



Published in final edited form as:

*Circ Res.* 2015 February 27; 116(5): 778–780. doi:10.1161/CIRCRESAHA.115.305923.

## Mechanisms of cardiac alternans in atrial cells: intracellular Ca<sup>2+</sup> disturbances lead the way

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### Keywords

atrial fibrillation; cardiac alternans; calcium signaling; myocardium; ryanodine receptor

Cardiac alternans, in the form of microvolt T wave alternans (TW-Alt), is an important clinical sign of heart disease that frequently emerges as a diagnostic manifestation of disease severity in cardiomyopathies such as heart failure, coronary artery disease, genetic and acquired channelopathies, and even in electrolyte disturbances of the body.<sup>1</sup> During cardiac alternans, there is a beat-to-beat oscillation between strong-weak contractions, and although the heart keeps a regular pace, cardiac alternans ultimately spawns lethal arrhythmias, serving as a valuable risk stratification factor for sudden cardiac death and helping guide arrhythmia treatment.<sup>2</sup>

At the cellular level, cardiac alternans manifests as a cyclic, simultaneous alternation of three of the fundamental events of cardiomyocyte physiology: action potential duration (APD), intracellular Ca<sup>2+</sup> transient (CaT) amplitude, and contraction amplitude. Because action potentials trigger cytosolic CaTs, and CaTs in turn influence the amplitude and duration of the action potentials through various Ca<sup>2+</sup>-dependent ionic currents, in this inescapable bidirectional coupling there is barely an instance in which APD alternans becomes dissociated from CaT alternans and in reality, one faithfully follows the other. But which one has the primary role? The question appears of fundamental importance if one is to understand the hierarchical role of distinct cellular processes and target the roots of this arrhythmogenic event through rationalized therapy.

The prominence of membrane currents in the genesis of cardiac alternans was demonstrated as early as 1968 by Nolasco & Dahlen<sup>3</sup> in their classical experiments of APD restitution. They observed that in electrically paced frog ventricular muscle strips, APD shortened as they accelerated the pacing rate. This phenomenon appeals to logic because the cardiac cycle length decreases considerably during increased metabolic demand, and it must do so by means of shortening both, APD and diastolic interval (DI). Typically, DI has a higher dynamic range because during a 1-s cardiac cycle it lasts ~700 ms (versus ~300 ms for

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*Conflict of interest statement:* HHV declares that he has no conflict of interest.

APD), giving the heart sufficient time to refill the ventricles with oxygenated blood before the next beat. If DI is longer than APD, then membrane currents that require transit through inactivated or refractory states, most notably L-type  $\text{Ca}^{2+}$  channels (LTCC) and voltage-dependent  $\text{Na}^+$  channels, have time to recover and get primed for full activation in the next cycle, effectively allowing for complete restitution of the APD. However, if DI shortens to the point of being briefer than APD, then incomplete recovery of membrane currents from inactivation can occur, providing a substrate for instabilities in AP dynamics. Thus, a linear relationship between APD and DI may be built, the slope of which (APD restitution) is lower than 1 for cells that are dynamically stable; that is, displaying uniform APD. On the other hand, if the slope steepens and is experimentally forced to be greater than 1, as in Nolasco & Dahlen,<sup>3</sup> then APD can oscillate in a short-long pattern on a beat-to-beat basis, giving rise to APD alternans. Hence, we can derive from these experiments an unequivocal participation of APD restitution in the genesis of cardiac alternans, but since APD is inextricably linked to CaT dynamics (see below), CaT alternans quickly follows APD alternans.

A strong case for CaT disorders being the primary force underlying cardiac alternans may be derived from physiological settings and the more common observation that APD alternans starts to develop *before* the DI becomes shorter than APD,<sup>4,5</sup> i.e. the slope of APD restitution is lower than 1 in most cases. DI lasts longer than the time that is presumably needed to re-prime all inactivated membrane currents. It follows therefore that the observed APD alternans must trail CaT disturbances (this is even more pronounced in pathological settings with prominent intracellular  $\text{Ca}^{2+}$  mishandling such as heart failure,<sup>6</sup> and underscores the power of cardiac alternans as an index of disease severity). Even more compelling is the fact that APD alternans quickly vanishes in ryanodine-treated cells,<sup>5,7</sup> which have negligible sarcoplasmic reticulum (SR)  $\text{Ca}^{2+}$  load and thus exhibit low-amplitude CaTs despite normal  $\text{Ca}^{2+}$  entry. That APD alternans disappears in the absence of intracellular  $\text{Ca}^{2+}$  release would be unexpected if the former originated from processes devised by, completely contained in, and inherent to, the electrical properties of the membrane. However, this observation is possible in cells where the information between membrane voltage ( $V_m$ ) and intracellular  $\text{Ca}^{2+}$  ( $[\text{Ca}^{2+}]_i$ ) flows both ways, and both processes are intertwined so radically that modifications to one inevitably affect the other. Thus, we are back to the original question because therein, in the bidirectional coupling between  $V_m$  and  $[\text{Ca}^{2+}]_i$  ( $V_m \leftrightarrow [\text{Ca}^{2+}]_i$ ), lies the classical conundrum of electromechanical alternans: does CaT alternans lead or lag APD alternans?

In this issue of *Circulation Research*, Kanaporis and Blatter<sup>8</sup> performed elegant experiments in isolated rabbit atrial and ventricular myocytes to determine whether failure of CaT regulation or disturbance in AP modulation is the primary instigator of cardiac alternans. As discussed in the preceding sections, the question has been addressed before in several experimental and computer modeling settings (for comprehensive reviews, see<sup>9-12</sup>), and although each study has taken the issue a step ahead, there are still nagging controversies, reflecting the complexity of the subject. Kanaporis & Blatter<sup>8</sup> used a clever approach wherein they first induced alternans by pacing cells at a progressively faster frequency, captured the AP waveform during the elicited APD alternans, and then applied identical

APD oscillations at various pacing rates to measure intracellular CaT dynamics. Besides determining the precise sequential order (if any) at which APD or CaT alternans first appeared, these technically-demanding experiments allowed them to test whether CaT alternans was “enslaved” to APD alternans, as would be indicated if a low-amplitude CaT alternans dependably proceeded from a short-duration APD alternans, and *vice versa*, a high-amplitude CaT alternans would be followed by a long-duration APD alternans. This would hint, albeit not exclusively, that LTCC current, which intervenes during the plateau of the AP and thus greatly influences APD, is a critical determinant of CaT amplitude by the classical mechanism of Ca<sup>2+</sup>-induced Ca<sup>2+</sup> release (CICR). However, LTCC amplitude was identical and inactivation only modestly prolonged, in low-amplitude CaT alternans,<sup>8</sup> suggesting that the feedback between LTCC currents and CICR plays a secondary role in the bidirectional  $V_m \leftrightarrow [Ca^{2+}]_i$  coupling of atrial and ventricular cells. Instead, Kanaporis & Blatter<sup>8</sup> found that CaT alternans can emerge even in the absence of APD alternans (by constant shape APs) and that AP clamp protocols in the form of APD alternans do not necessarily lead to CaT alternans. Moreover, when APD alternans did elicit CaT alternans, the amplitude of the latter could be completely divorced from the duration of the leading APD alternans, i.e., a high-amplitude or a low-amplitude CaT alternans could arbitrarily accompany a short APD alternans. Finally, APD alternans was shown to be dependent on CaT alternans in cells in which SR Ca<sup>2+</sup> release was suppressed by ryanodine.<sup>8</sup> Altogether, the results indicate that a CaT alternans can have a life of its own and take precedence as causative link to APD alternans.

To be real, the novelty of the experiments of Kanaporis & Blatter<sup>8</sup> resides in the analysis of alternans in atrial cells, which is newer, and their systematic comparison with those in ventricular cells, which stand on a more beaten path. Determining the mechanisms of alternans in atrial cells is timely now that atrial fibrillation has emerged as a disease of epidemic proportions.<sup>13</sup> Atrial cells are similar to ventricular cells in the three fundamental events of cardiomyocyte physiology, but finer structural and functional attributes may presage differences in the onset, intensity and duration of alternans between these two cell types. T-tubules are poorly organized or conspicuously absent in atrial cells,<sup>14</sup> and SERCA activity is higher due to lower expression of phospholamban.<sup>15</sup> These differences are expected to decrease the coupling efficiency between Ca<sup>2+</sup> release channels/ryanodine receptors (RyRs) and LTCCs and to refill the SR more rapidly, both of which should impinge profoundly on the aforementioned  $V_m \leftrightarrow [Ca^{2+}]_i$  coupling. In the experimental setting of Kanaporis & Blatter<sup>8</sup> atrial cells displayed more pronounced APD alternans than ventricular cells, and the latter exhibited lower alternans-induction threshold than the former, among the most notable variances. However, from a general perspective, differences were surprisingly minimal and incommensurable with the purportedly remarkable distinguishing characteristics of these two cell types, namely, rudimentary T-tubules, lower excitation-contraction coupling efficiency, and “signature” membrane currents in atrial cells ( $K_{ACh}$ , ultra-rapid rectifier  $K^+$  channels, higher density of small-conductance Ca<sup>2+</sup>-sensitive  $K^+$  channel, etc.). These subtle differences also do not seem to honor in full the dedicated computer models that predict significant differences in mechanisms of alternans in atrial cells.<sup>16</sup> Or perhaps the cellular experimental system of Kanaporis & Blatter<sup>8</sup> is too reduced to uncover tissue-level properties that may enhance the structural and functional disparities

between atrial and ventricular cells. However, it is remarkable that even at this level of integration, both cell types maintain a predictable, stereotyped pattern of alternans. This suggests that the mechanisms underlying cardiac alternans are primal to both cardiac cell types and that alternans is a multifactorial process that cannot be gleaned by addition or subtraction of unique constituents. Rather, alternans is quite possibly an emergent property of cardiomyocyte physiology where the whole is greater than the sum of the parts and where segregation of individual components deprives the cell of the integral phenomenon. Thus, the original question regarding the preponderance of  $V_m$  or  $[Ca^{2+}]_i$  as primary drivers of cardiac alternans, be it applied to atrial or ventricular cells, is perhaps an unrealistic one, and the relevant question has to lead to a comprehensive understanding of each and all of the molecular, subcellular and cellular processes involved, their timing and sequential contribution, their distinct hierarchical role, and their complex dynamic interplay. Even in the experiments of Kaporis & Blatter,<sup>8</sup> where CaT alternans could be dissociated from APD alternans, the latter inexorably followed the former, and the dissociation between the two was as fragile as elaborate was the experimental approach needed to separate them. In physiological and pathophysiological settings, APs are not clamped but free-running processes unencumbered from experimental manipulation, yet closely linked to  $[Ca^{2+}]_i$  dynamics. It is therefore expected that for every APD alternans detected as microvolt T-wave alternans in the ECG, a CaT alternans quietly lurks underneath.

Where do the results of Kaporis & Blatter<sup>8</sup> leave us? Although their study of atrial cells did not reveal radically new mechanisms of cardiac alternans, it did reaffirm the key role of bidirectional coupling between  $V_m$  and  $[Ca^{2+}]_i$  as causative factor and boosted the leading position of  $[Ca^{2+}]_i$  disturbances in electromechanical alternans. The exact mechanisms by which the CaT oscillates first were not addressed in this study, but given that LTCC current failed to alternate substantially during CaT alternans, they most likely point to altered SR behavior, including RyR refractoriness<sup>17,18</sup> and variable  $[Ca^{2+}]_{SR}$  load,<sup>19</sup> among the most prominent ones. Nevertheless, this study already illuminates mechanisms ripe for exploration: given the remarkable similitude between atrial and ventricular alternans found here, it is tempting to speculate that the preeminent role of RyR refractoriness recently uncovered in ventricular layers of intact hearts<sup>18</sup> may play an equally critical role in atrial tissue.

## Acknowledgments

*Sources of funding:* HHV is a recipient of National Institutes of Health grants RO1-HL055438, RO1-HL108175, and RO1-HL120108.

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