THERMODYNAMICS OF MYOPLASMIC CA^{2+} ALTERNANS REVEAL THE MOLECULAR MECHANISMS INVOLVED IN ITS GENESIS.

Maedeh Bazmi[1], Julio A. Copello[2] and Ariel L Escobar[3#]

[1]Quantitative Systems Biology Program, School of Natural Sciences, University of California, Merced, CA.
[2]Department of Pharmacology, School of Medicine, University of Southern Illinois, Springfield. IL.
[3]Department of Bioengineering, School of Engineering, University of California, Merced, CA.

[#]**Correspondence to:** aescobar4@ucmerced.edu

ABSTRACT

Myoplasmic Ca^{2+} alternans commonly occur during conditions such as tachycardia, ischemia, or hypothermia. This is a serious condition that can lead to sudden cardiac death. Myoplasmic Ca^{2+} alternans are alternating beat-to-beat changes in the amplitude of the Ca^{2+} transient. They typically arise from a variation in the amount of Ca^{2+} released from the sarcoplasmic reticulum (SR) between two consecutive heartbeats. This variability in the release of Ca^{2+} has previously been attributed to a delay in the recovery of the ryanodine receptor (RyR2), an incomplete Ca^{2+} refilling of the SR, or a change in the duration of the action potential. In each case, the RyR2 will mobilize Ca^{2+} from the SR in an alternating manner, thus generating Ca^{2+} alternans. To investigate the myoplasmic Ca^{2+} alternans in more depth, we utilized a novel experimental approach, Fluorescence Local Field Optical Mapping (FLOM), to record at the epicardial layer of an intact heart with subcellular resolution. These recordings were collected in conjunction with a local cold finger, where a temperature gradient was locally imposed on the tissue. In the colder regions, Ca^{2+} alternans were larger and occurred without changes in the duration of the action potential duration. Upon analyzing changes in the Q_{10} of several kinetic processes defining the intracellular Ca^{2+} dynamics, we found the imposed temperature gradient to have a significant effect on the relaxation of intracellular Ca^{2+} transients. The precipitous temperature dependency of Ca^{2+} alternans observed suggests they arise from an insufficient Ca^{2+} uptake into the SR by the ATPase of SR (SERCA2a). Interestingly, we found Ca^{2+} alternans to be heavily dependent on the SR Ca^{2+} and could be fostered with increased heart rate, which decreased the time for SERCA2a reuptake into SR beat to beat. Similarly, the partial pharmacological inhibition of SERCA2a with Thapsigargin increased the amplitude of myoplasmic Ca^{2+} alternans. Finally, the FLOM experimental approach is a valuable technique that can shed light on how arrhythmogenesis correlates with the spatial distribution of metabolically impaired myocytes along the myocardium.

Keywords: Myoplasmic Ca^{2+} alternans, FLOM, temperature dependency of Ca^{2+} transport mechanisms.

RESUMEN

Las alternancias de Ca^{2+} en el mioplasma suelen ocurrir durante una taquicardia, isquemia o hipotermia. Esta condición fisiopatológica puede desencadenar una muerte súbita. Las alternancias de Ca^{2+} mioplásmicas se expresan como un cambio latido a latido en la amplitud del Ca^{2+} sistólico. Estos cambios cíclicos en la amplitud del Ca^{2+} sistólico provienen de variaciones en la liberación de Ca^{2+} desde el retículo sarcoplasmático (RS) entre dos latidos consecutivos. Esta variabilidad en la liberación de Ca^{2+} desde el RS ha sido asociados con una incompleta recuperación del estado inactivado del receptor a rianodina tipo 2 (RyR2), un rellenado incompleto del RS o cambios en la duración del potencial de acción. En cualquier caso, el RyR_2 moviliza Ca^{2+} de manera alternadas, generando alternancias mioplásmicas de Ca^{2+}. Con la intención de estudiar las alternancias mioplásmicas de Ca^{2+} con mayor profundidad, hemos desarrollado una nueva aproximación experimental, el Mapeo Óptico Fluorescente de Campo Local (FLOM, por sus siglas en ingles). Esta técnica nos ha permitido registrar en la capa epicárdica del ventrículo izquierdo imágenes fluorescentes en un corazón intacto con resolución subcelular. Estas imágenes han sido obtenidas en conjunción con un dedo frio, que permite generar un gradiente de temperatura en el tejido. En las regiones más frías las alternancias mioplásmicas de Ca^{2+} son mayores y ocurren en ausencia de cambios en la duración del potencial de acción. Analizando el Q_{10} de algunos procesos cinéticos que determinan la dinámica de Ca^{2+} intracelular, encontramos que los gradientes de temperatura afectan la relajación de los transitorios de Ca^{2+}. Interesantemente, encontramos que la dependencia de temperatura de las alternancias mioplásmicas de Ca^{2+} provienen de una recaptura insuficiente de Ca^{2+} hacia el SR mediada por la bomba de Ca^{2+} SERCa2. Aún más, la inhibición de la bomba de Ca^{2+} con Thapsigargina ha mostrado que si la capacidad de transporte de Ca^{2+} hacia el RS es menor, la amplitud de las alternancias mioplásmicas de Ca^{2+} se incrementan. Finalmente, el desarrollo de FLOM como una nueva aproximación experimental nos ha permitido dilucidad como la arritmogénesis está correlacionada con la distribución espacial de disfunciones metabólicas en miocitos en el miocardio.

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Introduction

Cardiac alternans are described as beat-to-beat changes in the mechanical and electrical activity of the heart. Traub first described alternans in 1872 [1], as a strong contraction followed by a weak contraction. It was not until the invention of the electrocardiogram by Einthoven in 1903 [2] that the electrical alternans could be identified and further described by Lewis in 1910 [3]. Since then, it has been accepted that patients displaying with mechanical or electrical cardiac alternans exhibit very poor and unfavorable medical prognoses. Electrical alternans are usually reflected by the alternations in the T-wave of the EKG [4,5], but can also be present in the QRS complex [4, 6]. Currently, T-wave alternans are usually analyzed in microvolts as alternations in the amplitude of the T-wave[6].

Independently of the type of alternans, mechanical or electrical, it is widely accepted they occur as a consequence of beat-to-beat changes in the amount of Ca^{2+} released from the sarcoplasmic reticulum (SR). Different groups have postulated different mechanisms underlying this alternation in Ca^{2+} release. Ripplinger's laboratory [7] postulated alternans in the SR Ca^{2+} release to be produced by an incomplete recovery from inactivation of the ryanodine receptor (RyR2). On the other hand, our group postulated these alternans occur because of an incomplete Ca^{2+} refilling of the organelle under tachycardic conditions [8, 9, 10]. This review is concentrated on determining which hypothesis best describes the main cause of this Ca^{2+} alternans.

Our lab previously demonstrated knocking down calsequestrin in animals nearly abolished alternans [10]. Indeed, calsequestrin has two functions; first, it is the main Ca^{2+} buffering protein within the SR. Second, in conditions where the intra SR Ca^{2+} content has been diminished, it produces the inhibition of the RyR2 through two proteins: Triadin and Junctin [12]. Moreover, in a recent paper published in the Journal of General Physiology [12], our group demonstrated the temperature dependency of Ca^{2+} alternans is very similar to the uptake of Ca^{2+} by the SERCa2 pump into the SR. Moreover, if SERCa2 was partially inhibited by Thapsigargin, the amplitude of Ca^{2+} alternans was observed to dramatically increase.

One central question is how mechanical Ca^{2+} alternans is transduced as electrical alternans. Several groups have postulated different mechanisms, including Ca^{2+} dependent inactivation of the L-type Ca^{2+} channels[13, 14], activation of Ca^{2+} activated chloride channels[15, 16], or the activation of the Na^{+}-Ca^{2+} exchanger in its forward mode [17, 18]. In the two first cases, a larger Ca^{2+} release from the SR will induce a shorter action potential. In any case, if the Na^{+}-Ca^{2+} exchanger is also activated in the forward mode, the action potential will be longer, leading to a larger Ca^{2+} release.

Independently of the mechanisms by which Ca^{2+} alternates are transduced into electrical alternans to have T-wave alternans, there must be regions on the ventricular wall that alternate before others. The Ca^{2+} reuptake to the SR is less efficient in the endocardium compared to the epicardium. This is due to a significantly lower expression of SERCa2 in the endocardium than that of the epicardium [19, 20]. Moreover, the expression of phospholamban does not change between the endocardium and epicardium [19, 20]. This indicates the fraction of SERCa2 inhibited in the endocardium is larger than in the epicardium, suggesting electrical alternans occur first in the endocardium then move to the epicardium [18, 19]. As the T-wave reflects the difference in repolarization time between the endocardium and the epicardium, this indicates electrical alternans are induced by an alternating behavior of the endocardium.

Results

Figure 1 A-D illustrates the temperature and heart rate dependency of myoplasmic Ca^{2+} alternans. To record the changes in the myoplasmic Ca^{2+} concentration, intact hearts were loaded with the Ca^{2+} indicator Rhod-2-AM perfused through coronary vascularization. The recordings were performed with the newly developed Fluorescence Focal Field Optical Mapping (FLOM) instrument [12]. **Figure 1A** shows what happened when the heart was maintained at low temperature (20ºC) and paced at 2 Hz. Under these conditions, no Ca^{2+} alternans were observed. Interestingly, when the heart rate increases to 8 Hz, significant Ca^{2+} alternans were observed in the recording (**Figure 1B**). However, if the temperature was increased to 33ºC, no Ca^{2+} alternans were observed either at 2 Hz (**Figure 1C**) or 8 Hz (**Figure 1D**). **Figure 1 E** reveals the effect of local temperature cooling using a cold finger positioned in the periphery of the optical conduit used to record epicardial FLOM images. The heart was maintained at 33ºC, and at 4 Hz it was not possible to record myoplasmic Ca^{2+} alternans. However, when the heart rate was increased to 6 Hz, the Ca^{2+} alternans became larger in the cold region. Furthermore, when the heart rate was increased to 8 Hz, the difference in the amplitude of Ca^{2+} alternans became even larger. **Figure 1F** illustrates myoplasmic Ca^{2+} alternans were significantly larger in the cold region when compared to the warmer region. **Figure 1G** illustrates the effect of local cooling on the kinetics of the myoplasmic Ca^{2+} transients. In **Figure 2 A-C** we analyzed the global temperature dependency of myoplasmic Ca^{2+} transients using FLOM. **Figure 2A** illustrates a series of FLOM images at different temperatures when the hearts were paced at 2 Hz. At higher temperatures, the transients display a faster time to peak and faster relaxation times. **Figure 2B** shows the temperature dependency of myoplasmic Ca^{2+} transients recorded with FLOM at seven different temperatures. The relaxation of the myoplasmic Ca^{2+} transients was exponentially fitted to determine the relaxation time constant τ and the rate of relaxation k. The values for τ (green plot) and k are plotted in **Figure 2C**. There is a decrease in the relaxation time constant τ, as a function of the temperature in ºC, and an increase in the rate constant k also as a function of the increase in temperature.

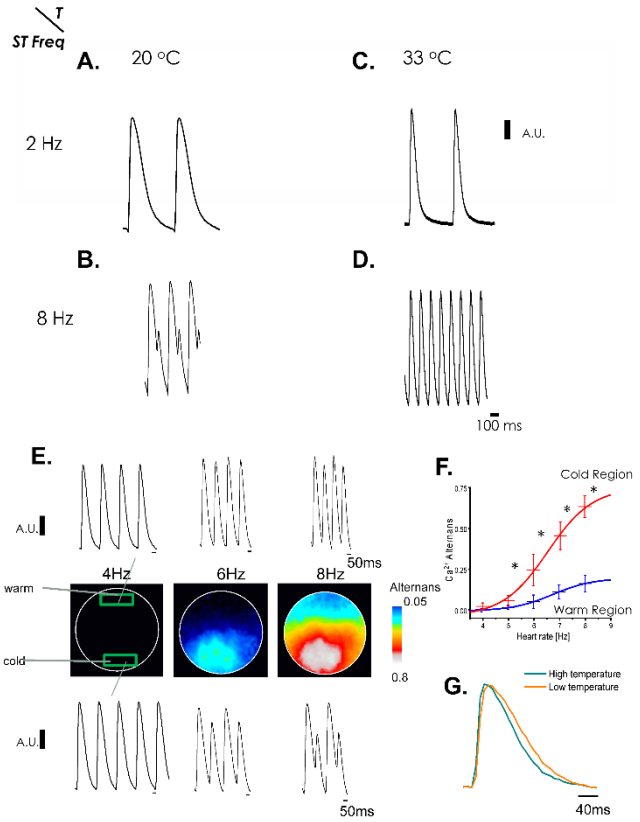

Figure 1. Global temperature dependency of myoplasmic Ca^{2+} alternans (A–D) Ca^{2+} transients measured with the FLOM apparatus at 20°C (global temperature of heart and bath) and an HR of 2 Hz (**A**); 20°C and 8 Hz (**B**); 33°C and 2 Hz (**C**); and 33°C and 8 Hz (**D**). Large Ca-Alts can be observed at 20°C and 8 Hz, which are removed by increasing the temperature to 33°C. **Local temperature dependency of myoplasmic Ca^{2+} alternans. E.** Cold and warm region spatial distribution of alternans versus heart pacing frequency (alternans maps computed as described in Results). Hearts were kept in a bath at 32°C, and the cold finger was set to 18°C. **F.** Effect of HR on the amplitude of myoplasmic Ca^{2+} alternans recorded in the warm and cold regions of the ventricular epicardium. Ca-Alts in the cold region is visible at 5 Hz and, at faster pacing (6–8 Hz), develop significantly larger than in the warm region. *, $P < 0.01$; $n = 5$ hearts. **G.** Comparison of Ca^{2+} transients at local high and low temperature.

The central idea of this experiment is to generate a temperature distribution plot based on the rate constants obtained from fitting the relaxation of the myoplasmic Ca^{2+} transients and the first derivative of the sequence of images using FLOM. **Figure 2D** shows a sequence of images and the time course of myoplasmic Ca^{2+} transients. **Figure 2E shows** a sequence of the images representing the first derivative calculated as the difference between two consecutive images divided by Δt. Also, we represent the time course for the first derivative. To calculate the maximum first derivative, we plotted the time course of the first derivative, and we used the images at the peak of the first derivative. **Figure 2F** shows the relationship between the maximum first derivative and the temperature map. To convert the maximum first derivative and the temperature map, we used the slope and the origin ordinate that we obtained in **Figure 2C** for the rates. When we computed the temperature map, we found the temperature difference between the cold and warm regions was 5ºC. Thus, using the rate *k* as a function of the temperatures and the first derivative, we were able to obtain a temperature map.

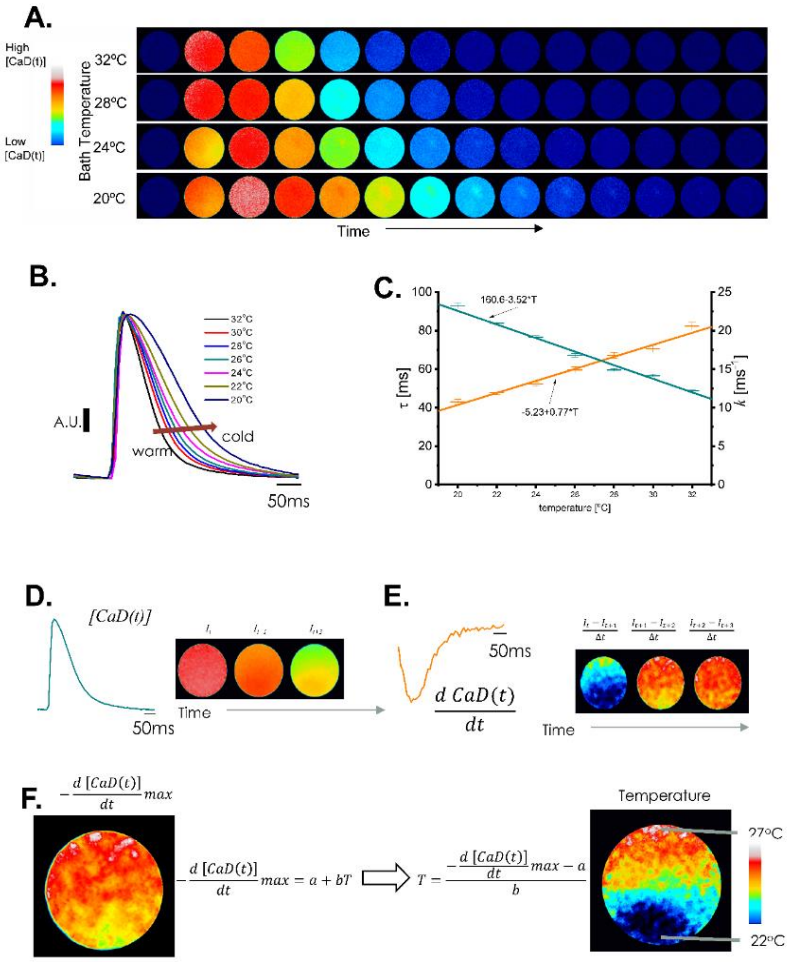

Figure 2. Global temperature dependency of Ca2+ transients' relaxation. (A) FLOM fluorescent images versus time from a heart loaded with Rhod-2 at 32°C, 28°C, 24°C, and 20°C when the heart was paced at 2 Hz. In the scale, blue and red indicate low and high levels of Ca2+ bound to dye (CaD), respectively. (B) Ca2+ transients averaged from FLOM fluorescent images of a heart loaded with Rhod-2 at decreasing bath temperatures. The temperature was dropped in steps of 2°C and the heart was stabilized for 5 min prior to each recording. (C) Fitted values for the relaxation time constant τ (tau) and the rate of relaxation k (n = 5 hearts). Generation of temperature maps. (D) Series of FLOM images from It minus to It+1 with its corresponding average Ca2+ transients in a heart externally paced at 5 Hz. (E) A derivative map is constructed from the rate of relaxation of the Ca2+ transients (F) Left: Map of maximum derivates (obtained from B). They represent the point in time at which the derivative is maximum.

Obtaining the temperature map is critical because it allowed us to determine the temperature dependency of myoplasmic Ca^{2+} alternans. **Figure 3A** shows the relationship between the local temperature and the amplitude of myoplasmic Ca^{2+} alternans. An increase in the temperature produced a decrease in the amplitude of the myoplasmic Ca^{2+} alternans as a function of the temperature. **Figure 3B** exemplifies the dual relationship between temperature and heart rate. The figure illustrates the myoplasmic Ca^{2+} alternans were larger at lower temperatures and at higher heart rates. This is highly related to the fact that with a higher heart rate, the degree of intra SR Ca^{2+} depletion is larger. **Figure 3C** shows the Q_{10} of the myoplasmic Ca^{2+} alternans at different heart rates where at higher heart rates, the amplitude of myoplasmic Ca^{2+} alternans became larger.

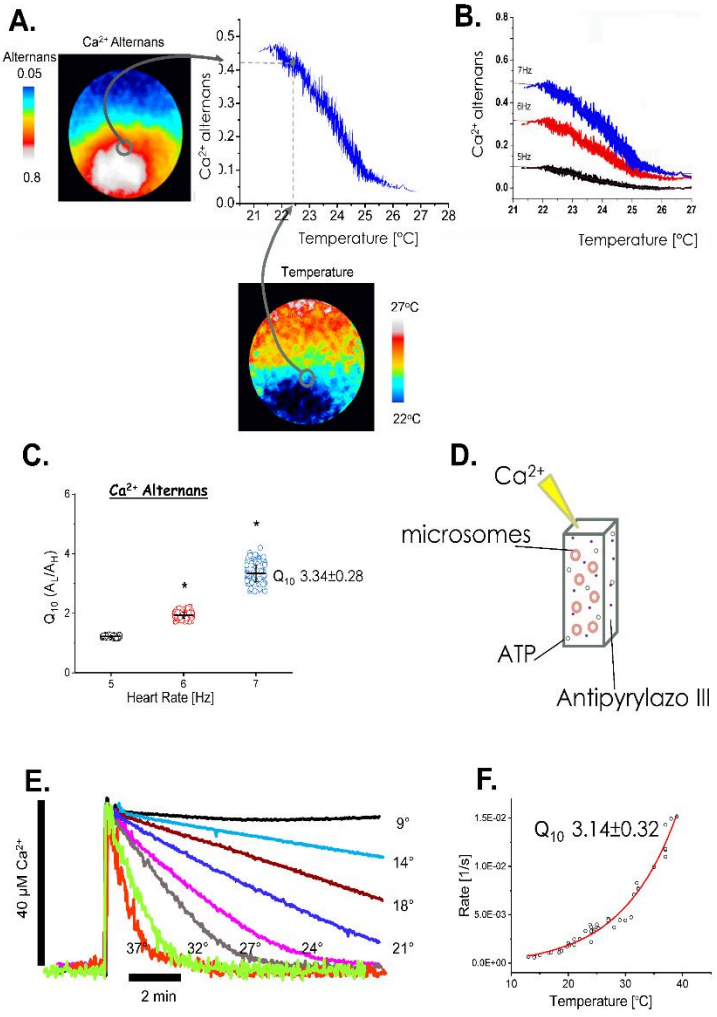

Figure 3. Relationship between myoplasmic Ca^{2+} alternans maps and temperature maps. (**A**) Distribution of myoplasmic Ca^{2+} alternans as a function of a temperature map. The curve was generated by plotting the myoplasmic Ca^{2+} alternans as a function of the temperature value at the same pixel on the FLOM image. (**B**) Temperature dependency of myoplasmic Ca^{2+} alternans when the heart was paced at 5 Hz, 6 Hz, and 7 Hz. **Figure 3C** illustrates the values of the Q_{10} for different heart rates. (**D**) Illustrates the procedure used to measure the uptake of Ca^{2+} by the microsomes that contain the SERCa2 pump. (**E**) Shows the kinetic of Ca^{2+} uptake by the microsomes at different temperatures. (**F**) shows how the rate of uptake changes as a function of the temperature. Moreover, the calculate Q_{10} for this process was 3.14± 0.32, a value very similar to the one we obtained for the Q_{10} of myoplasmic Ca^{2+} alternans at 7 Hz.

Our working hypothesis was that the myoplasmic Ca^{2+} alternans are produced due to an incomplete refilling of the SR mediated by the SERCa2 pump. Thus, we wanted to assess if the temperature dependency of the SERCa2 pump we utilized purified porcine ventricular SR microsomes. The temperature dependency of the SERCa2 pump was like the temperature dependency of myoplasmic Ca^{2+} alternans. To assess this question, we performed experiments evaluating the Ca^{2+} transport through the SERCa2 pump at different temperatures. These experiments were performed by incubating dog SR microsomes in a cuvette containing ATP, a low-affinity absorption dye Antipyrylazo III, and a cardiac intracellular solution. After incubation at different temperatures, 40 µM Ca^{2+} was added to the cuvette (**Figure 3D**), which triggers SERCa2a mediated Ca^{2+} uptake and the rate of relaxation was fitted with an exponential function (**Figure 3E**). **Figure 3F** illustrates the relaxation rate constants at different temperatures. Furthermore, the calculated Q_{10} (3.14±0.32) was very similar to the Q_{10} of myoplasmic Ca^{2+} alternans at 7 Hz. This result indicates the temperature dependency of the pump is very similar to the temperature dependency of myoplasmic Ca^{2+} alternans.

To further confirm that the uptake of Ca^{2+} to the SR was critical in defining myoplasmic Ca^{2+} alternans, we performed an intact perfused heart experiment in which the SERCa2 pump was partially inhibited by 200 nM of Thapsigargin. **Figure 4A** shows a control experiment in which the heart was paced at 9 Hz. The myoplasmic Ca^{2+} present a small degree of myoplasmic Ca^{2+} alternans. However, when the SERCa2 pump was partially inhibited by 200 nM of Thapsigargin, the amplitude of myoplasmic Ca^{2+} alternans increased significantly (**Figure 4B**). **Figure 4C** shows the result of four experiments in which we performed the same protocol. The inhibition with 200 Thapsigargin significantly increased the amplitude of myoplasmic Ca^{2+} alternans every time. This result further confirmed our hypothesis that myoplasmic Ca^{2+} alternans are produced by an incomplete refilling of the SR during a tachycardia episode.

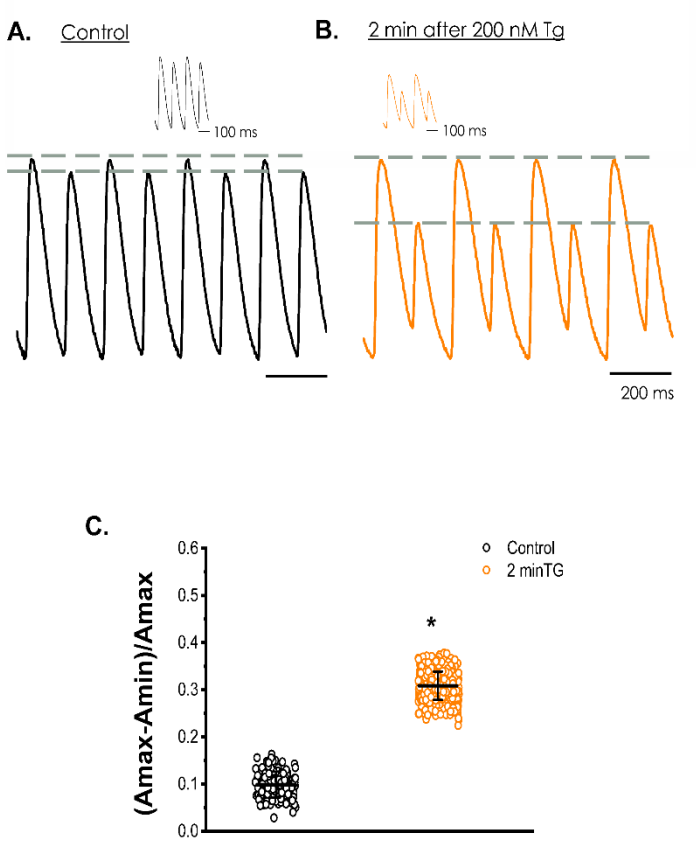

Figure 4. Effect of partially inhibiting SERCa2 pump with 200 nM of Thapsigargin. (A) control condition when the heart was paced at 9 Hz, a very small degree of myoplasmic Ca^{2+} alternans were observed. (B) Recording of myoplasmic Ca^{2+} alternans after perfusing the heart for 2 minutes with 200 nM of Thapsigargin. (**C**) Statistics of the effect of 200 nM Thapsigargin on the genesis of myoplasmic Ca^{2+} alternans (n = 4 hearts; *, $P < 0.01$)

Discussion

In this review, we used a FLOM instrument to measure local action potential and Ca^{2+} transients with intracellular spatial resolution. The FLOM instrument is extremely useful in that it can also detect images at high speed and allow us to record epicardial signals at the whole heart level.

Also, we used a cold finger to generate temperature gradients on the epicardial layer of the heart. Upon the generation of a temperature gradient, we were able to detect that in colder regions the amplitude of myoplasmic Ca^{2+} alternans was significantly larger. Interestingly these alternans occur in the absence of action potential alternans.

Several mechanisms have been suggested for the generation of Ca-Alts, involving alternans in the duration of APs which finally produce an alternation in the Ca^{2+} influx through L-type Ca^{2+} channels [21].

However, nowadays the central hypothesis is that myoplasmic Ca^{2+} alternans are produced by Ca^{2+} released from the sarcoplasmic reticulum [8, 9, 22]. As previously expressed in the introduction, two proposed mechanisms are describing the development of myoplasmic Ca^{2+} alternans.

The first hypothesis involves an incomplete recovery from the inactivation of RyR2. This possibility is highly unlikely to occur because when the RyR2 is activated by two consecutive pulses of Ca^{2+}, the channel can reopen. Moreover, when the channel is reactivated with a second Ca^{2+} pulse, there is a change in the modal gating of the protein which allows the channel to reopen [24, 25, 26]. The deactivation of RyR2 is very fast (~ 5 ms) [23] and this deactivation is even faster under physiological concentrations of Mg^{2+} (~ 3 ms) [27]. To have a Ca^{2+} dependent inactivation, the free Ca^{2+} in the dyadic space needs to be much higher than 1 mM, something that is highly unlikely.

The second hypothesis is related to an incomplete replenishment of the intra SR Ca^{2+} content during tachycardia. Data presented in this review shows the temperature dependency of the Ca^{2+} reuptake by the SERCa2 pump is very similar to the temperature dependency of myoplasmic Ca^{2+} alternans[12]. In a previous report, we show that increasing the magnitude of SR Ca^{2+} release as in hypercalcemia also associates with Ca^{2+} alternans [12]. Moreover, if the SERCa2 pump is partially inhibited by Thapsigargin, there is a significant increase in the amplitude of myoplasmic Ca^{2+} alternans. Our experimental evidence indicates that SERCA's inability to replenish Ca^{2+} into the SR is the stronger possibility. Furthermore, during tachycardia, the SERCa2 pump will not be able to replenish the intra SR Ca^{2+} content, an event that will not allow calsequestrin to dissociate from Triadin and Junctin leaving the RyR2 in a condition in which this protein cannot access its maximum open probability [11].

Role of temperature on the intracellular Ca^{2+} dynamics

In this review, we concurrently evaluated the impact of variations in the heart rate and temperature on the genesis of Ca^{2+} alternans. These effects can be observed in **Figure 1 A-D,** where the temperature not only had a significant effect on the activation and the relaxation of the Ca^{2+} transients but also set a condition in which myoplasmic Ca^{2+} alternans were more likely to be observed.

There is a large piece of evidence indicating hypothermia can produce cardiac arrest [28, 29, 30, 31, 32]. The link between hypothermia and cardiac arrest is centered on the effect low temperature can have on several physiological processes in the heart. Low temperatures can affect several key proteins involved in the excitation-contraction coupling mechanisms, such as the Na^{+}-Ca^{2+} exchanger [17, 33, 34], the SERCa2 [34]; and the L-type Ca^{2+} channels [14, 36]. Interestingly, there is little knowledge describing the regulation of RyR2 at low temperatures. For example, recordings of the activity of RyR2 in planar lipid bilayers indicate

there is an increase in the open probability of the channel at lower temperatures [37]. Furthermore, experiments, where Ca^{2+} sparks were recorded in isolated ventricular cardiac myocytes, indicate that in a range between 23°C and 32°C, there is not a big difference in the Ca^{2+} spark kinetic properties [38].

Effect of the temperature on the myocyte Ca^{2+} transport mechanisms.

We assessed kinetic and thermodynamic parameters of the Ca^{2+} transients and myoplasmic Ca^{2+} alternans by imposing a temperature gradient with the aid of a cold finger (**Figure 1E, 1F, and 1G**). In this figure, we not only found that the kinetics of Ca^{2+} transients to be faster at higher temperatures (**Figure 1G**), but also that myoplasmic Ca^{2+} alternans are smaller under this condition (**Figure 1E, and 1F**). These differences can be explained by the fact that the Na^{+}-Ca^{2+} exchanger [33, 34, 39, 40] and the SERCa2 pump [34] have a strong temperature dependency. Indeed, we found that the SERCa2 pump has a Q_{10} of 3.14. This further suggests the RyR2 is not directly involved in the genesis of myoplasmic Ca^{2+} alternans, but by another mechanism that regulates Ca^{2+} release from the sarcoplasmic reticulum having a strong temperature dependency.

The genesis of myoplasmic Ca^{2+} alternans under local cooling

We also studied the interplay between the relaxation kinetics of Ca^{2+} transients and the global temperature (**Figure 2A-C**). By fitting the relaxation kinetics of the Ca^{2+} transients (**Figure 2C**) and calculating the first derivative (**Figure 2E**) from Ca^{2+} transients (**Figure 2D**), we were able to compute a temperature map from the maximum first derivative and the parameters fitted to the rate of relaxation of Ca^{2+} transients. This is a key element in our approach because we want to correlate the temperature map (**Figure 2F**) with the amplitude of myoplasmic Ca^{2+} alternans.

Figure 3A shows the correlation between the myoplasmic Ca^{2+} alternans map and the temperature map. At higher temperatures less myoplasmic Ca^{2+} alternans are present than lower temperatures. Even more, **Figure 3B** illustrates that an increase in the heart rate increased the amplitude of myoplasmic Ca^{2+} alternans. This heart rate dependency can be correlated with the fact that at a higher heart rate, it is more difficult for the SERCa2 pump to replenish the intra sarcoplasmic reticulum Ca^{2+} content. Moreover, after we calculated the Q_{10} for myoplasmic Ca^{2+} alternans, we found that at 7 Hz the Q_{10} was 3.34±0.28. Furthermore, one way to determine if the thermodynamics of myoplasmic Ca^{2+} alternans was like the thermodynamics of the Ca^{2+} uptake to the sarcoplasmic reticulum is to compare the Q_{10} of both processes. This experiment is presented in **Figure 3 D-F**. As described in the **Results** section, the temperature dependency of the sarcoplasmic reticulum Ca^{2+} uptake was evaluated by adding 40 μM of Ca^{2+} to porcine cardiac microsomes in a cuvette that contains ATP and a low-affinity Ca^{2+} indicator (**Figure 3D**). **Figure 3E** shows at higher temperatures, the rate of Ca^{2+} uptake was faster. Even more, when we plotted the rates of uptake as a function of the temperature, we found that the Q_{10} of the pump was 3.14±0.32. This number is very similar to the Q_{10} of myoplasmic Ca^{2+} alternans at 7 Hz.

Although isolated RYR2 under steady-state in vitro conditions (bilayers) may not be significantly affected by changes in temperature, in the cardiomyocytes there are dynamic changes in cytosolic and luminal Ca^{2+} beat to beat. Here, the genesis of myoplasmic Ca^{2+} alternans in cold regions originated by an indirect effect of SERCA-mediated Ca^{2+} uptake and free diastolic Ca^{2+} in the SR on the RyR2 channel gating [41].

Pharmacological inhibition of SERCA2a increases the amplitude of myoplasmic Ca^{2+} alternans.

Figure 4 illustrates the effect of partially inhibiting SERCa2 with 200 nM of Thapsigargin. The impairment of the Ca^{2+} pump induced a significant increase in the amplitude of myoplasmic Ca^{2+} alternans (**Figure 4B**) when compared with the control condition (**Figure 4A**). **Figure 4C** shows the results of 4 experiments to verify that the increase in the amplitude of myoplasmic Ca^{2+} alternans was statistically different. This experiment boosts the idea that myoplasmic Ca^{2+} alternans are produced by an incomplete refilling of the sarcoplasmic reticulum mediated by the SERCa2 pump. [8, 12]

Conclusion

The experiments presented in this review use the new FLOM-based experimental approach and can further explain how arrhythmogenesis correlates with the spatial distribution of metabolically impaired myocytes along the epicardium. Finally, alternans in mechanical activity (Pulsus alternans) directly related to myoplasmic Ca^{2+} alternans can also develop under hypothermic conditions [42].

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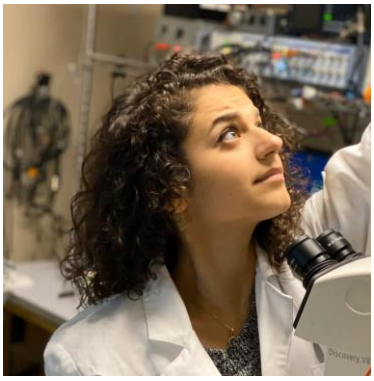
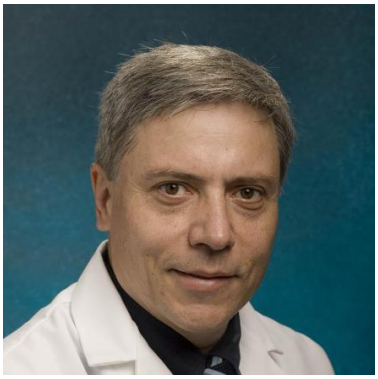
About authors

Ariel L Escobar Ph.D. Full Professor (Tenure), 2008-2021. Department of Bioengineering. School of Engineering, University of California Merced, Merced, California.

My Laboratory has been involved in the development of new optical, spectroscopic, and electrophysiological techniques for studying key aspects of striated muscle physiology. Some of these techniques, flash laser imaging and confocal spot detection, making it possible to define the spatial pattern of second messenger (i.e. Ca^{2+}) distribution in striated muscle. Recently, my laboratory developed a state-of-the-art technique called Loose Patch Photolysis and Florescence Local Field Optical Mapping that allow the measurements of Ionic Currents and Ca^{2+} spatial distribution in the intact heart. Specifically, we have been interested in how the Ca^{2+} released from the sarcoplasmic reticulum can define phase 2 in mouse hearts. Interestingly we discovered that phase 2 is generated by an influx of Na^{+} through the Na^{+}-Ca^{2+} exchanger (NCX) working in the forward mode. Thus, the regulation by Na^{+} of the NCX activity could be critical in defining the kinetic properties of phase 2. I have been performing optical and electrophysiological measurements using different approaches for the last 26 years.



Maedeh Bazmi BS. Ph.D. student in Quantitative Systems Biology. 2018-2021 Area of focus: Cardiac physiology Dr. Escobar, advisor School of Natural Sciences. University of California, Merced. Ph.D. thesis topic: Excitation Contraction Coupling in fish hearts. Teaching assistant for Physiology for Engineers, upper-division undergraduate bioengineering students. University of California, Merced. Teaching assistant for Cell Biology for Engineers, upper-division undergraduate bioengineering students. The University of California, Merced Teaching assistant for Contemporary Biology Laboratory, undergraduate biology students. University of California, Merced. Undergraduate Student Research Assistant in Neurobiology Dr. Norris, advisor California State University, San Marcos. San Diego, Ca.



Dr. Julio A. Copello is a Tenure Associate Professor in the Department of Pharmacology, School of Medicine, University of Southern Illinois, IL (2010-2021). Dr. Copello is also the graduate program director. Scientifically, Dr. Copello is a world leader in the study of Ca^{2+} transport in the sarcoplasmic reticulum. Indeed, he has made a central contribution to the kinetics of Ryanodine receptors incorporated in Planar Lipid Bilayers. Moreover, he was the first scientist that discovered that multiple RyR1 receptors can open and close synchronously by a process called coupled gating. Furthermore, he has also been interested in the SERCa2 pump transport, its kinetic features, and pharmacology.