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INTEGRATIVE ANALYSIS OF CALCIUM SIGNALLING IN CARDIAC MUSCLE

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1. ABSTRACT

This review discusses the control of the amplitude of the cardiac systolic Ca transient. The Ca transient arises largely from release from the sarcoplasmic reticulum (SR). Release is triggered by calcium-induced calcium release (CICR) whereby the entry of a small amount of Ca on the L-type Ca current, "the trigger", results in the release of much more Ca from the SR. There are three potential control points: (1) the Ca content of the SR; (2) the properties of the SR Ca release channel or ryanodine receptor (RyR); (3) the amplitude of the L-type Ca current. The data reviewed show that the Ca content of the SR has pronounced effects on systolic $[Ca^{2+}]_i$ and, reciprocally, the amount of Ca released from the SR affects sarcolemmal Ca fluxes thereby "autoregulating" SR content. Modulation of the ryanodine receptor has no steady-state effect due to compensating changes of SR Ca content. An increase of the L-type Ca current results in an abrupt increase of systolic $[Ca^{2+}]_i$ with little change of SR content. This is because of a coordinated increase of both the trigger and loading function of the Ca current. These results emphasise the importance of considering all aspects of Ca handling in the context of SR Ca release and thus the regulation of the systolic Ca transient and contraction in cardiac muscle.

2. INTRODUCTION

2.1. Overview of excitation contraction coupling

The other contributions in this volume deal largely with specific aspects of the Ca release process from the sarcoplasmic reticulum (SR). It is, however, important to note that the Ca release process is but one of many steps involved in Ca handling in cardiac cells. The function of this article is to integrate all these steps and show that, when considering the consequences of altering any one step (such as SR Ca release via the ryanodine receptor) effects of the other steps must also be considered.

The consensus scheme of excitation-contraction coupling (illustrated in Figure 1A) is that of calcium-induced calcium release (CICR) in which the entry of Ca ions through the surface L-type Ca channel produces a small "trigger" increase of calcium concentration ($[Ca^{2+}]_i$). This increase of $[Ca^{2+}]_i$ increases the probability that the sarcoplasmic reticulum (SR) Ca release channels or ryanodine receptors (RyR) are open. The increased opening of the RyRs results in efflux of Ca from the SR. Thus a small trigger entry of Ca^{2+} through the L-type channel is amplified by a larger release of Ca^{2+} from the SR. The "gain" of this amplification can be up to a factor of 10 (1,2).

It should be obvious that if the heart is to work effectively as a pump then this systolic rise of $[Ca^{2+}]_i$ must be returned to control levels on each beat. There are two major routes to do this. (1) Calcium can be pumped back out of the cell largely via the Na-Ca exchange (for reviews see (3-4)) with a smaller contribution from the plasma membrane Ca-ATPase (PMCA) (5-7). (2) Calcium can be taken back into the SR via the SR Ca-ATPase (SERCA). The coordination of all these processes is required to produce the normal Ca transient and contraction shown in Figure 1B.

2.2. Control Points

On this scheme for CICR, there are at least three potential points at which the amplitude of the systolic Ca transient can be regulated. (1). The amount of Ca entering

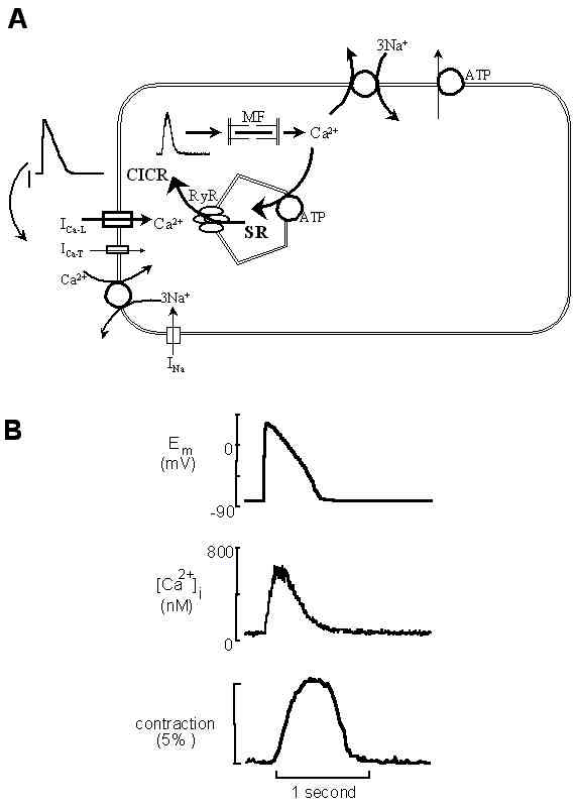

Figure 1. A. Schematic diagram of the processes involved in excitation-contraction coupling. The action potential activates Ca entry via the L-type Ca current. The resulting "trigger" increase of $[Ca^{2+}]_i$ then opens the SR Ca release channels (RyR). This released Ca activated the myofibrils (MF) resulting in contraction. For relaxation to occur Ca is removed from the cytoplasm. This occurs mainly via the SR Ca-ATPase (SERCA) and also by the sarcolemmal Na-Ca exchange with a small contribution from the sarcolemmal Ca-ATPase. B. Cellular measurements obtained from a single cardiac myocyte showing the relationship between the action potential (top), the systolic Ca transient (middle) and contraction (bottom).

the cell via the L-type Ca current; (2) the properties of the RyR; (3) the Ca content of the SR. In the remainder of this review we will show that, contrary to what has often been suggested, the RyR (2) is not a site for controlling the amplitude of the systolic Ca transient. Rather, co-ordinated control of 1 and 3 seem to be used by the cell.

2.3. Steady state Ca flux balance

As discussed above, there are two sources and sinks for calcium: the extracellular fluid and the SR. One would therefore expect that, in the steady state, on each beat exactly that amount of Ca which enters the cell from the extracellular fluid will be pumped back out of the cell and that amount which is released from the SR will be resequestered by the SR. If this condition did not hold then, inevitably, there would be a net gain or loss of Ca from the SR. An experimental demonstration of this steady state condition is shown in Figure 2. The top trace shows the Ca transient produced by a voltage clamp depolarisation. The middle trace shows the accompanying membrane current. On depolarisation, Ca enters via the L-type Ca current. The bottom trace shows the integral of this current demonstrating that about 4 µmol of Ca enter per l cell. On repolarization there is an inward Na-Ca exchange current that is more evident in the amplified record. Again this can be integrated and, assuming that the Na-Ca exchange transports 3 Na^+ per Ca^{2+} then the amount of Ca transported by the exchange can be calculated. Another correction must be made for the fact that the electroneutral sarcolemmal Ca-ATPase also transports Ca. With these corrections, the Ca efflux from the cell can be calculated (8). As shown by the integral, the Ca efflux exactly balances Ca entry. This therefore provides experimental evidence for the maintenance of Ca flux balance. As will be shown later in this article, the need for the cell to maintain Ca flux balance has profound implications for our understanding of cellular Ca handling.

3. CONTROL OF SR CA CONTENT AND IMPLICATIONS FOR THE REGULATION OF E-C COUPLING

SERCA is stimulated by an increase of cytoplasmic Ca and inhibited by an increase of lumenal Ca (9). In addition to this regulation by Ca concentration, there is a powerful effect of the inhibitory accessory protein phospholamban. Phosphorylation of phospholamban removes this inhibition and stimulates SERCA activity (10,11). This is partly responsible for the acceleration of the decay of the Ca transient on beta stimulation (12,13). Similarly, mice which have no phospholamban ("knock out" animals) have faster decaying Ca transients than controls and a smaller response to catecholamines (14,15). Importantly they also have greater SR Ca contents than control (16).

3.1. Autoregulation of sarcolemmal Ca fluxes and SR content

As mentioned above, SERCA activity is controlled, in part, by $[Ca^{2+}]_i$. This will include the levels of $[Ca^{2+}]_i$ throughout the entire cardiac cycle. A further complexity is provided by the fact that sarcolemmal Ca fluxes are affected by Ca release from the SR and thence by SR Ca content. This arises because an increase of systolic $[Ca^{2+}]_i$ increases Ca efflux on Na-Ca exchange (17) and decreases Ca entry on the L-type Ca current (18-20).

The interactions between these phenomena are shown in Figure 3 (8). Before the record began the SR had been emptied of Ca by the application of 10 mM caffeine. Stimulation initially resulted in very small Ca transients (Figure 3A) because the SR was depleted. However, with time the Ca transient increased in amplitude. Specimen membrane currents are shown in Figure 3B. In the steady state (trace *b*) the integrated current records show that Ca influx and efflux are equal. However a very different result is seen for the first transient (trace *a*). On this pulse, the Ca current inactivates more slowly than in the steady state (see Figure 3C, left) resulting in a greater calculated Ca entry. In contrast the Ca efflux (see Figure 3C, right) is less than in control. The second panel of Figure 3A shows the calculated Ca entry and efflux on each pulse.

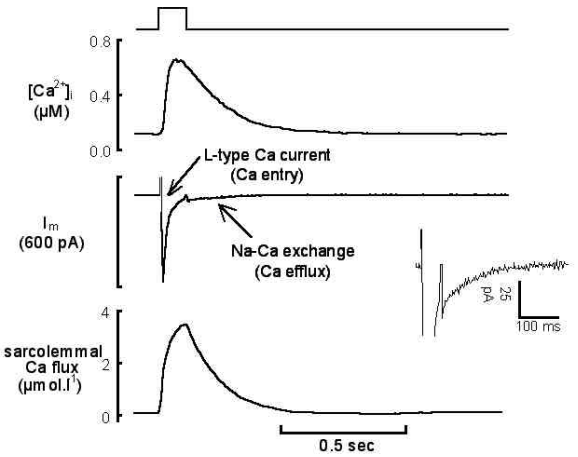

Figure 2. Equality of Ca influx and efflux in the steady state. A rat ventricular myocyte was stimulated with a 100 ms duration depolarizing pulse from –40 to 0 mV. The top trace shows $[Ca^{2+}]_i$; the middle membrane current and the bottom the integrated Ca fluxes. The inset shows an expanded version of the current on repolarization making it easier to see the Na-Ca exchange current.

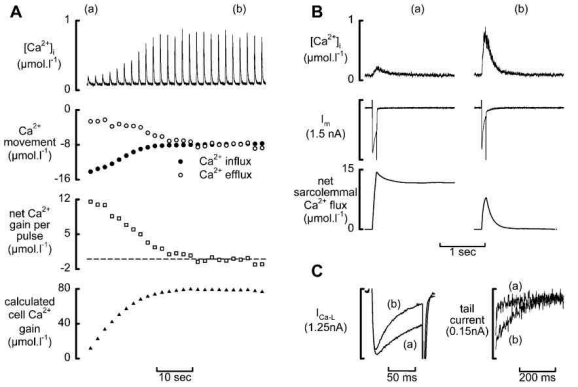

Figure 3. Interactions between SR Ca content and sarcolemmal Ca fluxes. A. Changes associated with refilling the SR. Before the record began the SR had been emptied by exposure to 10 mM caffeine. Following caffeine removal, the cell was stimulated with 100 ms duration pulses from –40 to 0 mV. Panels show (top to bottom): $[Ca^{2+}]_i$, calculated sarcolemmal fluxes – influx is from the L-type Ca current and efflux from Na-Ca exchange (note that larger fluxes are downward); net Ca gain per pulse calculated as influx – efflux; cumulative Ca gain. B. Specimen records obtained from the pulses (a & b) indicated on A. Traces show (top to bottom): $[Ca^{2+}]_i$; current; calculated fluxes. C Expanded records of (left) Ca current, (right) Na-Ca exchange tail current. Data modified from (8).

It is clear that entry is initially greater than efflux but, with time, entry decreases and exit increases until the two are in balance. This change in the balance between influx and efflux is emphasised in the third panel of Figure 3A. Finally the bottom panel shows the cumulative calculated Ca gain. In this particular experiment, the cell (and presumably therefore the SR), gains 80 μmol Ca per litre cell.

The effects of SR Ca release on sarcolemmal fluxes are important in controlling SR Ca content. Consider what happens if SR Ca content increases. This will result in an increase of Ca release from the SR, leading to an increase of Ca efflux from the cell and decrease of Ca influx, thereby decreasing SR Ca content. The steeper the dependence of sarcolemmal Ca fluxes on SR content, the more tightly controlled will be SR Ca content. It should, however, be noted that, as is the case for other feedback systems, excessive gain can result in instability. Thus, if the dependence of sarcolemmal fluxes on SR Ca is very steep, an increase of SR content may result in such a large effect on sarcolemmal fluxes that content decreases to a very low level at which the resulting small Ca transient results in a net cellular gain of Ca and thence an increased SR content. If this persists, alternation in the amplitude of the Ca transient and SR content would be expected (21).

3.2. The effect of SR Ca content on Ca release

Experiments such as that described in Figure 3 also provide information about the dependence of SR Ca release on content. As shown in Figure 4, the relationship is very steep (22,23).

At least 4 factors may contribute to this steepness. (1) The greater the SR Ca concentration, the larger the driving force for Ca efflux. If the intra-SR buffers tend to saturate then a given increase of total SR content will result in a larger fractional change of free SR Ca. (2) Increased Ca efflux will result in increased activation of adjacent RyRs (23,24). (3) An increase of $[Ca^{2+}]_i$ on the lumenal face of the SR increases the open probability of the RyR (25-27). In this context, increased SR content results in increased frequency of Ca sparks (28-30). (4) Finally, a tendency towards saturation of *cytoplasmic* Ca buffers would mean that, as $[Ca^{2+}]_i$ increases, a given further increase of total Ca will produce a larger fractional change of $[Ca^{2+}]_i$.

3.3. SR Ca content and contraction – an overview

The work described above shows that an intrinsic "autoregulation" mechanism allows the SR Ca content to modify sarcolemmal Ca fluxes and thereby control the SR content. This mechanism can be compared with the phenomenon of capacitative control of Ca entry which occurs in many other cell types. On this mechanism, a decrease of endoplasmic reticulum Ca content increases Ca influx into the cell through store operated Ca channels (for reviews see (31,32)). The control by SR Ca release of Na-Ca exchange and the L-type Ca current serves a similar function in the heart.

The previous discussion might make it seem that the SR Ca content could be a major site for modifying cardiac contraction. Indeed, over a certain range, experimental manoeuvres that increase SR Ca content do increase contraction (33-36). However, there are few measurements of SR content under physiologically relevant conditions. Furthermore, there is a limit to the extent to which SR Ca content can be increased. If the cell is overloaded with Ca then spontaneous release of this Ca occurs in the form of propagating waves of Ca release (37-

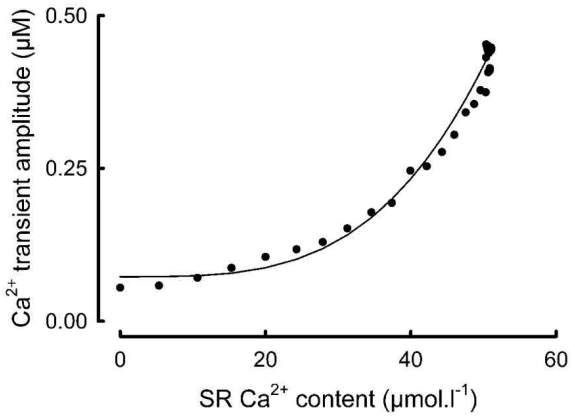

Figure 4. The relationship between the amplitude of the systolic Ca transient and the SR Ca content Data were obtained from an experiment similar to that of Figure 3. The points are the original data, the curve a best fit to: Ca transient = a*(SR Ca)^n where n = 3.4.

39). The tendency for such Ca release to occur limits the usefulness of SR Ca as a regulator of contractility. It is therefore important to consider now the effects of other control points.

4. MODULATION OF THE RYR

In this section we will consider the effects of modifying either the numbers or the properties of the RyRs. We do this in the context of suggestions that changes in these parameters may contribute to the depressed Ca transient seen in heart failure (40-43). In addition decreased coupling between the L-type Ca current and the RyR has also been suggested to contribute to failure (44-46). It has also been reported that cyclic ADP Ribose may be a natural regulator of contractility via its effects on regulating the RyR (47-50)

Many substances affect the properties of the RyR and these effects can be most simply studied by measuring the currents through isolated RyRs (for reviews see (51,52)). We have investigated the effects of four such substances on systolic Ca. These are caffeine and 2,3-butanedione monoxime (BDM) which increase the open probability of the isolated RyR (53,54) and tetracaine and intracellular acidification which decrease it (55-57). The effects of applying these agents are shown in Figure 5. It can be seen that although both caffeine (A) and BDM (B) produce an increase of the systolic Ca transient, this increase is only transient with the amplitude of the transient declining towards control in a few beats (58-60). Likewise acid (C) and tetracaine (D) decrease the amplitude of the transient in a purely transient manner (61,62).

An explanation of these transient effects is presented in Figure 6. Fig 6Aa shows that caffeine produces a transient increase of systolic $[Ca^{2+}]_i$. In the steady state in caffeine the amplitude of the systolic transient is identical to that in control (59). Specimen $[Ca^{2+}]_i$ and current records from (i) control and (ii) the first response in caffeine are presented in Figure 6B. As shown in Figure 2, in control Ca influx is equal to Ca efflux. In contrast the Na-Ca exchange current is larger for transient (ii) than in the control. This effect, which is presumably due to greater activation by the elevated systolic $[Ca^{2+}]_i$ means that the Ca efflux is now greater than the influx (6Bc). Figure 6Ab shows the net sarcolemmal Ca flux as a function of time. In control there is no net Ca flux. When caffeine is applied, the larger Ca transient results in net Ca efflux and this changes to no net Ca flux as the amplitude of the Ca transient declines. On removal of caffeine there is a calculated net Ca influx as the smaller Ca transient results in less Ca efflux. Figure 6Ac shows the calculated SR Ca content. The upper envelope of this record gives the SR Ca content before Ca release. It is clear that this is predicted to decrease during caffeine application The lower envelope was calculated by calculating the amount of Ca released from the SR (see (59,63)).

These data demonstrate, therefore, that the secondary fall of systolic $[Ca^{2+}]_i$ in caffeine is due to a decrease of SR Ca content. In caffeine, a potentiated RyR in combination with a decreased SR Ca content results in a transient of the same amplitude as the control. The fact that the amplitude of the transient in the steady state in caffeine is identical to that in control can be understood as follows. In the steady state Ca influx must equal efflux. So long as the manoeuvre being considered does not affect Ca influx then this condition requires that the Ca efflux in the steady state in caffeine be the same as in control. If we assume that the properties of Na-Ca exchange are not affected then the requirement for a constant Ca efflux means that the amplitude of the Ca transient must be constant. It can therefore be seen that manoeuvres which affect only the RyR will have no effect on the amplitude of the systolic Ca transient, rather they will affect the SR content. It should, however, be noted that the above analysis will not hold if the efflux of Ca from the SR during diastole is affected. For example high concentrations of caffeine make the SR release Ca even in the absence of stimulation thereby depleting it of Ca and greatly decreasing the amplitude of the transient (64,65). This circumstance may also occur in heart failure where it has been reported that the RyR is hyperphosphorylated and therefore leaky to Ca^{2+} thereby resulting in a depleted SR (66).

The above argument is difficult to reconcile with the fact that some agents which affect the RyR (e.g. phosphorylation) have maintained effects on the amplitude of the systolic Ca transient. This dichotomy can be resolved if the agent has other cellular targets as well as the RyR. For example the ability of cyclic ADP Ribose to increase the amplitude of the Ca transient has recently been suggested to result from stimulation of SERCA resulting in increased SR Ca content (67).

5. REGULATION OF THE CA TRANSIENT BY CA ENTRY INTO THE CELL

One of the earliest studies of cardiac contraction showed that external Ca was required for the heart to contract (68). Since then, much work has found that an increase of the L-type Ca current increases the systolic Ca transient and contraction (69-71). There are three possible explanations for this. (1) Increased Ca influx directly activates the

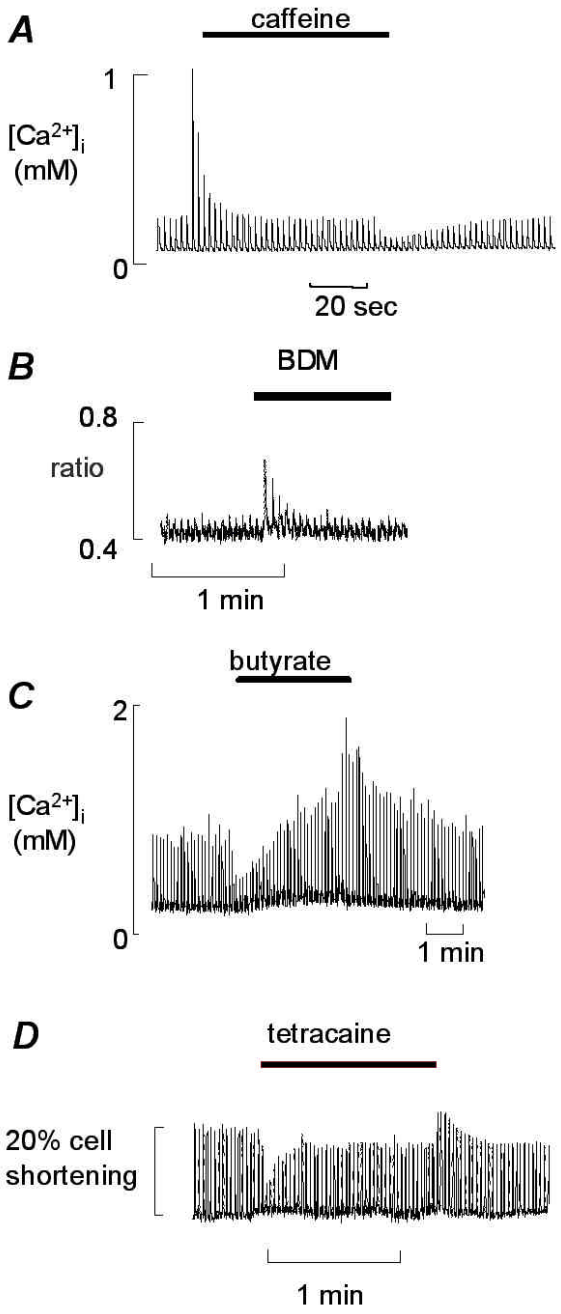

Figure 5. The effects of modifiers of RyR function on systolic $[Ca^{2+}]_i$. The panels show the effects of: A. caffeine (0.5 mM); B. BDM (butanedione monoxime), 5 mM; C. butyrate (30 mM at constant external pH); D, tetracaine (100 μM).

contractile machinery. Although this may make a small contribution, the fact that most of the Ca which activates contraction comes from the SR argues against this being the major factor. (2) An increase of Ca influx will increase the trigger releasing Ca from the SR and (3) the increase of Ca influx will increase the Ca content of the SR and thence the amount released. These latter two roles of the Ca current ("trigger" and "load") in excitation-contraction coupling have been identified previously (72). Our recent work has examined which of the two dominates in the inotropic response to increased sarcolemmal Ca current.

Figure 7 is a simple computer model of the effects of increasing Ca entry into the cell (74). Figure 7A shows the effects of only considering the loading property of the Ca current to be increased. In this case there is a gradual increase of the calculated SR Ca content because the Ca entry is greater than the Ca efflux. This results in an increase of the Ca transient as a result of which there is an increase in Ca efflux until a new steady state is reached where Ca entry and efflux are in balance, again, at a higher level. Therefore an increase of the Ca loading function, alone, would result in a positive inotropic effect. However this would be delayed in onset and would occur in conjunction with an increased SR Ca content. Figure 7B shows the effect of considering only the trigger function of the Ca current. This produces an immediate increase of the Ca transient. However, as the Ca efflux is now greater than the entry, this will result in a decrease of SR Ca and the amplitude of the Ca transient will decrease to basal levels. In other words the predicted response of increasing only the trigger function is identical to that of low concentrations of caffeine and is not expected to produce a maintained inotropic response. In the real case presumably both the loading and the trigger functions are increased and the expected response will be a combination of those seen in A and B. In Figure 7C the parameters were adjusted such that there is no change of SR Ca content. This can be seen to be accompanied by an abrupt and maintained increase of the Ca transient.

5.1. Coordinated trigger and load effects of increased L-type Ca current

The above model indicates the variety of responses predicted to accompany an increase in the L-type Ca current. Figure 8 presents an experimental test to identify which, if any, of these actually occur. In this experiment the amplitude of the L-type Ca current was altered by changing external Ca concentration ($[Ca^{2+}]_o$). Figure 8A confirms that lowering $[Ca^{2+}]_o$ from 1 to 0.2 mM decreases the systolic Ca transient whereas an increase to 2 mM increases $[Ca^{2+}]_i$. The records of Figure 8B show corresponding measurements of SR Ca content. The SR content is the same in 1 and 2 mM $[Ca^{2+}]_o$ but is slightly *increased* in 0.2 mM. The mean data of Fig 8C reveal that the amplitude of the systolic Ca transient is a linear function of $[Ca^{2+}]_o$. In contrast there is a much smaller change of SR content and, indeed, SR content increases at the lowest [Ca2+]o. To a first approximation, SR content is independent of [Ca2+]o. In other words the real cell behaves rather like the simulation of Figure 7C where an increase of Ca entry results in balanced effects on Ca entry and loading. This means that both the trigger and the loading functions of the Ca current increase by the same relative amount as [Ca2+]o is increased. This behaviour is required if the observed rapid and more or less maintained increase of the amplitude of the Ca transient is to be produced.

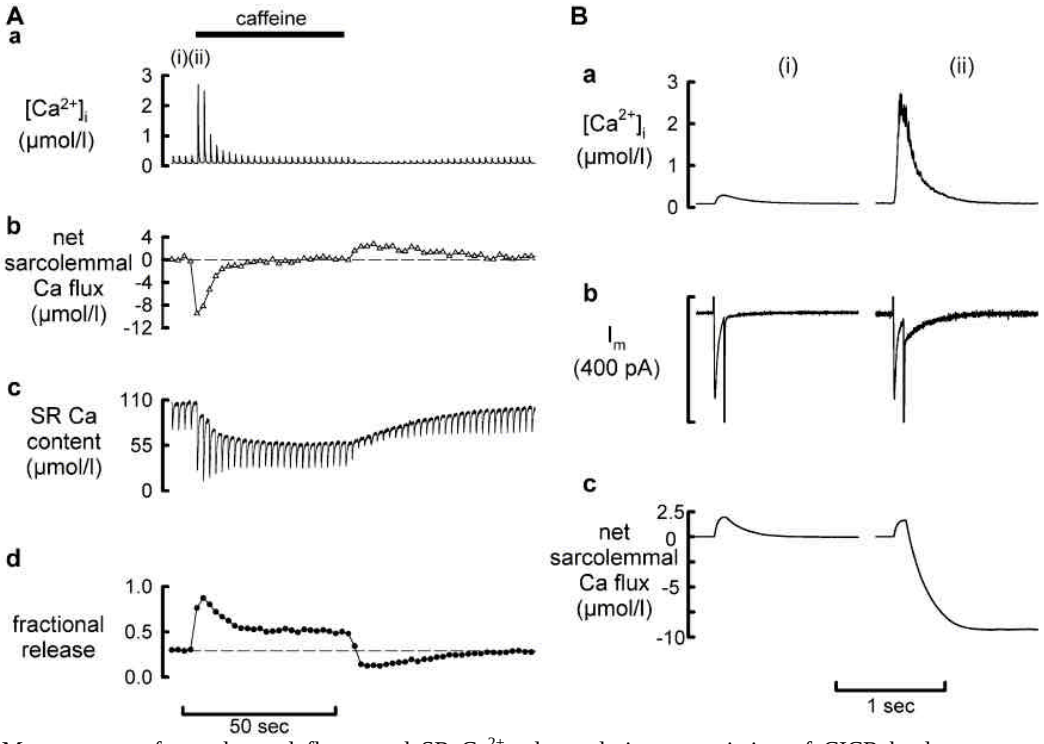

Figure 6. Measurments of sarcolemmal fluxes and SR Ca^{2+} release during potentiation of CICR by low concentrations of caffeine (500 μM). A. Time course. Traces show (from top to bottom): a, $[Ca^{2+}]_i$; b, net sarcolemmal flux (influx – efflux); c, time course of changes of SR Ca content; d, fraction of SR Ca released per pulse. The cell was held at –40 mV and 100 ms duration depolarizing pulses applied to 0 mV. B. Specimen records from the control transient (i) and the first in caffeine (ii). Traces show (from top to bottom): $[Ca^{2+}]_i$; membrane current; net sarcolemmal flux.

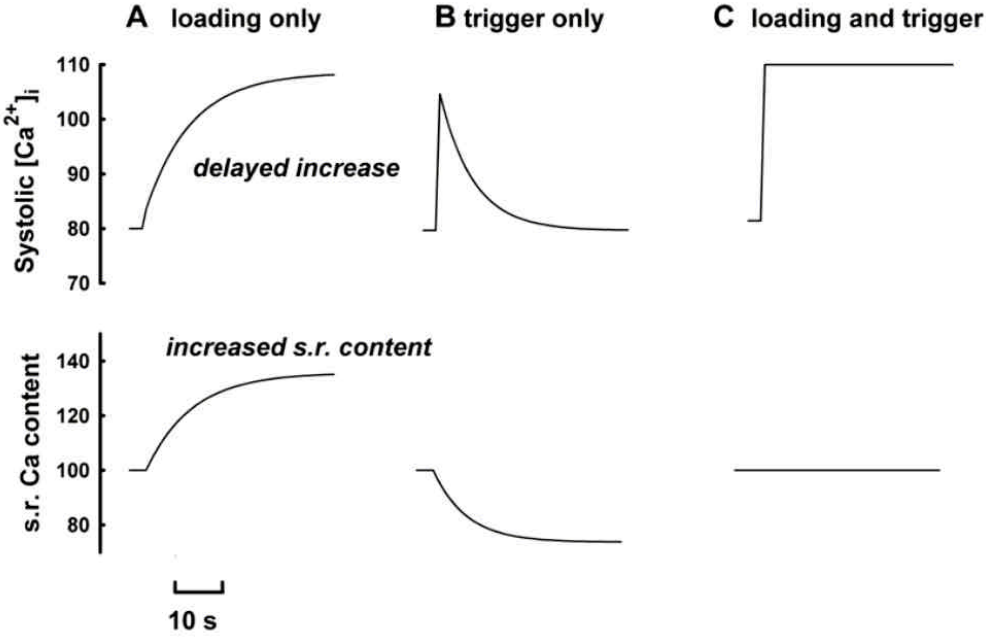

Figure 7. Simulation of the effects of increasing either or both of the loading or the trigger function of the L-type Ca current. The top shows the amplitude of the calculated systolic Ca transient and the bottom trace the SR content. A. The effects of increasing the loading function of the Ca current. B. The effects of increasing the trigger function; C. The effects of increasing trigger and loading. Reproduced from (73).

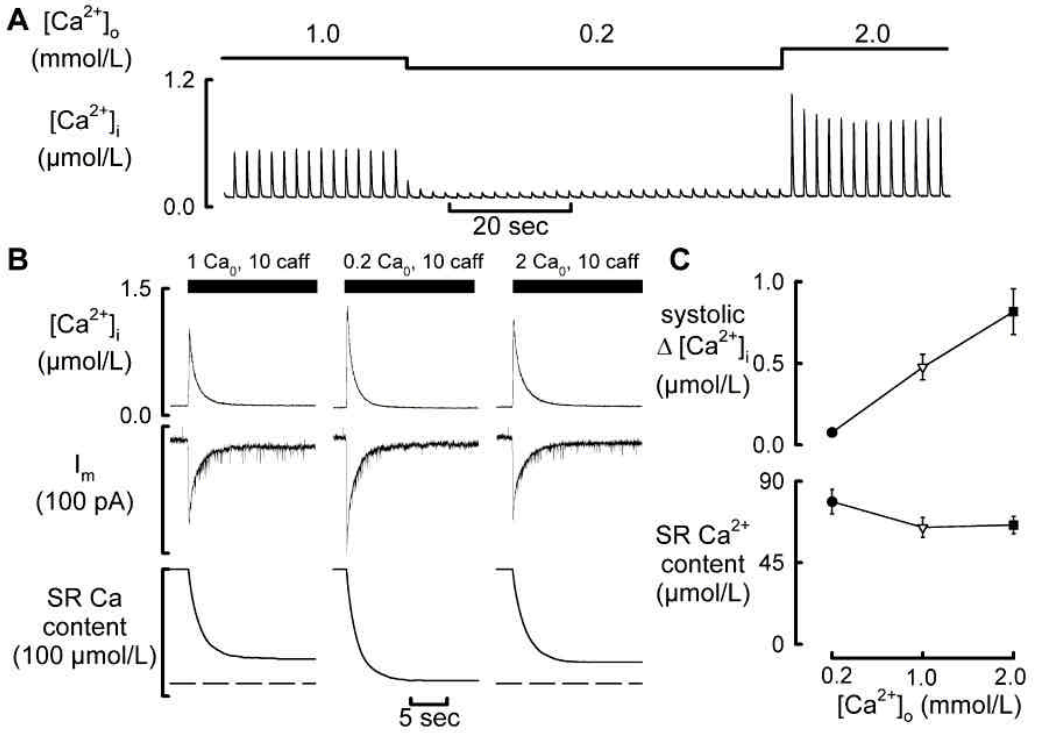

Figure 8. Changing Ca entry has a large effect on systolic $[Ca^{2+}]_i$ but little on SR content. A. Timecourse of change of systolic $[Ca^{2+}]_i$ in response to the indicated changes of external Ca concentration. B. SR Ca content following exposure to the indicated external Ca. In each panel 10 mM caffeine was applied to activate SR Ca release and the resulting electrogenic Na-Ca exchange used as an index of SR content. C. Mean data showing the dependence of: top, SR Ca content; bottom, systolic $[Ca^{2+}]_i$ on external Ca concentration. Taken from (75).

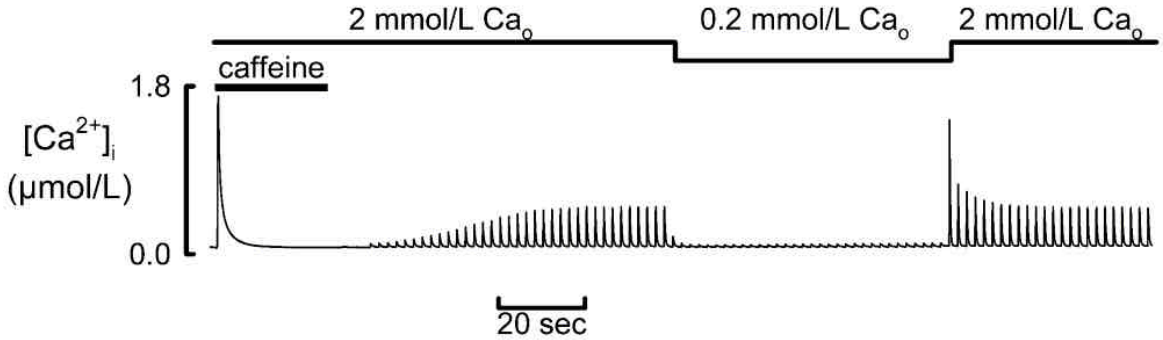

Figure 9. Comparison of the timecourse of the inotropic effects resulting from store refilling with those due to increased Ca current. In the record caffeine (10 mM) was initially applied to empty the SR. On removal of caffeine, the resulting recovery of the amplitude of the systolic Ca transient is much slower than that due to increased Ca current produced by raising external Ca concentration. Modified from (75).

The importance of the coordinated changes of trigger and load is shown in Figure 9. This compares two inotropic manoeuvres. (1) The recovery from an emptied SR. At the start of the record caffeine was applied to empty the SR. After removal of caffeine the empty SR results in a very small Ca transient. It then takes several beats for the control Ca transient to be reached; the slowness of the response being determined by sarcolemmal fluxes. (2) The second manoeuvre is the recovery from a reduction of $[Ca^{2+}]_o$. This is almost instantaneous and the speed of this response is due to the fact that there is no change of SR content.

Figure 10 considers what would happen if only loading changed. The experimental records show the timecourse of Ca transient (top) on increasing $[Ca^{2+}]_o$. Again, a rapid response is observed. The open circles show a simulation of what would happen if raising external Ca only increased the loading function of the Ca current. This would increase the SR Ca content and thence the Ca

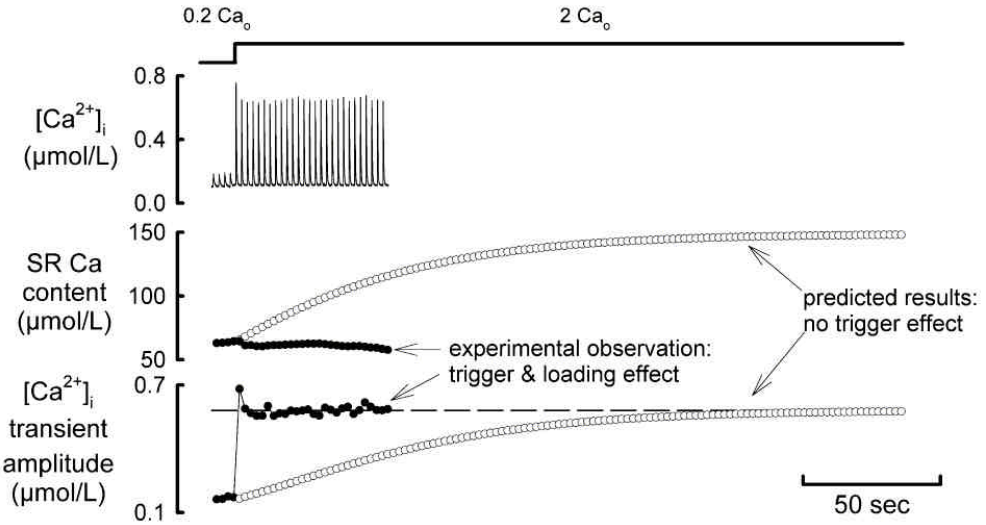

Figure 10. Comparison of the effects of increasing external Ca concentration with the effects expected if only the loading effect was present. External Ca concentration was increased from 0.2 to 2 mM for the period shown. The top trace shows the experimental record of $[Ca^{2+}]_i$. The solid symbols show the measured amplitude of systolic $[Ca^{2+}]_i$ and the calculated SR content. The open circles show the calculated results if there was no trigger effect. Modified from (75).

transient. The observed amplitude of the Ca transient could only be achieved with more than a doubling of SR content and after a considerable delay. The importance of the coordination of trigger and loading is clear from these data.

Close inspection of the data shows that the response of systolic Ca to an increase of $[Ca^{2+}]_o$ has a slight overshoot. There is also a small decrease of SR content (Figure 8). This presumably means that changing $[Ca^{2+}]_o$ over the range 0.2 to 1 mM has a slightly greater effect on the trigger than on the loading function. Nevertheless, to a first approximation, the overshoot and decrease of SR content are small and do not detract from the above conclusions.

Finally, it is worth noting that the design of Ca induced Ca release means that one process, the L-type Ca current, provides not only the trigger for Ca release but also the load which maintains the SR content. As we have seen above, this dual role for one channel is important in controlling the SR Ca content and thence contraction.

6. CONCLUSIONS

Work considered in the review emphasises the need to consider interactions between various Ca handling mechanisms. This should inform, not only consideration of normal excitation-contraction coupling but also those brought about by various inotropic manoeuvres or disease states e.g. in heart failure.

7. ACKNOWLEDGEMENTS

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