Ryanodine receptor dysfunction and triggered activity in the heart

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Submitted 25 August 2006; accepted in final form 18 December 2006

Katra RP, Oya T, Hoeker GS, Laurita KR. Ryanodine receptor dysfunction and triggered activity in the heart. Am J Physiol Heart Circ Physiol 292: H2144–H2151, 2007. First published December 22, 2006; doi:10.1152/ajpheart.00924.2006.—Arrhythmogenesis has been increasingly linked to cardiac ryanodine receptor (RyR) dysfunction. However, the mechanistic relationship between abnormal RyR function and arrhythmogenesis in the heart is not clear. We hypothesize that, under abnormal RyR conditions, triggered activity will be caused by spontaneous calcium release (SCR) events that depend on transmural heterogeneities of calcium handling. We performed high-resolution optical mapping of intracellular calcium and transmembrane potential in the canine left ventricular wedge preparation (n = 28). Rapid pacing was used to initiate triggered activity under normal and abnormal RyR conditions induced by FKBP12.6 dissociation and β-adrenergic stimulation (20–150 μM rapamycin, 0.2 μM isoproterenol). Under abnormal RyR conditions, almost all preparations experienced SCRs and triggered activity, in contrast to control, rapamycin, or isoproterenol conditions alone. Furthermore, under abnormal RyR conditions, complex arrhythmias (monomorphic and polymorphic tachycardia) were commonly observed. After washout of rapamycin and isoproterenol, no triggered activity was observed. Surprisingly, triggered activity and SCRs occurred preferentially near the epicardium but not the endocardium (P < 0.01). Interestingly, the occurrence of triggered activity and SCR events could not be explained by cytoplasmic calcium levels, but rather by fast calcium reuptake kinetics. These data suggest that, under abnormal RyR conditions, triggered activity is caused by multiple SCR events that depend on the faster calcium reuptake kinetics near the epicardium. Furthermore, multiple regions of SCR may be a mechanism for multifocal arrhythmias associated with RyR dysfunction.

ARRHYTHMIAS CAUSED BY ABNORMAL impulse formation (i.e., triggered activity) have been associated with (33, 34) and without (5, 9, 16, 27) structural heart disease. In addition, recent evidence suggests that such arrhythmias can be caused by spontaneous calcium release (SCR) from the sarcoplasmic reticulum (SR). SCR events may be a result of cardiac ryanodine receptor (RyR) dysfunction such as that associated with mutations in the human cardiac RyR channel (37), heart failure (25, 40), and/or alterations in the RyR (30) and accessory proteins (e.g., FKBP12.6) (45, 49); however, the underlying mechanisms are controversial. FKBP12.6 is an accessory protein to the tetrameric RyR channel with 4:1 stoichiometry that stabilizes the RyR in the closed/open state and prevents aberrant activation during diastole. Dissociation of the FKBP12.6 molecule is believed to cause RyR channels to gate independently and after individual RyR channel gating kinetics, rendering the channel prone to SCR events (28). Alternatively, SCR associated with abnormal RyR function may be mediated by SR calcium load (11, 23). Independent of the exact mechanism, the relationship between arrhythmogenesis and abnormal RyR function in the whole heart is not well understood. We have recently reported that, under calcium overload conditions with, otherwise, normal RyR function, ectopy and delayed afterdepolarization (DAD) activity are caused by nonelectrically driven SCR events that are associated with transmural heterogeneities of calcium handling (14). Recently, Nam et al. (30) reported, in a model of abnormal RyR function induced by caffeine and isoproterenol, that polymorphic ventricular tachycardia and ectopic activity are initiated by DAD activity. Interestingly, such activity occurred primarily from the epicardium, suggesting that underlying heterogeneities of calcium handling play an important role. We hypothesize that, under abnormal RyR conditions, triggered activity is caused by SCR events that depend on transmural heterogeneities of calcium handling. To test this hypothesis, ratiometric and dual calcium-voltage optical mapping techniques were used in a canine left ventricular wedge preparation model of RyR dysfunction induced by FKBP12.6 dissociation and β-adrenergic stimulation.

METHODS

Experimental preparation. Experiments were carried out in accordance with National Institutes of Health guidelines for the care and use of laboratory animals and were approved by the IACUC of Case Western Reserve University. Mongrel dogs (20–25 kg) were anticoagulated with heparin (2 ml) and anesthetized with pentobarbital sodium (30 mg/kg iv), and hearts were removed by a left lateral thoracotomy and placed in cold (4°C) cardioplegia solution. Transmural wedges of cardiac tissue [average dimension (in mm) 20 height × 10 width × 10 depth] surrounding and parallel to branches of the left anterior descending coronary artery and left circumflex artery were dissected from the left ventricular free wall (n = 28) (48). All preparations were taken near the base of the left ventricle, and any free running Purkinje fibers were removed from the endocardial surface. The coronary artery of each wedge was cannulated and perfused with oxygenated (95% O2–5% CO2) Tyrode solution containing (in mmol/l) 135 NaCl, 0.9 NaH2PO4, 0.492 MgSO4, 4.03 KCl, 5.5 dextrose, 1.8 CaCl2, and 10 HEPES (pH 7.40). Perfusion pressure was maintained between 50 and 70 mmHg by regulating coronary flow. For ratiometric intracellular calcium imaging (n = 22), wedges were loaded for 45 min at room temperature with the calcium-sensitive indicator indo 1-AM dissolved in 1 ml solution of DMSO and pluronic (20% wt/vol) at a final concentration in the perfusate of 10 μmol/l. The dye-loading period was followed by a 15-min washout period (15). For dual calcium-voltage optical mapping (n = 6), wedges were also loaded with the voltage-sensitive dye di-4-ANEPPS.
(15 μmol/l), in addition to indo 1-AM (14). In all experiments, 10–15 μmol/l of cytochalasin D was used to ensure that motion artifact, if present, did not influence results.

The perfused wedge was placed in a Lexan chamber where the transmural surface was gently positioned against an imaging window using a movable piston. To avoid surface cooling and desiccation, the wedge was immersed in the coronary effluent, which was maintained at a temperature equal to the perfusion temperature (36 ± 1°C). The volume-conducted ECG was monitored with the use of three silver disk electrodes fixed to the chamber. A fine-gauge silver unipolar electrode was inserted into the endocardial surface to stimulate at twice-diastolic threshold current. Physiological stability of the preparation was ensured by monitoring the ECG, coronary pressure, coronary flow, and perfusion temperature continuously throughout each experiment. Preparations remain viable for 2–3 h, but the entire experiment lasted no more than 2 h. All optical recordings were performed 1 h after cannulation to allow healing of the cut transmural surface.

**Optical mapping system.** To determine the amplitude of calcium transients, ratiometric imaging of intracellular calcium was performed (n = 22) (15). Briefly, excitation light was filtered at 350 ± 10 nm and directed to the preparation. Fluorescent light from the preparation was collected by a tandem lens assembly. A 445-nm dichroic long pass mirror was positioned in the tandem lens assembly at a 45° angle to transmit all wavelengths above 445 nm to a 16 × 16 element photodiode array and reflect all wavelengths below 445 nm to another 16 × 16 element photodiode array. All optical components were aligned with an accuracy of 35 μm (20). Ratiometric calcium transients were calculated by dividing the background-subtracted calcium transients at 405 nm by the background-subtracted calcium transients at 485 nm. To measure transmembrane voltage and intracellular calcium simultaneously (n = 6), we used dual calcium-voltage optical mapping techniques described previously (14).

All signals recorded from each photodiode and ECG signal were multiplexed and digitized with 12-bit precision at a sampling rate of 1,000 Hz per channel. For the present study, an optical magnification of ×1.24 was used, resulting in a total mapping field of 14 mm × 14 mm with 0.9-mm spatial resolution and 0.8-mm² pixel size. To view, digitize, and store the position of the mapping array relative to anatomic features, the dichroic mirror was rotated to reflect an image of the preparation to a charge-coupled device video camera.

**Experimental protocol.** In 28 wedges from 26 animals, rapamycin (22–150 μmol/l), an immunosuppressant agent known to dissociate FKBP12.6 from RyR macrocomplexes (1, 4, 13, 47), and isoproterenol (0.2 μmol/l), a β-adrenergic stimulant, were administered separately and then simultaneously to produce a model of RyR dysfunction induced by FKBP12.6 dissociation. This model has been previously shown to be associated with triggered activity (21, 31, 45). In four of the preparations, cyclopiazonic acid (CPA; 10 μM) was used during rapamycin and isoproterenol administration to reduce SR calcium uptake. All recordings were made under steady-state conditions. Ratiometric calcium transients (n = 22) or dual calcium-voltage (n = 6) recordings were made at constant baseline pacing [cycle length (CL) 600 ms] and during a momentary (5 s) step increase to CL = 100–200 ms with one-to-one capture, followed by a halt in pacing to elicit ectopic activity. Before optical recordings were made, all preparations were electrically quiescent, and some demonstrated a slight increase in automaticity due to β-adrenergic stimulation, similar to previous reports (14, 19). This increase in automaticity was abolished on rapid pacing. Finally, in the six preparations when dual calcium-voltage mapping was performed, the effects of rapamycin (150 μmol/l) alone (i.e., before the administration of isoproterenol) on action potential (AP) properties were studied, under steady-state pacing at a CL of 600 ms.

**Data analysis.** To quantify the rate of decrease of intracellular calcium to diastolic levels, the decay portion of the ratiometric calcium transient (from 30 to 100% of the decline phase) was measured by the time constant (τ) of a single exponential fit (14). Because noncalibrated ratiometric signals were used, the rate of calcium transient decline calculated (i.e., τ) may not exactly match the actual rate of cytoplasmic calcium decline during diastole. However, this limitation is unlikely to influence the main findings in this study because relative comparisons between regions in the same preparation were performed. When minimum diastolic calcium level alternated from beat to beat during rapid pacing, the average diastolic calcium level from four consecutive minima was used (14). To account for differences in baseline diastolic calcium levels, the elevation in diastolic calcium on rapid pacing (ΔCa, min) was measured as the difference in diastolic levels before and at the end of rapid pacing. SCR events were defined as a spontaneous increase in calcium levels during diastole (i.e., nonelectrically driven) that exceeded 10% of baseline calcium transient amplitude. SCR amplitude (SCRamp) was measured as the difference between minimum and maximum calcium levels during the SCR event at the center pixel. SCR onset at every site was calculated as the time of peak SCR level relative to the site of earliest SCR onset. Action potential duration (APD) was determined directly from optical APs using previously described algorithms (14). In the absence of AP data (i.e., when only ratiometric calcium transients were recorded), an ectopic beat was defined by evidence of a QRS deflection on the ECG and the presence of simultaneous, rapid, full-scale calcium transients. All measurements were made with automated algorithms with visual inspection by an experienced investigator. Levels of significance were determined with a Student’s t-test, ANOVA, and Fisher’s exact test where a value of P < 0.05 was considered statistically significant.

**RESULTS**

**Arrhythmias under abnormal RyR conditions.** Frequent arrhythmias were observed under abnormal RyR conditions. Shown in Fig. 1, top, are representative ECGs taken from the same wedge preparation under control conditions (left), during rapamycin + isoproterenol administration (middle), and after washout of rapamycin + isoproterenol (right). During identical pacing protocols, several ectopic beats were observed upon termination of rapid pacing only under rapamycin + isoproterenol conditions. In this example, ectopic beats with two different QRS morphologies (a and b in Fig. 1) were observed, indicating the existence of multiple foci. More complex arrhythmias were also observed. Figure 1, bottom, shows an
Ectopic activity was observed under Rap & Iso conditions (20/22) compared with control (2/22), Iso alone (7/22), Rap alone (3/22), and Rap & Iso washout (3/22). These data indicate a synergistic effect of Rap and Iso. B: representative example of the ECG and action potentials (AP) recorded under control and Rap (150 μmol/l) conditions in the wedge preparation (600 ms cycle length). Both the ECG and AP were not significantly influenced by Rap. Similar results (P = not significant) were obtained in 6 wedge preparations (C) at sites from the endocardium (Endo; n = 11), midmyocardium (Mid; n = 11), and epicardium (Epi; n = 11).

Figure 2 summarizes the occurrence of ectopic activity in 22 preparations, defined as at least one extra (nonstimulated) beat occurring immediately (<500 ms) after rapid pacing. Ectopic activity was observed much more frequently under rapamycin + isoproterenol conditions (91%) compared with control (9%) or washout (14%) conditions. In addition, the combined occurrence of ectopy under isoproterenol (32%) or rapamycin (14%) alone was much less than that during rapamycin + isoproterenol conditions. These data suggest that rapamycin + isoproterenol act synergistically to enhance susceptibility to ectopic activity. It is important to note that rapamycin alone did not significantly influence APD. Figure 2B shows a representative example of the ECG and AP recorded under control and rapamycin (150 μmol/l) conditions, in the wedge preparation during steady-state pacing (600 ms CL). Both the ECG and AP were not significantly influenced by rapamycin. Similar results were obtained for APs recorded at sites from the endocardium, midmyocardium, and epicardium, in six preparations (Fig. 2C).

Arrhythmia mechanism under abnormal RyR conditions. Ectopic activity under abnormal RyR conditions was associated with SCR events. Figure 3A shows the ECG and ratiometric calcium transients recorded from the same site under control conditions (left), during rapamycin + isoproterenol (middle), and after washout of rapamycin + isoproterenol (right). It is important to note that, during rapid steady-state pacing (4 beats total), alternans of calcium transient amplitude occurred, as observed in previous studies (38). The smaller calcium transients (2nd and 4th beats) actually correspond to captured beats (see ECG) and should not be confused with unstimulated SCR events. During control conditions, immediately on termination of rapid pacing, there was no evidence of SCR activity. During rapamycin + isoproterenol conditions, an ectopic beat followed by an SCR was induced by the same rapid pacing protocol. Interestingly, this ectopic activity was more likely to occur as the rate of rapid pacing increased (data not shown). On washout of rapamycin + isoproterenol, the same pacing protocol resulted in no ectopic or SCR activity. Figure 3B shows calcium and voltage signals recorded simul-
taneously under the same conditions but in a different preparation. In this example, however, a DAD (in $V_{na}$) and SCR occurred simultaneously in the absence of an ectopic beat. In the same preparation, but following a second induction protocol, an ectopic beat originated in the midmyocardium, without evidence of conduction block, as determined from its activation map (Fig. 3C). Notably, the origin of the ectopic beat occurred where DAD amplitude was greatest, which happens to be the same site for the signals shown in Fig. 3B. These data and the consistent occurrence of long isoelectric periods on the ECG preceding the first beat of ectopic activity (Fig. 3A) suggest that such ectopic activity is not reentrant. When multiple, consecutive ectopic beats were observed, the mechanism for this activity after the first beat was less clear (i.e., triggered or reentrant).

Figure 4A demonstrates a similar episode of ectopic and SCR activity under rapamycin + isoproterenol conditions in a different preparation. Shown are ratiometric calcium transients recorded near the epicardium (Fig. 4Aa) and endocardium (Fig. 4Ab). On termination of rapid pacing (last 6 beats shown), the calcium recording near the epicardium reveals an SCR just preceding the ectopic beat, a hallmark of DAD activity. After the ectopic beat is a second SCR event without evidence of ectopic activity. The endocardial calcium recording, however, showed no SCR activity. Figure 4B shows the activation pattern (as determined by the local time of rapid calcium release) of the ectopic beat shown in Fig. 4A. Earliest activation of the ectopic beat occurred near the epicardium (Fig. 4B, site a), where SCR activity was greatest. These data (Figs. 3 and 4) suggest that ectopic activity under rapamycin + isoproterenol conditions is calcium-mediated triggered activity.

**Mechanism of calcium-mediated triggered activity under abnormal RyR conditions.** Over all experiments, as shown in Fig. 5A, triggered activity originated most commonly from the epicardium ($P < 0.01$), under rapamycin + isoproterenol conditions. Interestingly, this pattern of triggered activity is consistent with results from Nam et al. (30) but is in stark contrast to what our group (14) reported previously in the same preparation during enhanced calcium entry conditions, where triggered activity originated mostly near the endocardium. Figure 5B indicates that most SCR events, as with ectopic activity, occurred near the epicardium ($P < 0.01$). In addition, SCR events near the epicardium had the largest amplitude (Fig. 5C) and earliest onset (Fig. 5D) compared with SCRs at other myocardial regions ($P < 0.01$). These data provide further evidence that SCR events are the mechanism of triggered activity, which occurs preferentially near the epicardium rather than the endocardium under abnormal RyR conditions.

To determine the mechanisms of SCR activity under rapamycin + isoproterenol conditions, the relationship between diastolic level of intracellular calcium and SCR activity was examined. Figure 6A shows calcium transients recorded from the endocardium (top) and epicardium (bottom) on termination of rapid pacing during rapamycin + isoproterenol conditions. The $ΔCa_{dia}$ during rapid pacing, measured as the difference between the average minimum diastolic levels just before and just after the termination of rapid pacing, is greater near the endocardium [99 ratio units (RU)] than near the epicardium (41 RU). In addition, the decay of the calcium transient (τ), as determined from the last paced beat, was slower near the endocardium than near the epicardium (128 vs. 93 ms, respectively). Figure 6 also presents summary data for all preparations demonstrating that $ΔCa_{dia}$ (Fig. 6B) was higher and the decay of the calcium transient (τ, Fig. 6C) was slower ($P < 0.01$) near the endocardium than near the epicardium under rapamycin + isoproterenol conditions. These data suggest that elevated diastolic calcium levels at the endocardium cannot explain the occurrence of SCR and triggered activity near the epicardium.

As shown in Fig. 7, transmural contour maps of $τ$, $ΔCa_{dia}$, and $SCR_{amp}$ further illustrate the weak relationship between elevated calcium levels and SCR activity. The map of $τ$ (Fig. 7A) reveals a distinct region of slow decay of the calcium transient near the endocardium (lighter shades). During rapid pacing, diastolic calcium levels (Fig. 7B) were, in general, greater near the endocardium where $τ$ was slow. After rapid pacing (Fig. 7C), three simultaneous SCR events occurred closer to the epicardium, distant from sites where diastolic calcium was elevated during rapid pacing. Triggered activity originated where $SCR_{amp}$ was greatest and SCR onset earliest. Interestingly, $SCR_{amp}$ appeared greatest where $τ$ was fastest. When correlated (Fig. 7D), $SCR_{amp}$ and $τ$ demonstrated a strong inverse relationship. Similar results were observed in six of seven preparations analyzed. Together, these data suggest

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**Fig. 4. A:** representative ratiometric calcium transients recorded during rapid pacing followed by an abrupt halt in pacing, from an epicardial site (a) and an endocardial site (b), under Rap & Iso conditions. RU, ratio units. After cessation of rapid pacing, an ectopic beat is evident (asterisks). The ectopic beat recorded near the epicardium (a) was preceded by a slow rise in cytoplasmic calcium (arrow), which was absent in the recording from the endocardium (b). Note that, during rapid pacing (6 beats), calcium release alternates in amplitude on every other beat. Furthermore, an SCR that did not elicit an ectopic beat was evident at the epicardium and not the endocardium. The activation map of the ectopic beat (B), as determined from the time of rapid calcium release, demonstrates a site of origin near the epicardium at site a. The preparation was paced from the endocardium (stimulus symbol) close to site b.
that the mechanism of ectopic activity under abnormal RyR conditions is not related to elevated cytoplasmic calcium, as previously shown under normal RyR conditions, but rather to fast SR uptake kinetics.

To further test the dependence of SCR activity on SR calcium handling, CPA was administered to reduce SR calcium reuptake kinetics (Fig. 8). As demonstrated in a representative example under rapamycin + isoproterenol conditions (Fig. 8A, top), rapid pacing initiated an SCR without a triggered beat. During administration of CPA under rapamycin + isoproterenol conditions (Fig. 8A, bottom), the same pacing protocol (rate and duration of rapid pacing) in the same preparation produced an SCR but with much lower amplitude than in the absence of CPA. Interestingly, $\text{SCR}_{\text{amp}}$ during CPA administration was smaller even though minimum cytoplasmic calcium during rapid pacing (94 RU) was larger than that in the absence of CPA (60 RU). These data are consistent with the notion that $\text{SCR}_{\text{amp}}$ depends on SR calcium kinetics under rapamycin and isoproterenol conditions.

**DISCUSSION**

In this study, we investigated the cellular mechanisms of calcium-mediated triggered activity under abnormal RyR conditions in an intact cardiac preparation. We observed triggered activity originating from SCR events, with largest amplitude and earliest onset located preferentially near the epicardium. Interestingly, SCR events were not associated with elevated cytoplasmic calcium levels as previously shown under normal RyR conditions, but rather with SR calcium reuptake kinetics. Importantly, we observed the occurrence of multiple simultaneous SCR events in myocardial tissue under abnormal RyR conditions, which may represent an important mechanism for...
Abnormal calcium release and arrhythmogenesis. Recent work suggests that disruption of the RyR complex can lead to arrhythmogenic right ventricular dysplasia type 2 and catecholaminergic and familial polymorphic ventricular tachycardia (5, 26, 27, 43). These lethal disruptions have been linked to genetic defects (13, 17, 27, 37, 43), transient pharmacological conditions. Finally, it is unlikely that the observed SCR, DAD, and triggered activity originated from Purkinje fibers, which are expected to be more abundant near the endocardium than near the epicardium. Moreover, the rather large spatial extent of triggered activity that is rate dependent and caused by multiple SCR events occur simultaneously from groups of myocardial cells. Under abnormal RyR conditions, we also observed triggered activity that is rate dependent and caused by multiple SCR events occurring from groups of myocardial cells.

Under abnormal RyR conditions, we demonstrate the mechanisms of SCR activity under enhanced calcium entry conditions are different from those under abnormal RyR conditions. Finally, it is unlikely that the observed SCR, DAD, and triggered activity originated from Purkinje fibers, which is known to dissociate FKBP12.6 from RyR and has been previously used to study the effects of abnormal RyR function (1, 4, 13, 47). Our data show that the arrhythmogenic phenotype is highly dependent on β-adrenergic stimulation combined with rapamycin (Fig. 2). This is in agreement with experimental findings (22, 45) and the clinical manifestation of the disease condition seen in patients (17, 26, 36, 39).

**SCRs and triggered activity.** We have reported previously that, under conditions of enhanced calcium entry with normal RyR, triggered activity and DADs occur in a rate-dependent fashion and are caused by SCR events (14, 19), similar to what has been reported under digitalis and calcium overload conditions (12). In addition, we have reported that multiple SCR events occur simultaneously from groups of myocardial cells. Under abnormal RyR conditions, we also observed triggered activity that is rate dependent and caused by multiple SCR events occurring from groups of myocardial cells. Therefore, in this regard, triggered activity and SCRs during enhanced calcium entry are similar to what occurs during abnormal RyR conditions. However, we did observe a striking difference. Under enhanced calcium entry conditions, triggered activity and SCR events occur near the endocardium where cytoplasmic levels of calcium were highest, whereas, under abnormal RyR conditions, triggered activity and SCR events occurred near the epicardium where calcium reuptake kinetics were fastest. Recently, Nam et al. (30) reported similar DAD and triggered activity in a canine left ventricular wedge preparation using glass floating microelectrodes. Interestingly, they also reported that ectopic activity occurred more frequently near the epicardium under abnormal RyR conditions. These data suggest that the mechanisms of SCR activity under enhanced calcium entry conditions are different from those under abnormal RyR conditions. Finally, it is unlikely that the observed SCR, DAD, and triggered activity originated from Purkinje fibers, which is expected to be more abundant near the endocardium than near the epicardium. However, the rather large spatial extent of triggered activity that we observed (>10 mm²) could not have occurred from a single Purkinje fiber. We cannot rule out the possibility that some triggered activity occurred in deeper layers below the mapping field. However, the SCR events that we observed most likely did originate near the surface of the mapping field because we saw no evidence of electrical propagation that could explain their occurrence.

**Cellular mechanism of SCR events under abnormal RyR conditions.** Under abnormal RyR conditions, we demonstrate the mechanisms of SCR activity under enhanced calcium entry conditions are different from those under abnormal RyR conditions. Finally, it is unlikely that the observed SCR, DAD, and triggered activity originated from Purkinje fibers, which is expected to be more abundant near the endocardium than near the epicardium. However, the rather large spatial extent of triggered activity that we observed (>10 mm²) could not have occurred from a single Purkinje fiber. We cannot rule out the possibility that some triggered activity occurred in deeper layers below the mapping field. However, the SCR events that we observed most likely did originate near the surface of the mapping field because we saw no evidence of electrical propagation that could explain their occurrence.
possible that a heterogeneous expression of other calcium handling proteins may play a role. We also found that SCR<sub>amp</sub> was strongly correlated with the rate of SR calcium uptake (Fig. 7), and CPA (which reduces SR calcium uptake) significantly reduced SCR<sub>amp</sub> despite an increase in cytoplasmic levels of calcium (Fig. 8). These results suggest that the mechanism of SCR activity when RyR is dysfunctional depends more on high SR calcium content than on high cytoplasmic calcium levels. We did not measure SR calcium content to confirm this result. However, Cordeiro et al. (6) reported higher SR calcium content in epicardial canine myocytes than in endocardial myocytes. Also, Diaz et al. (7) showed that increasing SR content increases the frequency of SCR activity, suggesting that there is a threshold value of SR content above which the SR will spontaneously release calcium. Using this same model of SCR activity, O’Neill et al. (32) showed that, by inhibiting SR calcium reuptake, spontaneous calcium waves were slowed, prolonged, and eventually abolished, which is consistent with our findings (Fig. 8).

Similarly, in studies conducted under abnormal RyR conditions, Jiang et al. (11) reported SCR governed by SR content. Together, these findings suggest that the mechanism of SCR activity under abnormal RyR conditions depends on fast calcium reuptake kinetics and, possibly, high SR calcium content. Given that abnormal RyR “leakiness” may reduce SR calcium content, recharging the SR with calcium may be necessary forSCRs to manifest (23, 24, 41), a process that occurs faster at the epicardium and is further accelerated by β-adrenergic stimulation. This may also explain why we observed a significant increase in triggered activity only under conditions of rapamycin + isoproterenol. However, the exact mechanism by which β-adrenergic stimulation contributes to the occurrence of SCR events is controversial, and our findings cannot provide a resolution, since it is possible for β-adrenergic stimulation to increase FKBP12.6 dissociation (45), as well as to enhance SR calcium load by enhanced calcium influx and SR uptake (11).

In this model, significant triggered activity was observed only under abnormal RyR conditions, which were produced by pharmacological perturbations using rapamycin and isoproterenol. Rapamycin inhibits the activity of FBKP12.6 by dissociating it from the RyR, which destabilizes its gating kinetics, leading to prolonged opening time and subconductance states, increased calcium leak, calcium spark frequency, and duration, and reduced calcium release amplitude and SR calcium content (1, 29, 42, 47). Several studies have reported rapamycin having no effect on resting potential, AP duration, L-type calcium channel current, or the sodium/calcium exchanger (42). However, rapamycin has been shown to affect potassium channels in mouse and rat myocytes and guinea pig smooth muscle cells (8, 46). Our data indicate no significant change in APD or APD transmural gradients with rapamycin at the concentration range used in this study (Fig. 2), which is higher than previously reported but expected given that our study was performed in intact preparations. Therefore, these data suggest that the nonspecific effects of rapamycin did not significantly influence our results.

Clinical implications. To date, >50 mutations in the RyR complex have been reported (2), and RyR dysfunction has been documented in conditions of heart failure (10, 25, 40, 49). The present study proposes a mechanism by which triggered activity occurs when FKBP12.6 is dissociated from RyR, which could be related to mutations or diseases that are also known to cause FKBP12.6 dissociation. Moreover, this study demonstrates that SCR events originating from ventricular myocytes are sufficient to cause triggered activity and do not require involvement of the Purkinje system. The results of this study may help identify novel targets for arrhythmogenesis that could, in turn, lead to novel therapy. In addition, it may also help provide a better understanding of the increasingly recognized relationship between abnormal mechanical and electrical function of the heart.

ACKNOWLEDGMENTS

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GRANTS

This work was supported by National Heart, Lung, and Blood Institute Grant HL-68877 and American Heart Association, Ohio Valley Affiliate predoctoral fellowship 0415213B (R. P. Katra).

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