Cardiac Alternans and Ventricular Fibrillation
A Bad Case of Ryanodine Receptors Reneging on Their Duty

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Since the first description in 1872 of cardiac alternans by Traube in a patient with alcoholic cardiomyopathy, there has been significant progress in understanding the significance of this clinical sign of heart disease. Traube reported this phenomenon before Einthoven invented the ECG in 1903. Thus, he actually described pulsus alternans in his patient, a strong–weak arterial pulse alternation perceptible by unaided finger tact. The patient died shortly after diagnosis, but because he had severe cardiomyopathy, cardiac alternans was not recognized as an ancillary index of disease severity or as a harbinger of mortality. Later, the introduction of ECGs in the routine examination of patients made it clear that cardiac alternans, in the form of T-wave alternans, was a common sign in several cardiomyopathies, such as heart failure, coronary artery disease, genetic and acquired channelopathies, and even in electrolyte disturbances of the body. However, an unequivocal association between cardiac alternans and arrhythmic risk was not recognized until the 1990s, and only recently a multicenter clinical trial established that patients with moderate cardiac dysfunction but lacking T-wave alternans may not need an implantable cardiac defibrillator to improve their odds of avoiding sudden cardiac death. Thus, from the peculiar weak–strong arterial pulse oscillation detected by Traube to a critical risk stratification factor for sudden cardiac death, cardiac alternans have come a long way as diagnostic and prognostic manifestation of cardiac disease.

If the pathway of cardiac alternans in the clinical arena has been a smoothly ascending line, explaining its precise cellular mechanisms has resulted in a more tortuous process, reflecting the complexity of the phenomenon. The ability to induce mechanical alternans by rapidly stimulating the heart reflects the complexity of the phenomenon. The ability to induce mechanical alternans by rapidly stimulating the heart was recognized early as an inherent ability of all mammalian ventricular muscles. Initially, researchers relied on traditional whole heart physiology and explained cardiac alternans based on the Frank–Starling relationship, wherein a strong beat, by expelling more blood, leaves a small residual end-diastolic volume, in turn reducing force development in the next beat. During the weak beat, the end-systolic volume increases because of decreased ejection, leading to a greater end-diastolic volume and thus stronger force in the next beat. However, it was quickly realized that cardiac alternans were more complex than apparently straightforward load–force relationships, as papillary muscles displayed alternans when contracting under a constant load (isotonically) or length (isometrically). Because isometric contractions could also be observed in isolated ventricular myocytes, it was therefore inferred that mechanisms intrinsic to the cardiac cell must account for the genesis of cardiac alternans. This conceptual framework was forged before the widespread use of intracellular Ca2+ imaging, and when Fura-2 and other fluorescent indicators interrupted in the scene, it became evident that the alternation in the force of contraction was mirrored with amazing faithfulness by alternations in the magnitude of the Ca2+ transient, or Ca2+ alternans (Ca-Alts). Now in the realm of subcellular mechanisms, Ca-Alts were first explained by a delay of Ca2+ transport from reuptake sites to release sites, but this idea has not gained traction as it has become increasingly evident that Ca2+ diffusion between these 2 compartments in a sarcomere is extremely fast. Instead, the availability of Ca2+ in the release sites (through the work of SERCA2a), more than diffusion from reuptake sites, was favored as a likely explanation for Ca-Alts. We will discuss now new data indicating that this limitation, sarcoplasmic reticulum (SR) Ca2+ load, is unlikely to be the first critical factor in the generation of Ca-Alts and their progression to ventricular fibrillation.

Because Ca-Alts may be detected in the absence of L-type Ca2+ current alternations and are abolished by ryanodine, there is compelling evidence that Ca-Alts are generated by SR behavior. With the focus squarely on this single organelle, the quest now is to delineate the hierarchical role of cardiac ryanodine receptors (RyR2) and the Ca2+-ATPase (SERCA2a) as molecular instigators of Ca-Alts. Thus, in historical terms, we are back to the former question, but with a molecular twist: is an intrinsic dysfunction of RyR2, or an alternating reduction of end-diastolic SR Ca2+ load (caused by an insufficient SERCA2a), that first intervenes to generate Ca-Alts? This central problem is elegantly addressed by Wang et al in the present issue of Circulation Research. Although studies with isolated ventricular cells may provide detailed examination of SR Ca2+ dynamics and RyR2 channel behavior, they are inherently incapable of providing the heterogeneity and complexity that sustains arrhythmogenic behavior such as spatially discordant alternans and other tissue-level phenomena. Thus, Wang et al developed a novel and sophisticated dual optical mapping strategy that allowed them to simultaneously...
measure action potential (AP) and intra-SR Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_{SR}\)) dynamics in epicardial layers of Langendorff-perfused intact rabbit hearts. Paired with electrocardiographic recordings, these technically challenging experiments stretched the physiological limitations of the system: hearts were pharmacologically immobilized to reduce the energetic demand and prevent motion artifacts and loaded with fluorescent dyes that absorbed light in the same spectral range but that have emission wavelengths apart from each other (RH237 for membrane potential and Fluo-5 N for intra-SR Ca\(^{2+}\)). Because the authors used hearts free from pathological conditions that normally favor the spontaneous appearance of cardiac alternans, the latter had to be induced by rapidly stimulating the apex of the heart, which necessarily short-circuits its normal conduction pathway. Nevertheless, there is much to be learned even from this not entirely physiological system, as hearts were unencumbered from altered protein expression, post-translational modifications, electric remodeling, etc, that are inherent to cardiac pathologies and that complicate interpretation of the role of critical players of cardiac alternans.

Data extracted from the above system both conformed to logical expectations and yielded surprising conclusions. Among the former, fast-pacing of the heart induced alternans both in the AP duration (APD) and in the magnitude of systolic [Ca\(^{2+}\)]\(_{SR}\) decay. This was expected from the inherent property of all mammalian hearts to generate alternans once a pacing threshold has been reached, although the virtue here is that now we are presented with windows into [Ca\(^{2+}\)]\(_{SR}\) and AP dynamics to better understand the subcellular mechanisms underlying this property. Another consistent observation was that the degree of alternation (Spectral Alternans Magnitude) was larger for the intra-SR signals than for the APD-Alts. Moreover, the alternations of [Ca\(^{2+}\)]\(_{SR}\) decay and APD were in-phase (greater Ca release=longer APD), producing concordant alternans. That a large Ca\(^{2+}\) release prolonged the APD was somewhat surprising if we consider that in mammals that have long APs, such as the rabbit, large SR Ca\(^{2+}\) releases are expected to shorten the APD by promoting Ca\(^{2+}\)-dependent inactivation of L-type Ca\(^{2+}\) channels, but the study underscores the preponderant effect of the Na–Ca exchanger (NCX) in prolonging the APD because of extrusion of the released Ca\(^{2+}\).

One of the most profitable advantages of the recording setup of Wang et al was its ability to resolve the subcellular events temporally that first led to alternans. Thus, as pacing frequencies increased (and cycle length decreased), [Ca\(^{2+}\)]\(_{SR}\) alternans preceded APD-Alts and clearly confirmed the SR as chief instigator of this phenomenon. Furthermore, the most critical player in the onset of [Ca\(^{2+}\)]\(_{SR}\) alternans seems to be the RyR2, as Ca\(^{2+}\) release alternans repeatedly proceeded without changes in the end-diastolic [Ca\(^{2+}\)]\(_{SR}\).

What causes a pool of RyR2s to default on their duty and seem insensible to triggering signals (SR load, I\(_{Ca}\)) that normally induce their opening? Wang et al offers RyR2 refractoriness, an operational term indicating resistance of the RyR2 channel to open after a Ca\(^{2+}\) release event, to explain their results. In other words, because the first event is the recalcitrance of RyR2s to open despite triggering stimuli, Wang et al propose that RyR2 refractoriness is the mechanism that

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**Figure.** Ca\(^{2+}\) alternans and their subcellular origins.

A, Alternans promoted by insufficient sarcoplasmic reticulum (SR) Ca\(^{2+}\) load. An increase in the heart rate prevents the end-diastolic [Ca\(^{2+}\)]\(_{SR}\) to reach the previous beat diastolic level (1a). The reduced SR Ca\(^{2+}\) then reduces ryanodine receptor (RyR2) Ca\(^{2+}\) release, leading to a smaller SR Ca\(^{2+}\) decay (2a) and a reduction in [Ca\(^{2+}\)]\(_{sr}\) transients (3a). Smaller [Ca\(^{2+}\)]\(_{SR}\) transient promotes a decrease in the activation of Na–Ca exchange (NCX) forward mode (4a). The reduction of Na\(^{+}\) influx through the NCX (4a) leads to a faster action potential (AP) repolarization (5a). B, Alternans promoted by slow RyR2 recovery from inactivation. In this case, an increase in the heart rate does not allow RyR2 to recover from inactivation. A distinct signature of RyR2 behavior is its modal gating.\(^{16}\) This modal gating allows the channel to open in a high open probability (P\(_o\)) mode (HP\(_o\), C\(_{3}\)–C\(_{4}\)–O\(_{1}\)–O\(_{2}\)) or in a low P\(_o\) mode (LP\(_o\), C\(_{3}\)–O\(_{3}\)). For a fast change in the cytosolic [Ca\(^{2+}\)]\(_{SR}\), the probability of visiting the HP\(_o\) is higher than visiting the LP\(_o\). After populating the O\(_2\) state of the HP\(_o\), the channel will decrease its P\(_o\) by visiting a Ca\(^{2+}\)-dependent inactivated state (C\(_{7}\)) or a set of adapted closed states (C\(_{5}\)–C\(_{6}\)). The process of coming back from these closed states (C\(_{5}\), C\(_{6}\), and C\(_{7}\)) defines the intrinsic refractoriness of the RyR2 channel. If the backward pathways (green and yellow lines on the Markovian scheme) that lead to the initial closed states (C\(_{1}\), C\(_{2}\), and C\(_{3}\)) are slow in comparison with the heart rate, the channel will be partially refractory for a new cytosolic Ca\(^{2+}\) stimulus. This refractoriness will finally reduce the mean P\(_o\) of RyR2 and the Ca\(^{2+}\) influx (J\(_{RyR}\)) through the RyR2 (1b). The reduction of the Ca\(^{2+}\) influx from the SR will lead to a smaller [Ca\(^{2+}\)]\(_{SR}\) decay with no changes in the end-diastolic [Ca\(^{2+}\)]\(_{SR}\) (2b). This will subsequently reduce both the amplitude of the cytosolic Ca\(^{2+}\) transient (3b) and the size of the Na\(^{+}\) influx through the NCX (4b) leading to a shorter AP (5b). Finally, if the recovery from inactivation is highly cooperative this can lead to a nonlinear refractoriness that can set a substrate for Ca\(^{2+}\) alternanses. CSQ indicates calsequestrin; and PLN, phospholamban.
is first encroached upon for the development of SR Ca-Alts. Because RyR2 refractoriness has been recognized as an outstanding factor in several cardiomyopathies, the results nicely add to the emergent notion that RyR2 refractoriness is a finely regulated process: deleterious consequences emerge when it is too short or too long. In fact, continuing with the results of Wang et al at fast stimulation rates that drive the heart into ventricular fibrillation, there seems to be a steady-fast reluctance of RyR2s to release Ca$^{2+}$, despite [Ca$^{2+}$]$_{SR}$ being high, that is, RyR2s remain nearly continuously refractory. Thus, a translational value of these results is that, driven to extreme, the persistence absorption of RyR2s into this refractory state may ignite various mechanisms that, in unison, ultimately lead to life-threatening arrhythmias and sudden death. This underscores the importance of defining the molecular underpinnings of RyR2 refractoriness (RyR2 hyperphosphorylation, oxidation, luminal Ca$^{2+}$ desensitization, Ca$^{2+}$-dependent inactivation, etc), which at present are not clearly understood.

The results of Wang et al are illuminating but at the same time humbling because they expose the intricacies of cellular Ca$^{2+}$ handling and the necessity to comprehend the hierarchy of multi-check systems in the control of many Ca$^{2+}$-dependent processes. For instance, low doses of caffeine and $\beta$-adrenergic stimulation are both clearly arrhythmogenic under conditions where RyR2s are trigger-ready (RyR2 gain-of-function mutation) and spontaneously release Ca$^{2+}$ to promote delayed afterdepolarizations, but caffeine and $\beta$-adrenergic stimulation in this study seem to disperse the arrhythmogenic substrate of cardiac alternans by sensitizing RyR2s and rescuing them from their refractory state. This pro- or antiarrhythmic paradox, where an exact same maneuver suppresses one type of arrhythmia while promoting another, stands as the most challenging obstacle for the pharmacological treatment of arrhythmias. In the end, a clear understanding of the multi-faceted nature of cardiac pathologies is key to design rationalized therapies. Returning to the results of Wang et al, they have shown that RyR2 refractoriness is the first mechanism to be encroached upon in the development of cardiac alternans, making it a potential target for intervention, but they have also made clear that cardiac alternans emerge as a continuum of mechanisms where many cellular processes synergize, support, or antagonize each other to produce this macroscopic phenomenon (Figure). From that multi-process viewpoint, if persistent RyR2 refractoriness could be effectively abolished in the face of prolonged cardiac insult, then it is possible that other processes would come to the fore and trigger cardiac alternans. It seems therefore impossible to erect cardiac alternans solely on the legs of RyR2s. Quoting Einstein in his principle of parsimony, “a hypothesis should be kept as simple as possible, but not simpler.” The role of SERCA2a, Na–Ca exchanger, sarcosomal membrane currents, intra-SR Ca$^{2+}$-binding proteins, etc, cannot be disregarded. Finally, these novel results will surely invite the evaluation of RyR2 behavior in in silico frameworks to determine whether the detailed single channel kinetic information already available for RyR2 (Figure) may reproduce the conclusive experimental evidence presented in this article.

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References

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