the release mechanism by thapsigargin, we examined the effect of thapsigargin on SR Ca\(^{2+}\) release channels incorporated into lipid bilayers (not shown). In four channels the open probability (\(P_o\)) measured before and after application of 10 μM thapsigargin to the cytosolic side of the channel was 0.21 ± 0.03 and 0.26 ± 0.04, respectively. This result is consistent with the lack of effect of thapsigargin (10 μM) on Ca\(^{2+}\) release in purified muscle vesicles (Kirby et al. 1992). Taken together, these results suggest that Ca\(^{2+}\) uptake from the approaching wave front is not required for wave propagation.
Effects of low caffeine concentrations

It has been demonstrated that elevation of luminal Ca²⁺ increases the sensitivity of the Ca²⁺ release channel to cytosolic Ca²⁺ (Gyorke & Gyorke, 1998). Such an increase in Ca²⁺ sensitivity of the Ca²⁺ release mechanism, expected as a result of enhanced Ca²⁺ accumulation in the SR, could represent another mechanism for Ca²⁺ wave propagation in Ca²⁺-overloaded myocytes. To investigate whether the conditions for propagation of Ca²⁺ waves can be created ‘artificially’ by sensitizing the release channels to cytosolic Ca²⁺ (i.e. without increasing the SR Ca²⁺ load), we tested the effects of low concentrations of caffeine on the ability of local caffeine pulses to trigger propagating releases. Caffeine is a known potentiator of CICR, which is believed to act by enhancing the sensitivity of the Ca²⁺ release channel to cytosolic Ca²⁺ (Endo, 1977). Figure 4 illustrates the results of exposure of cells to 0·5 mM caffeine under the conditions of reduced ([Ca²⁺]ₒ = 1 mM) or elevated ([Ca²⁺]ₒ = 3 mM) SR Ca²⁺ loads employed beforehand. Caffeine was applied to the whole bath 20 s prior to delivery of the focal 10 mM

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Figure 2. The effects of [Ca²⁺]ₒ on [Ca²⁺], responses to focal applications of caffeine in cells pretreated with thapsigargin

A, linescan images of fluorescence changes induced by focal applications of caffeine (10 mM) in the presence of 1 mM [Ca²⁺]ₜ (a) and 2 min after increasing [Ca²⁺]ₜ to 3 mM (b). Thapsigargin (10 μM) was introduced into the bath 1 min prior to elevating [Ca²⁺]ₜ. The experimental protocol is presented schematically at the top. Calibration bars: horizontal, 18 μm; vertical, 0·64 s. B, time-dependent changes in fluorescence at the site of caffeine applications (marked in Aa and b by bars) at 1 mM [Ca²⁺]ₜ (black trace) and 3 mM [Ca²⁺]ₜ (grey trace). C, caffeine-induced Ca²⁺ transients measured before and after successive applications of thapsigargin and 3 mM Ca²⁺ to the bath. Caffeine (20 mM) was applied for 2 s. The lefthand trace is the control ([Ca²⁺]ₜ = 1 mM). The middle trace was recorded 3 and 2 min after introduction to the bath of thapsigargin (10 μM) and elevated Ca²⁺ (3 mM), respectively. The righthand trace was acquired 2 min after the middle trace in the continuous presence of thapsigargin and 3 mM Ca²⁺.
caffeine pulse. At this combination of concentration and exposure time, caffeine used for conditioning of the release channels could effectively reach the channels inside the cell but at the same time did not cause premature Ca\(^{2+}\) release on a massive scale. It can be seen that with 1 mM \([\text{Ca}^{2+}]_o\) the 10 mM caffeine pulse again induced only an abortive Ca\(^{2+}\) response, which died out within 30 \(\mu\text{m}\) of the site of caffeine application (Fig. 4Aa). However, following pre-exposure of the cell to 0·5 mM caffeine the release became regenerative and propagated through the entire cell (Fig. 4Ab). This was despite the fact that the SR Ca\(^{2+}\) content might have been slightly reduced in the presence of 0·5 mM caffeine, as suggested by the decreased amplitude of the local caffeine-induced \([\text{Ca}^{2+}]\) transient (Fig. 4C). Interestingly, the velocity of the waves recorded under these conditions (i.e. 0·5 mM caffeine and 1 mM \([\text{Ca}^{2+}]_o\)) was much lower than under our standard conditions with no caffeine and 3 mM \([\text{Ca}^{2+}]_o\) (31·4 ± 2·9 \((n = 12)\) vs. 46·0 ± 2·3 \((n = 25)\), \(P < 0·05\); Fig. 4B). Such low wave velocities at reduced Ca\(^{2+}\) loads evidently result from reduced diffusion of Ca\(^{2+}\) out of the

Figure 3. The effects of thapsigargin on \([\text{Ca}^{2+}]\) responses to focal applications of caffeine in cells preloaded with Ca\(^{2+}\) above normal levels

A, linescan images of fluorescence changes induced by focal applications of caffeine (10 mM) before (a) and 2 min after introducing of 10 \(\mu\text{M}\) thapsigargin to the bath (b). \([\text{Ca}^{2+}]_o\) was 3 mM. Calibration bars: horizontal, 18 \(\mu\text{m}\); vertical, 0·66 s. B, linear fits to the positions of the wave in Aa (●) and Ab (○) in sections defined by the arrows; the values of mean wave velocities are indicated next to the respective linear fits. C, time-dependent changes in fluorescence at the sites of caffeine application (marked in Aa and Ab by bars) in the presence (black trace) and absence (grey trace) of thapsigargin. D, \(\text{Ca}^{2+}\) transients induced by applications of caffeine before (lefthand trace) and after introduction to the bath of 10 \(\mu\text{M}\) thapsigargin (middle and righthand traces). Caffeine (20mM) was applied to the bath for 2 s at 2 min intervals; thapsigargin was applied 1 min prior to the second addition of caffeine.
wavefront due to a smaller wave amplitude (Stern, 1992). With 3 mM [Ca\(^{2+}\)]\(_{i}\), propagating Ca\(^{2+}\) waves could be triggered under control conditions, but preconditioning with 0.5 mM caffeine caused a significant increase in the velocity of the wave (51.0 ± 3.4 vs. 65.2 ± 3.8, n = 9, P < 0.05; Fig. 5A and B). Exposure to 0.5 mM caffeine for periods longer than 0.5–1 min resulted in a decrease in both propagation ability and magnitude of the Ca\(^{2+}\) responses below their respective control levels (not shown). These changes were evidently caused by a progressive loss of the SR Ca\(^{2+}\) content in the presence of caffeine.

To quantify the potentiatory effects of 0.5 mM caffeine on the release mechanism, we performed measurements from single cardiac SR Ca\(^{2+}\) release channels incorporated into lipid bilayers. Ca\(^{2+}\) release channels were recorded using symmetrical 300 mM CsMeSO\(_4\) in the presence of 3 mM cytosolic MgATP with 2 μM and 1 mM Ca\(^{2+}\) at the cytosolic and luminal sides of the channel, respectively. Figure 6 shows examples of traces acquired from a single channel before and after the addition of 0.5 mM caffeine to the cytosolic side of the channel. It can be seen that caffeine caused a dramatic potentiation of channel activity. On average, 0.5 mM caffeine increased the channel P\(_i\) measured for 1 min after addition of the drug by 400% (0.014 ± 0.003 vs. 0.056 ± 0.006, n = 5, P < 0.05). For comparison, increasing luminal Ca\(^{2+}\) from 20 μM to 2 mM under similar ionic conditions has been shown to increase P\(_i\) by 700% (Lukyanenko et al. 1996). Therefore, using 0.5 mM caffeine can indeed be regarded as a reasonable approximation of the effects of increased luminal Ca\(^{2+}\) on Ca\(^{2+}\) release channel activity.

**Effects of tetracaine**

To probe further the role of luminal Ca\(^{2+}\) in Ca\(^{2+}\) wave propagation, we examined the effects of the allosteric inhibitor of the RyR channel, tetracaine, on responses induced by focal caffeine applications. It has been shown that submaximal tetracaine concentrations have only a transient suppressing effect on spontaneous (i.e. Ca\(^{2+}\) sparks and waves) and electrically evoked Ca\(^{2+}\) release (Györke et al. 1997; Overend et al. 1997, 1998). The temporary nature of tetracaine inhibition has been attributed to the increased SR Ca\(^{2+}\) accumulation caused by the drug (presumably via inhibition of Ca\(^{2+}\) leakage through the Ca\(^{2+}\) release channels).

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**Figure 4.** The effects of sensitizing the release channels to cytosolic Ca\(^{2+}\) by low doses of caffeine on the ability of local releases to induce propagating Ca\(^{2+}\) waves under conditions of reduced SR Ca\(^{2+}\) load

A, linescan images of fluorescence changes induced by focal applications of caffeine (10 mM) before (a) and 20 s after introducing 0.5 mM caffeine to the bath (b). The experimental protocol is illustrated by the scheme at the top. [Ca\(^{2+}\)]\(_{i}\), was 1 mM. Calibration bars: horizontal, 18 μm; vertical, 0.66 s. B, linear fit to the position of the propagating Ca\(^{2+}\) wave for the section in A defined by the arrows; the value of the mean wave velocity (v) is indicated. C, time-dependent changes in fluorescence at the sites of caffeine application (marked in corresponding images in A by bars) with (black traces) and without (grey traces) conditioning by 0.5 mM caffeine.
One possibility is that the recovery of release in the presence of tetracaine is due to the increased amount of releasable Ca²⁺ (Györke et al. 1997; Overend et al. 1997). Such a compensation of blockage could only occur when the inhibition by the drug is partial and at least some release sites remain available for liberation of Ca²⁺. Alternatively, luminal Ca²⁺ alters the tetracaine sensitivity of the Ca²⁺ release channels through an allosteric mechanism, making the channels more resistant to tetracaine (Györke et al. 1997). With such a removal of blockage, a recovery of release

Figure 5. The effects of low doses of caffeine on Ca²⁺ wave propagation at increased SR Ca²⁺ load

A, linescan images of fluorescence changes induced by focal applications of caffeine (10 mM) before (a) and 20 s after introducing 0.5 mM caffeine to the bath (b), as illustrated by the scheme at the top. [Ca²⁺] was 3 mM. Calibration bars: horizontal, 18 μm; vertical, 0.66 s. B, linear fits to the positions of the Ca²⁺ waves for sections defined by arrows in corresponding images; the values of mean wave velocities are indicated. C, time-dependent changes in fluorescence at the sites of caffeine applications (marked in the corresponding images by bars) with (black traces) and without (grey traces) conditioning by 0.5 mM caffeine.

Figure 6. The effects of 0.5 mM caffeine on the activity of single cardiac RyR channels incorporated into lipid bilayers

A and B, representative single channel traces recorded before (A) and after (B) introduction of 0.5 mM caffeine into the cis chamber. Current recordings were obtained at −40 mV holding potential in symmetrical 350 mM CsCl·SO₄, pH 7.4. The cis chamber contained 3 mM MgATP and 2 μM free [Ca²⁺]. The trans chamber contained 1 mM Ca²⁺.
could occur even following a complete initial inhibition. The effects of tetracaine on caffeine-induced Ca²⁺ waves are summarized in Fig. 7. Exposure of the cells to 0.75 mM tetracaine for 1 min resulted in a complete inhibition of caffeine-induced Ca²⁺ release of all forms (Fig. 7Aa and b). However, further continuous incubation of the cells with the drug resulted in re-emergence of first localized (1 min, Fig. 7Ac) and then propagating Ca²⁺ releases (3 min, Fig. 7Ad). The amplitude of the fluorescence signal at the site of caffeine application was significantly increased compared with the reference situation (Fig. 7B). Similar results were obtained in eight other experiments. This is consistent with an increase in the SR Ca²⁺ content caused by tetracaine (Gyorke et al. 1997; Overend et al. 1997). The increased accumulation of Ca²⁺ in the SR in the presence of tetracaine was confirmed in experiments with application of caffeine to the entire cell (Fig. 7C). The magnitude of the caffeine-induced Ca²⁺ transients increased by 39% ± 7% (n = 4) after 3 min of incubation with 0.75 mM tetracaine. Based on these results we conclude that the recovery of release from inhibition in the presence of tetracaine is not simply due to a compensation of blockage by increased SR Ca²⁺ load. Instead, the release mechanism itself becomes altered in a way that makes it less sensitive to inhibition by tetracaine. These results further support the possibility that a modulation of the release mechanism by luminal Ca²⁺ may be involved in the generation of Ca²⁺ waves in cardiac myocytes.

**DISCUSSION**

Growing evidence suggests that the activity of Ca²⁺ release channels of the SR can be influenced by luminal Ca²⁺ (Fabiato, 1992; Sitsapesan & Williams, 1994; Bassani et al. 1995; Lukyanenko et al. 1996; Györke et al. 1997; Santana et al. 1997; Györke & Györke, 1998). The present study is the first systematic attempt to define the potential role of this mechanism in the generation and propagation of Ca²⁺ waves in cardiac myocytes under conditions of increased Ca²⁺ loading. Two specific hypotheses were considered: (1) Increased luminal Ca²⁺ sensitizes the Ca²⁺ release channels to cytosolic Ca²⁺, enhancing the ability of cytosolic Ca²⁺ to activate adjacent release sites via CICR. (2) Ca²⁺ transported from the wavefront into the adjacent SR elements raises luminal Ca²⁺ above a critical threshold level, resulting in activation of the release channels at a luminal site.

**Figure 7. The effect of tetracaine on [Ca] responses induced by focal caffeine applications**

*Panel A:* Time linescans of fluorescence changes acquired under control conditions (a) and at different times (1, 2 and 3 min; b, c and d, respectively) after addition to the bath of 0.75 mM tetracaine. [Ca²⁺]₀ was 3 mM. Calibration bars: horizontal, 10 μm; vertical, 0.33 s. *Panel B:* Time-dependent changes in fluorescence at the site of caffeine applications (marked by bars) for the images in A, as indicated. C, Ca²⁺ transients elicited by application of 20 mM caffeine to the whole bath before (lefthand trace) and 3 min after exposure of the cell to 0.75 mM tetracaine (righthand trace).
To trigger and measure propagating Ca\(^{2+}\) waves we used focal caffeine application in combination with linescan confocal Ca\(^{2+}\) imaging. We created conditions for the transition of localized Ca\(^{2+}\) releases into propagating Ca\(^{2+}\) waves by increasing the cellular Ca\(^{2+}\) load via elevation of extracellular [Ca\(^{2+}\)]. This procedure is known to increase [Ca\(^{2+}\)]\(_i\) in both cytosolic and SR luminal compartments of cardiac myocytes (Wier et al. 1987; Lukyanenko et al. 1996; and the present study). We found that selectively inhibiting the accumulation of extra Ca\(^{2+}\) into the luminal compartment by thapsigargin prevented the transition of non-propagating Ca\(^{2+}\) responses to propagating Ca\(^{2+}\) waves upon elevation of [Ca\(^{2+}\)]\(_i\). This result provides a direct experimental confirmation of the common assumption that increased SR Ca\(^{2+}\) content plays a critical role in the generation of propagating Ca\(^{2+}\) waves in Ca\(^{2+}\)-overloaded cardiac myocytes. We further found that inhibition of the SR Ca\(^{2+}\) uptake into the SR with thapsigargin did not prevent Ca\(^{2+}\) wave propagation in cells already preloaded with Ca\(^{2+}\) above normal levels. Moreover, the velocity of the waves was accelerated relative to the control conditions (Fig. 3). This result shows unequivocally that propagation of Ca\(^{2+}\) waves does not require Ca\(^{2+}\) uptake from the approaching wave front. This conclusion is consistent with the relatively weak temperature dependency of Ca\(^{2+}\) wave propagation (Engel et al. 1995) and the rapid kinetics of local [Ca\(^{2+}\)]\(_i\) rise during the wave (Cheng et al. 1996) shown recently in rat ventricular myocytes.

Another important finding of the present study was that sensitizing the release channels to cytosolic Ca\(^{2+}\) by low concentrations of caffeine dramatically augmented the ability of local releases to induce propagating Ca\(^{2+}\) waves. These results showed for the first time that propagating Ca\(^{2+}\) waves could be produced solely by enhancing the activity of the Ca\(^{2+}\) release channels without increasing the cellular Ca\(^{2+}\) load. These results are consistent with the hypothesis that potentiation of the Ca\(^{2+}\) release channel activity by elevated luminal Ca\(^{2+}\) may be involved in propagation of Ca\(^{2+}\) waves in cardiac myocytes. This hypothesis was further supported by the results of our experiments with the allosteric blocker of the RyR channel, tetracaine. We have recently demonstrated in lipid bilayer experiments that increased luminal Ca\(^{2+}\) modifies the RyRs in a fashion that makes them more sensitive to activation by cytosolic Ca\(^{2+}\) and more resistant to inhibition by cytosolic Ca\(^{2+}\) and tetracaine (Gyorke et al. 1997; Gyorke & Gyorke, 1998). In the present study we used a tetracaine resistance test to confirm that RyRs also become allosterically altered in intact myocytes upon increasing the SR Ca\(^{2+}\) load. We showed that tetracaine (0·75 mm) initially inhibited fully both localized and propagating responses triggered by focal caffeine applications. This inhibition was, however, completely reversed by the increased accumulation of Ca\(^{2+}\) in the SR caused by the drug (Fig. 7). We speculate that the tetracaine-resistant conformation with altered Ca\(^{2+}\) gating is the form of the channel that is involved in Ca\(^{2+}\) wave generation in cardiac myocytes.
normal E–C coupling and Ca\textsuperscript{2+} waves has been described previously (Cheng et al. 1993, 1996; Cannel et al. 1994). It has been shown that image enhancement analysis can reveal Ca\textsuperscript{2+} wave propagation by sequential activation of release sites (Cheng et al. 1996).

It is interesting to note that in Fig. 8b, the wave propagated only 10 μm when the threshold was 2 μm. The reason for termination is that there is a net depletion of calcium in the wavefront, i.e. \( q_{\text{rel}} - q_{\text{rem}} < 0 \). The wave will terminate when the local free calcium concentration falls below the firing threshold. Increasing the magnitude of the initial trigger signal while holding the firing threshold fixed resulted in increased wave propagation length (not shown). Our model therefore predicts that an abortive (i.e. intrinsically not self-sustaining) wave propagates only over the region in which the initial calcium release has a diffusive influence.

Our computer simulations indicate that even with fairly large Ca\textsuperscript{2+} releases and with parameters of the model favouring Ca\textsuperscript{2+} diffusion, a localized release fails to initiate a self-sustaining Ca\textsuperscript{2+} wave for thresholds \( \geq 1.3 \) μm. In rat ventricular myocytes under normal SR Ca\textsuperscript{2+} loading conditions, the cytosolic Ca\textsuperscript{2+} concentration required for activation of a Ca\textsuperscript{2+} spark has been estimated to be close to 15 μm (Cannel et al. 1994). This value is consistent with the low Ca\textsuperscript{2+} sensitivity of the RyR channels (\( K_D \approx 30 \) μm; Gyorke & Gyorke, 1998) in reconstitution experiments in the presence of physiological concentrations of Mg\textsuperscript{2+} and ATP. Studies from our laboratory have shown that increased luminal [Ca\textsuperscript{2+}] enhances the sensitivity of the RyR to cytosolic [Ca\textsuperscript{2+}] (Lukyanenko et al. 1996; Gyorke & Gyorke, 1998). In particular, elevating luminal Ca\textsuperscript{2+} from 0.2–1 to 5–10 mM induced a 2– to 7-fold increase in the \( P_o \) of RyRs activated by cytosolic [Ca\textsuperscript{2+}] (Gyorke & Gyorke, 1998). Values for resting free [Ca\textsuperscript{2+}]\textsubscript{SR} have been estimated to be in the 0.7–1.5 mM range at loading conditions that can be considered normal (Chen et al. 1996; Shannon & Bers, 1997). Under conditions of increased cellular Ca\textsuperscript{2+} load, free [Ca\textsuperscript{2+}]\textsubscript{SR} can rise to 5 mM (Chen et al. 1996). Such an elevation in [Ca\textsuperscript{2+}]\textsubscript{SR} would be expected to augment RyR channel activity, bringing the threshold of activation of the RyRs by cytosolic Ca\textsuperscript{2+} closer to the range that is required for Ca\textsuperscript{2+} wave propagation according to our simulations. Therefore, the results of our Ca\textsuperscript{2+} wave simulations suggest that increased amounts of releasable Ca\textsuperscript{2+} alone may not be sufficient to account for the transition of local releases into propagating Ca\textsuperscript{2+} waves under conditions of increased SR Ca\textsuperscript{2+} loads. However, sensitization of the release sites to activating Ca\textsuperscript{2+} can effectively promote the generation of self-sustaining Ca\textsuperscript{2+} waves.

Taken together, the results of the present study suggest that the potentiation of SR Ca\textsuperscript{2+} release channels by luminal Ca\textsuperscript{2+} is an important factor in the generation of self-sustaining Ca\textsuperscript{2+} waves in rat ventricular myocytes. Ca\textsuperscript{2+} wave propagation does not require the translocation of Ca\textsuperscript{2+} from

![Figure 8. The dependence of theoretical [Ca\textsuperscript{2+}], responses on the threshold of release site Ca\textsuperscript{2+} activation](image-url)

\( a-d \), simulated linescan images of [Ca\textsuperscript{2+}], responses at different Ca\textsuperscript{2+} activation thresholds (5, 2, 1.3 and 0.5 μm, respectively). The [Ca\textsuperscript{2+}], responses were triggered by a Ca\textsuperscript{2+} elevation produced by an exponentially decaying release flux with a peak amplitude of 30 mM s\textsuperscript{-1} and decay time constant of 2 ms in a region with a diameter of 6 μm. The simulation included 50 individual release sites with a diameter of 0.1 μm each spaced at intervals of 2 μm, 2 spatially homogeneous removal fluxes (SR Ca\textsuperscript{2+} pumps and soluble buffers), diffusible dye and the Ca\textsuperscript{2+} dye complex. The values of the mean wave velocities (μm s\textsuperscript{-1}) in \( c \) and \( d \) are indicated.
the spreading wave front into the SR. Instead it appears to rely on the steadier effects of luminal Ca\(^{2+}\), consisting of sensitization of the Ca\(^{2+}\) release channels to cytosolic Ca\(^{2+}\). These results may have implications for understanding mechanisms of Ca\(^{2+}\) waves in other cell types where modulatory effects of luminal Ca\(^{2+}\) on the release mechanism have been implicated (Nunn & Taylor, 1992).

**References**


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