Local control of excitation–contraction coupling in rat heart cells

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1. Cytosolic free calcium ion concentration ($[Ca^{2+}]_i$) and whole-cell L-type Ca^{2+} channel currents were measured during excitation–contraction (E–C) coupling in single voltage-clamped rat cardiac ventricular cells. The measurements were used to compute the total cellular efflux of calcium ions through sarcoplasmic reticulum (SR) Ca^{2+} release channels ($F_{SR,rel}$) and the influx of Ca^{2+} via L-type Ca^{2+} channels ($F_{I_{Ca}}$).

2. $F_{SR,rel}$ was elicited by depolarizing voltage-clamp pulses 200 ms in duration to membrane potentials from −30 to +80 mV. Over this range, peak $F_{SR,rel}$ had a bell-shaped dependence on clamp pulse potential. In all cells, the 'gain' of the system, measured as the ratio, $F_{SR,rel(max)}/F_{I_{Ca}(max)}$, declined from about 16, at 0 mV, to much lower values as clamp pulse voltage was made progressively more positive. We named this phenomenon of change in gain as a function of membrane potential, 'variable gain'. At clamp pulse potentials in the range −30 to 0 mV, the gain differed from cell to cell, being constant at about 16 in some cells, but decreasing from high values (≈65) at −20 mV in others.

3. At clamp pulse potentials at which Ca^{2+} influx ($F_{I_{Ca}}$) was maintained, $F_{SR,rel}$ also had a small maintained component. When macroscopic Ca^{2+} influx was brief (1–2 ms, during 'tails' of $F_{I_{Ca}}$), $F_{SR,rel}$ rose rapidly to a peak after repolarization and then declined with a half-time of about 9 ms (typically).

4. The rising phase of $[Ca^{2+}]_i$ transients could be interrupted by stopping Ca^{2+} influx rapidly (by voltage clamp). We therefore termed this phenomenon 'interrupted SR Ca^{2+} release'.

5. 'Variable gain', high gain in a stable system, and 'interrupted SR Ca^{2+} release' are not consistent with 'common pool' theories of E–C coupling. Therefore the results are discussed in terms of a theory in which SR Ca^{2+} release channels are controlled locally by Ca^{2+} influx through co-associated L-type Ca^{2+} channels and by Ca^{2+} released from neighbouring SR release channels.

Calcium ions (Ca^{2+}) entering mammalian cardiac cells through L-type Ca^{2+} channels control the release of Ca^{2+} from the sarcoplasmic reticulum (SR) during excitation–contraction coupling (recent reviews: Wier, 1990; Bers, 1991; Stern, 1992*a*). Ca^{2+}-induced Ca^{2+} release has been characterized in skinned cardiac cells (Fabiato, 1983, 1985*a*, *b*) and, through flash photolysis of caged Ca^{2+}, in intact cardiac cells (Valdeomillos, O'Neill, Smith & Eisner, 1989; Naebauer & Morad, 1990). Observations of intracellular $[Ca^{2+}]_i$ transients in intact cardiac cells also provide evidence for Ca^{2+}-induced Ca^{2+} release (Barcenas-Ruiz & Wier, 1987; Beuckelmann & Wier, 1988; Naebauer, Callewaert, Cleemann & Morad, 1989; Cleemann & Morad, 1991). Presumably, Ca^{2+}-induced Ca^{2+} release occurs as the result of the binding of Ca^{2+} to activating sites on Ca^{2+} channels (ryanodine receptors) in the membrane of the SR (Meissner & Henderson, 1987; Ashley & Williams, 1990). Much indirect evidence indicates that the process of release is characterized by high gain or amplification, in that much more Ca^{2+} can be released from the SR than enters the cell via the L-type Ca^{2+} channels (Wier, 1990; Bers, 1991). Thus, a major question remains in the study of E–C coupling: how can SR Ca^{2+} release be controlled by the relatively small amount of Ca^{2+} that enters, given that Ca^{2+} released from the SR might activate further release in a positive feedback loop? Attempts to answer this question have been impeded by the problem that although $[Ca^{2+}]_i$ transients have been measured and, recently, even the net SR Ca^{2+} flux has been measured (Sipido & Wier, 1991), the actual SR Ca^{2+} release flux, through the Ca^{2+}-activated ryanodine-sensitive Ca^{2+} release

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channels, had not. (This quantity has been measured in frog skeletal muscle fibres and knowledge of it is central to theories of E–C coupling in that tissue; Rios & Pizarro, 1989.) We now report such measurements in intact cardiac cells, with the quantity symbolized as $F_{SR,rel}$. These measurements, together with precise measurements of Ca^{2+} influx via L-type Ca^{2+} channels (symbolized as $F_{I_{Ca}}$) have permitted us to test certain new ideas (termed 'local control'; Stern, 1992*a*) on E–C coupling in mammalian cardiac muscle. 'Local control' theories of E–C coupling in mammalian heart are more consistent with certain experimental phenomena than 'common pool' models of E–C coupling, as discussed further below. The essential feature of a 'common pool' model of E–C coupling is that the entering Ca^{2+} and released Ca^{2+} occupy a common cytosolic space (i.e. there is no spatial localization of function). The essential feature of 'local control' models is that SR Ca^{2+} release is controlled by events or phenomena localized to the region of the SR Ca^{2+} release channels, and these events (such as local $[Ca^{2+}]_i$ transients) may be quite different from those occurring macroscopically. For example, the macroscopic Ca^{2+} current has been viewed (conventionally) as an adequate representation of the signal for SR Ca^{2+} release at the transverse tubule–SR junction. Recently, however, we (Rose, Balke, Wier & Marban, 1992) have pointed out that, for a number of reasons, this is probably not the case. Most importantly, it is now known (Mazzanti & DeFelice, 1990; Rose *et al.* 1992) that L-type Ca^{2+} channels open only very briefly (0·2 ms) and extremely infrequently (probability of channel opening, P_o, of about 0·04 at the time of maximal macroscopic current), that the current is constant during this brief opening, and that $[Ca^{2+}]_i$ probably rises quickly to levels of several hundred millimolar near the mouth of the channel when the channel is open, but falls extremely quickly (less than 1 ms) when the channel closes (Bers & Peskoff, 1991; Stern, 1992*b*). Given this, it seems virtually certain that local $[Ca^{2+}]_i$ is quite different from that which would be predicted by considering the macroscopic Ca^{2+} current. Similarly, the opening of a large-conductance SR Ca^{2+} release channel will almost certainly result in local $[Ca^{2+}]_i$ that is quite different from macroscopic. Yet it is the $[Ca^{2+}]_i$ in this region (i.e. the 'local $[Ca^{2+}]_i$ transient') that presumably controls SR Ca^{2+} release through Ca^{2+}-induced Ca^{2+} release (Fabiato, 1983, 1985*a*, *b*; Beuckelmann & Wier, 1988; Naebauer & Morad, 1990).

While the observation of 'local $[Ca^{2+}]_i$ transients' is not feasible technically, consideration of the theory of Ca^{2+}-induced release of Ca^{2+} leads to certain experimental observations that can distinguish 'local control models' from 'common pool' models. In particular, Stern (1992*a*) has pointed out that 'common pool' models tend to give nearly 'all-or-none' regenerative Ca^{2+} release, unless 'gain' is very low. Such models tend to 'latch up' in continuous release, with release escaping control by the entering Ca^{2+}. Although all possible 'common pool' models cannot be tested, Stern (1992*a*) has proved rigorously that no common pool model can give graded SR Ca^{2+} release with high gain unless it is on the verge of spontaneous oscillation. In this report, therefore, we use our measurements of $[Ca^{2+}]_i$ transients, Ca^{2+} currents, and of SR Ca^{2+} release flux to quantify the 'gain' of SR Ca^{2+} release during E–C coupling. We show that SR Ca^{2+} release can be interrupted by stopping Ca^{2+} current, and that Ca^{2+} currents are not all equally effective in eliciting SR Ca^{2+} release (i.e. the 'gain' of the system varies). These results are not consistent with 'common pool' models, particularly the findings that the 'gain' of SR Ca^{2+} release can be high and is variable, but is still controlled by entering Ca^{2+} (i.e. it is not highly regenerative and the system is stable despite changes in loading of the SR with Ca^{2+}). Although gain had not actually been measured previous to this study, the idea of a variation in gain has been suggested, based on observation of $[Ca^{2+}]_i$ transients elicited by voltage clamp depolarization (Cannell, Berlin & Lederer, 1987) and on observation of twitch contractions elicited by flash photolysis of caged Ca^{2+} (Niggli & Lederer, 1990). Our results are discussed in terms of a 'local control' model of E–C coupling which takes into account the stochastic nature of L-type Ca^{2+} channel gating, as we have observed it previously (Rose *et al.* 1992).

METHODS

Single rat ventricular myocytes were subjected to whole-cell voltage clamp and internal perfusion with the Ca^{2+} indicator, indo-1. Conditions were used that eliminated sodium (Na^+) currents, Na^+–Ca^{2+} exchange, and potassium (K^+) currents that would interfere with the measurement of Ca^{2+} influx and SR Ca^{2+} release as in the preceding report (Balke, Egan & Wier, 1994). Single rat ventricular cells were obtained by an enzymatic dispersion technique described in detail previously (Balke, Rose, Marban & Wier, 1992). The external solution used during giga-seal formation, break-in, and control recordings was a physiological salt-containing solution composed of (mM): NaCl, 140; dextrose, 10; Hepes, 10; CsCl, 10; $MgCl_2$, 1; $CaCl_2$, 1; pH adjusted to 7·3–7·4 with CsOH. The Na^+-free external solution was composed of (mM): CsCl, 130 or 20; tetraethlyammonium chloride (TEA-Cl), 20 or 130; dextrose, 10; Hepes, 10; $MgCl_2$, 1; pH adjusted to 7·3–7·4 with CsOH. The electrode-filling solution was Na^+ free and was composed of (mM): caesium glutamate, 130; Hepes, 10; CsCl, 20 or TEA-Cl, 20; $MgCl_2$, 0·33; Mg_2ATP, 4; indo-1 (pentapotassium salt), 0·1 or 0·05; pH adjusted to 7·3–7·4 with CsOH. In selected experiments, K^+ channels were blocked with the addition of 4-aminopyridine (1–2 mM) to all the external solutions. Ca^{2+} current (I_{Ca}) was taken to be the Cd^{2+}-sensitive current (0·1 mM Cd^{2+}), as described previously (Balke *et al.* 1994). The holding potential was −40 mV. All experiments were performed at 23 °C. Whole-cell currents were recorded using standard methods. The filled micropipette electrodes had resistances of 1·0–4·0 MΩ. Current was digitized at 2 kHz with 12-bit resolution. $[Ca^{2+}]_i$ was calculated from the indo-1 fluorescence through the use of 'calibration parameters' obtained *in situ* (Balke *et al.* 1994) and was corrected for the kinetics of indo-1, as described previously (Sipido & Wier, 1991).

RESULTS

SR Ca^{2+} fluxes

The first step in computing the SR Ca^{2+} release flux is to account quantitatively for the uptake of Ca^{2+} by the SR Ca^{2+}-pumping ATPase, so that the flux through it (ATPase)

can be subtracted from the net SR Ca^{2+} flux (Sipido & Wier, 1991). The methods for characterizing the functioning of the SR Ca^{2+}-pumping ATPase, during the declining phase of the $[Ca^{2+}]_i$ transient, have been described in detail in the preceding report (Balke *et al.* 1994). Briefly, analysis of the declining phase of the $[Ca^{2+}]_i$ transient results in estimations of the parameters, K_M (Michaelis–Menten constant), V_{max} (maximum rate), and $F_{SR,leak}$ (leak of Ca^{2+} into the cytoplasm from the SR). The smooth line through the declining phase of the $[Ca^{2+}]_i$ transient illustrated in Fig. 1 has been calculated according to the methods presented previously (Balke *et al.* 1994) with V_{max} of 0·14 mM s^{-1}, K_M of 0·24 mM, and $F_{SR,leak}$ of 0·018 mM s^{-1} with the assumption that 2 Ca^{2+} ions bind to each ATPase and that 2 ATPases co-operate in the transport of 4 Ca^{2+} ions (Klein, Kovacs, Simon & Schneider, 1991). In order to calculate $F_{SR,rel}$, however, we then assume that this characterization of the processes removing Ca^{2+} from the cytoplasm applies, with the same parameters, throughout the entire $[Ca^{2+}]_i$ transient, not just its declining phase. $F_{SR,rel}$ is then calculated using eqn (1):

$$F_{SR,rel} = \frac{d}{dt}[Ca^{2+}]_i - F_{SR,pump} - F_{SR,leak} - F_{I_{Ca}} + \sum_{n=1}^{N} \frac{d}{dt}[CaL]_n \quad (1)$$

where $F_{I_{Ca}}$ is calculated from the measured Ca^{2+} current and other measured parameters according to methods described earlier by Sipido & Wier (1991) and, in rat cells, by Balke *et al.* (1994). (Note that we follow the convention that a cellular flux producing a rate of change in $[Ca^{2+}]_i$, symbolized as F, will be positive in sign if it tends to increase $[Ca^{2+}]_i$, and will have the units millimolar per second (mM s^{-1}). Thus, $F_{SR,pump}$ is negative in sign, while $F_{SR,leak}$ and $F_{I_{Ca}}$ are positive in sign). The $[Ca^{2+}]_i$ transient, $F_{I_{Ca}}$, $F_{SR,rel}$, $F_{SR,leak}$ and $F_{SR,pump}$ shown in Fig. 1 are representative of those obtained in more than twenty-five different cells, although the full analysis to be presented below was carried out in only nine (Fig. 3, panels *c*).

$F_{SR,rel}$ and $F_{I_{Ca}}$ elicited by voltage-clamp pulses 200 ms in duration over the range of membrane potential of −30 to +80 mV are illustrated in Fig. 2. It can be seen that peak $F_{SR,rel}$ was maximal at a clamp pulse potential of 0 mV, and that $F_{SR,rel}$ was elicited upon *repolarization* from very positive membrane potentials, as expected from the existence of 'tail $[Ca^{2+}]_i$ transients'. This dependence on clamp pulse potential is similar to that of the $[Ca^{2+}]_i$ transient, as demonstrated previously (Barcenas-Ruiz & Wier, 1987; Cannell *et al.* 1987; Beuckelmann & Wier, 1988).

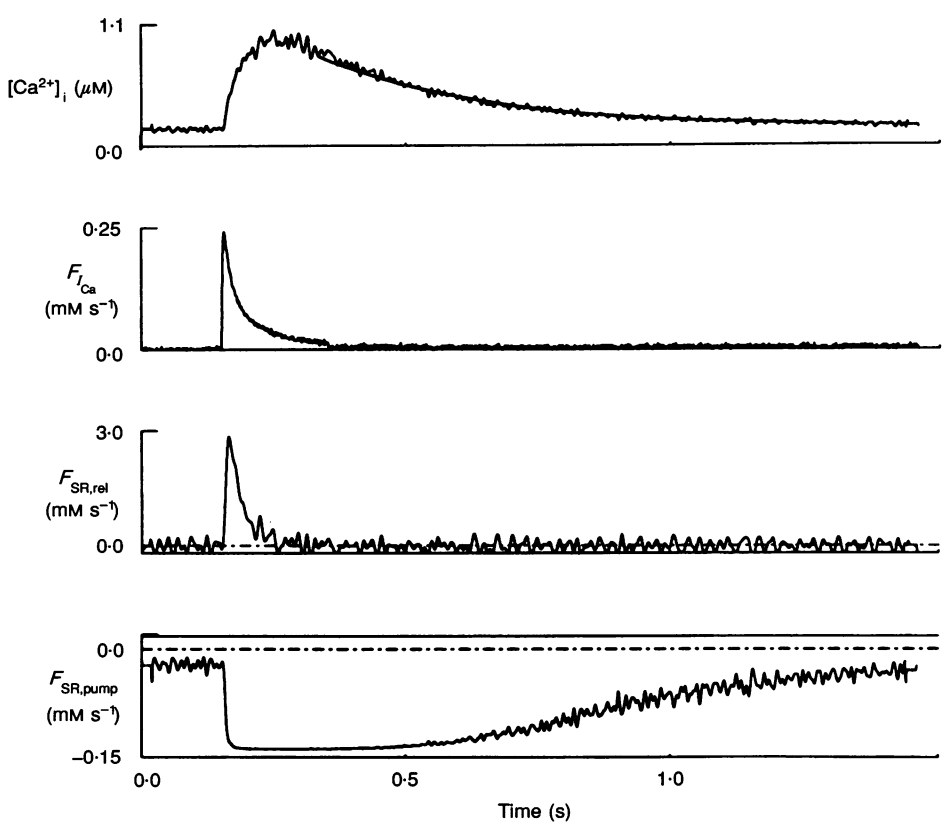

Figure 1. Computation of SR Ca^{2+} fluxes from measured $[Ca^{2+}]_i$ transient and Ca^{2+} current
From top to bottom, records are: $[Ca^{2+}]_i$ transient, flux through L-type Ca^{2+} channels ($F_{I_{Ca}}$), calculated SR release flux ($F_{SR,rel}$), and calculated SR pump flux ($F_{SR,pump}$). Interrupted lines (—·—·) are at zero. The continuous line in the lowest record indicates the level of the constant leak into the cytoplasm, $F_{SR,leak}$. Smooth line superimposed on the $[Ca^{2+}]_i$ transient (top trace) is the Ca^{2+} removal function, as described in the text.

Since Na^+ was absent entirely, there was no possible triggering of SR Ca^{2+} release by Ca^{2+} entering via the Na^+–Ca^{2+} exchanger (LeBlanc & Hume, 1990; Sham, Cleemann & Morad, 1992).

Detailed comparison of the rising phases of the two fluxes ($F_{I_{Ca}}$ and $F_{SR,rel}$) is problematic, given uncertainties about the kinetics of the indo-1 signal, the limited speed of the clamp, and the fact that $[Ca^{2+}]_i$ gradients may exist early during the $[Ca^{2+}]_i$ transient (Cannell & Allen, 1984; Wier & Yue, 1986). For small depolarizations, to −30 and −20 mV (Fig. 2), peak $F_{I_{Ca}}$ is relatively small and $F_{I_{Ca}}$ has a relatively large maintained component. This maintained component results from an 'active-late' pattern of channel gating and multiple re-openings (Rose *et al.* 1992). The time courses of the two fluxes are similar, but not identical. For larger depolarizations, to 0 mV (Fig. 2), the same is true, with the maintained component of both fluxes being relatively much less. $F_{I_{Ca}}$ elicited upon repolarization from very positive clamp pulse potentials (+80 mV, Fig. 2) is extremely brief (< 2 ms), but $F_{SR,rel}$ is much longer, and lacks a 'maintained component'. $F_{SR,rel}$ elicited by repolarization from +80 mV (Fig. 2) declines with a half-time of about 9 ms. $F_{SR,rel}$ elicited by repolarization declined more rapidly than that elicited by any other clamp pulse protocol.

Graphs are presented in Fig. 3 of $F_{SR,rel(max)}$ and $F_{I_{Ca}(max)}$ ('max' means the maximum or peak value attained) for the experiment shown in Fig. 2 (Fig. 3*A a*), another representative cell (Fig. 3*A b*) that gave slightly different results, and the average results in nine cells (Fig. 3*A c*).

Test of 'local control' theories of E–C coupling

'Gain' of SR Ca^{2+} release

Stern (1992*a*) has analysed the dependencies of the amounts of Ca^{2+} entering via L-type Ca^{2+} channels and the amounts of Ca^{2+} released from the SR on membrane potential for two explicit local control models of E–C coupling that he introduced. Both models predict that there can be a significant difference between the voltage dependence of $F_{I_{Ca}}$ and $F_{SR,rel}$ even though, in the models, SR Ca^{2+} release is being controlled by Ca^{2+} entry via the L-type Ca^{2+} current. Furthermore, the apparent 'gain' of the system will vary with membrane voltage, where gain is defined here as the ratio, $F_{SR,rel(max)}/F_{I_{Ca}(max)}$. We have analysed our data in a similar fashion, and the results are presented in Fig. 3*B* and *C*. In Fig. 3*B* we have plotted the normalized peak fluxes as a function of clamp pulse potential and in *C* we have plotted

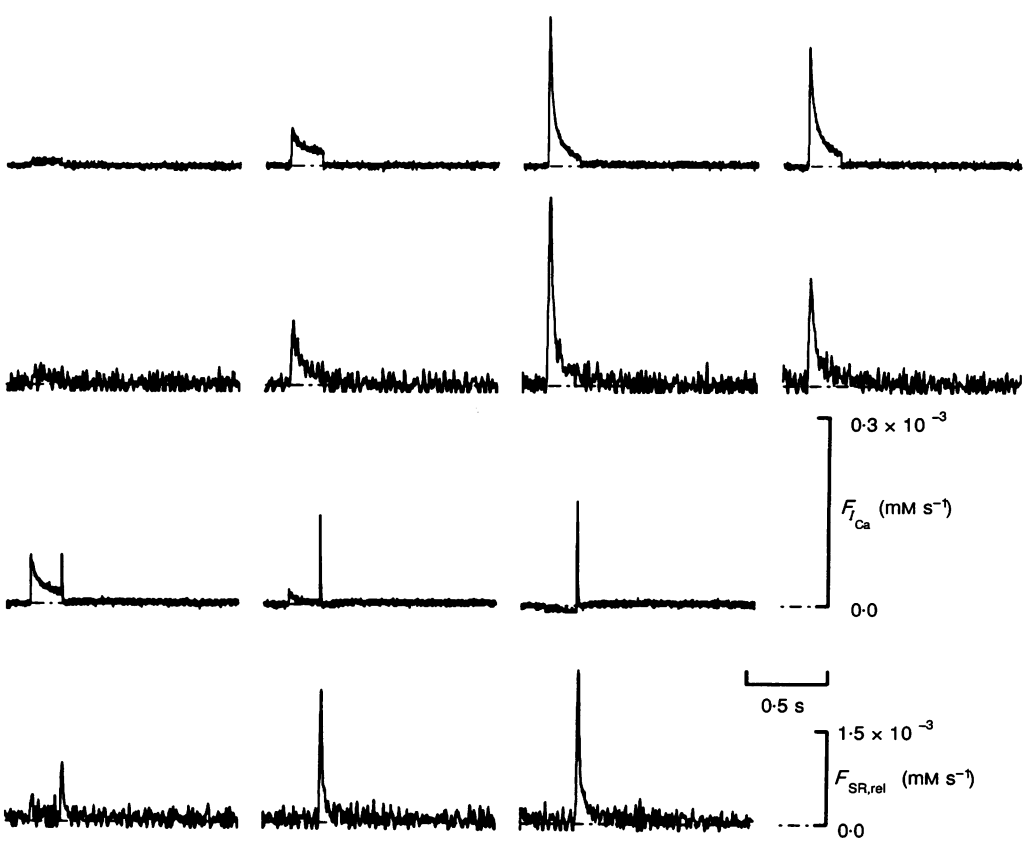

Figure 2. SR Ca^{2+} release flux and Ca^{2+} influx elicited by voltage clamp pulses over the range −30 to +80 mV

Upper trace of each pair, $F_{I_{Ca}}$; lower trace of each pair, $F_{SR,rel}$. From left to right, top to bottom, clamp pulse voltage was −30, −20, 0, 20, 40, 60 and 80 mV for 200 ms from a holding voltage of −40 mV.

'gain', as defined above, for the two representative cells (*a* and *b*) and for nine cells (*c*). On average, gain in the nine cells is relatively high at −20 mV and falls progressively as clamp pulse potential is made more positive. In some cells, (Figs 2 and 3*a*) the 'gain' seemed relatively constant over the clamp pulse potential range −30 to 0 mV. In all cells, however, the gain fell progressively as clamp pulse potential was made more positive than 0 mV. We call the decrease in gain 'variable gain'.

According to the analysis of Stern (1992*a*) a common pool model with gain as high as 15 would become unstable for only very slightly increased SR Ca^{2+} loading, a phenomenon that is not observed. (Instability is observed with very high SR Ca^{2+} loading, as discussed later.) Although variable gain might be observed in a common pool model of E–C coupling, it would be impossible for a common pool model to have different gain for *Ca^{2+} currents of the same amplitude.* This phenomenon is shown clearly in Fig. 4, which compares Ca^{2+} currents of quite similar amplitude and time course, but which elicit very different SR Ca^{2+} release. All cells, whether or not the gain varied over the range of −30 to 0 mV, showed the phenomenon of variable gain, in that gain at membrane potentials greater than 0 mV was always less than that at membrane potentials less than 0 mV.

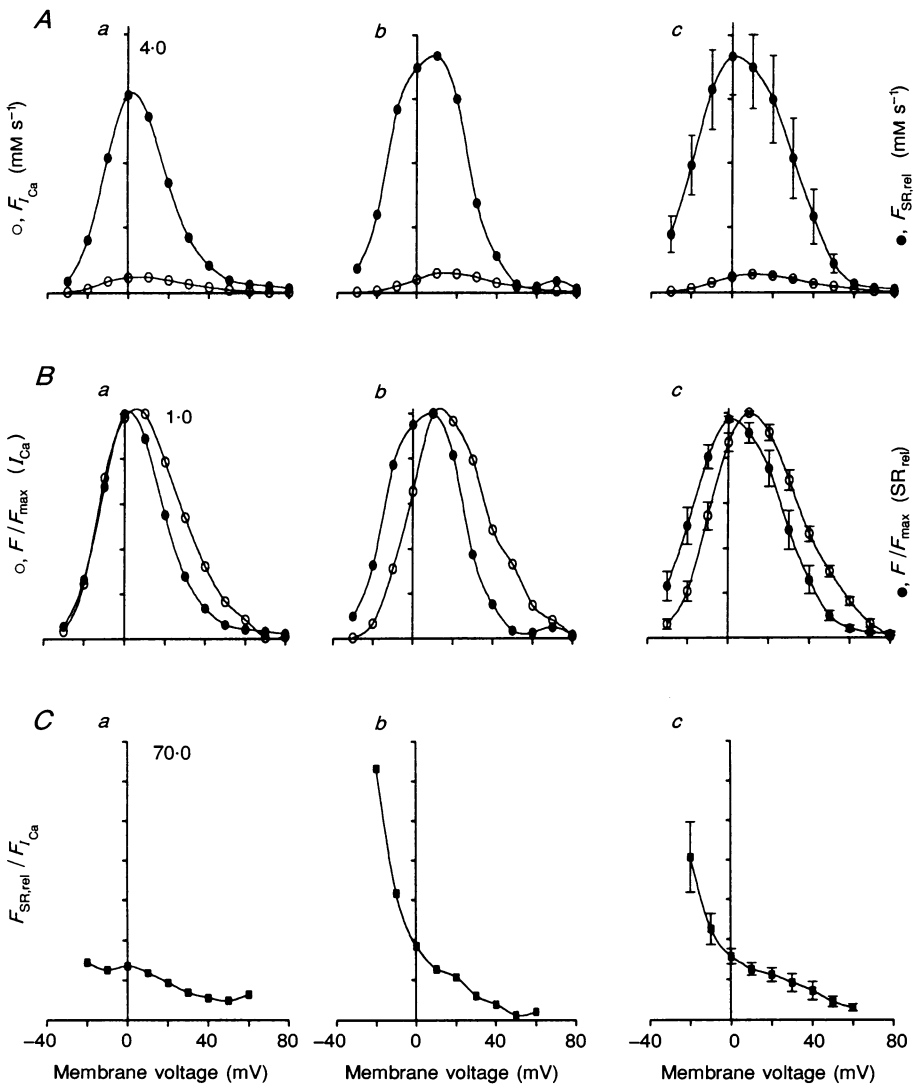

Figure 3. Variable gain in SR Ca^{2+} release: voltage dependence

A, peak amplitudes of $F_{I_{Ca}}$ (○) and $F_{SR,rel}$ (●) plotted against clamp pulse voltage (membrane voltage) for two representative cells (*a* and *b*) and the mean ± S.E.M. for nine cells (*c*). Data in panels *a* is from the experiment illustrated in Figs 1 and 2. *B*, normalized fluxes; in each graph, each point was obtained by dividing the original value of *F* (either $F_{SR,rel}$ or $F_{I_{Ca}}$ from *A*) by the corresponding maximum of that quantity (F_{max}). *C*, the 'gain' of SR Ca^{2+} release as a function of clamp pulse potential, where gain is the ratio of the absolute amplitudes, $F_{SR,rel(peak)}/F_{I_{Ca}(peak)}$, from *A*. The line through the points has been drawn by eye and the points for voltages greater than +50 mV have been omitted because $F_{SR,rel}$ was too small to measure reliably.

Effects of stopping $F_{I_{Ca}}$ on $F_{SR,rel}$

In common pool models, SR Ca^{2+} release tends to be highly regenerative, and SR Ca^{2+} release tends to 'latch up' in a state of continuous release. If it can be shown that SR Ca^{2+} release can be stopped, once it has started, then it suggests that common pool models are not appropriate. In particular, it is of interest to determine whether or not stopping the presumed signal for release, $F_{I_{Ca}}$, stops or affects SR Ca^{2+} release. It must be determined whether SR Ca^{2+} release is highly regenerative or whether it is under control of the Ca^{2+} current. Previous results have suggested that SR Ca^{2+} release can be stopped (Cannell *et al.* 1987; Cleemann & Morad, 1991; Wier & Balke, 1991). For this purpose, the best way to stop or interrupt $F_{I_{Ca}}$, once the L-type Ca^{2+} channels have opened and influx is occurring, is to change the membrane potential instantaneously to one (e.g. +80 mV) at which the driving force for influx of Ca^{2+} is negligibly small. $[Ca^{2+}]_i$ transients and Ca^{2+} currents from such experiments are illustrated in Fig. 5 (control, *a*; interrupted, *c*). The result of stopping $F_{I_{Ca}}$ just before its peak was that the rising phase of the $[Ca^{2+}]_i$ transient (which reflects SR Ca^{2+} release under these circumstances) was interrupted. The peak of the $[Ca^{2+}]_i$ transient was reduced to less than one-half (of that elicited by uninterrupted $F_{I_{Ca}}$). We term this phenomenon 'interrupted SR Ca^{2+} release', and its existence is an important indication against 'common pool' models of E–C coupling. Ca^{2+} current ($F_{I_{Ca}}$) can also be turned off quickly by repolarizing to −40 mV where the probability of L-type Ca^{2+} channels being open is very low. This type of experiment is also shown in Fig. 5 (traces labelled *b*). This protocol produces a tail of Ca^{2+} current. Nevertheless, the $[Ca^{2+}]_i$ transient is interrupted, much as in the protocol in which Ca^{2+}current is interrupted or stopped without a tail. Thus tails of Ca^{2+} current produced in this way seem to be less efficacious than those occurring after long depolarizing pulses to positive membrane potentials (Figs 2 and 4).

DISCUSSION

These are the first computations of the time course and magnitude of the unidirectional efflux of Ca^{2+} from the SR during E–C coupling in intact cardiac cells. One notable feature of the release flux in mammalian cardiac muscle is the lack of a prominent maintained component, a feature that is characteristic of the release flux in amphibian skeletal muscle. This maintained release has been attributed to SR Ca^{2+} release channels that are activated directly by depolarization, through interaction with voltage sensors in the surface membrane (DHP receptors) (Rios & Pizarro, 1989). In our computations (e.g. Fig. 2) release does occur throughout the duration of the depolarizing pulse, but it becomes relatively very small, and its relative amplitude appears to be related to the relative amplitude of the slowly inactivating Ca^{2+} current, rather than to membrane voltage. For example, continued release flux is relatively larger for the depolarization to −20 mV than to +20 mV,

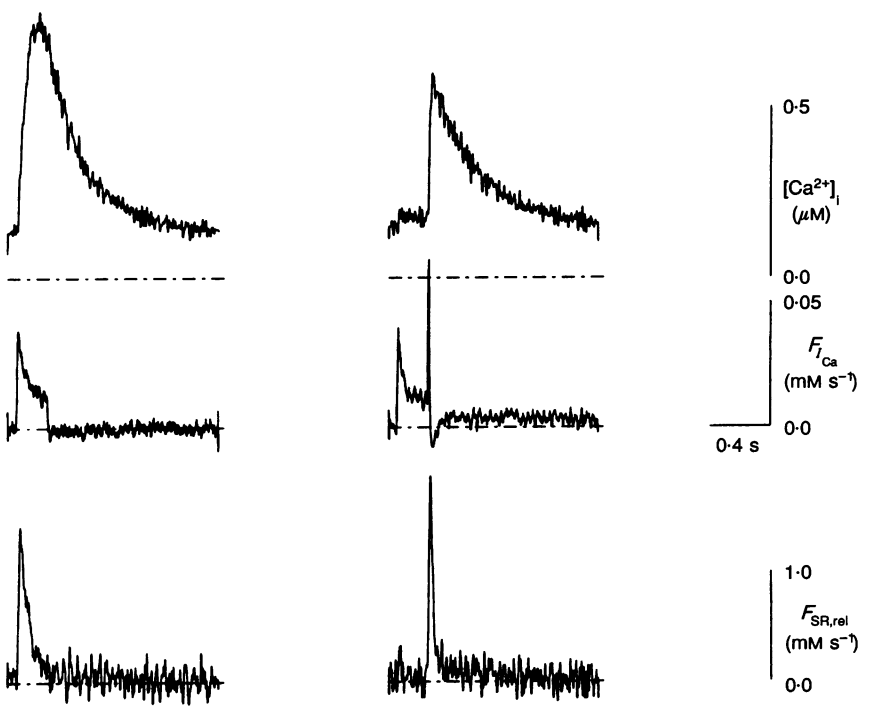

Figure 4. Variable gain in SR Ca^{2+} release: tail currents
Traces, from top to bottom, are $[Ca^{2+}]_i$ transient, Ca^{2+} influx ($F_{I_{Ca}}$), and SR Ca^{2+} release flux ($F_{SR,rel}$). Voltage-clamp pulses were to −20 mV (left-hand column) and to +60 mV (right-hand column). Although the Ca^{2+} influxes through L-type Ca^{2+}channels ($F_{I_{Ca}}$) elicited on depolarization to the two membrane potentials are similar, their efficacy in eliciting SR Ca^{2+} release is very different. Note also the large release of Ca^{2+} elicited by the very brief tail of Ca^{2+} influx upon repolarization from +60 mV.

which would be opposite to the voltage-dependent release in amphibian skeletal muscle. Thus the present results support the concept that changes in membrane voltage have a fundamentally different role in E–C coupling in mammalian cardiac muscle and amphibian skeletal muscle.

Several of our results support 'local control' models in general, as opposed to 'common pool' models. (1) We have demonstrated that the system has high gain, but is not unstable. Stern (1992*a*) showed rigorously for all common pool models that for a gain even of 10, an 11 % change in SR loading would result in instability. Although we did not change SR loading in this study specifically, increases of 11 % in the $[Ca^{2+}]_i$ transient are well within the physiological range over which SR Ca^{2+} release is still stable (graded). For example, isoprenaline may increase the $[Ca^{2+}]_i$ transient several-fold (Balke *et al.* 1994) without instability, although instability certainly does result when SR Ca^{2+} loads are high (Orchard, Eisner & Allen, 1983; Wier, Kort, Stern, Lakatta & Marban, 1983). (2) We have shown (see also Cleemann & Morad, 1991) that SR Ca^{2+} release can be terminated once it has started, a phenomenon that is difficult to reconcile with common pool models (Cannell *et al.* 1987; Stern, 1992*a*) but which indicates clearly that SR Ca^{2+} release is controlled by the Ca^{2+} current. (3) We have demonstrated 'variable gain' which is difficult to reconcile with common pool models, as already discussed.

These results, and others, necessitate new concepts on E–C coupling in mammalian cardiac muscle. Most important is that the macroscopically observable Ca^{2+} currents and $[Ca^{2+}]_i$ transients may not be representative of 'local events' (viz local $[Ca^{2+}]_i$, and unitary L-type and SR Ca^{2+} channel currents) that actually determine the course of E–C coupling. This concept may be referred to as 'local control'. In general, it may be hypothesized that the macroscopic Ca^{2+} current does not provide a good basis for estimating 'local calcium' that may activate release, by binding to activating sites on SR Ca^{2+}channels, because: (1) under physiological conditions the mean open time of L-type Ca^{2+} channels is extremely brief, compared to the time course of decay of the macroscopic current (Rose *et al.* 1992); (2) at the time of peak current, the maximum probability of being open (P_o) is very low; (3) the current through such channels is constant when the channel is open; and (4) local calcium concentration will also be determined by Ca^{2+} released through the SR release channels. The macroscopically observable Ca^{2+} currents and $[Ca^{2+}]_i$ transient are nevertheless nothing more than the

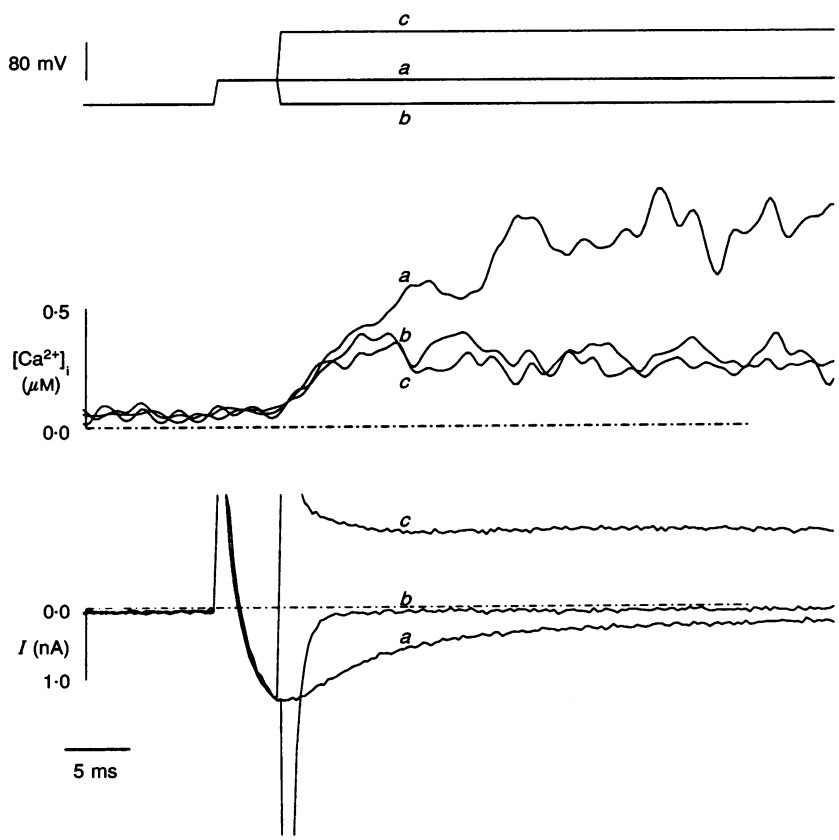

Figure 5. Interrupted SR Ca^{2+} release
Upper traces are superimpositions of three voltage-clamp pulse depolarizations: *a*, control, from −40 to 0 mV for 5 ms; *b*, brief depolarization, from −40 to 0 mV for 5 ms; *c*, further depolarization; brief depolarization from −40 to 0 mV for 5 ms, followed by further depolarization to +80 mV for 50 ms before repolarization to −40 mV. $[Ca^{2+}]_i$ transients and currents elicited by these clamp pulse depolarizations are labelled correspondingly in the lower traces.

sum of the local events, as is well established in the case of unitary currents and macroscopic (whole-cell) currents.

Local control theory of E–C coupling

Local control of E–C coupling may be described as follows. On the ascending limb of the $F_{I_{Ca}}$–V and $F_{SR,rel}$–V relations (Fig. 3*A* and *B*), the macroscopic Ca^{2+} current elicited by depolarization results from the relatively infrequent and very brief opening (and re-opening) of L-type Ca^{2+} channels. Consequently, open channels will be quite distant from each other (a factor that may contribute to local control). The overall time course of the macroscopic current is given by a combination of the first-latency density function and a decline in the conditional open probability (probability of re-opening) as we have shown previously (Rose *et al.* 1992). As pulse voltage is made more positive on this limb of the relations, P_o of L-type Ca^{2+} channels increases, and single-channel current decreases. When an L-type Ca^{2+} channel does open and influx occurs, nearby SR Ca^{2+} release channels are presumably activated. Then some rapid, and yet unknown, mechanism turns the release off (Figs 1, 2 and 4). On the *descending* limb of this relationship, events are quite different. As pulse voltage is made more positive, P_o of the L-type Ca^{2+} channels increases to a maximum, but the current through the open channels decreases to low levels, as the reversal potential is approached. The continuous variation (decline) in gain over both the ascending and descending limbs must somehow be related to these phenomena, since macroscopic Ca^{2+} current can be similar on the two limbs (e.g. Fig. 4). The obvious hypothesis is that the fall in gain is related to the amplitude of the single-channel current, since that is the quantity that decreases continuously just as does gain. The fundamental mechanism by which this could occur, however, remains unknown.

It must be remembered that we studied cells in the absence of Na^+, a condition which can lead to a highly loaded SR. This may be important because these are the conditions that can lead to unstable SR Ca^{2+} release, such as the well-known Ca^{2+} oscillations and Ca^{2+} waves. When such phenomena are present, SR Ca^{2+} release is not controlled by Ca^{2+} influx nor is it spatially confined, as under normal conditions (O'Neill, Mill & Eisner, 1990). Thus the loading of the SR, which is not a factor in the model discussed above, is clearly an important factor in the mechanism of E–C coupling, even if only when the SR is highly loaded. Although cells exhibiting unstable SR Ca^{2+} release were excluded from the present study, it is conceivable that, under the conditions of this study, some of the cells were more highly loaded with Ca^{2+} and were near this type of behaviour. We did observe that in cells in which spontaneous SR Ca^{2+} release was observed unequivocally the gain at negative membrane potentials (−30 mV) was very high; such cells were not included in the present report.

In summary, our results cannot be reconciled with a 'common pool' model of E–C coupling in mammalian cardiac muscle, primarily because of the phenomenon of variable gain, in which the same or similar macroscopic Ca^{2+} currents elicited at different membrane potentials elicit very different SR Ca^{2+} release or in which very brief 'tails' of Ca^{2+} current were either very effective in eliciting SR Ca^{2+} release or completely ineffecitive (Fig. 5). We postulate that these phenomena are the result of local events that are different from those observed macroscopically (i.e. 'local control').

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