Decreased RyR2 refractoriness determines myocardial synchronization of aberrant Ca^{2+} release in a genetic model of arrhythmia

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Edited by Gerhard Meissner, University of North Carolina at Chapel Hill, Chapel Hill, NC, and accepted by the Editorial Board May 6, 2013 (received for review January 2, 2013)

Dysregulated intracellular Ca^{2+} signaling is implicated in a variety of cardiac arrhythmias, including catecholaminergic polymorphic ventricular tachycardia. Spontaneous diastolic Ca^{2+} release (DCR) can induce arrhythmogenic plasma membrane depolarizations, although the mechanism responsible for DCR synchronization among adjacent myocytes required for ectopic activity remains unclear. We investigated the synchronization mechanism(s) of DCR underlying untimely action potentials and diastolic contractions (DCs) in a catecholaminergic polymorphic ventricular tachycardia mouse model with a mutation in cardiac calsequestrin. We used a combination of different approaches including single ryanodine receptor channel recording, optical imaging (Ca^{2+} and membrane potential), and contractile force measurements in ventricular myocytes and intact cardiac muscles. We demonstrate that DCR occurs in a temporally and spatially uniform manner in both myocytes and intact myocardial tissue isolated from cardiac calsequestrin mutation mice. Such synchronized DCR events give rise to triggered electrical activity that results in synchronous DCs in the myocardium. Importantly, we establish that synchronization of DCR is a result of a combination of abbreviated ryanodine receptor channel refractoriness and the preceding synchronous stimulated Ca^{2+} release/reuptake dynamics. Our study reveals how aberrant DCR events can become synchronized in the intact myocardium, leading to triggered activity and the resultant DCs in the settings of a cardiac rhythm disorder.

calcium-induced calcium release | luminal calcium | RyR2 deactivation | sarcoplasmic reticulum

Cardiac arrhythmias are the most common and lethal cardiac diseases, accounting for around 450,000 deaths annually in the United States alone (1). The majority of these deaths are caused by irregular ectopic beats that lead to ventricular tachycardia and fibrillation. The normal rhythmic beating of the heart is maintained by the cross-talk between the action potential (AP) and self-regenerating Ca^{2+} -induced Ca^{2+} release (CICR), where Ca^{2+} entry during the cardiac AP ignites CICR. The CICR is mediated by the ryanodine receptor (RyR2) on the sarcoplasmic reticulum (SR) and contributes to the global intracellular Ca^{2+} transient that drives cardiomyocyte contraction. Following Ca^{2+} release and contraction, relaxation ensues as Ca^{2+} is resequenced back into the SR by the SR Ca^{2+} pump (2). Importantly for normal Ca^{2+} cycling, the SR becomes refractory after each systolic Ca^{2+} release, thereby preventing spontaneous reactivation of CICR during the diastolic period (3–5). This temporal restraint of Ca^{2+} signaling is thought to be a consequence of the local decrease in intra-SR Ca^{2+} that in turn can inhibit CICR either by reducing RyR2 Ca^{2+} efflux (6) and/or by deactivating RyR2s via a mechanism that involves the SR Ca^{2+} binding protein calsequestrin 2 (CASQ2) (7–9).

In various disease settings, aberrant diastolic Ca^{2+} release (DCR) from the SR gives rise to arrhythmic ectopic activity (10–14). The pathologic link between diastolic dysregulated Ca^{2+} release and arrhythmia is particularly evident in catecholaminergic polymorphic ventricular tachycardia (CPVT), a familial form of arrhythmia caused by mutations in the components of the RyR2 complex, including RyR2 and CASQ2 (15, 16). In myocytes harboring CPVT-associated CASQ2 mutations, altered CASQ2-dependent RyR2 modulation predisposes the SR to spontaneous DCR. DCR occurs in the form of waves of self-propagating CICR. These waves result in Ca^{2+} -dependent depolarizing currents generated by the electrogenic $\text{Na}^{+}/\text{Ca}^{2+}$ exchanger activity on the cell membrane giving rise to delayed afterdepolarizations (DADs) and consequently to triggered arrhythmias (17–19).

Despite the fact that the mechanisms of triggered arrhythmias have been well established on the cellular level, the relationship between abnormal myocyte Ca^{2+} cycling and focal excitation at the tissue level remains poorly understood. A key unresolved question is how spontaneous Ca^{2+} release in individual myocytes can cause a sufficiently large membrane depolarization to elicit an ectopic event across the whole of myocardium, particularly because a local depolarizing current is expected to be absorbed by the electrically connected neighboring myocytes serving as current sinks (20). Consequently, it has been suggested that ectopic activity, rather than arising in individual ventricular myocytes, originates in the Purkinje system, which has a smaller source-sink mismatch relative to the 3D ventricular network (21). Thus, there is uncertainty whether and how focally activated arrhythmias such as CPVT can originate in the ventricular myocardium. Here, our focus is a murine model of CPVT with a point mutation in CASQ2 (CASQ2^{R33Q}) that causes an impairment of store-dependent RyR2 deactivation (22). We demonstrate that this impairment alters refractoriness of Ca^{2+} release, which in turn provides a mechanism for synchronization of DCR, DADs, and diastolic contractions (DCs) across intact myocardium. Importantly, our study provides a conceptual framework in which

Author contributions: L.B., P.B.R., A.E.B., P.V., M.F., P.M.L.J., and S.G. designed research; L.B., J.L.S., P.B.R., H.-T.H., A.E.B., Q.L., and H.C. performed research; Q.L., C.N., F.L., S.G.P., V.V.F., and P.M.L.J. contributed new reagents/analytic tools; L.B., J.L.S., P.B.R., A.E.B., Q.L., and H.C. analyzed data; and L.B., P.B.R., P.V., M.F., P.M.L.J., and S.G. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission. G.M. is a guest editor invited by the Editorial Board.

Freely available online through the PNAS open access option.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1300052110/-DCSupplemental.

store-dependent RyR2 deactivation on the cellular level serves as a substrate for triggered arrhythmias at the whole heart level.

Results

Spontaneous Ca^{2+} Release Is Associated with Reduced RyR2 Refractoriness in $\text{CASQ2}^{\text{R33Q}}$ Myocytes. Myocytes isolated from WT and $\text{CASQ2}^{\text{R33Q}}$ hearts were paced at 1 Hz in the presence of the β -adrenergic agonist isoproterenol (ISO, 100 nM, Fig. 1A). Under these conditions, DCR (waves and/or wavelets) occurred at a frequency of 0.24 ± 0.06 events per cycle in $\text{CASQ2}^{\text{R33Q}}$ myocytes, which was significantly higher than those in WT myocytes (0.03 ± 0.01 events per cycle, Fig. 1C). The few DCRs observed in WT myocytes were randomly distributed through diastole. In contrast, DCRs in $\text{CASQ2}^{\text{R33Q}}$ cells had a high probability of occurrence around three distinct time points consistent with the development of up to three DCRs during the diastolic period. The distribution of DCRs reveals a well-defined periodicity with a fixed interrelease interval of ~ 260 ms (Fig. 1D). Of note, the occurrence of DCR displayed a clear rate dependency such that the number of events per cycle decreased and their distribution narrowed at faster pacing rates (Fig. S1).

To gain further insights into the mechanisms of DCR, we examined more closely the distribution of the latencies to the first DCR. In $\text{CASQ2}^{\text{R33Q}}$ myocytes, the histogram after an initial time lag of ~ 200 ms showed a sharp peak followed by a gradual, close to exponential decay (Fig. 1E). As demonstrated by Maltsev et al. (23) in pacemaker cells with robust Ca^{2+} oscillations, the high synchronicity of spontaneous firing of release sites is attributable to synchronization of refractory periods. Thus, the 200-ms time lag before the first DCR in $\text{CASQ2}^{\text{R33Q}}$ myocytes provides a metric consistent with the RyR2 refractory period. In contrast, DCRs in WT myocytes were less frequent and occurred more randomly, with a median time of onset occurring at 751 ms, which was distinctly longer relative to $\text{CASQ2}^{\text{R33Q}}$ myocytes (Fig. 1F). Based on previous work by Maltsev et al. (23), the increased synchronicity of release events in $\text{CASQ2}^{\text{R33Q}}$ cells could be ascribed to either an increased magnitude of Ca^{2+} release through RyR2s and/or altered refractory properties of RyR2s (facilitating CICR). Because the amplitude of Ca^{2+} transients decreased in $\text{CASQ2}^{\text{R33Q}}$ compared with WT myocytes (Fig. 1B), an alteration in refractoriness of release is the likely factor behind the increased synchronicity of DCR during CPVT.

To more closely assess the refractory properties of Ca^{2+} signaling in $\text{CASQ2}^{\text{R33Q}}$ and WT myocytes, we measured the restitution of SR Ca^{2+} release using an established S1-S2 protocol in which the first stimulus is followed by a second one after a varying time interval (Fig. 2A). This protocol revealed that the restitution of Ca^{2+} release, as evidenced by the rate of recovery of Ca^{2+}

transient amplitude, was significantly accelerated in $\text{CASQ2}^{\text{R33Q}}$ myocytes, consistent with impaired Ca^{2+} signaling refractoriness in these cells (Fig. 2B). Furthermore, to examine whether these differences in recovery of Ca^{2+} release are caused by potential alterations in Ca^{2+} currents (I_{Ca}), we performed measurements of I_{Ca} restitution in patch-clamped myocytes following the same S1-S2 protocol as mentioned previously. The recovery of the peak I_{Ca} was similar in both myocyte groups, suggesting that I_{Ca} is unlikely to underlie the differences in release restitution (Fig. S2).

To examine whether the altered Ca^{2+} signaling in $\text{CASQ2}^{\text{R33Q}}$ myocytes was associated with altered RyR2 function, we performed single-channel measurements in RyR2s incorporated into lipid bilayers. Previously, we demonstrated that the R33Q CASQ2 variant lacks the ability of its WT counterpart to inhibit RyR2 at low luminal Ca^{2+} (20 μM) (24). Here we examined whether altered modulation of RyR2 activity by $\text{CASQ2}^{\text{R33Q}}$ is present at a higher, near-diastolic luminal $[\text{Ca}^{2+}]$ (1 mM). Indeed, at this Ca^{2+} , RyR2 open probability (P_o) was significantly higher, whereas the mean closed time (MCT) was significantly shorter in $\text{CASQ2}^{\text{R33Q}}$ RyR2s compared with WT channels (Fig. 2C–F). Consistent with these results in single RyR2s, diastolic Ca^{2+} sparks occurred more frequently in $\text{CASQ2}^{\text{R33Q}}$ than in WT myocytes (4.4 ± 1.1 vs. 2.5 ± 0.3 $0.1 \text{ mm}^{-1} \cdot \text{s}^{-1}$; $n = 74$ –621 events; $P < 0.05$). Collectively, these data suggest that impaired refractory behavior of individual RyR2s leads to temporally aligned DCR in $\text{CASQ2}^{\text{R33Q}}$ myocytes.

Highly Synchronized DCs in Intact $\text{CASQ2}^{\text{R33Q}}$ Muscle. To test whether the temporal synchronization of spontaneous DCR in myocytes isolated from $\text{CASQ2}^{\text{R33Q}}$ mice gives rise to synchronous DCs in cardiac muscle, we performed force measurements in multicellular papillary muscle and trabeculae preparations. Muscles from WT and $\text{CASQ2}^{\text{R33Q}}$ mice were electrically paced at 1 Hz, and mechanical force was measured before and after exposure to ISO (Fig. 3A). Under baseline conditions (upper traces), WT and $\text{CASQ2}^{\text{R33Q}}$ muscles exhibited regular twitches of similar amplitude and time course (Fig. 3A, E, and F). Exposure to ISO (30–300 nM; lower traces in Fig. 3A) effectively tripled the frequency of contractions because of the occurrence of unprovoked diastolic events in $\text{CASQ2}^{\text{R33Q}}$ but not in WT muscles. As expected, ISO significantly increased average twitch force amplitude in WT muscles. Interestingly, the average twitch force amplitude at the peak ISO effect remained unchanged in $\text{CASQ2}^{\text{R33Q}}$ muscles. This is mainly due to the increased DCR frequency depleting the SR Ca^{2+} store (Fig. 3E). Superimposed traces from different $\text{CASQ2}^{\text{R33Q}}$ muscles challenged with ISO are shown in Fig. 3B. As in myocyte experiments, the DCs consistently arose

Fig. 1. Frequency and timing of spontaneous Ca^{2+} release in $\text{CASQ2}^{\text{R33Q}}$ isolated ventricular myocytes. (A) Representative line-scan images of WT and $\text{CASQ2}^{\text{R33Q}}$ isolated cardiomyocytes paced at 1 Hz (dots) in the presence of ISO (100 nM) show increased incidence of DCR events in $\text{CASQ2}^{\text{R33Q}}$ cells. (B) Stimulated Ca^{2+} transient amplitude in $\text{CASQ2}^{\text{R33Q}}$ and WT myocytes paced at 2 Hz in the presence of 100 nM ISO. F/F_0 (in which F is the fluorescence at time t and F_0 represents the background signal) is 2.56 ± 0.06 vs. 2.75 ± 0.06 , respectively. Summary of DCR frequency (C) and DCR temporal distribution (D): DCRs are more common in $\text{CASQ2}^{\text{R33Q}}$ myocytes and occur at three distinct time points (interrelease latencies 337, 259, and 269 ms, respectively). (E) Distribution of the latency to the first DCR following a delay displays a prominent early peak. (F) The median of the first DCR latency occurs earlier in $\text{CASQ2}^{\text{R33Q}}$ cells relative to WT. (B) $n = 24$ and 10 cells for $\text{CASQ2}^{\text{R33Q}}$ and WT, respectively; (C–F) $n = 59$ and 30 cells for $\text{CASQ2}^{\text{R33Q}}$ and WT from five and three animals, respectively. $*P < 0.05$; $**P < 0.01$.

Fig. 2. Abbreviated RyR2 refractoriness underlies spontaneous Ca^{2+} release: single myocytes and single RyR2 channels experiments. (A) Representative traces of SR Ca^{2+} release attained during an S1-S2 restitution protocol show faster release recovery in $\text{CASQ2}^{\text{R33Q}}$ cardiomyocytes. Experiments were carried out in the presence of 100 nM ISO. (B) Average time course of the restitution of Ca^{2+} transients shows faster recovery of SR Ca^{2+} release in $\text{CASQ2}^{\text{R33Q}}$ myocytes relative to WT cells (time to half-maximal recovery was 134 ± 5 vs. 166 ± 2 ms respectively, $P < 0.001$). Each data point was recorded in 12–29 $\text{CASQ2}^{\text{R33Q}}$ and 6–11 WT myocytes, respectively. Restitution of Ca^{2+} transients in each group was fitted to logistic functions. (C) Sample recordings of RyR2 channels obtained from cardiac SR microsomes of WT and $\text{CASQ2}^{\text{R33Q}}$ mice. Open events are shown as upward deflections from the marked zero current level. $\text{CASQ2}^{\text{R33Q}}$ single RyR2s were more active. Bar graphs showing the P_o (D), mean open time (MOT) (E), and MCT (F) of 8 WT and 4 $\text{CASQ2}^{\text{R33Q}}$ RyR2 channels. Single RyR2 P_o was significantly greater and MCT significantly smaller in $\text{CASQ2}^{\text{R33Q}}$ mice compared with WT mice in a one-tail unpaired Student t test. * $P < 0.05$; ** $P < 0.01$.

around three different diastolic time points as illustrated in Fig. 3C. These results suggest that DCs result from highly synchronized cellular events consistent with the temporal alignment of DCR observed in isolated myocytes.

To further assess the cellular synchronicity underlying the observed DCs, we examined the amplitude and decay rate of developed force during stimulated and spontaneous Ca^{2+} release (Fig. 3E and F). Stimulated contraction (SC) of myocardium is maximally synchronized by electrical stimulus. Thus, we compared the amplitude and decay rate of DCs with SCs to determine the intrinsic synchronicity of the DCs. As demonstrated in Fig. 3D, a significant pool of DCs was similar in size or was even higher than SCs. Taken together, this suggests that most DCs recruited the vast majority of muscle cells and were likely associated with an ectopic AP.

DCR Is Synchronized in Intact $\text{CASQ2}^{\text{R33Q}}$ Cardiac Muscle. To directly examine synchronization of DCR between different myocytes in myocardium of $\text{CASQ2}^{\text{R33Q}}$ mice, we performed confocal Ca^{2+} imaging in cardiac muscle preparations. Muscles loaded with the cytosolic Ca^{2+} indicator Rhod-2 were paced at 1 Hz in the presence of ISO (Fig. 4). Consistent with the results in isolated myocytes, DCR in the form of Ca^{2+} waves occurred regularly between pacing-induced Ca^{2+} transients in the vast majority of $\text{CASQ2}^{\text{R33Q}}$ cells within the field of view (Fig. 4B and C) but not in WT muscles (Fig. 4A). Once again, when we examined the timing of cellular DCRs within cardiac muscles, we observed three distinct time peaks similar to those observed in isolated myocytes (Fig. 4D). As in isolated myocytes, the distribution of DCRs and DCs in cardiac muscles was rate-dependent (Fig. S3). Furthermore, similar to the results of force measurements, the amplitudes of DCRs from a significant portion of myocytes were similar to those of Ca^{2+} transients generated by electrical stimulation (Fig. 4E).

Experiments conducted in isolated myocytes suggest that the temporal alignment of DCR arises from the shortened RyR2 refractory period following temporally uniform stimulated Ca^{2+} release and the resultant synchronized reuptake. To test the role

of the stimulated Ca^{2+} release/reuptake in DCR synchronization, we examined the effects of a single electrical stimulation on the timing of DCR (Fig. 5A, Left). At rest, the muscles exhibited occasional Ca^{2+} waves that randomly occurred in different myocytes. Following an electrically induced Ca^{2+} transient, several sequential oscillations of Ca^{2+} release occurred in multiple adjacent cells, which gradually decreased in amplitude and in the degree of cohesiveness (Fig. 5B). Application of additional electrical stimuli once again resynchronized the diastolic release (Fig. 5A, Right). To quantify the degree of synchronicity of DCR, we measured the time interval (i.e., latency) to the first DCR following stimulated Ca^{2+} release and the time intervals between consecutive DCRs (Fig. 5C). The first DCR evidenced the shortest mean latency and smallest corresponding standard deviation (SD), suggesting the highest level of synchronicity. The subsequent DCRs evidenced longer latencies that were coupled to a greater variance in their occurrence. These results suggest that temporally uniform stimulated Ca^{2+} release/reuptake dynamics plays a key role in synchronization of DCR in $\text{CASQ2}^{\text{R33Q}}$ muscle. Of note, Ca^{2+} waves are capable of crossing cell boundaries apparently via gap junctions (25). However, the rate of propagation of isolated Ca^{2+} waves ($\sim 50 \mu\text{m/s}$) in our study in the absence of pacing seems too slow to account for the high synchronicity of release in the wake of a temporally aligned pacing-induced Ca^{2+} transient.

For spontaneous Ca^{2+} release to become proarrhythmic, it must cause an ectopic AP that triggers an arrhythmia. As mentioned previously, the high amplitude of DCs and that of DCR events in intact tissue suggest an accompanying muscle-wide triggered electrical depolarization (i.e., extrasystolic AP). To test directly whether synchronous DCR can result in extrasystolic electrical activity in $\text{CASQ2}^{\text{R33Q}}$ muscles, we performed simultaneous Ca^{2+} and membrane potential (MP) imaging. Muscles were dual-labeled with Rhod-2 and RH237 or di-4-ANBDQBS for simultaneous Ca^{2+} and MP measurements, respectively, and stimulated at

Fig. 3. $\text{CASQ2}^{\text{R33Q}}$ intact cardiac muscles show highly synchronized DCs. (A) Representative recordings of developed (Dev) force from WT and $\text{CASQ2}^{\text{R33Q}}$ cardiac muscles electrically stimulated at 1 Hz (dots). Superfusion with ISO (30–300 nM) resulted in DCs (red asterisks) in $\text{CASQ2}^{\text{R33Q}}$ but not in WT preparations. (B) Superimposed twitch recordings from four $\text{CASQ2}^{\text{R33Q}}$ muscles in the presence of ISO (30–300 nM) paced at 1 Hz (black dots) show that DCs consistently occur around the same time points (not to scale). (C) Cumulative temporal distribution of DCs in $\text{CASQ2}^{\text{R33Q}}$ muscles shows three probability peaks. (D) Distribution of DC amplitudes (as % of the mean value of the corresponding SC) in $\text{CASQ2}^{\text{R33Q}}$ muscles. Average amplitude (E) and decay rate (F) of WT and $\text{CASQ2}^{\text{R33Q}}$ contractions under baseline (BSL) conditions (– ISO) and under maximum effect of adrenergic stimulation (+ ISO, 30–300 nM). $n = 5$ muscles for both $\text{CASQ2}^{\text{R33Q}}$ and WT. * $P < 0.05$. $-dF/dt$, maximum of the first derivative of the developed force.

pharmacological stabilization of the RyR2 complex and subsequent prolongation of SR Ca^{2+} release refractoriness.

Summary. We show that impaired Ca^{2+} release refractoriness combined with uniform SR Ca^{2+} release in intact cardiac muscle synchronizes the DCR that underlies triggered arrhythmias in CPVT. These aberrant DCRs can be mitigated by pharmacological stabilization of the release machinery that improves abnormally abbreviated SR Ca^{2+} release refractoriness. It remains to be determined whether, and to what extent, such synchronization of Ca^{2+} release in ventricular myocardium as opposed to other tissue types (i.e., Purkinje fibers) contributes to clinically relevant arrhythmic episodes in CPVT and other forms of Ca^{2+} -dependent arrhythmias in the intact heart. Targeting DCR synchronization through reagents affecting RyR2 refractory behavior such as dantrolene may be an innovative strategy for development of mechanism-based antiarrhythmia therapies.

Materials and Methods

All animal procedures were approved by The Ohio State University Institutional Animal Care and Use Committee and conformed to the Guide for

the Care and Use of Laboratory Animals published by the US National Institutes of Health [(NIH) Publication No. 85-23, revised 1996], and by the Animal Care and Use Committee of the University of Padova. Ventricular myocytes were obtained by enzymatic isolation as previously described (39). I_{Ca} were recorded using whole-cell patch-clamp technique. Fluo-3 (an intracellular Ca^{2+} indicator) fluorescence was recorded in the line scan mode of the confocal microscope. SR microsomes were prepared from homogenized WT and CASQ2^{R33Q} mouse ventricular muscle, and single channels were recorded as previously described (24). Trabeculae or papillary muscles were used for force measurement as described previously (40). For confocal Ca^{2+} and MP imaging, muscles were loaded with the cytosolic Ca^{2+} indicator Rhod-2 AM (40 μM). For some experiments, the muscles were also stained with MP indicator RH237 or di-4-ANBDQBS (Richard D. Berlin Center for Cell Analysis and Modeling, University of Connecticut Health Center). Detailed material and methods can be found in *SI Materials and Methods*.

ACKNOWLEDGMENTS. This work was supported by NIH Grants HL074045 and HL063043 (to S.G.), NIH Grants HL07832 and AR054098 (to M.F.), and Italian Telethon Organizzazione Non Lucrativa di Utilità Sociale (ONLUS) Foundation Grant GGP11141 and Fondazione Cassa di Risparmio delle Province Lombarde (Cariplo) Grant p2008.2275 (to P.V.). L.B. is supported by the Great Rivers Affiliate, American Heart Association Postdoctoral Fellowship 13POST14050001.

- Roger VL, et al.; American Heart Association Statistics Committee and Stroke Statistics Subcommittee (2012) Heart disease and stroke statistics—2012 update: A report from the American Heart Association. *Circulation* 125(1):e2–e220.
- Bers DM (2001) *Excitation-Contraction Coupling and Cardiac Contractile Force* (Springer, Dordrecht), 2nd Ed.
- Terentyev D, Viatchenko-Karpinski S, Valdivia HH, Escobar AL, Györke S (2002) Luminal Ca^{2+} controls termination and refractory behavior of Ca^{2+} -induced Ca^{2+} release in cardiac myocytes. *Circ Res* 91(5):414–420.
- Szentési P, Pignier C, Egger M, Kranias EG, Niggli E (2004) Sarcoplasmic reticulum Ca^{2+} refilling controls recovery from Ca^{2+} -induced Ca^{2+} release refractoriness in heart muscle. *Circ Res* 95(8):807–813.
- Sobie EA, Song L-S, Lederer WJ (2005) Local recovery of Ca^{2+} release in rat ventricular myocytes. *J Physiol* 565(Pt 2):441–447.
- Guo T, Gillespie D, Fill M (2012) Ryanodine receptor current amplitude controls Ca^{2+} sparks in cardiac muscle. *Circ Res* 111(1):28–36.
- Györke S, Terentyev D (2008) Modulation of ryanodine receptor by luminal calcium and accessory proteins in health and cardiac disease. *Cardiovasc Res* 77(2):245–255.
- Qin J, et al. (2008) Luminal Ca^{2+} regulation of single cardiac ryanodine receptors: Insights provided by calsequestrin and its mutants. *J Gen Physiol* 131(4):325–334.
- Györke S, Hester N, Jones LR, Györke S (2004) The role of calsequestrin, triadin, and junctin in conferring cardiac ryanodine receptor responsiveness to luminal calcium. *Biophys J* 86(4):2121–2128.
- Ter Keurs HEDJ, Boyden PA (2007) Calcium and arrhythmogenesis. *Physiol Rev* 87(2):457–506.
- Laurita KR, Rosenbaum DS (2008) Mechanisms and potential therapeutic targets for ventricular arrhythmias associated with impaired cardiac calcium cycling. *J Mol Cell Cardiol* 44(1):31–43.
- Weiss JN, Nivala M, Garfinkel A, Qu Z (2011) Alternans and arrhythmias: From cell to heart. *Circ Res* 108(1):98–112.
- Venetucci LA, Trafford AW, O'Neill SC, Eisner DA (2008) The sarcoplasmic reticulum and arrhythmogenic calcium release. *Cardiovasc Res* 77(2):285–292.
- Györke S, Carnes C (2008) Dysregulated sarcoplasmic reticulum calcium release: Potential pharmacological target in cardiac disease. *Pharmacol Ther* 119(3):340–354.
- Györke S (2009) Molecular basis of catecholaminergic polymorphic ventricular tachycardia. *Heart Rhythm* 6(1):123–129.
- Napolitano C, Priori SG (2007) Diagnosis and treatment of catecholaminergic polymorphic ventricular tachycardia. *Heart Rhythm* 4(5):675–678.
- Lederer WJ, Tsien RW (1976) Transient inward current underlying arrhythmogenic effects of cardiotonic steroids in Purkinje fibres. *J Physiol* 263(2):73–100.
- Radwański PB, Poelzing S (2011) NCX is an important determinant for premature ventricular activity in a drug-induced model of Andersen-Tawil syndrome. *Cardiovasc Res* 92(1):57–66.
- Radwański PB, Veeraraghavan R, Poelzing S (2010) Cytosolic calcium accumulation and delayed repolarization associated with ventricular arrhythmias in a guinea pig model of Andersen-Tawil syndrome. *Heart Rhythm* 7(10):1428–1435.e1.
- Xie Y, Sato D, Garfinkel A, Qu Z, Weiss JN (2010) So little source, so much sink: Requirements for afterdepolarizations to propagate in tissue. *Biophys J* 99(5):1408–1415.
- Herron TJ, Milstein ML, Anumonwo J, Priori SG, Jalife J (2010) Purkinje cell calcium dysregulation is the cellular mechanism that underlies catecholaminergic polymorphic ventricular tachycardia. *Heart Rhythm* 7(8):1122–1128.
- Rizzi N, et al. (2008) Unexpected structural and functional consequences of the R33Q homozygous mutation in cardiac calsequestrin: A complex arrhythmogenic cascade in a knock in mouse model. *Circ Res* 103(3):298–306.
- Maltsev AV, et al. (2011) Synchronization of stochastic Ca^{2+} release units creates a rhythmic Ca^{2+} clock in cardiac pacemaker cells. *Biophys J* 100(2):271–283.
- Terentyev D, et al. (2008) Modulation of SR Ca release by luminal Ca and calsequestrin in cardiac myocytes: Effects of CASQ2 mutations linked to sudden cardiac death. *Biophys J* 95(4):2037–2048.
- Lamont C, Luther PW, Balke CW, Wier WG (1998) Intercellular Ca^{2+} waves in rat heart muscle. *J Physiol* 512(Pt 3):669–676.
- Kobayashi S, et al. (2010) Dantrolene, a therapeutic agent for malignant hyperthermia, inhibits catecholaminergic polymorphic ventricular tachycardia in a RyR2 (R2474S/+) knock-in mouse model. *Circ J* 74(12):2579–2584.
- Maxwell JT, Domeier TL, Blatter LA (2012) Dantrolene prevents arrhythmogenic Ca^{2+} release in heart failure. *Am J Physiol Heart Circ Physiol* 302(4):H953–H963.
- Terentyev D, et al. (2006) Abnormal interactions of calsequestrin with the ryanodine receptor calcium release channel complex linked to exercise-induced sudden cardiac death. *Circ Res* 98(9):1151–1158.
- di Barletta MR, et al. (2006) Clinical phenotype and functional characterization of CASQ2 mutations associated with catecholaminergic polymorphic ventricular tachycardia. *Circulation* 114(10):1012–1019.
- Terentyev D, et al. (2003) Calsequestrin determines the functional size and stability of cardiac intracellular calcium stores: Mechanism for hereditary arrhythmia. *Proc Natl Acad Sci USA* 100(20):11759–11764.
- Knollmann BC, et al. (2006) Casq2 deletion causes sarcoplasmic reticulum volume increase, premature Ca^{2+} release, and catecholaminergic polymorphic ventricular tachycardia. *J Clin Invest* 116(9):2510–2520.
- Stern MD, Kort AA, Bhatnagar GM, Lakatta EG (1983) Scattered-light intensity fluctuations in diastolic rat cardiac muscle caused by spontaneous Ca^{++} -dependent cellular mechanical oscillations. *J Gen Physiol* 82(1):119–153.
- Radwański PB, Belevych AE, Brunello L, Carnes CA, Györke S (2013) Store-dependent deactivation: Cooling the chain-reaction of myocardial calcium signaling. *J Mol Cell Cardiol* 58:77–83.
- Belevych AE, et al. (2011) The relationship between arrhythmogenesis and impaired contractility in heart failure: Role of altered ryanodine receptor function. *Cardiovasc Res* 90(3):493–502.
- Ramay HR, Liu OZ, Sobie EA (2011) Recovery of cardiac calcium release is controlled by sarcoplasmic reticulum refilling and ryanodine receptor sensitivity. *Cardiovasc Res* 91(4):598–605.
- Belevych AE, et al. (2012) Shortened Ca^{2+} signaling refractoriness underlies cellular arrhythmogenesis in a postinfarction model of sudden cardiac death. *Circ Res* 110(4):569–577.
- Korneyev D, et al. (2012) Calsequestrin 2 deletion shortens the refractoriness of Ca^{2+} release and reduces rate-dependent Ca^{2+} -alternans in intact mouse hearts. *J Mol Cell Cardiol* 52(1):21–31.
- Wasserstrom JA, et al. (2010) Variability in timing of spontaneous calcium release in the intact rat heart is determined by the time course of sarcoplasmic reticulum calcium load. *Circ Res* 107(9):1117–1126.
- Zhao W, et al. (2003) Combined phospholamban ablation and SERCA1a overexpression result in a new hyperdynamic cardiac state. *Cardiovasc Res* 57(1):71–81.
- Hiranandani N, Bupha-Intr T, Janssen PML (2006) SERCA overexpression reduces hydroxyl radical injury in murine myocardium. *Am J Physiol Heart Circ Physiol* 291(6):H3130–H3135.