

Alternans Goes Subcellular A “Disease” of the Ryanodine Receptor?

Burkert Pieske, Jens Kockskämper

In 1872 Traube¹ first described pulsus alternans, a regular beat-to-beat alternation of the strength of the heartbeat. Since then, cardiologists and physiologists have learned that cardiac alternans can come in many flavors: as mechanical, electrical, or $[Ca^{2+}]_i$ transient alternans (Ca^{2+} alternans).² They also had to realize that alternans is a life-threatening condition, less so because of impaired cardiac output but because it can lead to ventricular fibrillation (VF) and sudden cardiac death. How exactly electromechanical alternations of the heartbeat can cause VF has long been an open question. A leap forward came recently with an elegant study on electrical (T-wave) alternans in guinea-pig hearts.³ It was shown that during alternans, neighboring regions within the heart started to alternate out-of-phase with each other (discordant alternans). Such discordant electrical alternans, if sufficient in magnitude, led to unidirectional block and reentry, thereby causing VF.

Ca^{2+} Alternans: The Heart of the Problem

Despite recent advances in our understanding of the mechanisms linking electromechanical alternans to VF, the crucial question still remains: how does alternans develop in the first place? The study of cellular Ca^{2+} alternans might help answer this question because Ca^{2+} alternans lies at the heart of the problem. It causes both mechanical alternans (by activation of the myofilaments) and electrical alternans (by modulation of Ca^{2+} -dependent membrane currents). Experimental interventions aimed at disabling sarcoplasmic reticulum (SR) Ca^{2+} release abolish electromechanical alternans.⁴ Furthermore, enhancement of sarcolemmal Ca^{2+} influx and/or SR Ca^{2+} load and release can reverse alternans.^{5,6} Thus, modulation of the SR Ca^{2+} release process is somehow critically involved in the generation of alternans and only a detailed study of this process can help elucidate the underlying mechanisms.

In the last 20 years, scientists have learned a tremendous deal about the subcellular and even molecular events taking place during SR Ca^{2+} release.⁷ In the heart, Ca^{2+} entering during an action potential (AP) through L-type Ca^{2+} channels (I_{Ca}) triggers the release of Ca^{2+} from the SR via activation of Ca^{2+} release channels or ryanodine receptors (RyRs) (Figure,

Top). This Ca^{2+} -induced Ca^{2+} release (CICR) mechanism is essential for cardiac contraction and, hence, highly regulated. In ventricular myocytes, an AP triggers CICR almost instantaneously and synchronously throughout the entire cell. This is because of the transverse (T) tubular membrane system extending deep into the cell and the close apposition of T tubular and SR membranes containing adjacent Ca^{2+} channels and RyRs, respectively. Synchronicity is important. It ensures a fast upstroke of the global $[Ca^{2+}]_i$ transient and synchronous activation of the myofilaments for efficient contraction of the myocyte (Figure, A). Alterations of this fine-tuned process of CICR may underlie defects of contraction in cardiac disease.^{8,9}

In an article in this issue of *Circulation Research*, Díaz and colleagues¹⁰ pursue the question of what consequences depression of RyR function only might have on the complex process of CICR. Using single rat ventricular myocytes, they used confocal microscopy and a fluorescent Ca^{2+} indicator to image local Ca^{2+} release and simultaneous voltage clamp to control membrane potential and I_{Ca} . In order to depress RyRs, they challenged the myocytes with either the local anesthetic tetracaine or the fatty acid butyrate. The former is a known blocker of RyRs, whereas the latter may inhibit RyRs through intracellular acidosis. Expectedly, tetracaine and butyrate decreased the amplitudes of $[Ca^{2+}]_i$ transients and contractions. Interestingly, however, there was large regional variability in the effects of both substances. Even more surprisingly, some regions started to develop alternans. The subcellular regions exhibiting Ca^{2+} alternans were 10 to 60 μm wide. Neighboring regions could alternate in-phase (Figure, B) or out-of-phase with each other (Figure, C). This adds new complexity to the phenomenon of cardiac alternans: alternans has gone subcellular. During subcellular Ca^{2+} alternans, activation of SR Ca^{2+} release was no longer synchronized. Local $[Ca^{2+}]_i$ transients were biphasic. The first phase was due to some initial Ca^{2+} release, whereas the second phase was caused by miniwaves of Ca^{2+} spreading away from a neighboring initiation site into regions of low $[Ca^{2+}]_i$. Consequently, spatially inhomogeneous subcellular Ca^{2+} alternans caused prolonged and diminished global $[Ca^{2+}]_i$ transients and uncoordinated, inefficient contraction of the myocytes. But how common is this novel form of subcellular Ca^{2+} alternans? A recent study in cat atrial myocytes has made strikingly similar observations on Ca^{2+} alternans, including large spatiotemporal heterogeneities and biphasic local $[Ca^{2+}]_i$ transients caused by delayed Ca^{2+} waves.¹¹ The fact that subcellular alternans occurs in both atrial and ventricular myocytes in two different species and, in addition, that it can be induced by a variety of interventions,^{10,11} may suggest (1) that it is a multifactorial process and (2) that it

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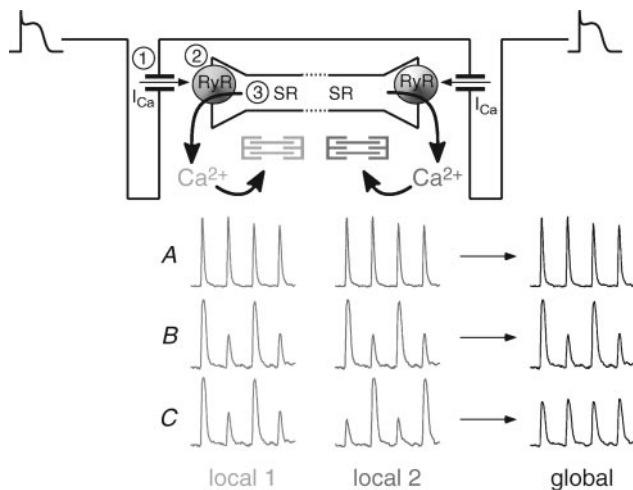
From the Department of Cardiology and Pneumology, University of Göttingen, Germany.

Correspondence to Priv-Doz Dr Burkert Pieske, Dept of Cardiology and Pneumology, University of Göttingen, Robert-Koch-Str 40, 37075 Göttingen, Germany. E-mail pieske@med.uni-goettingen.de

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Top, Ca^{2+} -induced Ca^{2+} release (CICR) in two neighboring dyads in a ventricular myocyte. A, Identical local $[\text{Ca}^{2+}]_i$ transients during normal CICR. B and C, Local $[\text{Ca}^{2+}]_i$ transients during subcellular Ca^{2+} alternans can alternate in-phase (B) or out-of-phase (C). In the former case, global Ca^{2+} alternans develops, whereas in the latter case, global $[\text{Ca}^{2+}]_i$ transients are diminished and prolonged.

may be more common than previously recognized. Further studies, however, will have to clarify this issue. More importantly, it will have to be demonstrated that subcellular Ca^{2+} alternans is also present in the intact heart (not only in isolated cells) and that it is somehow capable of eliciting discordant regional Ca^{2+} alternans of the type recently imaged in ischemic rabbit heart.¹²

What Do We Learn About the Underlying Mechanisms of Alternans?

In addition to revealing exciting new insights into the subcellular features of Ca^{2+} alternans, Díaz and colleagues also offer suggestions as to the underlying mechanisms. They found that global Ca^{2+} influx was unchanged during alternans. Furthermore, global Ca^{2+} efflux was little altered, implying that global SR Ca^{2+} content and release were little changed as well. These results fit well with previous studies indicating that neither I_{Ca} nor SR Ca^{2+} load alternate significantly during alternans.⁶ Because Ca^{2+} alternans is a local phenomenon, it is important to consider the possibility of local (subcellular) rather than global (cellular) alternations of SR Ca^{2+} release. Consequently, the authors calculated local Ca^{2+} fluxes and found that the local loss of Ca^{2+} during alternans was about 2 to 3 times larger than indicated by the global loss. Importantly, however, this local loss of Ca^{2+} was still so small that it was unlikely to account for the large alternations of the local $[\text{Ca}^{2+}]_i$ transients. For their calculations of local Ca^{2+} fluxes Díaz et al¹⁰ used the relationship between the global Na^+ - Ca^{2+} exchange current and the “global” $[\text{Ca}^{2+}]_i$ transient (ie, $[\text{Ca}^{2+}]_i$ averaged over the whole line scan) and, in addition, assumed that the SR network is discontinuous so that neighboring regions of SR would not communicate with each other. In other words, they assumed that there is no redistribution of intraluminal Ca^{2+} between neighboring SR elements. This assumption, although conve-

nient for the calculations, might not hold true. Recent functional studies on local CICR in ventricular myocytes favor a continuous SR network in which redistribution of Ca^{2+} can occur readily.¹³ Intuitively, however, this would suggest that the local loss of Ca^{2+} during alternans might be even smaller than calculated. Clearly, refined models have to test this prediction. Nevertheless, evidence is mounting suggesting that alternations of cellular Ca^{2+} , globally or locally, caused by changes of either I_{Ca} or SR Ca^{2+} content (Figure, Top, ① and ③) are unlikely to be the primary mechanism underlying Ca^{2+} alternans in cardiac myocytes.

This leaves the RyR (Figure, Top, ②) as the prime candidate responsible for the observed alternations of SR Ca^{2+} release during alternans, leading us back to the initial rationale of the study by Díaz et al¹⁰: to depress selectively RyR function and examine the consequences on CICR. But do tetracaine and butyrate depress RyR function selectively? Butyrate, a short chain fatty acid, is certainly not a specific inhibitor of RyRs. Apart from inducing acidosis, it feeds into the mitochondria and may alter metabolism. Moreover, acidosis itself does not depress RyRs selectively but rather has multiple effects on cellular Ca^{2+} handling.⁷ Likewise, tetracaine not only depresses RyRs but also inhibits sarcolemmal ion channels. Under the present experimental conditions (voltage clamp, I_{Na} inactivated, and I_{Ca} constant), however, the latter should not be a factor, and it appears reasonable to assume that tetracaine depresses RyRs rather specifically. This raises the provoking hypothesis that inhibition of RyRs may be sufficient to induce subcellular Ca^{2+} alternans.

RyR as a Potential Therapeutic Target?

It is clear that the incidence of cardiac alternans is increased in cardiovascular disease² and that ischemic and failing myocardium is particularly vulnerable to alternans. The study by Díaz and coworkers in the present issue of *Circulation Research* could indicate that subcellular Ca^{2+} alternans may be a common condition associated with depressed RyR function, present long before electromechanical alternans becomes evident. Besides its potential arrhythmogenicity, subcellular Ca^{2+} alternans, by distorting normal $[\text{Ca}^{2+}]_i$ transients and contractions, may result in futile Ca^{2+} cycling and waste of energy, which may be especially disadvantageous in ischemic or failing myocardium. Indeed, Díaz et al¹⁰ observed diminished and prolonged $[\text{Ca}^{2+}]_i$ transients typical for failing human myocardium.^{14,15} Depression of RyRs may, by spatial and temporal desynchronization of SR Ca^{2+} release, cause Ca^{2+} alternans and contribute to arrhythmias as well as systolic and diastolic contractile dysfunction in cardiac disease. On the other hand, hyperphosphorylation of RyRs in heart failure causes dissociation of FKBP12.6 and increases SR Ca^{2+} leak,¹⁶ whereas overexpression of FKBP12.6 improves SR Ca^{2+} handling by stabilizing RyRs in their closed conformation.¹⁷ Thus, a growing body of evidence implicates defective regulation of RyRs as a cause of dysfunctional Ca^{2+} handling. If this concept is supported by further studies, future therapeutic strategies might be directed towards modulation of the gating behavior of cardiac RyRs.

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