Temperature Dependence of Ca^{2+} Wave Properties in Cardiomyocytes: Implications for the Mechanism of Autocatalytic Ca^{2+} Release in Wave Propagation

Jutta Engel, Andrew J. Sowerby, Simon A. E. Finch, Martin Fechner, and Anton Stier
Max Planck Institute for Biophysical Chemistry, Department of Spectroscopy, 37018 Göttingen, Germany

ABSTRACT Digital imaging microscopy of fluo-3 fluorescence was used to study the velocity and shape of intracellular Ca^{2+} waves in isolated rat cardiomyocytes as a function of temperature. Decreasing the temperature from 37 to 17°C reduced the longitudinal wave velocity by a factor of 1.8 and remarkably slowed the decay of $[Ca^{2+}]_i$ in the trailing flank of a wave. Using image analysis, rise times, and half-maximum decay times of local Ca^{2+} transients, which characterize the processes of local Ca^{2+} release and removal, were determined as a function of temperature. Apparent activation energies for wave front propagation, local Ca^{2+} release, and local Ca^{2+} removal were derived from Arrhenius plots and amounted to −23, −28, and −46 kJ/mol, respectively. The high activation energy of Ca^{2+} removal, which arises from the activity of the sarcoplasmic reticulum (SR) Ca^{2+} ATPase, relative to those of longitudinal wave propagation and local Ca^{2+} release excludes the hypothetical mechanism of regenerative "spontaneous Ca^{2+} release," in which Ca^{2+} that has been taken up from the approaching wavefront triggers Ca^{2+} release at a luminal site of the SR. It is consistent, however, with the hypothesis that Ca^{2+} wave propagation is based on Ca^{2+}-induced Ca^{2+} release where Ca^{2+} triggers release on the cytosolic face of the SR.

INTRODUCTION

During the last few years, considerable progress has been made in the understanding of spatio-temporal aspects of Ca^{2+} signaling because of the development of fluorescent Ca^{2+} dyes and the improvement of fluorescence imaging techniques. Propagating sites of elevated free Ca^{2+} concentration ($[Ca^{2+}]_i$), so called Ca^{2+} waves, have been found in many different cell types (for review see Jaffe, 1993), among which are cardiomyocytes. Although it has remained unclear whether Ca^{2+} waves occur in normal cardiomyocytes in vivo (Lakatta, 1992) they have been intensively investigated (Takamatsu and Wier, 1990; Ishide et al., 1990, 1992; Wier and Blatter, 1991; Grouselle et al., 1991; Williams et al., 1992; Williams, 1993; Lipp and Niggli, 1993; Engel et al., 1994) because they yield information about the Ca^{2+} homeostasis of these cells. Another reason for studying Ca^{2+} waves is their possible involvement in pathological phenomena such as ischemia or arrhythmia (Stern et al., 1988; Lakatta, 1992; Fabiato, 1992).

Ca^{2+} waves are generally assumed to propagate by regenerative coupling of autocatalytic release of Ca^{2+} from intracellular stores and Ca^{2+} diffusion (for review see Jaffe, 1993). The exact mechanism, however, by which Ca^{2+} waves propagate through cardiomyocytes has not been resolved. Two models of wave propagation (Wier and Blatter, 1991) based on two types of autocatalytic Ca^{2+} release from the sarcoplasmic reticulum (SR), which were first observed in skinned cardiac cells and termed Ca^{2+}-induced Ca^{2+} release (CICR) and spontaneous Ca^{2+} release (SCR) (Fabiato, 1985a, b), exist.

CICR was defined as "Ca^{2+} release triggered by a rapid increase of $[Ca^{2+}]_i$ to about 0.6 μM at the outer surface of the SR of a previously quiescent skinned cell" (Fabiato, 1985a, b; 1992). The amount of Ca^{2+} released by CICR was found to be proportional to the rate of change of $[Ca^{2+}]_i$ and to be inhibited at Ca^{2+} concentrations above 1 μM (Fabiato, 1985a).

The second type of autocatalytic release of Ca^{2+} from the SR was observed when a skinned cell was exposed to a solution containing 7 μM Ca^{2+} (Fabiato, 1985b). In contrast to CICR, SCR occurred after a delay of up to several hundred milliseconds after increasing the Ca^{2+} concentration of the bath solution. SCR was thus explained to result from an overloaded SR in which Ca^{2+} triggered release at a luminal site of the SR. The delay in release was attributed to pumping Ca^{2+} inside the lumen by the SR Ca^{2+} ATPase (Fabiato, 1985a, b, 1992).

From these observations, it was concluded that "SCR and CICR do not occur through the same mechanism" (Fabiato, 1985b, 1992). The fact that the frequency of Ca^{2+} waves rises with increasing $[Ca^{2+}]_i$ (Fabiato, 1985b, 1992; Stern et al., 1988, Grouselle et al., 1991; Williams et al., 1992) led to the interpretation that Ca^{2+} waves are merely a consequence of SR overload, i.e., based on SCR/diffusion coupling (Fabiato, 1985b, 1992). The mechanism of a wave relayed by SCR would be the following (Wier and Blatter, 1991):

1) Locally, overloaded SR spontaneously releases Ca^{2+};
2) Ca^{2+} released diffuses radially and is taken up by Ca^{2+} pumps into SR that is almost overloaded;

Received for publication 25 May 1994 and in final form 16 September 1994.

Address reprint requests to Dr. Jutta Engel, Georg August University, Institute of Physiology, Department of Neurophysiology, Humboldtallee 23, 37073 Göttingen, Germany. Tel.: 49-551-395913; Fax: 49-551-395923; E-mail: je@helena.ukps.gwdg.de.

Dr. Engel's current address: Georg August University, Institute of Physiology, Dept. of Neurophysiology, Humboldtallee 23, 37073 Göttingen, Germany.

0006-3495/95/01/40/06 $2.00

3) In these parts of the SR, SCR is triggered, and steps 1–3 repeat;
4) Ca^{2+} is pumped back into the SR.

The alternative model for Ca^{2+} wave propagation is based on the coupling of CICR and diffusion as follows:

1) Locally, $[Ca^{2+}]_i$ rises because of influx through the sarcolemma, or an overloaded part of the SR spontaneously releases Ca^{2+};
2) This Ca^{2+} reaches SR release channels by diffusion, triggers Ca^{2+} release by acting at the outside of the SR, and the whole process repeats;
3) Ca^{2+} is pumped back into SR by Ca^{2+} ATPases.

The SCR hypothesis has been a matter of controversy (Wier and Blatter, 1991; Stern, 1992; Jaffe, 1993; Williams, 1993). Unfortunately, it cannot be proven directly, because no technique of imaging the Ca^{2+} concentration inside the SR in situ with high temporal resolution is available so far. Recently, we have studied the anisotropy of Ca^{2+} wave propagation in cardiomyocytes (Engel et al., 1994). By investigating the temperature dependence of transverse and longitudinal wave velocity, we tried to find out whether different direction-dependent processes cause anisotropic wave propagation. Here we characterize several wave parameters in more detail to compare their temperature dependence and to gain support for one of the above delineated models of wave propagation. It turns out that the velocity of wave propagation is much less affected by a decrease in temperature than is the rate of Ca^{2+} reuptake into the SR, suggesting that Ca^{2+} triggers autocatalytic Ca^{2+} release on a cytosolic rather than luminal site of the SR.

MATERIALS AND METHODS

Preparation of cells and labeling

Ventricular cardiomyocytes were isolated from adult Sprague-Dawley rats by Langendorff perfusion with collagenase solution as described previously (Engel et al., 1994). Cells were plated into plastic dishes where they attached to glass coverslips; they were used within 30 h. The cells were loaded with 2 μM Fluo-3 AM (Molecular Probes, Eugene, OR) for 30 min at 22°C, washed, and incubated for 1 h to allow for dye hydrolysis. Coverslips were mounted into a 20 μl perfusion chamber and continuously superfused with a HEPES buffer (in mM): NaCl 125, KCl 2.6, $CaCl_2$ 1, $MgSO_4$ 1.2, glucose 10, HEPES 20, pH 7.4, 300 mOsm. For measurements at 17°C, the room was cooled by air conditioning. Otherwise, chamber and microscope stage were heated to 27 or 37°C with an air stream incubator (Zeiss, Oberkochen, Germany).

Ca^{2+} measurements

On binding Ca^{2+}, the fluorescence intensity of fluo-3 changes by a factor of 40 (Minta et al., 1989). Therefore, an increase in fluorescence intensity corresponds to an increase in $[Ca^{2+}]_i$. Measurements of fluo-3 fluorescence were performed as described previously (Engel et al., 1994). Briefly, a Zeiss Axiomat was used in the epifluorescence mode with the 488 nm line of an argon laser (Series 2000, Spectra Physics, Mountain View, CA) as light source. Emitted fluorescence was detected by a low light level intensifying video camera (VIM C2400, Hamamatsu Photonics Europa GmbH, Seefeld, Germany). Images were acquired at video rate (25 Hz interlaced) and recorded on video tape by a SuperVHS video recorder (Panasonic; Matsushita Electric Industrial Co., Osaka, Japan). Video frames were digitized by a frame grabber (VTE Digital Braunschweig, Germany) and transferred to a Silicon Graphics Workstation 4D/35 (Silicon Graphics Inc., Mountain View, CA). Processing of the images (512x795 pixels, 8 bit) was carried out with user-written routines based on the software IDL (Research Systems Inc., Boulder, CO). Each series of images displaying a Ca^{2+} wave (varying from 25 to 65 images) was scaled between pixel intensity 0 (background fluorescence) and 255 (maximum fluorescence in that series). Shaded surface plots and fluorescence intensity profiles were obtained after smoothing the images using a box car averaging procedure of 8 or 11 pixels (1.4 or 1.9 μm) width, respectively.

RESULTS

Fluo-3-loaded myocytes were monitored for spontaneously generated Ca^{2+} waves at 17, 27 or 37°C for 2 min. Each cell was subjected to only one temperature to exclude possible side effects of fast temperature changes on its Ca^{2+} homeostasis. At each temperature, cells of at least three different preparations were examined. Only cells with clear morphology (without branches) that exhibited linear, longitudinal Ca^{2+} waves were selected for evaluation.

Ca^{2+} waves were observed at all temperatures examined. Changing the temperature from 37 to 17°C affected both wave velocity and shape. Fig. 1 shows longitudinal Ca^{2+} waves in isolated myocytes recorded at 37°C and at 17°C as surface plots i.e. the fluorescence intensity of fluo-3 is encoded in the height of the surface. Both Ca^{2+} waves started at one end of the cell and traveled to the opposite end where they disappeared. The wave recorded at 37°C traveled much faster than that recorded at 17°C (93 μm/s versus 46 μm/s). In addition, the waves differed markedly in their length: the wave at 17°C was much longer than that at 37°C, indicating that the removal of Ca^{2+} from the cytosol was considerably slower at 17°C.

In order to compare wave shapes as a function of temperature, longitudinal sections through fluorescence images of cells exhibiting linear Ca^{2+} waves from one end to the other were made. Fig. 2 shows wave propagation in terms of series of longitudinal fluorescence intensity profiles in three cells at 17, 27, and 37°C. The temporal distance between consecutive fluorescence profiles varies from 120 ms for the wave recorded at 37°C (A) to 240 ms for the one recorded at 17°C (C) because of the temperature-dependent slowing of wave propagation. Wave velocities are 99, 86, and 63 μm/s at 37, 27, and 17°C, respectively.

Changing the temperature from 37 to 17°C decreased the slope of the $[Ca^{2+}]_i$ increase at the wave front only slightly. However, the extension of the wave crest increased remarkably. Having left the focus, all three waves traveled at relatively constant width—30, 46, and 72 μm at 37, 27, and 17°C, respectively—through the cell. This indicates that Ca^{2+} removal on the trailing edge of the wave is very sensitive to changes in temperature.

To quantify the processes of Ca^{2+} release and removal in Ca^{2+} waves, we analyzed the kinetics of local Ca^{2+} transients (Fig. 3). For this purpose, cross sections through fluorescence images of cardiomyocytes were defined and the fluorescence intensity was integrated along this line (width 1

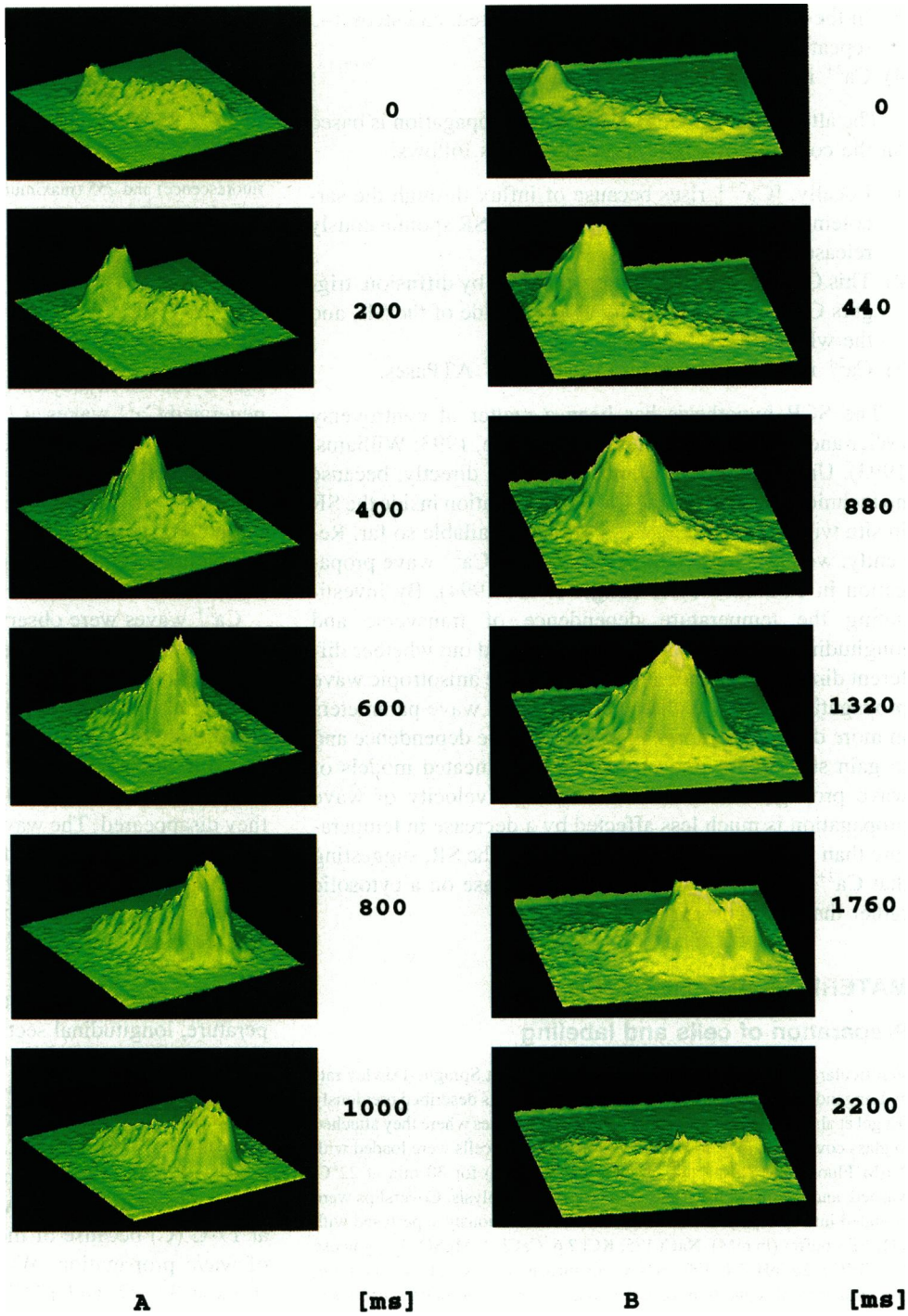

FIGURE 1 Propagation of Ca^{2+} waves in fluo-3-loaded cardiomyocytes under normothermic (37°C, *A*) and hypothermic (17°C, *B*) conditions. Consecutive surface plots of fluorescence intensity of scaled images are shown. Times (in ms) are respectively indicated in the right margin. Side lengths of the image planes are 140 and 94 μm.

pixel, Fig. 3 *A*, *inset*) as a function of time. This yielded the local kinetics of Ca^{2+} release and removal. Fig. 3 *A* shows local transients of three typical Ca^{2+} waves at 37, 27, and 17°C. The rate of Ca^{2+} release was less affected by a temperature change than that of Ca^{2+} removal. To describe the process of Ca^{2+} release, we used the local rise time, t_r, which is the time span from the beginning of fluorescence increase to maximum fluorescence as shown in the sketch in Fig. 3 *B*. To characterize the process of Ca^{2+} removal, the half-maximum decay time of fluorescence intensity, $t_{1/2}$, was chosen (Fig. 3 *B*).

A summary of the dependences of characteristic Ca^{2+} wave parameters such as longitudinal wave velocity, v_l, local rise time, t_r, and local half-maximum decay times, $t_{1/2}$, upon temperature is given in Table 1. Wave velocities were obtained as described previously (Engel et al., 1994). Rise time and half-maximum decay time were determined in triplicate from each cell by using fluorescence integrals from three equidistant transverse sections (which divided a cell in 4 parts) and were averaged. From Table 1 follows that propagation velocity decreased by a factor of 1.8 upon decreasing the temperature from 37 to

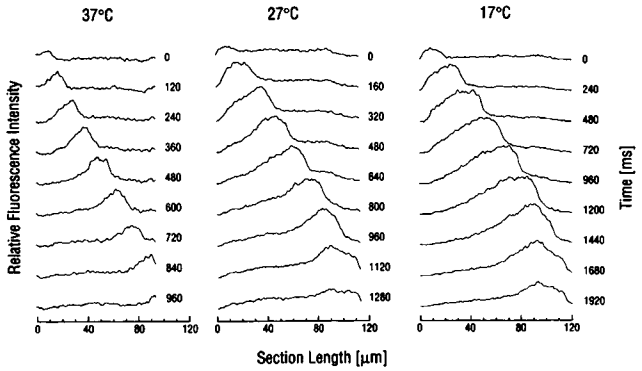

FIGURE 2 Longitudinal propagation of three representative Ca^{2+} waves recorded at 37, 27, and 17°C (*A–C*). Fluorescence intensity profiles obtained along a central longitudinal section in every cell are plotted for different times. Note that the temporal distance between consecutive intensity profiles is different for the three temperatures because of considerable slowing of both wave velocity and wave decay on lowering the temperature.

17°C, whereas the local decay time was much more affected, being 3.5-fold increased.

To compare the dependences of wave propagation velocity, Ca^{2+} release, and Ca^{2+} removal upon temperature, Arrhenius plots were derived from the data given in Table 1. In general, an Arrhenius plot shows the dependence of a reaction time constant on a logarithmic scale upon the inverse temperature. Unfortunately, the processes mentioned above are rather complex and cannot be described by single reaction time constants. Nevertheless, an Arrhenius plot of a catenated series of reactions can often yield useful information about rate-limiting reaction steps (Johnson et al., 1974). Here we used the longitudinal wave velocity, the rise time constant, $k_r = 1/t_r$, and the half-maximum decay time constant, $k_{1/2} = 1/t_{1/2}$, to describe the complex processes of wave propagation, local Ca^{2+} release, and local Ca^{2+} removal. Fig. 4 shows that the logarithms of the mean values of v_l, k_r, and $k_{1/2}$ could be approximated by straight lines in the temperature range 17–37°C. Apparent activation energies E_a were calculated from the slope of the regression line according to the Arrhenius equation:

$$k = k_0 \exp(-\Delta E_a/RT), \qquad (1)$$

with k being the time constant of the respective process, k_0 a constant, R the gas constant, and T the absolute temperature (Johnson et al., 1974). Apparent activation energies were −23 kJ/mol for longitudinal wave propagation, −28 kJ/mol for local Ca^{2+} release, and −46 kJ/mol for local Ca^{2+} removal.

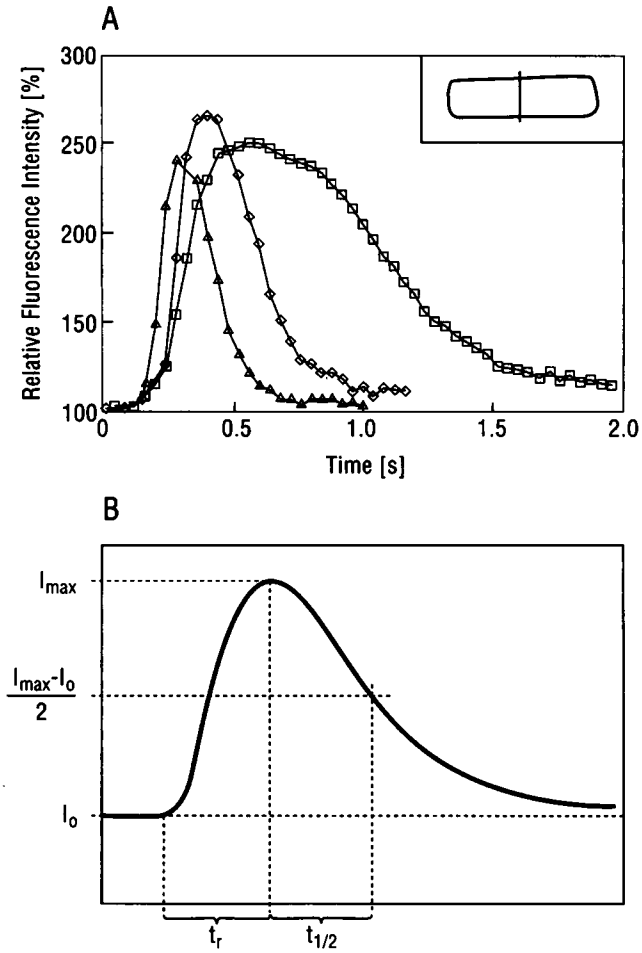

FIGURE 3 Temperature dependence of local Ca^{2+} release and reuptake kinetics in Ca^{2+} waves. (*A*) In three representative cells, the fluorescence intensity was integrated along a central cross section of the cell (see *insert*) as a function of time. Symbols refer to different temperatures: (△) 37, (◇) 27, and (□) 17°C. (*B*) Schematic representation of a local Ca^{2+} transient in *A* to illustrate the definition of local rise time, t_r, and half-maximum decay time, $t_{1/2}$.

TABLE 1 Temperature dependence of mean longitudinal wave velocity, mean rise time, and mean half-maximum decay time of the local Ca^{2+} transient

Temperature (°C)	Longitudinal velocity (v_l) (μm/s)	Rise time (t_r) (ms)	Half-maximum decay time ($t_{1/2}$) (ms)
17	56 ± 16 (18)	356 ± 31 (13)	597 ± 169 (13)
27	75 ± 11 (20)	259 ± 45 (14)	288 ± 79 (14)
37	103 ± 14 (17)	169 ± 24 (15)	172 ± 34 (15)

For definition of t_r and $t_{1/2}$, see Fig. 3 *B*. Data represent mean ± SD (number of waves).

DISCUSSION

In this study we have analyzed the temperature dependence of three parameters of a Ca^{2+} wave: longitudinal wave velocity, rise time constant, and half-maximum decay time constant of the local Ca^{2+} transient. Our aim was to compare the temperature dependences of wave front propagation velocity and of the process of local Ca^{2+} release with the process of active Ca^{2+} reuptake by the SR, and to decide whether Ca^{2+} waves propagate by a mechanism in which Ca^{2+} regeneratively triggers Ca^{2+} release from an overloaded SR at a luminal site.

Each of the three wave parameters arises from several elementary processes rather than describing a single

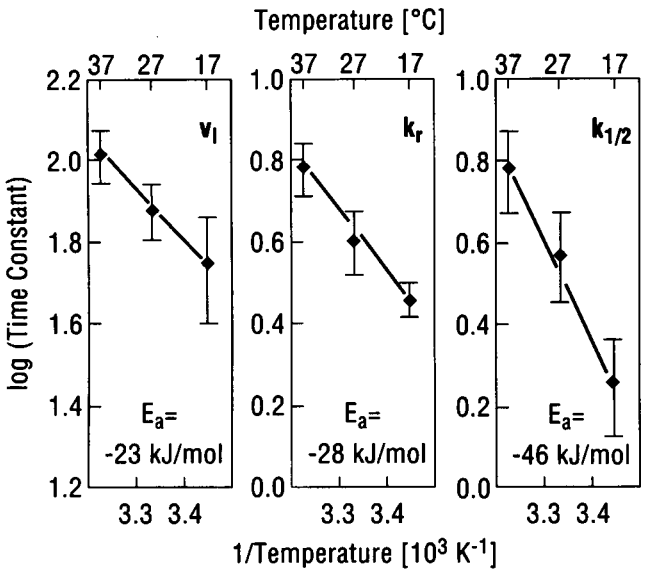

FIGURE 4 Arrhenius plots of longitudinal wave velocity v_l (*left*), rise time constant k_r (*center*), and decay time constant $k_{1/2}$ (*right*). Linear regression yielded the apparent activation energies indicated in the plots.

chemical reaction: the rise time constant, k_r, mainly reflects local Ca^{2+} release from the SR caused by previous binding of Ca^{2+} to a catalytic (cytosolic or luminal) site of the ryanodine receptor. Its apparent activation energy of −28 kJ/mol arises from the combination of at least two processes: channel opening and ion flow. Activation energies of channel opening, which involve conformational changes, may be quite high (−80 kJ/mol, Hille, 1992). In contrast, ion flow through an ion channel has a comparatively low activation energy of about −20 to −30 kJ/mol (Hille, 1992). This suggests that the flow of Ca^{2+} ions through the channel is the rate-limiting step in the temperature range considered.

The second local parameter, $k_{1/2}$, summarizes Ca^{2+} reuptake into the SR by the SR Ca^{2+} ATPase and Ca^{2+} extrusion out of the cell. The latter process occurs mainly via the Na^+/Ca^{2+} exchanger (Sipido and Wier, 1991). Unfortunately, the ratio of the transport capacities of the two processes in a Ca^{2+} wave is not known. From experiments with electrically stimulated cardiomyocytes, it has been estimated that less than 25% of the total Ca^{2+} in a Ca^{2+} transient enters the cell during depolarization, whereas 75–90% is released from the SR (Bers and Bridge, 1989; Sipido and Wier, 1991). Hence, in the steady state about 20% of the Ca^{2+} must be extruded from the cell after each stimulation (Powell and Noble, 1989). This percentage is probably even less in a Ca^{2+} wave. For simplicity, we will neglect Ca^{2+} extrusion from the cell in the following discussion, so $k_{1/2}$ is assumed to reflect only the activity of the Ca^{2+} ATPase of the SR. This is supported by the notion that its activation energy is in the same range as that determined for pumping Ca^{2+} by the SR Ca^{2+} ATPase (−49 kJ/mol, Squier et al., 1988), suggesting that $k_{1/2}$ reflects the activity of this enzyme.

Finally, the longitudinal velocity of a Ca^{2+} wave depends on the rate of Ca^{2+} release and the amount of Ca^{2+} released at each site, on the distance between successive release regions, and on the effective diffusion coefficient for Ca^{2+} in the myoplasm (Stern, 1992). Unfortunately, quantitative data regarding the regulation of the release channels and their ultrastructural organization are still rather limited. For simplicity, we consider a Ca^{2+} wave in a myocyte as a chemical wave in an excitable medium. The longitudinal velocity of such a wave is given approximately by

$$v_l \sim (k_{as} D)^{1/2}, \tag{2}$$

where k_{as} is the rate constant of the autocatalytic step and D is the diffusion coefficient of the autocatalytic species (Tilden, 1974; Kuhnert et al., 1985; Foerster et al., 1990). Taking into account the temperature dependence of D and k_a, respectively, one arrives at

$$E_v = \tfrac{1}{2}(E_d + E_{as}), \tag{3}$$

where E_v is the apparent activation energy of longitudinal wave propagation, E_d the activation energy of diffusion, and E_{as} the activation energy of the autocatalytic step (Kuhnert et al., 1985; Foerster et al., 1990). It is interesting to note that when inserting $E_v = -23$ kJ/mol from the Arrhenius plot (Fig. 4 *A*) and $E_d = -18$ kJ/mol for Ca^{2+} diffusion (Erdey-Grüz, 1974) in Eq. 3, one arrives at $E_{as} = -28$ kJ/mol, a value we found experimentally for the apparent activation energy of local Ca^{2+} release (Fig. 4 *B*). This suggests that despite the complex ultrastructure of the cytoplasm, it may be useful to apply the theory of chemical waves to cytoplasmic second messenger waves, as has been done successfully for Ca^{2+} waves in oocytes (Lechleiter and Clapham, 1992).

In the SCR model, autocatalytic Ca^{2+} release is triggered at a luminal site of the SR. This requires that at the front of the Ca^{2+} wave, Ca^{2+} has first to be taken up by the SR, where it triggers release above a certain threshold concentration of luminal Ca^{2+}. According to this model and in view of the high activation energy of Ca^{2+} pumping into the SR, one expects that both local Ca^{2+} release and wave velocity are similarly affected by a decrease in temperature as is the rate of Ca^{2+} reuptake. However, this is not the case: propagation velocity decreases by a factor of only 1.8 and the rate of local Ca^{2+} release by a factor of 2.1, whereas the reuptake rate decreases by a factor of 3.5 on changing the temperature from 37 to 17°C. We thus conclude that SCR is not the mechanism of regenerative Ca^{2+} wave propagation. In contrast, the results presented here support the CICR hypothesis, i.e., that Ca^{2+} release is triggered at a cytosolic site of the SR release channel.

The experiments with skinned cardiac cells (Fabiato, 1985b, 1992) mentioned in the Introduction, as well as the fact that the frequency of Ca^{2+} waves rises with an increasing cytosolic Ca^{2+} concentration—which certainly also leads to a higher luminal Ca^{2+} concentration (Grouselle et al., 1991; Williams et al., 1992)—suggest that SCR does exist in cardiomyocytes. It seems likely that SCR initiates Ca^{2+} waves by spontaneous local Ca^{2+} release from an overloaded part of the SR, whereas propagation of the wave is based on regenerative CICR. Interestingly, CICR does not seem to be

independent of the luminal Ca^{2+} concentration. A certain supranormal Ca^{2+} SR load has been reported to be necessary to support wave propagation (Trafford et al., 1993). Theoretical considerations (Stern, 1992) indicate that the loading state of the SR might also modulate CICR in that differences in the loading state of the SR may be responsible for the variety of Ca^{2+} amplitudes and propagation velocities seen in Ca^{2+} waves at a given temperature.

We wish to thank Bianca Kellner and Caterina Habermann for excellent technical assistance and Stefan C. Müller, Thomas Heimburg, and Detlev Schild for stimulating discussions.

This work was supported by the Deutsche Forschungsgemeinschaft (SFB 330) and the Association for International Cancer Research (U.K.).

REFERENCES

Bers, D. M., and J. H. Bridge. 1989. Relaxation of rabbit ventricular muscle by Na-Ca exchange and sarcoplasmic reticulum calcium pump. *Circ. Res.* 65:334–342.

Engel, J., M. Fechner, A. J. S. Sowerby, S. A. E. Finch, and A. Stier. 1994. Anisotropic propagation of Ca^{2+} waves in isolated cardiomyocytes. *Biophys. J.* 66:1756–1762.

Erdey-Grüz, T. 1974. Transport Phenomena in Aqueous Solutions. Adam Hilger, London. 194 pp.

Fabiato, A. 1985a. Time- and calcium dependence of activation and inactivation of calcium-induced release of calcium from the sarcoplasmic reticulum of a skinned canine cardiac Purkinje cell. *J. Gen. Physiol.* 85: 247–289.

Fabiato, A. 1985b. Simulated calcium current can both cause calcium loading in and trigger calcium release from the sarcoplasmic reticulum of a skinned canine cardiac Purkinje cell. *J. Gen. Physiol.* 85:291–320.

Fabiato, A. 1992. Two kinds of calcium-induced release of calcium from the sarcoplasmic reticulum of skinnèd cardiac cells. *In* Excitation-Contraction Coupling in Skeletal, Cardiac and Smooth Muscle. G. B. Frank, editor. Plenum Press, New York. 245–262.

Foerster, P., S. C. Müller, and B. Hess. 1990. Temperature dependence of curvature-velocity relationship in an excitable Belousov-Zhabotinskii reaction. *J. Phys. Chem.* 94:8859–8861.

Grouselle, M., B. Stuyvers, S. Bonoron-Adele, P. Besse, and D. Georgescauld. 1991. Digital-imaging microscopy analysis of calcium release from sarcoplasmic reticulum in single rat cardiac myocytes. *Pflügers Arch.* 418:109–119.

Hille, B. 1992. Ionic Channels of Excitable Membranes. Sinauer Associates Inc., Sunderland, MA. 50 pp., 329 pp.

Ishide, N., M. Miura, M. Sakurai, and T. Takishima. 1992. Initiation and development of calcium waves in rat myocytes. *Am. J. Physiol.* 263: H327–H332.

Ishide, N., T. Urayama, K. I. Inoue, T. Komaru, and T. Takishima. 1990. Propagation and collision characteristics of calcium waves in rat myocytes. *Am. J. Physiol.* 259:H940–H950.

Jaffe, L. F. 1993. Classes and mechanisms of calcium waves. *Cell Calcium* 14:736–745.

Johnson, F. H., H. Eyring, and B. J. Stover. 1974. The Theory of Rate Processes in Biology and Medicine. John Wiley & Sons, New York. 169 pp.

Kuhnert, L., H.-J. Krug, and L. Pohlmann. 1985. Velocity of trigger waves and temperature dependence of autowave processes in the Belousov-Zhabotinsky reaction. *J. Phys. Chem.* 89:2022–2026.

Lakatta, E. 1992. Functional implications of spontaneous sarcoplasmic reticulum Ca^{2+} release in the heart. *Cardiovasc. Res.* 26:193–214.

Lechleiter, J. D., and D. Clapham. 1992. Molecular mechanisms of intracellular calcium excitability in *X. laevis* oocytes. *Cell.* 69:283–294.

Lipp, P., and E. Niggli. 1993. Microscopic spiral waves reveal positive feedback in subcellular calcium signaling. *Biophys. J.* 65: 2272–2276.

Minta, A., J. P. Y. Kao, and R. Y. Tsien. 1989. Fluorescent indicators for cytosolic calcium based on rhodamine and fluorescein chromophores. *J. Biol. Chem.* 264:8171–817.

Powell, T., and D. Noble. 1989. Calcium movements during each heart beat. *Mol. Cell. Biochem.* 89:103–108.

Sipido, K. R., and W. G. Wier. 1991. Flux of Ca^{2+} across the sarcoplasmic reticulum of guinea-pig cardiac cells during excitation-contraction coupling. *J. Physiol.* 435:605–630.

Squier, T. C., D. J. Bigelow, and D. D. Thomas. 1988. Lipid fluidity directly modulates the overall protein rotational mobility of the Ca-ATPase in sarcoplasmic reticulum. *J. Biol. Chem.* 263:9178–9186.

Stern, M. D. 1992. Theory of excitation-contraction coupling in cardiac muscle. *Biophys. J.* 63:497–517.

Stern, M. D., M. C. Capogrossi, and E. G. Lakatta. 1988. Spontaneous calcium release from the sarcoplasmic reticulum in myocardial cells: mechanisms and consequences. *Cell Calcium.* 9:247–256.

Takamatsu, T., and W. G. Wier. 1990. Calcium waves in mammalian heart: quantification of origin, magnitude, waveform, and velocity. *FASEB J.* 4:1519–1525.

Tilden, J. 1974. On the velocity of spatial wave propagation in the Belousov reaction. *J. Chem. Phys.* 60:3349–3350.

Trafford, A. W., S. C. O. O'Neill, and D. A. Eisner. 1993. Factors affecting the propagation of locally activated systolic Ca transients in rat ventricular myocytes. *Pflügers Arch.* 425:181–183.

Wier, W. G., and L. A. Blatter. 1991. Ca^{2+}-oscillations and Ca^{2+}-waves in mammalian cardiac and vascular smooth muscle cells. *Cell Calcium.* 12:241–254.

Williams, D. A. 1993. Mechanisms of calcium release and propagation in cardiac cells. Do studies with confocal microscopy add to our understanding? *Cell Calcium.* 14:724–735.

Williams, D. A., L. M. Delbridge, S. H. Cody, P. J. Harris, and T. O. Morgan. 1992. Spontaneous and propagated calcium release in isolated cardiac myocytes viewed by confocal microscopy. *Am. J. Physiol.* 262: C731–C742.