No Role for the Ryanodine Receptor in Regulating Cardiac Contraction?

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Cardiac contraction is initiated by Ca\textsuperscript{2+} leaving the sarcoplasmic reticulum through the ryanodine receptor (RyR). Although opening of the RyR can be modified by various ligands, these have no maintained effect on contraction. We conclude that modulation of the RyR controls sarcoplasmic reticulum Ca\textsuperscript{2+} content rather than cytoplasmic Ca\textsuperscript{2+} concentration.

Contraction of cardiac muscle results from an increase of intracellular Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]) from resting or diastolic levels of 100 nM to a systolic level on the order of 1 \mu M. Varying the magnitude of this systolic increase of Ca\textsuperscript{2+} alters the degree of activation of the contractile proteins and therefore force production (4). As shown in Fig. 1A, the systolic Ca\textsuperscript{2+} transient has two sources: 1) Ca\textsuperscript{2+} enters the cell from the extracellular fluid via the L-type Ca\textsuperscript{2+} current, and 2) Ca\textsuperscript{2+} is released from an intracellular store, the sarcoplasmic reticulum (SR). In mammalian cardiac muscle, the release from the SR contributes the bulk of the increase of [Ca\textsuperscript{2+}]. Ca\textsuperscript{2+} leaves the SR down a concentration gradient through the Ca\textsuperscript{2+} release channel or ryanodine receptor (RyR). The probability that the channel is open is increased by an increase of the cytoplasmic Ca\textsuperscript{2+} concentration. This results in the process of Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+} release (CICR), in which the opening of a single L-type Ca\textsuperscript{2+} channel results in the release of a much larger amount of Ca\textsuperscript{2+} from the SR. Recent work using confocal microscopy has led to the suggestion that the elementary event of CICR is a Ca\textsuperscript{2+} “spark,” in which the opening of a single L-type Ca\textsuperscript{2+} channel results in the opening of one or a small number of RyRs. Further information can be found in recent reviews (5, 11).

Ca\textsuperscript{2+} influx and efflux are equal over the cardiac cycle

For the heart to function as a pump, it must relax as well as contract. This relaxation requires that Ca\textsuperscript{2+} be removed from the cytoplasm either by reuptake into the SR (by the SR Ca\textsuperscript{2+}-ATPase; Fig. 1A) or by pumping out of the cell (by the combined actions of the sarcolemmal Ca\textsuperscript{2+}-ATPase and Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange; Fig. 1A). It is important to note that, for the heart to be in a steady state, the amount of Ca\textsuperscript{2+} that enters the cytoplasm on each beat must exactly equal that which is removed. Furthermore, the amount released from the SR must balance that taken back up into the SR and that entering the cell from outside must be pumped back out of the cell. This cellular Ca\textsuperscript{2+} balance is demonstrated in Fig. 1B. Ca\textsuperscript{2+} entry occurs during the depolarizing pulse via the L-type Ca\textsuperscript{2+} current. The magnitude of this entry can be measured by integrating the current and, in this case, corresponds to 3.5 \mu mol/l. The efflux from the cell can be measured from the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange current on repolarization (after correcting for the electroneutral Ca\textsuperscript{2+}-ATPase; for methods and so forth see Ref. 9). The cumulative integral shows that efflux equals influx. As will be reviewed below, this condition of cellular Ca\textsuperscript{2+} balance has important consequences for maneuvers that affect systolic [Ca\textsuperscript{2+}]i.

The above description provides an overview of the origin of the systolic increase of [Ca\textsuperscript{2+}]i. It does not, however, address the physiologically important question of how the magnitude of the transient is regulated to control cardiac output. The following are among the potential control points: 1) The magnitude of the Ca\textsuperscript{2+} influx. The larger this is the greater the trigger will be, leading to increased RyR opening. 2) The properties of the RyR. In particular, the dependence of the opening probability of the RyR on the trigger [Ca\textsuperscript{2+}]i is important. 3) The Ca\textsuperscript{2+} content of the SR. This will determine how much Ca\textsuperscript{2+} is released when a given number of RyRs open. The importance of 1 and 3 are well established; both an increase of the magnitude of the L-type Ca\textsuperscript{2+} current and of SR Ca\textsuperscript{2+} content increase systolic Ca\textsuperscript{2+}. Indeed, the magnitude of the systolic Ca\textsuperscript{2+} transient depends very steeply on SR Ca\textsuperscript{2+} content (3). In contrast, as will be shown below, the effects of 2 are much more complicated.

The regulation of SR Ca\textsuperscript{2+} content ultimately depends on the control of surface membrane Ca\textsuperscript{2+} fluxes: control by release of Ca\textsuperscript{2+} from the SR

Given that SR Ca\textsuperscript{2+} content affects the size of the systolic Ca\textsuperscript{2+} transient, it is important that the SR Ca\textsuperscript{2+} content is regulated. It is therefore important to understand the mechanisms controlling the SR Ca\textsuperscript{2+} content. The immediate control point is the balance of Ca\textsuperscript{2+} uptake into the SR and Ca\textsuperscript{2+} release. Thus maneuvers that stimulate the SR Ca\textsuperscript{2+}-ATPase (such as phosphorylation of phospholamban) will increase the SR Ca\textsuperscript{2+} content, whereas stimulation of Ca\textsuperscript{2+} release will decrease the content. It should also be noted that sarcolemmal Ca\textsuperscript{2+} fluxes play a major but indirect role in the control of the SR Ca\textsuperscript{2+} content. If the SR is emptied, then the rate and extent of refilling are both increased by electrical stimulation (9).

At first sight, it might seem obvious that stimulation increases the rate of refilling of the SR. One would expect
that the Ca\textsuperscript{2+} entry produced by each depolarization would lead to an increase of cell and therefore SR Ca\textsuperscript{2+} content. The situation is, however, more complex than this. The experiment illustrated in Fig. 2 measures the Ca\textsuperscript{2+} influx and efflux produced by each pulse during refilling. Immediately after removal of caffeine, the systolic Ca\textsuperscript{2+} transient is very small, presumably due to the reduced SR Ca\textsuperscript{2+} content. This small Ca\textsuperscript{2+} transient is accompanied by an entry of Ca\textsuperscript{2+} on the Ca\textsuperscript{2+} current, which is larger than that observed in the steady state (when the systolic Ca\textsuperscript{2+} transient has recovered). The increase of Ca\textsuperscript{2+} entry is presumably due to the fact that the smaller Ca\textsuperscript{2+} transient results in less Ca\textsuperscript{2+}-dependent inactivation of the L-type Ca\textsuperscript{2+} current (1). In addition, the efflux of Ca\textsuperscript{2+} on the first transient is less than that in the steady state, because the small Ca\textsuperscript{2+} transient produces a reduced activation of the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange. The data in this figure show that, when caffeine is initially removed, the Ca\textsuperscript{2+} entry on the L-type Ca\textsuperscript{2+} current is larger...
than the efflux on the exchanger. This results in a net gain of Ca$^{2+}$ by the cell and therefore by the SR. As the systolic Ca$^{2+}$ transient increases in size, the influx gradually decreases and the efflux increases until they are once more in balance. This result therefore shows two important negative feedback systems. An increase of the Ca$^{2+}$ transient results in 1) a decrease of Ca$^{2+}$ entry into the cell and 2) an increase of efflux such that the SR Ca$^{2+}$ content will decrease. This, in turn, will decrease the systolic Ca$^{2+}$ transient. Therefore, the effect of systolic Ca$^{2+}$ on sarcolemmal Ca$^{2+}$ fluxes serves to control the SR Ca$^{2+}$ content.

Two interesting conclusions can be drawn from the above result. The first is that without the feedback of SR Ca$^{2+}$ release onto the sarcolemmal fluxes, it would be very hard to regulate cell Ca$^{2+}$ balance. Any small imbalance between Ca$^{2+}$ entry and efflux across the sarcolemma would persist, and the cell would either gain or lose Ca$^{2+}$. Although mechanisms exist to regulate the rate of the SR Ca$^{2+}$-ATPase, these cannot compensate for a net imbalance of Ca$^{2+}$ flux into the cell. The second point comes from a comparison with the control of endoplasmic reticulum Ca$^{2+}$ content in nonexcitable cells. This involves the process of capacitative Ca$^{2+}$ influx, in which the state of filling of the endoplasmic reticulum controls a dedicated Ca$^{2+}$ entry across the surface membrane (for review see Ref. 2). Such a mechanism would not be effective in cardiac muscle, because it would be swamped by the large sarcolemmal fluxes. Instead, a similar function is obtained by allowing Ca$^{2+}$ release from the SR to regulate the L-type Ca$^{2+}$ current and the Na$^{+}$/Ca$^{2+}$ exchange.

The properties of RyR do not regulate systolic Ca$^{2+}$

As well as being regulated by cytoplasmic Ca$^{2+}$ concentration, the properties of RyR can be influenced by a variety of other agents and conditions (for review see Refs. 7 and 8). Pharmacologically, caffeine increases the open probability and the local anesthetic tetracaine decreases it. Of particular note under physiological conditions is the fact that the opening probability is enhanced by phosphorylation and cADP ribose. A decrease of ATP concentration or acidification (as occur during ischemia) reduce the open probability, whereas an increase of the concentration of free radicals (as happens on reperfusion) will increase the open probability of the RyR. Finally, it has also been suggested that one explanation for the changes of the systolic Ca$^{2+}$ transient in cardiac hypertrophy and failure is an effect on the coupling between the L-type Ca$^{2+}$ current and the RyR, leading to a decrease of open probability (6). To investigate the role of modulation of the RyR, we have carried out experiments in which the SR Ca$^{2+}$ content, sarcolemmal fluxes, and systolic [Ca$^{2+}$] were measured. We find that pharmacological modification of the RyR has no steady state effect on contraction (10). This is demonstrated in Fig. 3, which shows the effects of applying a low concentration of caffeine to increase the open probability of the RyR. However, the effect on systolic Ca$^{2+}$ is...
purely transient and, in the steady state, the contraction in the presence of caffeine is the same magnitude as that under control conditions. On removal of caffeine, there is an undershoot before contraction recovers. The explanation of this transient effect is that, as shown above, in the steady state, Ca\textsuperscript{2+} efflux must equal Ca\textsuperscript{2+} influx. Since caffeine has no effect on Ca\textsuperscript{2+} influx, in the steady state it cannot affect the efflux. Because the efflux is determined by the magnitude of the systolic Ca\textsuperscript{2+} transient, this requires that the amplitude of the systolic Ca\textsuperscript{2+} transient be unaffected by manipulation of the RyR. The transient response arises because caffeine initially increases the fraction of the SR Ca\textsuperscript{2+} content that is released. This therefore increases the efflux of Ca\textsuperscript{2+} from the cell and results in a decrease of SR Ca\textsuperscript{2+} content. This, in turn, decreases the amplitude of the next systolic Ca\textsuperscript{2+} transient. In the steady state in caffeine, the Ca\textsuperscript{2+} transient has the same amplitude as the control one as a result of the opposing effects of an increase in the fractional release of Ca\textsuperscript{2+} and a decrease of SR Ca\textsuperscript{2+} content.

If the modification of the RyR has no steady state effect on systolic Ca\textsuperscript{2+}, why is it regulated?

The above discussion has shown that modification of the RyR has only a transient effect on systolic Ca\textsuperscript{2+}. The question therefore arises as to why the RyR is regulated. Two possible explanations are illustrated in Fig. 4. This shows a simulation of the effects of increasing the magnitude of the L-type Ca\textsuperscript{2+} current as occurs, for example, during β-sympathetic stimulation. This has two effects on excitation-contraction coupling: 1) It will increase the Ca\textsuperscript{2+} loading of the cell and 2) it will increase the trigger Ca\textsuperscript{2+}, thereby increasing the number of RyRs that open. These two consequences are modeled individually in Fig. 4, A and B. Figure 4A shows the effects of only increasing the loading function. This produces a maintained increase of systolic Ca\textsuperscript{2+} accompanied by an increase of SR Ca\textsuperscript{2+} content. The increase of systolic Ca\textsuperscript{2+} takes time to develop because the SR accumulates Ca\textsuperscript{2+} on a beat-to-beat basis. The effects of only increasing the trigger are shown in Fig. 4B. Similar to the effects of caffeine, this produces a transient increase of systolic Ca\textsuperscript{2+} accompanied by a decrease of SR Ca\textsuperscript{2+} content. There is no maintained effect on systolic Ca\textsuperscript{2+}. The final simulation (Fig. 4C) shows the effect of simultaneously increasing both the loading and trigger functions of the L-type Ca\textsuperscript{2+} current (as will actually occur). This results in an immediate and maintained increase of systolic Ca\textsuperscript{2+} with no change of SR Ca\textsuperscript{2+} content. Coordinated stimulation of both loading and trigger functions is therefore essential if an increase of Ca\textsuperscript{2+} current is to produce both a rapid onset and
a maintained response. Another potential benefit is related to the changes of SR Ca\(^{2+}\) content that accompany the increase of systolic Ca\(^{2+}\). When the loading alone is stimulated, an increase of SR Ca\(^{2+}\) content accompanies the increase of systolic Ca\(^{2+}\). This means that the ATPase has to pump against an increased Ca\(^{2+}\) concentration gradient, and this may become limiting. In addition, excessive filling of the SR results in spontaneous release of Ca\(^{2+}\) from the SR (12), and this may be arrhythmogenic. Further experimental work will be required to see whether, as suggested by the model, SR Ca\(^{2+}\) content is fixed during such simple inotropic interventions.

**Conclusion**

The arguments above suggest that, although RyR is essential for normal excitation-contraction coupling in the heart, it does not constitute a locus for regulation of contraction. This is because changes in SR Ca\(^{2+}\) content compensate for effects of modification of the RyR. Rather, modulation of the properties of the RyR may serve to allow rapid changes of contraction and, also, to control the Ca\(^{2+}\) content of the SR.

**References**