Integrative Physiology

In the RyR2^{R4496C} Mouse Model of CPVT, β-Adrenergic Stimulation Induces Ca Waves by Increasing SR Ca Content and Not by Decreasing the Threshold for Ca Waves

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Rationale: **Mutations of the ryanodine receptor (RyR) cause catecholaminergic polymorphic ventricular tachycardia (CPVT). These mutations predispose to the generation of Ca waves and delayed afterdepolarizations during adrenergic stimulation. Ca waves occur when either sarcoplasmic reticulum (SR) Ca content is elevated above a threshold or the threshold is decreased. Which of these occurs in cardiac myocytes expressing CPVT mutations is unknown.**

Objective: **We tested whether the threshold SR Ca content is different between control and CPVT and how it relates to SR Ca content during β-adrenergic stimulation.**

Methods and Results: **Ventricular myocytes from the RyR2 R4496C$^{+/-}$ mouse model of CPVT and wild-type (WT) controls were voltage-clamped; diastolic SR Ca content was measured and compared with the Ca wave threshold. The results showed the following. (1) In 1 mmol/L [Ca^{2+}]$_o$, β-adrenergic stimulation with isoproterenol (1μmol/L) caused Ca waves only in R4496C. (2) SR Ca content and Ca wave threshold in R4496C were lower than those in WT. (3) β-Adrenergic stimulation increased SR Ca content by a similar amount in both R4496C and WT. (4) β-Adrenergic stimulation increased the threshold for Ca waves. (5) During β-adrenergic stimulation in R4496C, but not WT, the increase of SR Ca was sufficient to reach threshold and produce Ca waves.**

Conclusions: **In the R4496C CPVT model, the RyR is leaky, and this lowers both SR Ca content and the threshold for waves. β-Adrenergic stimulation produces Ca waves by increasing SR Ca content and not by lowering threshold.** (***Circ Res***. 2010;107:1483-1489.)

Key Words: sarcoplasmic reticulum ■ ryanodine receptor ■ arrhythmia

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited arrhythmia syndrome causing syncope and sudden death during physical and emotional stress in young patients with structurally normal hearts.[1] Early studies drew attention to the fact that the electrocardiographic changes in CPVT are similar to those accompanying the arrhythmias observed in digitalis intoxication,[2] which are known to result from delayed afterdepolarizations (DADs).[3,4] These, in turn, are produced by intracellular waves of calcium release from the sarcoplasmic reticulum activating the inward Na/Ca exchanger (NCX) current (recently reviewed[5]). Ca waves occur when the sarcoplasmic reticulum (SR) Ca content exceeds a threshold level[6,7] and have therefore been termed store overload–induced calcium release.[7] DADs are also seen in CPVT patients,[8,9] and cellular studies have shown the presence of the DADs[10] and underlying diastolic Ca waves in animal models.[11,12]

Ca is released from the sarcoplasmic reticulum through a channel known as the ryanodine receptor (RyR). Many CPVT patients have mutations in the RyR.[13,14] Studies have shown that mutations in the RyR2 gene cause Ca leak during adrenergic stimulation[15,16] and have suggested that the enhanced leak eventually leads to diastolic Ca waves and arrhythmias. However, how the mutations and adrenergic stimulation interact to cause diastolic Ca waves remains unclear. One explanation is that the mutation increases the open probability (P_o) of the RyR and decreases the SR Ca content required for Ca waves (threshold).[16] There is, however, an important complication: although making the RyR leaky will decrease Ca wave threshold, the resulting Ca waves will decrease SR Ca content and abolish waves.[17] Another question is why the arrhythmias only develop during β-adrenergic stimulation. Two explanations are that β-adrenergic stimulation either (1) increases SR Ca content by activating SERCA and the L-type Ca current or (2) phosphorylates the RyR, thereby decreasing the threshold for Ca waves. As mentioned above, the latter hypothesis suffers from the limitation that purely increasing RyR opening would be expected to

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Non-standard Abbreviations and Acronyms	
BDM	2,3-butanedione monoxime
CPVT	catecholaminergic polymorphic ventricular tachycardia
DAD	delayed afterdepolarization
NCX	sodium calcium exchange
P_o	open probability
RyR	ryanodine receptor
SR	sarcoplasmic reticulum
WT	wild type

Figure 1. Induction of diastolic Ca waves in physiological external calcium concentration. In both WT and R4496C, traces show fluorescence intensity in response to voltage step stimulation applied from a holding potential at -80 mV to $+20$ mV at 0.5 Hz in physiological $[Ca^{2+}]_o$ (1 mmol/L). The **traces** show data from WT **(top)** and R4496C **(bottom)** myocytes at baseline **(left)** and after 2 minutes exposure to ISO **(right)**. **Arrows** indicate diastolic Ca waves.
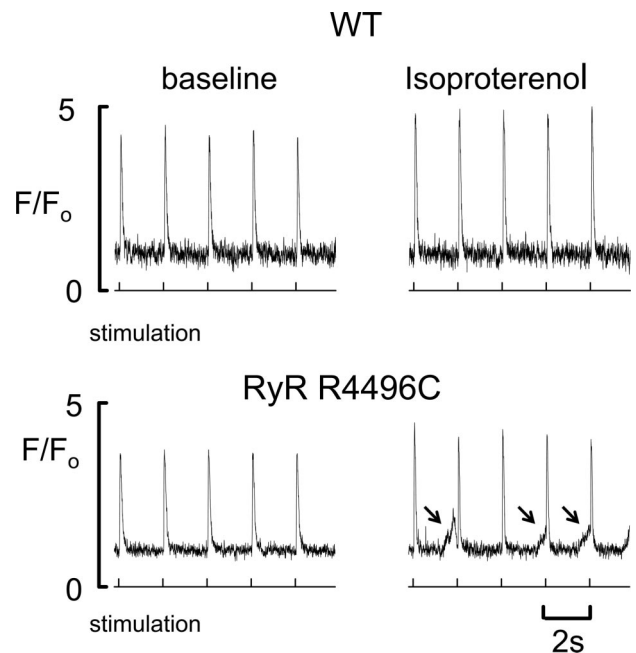
further decrease SR Ca content.[17] A recent study has found that mouse myocytes expressing a mutant RyR show increased Ca spark frequency and decreased SR Ca content indicative of an increase of RyR opening.[12] That study, however, did not compare the threshold and SR content nor the effects of β-adrenergic stimulation on these parameters. Therefore, in this study in the RyR2 R4496C mutant mouse, we explored the underlying mechanism of diastolic Ca wave development in CPVT by measuring SR Ca content and Ca wave threshold. We found that the threshold for Ca waves was reduced in the RyR2 mutant and that β-adrenergic stimulation increased SR Ca content to this threshold, thereby producing waves.

Methods

Ventricular myocytes were isolated from 3- to 4-month-old male RyR2 R4496C$^{+/-}$ mice (R4496C) and their wild-type (WT) littermates using an established enzymatic digestion technique.[18]

Voltage clamp was imposed using the perforated patch technique with amphotericin-B (240 μg/mL). The patch pipette (2 to 3 MΩ) contained (in mmol/L): KCH$_3$O$_3$S 125, KCl 10, NaCl 20, HEPES 10, MgCl$_2$ 5, K$_2$EGTA 0.1, titrated to 7.2 with KOH. The final access resistance was typically $\approx$20 MΩ and was overcome using the switch-clamp facility of an Axoclamp-2A amplifier (Axon Instruments). Cells were stimulated with 50-ms pulses (from -80 to $+20$mV) at 0.5 Hz. The superfusing solution contained (in mmol/L): NaCl 134, HEPES 10, glucose 11, MgCl$_2$ 1.2, 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 indicator Fluo-3 (Molecular Probes). All experiments were performed at 37°C. To induce β-adrenergic stimulation, isoproterenol (1 μmol/L) was applied for at least 2 minutes before making measurements.

The SR Ca content was quantified by releasing all of the Ca and integrating the resulting NCX current as the Ca was pumped out of the cell.[19] In previous work, this was performed using 10 mmol/L caffeine. However, early experiments showed that the resulting contraction frequently ruptured the seal and resulted in the death of the mouse myocytes. We therefore used 5 mmol/L caffeine and 20 mmol/L 2,3-butanedione monoxime (BDM). The rationale behind this is that BDM also releases Ca from the SR[20] yet inhibits the resulting contraction.[21] For simplicity, the traces are all labeled simply as "caffeine." We confirmed that the BDM/caffeine mixture releases all the calcium by demonstrating that subsequent addition of 10 mmol/L caffeine produced no rise of $[Ca^{2+}]_i$ (Figure I in the Online Data Supplement, available at http://circres.ahajournals.org). The Ca efflux associated with the Ca wave was also quantified by integrating the inward NCX current.[22,23] This was then corrected for non–NCX-mediated Ca efflux as described previously.[19] The correction factor was 1.2 in both WT and R4496C and was unaffected by isoproterenol (data not shown). The repeatability of SR Ca content measurements is shown in Online Figure III. SERCA activity was assessed by subtracting the rate constant of decay of the caffeine

response (k_{caff}) from that of the systolic Ca transient (k_{sys}) assuming that in caffeine, SERCA does not contribute to Ca removal.[24]

Where applicable, the data are reported as the means$\pm$SEM of n experiments. Significance was tested using Fischer's exact test for categorical values, and either t test or paired t test for normally distributed variables. When the normality test failed, Mann–Whitney rank sum test was used. Differences with values of $P<0.05$ were considered statistically significant.

Results

Ca Waves in R4496C Cells

The inducibility of diastolic Ca waves was examined in both WT and R4496C in physiological $[Ca^{2+}]_o$ (1 mmol/L). Cells were stimulated with depolarizing voltage-clamp pulses from -80 to $+20$ mV at 0.5 Hz (Figure 1). In the absence of isoproterenol, no myocytes (from either WT or R4496C) showed diastolic Ca waves. After at least 2 minutes of application of isoproterenol (1 μmol/L), none of the 9 WT cells had Ca waves; in contrast, 6 of 12 R4496C showed diastolic waves ($P<0.05$).

The observation that both β-adrenergic stimulation and a RyR mutation are required to produce waves is consistent with both the clinical phenotype of CPVT and previous cellular studies.[12,25,26] The purpose of the next series of experiments was therefore to examine whether the requirement for both the RyR mutation and β-adrenergic stimulation could be explained in terms of changes of SR Ca content and/or the threshold for Ca waves.

Measurement of Ca Transient Amplitude and Diastolic SR Ca Content

When cells were stimulated at 0.5 Hz, there was no difference in the amplitude of the Ca transient between WT and R4496C
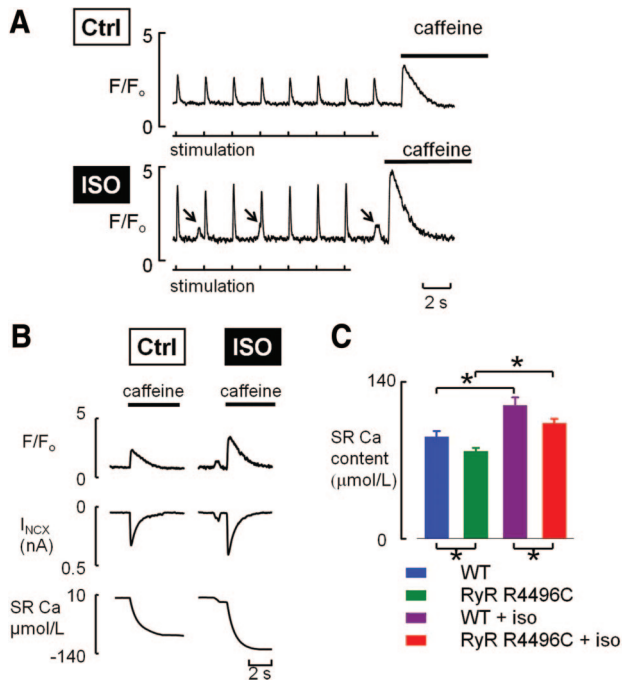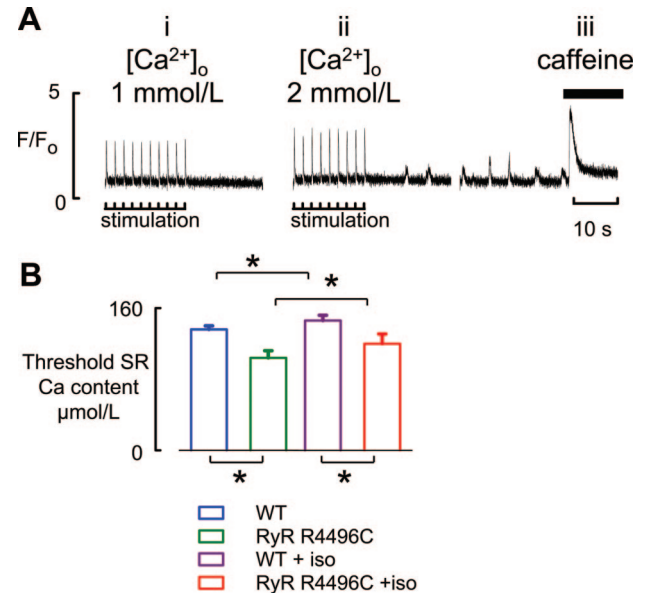
Figure 2. Measurement of diastolic SR Ca content in physiological external Ca concentration. **A,** Experimental time course. After stopping steady-state stimulation in control condition (Ctrl), caffeine was applied to quantify SR Ca content, and then the same protocol was repeated in the presence of isoproterenol (ISO). The example shows data from a R4496C cell. **Arrows** indicate diastolic Ca waves. **B,** Measurement of SR Ca content. **Traces** show fluorescence intensity **(top)**, NCX current **(middle)**, and integrated NCX current as calculated SR content **(bottom)**. **C,** Mean data. Diastolic SR Ca contents before and after isoproterenol were compared between WT and R4496C before and after application of isoproterenol. *$P<0.05$.

Figure 3. Measurement of Ca wave threshold in high $[Ca^{2+}]_o$. **A,** Experimental time course. **i through iii,** Stimulation in 1 mmol/L $[Ca^{2+}]_o$ **(i)**, stimulation in 2 mmol/L $[Ca^{2+}]_o$ **(ii)**, SR threshold was quantified by application of caffeine immediately after a Ca wave **(iii)**. **B,** Mean data for SR Ca threshold. *$P<0.05$ for indicated comparisons.

(Online Figure II) (752 ± 50 versus 792 ± 161 nmol/L; 8 WT and 10 R4496C cells).

We next measured diastolic SR Ca content during steady-state stimulation in the presence of 1 mmol/L $[Ca^{2+}]_o$. After stopping stimulation, caffeine was applied at an interval similar to the previous stimulation interval (Figure 2A, Ctrl). After application of isoproterenol, SR Ca content was measured in the same way. For R4496C myocytes producing diastolic Ca waves with isoproterenol, caffeine was applied just after a wave (Figure 2A, ISO). The resulting inward NCX current was integrated to quantify diastolic SR Ca content (Figure 2B). For myocytes with diastolic Ca waves, the integral of the inward current caused by the wave was added to that produced by caffeine to quantify diastolic Ca content. As shown in Figure 2C, in the absence of isoproterenol, the SR Ca content in R4496C (78.3 ± 3.3 μmol/L) was lower ($P<0.05$) than that in WT (91.4 ± 5.3 μmol/L). Isoproterenol increased SR content in both WT (119.3 ± 6.5 μmol/L; $P<0.001$) and R4496C (102.9 ± 4.4; $P<0.001$). The mean increase of SR Ca content produced by isoproterenol was the same in WT ($31.1\pm4.4\%$) and R4496C ($32.6\pm5.6\%$). Importantly, in the presence of isoproterenol, the diastolic SR Ca content of the R4496C myocyte was still lower than that of WT ($P<0.05$).

SR Ca content is determined by SR Ca leak-load balance[27] and is ultimately controlled by sarcolemmal Ca fluxes.[28] Although the lower SR Ca content in R4496C myocytes is

consistent with reported increased Ca leak through the RyR in this mutation, it could possibly be related to compensatory changes in Ca handling secondary to the mutation. Therefore, we characterized Ca removal mechanisms by obtaining single exponential rates of decay for both the systolic Ca transient (the sum of SR Ca uptake and sarcolemmal Ca efflux) and the caffeine-evoked transient (sarcolemmal Ca efflux only). The rate constant of decay of the systolic Ca transient (k_{sys}) of R4496C was identical to that of WT in control (WT, 13.70 ± 0.92 sec^{-1}, n=9; R4496C, 13.96 ± 0.71 sec^{-1}, n=12) and in isoproterenol (WT, 22.13 ± 0.59 sec^{-1}; R4496C, 22.12 ± 0.70 sec^{-1}). The rate constant of decay of the caffeine-evoked transient (k_{caff}) was also identical in R4496C and WT (WT, 1.323 ± 0.073 sec^{-1}; R4496C, 1.260 ± 0.066 sec^{-1}) and was also unaffected by isoproterenol (WT, 1.386 ± 0.054 sec^{-1}; R4496C, 1.297 ± 0.048 sec^{-1}). These results confirm that the lower SR Ca content in R4496 is related to SR Ca leak rather than altered SERCA or NCX function.

Measurement of the Threshold for Ca Waves

In this series of experiments, we elevated the external Ca concentration from 1 to 2 mmol/L and stopped electric stimulation. Under these conditions, all cells developed Ca waves (Figure 3). We define the threshold as the minimum amount of SR Ca required for a wave to be activated. In unstimulated cells, each wave will deplete the SR and the next wave will occur only once the SR has refilled back to the threshold level.[29] As shown in Figure 3A (iii), caffeine was added immediately after a wave. We assume that the sum of the Ca lost from the cell during the caffeine response and the preceding Ca wave gives a measure of the amount of Ca in the SR before the wave and therefore the SR threshold for Ca waves.[22] Figure 3B shows that the threshold SR Ca content in R4496C (103.6 ± 7.8 μmol/L) was lower ($P<0.01$) than that in WT (136.0 ± 4.3 μmol/L). Interestingly,

isoproterenol increased threshold in both WT and R4496C (WT, 146.3 ± 5.5 μmol/L; R4496C, 120.1 ± 10.8 μmol/L; both $P < 0.05$ compared to before isoproterenol). Importantly, however, in the presence of isoproterenol, the SR wave threshold of R4496C remained lower than that of WT ($P < 0.05$).

The experiments illustrated above clearly demonstrate that the R4496C mutation lowers the threshold for diastolic Ca release and that β-adrenergic stimulation induces Ca waves by raising SR Ca content and not by lowering the threshold. The main limitations of these experiments is that the threshold and SR Ca content were measured in different groups of myocytes and in different concentrations of $[Ca^{2+}]_o$. (1 mmol/L for SR Ca content and 2 mmol/L for threshold). We cannot, therefore, be certain what the relationship is between threshold and content during electric stimulation. Therefore, subsequent experiments were designed to compare threshold and content in the same cells and, specifically, to relate this to whether or not the cells showed waves.

Comparison of SR Ca Content and Ca Wave Threshold in Elevated $[Ca^{2+}]_o$

The aim of these experiments was to focus on the R4496C cells and determine why some cells showed Ca waves under a given condition when others did not. In these experiments, we measured both SR Ca content and Ca wave threshold in the same cell in 2 mmol/L $[Ca^{2+}]_o$. As shown in Figure 4A (Ctrl), cells were first stimulated to see whether waves occurred during stimulation and then SR Ca content was measured. Subsequently, after a short period of stimulation, the myocyte was left unstimulated until Ca waves developed. Importantly, all cells showed waves in the absence of stimulation, thereby allowing the threshold to be measured and compared directly to the diastolic SR Ca content. This protocol was repeated in the same cells in the presence of isoproterenol (Figure 4A, ISO).

In response to voltage-clamp stimulation, of the 14 R4496C cells studied, 6 showed no waves either in control or isoproterenol, 5 showed waves only in the presence of isoproterenol (as for the example shown in Figure 4A), and 3 showed waves both in control and with isoproterenol.

In the 6 myocytes that never had waves during stimulation (even in ISO), the SR Ca content was lower than the threshold. This was the case in both control (SR content, 89.4 ± 6.9 μmol/L; threshold, 114.0 ± 7.0 μmol/L , $P = 0.01$) and in ISO (SR content, 109.9 ± 7.8; threshold, 132.0 ± 8.9; $P < 0.05$) (Figure 4B, top). In the 5 R4496C myocytes that showed Ca waves only in the presence of isoproterenol, the SR Ca content was lower than the threshold in control conditions (SR content, 94.7 ± 9.2 μmol/L; threshold, 111.9 ± 5.9; $P < 0.05$). However, in these cells, there was no difference between content and threshold in isoproterenol when waves were observed (SR Ca content, 124.8 ± 6.1 μmol/L; threshold, 124.9 ± 3.6 μmol/L; Figure 4B, middle). In the 3 remaining myocytes (which had diastolic waves both in control and in isoproterenol), the SR Ca content was not different from threshold in either control (respectively, 96.1 ± 7.7 and 95.5 ± 9.6 μmol/L) or isoproterenol (respectively, 120.1 ± 6.2 μmol/L and 120.9 ± 4.5 μmol/L). These results demonstrate that, irrespective of the conditions, Ca waves only occur when the SR Ca content reaches the threshold. In these cells, we also measured the effects of β-adrenergic stimulation
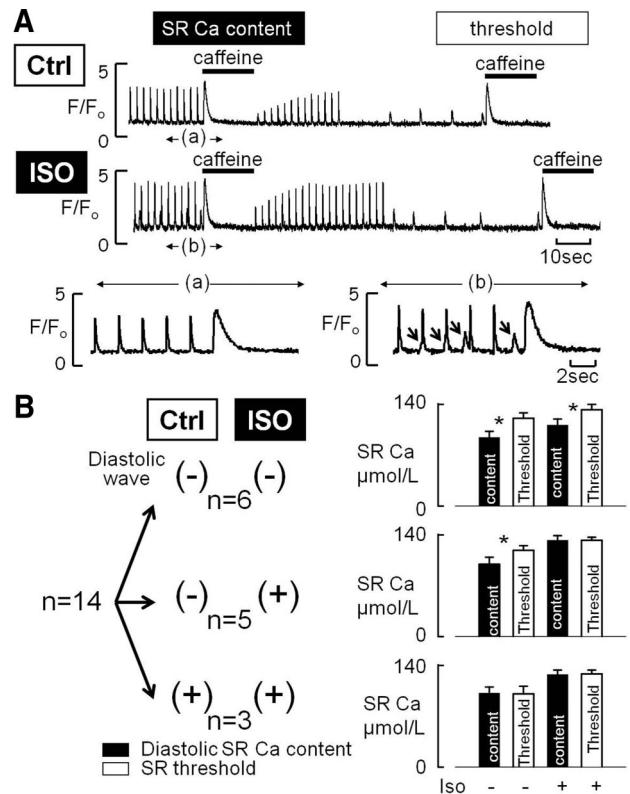
Figure 4. Direct comparison of diastolic SR Ca content and Ca wave threshold of R4496C myocytes. A, Experimental time course. In the presence of 2 mmol/L $[Ca^{2+}]_o$, diastolic SR Ca content and Ca wave threshold were measured in both the absence (Ctrl) and presence of isoproterenol (ISO). In the example illustrated, there were no diastolic Ca waves in Ctrl **(a)**, but Ca waves were present in ISO **(b)**. The **lower traces** show magnified sections of **a** in Ctrl and **b** in ISO. **B,** Mean data. Fourteen cells tested were classified according to the presence or absence of waves in Ctrl and in ISO **(left)**. In 6 cells without waves even in ISO **(top)**, SR Ca content was always lower than the threshold. In 5 cells without waves in Ctrl and with waves in ISO **(middle)**, SR Ca content was lower than the threshold in Ctrl, but identical to the threshold in ISO. In 3 cells with waves even in Ctrl **(bottom)**, SR Ca content was identical to the threshold. *$P < 0.05$.

on diastolic $[Ca^{2+}]_i$; this was unchanged (124 ± 10 nmol/L at baseline 108 ± 9 after isoproterenol; $P = 0.126$).

The Increase in Threshold After β-Adrenergic Stimulation Is Independent of SERCA Activity

One of the most unexpected results above is the increase of threshold during β-adrenergic stimulation. Previous work has shown that altering SERCA activity changes threshold,[29a] and we sought to determine whether the increase of threshold is, indeed, attributable to enhanced SERCA activity. The regression analysis of Figure 5 relates the change of threshold produced by isoproterenol to the change of SERCA activity (see Methods for measurement details). It is clear that there is no correlation. On the basis of this observation, we conclude that the increase in threshold produced by β-adrenergic stimulation is not attributable to an increase in SERCA activity.

Discussion

In the present article, we have made 3 major observations. (1) The increased probability of occurrence of Ca waves in the
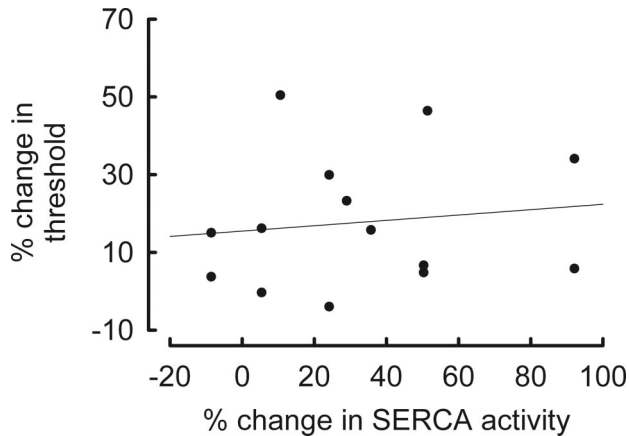
Figure 5. Lack of correlation between change of SERCA activity and threshold. The data show threshold (**ordinate**) as a function of SERCA activity (**abscissa**). The **solid line** through the data is a linear regression between change in SERCA activity and change in SR threshold after *β*-adrenergic stimulation ($P=0.66$). SERCA activity was calculated by subtracting the rate constant of decay of the caffeine-evoked Ca transient from that of the systolic (see the Online Data Supplement).[24]

R4496C RyR mouse (relative to WT) is attributable to a lower SR Ca threshold for the initiation of these waves. (2) When Ca waves are observed during electric stimulation in the R4496C mouse, the SR Ca content is equal to the threshold for Ca wave initiation, and, correspondingly, when waves are not seen, the SR content is below this threshold. (3) *β*-Adrenergic stimulation produces Ca waves in R4496C by increasing SR Ca content and not by decreasing threshold. Indeed, even in the R4496C cells, *β*-adrenergic stimulation increases threshold.

SR Ca Content in R4496C

Previous work qualitatively assessing the caffeine-evoked increase of $[Ca^{2+}]_i$ found that there was no difference in SR Ca content between WT and R4496C when myocytes were stimulated at 2 Hz. However, as the frequency of stimulation was increased, SR content fell more in R4496C than in WT.[12] In contrast, we found that SR content was lower in R4496C cells stimulated at 0.5 Hz and even in the absence of stimulation. This may reflect the greater sensitivity of the quantitative methods used to measure SR Ca content in the present study (integrating NCX current activated by caffeine-induced Ca release as opposed to simply measuring the amplitude of the caffeine-evoked rise of $[Ca^{2+}]_i$).

The lower SR Ca content observed in the R4496C cells is presumably attributable to increased Ca efflux from the SR as is also the case when RyR P_o is increased pharmacologically with caffeine.[30] This is confirmed by the observation that both SERCA activity and NCX activity are identical in WT and RyR R4496C mouse. The reduction of SR Ca content might, at first sight, seem inconsistent with the fact that patients with CPVT have normal systolic function. However, as is the case with the application of low concentrations of caffeine to increase RyR P_o, the decrease of SR Ca content will exactly compensate for the increased P_o, and no net change of systolic Ca or contractility is to be expected.[31]

Ca Wave Threshold in R4496C

The present results show that the threshold for Ca waves is lower in the R4496C cells compared to WT. It is important to

note that although such a decrease of threshold has been suggested to be produced by CPVT mutations, previous studies have inferred, rather than directly measured, it. Thus, Jiang et al found an increased frequency of waves in HEK cells expressing various mutant RyRs compared to those expressing control RyR.[7] The R4496C mouse has also been shown to have a higher frequency of waves compared to WT.[12] We now demonstrate directly that the RyR mutation makes Ca waves occur at a lower SR content.

β-Adrenergic Effect on SR Ca Content

We found that *β*-adrenergic stimulation increased the SR Ca content by a similar amount in both WT and R4496C cells. It is noteworthy that during *β*-adrenergic stimulation, the SR Ca content remains lower in R4496C cells compared to WT. The increase of SR Ca content is presumably largely because of increased phosphorylation of phospholamban increasing SERCA activity.[32] The L-type Ca channel will also be phosphorylated, and although the increased Ca influx might be expected to increase SR Ca content, it will also increase Ca release from the SR, thereby tending to decrease SR content. It is therefore difficult to predict the overall effect of increased L-type current on SR content.[33] Most studies have found that phosphorylation of the RyR increases P_o[34–36] and makes it leaky.[37] This effect, if anything, will tend to decrease SR Ca content. The fact that isoproterenol produces an increase of SR Ca content that is quantitatively similar in WT and R4496C is more easily accounted for by an effect on SERCA with little dependence on phosphorylation of the RyR.

β-Adrenergic Effect on Ca Wave Threshold in R4496C

We found that *β*-adrenergic stimulation increased Ca wave threshold in both WT and R4496C cells. If R4496C was highly susceptible to *β*-adrenergic stimulation and the RyR became much leakier with isoproterenol, its threshold should have decreased or at least increased less than in WT. This was not the case. These data suggest that the R4496C mutation of RyR2 does not make the channel more sensitive to *β*-adrenergic stimulation than WT.

The question that remains unresolved is what causes the increase in threshold after *β*- adrenergic stimulation. Our regression analysis clearly demonstrates that the increase in threshold does not correlate with the change in SERCA activity produced by *β*-adrenergic stimulation. We conclude that stimulation of SERCA is not responsible for the change in threshold and that some other target must be involved. Other obvious phosphorylation candidates include the L-type Ca channel and troponin I. It is not, however, obvious how phosphorylation of either of these would increase the threshold. It should also be noted that, at least in skeletal muscle, the intra-SR Ca buffer calsequestrin can also be phosphorylated.[38]

Recently Uchinoumi et al[39] have reported that a different mutation of RyR (R2474S) makes the RyR more sensitive to adrenergic stimulation by destabilizing interdomain interaction within RyR. This raises the possibility that different mutations of RyR respond differently to adrenergic stimulation; this in turn could have implications on the severity of the phenotype. Another interesting observation in the present study is the variability in the changes in threshold produced by *β*-adrenergic

stimulation. In particular, the cells with the lower threshold seem to experience the greatest change in threshold during β-adrenergic stimulation. The variability in baseline threshold and its change in isoproterenol could be explained by the fact that our population of cells is derived both from the right and left ventricle and possibly from the conduction system,[40–42] and this could result in this variability in baseline threshold and response of threshold to adrenergic stimulation.

Mechanism of Induction of Diastolic Ca Waves in R4496C

This study clearly demonstrates the importance of SR Ca content in the occurrence of Ca waves in CPVT. In 2 mmol/L $[Ca^{2+}]_o$ in the absence of isoproterenol, the majority of R4496C cells (11/14) do not show waves, because the SR Ca content is below the threshold level. In these cells, Ca waves can be produced when the SR Ca content is increased either by β-adrenergic stimulation or by increasing external Ca concentration. The importance of SR Ca load in the genesis of Ca waves and arrhythmias in CPVT patients is further supported by a recent article from Sedej et al.[43] The authors clearly demonstrate that, in the same mouse model of CPVT as used in the present work, Ca waves and arrhythmias can be induced by raising SR Ca load using ouabain. The effects of ouabain are attributable to an increase in SR Ca content.

Implications for CPVT Treatment

β-Blockers effectively prevent arrhythmias in 70% of patients. Many patients have an internal cardiac defibrillator implanted to prevent sudden death. At present, there is an active interest in finding new treatments for CPVT. The observations that CPVT mutations reduce the SR threshold for Ca waves and adrenergic stimulation induces Ca waves and arrhythmias by increasing SR Ca content suggest that Ca waves and arrhythmias can be prevented either by increasing the SR threshold or by reducing SR Ca content. Initial therapeutic success has recently come with the introduction of flecainide to decrease RyR P_o.[44] β-Blockers will presumably attenuate the increase of SR Ca content. The most effective treatments will probably combine these 2 effects, and the search for new therapeutic agents should concentrate on agents that combine these effects.

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Disclosures

None.

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Novelty and Significance

What Is Known?

- Patients with mutations in the sarcoplasmic reticulum (SR) Ca release channel (ryanodine receptor [RyR]) are predisposed to a lethal arrhythmia (catecholaminergic polymorphic ventricular tachycardia [CPVT]) during exercise.
- This arrhythmia is a consequence of intracellular Ca waves resulting from spontaneous SR Ca release that produce delayed after depolarizations (DADs).
- Ca waves occur when the SR Ca content exceeds a threshold level.

What New Information Does This Article Contribute?

- The threshold SR Ca content for Ca wave production is lower in mice expressing the RyR mutation than in wild-type littermates.
- The decreased threshold explains why mice expressing the RyR mutation are more likely to develop Ca waves, DADs and arrhythmias.
- The induction of Ca waves by β-adrenergic stimulation is attributable to an increase of SR Ca content.
- In contrast to previous suggestions, β-adrenergic stimulation increases the SR threshold for Ca waves.

DADs resulting from intracellular Ca waves have been implicated in the genesis of ventricular arrhythmias. These are thought to occur when the SR Ca content exceeds a threshold level and opening of RyR results in spontaneous release of Ca. This can occur (as is the case in digitalis intoxication) any time the SR Ca content increases. We investigated the role of changes of SR Ca content and threshold in CPVT and, specifically, in the arrhythmias produced by mutations in the RyR. We found that the R4496C mutation reduced the SR Ca threshold and decreased the SR Ca content. In contrast to previous suggestions, the present results demonstrate that β-adrenergic stimulation produces Ca waves by increasing the SR Ca content and not by decreasing the threshold. Indeed β-adrenergic stimulation caused an increase of threshold for spontaneous SR Ca release in both wild-type and R4496C myocytes. Our finding shows that β-adrenergic–mediated phosphorylation of normal and R4496C RyR actually reduces rather than increases the likelihood for SR-dependent arrhythmias. This work clearly highlights the fundamental importance of SR Ca content in the genesis of Ca waves and arrhythmias. It emphasizes that, in addition to targeting the RyR, treatment of CPVT should aim to reduce SR Ca content.