Increasing Ryanodine Receptor Open Probability Alone Does Not Produce Arrhythmogenic Calcium Waves
Threshold Sarcoplasmic Reticulum Calcium Content Is Required

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Abstract—Diastolic waves of Ca\(^{2+}\) release have been shown to activate delayed afterdepolarizations as well as some cardiac arrhythmias. The aim of this study was to investigate whether increasing ryanodine receptor open probability alone or in the presence of β-adrenergic stimulation produces diastolic Ca release from the sarcoplasmic reticulum (SR). When voltage-clamped rat ventricular myocytes were exposed to caffeine (0.5 to 1.0 mmol), diastolic Ca\(^{2+}\) release was seen to accompany the first few stimuli but was never observed in the steady state. We attribute the initial phase of diastolic Ca\(^{2+}\) release to a decrease in the threshold SR Ca\(^{2+}\) content required to activate Ca\(^{2+}\) waves and its subsequent disappearance to a decrease of SR content below this threshold. Application of isoproterenol (1 μmol/L) increased the amplitude of the systolic Ca\(^{2+}\) transient and also the SR Ca\(^{2+}\) content but did not usually produce diastolic Ca\(^{2+}\) release. Subsequent addition of caffeine, however, resulted in diastolic Ca\(^{2+}\) release. We estimated the time course of recovery of SR Ca\(^{2+}\) content following recovery from emptying with a high (10 mmol/L) concentration of caffeine. Diastolic Ca\(^{2+}\) release recommenced only when SR content had increased back to its final level. We conclude that increasing ryanodine receptor open probability alone does not produce arrhythmogenic diastolic Ca\(^{2+}\) release because of the accompanying decrease of SR Ca\(^{2+}\) content. β-Adrenergic stimulation increases SR content and thereby allows the increased ryanodine receptor open probability to produce diastolic Ca\(^{2+}\) release. The implications of these results for arrhythmias associated with abnormal ryanodine receptors are discussed. (Circ Res. 2007;100:105-111.)

Key Words: ryanodine receptor ■ Ca\(^{2+}\) wave ■ arrhythmias

In cardiac muscle, calcium ions are released from the sarcoplasmic reticulum (SR) through a specialized release channel known as the ryanodine receptor (RyR) via the process of calcium-induced calcium release. An influx of calcium into the cell on the L-type Ca\(^{2+}\) current makes the RyR open resulting in the release of much more Ca\(^{2+}\) from the SR (see Bers for review). However, under some abnormal conditions, calcium can be released from the SR during diastole in the absence of Ca\(^{2+}\) influx. Such diastolic release propagates along cells as waves of calcium-induced calcium release. This diastolic Ca\(^{2+}\) release activates the electrogenic Na\(^{+}/\)Ca\(^{2+}\) exchange (NCX), and the resulting inward current can produce a delayed afterdepolarization as well as ectopic pacemaker activity. At least 2 factors have been suggested to be involved in producing this diastolic release; (1) an increase of SR Ca\(^{2+}\) content (calcium overload) results in increased frequency of RyR opening until the point that Ca\(^{2+}\) waves are produced. This is thought to be responsible for the arrhythmogenic diastolic Ca\(^{2+}\) release in conditions such as digitalis intoxication and reperfusion following ischemia; and (2) changes in the properties of the RyR. Mutations in the human RyR or accessory proteins such as calsequestrin increase susceptibility to lethal arrhythmias such as catecholaminergic polymorphic ventricular tachycardia. It has also been suggested that in heart failure, the open probability (P\(_o\)) of the normal RyR may be increased by protein kinase A–dependent phosphorylation and that this may be arrhythmogenic. However, the regulatory protein of the SR Ca\(^{2+}\)-ATPase (SERCA) phospholamban is also a target for protein kinase A, and this work has not distinguished between the roles of (1) changes of RyR properties and (2) change of SR Ca\(^{2+}\) content in arrhythmogenesis. Although, acute sensitization of the RyR produces an abrupt Ca\(^{2+}\) release from the SR, prolonged exposure does not result in diastolic waves of Ca\(^{2+}\) release. The fact that humans with mutant RyRs develop arrhythmias in the presence of catecholaminergic stimulation is consistent with the idea that affecting the RyR alone is insufficient to cause arrhythmias, but the intact human is a complicated system to analyze. Work on ventricular wedge preparations found that increasing RyR probability (with caffeine) did not produce arrhythmias unless catecholaminergic stimulation was also present. This study, however, did not investigate cellular Ca\(^{2+}\) handling.

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The aim of the present work was to see whether increasing RyR P, alone could produce diastolic Ca\(^{2+}\) release. We did this by adding caffeine and found that (in the steady state) diastolic Ca\(^{2+}\) release was only observed when β-adrenergic stimulation was also present. We attribute the effects of β-adrenergic stimulation to the fact that it increases SR content to a level at which diastolic Ca\(^{2+}\) release can occur and conclude that increasing RyR leak per se is not necessarily arrhythmogenic.

**Materials and Methods**

**Voltage Clamp and [Ca\(^{2+}\)]\(_i\), Measurements**

Single ventricular myocytes were isolated from male Wistar rats by collagenase/protease digestion as described previously.\(^{14}\) The perforated patch technique with amphotericin-B (240 μg/mL) was used to impose voltage clamp on the cells. The patch pipettes (1.5 to 3 Ω resistance) contained (in mmol/L): CsCH\(_3\)O\(_3\) 115, CsCl 20, NaCl 12, HEPES 10, CsEGTA 0.1, and MgCl\(_2\) 5, titrated to pH 7.2 with CsOH. Access resistance was ~20 Ω, and the switch-clamp facility of the Axoclamp-2A amplifier (Axon Instruments) was used. Cells were stimulated with depolarizing pulses (of 75- to 100-ms duration from −40 to 0mV) at 0.5 Hz. The control superfusing solution contained (in mmol/L) NaCl 135, glucose 11, Ca\(^{2+}\)Cl\(_2\) 1 to 2, HEPES 10, MgCl\(_2\) 1, KCl 4, 4-aminopyridine 5, BaCl\(_2\) 0.1, probenecid 2, titrated to pH 7.4 with NaOH. Cells were loaded with the acetoxymethyl ester of the low-affinity (K\(_o\), 2.3 μmol/L) indicator Fluo 5F (Molecular Probes) to provide a wide range of sensitivity to changes of [Ca\(^{2+}\)]\(_i\).\(^{16}20\) Fluorescence was normalized to resting levels (F/F\(_o\)). Experiments were performed at room temperature (24°C). RyR \(P\), was increased using 2 different caffeine concentrations, 0.5 and 1 mmol/L. Isoproterenol (1 μmol/L; Sigma) or elevated (5 mmol/L) Ca\(^{2+}\) were applied as indicated.

**Calculation of Ca\(^{2+}\) Fluxes**

Ca\(^{2+}\) fluxes were calculated by integration of membrane currents as described previously.\(^{16,20}\) Ca\(^{2+}\) influx through the Ca\(^{2+}\) current was calculated by integrating the L-type Ca\(^{2+}\) current and efflux associated with the Ca\(^{2+}\) transient by integration of the NCX current immediately after repolarization (tail current). The efflux mediated by Ca\(^{2+}\) was quantified by integrating the associated inward NCX current. For these calculations, the current value corresponding to the minimum [Ca\(_i\)], reached after systole was used as the baseline current level.

Where applicable, the data are reported as the mean±SEM of n experiments. Significance was tested using either t test or 1-way ANOVA.

**Results**

Initial experiments investigated the effects of adding caffeine to cells superfused with control solution. As shown in Figure 1A, diastolic Ca\(^{2+}\) release was seen during the first few stimuli after addition of caffeine. However, in this and all the other 20 cells studied, the application of caffeine (at 0.5 or 1 mmol/L) never produced diastolic Ca\(^{2+}\) release in the steady state. Rather, caffeine decreased both the amplitude and the rate constant of decay of the systolic Ca\(^{2+}\) transient (Figure 1B and 1C). By integrating the Ca\(^{2+}\) influx (on the L-type Ca\(^{2+}\) current) and efflux (on NCX),\(^{16}\) we can calculate the change of SR Ca\(^{2+}\) content (Figure 1A, bottom). In agreement with previous work,\(^{16,21}\) caffeine decreases the SR Ca\(^{2+}\) content and this decrease is associated with the disappearance of diastolic Ca\(^{2+}\) release.

Qualitatively different results were seen in the presence of isoproterenol (1 μmol/L). In 4 of 21 cells studied, isoproterenol resulted in diastolic waves of Ca\(^{2+}\) release. Caffeine (0.5 mmol/L) increased the frequency of occurrence of such waves in 3 of these 4 cells (Figure 2Bc). Of more relevance to the present report are the 17 cells in which isoproterenol alone did not produce waves, Caffeine (0.5 mmol/L) produced waves in 12 cells, and a typical result is shown in Figure 2A. Although the effect of caffeine on diastolic waves is greatest immediately after its application, waves are now seen throughout the duration of exposure to caffeine. The decreased frequency of waves is associated with a decrease in the calculated SR content (Figure 2A, bottom). The data also showed that higher concentrations of caffeine were less effective at producing waves; only 6 of the 12 cells that produced waves in 0.5 mmol/L caffeine had waves in 1 mmol/L caffeine (eg, Figure 2Bb). In those cells where waves persisted in 1 mmol/L caffeine, they were of smaller amplitude than in 0.5 mmol/L (Figure 2Ba). It is also noteworthy that increasing the caffeine concentration slowed...
the rate constant of decay of the systolic Ca\textsuperscript{2+} transient (see Figure 5).

The question then arises as to why caffeine only produces diastolic release in the presence of isoproterenol? A possible answer lies in the interplay between SR Ca\textsuperscript{2+} content and the SR threshold for Ca\textsuperscript{2+} waves. Previous work has shown that in resting cells waves occur when SR Ca\textsuperscript{2+} content reaches a threshold\textsuperscript{22,23} and that caffeine reduces this threshold and SR content.\textsuperscript{24} In addition isoproterenol can increase SR Ca\textsuperscript{2+} content.\textsuperscript{25,26} We therefore hypothesized that caffeine in isolation reduces both SR Ca\textsuperscript{2+} content and threshold for waves, so the SR content falls below the threshold for Ca\textsuperscript{2+} waves. Isoproterenol may therefore act by increasing the SR Ca\textsuperscript{2+} content above the threshold. To test this, we measured SR Ca\textsuperscript{2+} by using the integral of the NCX current produced by application of 10 mmol/L caffeine.\textsuperscript{27} In the example shown in Figure 3A, caffeine (0.5 mmol/L) did not produce waves. The addition of isoproterenol (in the presence of caffeine) increased SR Ca\textsuperscript{2+} content and initiated Ca\textsuperscript{2+} waves. Removal of caffeine (in the maintained presence of isoproterenol) resulted in a further increase of SR Ca\textsuperscript{2+} content and the abolition of Ca\textsuperscript{2+} waves. Figure 3B shows the SR Ca\textsuperscript{2+} content as a function of caffeine concentration in the presence and absence of isoproterenol. It is clear that caffeine decreases and isoproterenol increases SR content. The importance of SR Ca\textsuperscript{2+} content in the genesis of Ca\textsuperscript{2+} waves even in the presence of caffeine is confirmed by the data in Figure 3C. In both 0.5 and 1.0 mmol/L caffeine, those cells that show waves have higher SR Ca\textsuperscript{2+} content than those that do not. These data explain the requirement for both caffeine and isoproterenol to generate Ca\textsuperscript{2+} waves: caffeine decreases the SR threshold for release, whereas isoproterenol increases Ca\textsuperscript{2+} content.
Further evidence in support of the importance of SR Ca\(^{2+}\) content is provided by the data of Figure 4A, which show the effect of decreasing caffeine concentration from 1 to 0.5 mmol/L. The cell was exposed to isoproterenol and caffeine (0.5 mmol/L) throughout. In 1 mmol/L caffeine, diastolic Ca\(^{2+}\) release followed each stimulus. Reduction of caffeine to 0.5 mmol/L immediately abolished diastolic Ca\(^{2+}\) release. This was followed by an increase of the amplitude of the systolic Ca\(^{2+}\) transient over the next few beats before diastolic Ca\(^{2+}\) release redeveloped. The redevelopment of diastolic Ca\(^{2+}\) release is associated with an increase of SR Ca\(^{2+}\) content as calculated in the lower trace. The simplest explanation of these data are that in 1 mmol/L caffeine, the SR Ca\(^{2+}\) content reaches the threshold at which diastolic Ca\(^{2+}\) release occurs (with the RyR sensitized by 1 mmol/L caffeine). When caffeine is decreased to 0.5 mmol/L, then the decrease of RyR sensitization suddenly increases the SR threshold for waves to above the SR Ca\(^{2+}\) content and diastolic release ceases. The abolition of diastolic release will then increase SR Ca\(^{2+}\) content until the new threshold Ca\(^{2+}\) content is reached and Ca\(^{2+}\) waves recommence.

In Figure 4B (isoproterenol present throughout), the cell had previously been exposed to 0.5 mmol/L caffeine and diastolic release was observed. Caffeine at a concentration of 10 mmol/L was then used to empty the SR. After washing off the high concentration of caffeine, 0.5 mmol/L caffeine was reapplied and stimulation was recommenced at the start of the period shown. It is clear that the recovery of SR content is paralleled by that of the systolic Ca\(^{2+}\) transient and, more pertinently, that the diastolic release resumes only when SR content has almost reached its final level. Figure 4C shows the frequency of occurrence of diastolic Ca\(^{2+}\) waves as a function of SR content measured in the same experiment. In this cell in the absence of caffeine there were no waves over the range of SR Ca\(^{2+}\) content measured. 0.5 mmol/L caffeine results in waves above a threshold SR Ca\(^{2+}\) and increasing caffeine to 1.0 mmol/L shifts the relationship to the left.

As mentioned above (eg, Figure 2), in many experiments it was apparent that Ca\(^{2+}\) waves appeared at 0.5 mmol/L and disappered or become less prominent at higher caffeine concentrations, and we have investigated the reasons for this. Figure 5 shows a comparison of membrane currents and calculated sarcolemmal fluxes in a cell exposed to isoproterenol in 0, 0.5, and 1 mmol/L caffeine. In 0 mmol/L caffeine, there is an influx on the L-type current, and the large systolic Ca\(^{2+}\) transient is accompanied by a large systolic efflux and no Ca\(^{2+}\) wave or diastolic efflux. In 0.5 mmol/L caffeine, there is an increase in the Ca\(^{2+}\) influx, probably resulting from decreased inactivation of the L-type current as a result of the decreased Ca\(^{2+}\) transient. This decreased transient also decreased the systolic efflux and the Ca\(^{2+}\) efflux is maintained by the diastolic efflux accompanying the diastolic wave. In 1.0 mmol/L caffeine, systolic efflux is increased because of the prolongation of the decay of the Ca\(^{2+}\) transient. This means that less diastolic efflux is required for Ca\(^{2+}\) flux balance. In a total of 9 cells studied in isoproterenol, increasing caffeine concentration from 0.5 to 1.0 mmol/L decreased the diastolic component of Ca\(^{2+}\) efflux from 6.5±0.5 to 2.5±0.6 μmol/L (P<0.001) and increased the systolic component from 6.5±0.6 to 10.6±0.5 μmol/L (P<0.01).

The above suggests that the reason that the application of caffeine only produces diastolic Ca\(^{2+}\) release in the presence of isoproterenol is because isoproterenol increases SR content to above a threshold level. However, there are other potential phosphorylation targets for β-adrenergic stimulation including the RyR. If the effects of isoproterenol were simply attributable to increased SR content, then other maneuvers that also load the cell with Ca\(^{2+}\) should allow caffeine to produce diastolic release. In Figure 5B, the cell was exposed to elevated (5 mmol/L) extracellular Ca\(^{2+}\). Under these conditions, diastolic Ca\(^{2+}\) release was not observed in the absence of caffeine, but it was induced by caffeine, support-
Discussion

The results show that increasing the RyR $P_o$ with caffeine does not result in waves of diastolic Ca$^{2+}$ release in the steady state under control conditions. Waves are, however, seen if caffeine is applied in the presence of isoproterenol. Before discussing these results, it is necessary to consider whether the effects of low concentrations of caffeine can be attributed entirely to activation of the RyR. Caffeine has 2 other actions. It is a phosphodiesterase inhibitor and might thereby increase cAMP concentration, both at rest and during application of isoproterenol. However, with the low concentrations of caffeine used, we did not find (either in the absence or presence of isoproterenol) the expected effects of an increase of cAMP, specifically an increase in the amplitude of the L-type Ca$^{2+}$ current and an acceleration of the rate of decay of the transient caused by phospholamban phosphorylation and stimulation of SERCA. Furthermore, the effects of caffeine occur and disappear within 1 or 2 stimuli a time course, which is much faster than that of cAMP-dependent changes produced by adding or removing phosphodiesterase inhibitors. Caffeine also changes the relationship between [Ca$^{2+}$]$_i$ and contraction, but this does not occur at concentrations of 1 mmol/L and below, and it is also not obvious how such an effect could affect diastolic Ca$^{2+}$ release.

Previous work has shown that Ca$^{2+}$ waves are observed when the SR Ca$^{2+}$ content exceeds a threshold level. This can occur either by an increase of SR content or by decreasing the threshold SR Ca$^{2+}$ content by affecting the properties of the RyR. In agreement with previous work, we found that when caffeine was applied under control conditions, Ca$^{2+}$ waves were not seen in the steady state. In a fraction of cells, Ca$^{2+}$ waves were seen immediately after caffeine application. An explanation of this result is that caffeine increases RyR $P_o$, and thereby lowers the threshold SR Ca$^{2+}$ content at which waves occur. However, as a result of these waves, the cell and therefore the SR loses Ca$^{2+}$ (as shown both by the flux measurements of Figure 1 and the direct measurements of Figure 3B) and the SR Ca$^{2+}$ content decreases to below the threshold for Ca$^{2+}$ waves.

We find that caffeine can produce Ca$^{2+}$ waves in the steady state in the presence of isoproterenol. The most straightforward explanation of this result is that it results from isoproterenol increasing SR content to a level at which it is above the threshold for Ca$^{2+}$ waves in the presence of caffeine. It might be suggested that the increase of SR Ca$^{2+}$ content produced by isoproterenol is not necessary to produce spontaneous Ca$^{2+}$ waves but simply accompanies them. However, the fact that even modest decrease of SR Ca$^{2+}$ (following recovery from an empty SR, or after spontaneous SR Ca$^{2+}$ release following a loading protocol) with no other changes can abolish waves suggests that the effect on content is essential. It is also relevant (Figure 5B) that another maneuver that increases SR Ca$^{2+}$ content (elevating external Ca$^{2+}$ to 5 mmol/L) allows caffeine to produce diastolic Ca$^{2+}$ waves, suggesting that it is the effect on SR content that is important.

The above arguments have focused on the need for SR Ca$^{2+}$ content to be above a threshold value for diastolic Ca$^{2+}$ release to occur. Another related argument to explain why diastolic Ca$^{2+}$ waves are not seen under control conditions considers the Ca$^{2+}$ fluxes across the membrane. In the steady state, the Ca$^{2+}$ influx into the cell (largely via the L-type Ca$^{2+}$ current) must equal the sum of the systolic and diastolic effluxes. The Ca$^{2+}$ efflux produced by a wave is $\approx 7 \mu$mol/L. In contrast, the Ca$^{2+}$ influx in the absence of isoproterenol is only 4 $\mu$mol/L. Therefore even if it were possible to avoid systolic efflux, it would be impossible to provide enough Ca$^{2+}$ entry to support diastolic waves. The situation changes in the presence of isoproterenol or elevated Ca$^{2+}$ when the Ca$^{2+}$ influx increases to levels when both systolic and diastolic efflux can be supported. We conclude therefore that RyR potentiation can only result in Ca$^{2+}$ waves in the

![Figure 3A](http://circres.ahajournals.org/)

Figure 3A. A fluxes underlying the reduced ability of higher concentrations of caffeine to produce Ca$^{2+}$ waves. The cell was exposed to isoproterenol in the presence of 0 (left), 0.5 (middle), or 1 mmol/L (right) caffeine. Traces show [Ca$^{2+}$]$_i$ (top), current (note the high-gain trace: amplified $\times 5$ on repolarization) (middle), and calculated Ca$^{2+}$ flux (bottom). The upward deflection shows Ca$^{2+}$ entry via the L-type Ca$^{2+}$ current (i). The initial downward deflection is the systolic Ca$^{2+}$ efflux (SE) and the final downward deflection represents Ca$^{2+}$ lost during diastole (DE). $I_n$ indicates membrane current. B, Caffeine can produce waves in the presence of elevated Ca$^{2+}$. External Ca$^{2+}$ concentration was 5 mmol/L, and isoproterenol was not present. The traces were obtained in the presence of the following concentrations of caffeine (mmol/L): 0 (left), 0.25 (middle), 0.5 (right).

![Figure 5](http://circres.ahajournals.org/)

Figure 5. A. Fluxes underlying the reduced ability of higher concentrations of caffeine to produce Ca$^{2+}$ waves. The cell was exposed to isoproterenol in the presence of 0 (left), 0.5 (middle), or 1 mmol/L (right) caffeine. Traces show [Ca$^{2+}$]$_i$ (top), current (note the high-gain trace: amplified $\times 5$ on repolarization) (middle), and calculated Ca$^{2+}$ flux (bottom). The upward deflection shows Ca$^{2+}$ entry via the L-type Ca$^{2+}$ current (i). The initial downward deflection is the systolic Ca$^{2+}$ efflux (SE) and the final downward deflection represents Ca$^{2+}$ lost during diastole (DE). $I_n$ indicates membrane current. B, Caffeine can produce waves in the presence of elevated Ca$^{2+}$. External Ca$^{2+}$ concentration was 5 mmol/L, and isoproterenol was not present. The traces were obtained in the presence of the following concentrations of caffeine (mmol/L): 0 (left), 0.25 (middle), 0.5 (right).
steady state if the Ca\(^{2+}\) influx into the cell minus the systolic efflux results in a large enough net influx to support the Ca\(^{2+}\) efflux during diastole.

Many experiments showed that 1.0 mmol/L caffeine was less effective than 0.5 mmol/L at producing diastolic Ca\(^{2+}\) release. It is well known that even higher caffeine concentrations abolish diastolic (and systolic) Ca\(^{2+}\) release.\(^{30}\) As shown in Figure 5A, the slowing of the systolic Ca\(^{2+}\) transient increases the systolic efflux. This presumably decreases SR Ca\(^{2+}\) to a level below the threshold for Ca\(^{2+}\) release. In other words, high concentrations of caffeine oppose the ability of SERCA to raise SR content to threshold levels. Figure 5 also shows the changes of Ca\(^{2+}\) fluxes that occur when Ca\(^{2+}\) waves are induced by caffeine. The increase of Ca\(^{2+}\) efflux accompanying the wave is largely compensated for by a decrease of systolic efflux attributable to a smaller Ca\(^{2+}\) transient.

**Are the Effects of Isoproterenol Solely Attributable to Changes of SR Ca\(^{2+}\) Content?**

Some previous work has suggested that phosphorylation of the RyR can increase its \(P_o\), possibly by promoting dissociation of the FKBP12.6 accessory protein.\(^{15,37}\) It is therefore important to consider whether the effects of isoproterenol are attributable to (1) an increase of SR Ca\(^{2+}\) content or (2) an effect on the RyR. The discussion above argues that the effect on SR content is very important but does not exclude a role for an effect on the RyR. If an effect of \(\beta\)-adrenergic stimulation on the RyR contributes to diastolic release, then one would expect that it would decrease the threshold for diastolic Ca\(^{2+}\) release. That this is not the case is shown by the fact that in the majority of cells exposed to isoproterenol alone, despite the fact that SR Ca\(^{2+}\) is increased to above control levels, no diastolic release was observed. It should also be noted that (eg, Figure 1) when caffeine is applied in the absence of isoproterenol, the initial diastolic release shows that the threshold for diastolic Ca\(^{2+}\) release in caffeine is less than the initial SR content. Diastolic Ca\(^{2+}\) release ceases when SR Ca\(^{2+}\) has fallen below the threshold level consistent with the calculated dependence of RyR Ca\(^{2+}\) leak on SR content noted by Shannon et al\(^{38}\) and that of Ca\(^{2+}\) spark frequency on SR Ca\(^{2+}\) content during \(\beta\)-adrenergic stimulation.\(^{39}\) In other words, to obtain diastolic release in caffeine, it is sufficient to increase SR Ca\(^{2+}\) content, thereby arguing that the major effect of \(\beta\)-adrenergic stimulation is by increasing SR content.

Previous work has found that mice deficient in FKBP12.6 which have leaky RyRs show exercise induced arrhythmias. Cells from these animals have delayed afterdepolarizations in the presence of \(\beta\) stimulation.\(^{15}\) That study focused on the hypothesis that the effects of \(\beta\)-adrenergic stimulation were caused by phosphorylation of the RyR. However, in light of the present data, one should consider a role for effects on SERCA activity and/or the L-type current as well as on SR content. One way to address this question would be to examine whether phosphorylation decreases the SR Ca\(^{2+}\) content at which Ca\(^{2+}\) waves occur (as shown for caffeine in the present work).

**Implications for Arrhythmogenesis**

The present results show that simply increasing the \(P_o\) of the RyR by itself will not cause diastolic Ca\(^{2+}\) waves in the steady state. These findings therefore are in accord with the clinical observation that patients with mutuated RyRs display a catecholaminergic polymorphic ventricular tachycardia and therefore show arrhythmias only during adrenergic stimulation.\(^{11,12}\) Our results show that an acute increase of RyR opening can cause diastolic Ca\(^{2+}\) release until SR Ca\(^{2+}\) decreases. It is therefore possible that clinically an acute sensitization of the RyR would be arrhythmogenic. This, however, would require that the RyR be sensitized very rapidly relative to the interval between beats. One implication of these finding is that there are 2 possible treatment strategies for catecholaminergic polymorphic ventricular tachycardia. The first would be to use an agent that reduces RyR \(P_o\) and increases SR threshold for Ca\(^{2+}\) waves. Mixed results have been obtained in cellular studies using JTV 519,\(^{40,41}\) and no clinical study has at present been performed. We have recently shown in cellular studies that decreasing RyR opening with tetracaine can abolish Ca\(^{2+}\) waves and have argued that a more selective version of tetracaine could be clinically useful.\(^{19}\) A second possible strategy would be to limit the increase in Ca\(^{2+}\) influx and therefore SR Ca\(^{2+}\) content produced by \(\beta\)-adrenergic stimulation to a level at which Ca\(^{2+}\) waves cannot be supported.

In conclusion, this report has shown that simply increasing RyR open probability does not produce diastolic Ca\(^{2+}\) release and that SR Ca\(^{2+}\) content must be maintained.

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**Disclosures**

None.

**References**


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