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Review Article

The control of calcium signaling in the heart

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ABSTRACT

Work on the role of calcium in the heart began in the nineteenth century with Ringer's demonstration that calcium is essential for cardiac contraction. This article provides a brief overview of the regulation of cardiac calcium signalling. Contraction results from the systolic rise of Ca concentration (the Ca transient). This occurs by the process of calcium induced Ca release in which Ca entry into the cell results in the opening of the sarcoplasmic reticulum Ca release channel (Ryanodine Receptor) releasing a much larger quantity of Ca into the cytoplasm. The Ca concentration in the sarcoplasmic reticulum is a major factor determining the amplitude of the Ca transient. The remainder of the article discusses how sarcoplasmic reticulum Ca content is regulated and the consequences this has for regulation of systolic Ca.

INTRODUCTION

The origin of ideas on calcium and the heart

The story of calcium and the heart began with Sidney Ringer (1836-1910) who was a Physician at University College Hospital, London. He set out to investigate the effects of various inorganic compounds on the contractility of the isolated heart. In 1882 he reported that a simple solution of sodium chloride was sufficient to sustain cardiac contraction (Ringer 1882). A year later, however, he wrote what is one of the most famous retractions in the field of physiology (Ringer 1883). The most important paragraph in this paper is. "*I discovered, that the saline solution which I had used had not been prepared with distilled water, but with pipe water supplied by the New River Water Company. As this water contains minute traces of various inorganic substances, I at once tested the action of saline solution made with distilled water and I found that I did not get the effects described in the paper referred to. It is obvious therefore that the effects I had obtained are due to some of the inorganic constituents of the pipe water."*

130 years later one can still admire the mind that, having discovered that the previous year's result was not reproducible, realized that the problem was due to a contaminant and then correctly identified calcium as the culprit. Several other things are worth noting in this anecdote. (1) Ringer's exhaustive work provided the evidence required to design intravenous solution as illustrated by the term "Ringers" or "Ringers-lactate". (2) It is interesting to focus on the language of his paper. The use of the active voice as in "*I discovered"* and *"I had used"*emphasises his leading role in the work and contrasts with the passive *had not been prepared"* indicating that someone else had erred in making the solution. (3) It is also worth noting that the calcium concentration in London pipe water is of the order of 1 mM. If Ringer had done his 1882 experiments in an area with softer water then history would have been very different!

Another important milestone came almost one hundred years later in 1978 with the recording by Allen and Blinks of the transient increase of cytoplasmic calcium that activates cardiac contraction (Allen and Blinks 1978). The subsequent introduction by Tsien(Grynkiewicz et al. 1985) of calcium-sensitive fluorescent indicators has revolutionized studies of

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cardiac calcium signalling. As a result, calcium signalling in the heart has expanded into an enormous field, which is impossible to summarize in one brief review. This article will therefore focus on recent work from our laboratory.

Calcium signaling in the heart

Fig. 1 shows the steps involved in linking cardiac excitation to contraction ("excitation-contraction coupling"). This process depends on the precise anatomical subcellular arrangement. Most of the calcium that makes up the systolic Ca transient comes from the sarcoplasmic reticulum (SR). Ca^{2+} ions are accumulated in the SR by the SR Ca-ATPase (SERCA) and are released into the cytoplasm by a release channel known as the Ryanodine Receptor (RyR). The probability that the RyR is open (and can therefore allow Ca^{2+} ions to be released from the SR is increased by both cytoplasmic and SR Ca concentration (Rousseau et al. 1986;Xu and Meissner 1998). Critically, the RyRs in the SR membrane are very close to the invaginations of the cell surface membrane known as transverse (t)-tubules. These contain many of the ion channels involved in excitation-contraction coupling (for review see (Orchard and Brette 2008)). During the heart beat, opening of the L-type Ca channels in the sarcolemma produces a large increase of cytoplasmic Ca concentration in the space between the surface membrane and SR. This results in opening of the RyRs leading to Ca release from the SR. This mechanism is known as "Ca-induced Ca release" (CICR) (Fabiato 1985). This release of Ca produces the upstroke of the systolic Ca. For the heart to work efficiently as a pump, it must relax in order to refill with blood. This relaxation is achieved by a combination of the RyRs closing and active uptake of Ca into the SR by SERCA. Ca is also removed from the cytoplasm by the Na-Caexchange (NCX). This uses the energy provided by 3 Na^{+} entering the cell to pump one Ca^{2+} out and thereby generates a depolarizing current as calcium is pumped out of the cell.

It should be noted that, in the steady state, the amount of Ca that enters the cell on each beat (via the L-type Ca current) must equal that which is pumped out (largely on NCX). In addition the amount released from the SR (through the RyR) must equal that taken back into the SR on SERCA. This condition of flux balance is vital for cardiac function and has important consequences for understanding the control of contraction (Eisner et al. 1998;Eisner et al. 2000;Eisner et al. 2009).

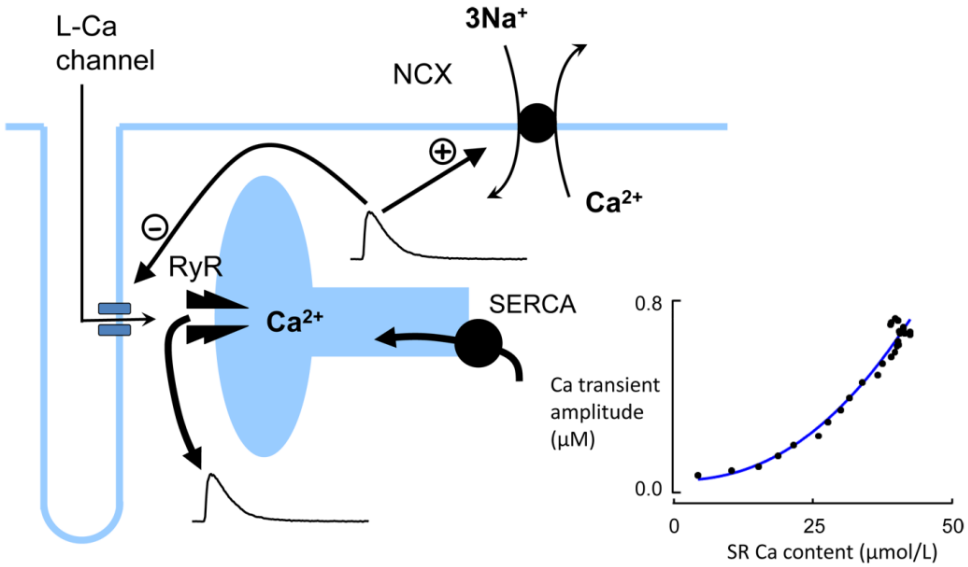

Fig. 1.
Calcium fluxes in the ventricular myocytes and control of SR Ca. The systolic Ca transient results from an influx of Ca into the cell (L-type Ca current) opening Ryanodine Receptors (RyR).Ca is then removed from the cytoplasm by SERCA and Na-Caexchange (NCX). An increase of the Ca transient decreases Ca entry on the L-type current and increases efflux on NCX. See main text for how this contributes to control of SR Ca content. The inset shows the steep dependence of the systolic Ca transient on SR Ca content. Figure modified from (Eisner, Bode, Venetucci, & Trafford 2012).

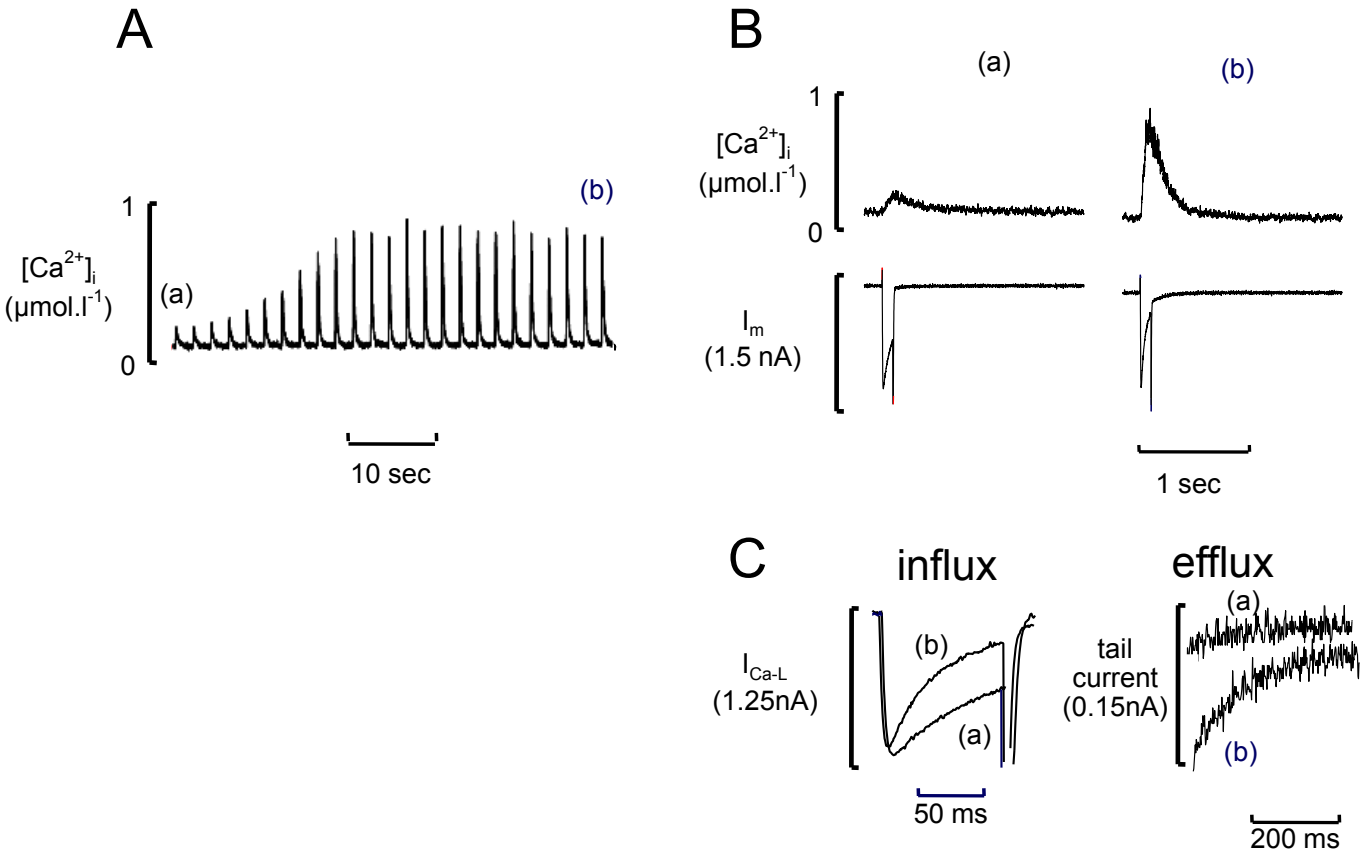

Fig. 2.
The effects of the systolic Ca transient on sarcolemmal Ca fluxes. **A**. Timecourse. The trace shows intracellular Ca transients in response to voltage clamp stimulation. The SR had previously been emptied by exposure to 10 mM caffeine. Caffeine was then removed and stimulation begun. **B**. Expanded records of $[Ca^{2+}]_i$ (top) and membrane current (below) from the transients identified in A with SR empty (a) and full (b). **C**. Superimposed records of Ca entry on L-type Ca current (influx) and efflux on NCX. Modified from (Trafford, Díaz, Negretti, & Eisner 1997).

The amount of Ca released from the SR and therefore the amplitude of the systolic Ca transient depend on (i) the size of the triggering L-type current; (ii) the properties of the RyR and (iii) the amount of Ca in the SR. The importance of SR Ca is emphasised by the inset of Fig 1 which shows the steep dependence of the amplitude of the systolic Ca transient on the SR Ca content (Shannon et al. 2000;Trafford et al. 1997;Trafford et al. 2000). This arises, at least in part, from the fact that increasing SR Ca content increases the open probability of the RyR.

This steep dependence of systolic Ca on the SR Ca content has two important implications. (1) It means that even modest changes of SR Ca can have important effects on contractility. Not only is this potentially physiologically important but the decline of the systolic Ca transient seen in cardiac hypertrophy or failure is contributed to by a decrease of SR Ca content and even a modest decrease of SR Ca can produce a significant decrease of the systolic Ca transient(Díaz et al. 2004). (2) Stable beat to beat control of the amplitude of the systolic Ca transient means that SR Ca content must be regulated very precisely. It is therefore essential to understand the mechanisms that contribute to the stable regulation of SR Ca content. We have investigated this by deliberately emptying the SR and studying how it

refills. In the experiment illustrated in Fig 2A the SR had been emptied by exposing the cell to caffeine (10 mM). (Caffeine makes the RyRs open thereby releasing Ca into the cytoplasm whence it is pumped out of the cell). Caffeine was removed and the cell stimulated. As shown, the Ca transient is initially very small as the SR is empty. The subsequent recovery of the amplitude of the Ca transient is due to the SR regaining Ca. The mechanism by which the SR Cacontent recovers is illustrated in Fig 2B. The left hand traces show Ca transient and membrane current when the SR is empty (a) and the right hand one when it is full (b). These current traces are shown in even more detail in Fig. 2C. The left hand traces show the L-type Ca currents superimposed (Ca influx). Both currents have the same amplitude but the one with the SR full (b) decays (inactivates) more quickly. This is because inactivation of the L-type Ca current is a Ca-dependent process and the larger systolic Ca transient leads to faster inactivation. The Ca efflux (on Na-Ca exchange) is illustrated in the right hand panel of Fig. 2C. The size of the current (and therefore the Ca efflux) is greater for the larger (b) than the smaller (a) Ca transient. This is a simple consequence of the fact that NCX is activated by the Ca transient.

The effects of this are shown in Fig. 1. The Ca transient acts to inactivate the L-type Ca current and thereby decrease Ca influx and increase Ca efflux on NCX and thereby increasesCa efflux from the cell. This leads to a mechanism for regulating SR Ca content as follows. An increase of SR Ca content will increase the size of the systolic Ca transient. This will decrease Ca entry and increase Ca efflux thereby decreasing the Ca content of the cell and, consequently, of the SR. It is this negative feedback system which is responsible for beat to beat regulation of the Ca content of the SR.

It is important to emphasize that the exact steady state level of SR Ca which is attained depends on the relative activity of the four major Ca handling proteins: L-type Ca current, NCX, RyR and SERCA. A change of any single one of these will result in a change of SR Ca content. In heart failure, for example, the increase of NCX(Hasenfuss et al. 1999) , decrease of SERCA(Hasenfuss 1998) and increase of RyR leak (Marx et al. 2000;Shannon et al. 2003;Terentyev et al. 2008) will result in a decrease of SR Ca content.

Consequences of feedback control of SR Ca content

In the remainder of this review we consider examples showing the importance of this feedback control of SR Ca content.

The effects of modifying systolic opening of the RyR

A good example of the importance of this feedback regulation of SR Ca content is provided by considering the effects of increasing the open probability of the RyR(Trafford et al. 1998; Trafford, Díaz, Sibbring, & Eisner 2000). One might expect that this would increase the amount of calcium released from the SR and therefore the amplitude of the systolic Ca transient.

That things are not so simple is shown by the trace shown in the top panel of Fig. 3. Here the open probability of the RyR was increased with a low concentration of caffeine (0.5 mM). This effectively sensitizes calcium induced calcium to activation by the local rise of $[Ca^{2+}]_i$ produced by Ca entry on the L-type Ca current. Consequently, the amplitude of the first systolic Ca transient after caffeine application is much larger than that of the control.

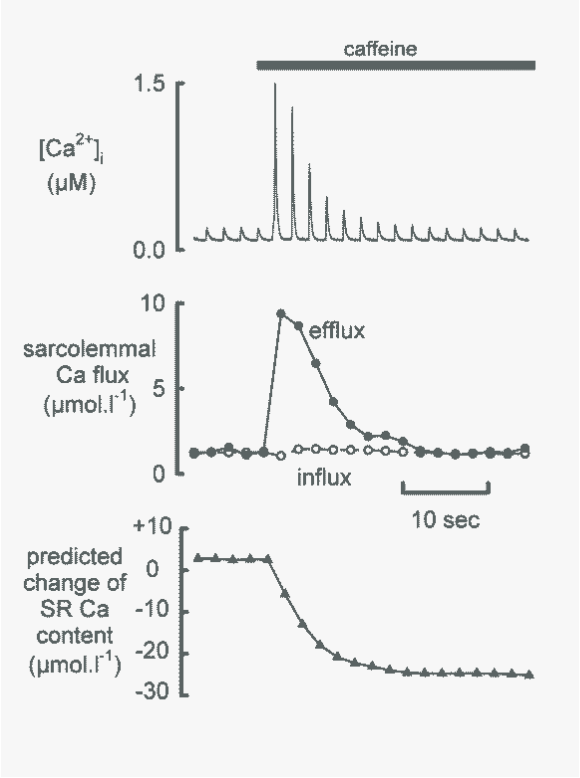

Fig. 3.
The effects of potentiation of the RyR on systolic $[Ca^{2+}]_i$. The top trace shows the time course of $[Ca^{2+}]_i$. The cell was stimulated with depolarizing pulses and caffeine applied as shown. The middle panel shows the calculated influx (open circles) and efflux (closed circles) on each beat. The bottom panel shows the calculated changes of SR Ca content. Modified from (Trafford, Díaz, Sibbring, & Eisner 2000).

However this increase is not maintained and the amplitude decreases over the next few transients such that, in the steady state, the amplitude is identical to that of the control. In other words, despite the maintained presence of caffeine and therefore of its effect on the RyR, the effect on the systolic Ca transient fades quickly.

The explanation of this effect is provided by the middle panel of Fig. 3 which shows the calculated Ca entry (via the L-type channel) and efflux (NCX) on each beat. At the start of the period shown the two fluxes are equal and the cell is therefore in a steady state. Consequently the Ca content of the cell and therefore the SR is steady (see bottom panel). However, when the amplitude of the Ca transient increases so does the Ca efflux on NCX. As a result, Ca efflux exceeds Ca influx and the cell is no longer in a steady state and SR Ca content decreases. This continues until the SR Ca content has declined sufficiently that the amplitude of the systolic Ca transient has fallen to control levels. Consequently the Ca efflux will be the same as in control and Ca efflux and influx are back in balance.

The negative feedback regulation of SR Ca content therefore accounts for the previously perplexing observation that simply increasing the systolic opening of the RyR has no effect on the amplitude of the systolic Ca transient in the steady state. These results have implications for the general issue of the effects of regulating the RyR. For example it has been suggested that some of the positive inotropic effects of β-adrenergic stimulation are due to phosphorylation of the RyR increasing its open probability(Shan et al. 2010). Phosphorylation of the RyR by CaM-Kinase II has also been suggested to increase contraction(Kushnir et al. 2010). These experimental observations are strongly contested(MacDonnell et al. 2008) (see (Eschenhagen 2010) for review). Our view is that the results of Fig. 3 make it unlikely that opening of the RyR will produce a maintained positive inotropic effect (Eisner et al. 2012;Eisner, Kashimura, O'Neill, Venetucci, & Trafford 2009).

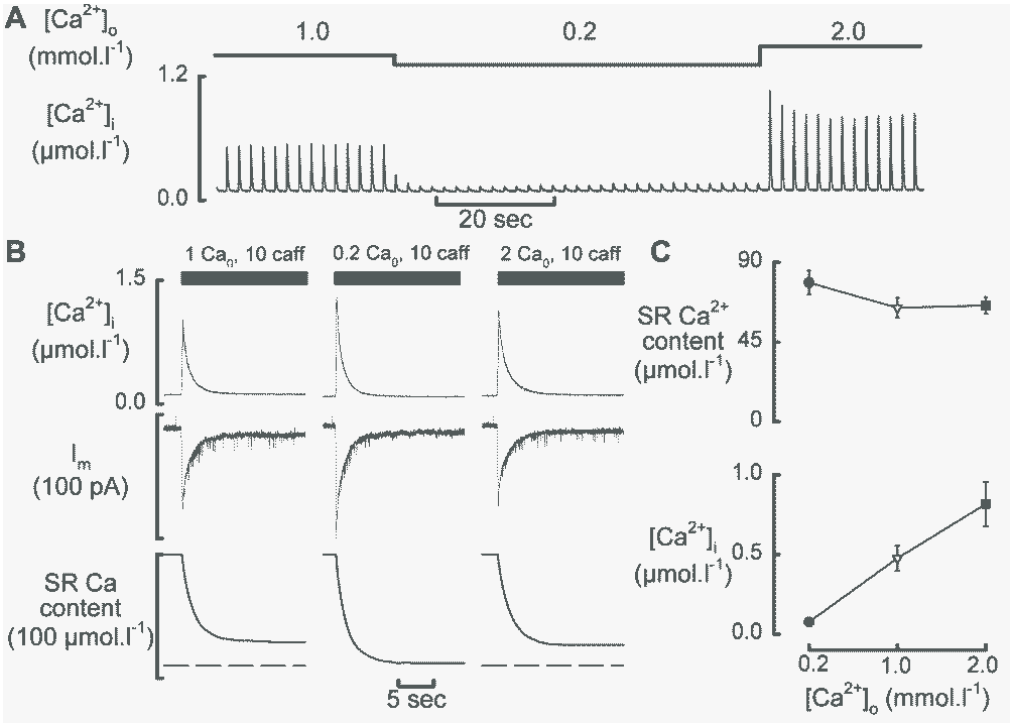

Fig. 4.
The effects of extracellular Ca concentration on the systolic Ca transient and SR Ca content. **A**. Timecourse of effects of changes of external Ca on systolic Ca. **B**. Measurement of SR Ca content. Traces show (from top to bottom): $[Ca^{2+}]_i$; membrane current, integral of current – a measure of SR Ca content. In each part the cell had been stimulated in the presence of the external Ca concentration indicated and then caffeine (10 mM) was applied to release Ca from the SR. **C**. Relationship between SR Ca content (top), systolic $[Ca^{2+}]_i$(bottom) and external Ca concentration. Taken from (Trafford, Díaz, & Eisner 2001).

The effects of modifying the L-type Ca current

As reviewed at the beginning of this article, our knowledge of the role of calcium in the heart began with Ringer's experiment in which he accidentally removed external calcium and thereby abolished the calcium current. We now know that the calcium current has two roles in cardiac excitation-contraction coupling (Fabiato 1985). (1) It triggers release of calcium from the SR and (2) it reloads the cell (and therefore the SR) with calcium. In terms of the feedback mechanism for control of SR Ca content discussed above, these two roles of Ca have complementary effects on SR Ca. The triggering role will lead to a decrease of SR Ca as some of the released Ca is pumped out of the cell; the loading function will restore SR Ca. The consequences of this can be seen in Fig. 4. This shows the effects of changing the calcium current by altering external Ca from 1 to 0.2 to 2 mM. These changes of Ca result in corresponding changes of the amplitude of the systolic Ca transient(Trafford et al. 2001) (Fig. 4A). We next addressed the question ofwhat role do changes of SR Ca content play in these changes? SR Ca was measured by applying 10 mM caffeine to release Ca from the SR. This results in an increase of $[Ca^{2+}]_i$ (Fig. 4B) which decays as Ca is pumped out of the cell on NCX. The stimulation of NCX activity is reflected by the inward NCX current and this can be integrated to give a measure of the amount of Ca released from the SR(Varro et al. 1993). Fig. 4C shows mean data. An increase of external Ca concentration produces large effects on the systolic Ca transient but much smaller effects on SR Ca content. Our explanation of this result is that the effects of Ca current to release Ca from the SR are balanced with those to load the cell with calcium. Indeed, at least under the conditions of the experiment of Fig. 4., the ability to trigger release and therefore deplete the SR is actually slightly more powerful than to load the SR as shown by the fact that an increase of external Ca actually produces a small decrease of SR Ca content.

The fact that changes of calcium current have little or no effect on SR Ca content allows a change of Ca current to have an instantaneous effect on the amplitude of the systolic Ca transient without the delay that would be introduced if changes of SR content were involved(Trafford, Díaz, & Eisner 2001).

Conclusions

This brief review has highlighted the importance of the calcium content of the SR in the regulation of contraction of the heart. Furthermore, we have demonstrated how SR Ca content is controlled and how this regulatory mechanism impacts on the effects of inotropic manoeuvres.

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