Are SR Ca content fluctuations or SR refractoriness the key to atrial cardiac alternans?: insights from a human atrial model

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Lugo CA, Cantalapiedra IR, Peñaranda A, Hove-Madsen L, Echebarria B. Are SR Ca content fluctuations or SR refractoriness the key to atrial cardiac alternans?: insights from a human atrial model. Am J Physiol Heart Circ Physiol 306: H1540–H1552, 2014. First published March 7, 2014; doi:10.1152/ajpheart.00515.2013.—Despite the important role of electromechanical alternans in cardiac arrhythmogenesis, its molecular origin is not well understood. The appearance of calcium alternans has often been associated to fluctuations in the sarcoplasmic reticulum (SR) Ca2+ loading. However, cytosolic calcium alternans observed without concurrent oscillations in the SR Ca content suggests an alternative mechanism related to a dysfunction in the dynamics of the ryanodine receptor (RyR2). We have investigated the effect of SR release refractoriness in the appearance of alternans, using a mathematical model of a single human atrial cell, based on the model by Nygren et al. (30), where we modified the dynamics of the RyR2 and of SR Ca release. The genesis of calcium alternans was studied stimulating the cell for different periods and values of the RyR2 recovery time from inactivation. At fast rates cytosolic calcium alternans were obtained without concurrent SR Ca content fluctuations. A transition from regular response to alternans was also observed, changing the recovery time from inactivation of the RyR2. This transition was found to be hysteretic, so for a given set of parameters different responses were observed. We then studied the relevance of RyR2 refractoriness for the generation of alternans, reproducing the same protocols as in recent experiments. In particular, the generation of alternans was studied using a S1S2 protocol, obtaining a different response if the S2 stimulation was given after a long or a short release. We show that the experimental results can be explained by RyR2 refractoriness, arising from a slow RyR2 recovery from inactivation, stressing the role of the RyR2 in the genesis of alternans.

electromechanical alternans; arrhythmias; calcium dynamics; ryanodine receptor; SR calcium release refractoriness

THE OCCURRENCE OF CARDIAC arrhythmias has often been associated to irregularities in intracellular calcium dynamics (43). An important example is calcium alternans (6), a beat-to-beat alternation in the intracellular Ca transient of cardiac cells (14, 45) that develops when the cells are paced rapidly or pharmacologically stressed. Alternans has been shown to precede episodes of atrial fibrillation (AF) and to appear at slower pacing times in patients with persistent AF (29).

Despite the important role of electromechanical alternans in cardiac arrhythmogenesis, its molecular origin is not well understood. An explanation for the emergence of calcium alternans has been given in terms of fluctuations in the Ca content of the sarcoplasmic reticulum (SR) at fast pacing rates, where the sarco(endo)plasmic reticulum Ca2+-ATPase (SERCA) does not have enough time to refill completely the SR before the next excitation. This situation, coupled with a steep SR calcium load/calcium release relationship (3), has been shown to give rise to sustained alternans (12, 37). However, calcium alternans has also been observed in experiments where the SR content remains constant in successive beats (31), prompting the quest of alternative mechanisms.

In this regard, dysfunctions of the ryanodine receptor (RyR2) have been suggested to play an important role. In particular, its long latency period (4, 5) gives rise to SR refractoriness, which has been related to the onset of alternans (1, 31, 32, 38, 42). This mechanism is particularly relevant in situations when the SERCA pump is able to rapidly eliminate calcium from the cytoplasm and fully recover the same end-diastolic SR calcium concentration at every beat. In this respect, atrial cells are known to have faster SERCA than ventricular cells (8, 28). This seems to agree with recent experiments in human atrial cells (25) where a slow recovery of refractoriness in release has been proposed as a mechanism for alternans. This scenario has also been studied in rabbit atrial myocytes (38), showing both the absence of significant diastolic SR oscillations during alternans and the presence of release refractoriness.

In this work, we show that calcium alternans occurs under the above-mentioned circumstances if the recovery from inactivation rate of the RyR2s malfunctions and becomes slow enough. To this end, we perform simulations using a numerical model of a human atrial myocyte. In particular, we have considered a well-established model by Nygren et al. (30), introducing the following major distinctions: 1) we incorporate a four state description of the RyR2s dynamics, following Stern et al. (40, 41) and 2) we define a compartmental distinction between dyadic, subsarcolemmal, and intracellular spaces [similar to Leem et al. (20)]. We show that with these modifications, the model gives rise to calcium alternans at fast pacing rates. Typically, alternans appears without SR calcium content oscillations, stressing the importance of calcium release refractoriness as an alternative mechanism. In fact, we find that the onset of alternans is dependent on the recovery time of the RyR2s, which we propose as the origin of refractoriness, as has been discussed recently with the use of a model for rabbit ventricular cells (1). This relation has been further investigated showing that a slow recovery of the RyR2s can explain all the experimental results in Shkryl et al. (38).
Glossary

- \([Ca^{2+}]_c\) concentration in the cleft space
- \([Ca^{2+}]_d\) concentration in the dyadic space
- \([Ca^{2+}]_i\) concentration in the intracellular cytosolic space
- \([Ca^{2+}]_{rel,d}\), \([Ca^{2+}]_{rel,i}\) concentration in the junctional sarcoplasmic reticulum (JSR) and cytolsic sarcoplasmic reticulum (CSR)
- \([Ca^{2+}]_s\) concentration in the subsarcolemmal space
- \([Ca^{2+}]_lup\) concentration in the network SR (NSR)
- \(I_{BaCa}, I_{BaNa}\) Ca\(^{2+}\) and Na\(^{+}\) background currents
- \(I_{Ca,L}\) L-type Ca\(^{2+}\) current
- \(I_{Ca,P}\) Subsarcolemmal space Ca\(^{2+}\) pump current
- \(I_{ds}\) Ca\(^{2+}\) diffusive current between dyadic-subsarcolemmal spaces
- \(I_{K_1}\) Inward K\(^{+}\) rectifier current
- \(I_{Kr}, I_{Ks}\) Delayed rectifier slow and fast K\(^{+}\) currents
- \(I_{Na}\) Na\(^{+}\) current
- \(I_{NaCa}\) Sodium-calcium membrane exchanger current
- \(I_{NaK}\) Na\(^{+}\)-K\(^{+}\) pump current
- \(I_{rel,d}, I_{rel,i}\) JSR and CSR Ca\(^{2+}\) release currents
- \(I_{si}\) Ca\(^{2+}\) diffusive current between subsarcolemmal-intracellular spaces
- \(I_{stim}\) External stimulus current
- \(I_{t}, I_{tas}\) Transient and sustained outward K\(^{+}\) currents
- \(I_{tr,d}, I_{tr,i}\) Ca\(^{2+}\) translocation currents between network SR and CSR or JSR spaces
- \(I_{up}\) Ca\(^{2+}\) uptake current
- \(O\) Buffer occupancy

\(\text{OCSQ,i}, \text{OCSQ,d}\) Calsequestrin occupational fractions by Ca\(^{2+}\) in CSR and JSR

\(V_m\) Membrane potential

\(X_{Cl}, X_{Cd}\) Fraction of closed (C) RyR2s channels in the CSR and JSR

\(X_{II1,i}, X_{II1,d}\) Fraction of RyR2s channels in state I\(_{1}\) in the CSR and JSR

\(X_{II2,i}, X_{II2,d}\) Fraction of RyR2s channels in state I\(_{2}\) in the CSR and JSR

\(X_{O,i}, X_{O,d}\) Fraction of open (O) RyR2s channels in the CSR and JSR

\(OCSQ,i, OCSQ,d\) Calsequestrin occupational fractions by Ca\(^{2+}\) in CSR and JSR

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**METHODS**

Human atrial model. We have considered a human atrial cell of volume 12,354 \(\mu\)m\(^3\) and capacitance \(C_m = 50\) pF, as in the original model by Nygren et al. (30). However, we have made several modifications, to adapt it to recent experiments. In the model by Nygren et al. (30), calcium is released from the SR directly to the cytosolic space. In atrial cells without t-tubules, immunolabeled images of RyRs in atrial myocytes (18, 27) suggest a distribution of a junctional SR close to the L-type calcium channels (LCC) near the sarcolemma, and a nonjunctional SR (CSR) at the cytosol (16, 18, 26, 27). This results in a release that starts at the peripheral region close to the cell membrane, with the calcium transient at the central space starting after a delay (16), contrary to ventricular cell or atrial cells with t-tubules, where the rise in calcium is produced simultaneously in the whole cell (7). The presence of t-tubules in human atrial cells is controvesial (21, 33). In this article we will thus consider the general case of both junctional and nonjunctional SR spaces. In this we follow recent models that introduce a distinction between release to the dyadic and the cytosolic space (19, 20). The amount or lack of t-tubules can be regulated changing the relative ratio of the volumes of these two spaces. In addition, we split the submembrane space into a proximal (dyadic) and nonproximal (subsarcolemmal) space (39). This, together with a modified RyR2 dynamics, following Stern et al. (40, 41), permitted to reproduce calcium alternans at high pacing rhythms, absent in the original model by Nygren et al. (30).

As illustrated in Fig. 1A, we consider a distinction between three different compartments: 1) a dyadic space of volume \(Vol_d\), with the presence of LCC and RyR2 channels, in which only three currents contribute to changes in the dyadic calcium concentration \([Ca^{2+}]_d\). namely, the L-type Ca current \(I_{Ca,L}\), the Ca release current into the...
dyadic volume \( I_{rel,d} \), and the dyadic-subsarcolemmal diffusive Ca flux \( I_{rel} \), 2) a subsarcolemmal space of volume \( V_{rel,s} \), where the other calcium transmembrane currents \( I_{NaCa}, I_{Ca}, \) and \( I_{NaCa}, \) act on and contribute to the subsarcolemmal concentration \([Ca^{2+}]_{s}\), along with the diffusive fluxes between this compartment and both the cytosolic space, \( I_{rel} \), and the previously mentioned dyadic space, \( I_{rel} \), the bulk cytosolic compartment of volume \( V_{cal} \), where the calcium concentration transient \([Ca^{2+}]_{t}\) is affected by diffusion from the subsarcolemmal, luminal calcium efflux \( I_{rel,s} \), and the SR Ca uptake \( I_{rel} \), alongside a number of buffers, as described in Nygren et al. (30). This same compartmental division was recently used by Leem et al. (20) in a model of cardiomyocytes in rabbit pulmonary vein. Following this reference we divided the release from the SR into two currents, one going to the dyadic space, through the JSR, \( I_{rel,d} \), and the other one going directly to the cytosol, through the CSR, \( I_{rel,s} \). For the compartmental volumes we take \( V_{rel} = 5 V_{rel,s}, V_{rel,d} = 10 V_{rel,s} \), and the rest as in Nygren et al. (30). Besides, we have the intercompartment currents: \( I_{rel} = 2 F V_{rel}([Ca^{2+}]_{l} - [Ca^{2+}]_{s})/\tau_{rel} \) and \( I_{rel,s} = 2 F V_{rel}([Ca^{2+}]_{l} - [Ca^{2+}]_{s})/\tau_{rel} \), with \( \tau_{rel} = 0.01 \) ms and \( \tau_{rel} = 0.1 \) ms. Due to the different release dynamics, the conductance of the L-type calcium current was reduced to 70% of the original value. To study the effect of t-tubules, we also performed simulations in a modified model, where we simulated the presence of t-tubules by changing by a factor of two and a half the ratio of JSR and CSR volumes.

Besides the cellular structure, we have also modified the dynamics of the RyR2. For this, we assumed a four state model based on the one introduced by Stern et al. (40, 41). It consists of a set of four rate equations that describe the reversible transitions between one open (O), one closed (C), and two inactive states (I1 and I2) for the RyR2s (see Fig. 1B). We denote by \( X_{O}, X_{C}, X_{I1}, \) and \( X_{I2} \) the corresponding fraction of RyR2s in each state, with \( l = d \) indicating the respective compartment (dyadic or cytosolic). This more detailed description of a peripheral (sarcotubular) and inner cell (cytosolic) distribution of RyR2s captures the essence of atrial myocyte physiology, in the case that t-tubules are absent. The transition rate coefficients represent the activation \( k_{a} \), inactivation \( k_{s} \), closing \( k_{c} \), and recovery from inactivation \( k_{b} \) of the RyR2. As standard values we consider \( 40, 41) k_{-A} = 0.06 \) ms\(^{-1} \), \( k_{A} = 22 \times 10^{6} \) M\(^{-1}\)s\(^{-1} \), \( k_{-B} = 1/200 \) ms\(^{-1} \), and \( k_{B} = 21 \) mM\(^{-1}\)s\(^{-1} \). The activation rate was chosen so the RyR2 opens for the typical concentration values at the interior of the cell. In this work we are particularly interested in the role played by the refractoriness necessary for the generation of alternans. In particular, we performed a series of two numerical experiments: 1) the cell was stimulated at a constant period S1 until it reached steady state. An extra stimulus S2 was then given at increasing time intervals \( \Delta T \), after either the long or the short calcium transient. From this, we computed the cytosolic calcium peak and the fractional release as a function of the delay time \( \Delta T \); and 2) in Skryl et al. (38) RyR2 refractoriness was studied increasing the \([Ca^{2+}]_{l}\), by photolytical release of Ca (referred as photolysis-induced Ca release, or PICR). We mimicked this effect by adding a photolysis rate \( k_{ph} \) to the terms involved in the opening of the RyR2 in both compartments, i.e., \( cj\times X_{C} \rightarrow cj\times X_{C} + k_{ph} \), \( j = d,i \). This was done only during a brief window of time \( t_{ph} = 2\tau_{rel} \) ms. The value used for \( k_{ph} = 0.01 \) ms\(^{-1} \) was chosen so it gave an increase in release similar to that observed in Skryl et al. (38). The PICR simulations were then performed for a variety of delay times after normal depolarization, both after a long and a short beat of the calcium transients, measuring the corresponding Ca\(^{2+}\) concentration peak \((Ca_{Peak}, Ca_{Peak})\) respectively. From this, we calculated the ratio \( Ca_{Peak}/Ca_{Peak} \) as a function of the delay during repolarization.

**Ventricular cells.** To compare the characteristics of alternans in atrial and ventricular cells we have performed simulations in a ventricular myocyte, following the model by Shannon et al. (36), as specified in Alvarez-Lacalle et al. (1). Following this latter work, we have performed clamping protocols for both SR Ca content and number of recovered RyR2s and chosen RyR2 parameters for which alternans are due to a slow recovery from inactivation (specifically, \( k_{s} = 0.3 \) mM\(^{-1}\)s\(^{-1} \), \( k_{i} = 0.25 \) mM\(^{-1}\)s\(^{-1} \)), in the notation of Ref. 1).

### RESULTS

**Comparison with other human atrial models.** As we have explained in METHODS, we considered the model by Nygren et al. (30), with different kinetics for the RyR2, and a release in both the dyadic and intracellular spaces. In Fig. 2A, we show a comparison between our model and the original model by Nygren et al. For completeness, we also show the results from the Courtemanche et al. (11) human atrial model. The action potential (AP) in our model is intermediate between both (Fig. 2A) and compares well with experimental APs (Fig. 2B) (46, 47). The intracellular calcium transient is broader than in the model by Nygren et al. (Fig. 2C). In our model this is due to the delay between release in the dyadic space and in the cytosol. In contrast with ventricular cells or atrial cells with t-tubules, where the rise of calcium is synchronous in the whole cell (13), in atrial cells without or with reduced t-tubules (13) as considered in the present model, the rise of calcium in the cytosol starts a few milliseconds after the subsarcolemma, with a shift between both peaks of about 50 ms (7). As a result of this, inactivation of \( I_{calc} \) is also slower, compared with the original model by Nygren et al. (Fig. 2E). In Table 1, we compare the main properties of the AP and calcium transient between our model and different human atrial models in the literature, as given in Wilhelms et al. (48).

**Onset of alternans.** Increasing the RyR2 recovery time from inactivation from its standard value (\( \tau_{i} = 200 \) ms), the dynamics of intracellular calcium became unstable, and alternans appeared, with concordant oscillations in voltage and calcium (Fig. 3). As also observed in experiments (7), alternans in our model are stronger in the interior of the cell than in the
submembrane. We typically found cytosolic Ca alternans without concurrent oscillations in SR Ca content (Fig. 4A). Alternans resulted in oscillations in the calcium flux through the cell membrane. However, the total number of Ca$^{2+}$ ions transported across the membrane is the same every beat (Fig. 4A). This is in contrast with the case of our model of an atrial cell with increased t-tubules, where there are oscillations in both SR Ca content and total Ca$^{2+}$ ions transported at every beat (Fig. 4B), similar to the results obtained with the Shannon et al. (36) ventricular myocyte model, shown in Fig. 4C. In this latter case we considered slower pacing periods (TS = 600 ms) to make sure that SERCA has enough time to reach its equilibrium value. Besides, using the clamping protocols introduced in Alvarez-Lacalle et al. (1), we have checked the mechanism behind the onset of alternans in this latter case, verifying that alternans is due to RyR2 refractoriness even if there are oscillations in end-diastolic SR content.

The onset of alternans occurred at larger values of the critical pacing rate as $r$ was increased. In Fig. 5, A and C, we show the peak intracellular calcium at two consecutive beats as a function of the pacing period and RyR2 recovery time, respectively. As expected, alternans appeared at fast pacing

Table 1. Comparison of the results in this article and other models for human atrial cells, adapted from Wilhelms et al. (48)

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<td>Diastolic</td>
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<td>0.065</td>
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<td>1.227</td>
<td>0.496</td>
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rates, of about 2 Hz (Fig. 5A), without concurrent SR Ca content oscillations (Fig. 5B). More interestingly, a change in the recovery time of the RyR2 made alternans disappear (Fig. 5C), suggesting recovery of the RyR2 from inactivation as the originating mechanism in the cases where SR Ca fluctuations are absent (Fig. 5D). At some pacing rates the transition to alternans was subcritical; that is, there was a hysteretic region where either alternans or a constant response could be observed (Fig. 5E). In this region a sudden change in intracellular calcium concentration was able to change the response of the system, from normal rhythm to alternans (Fig. 5F). The presence of a hysteretic region can explain the results obtained by Shkryl et al. (38), where PICR could result in a change in the phase of alternans at constant pacing period but also in a regularization of the alternans response or in the generation of alternans from a regular calcium transient.

**Influence of RyR2 dynamics on the appearance of alternans.**

As stated previously, the results in Fig. 5C seem to suggest an important role of the RyR2 recovery from inactivation time in the onset of alternans. This is in agreement with recent experiments, showing that alternans may appear due to RyR2 refractoriness (38). We show that, in fact, time-dependent refractoriness appears due to a slow recovery from inactivation of the RyR2. Repeating numerically the experimental protocols in Shkryl et al. (38), we were able to relate the SR release with the level of recovered RyR2s in each case.

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**Fig. 3.** Electromechanical alternans, at a pacing period of $T_S = 400$ ms. The recovery time of the RyR2 is set to $\tau_r = 650$ ms. Here and in the following figures $g_{CaL}$ is reduced to 60% of the original value in Nygren et al. A: transmembrane voltage. B: cytosolic calcium transient. For comparison we show above experimental alternans observed in cat atrial cells (7).

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**Fig. 4.** Characteristics of alternans in our model, with $\tau_r = 700$ ms, for atrial cells (A), at a pacing period of $T_S = 400$ ms (alternans appearing between pacings of 270 and 500 ms), and atrial cells with t-tubules (B; with $g_{CaL}$ 50% of the original value), at $T_S = 300$ ms (alternans between 240 and 300 ms). C: for comparison we also show the results from a ventricular myocyte model (36), for parameters at which alternans are due to RyR2 recovery from inactivation (1), and a pacing period of $T_S = 600$ ms. At this pacing rate it is clearly seen that the SR Ca content has had enough time to reach its equilibrium value at every beat. In each case we show intracellular and SR calcium concentration, the net flux of calcium ions through the transmembrane, normalized by cytosol volume, and its cumulative value.
First, we studied the effect of an extra stimulation after a long or short Ca transient during alternans, with a slow RyR2 recovery from inactivation. We reproduced the same protocol in Shkryl et al. (38), applying premature stimuli after large and small Ca transients and measuring the magnitude of the intracellular calcium increase (Fig. 6A) and SR Ca fractional release (Fig. 6B). As expected, after a short calcium transient, the release is higher than after a long one. This is mainly due to the slow recovery of the RyR2, since the SR Ca concentration rapidly goes back to its presystolic equilibrium value. At a delay of $\Delta T = 350$ ms (Fig. 6C), neither the SR Ca concentration nor the level of recovered RyR2s have attained the equilibrium values. Thus each one contributes to a larger release after a short transient. However, for a delay of $\Delta T = 550$ ms (Fig. 6D), the SR Ca concentration has reached its equilibrium value after both the short and the long transients and, therefore, is not expected to contribute to a different release. The difference in release still observed must then be

Fig. 5. Amplitude of alternans (A) and SR Ca concentration (B) as a function of pacing period $T_S$, for $\tau_r = 700$ ms. Amplitude of alternans (C) and SR Ca concentration (D) as a function of the RyR2 recovery time $\tau_r$, at a pacing period of $T_S = 400$ ms, showing that the bifurcation is subcritical, and presents hysteresis. E: calcium transient at different pacing rates, for a RyR2 recovery time of $\tau_r = 380$ ms and obtained alternans (corresponding to crosses in C). Then we decreased $\tau_r$ to 370 ms, producing a transition to a uniform response, corresponding to a jump to the upper branch in C. When $\tau_r$ was set back to the original value, the regular rhythm was maintained, showing bistability for those parameters. The transition is showed in more detail in the insets.

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due to an incomplete recovery of the RyR2, as shown in Fig. 6D, b and d.

Again, in Shkryl et al. (38), the effect of recovery from inactivation of SR Ca release was studied imposing step-like increases of [Ca$^{2+}$], during alternans using a photolytical release of Ca, termed PICR. We reproduced this protocol, as explained in METHODS, and changed the opening probability of the RyR2 at given intervals after depolarization, after long and short Ca transients. As observed in the experiments, at short delays the amplitude of the Ca transient elicited by the combination of membrane depolarization and PICR was larger after a small transient than after a large transient. We then repeated this protocol at increasing delays from the beginning of the Ca transient and measured the peak cytosolic calcium. In Fig. 7A
Fig. 7. A, left: ratio of peak cytosolic Ca during a large \( \text{C}_{\text{peak,L}} \) and a small beat \( \text{C}_{\text{peak,S}} \), as a function of the delay between \( \text{Ca}^{2+} \)-induced Ca release and photolysis-induced Ca release (PICR). A, right: results by Shkryl et al. (38) showing the PICR ratio during large \( \text{PICR}_L \) and small \( \text{PICR}_S \) transients. B and C: typical examples of the results of photolysis of Ca after a long and a short Ca peak for two different delay times, \( \Delta T = 50 \text{ ms} \) (B) and \( \Delta T = 150 \text{ ms} \) (C). In each row we show the intracellular calcium transient, the SR Ca concentration, and the percentage of recovered RyR2s. For comparison, with a dotted line, we show how the time trace would have continued without the PICR.
we plot the ratio of peak Ca after a long and a short beat, as a function of the delay between Ca\(^{2+}\)-induced Ca release and PICR. As observed by Shkryl et al. (38), this ratio is larger than one at short delays, becoming lower than one for delays above \(\sim 70-80\) ms and reaching one asymptotically for long delays. This can be explained in the following terms: after a large transient, a large number of RyR2 is inactivated and has not fully recovered at the following stimulation. This effect is much less pronounced after a small transient, resulting in a larger number of available RyR2s. If the PICR is applied at short delays after depolarization compared with the inactivation time (Fig. 7B), the release is directly related with the number of available RyR2s and, therefore, larger after a short transient than after a large one. On the contrary, at longer delays between depolarization and PICR (Fig. 7C), the level of recovered RyR2 is lower for the large transient than for the small one. This results in a smaller PICR-induced release in the former case than in the latter one, or \(\text{Ca}^{\text{peak}}_{\text{L}}/\text{Ca}^{\text{peak}}_{\text{S}} < 1\). At very long delays, the RyR2s always recover, independently if the previous beat was a long or short Ca transient, and therefore, this ratio must asymptote to one. Notice that the SR Ca content always reaches the same level and, therefore, does not play a role in the previous explanation.

**DISCUSSION**

*Human atrial models.* The models by Nygren et al. (30) and by Courtemanche et al. (11) have been amply used to study human atrial cells. Although both deal with the same type of myocytes, their dynamical properties have been shown to differ considerably (10). These models focus primarily on the effect of ionic currents on the AP, and not so much on the calcium handling. To better model calcium release, we modified the dynamics of the RyR2 (Fig. 1B). In the original model by Nygren et al. (30) it consisted of a three state model, with transitions from one state to the other mediated by cytosolic and dyadic calcium. The RyR2 can be in an activated, inactivated, or recovered states, and the transition is always one way. Thus it does not fulfill detailed balance, and it can be considered as a phenomenological description of an excitable system (24). Using the original formulation we were unable to obtain calcium alternans under voltage-clamped conditions, despite a broad change in the parameters of the RyR2. We thus introduced a standard model for the RyR2, proposed by Stern et al. (40, 41) and used in several models showing calcium alternans (1, 34). Besides this, we considered the existence of junctional and nonjunctional SR release sites, consistent with cells lacking t-tubules, as in other atrial models (19, 20). For this, we introduced a subsarcolemmal space such that the current \(I_{\text{CaL}}\) and the release \(I_{\text{rel}}\) go to the dyadic space, but \(I_{\text{CaS}}\) and the other transmembrane currents act in the subsarcolemmal space (Fig. 1A). Besides, we divided the release from the SR into two parts, one going to the dyadic space and the other to the cytosol. These changes prompted us to modify some of the parameters in the original model by Nygren et al. (30), as explained in the Appendix. The resulting AP properties and calcium transient agree well with the original Nygren and other human atrial models (Table 1).

Recently, a model for human atrial myocytes has been developed by Grandi et al. (15), based on the ventricular myocyte model by Shannon et al. (36). Although we have not tested if calcium alternans appears in this atrial model, we would assume so, because it presents a dynamics of the RyR2s and distribution of compartments similar to the model we developed here. Because the model by Grandi et al. (15) is based on a ventricular model, it considers t-tubules and presents synchronous release of calcium in the whole cell. In human atrial cells, the presence of t-tubules is not clear and seems to depend on the size and location of the selected myocytes in the atria (21, 25, 33). Moreover, in animals where t-tubules have been observed, their number is depleted under abnormal conditions, as atrial fibrillation (22) or heart failure (2, 13). A reduced t-tubule structure is also consistent with the delay observed in atrial cells between the rise of calcium close to the cell membrane and the interior of the cell (16). In our model, this is achieved considering two releases from the SR, one going into the dyadic cleft and the other directly to the cytosol. Although the majority of our study has been performed for cells without t-tubules, we have also considered the presence of t-tubules in our model changing the relative volume ratio between the JSR and CSR SR spaces.

**Mechanisms for calcium alternans.** The standard mechanism that has been proposed for calcium alternans is based on the assumption of a steep relation between calcium load and calcium release. Thus, if reuptake is not fast enough, after a strong release, the SR content does not reach its previous value, and release is smaller. If this relation is strong enough it can give rise to sustained alternans, which appears through a period doubling instability of the calcium transient, as explained in detail by Shiferaw et al. (37). This mechanism has been observed also by Li et al. (23), in an atrial myocyte model that lacks t-tubules. Notice that this latter model does not present dynamics for the RyR2, and therefore cannot account for a mechanism involving refractoriness of the RyR2. An alternative explanation of this mechanism based on the dynamics of the RyR2 has been given by Restrepo et al. (32), who assumed that the RyR2 presents two open states, corresponding to fast and slow release, and the transition between both is mediated by calsequestrin (and consequently, dependent of SR Ca content). They showed that this dynamics of the RyR2, when implemented in a full calcium model, did indeed give rise to a strong calcium load-calcium release relation and to instability to alternans.

However, some experiments have shown calcium alternans without concurrent SR content fluctuations (17, 31), thus suggesting that the previous mechanism is, at least, not always, responsible for the appearance of alternans. The relevance of RyR2 refractoriness was manifested by Restrepo et al. (32), who obtained calcium alternans without SR Ca oscillations in a distributed model of calcium release units. A similar result was obtained by Rossetti et al. (34), explaining the appearance of alternans as a combination of slow RyR2 recovery, recruitment, and release. Later on, it was shown by Alvarez-Lacalle et al. (1) that a slow recovery of the RyR2 could be responsible for alternans, even in a common pool model, thus stressing that this mechanism could be observed in fully synchronized alternans. Besides, they found that, depending on the dynamics of the RyR2 (its activation, inactivation, or recovery from inactivation), a mechanism of load, or other due to a slow recovery, could be present.

Recently, Shkryl et al. (38) have performed experiments that reveal an important role of the refractoriness in SR Ca release,
suggesting a time-dependent change in refractoriness. By reproducing their experimental results, we have shown that a slow recovery from inactivation of the RyR2 could, in fact, be responsible for this change in refractoriness, which can explain the onset of alternans when SR Ca content does not change.

**RyR2 recovery from inactivation.** Using our model, we have found calcium alternans that typically appears with constant SR Ca content (see Fig. 4A and Fig. 5, B and D), for a broad variety of pacing rates (Fig. 5A) and RyR2 recovery from inactivation rates (Fig. 5C). This is probably the most common situation in atria, since SERCA is faster than in ventricle (44), and typically it has enough time to refill the SR at the typical rates at which alternans occur. Notice that alternans in our model is produced at the cytosolic space, with smaller fluctuations in calcium concentration at the subsarcolemma. This is in contrast with alternans appearing in ventricle or in atrial cells with t-tubules (Fig. 4, B and D). A change in the relative JSR and CSR volumes in our model, simulating an increase in the amount of t-tubules, modifies the appearance of alternans, which appears only at fast stimulation rates and presents larger SR calcium content oscillations.

In addition, we have shown that a change in the recovery time of the RyR2 affects crucially the dynamics of alternans, so it disappears when recovery is faster (Fig. 5). Interestingly, we found the transition to alternans to be hysteretic, so for a given recovery time and/or pacing period either alternans or a uniform response could appear. These results could explain the observation by Shkryl et al. (38) that a large increase in Ca induced by photolysis of caged Ca, coincident with the electrical impulse, when applied during a uniform response could produce a transition to alternans, and, on the contrary, when applied during calcium alternans, could result in a uniform response. In fact, we found that a slow recovery from inactivation of the RyR2 suffices to explain all the results observed experimentally by Shkryl et al. (38). A different Ca response is obtained during alternans if a stimulation is given after a long or short calcium transient (Fig. 6). In our simulations, it is clearly demonstrated that the responsibility for this is not an incomplete Ca loading, since SR Ca reaches its equilibrium value on a fast time scale (Fig. 6D). Rather, a slow recovery results in a lower level of available RyR2 after a large calcium transient. If the premature stimulation is given during the transient (similar to PICR stimulation) (38), the calcium response depends on the exact timing of the stimulus. At the beginning of the transient, the level of available RyR2s is larger for a large rather than for a small transient, resulting in a larger response in the former case (Fig. 7A). However, during the large transient a bigger number of RyR2s are inactivated, so very rapidly the response with an extra stimulation becomes so small that a bigger number of RyR2s are inactivated, thus a lack of available calcium to refill the SR at the next stimulation (Fig. 4C). This gives rise to SR Ca content oscillations during alternans, even if SR Ca fluctuations are not an essential ingredient for it. For instance, in Fig. 4C the alternans shown in ventricle is caused by RyR2 refractoriness but, nevertheless, results in a strong SR Ca content fluctuation. By the contrary, in our atrial model alternans appears first in the intracellular calcium transient, whereas the junctional SR Ca release presents very small beat-to-beat oscillations. Hence, at the subsarcolemma the oscillations are typically smaller than those found in ventricular cells and mostly due to the beat-to-beat change in concentration gradient with the interior of the cell. In this situation, the calcium transport across the cell membrane is nearly the same at every beat (Fig. 4A). Thus the amount of available calcium at every beat within the cell remains almost constant. If SERCA is fast enough, this makes it possible to refill the SR to the same value at every beat. Interestingly, a modification of the relative volumes of the JSR and CSR in our model produces alternans similar to those in ventricle, with alternations in the transmembrane Ca$^{2+}$ flux (Fig. 4C), as one would expect in atrial cell with t-tubules. Our results suggest thus an important role of transmembrane currents in determining the presence or not of SR Ca oscillations during alternans. One should notice, however, that there are experiments in ventricular cells that show alternans without appreciably SR Ca content fluctuations (31). A possible explanation, suggested by Picht et al. (31), would be related to a small $I_{NaCa}$ mediated Ca removal compared with SR reuptake, which would permit to reach the same level of SR Ca concentration at every beat.

**Limitations.** The current model, being a whole cell model, cannot account for subcellular properties, i.e., a spatial distribution of t-tubules within the cell that could give rise to subcellular discordant or inhomogeneous alternans. It does not reproduce voltage and calcium discordant alternans, as observed in several experiments (25).

**Conclusions**

In this article we have studied the onset of alternans in a human atrial model. To obtain alternans, we have modified a previous model (30), introducing a further compartment, the subsarcolemmal space, and modifying the dynamics of the RyR2. With these modifications we find alternans at constant SR Ca content and study the possible mechanisms for this. We have shown that a slow recovery from inactiv-
calcium alternans in this case, and further demonstrated it by comparison with recent experiments. This stresses the importance of studying the dynamics of the RyR2 to understand arrhythmias and, in particular, the origin of RyR2 refractoriness that gives rise to calcium alternans.

### APPENDIX

Here we present all the currents that have been changed with respect to the original model by Nygren et al. (30), following its same notation for coherence with the original model.

**Membrane Voltage**

\[
V_m = -I_{Na} + I_{Cal.} + I_I + I_{\text{mus}} + I_R + I_{K} + I_{K_a} + I_{B,Na} + I_{B,Ca} + I_{Na} + I_{Ca} + I_{NaCa} - I_{\text{stim}}
\]

**Intracellular Space**

\[
\frac{d[Ca^{2+}]}{dt} = \frac{I_{st} + I_{rel,d} - I_{ap}}{2FVol_i} - 0.454 \frac{dO}{dt}
\]

**JSR and CSR Spaces**

\[
\frac{d[Ca^{2+}]_{rel,k}}{dt} = \frac{I_{rel,k} - I_{rel,k} - dO_{C\text{SO},k}}{2FVol_{rel,k}} - \text{where } k = i, d.
\]

\[
\frac{dO_{C\text{SO},k}}{dt} = 0.48 \left[ Ca^{2+} \right]_{rel,k} (1.0 - O_{C\text{SO},k}) - 0.40 C_{SO,k}, \text{ where } k = i, d.
\]

**Network SR Space**

\[
\frac{d[Ca^{2+}]_{ap}}{dt} = \frac{I_{up} - I_{tr,i} - I_{tr,d}}{2FVol_{ap}}
\]

**Interspace Currents**

\[
I_{ds} = \frac{2FVol_{d} \left[ Ca^{2+} \right]_{d} - \left[ Ca^{2+} \right]_{i}}{\tau_{di}}
\]

\[
I_{st} = \frac{2FVol_{i} (\left[ Ca^{2+} \right]_{i} - \left[ Ca^{2+} \right]_{d})}{\tau_{si}}
\]

\[
I_{rel,k} = a_{rel} 2FVol_{rel,k} X_{Ok} \left( \left[ Ca^{2+} \right]_{rel,k} - \left[ Ca^{2+} \right]_{i} \right),
\]

\[
I_{rel,k} = \frac{2FVol_{rel,k} \left[ Ca^{2+} \right]_{ap} - \left[ Ca^{2+} \right]_{rel,k}}{\tau_{r}}\text{, where } k = i, d.
\]

The rest of the currents are the same as in the Nygren et al. model, with \( I_{CaP} \) and \( I_{NaCa} \) depending now on the calcium concentration at the subsarcolemmal space. The parameters of the model are given in Table A. 1.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

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