Discordant Calcium Transient and Action Potential Alternans in a Canine Left-Ventricular Myocyte

The MIT Faculty has made this article openly available. Please share how this access benefits you. Your story matters.

<table>
<thead>
<tr>
<th>Citation</th>
<th>Armoundas, A.A. “Discordant Calcium Transient and Action Potential Alternans in a Canine Left-Ventricular Myocyte.” Biomedical Engineering, IEEE Transactions on 56.9 (2009): 2340-2344. © 2009 Institute of Electrical and Electronics Engineers</th>
</tr>
</thead>
<tbody>
<tr>
<td>As Published</td>
<td><a href="http://dx.doi.org/10.1109/tbme.2009.2023671">http://dx.doi.org/10.1109/tbme.2009.2023671</a></td>
</tr>
<tr>
<td>Publisher</td>
<td>Institute of Electrical and Electronics Engineers</td>
</tr>
<tr>
<td>Version</td>
<td>Final published version</td>
</tr>
<tr>
<td>Accessed</td>
<td>Thu Dec 06 06:14:43 EST 2018</td>
</tr>
<tr>
<td>Citable Link</td>
<td><a href="http://hdl.handle.net/1721.1/52424">http://hdl.handle.net/1721.1/52424</a></td>
</tr>
<tr>
<td>Terms of Use</td>
<td>Article is made available in accordance with the publisher’s policy and may be subject to US copyright law. Please refer to the publisher’s site for terms of use.</td>
</tr>
<tr>
<td>Detailed Terms</td>
<td></td>
</tr>
</tbody>
</table>
Discordant CalciumTransient and Action Potential Alternans in a Canine Left-Ventricular Myocyte

Antonis A. Armoundas, Senior Member, IEEE

Abstract—Electrocardiographic alternans is known to predispose to increased susceptibility to life-threatening arrhythmias and sudden cardiac death. While this decreased level of cardiac electrical stability is often due to the presence of discordant action potential (AP) alternans in the heart, the mechanism of discordant cardiac alternans remains unknown. This study presents a case report of cellular discordant cardiac alternans between AP and [Ca\(^{2+}\)], and employs a novel reverse engineering approach that applies a simultaneous AP and [Ca\(^{2+}\)] clamp of experimentally obtained data to a left-ventricular canine myocyte model, to probe its underlying mechanism. The model results indicate that during alternans, the increased sarcoplasmic reticulum Ca\(^{2+}\), triggers multiple ryanodine receptor (RyR) channel openings and delayed Ca\(^{2+}\) release, which subsequently triggers an inward depolarizing current, a subthreshold early after-depolarization, and AP prolongation. The amplitude of [Ca\(^{2+}\)], plays a critical role in defining the discordant or concordant relationship between the [Ca\(^{2+}\)], and AP at the myocyte level. In conclusion, the results presented in this study support the idea that aberrant RyR openings on alternate beats are responsible for the [Ca\(^{2+}\)]-type oscillations, which, in turn, give rise to an in- or out-of-phase relationship between [Ca\(^{2+}\)], and AP alternans.

Index Terms—Cellular alternans, model, myocyte, ryanodine receptor (RyR), sarcoplasmic reticulum (SR).

I. INTRODUCTION

T-WAVE alternans has been associated with an increased risk to arrhythmias [1] and sudden cardiac death (SCD) [2], [3]. However, at the myocyte level, it is still unclear what triggers action potential (AP) alternans. It was recently shown that the morphology of the AP (through its modulation by sarcolemmal Ca\(^{2+}\) [4] and K\(^+\) [5], [6] currents) has a significant effect on the stability of the Ca\(^{2+}\) handling processes and the transition from normal intracellular Ca\(^{2+}\) ([Ca\(^{2+}\)]), to stable [Ca\(^{2+}\)] alternans [7]. However, other studies have suggested that [Ca\(^{2+}\)], alternans give rise to AP alternans [7]–[15]; thus, according to this hypothesis, [Ca\(^{2+}\)], alternans resulting from stress-induced [1], [8] deficiencies in any number of Ca\(^{2+}\) transport processes may, in turn, give rise to AP alternans. Irrespective of the proposed hypothesis, common characteristic of all studies was that the [Ca\(^{2+}\)], and AP oscillated in phase (concordant alternans; for definitions, see Section II).

This study presents the first report of discordant (out of phase) [Ca\(^{2+}\)], and AP alternans at the myocyte level and employs a novel hybrid experimental–computational approach to probe the mechanisms underlying the relationship between [Ca\(^{2+}\)], and AP alternans.

II. METHODS

A. Definitions

Concordant and discordant alternans at the whole heart as well as the myocyte level are defined as follows.

1) Large/small [Ca\(^{2+}\)], during alternans, refers to [Ca\(^{2+}\)], amplitude that is larger/smaller than its immediately preceding [Ca\(^{2+}\)].

2) Long/short AP duration (APD) during alternans, refers to APD that is longer/shorter than its immediately preceding APD.

3) Concordant APD alternans between two areas in the heart refer to APD oscillations that are in-phase, i.e., both areas exhibit either long or short APDs.

4) Discordant APD alternans between two areas in the heart refer to APD oscillations that are out-of-phase, i.e., one area exhibits long APDs, while the other exhibits short APDs.

5) Concordant alternans between [Ca\(^{2+}\)], and AP at the single myocyte refer to oscillations of these signals that are in-phase, i.e., a large [Ca\(^{2+}\)], corresponds to a long APD and vice versa.

6) Discordant alternans between [Ca\(^{2+}\)], and AP at the single myocyte refer to oscillations of these signals that are out-of-phase, i.e., a large [Ca\(^{2+}\)], corresponds to a short APD and vice versa.

B. Myocyte Isolation and Electrophysiological Studies

A canine-isolated left-ventricular myocyte [15] was whole-cell patch-clamped at 37 °C in a heated chamber on the stage of an inverted fluorescence microscope (Olympus IX70). Borosilicate glass pipettes of 3–5 MΩ tip resistance were used for whole-cell recording of APs or membrane currents with an Axopatch 200B amplifier digitized via a Digidata 1200A (Axon Instruments) personal computer interface.

A xenon arc lamp was used to excite indo-1 fluorescence at 365 nm (390 nm dichroic mirror), and the emitted fluorescence was recorded using a dual-channel photomultiplier tube assembly (ESP associates, Toronto, ON, Canada) at wavelengths of 405 and 495 nm. Cellular autofluorescence at both emission

Manuscript received December 14, 2008; revised February 17, 2009. First published June 2, 2009; current version published August 14, 2009. This work was supported by the American Heart Association awards under Beginning Grant-in-Aid 0365304U and under Scientist Development Grant 0635127N.

The author is with the Cardiovascular Research Center, Massachusetts General Hospital, Boston, MA 02129 USA, and also with Massachusetts Institute of Technology, Cambridge, MA 02139 USA (e-mail: armoundas@partners.org).

Digital Object Identifier 10.1109/TBME.2009.2023671
wavelengths was recorded before rupturing the cell-attached patch. The ratio of indo-1 fluorescence \( R = \frac{F_{405 \text{ nm}}}{F_{495 \text{ nm}}} \) was determined after subtraction of cellular autofluorescence, and was used to calculate free intracellular \( \text{Ca}^{2+} \) according to the equation \( [\text{Ca}^{2+}]_i = K_d \beta ([R - R_{\text{min}}]/(R_{\text{max}} - R)) \), using a \( K_d \) of 844 nmol/L [16]. The \( R_{\text{min}}, R_{\text{max}}, \) and \( \beta \) for the fluorescence system were determined to be 0.45, 2.4, and 3.8, respectively [16]. Electrophysiological and fluorescence signals were acquired simultaneously and analyzed offline.

The myocyte was patched using physiological extracellular solution containing (in millimoles per liter): NaCl 138, KCl 4, MgCl\(_2\) 1, CaCl\(_2\) 2, NaH\(_2\)PO\(_4\) 0.33, glucose 10, and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, (HEPES) 10; pH 7.4 with NaOH, and intracellular solution containing (in millimoles per liter): potassium glutamate 130, KCl 10, MgCl\(_2\) 0.5, MgATP 5, and HEPES 10; pH 7.2 with KOH and 50 \( \mu \text{mol/L} \) indo-1 (Molecular Probes). The pipette-to-bath liquid junction potential was \(-17\) mV, and was corrected.

The myocyte was stimulated in current clamp at progressively faster frequencies until alternans was elicited.

C. Canine Left-Ventricular Myocyte Model

A simultaneous \( [\text{Ca}^{2+}]_i \) and AP-clamp variation of the canine left-ventricular myocyte computer model [15]–[18] was developed, which permitted to input experimentally obtained records of \( [\text{Ca}^{2+}]_i \), and AP as a driving function, and compute, in isolation, the intracellular compartments’ \( \text{Ca}^{2+} \) concentration and AP alternans leads to the equation of the beat-to-beat fluctuations of the correspond-

III. RESULTS

A. Concordant and Discordant \( [\text{Ca}^{2+}]_i \) and AP Alternans

Fig. 1(a) presents sustained concordant (in phase) \( [\text{Ca}^{2+}]_i \) and AP alternans recorded from an epicardial left-ventricular myocyte stimulated every 0.8 s. After some period of sustained concordant \( [\text{Ca}^{2+}]_i \), and AP alternans in the same data record, in Fig. 1(b), one notices examples of discordant \( [\text{Ca}^{2+}]_i \) and AP alternans; specifically, one observes that the in-phase relationship between \( [\text{Ca}^{2+}]_i \) and AP is altered at point (a) to become discordant (out-of-phase), becomes again concurrent at point (b), changes to discordant at point (c), and finally, becomes concurrent at (d).

To investigate the complex interplay between the sarco-

Fig. 2(b), the left axis presents the time-dependent beat-to-beat current attributed to both the \( L \)-type calcium channel (LTCC) \((I_{\text{Ca},L})\) and the Na/Ca exchanger \((I_{\text{NCX}})\), while the right axis presents the ryanodine receptor (RyR) open probability of the same beats presented in Fig. 1(b); in this figure, the choice of lumping the two currents is based on the hypothesis that if \( [\text{Ca}^{2+}]_i \) and AP alternans are linked, it is likely that the balance (reflected in the sum) of these two currents that control the depolarization versus repolarization of the membrane, will determine their effect on the APD.

One observes that the APD prolongation observed in Fig. 2(a) is associated with the larger inward depolarizing current attributed to the LTCC and the NCX seen in Fig. 2(b); this depolarizing current is a result of secondary, much smaller SR \( \text{Ca}^{2+} \) release events, reflected at the RyR state-1 open probability \( P_{\text{O1}} \) [see Fig. 2(b)] on an every other beat basis indicated by an “*,” while the primary SR \( \text{Ca}^{2+} \) release events are represented by an “+.” This depolarizing current is associated with a small deflection on the AP [also seen after careful inspection in Fig. 1(b)],
which is a subthreshold early after-depolarization (sEAD). This secondary Ca$^{2+}$ release also results in a longer time for [Ca$^{2+}$], to reach its peak value, and is seen only in beats associated with a large [Ca$^{2+}$], that is on every other beat basis [see Fig. 2(b)].

Significantly, in Fig. 2(b), after sustained concordant alternans [seen in Fig. 1(b)] between the ∆[Ca$^{2+}$], and APD in which a large/small (indicated as A/B) ∆[Ca$^{2+}$], corresponds to a long/short (indicated as A/B) APD, in beat 3, one observes that a phase reversal between ∆[Ca$^{2+}$], and APD occurs (i.e., a small ∆[Ca$^{2+}$], is associated with a long APD). It is likely that what causes the phase reversal between [Ca$^{2+}$], and APD is the ∆[Ca$^{2+}$], amplitude, which in this beat is larger than the amplitude of the previous small ∆[Ca$^{2+}$], and is smaller than the previous large ∆[Ca$^{2+}$], [see Fig. 1(b), and also Fig. 3(a) and (b)]; ∆[Ca$^{2+}$], influences the balance of the depolarizing and repolarizing currents through the LTCC and NCX, and their effect on APD.

In beat 4, one observes that the ∆[Ca$^{2+}$], and APD continue to be out of phase, in which case, a large ∆[Ca$^{2+}$], coincides with a short APD. In beats 5 and 6, one sees that the ∆[Ca$^{2+}$], and APD are again in-phase, but because ∆[Ca$^{2+}$], is still within a critical range (see Fig. 3(a) and (b) later) between a small and a large ∆[Ca$^{2+}$], (i.e., of the first, eighth, ninth, and tenth beat), the APD can be either short or long, which is likely to result in a phase reversal between ∆[Ca$^{2+}$], and APD. Indeed, in beat 7, there is a phase reversal between ∆[Ca$^{2+}$], and APD, that is a large ∆[Ca$^{2+}$], (larger than that in beat 6), corresponds to a short APD.

Thereafter, in beat 8, phase reversal again results in in-phase alternans of ∆[Ca$^{2+}$], and APD, which is maintained in subsequent beats.

Since reduced SR Ca$^{2+}$ uptake alone could lead to smaller Ca$^{2+}$ release and [Ca$^{2+}$], the beat-to-beat ∆[Ca$^{2+}$],SR (defined as the diastolic minus the systolic [Ca$^{2+}$],SR oscillations have been quantified from the computer-generated data (using the same simultaneous AP- and [Ca$^{2+}$],SR clamp approach). It was observed that ∆[Ca$^{2+}$],SR alternated between beats corresponding to small and large [Ca$^{2+}$], during alternans [see Fig. 2(c)]; however, ∆[Ca$^{2+}$],SR was almost constant in beats corresponding to nonchanging [Ca$^{2+}$].

B. Relationship of Beat-to-Beat [Ca$^{2+}$], and APD During Alternans

Analysis of the beat-to-beat ∆[Ca$^{2+}$], and APD for the whole-data record revealed that discordant alternans occurred four times [red pixels in Fig. 3(a) and (b)]. Interestingly, similarly to the example presented in Fig. 1(b), all four phase transitions of concordant to discordant alternans were transient, which resulted into discordant alternans to be reverted back to sustained concordant alternans, within a few beats.

Fig. 3(b) which presents the relationship between the ∆[Ca$^{2+}$], and APD. One observes that phase reversal occurs for APD values that fall within ∆[Ca$^{2+}$],SR ≤ 84%, where APD$\text{rev}$ is the APD value in which a phase reversal occurs, and APD$\text{max}$ is the maximum APD value in the APD time series. APD$\text{min}$ is the minimum APD value in the APD time series, and ∆[Ca$^{2+}$],SR is the maximum ∆[Ca$^{2+}$], value in which a phase reversal occurs. ∆[Ca$^{2+}$],SR is the minimum ∆[Ca$^{2+}$], value in the ∆[Ca$^{2+}$], time series, and ∆[Ca$^{2+}$],SR is the maximum ∆[Ca$^{2+}$], value in the ∆[Ca$^{2+}$], time series.

Fig. 3. Presence of phase transitions between [Ca$^{2+}$]i and AP (red pixels) in experimentally obtained ∆[Ca$^{2+}$], and APD time series. (a) The relationship between the APD and ∆[Ca$^{2+}$]i (systolic minus diastolic [Ca$^{2+}$]i), which indicates that phase reversal occurs when the APD falls within a small range of its minimum and maximum values (113% ≤ APD$\text{rev}$/APD$\text{min}$ ≤ 117% and 81% ≤ APD$\text{rev}$/APD$\text{max}$ ≤ 84%). (b) Relationship between the ∆[Ca$^{2+}$], of the (N + 1)st-beat versus the ∆[Ca$^{2+}$], of the (N)th-beat, indicates that phase reversal occurs when ∆[Ca$^{2+}$], falls within a small range of its minimum and maximum values of the ∆[Ca$^{2+}$], time series.
The apparent inter-dependence of the shape of the AP waveform on the NCX which contributes either a depolarizing or repolarizing current during the AP and the LTCC that contributes a depolarizing current. Since both the NCX and LTCC are directly mediated by \([Ca^{2+}]_i\), a large calcium transient (in black) first causes the NCX to reverse earlier, thus contributing a smaller repolarizing current, and then, contributing to AP prolongation, and second causes acceleration of the LTCC \(Ca^{2+}\)-mediated inactivation that causes AP shortening; one expects the opposite results for a small calcium transient (in red). Thus, the effect of \([Ca^{2+}]_i\), on the membrane potential is defined by the net balance of these two currents.

In summary, \(SR Ca^{2+}\) overload that results in spontaneous \(SR Ca^{2+}\) release and stimulates additional \(Ca^{2+}\) extrusion via the NCX which in turn produces an inward, depolarizing current and sub-threshold triggered depolarizations, are the underlying events for \([Ca^{2+}]_i\), and AP alternans. Furthermore, the finding that the \([Ca^{2+}]_i\), and APD can oscillate in an uncorrelated manner is likely to constitute the ventricular myocyte as the smallest unit underlying cardiac alternans and increased susceptibility to arrhythmogene

### References


