Investigation of the Mechanisms Underlying Cardiac Alternans – Insights from a Computational Study

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Abstract

Cardiac alternans is potentially associated with cardiac arrhythmia. Two forms of Cardiac alternans, action potential (AP) and Ca^{2+} transient (CaT) alternans are believed to be strongly correlated; however, the primary mechanisms underlying the occurrence of cardiac alternans are still unclear. In this study, a computational model of human ventricular cells was implemented to simulate cardiac alternans. By utilizing AP-clamp protocol, results showed that CaT alternans were generated at fast pacing rates, but absence at slow rates irrespective of AP alternans, suggesting that CaT alternans proceeded before AP alternans. By inhibiting the intracellular Ca^{2+} release from the sarcoplasmic reticulum (SR), CaT alternans were abolished, demonstrating a crucial role of the intracellular Ca^{2+} handling dynamics in genesis of cardiac alternans. Furthermore, I_{CaL} and the SR content were analysed during the time courses of AP alternans. CaT alternans were observed without apparent association with alternating I_{CaL}, but closely related to the alteration of the SR content. Our simulation data suggests that the leading cause of cardiac alternans in the human ventricle is CaT alternans, while AP alternans is the secondary result.

1. Introduction

Cardiac alternans, whose major clinical manifestation is T-wave alternans, is an important sign to predict cardiac arrhythmia including ventricle fibrillation (VF). At cellular level, cardiac alternans manifest as cyclical oscillations in action potential duration (APD), the magnitude of Ca^{2+} transient and the amplitude of muscle contraction that has been proved to be highly related to CaT. It is generally believed that APD and CaT alternans have a tight link between each other through a complicated feedback mechanism, mainly connected by intracellular Ca^{2+} concentration. Due to the bidirectional coupling of AP and CaT, investigating which one ($[Ca^{2+}]_i \rightarrow V_m$ or $V_m \rightarrow [Ca^{2+}]_i$) is the primary mechanism for cardiac alternans becomes an interesting scientific issue, which has not been fully elucidated to date.

$V_m \rightarrow [Ca^{2+}]_i$ hypothesis says that an increased pacing rate leads a decreased diastolic interval (DI), which results in some membrane currents, notably I_{CaL} and I_{Na}, not having enough time to recovery from their inactivated states before the next AP. A previous study has shown that when the slop of APD restitution is steep enough (>1 in most cases), APD alternans occurs [1]. In this case, the APD restitution is the main cause for generating APD and CaT alternans. However, there are some other studies demonstrating APD alternans when the slop of the restitution curve is lower than 1, and some others even found CaT alternans before apparently APD alternans occurred [2,3]. These studies supported that CaT alternans were caused by instable intracellular Ca^{2+} cycling process, which are the results of imbalanced SR release and uptake current. In this circumstance, $[Ca^{2+}]_i$ would influence Ca^{2+}-dependent membrane current and then affect cell membrane voltage, therefore, forming the basis for the $[Ca^{2+}]_i \rightarrow V_m$ hypothesis.

The aim of this study was to determine the potential causes of cardiac alternans by using a human ventricular cell model. Simulation results provided a great support to the $[Ca^{2+}]_i \rightarrow V_m$ theory and indicated that the SR content had great effects on the genesis of CaT alternans.

2. Methods

The O'Hara-Rudy model [4] of human ventricular cells was implemented to simulate cardiac alternans. Cardiac alternans (APD and CaT alternans) were evoked by decreasing the pacing basic cycle length (BCL) from 1000 to 245ms. Specifically, we analysed the alternative APs generated at BCL = 250ms, which were stable and had relatively obvious AP and CaT alternans.

To investigate whether AP alternans gave rise to CaT alternans or intracellular Ca^{2+} instabilities lead to the AP variations, similar AP-clamp protocols as used by Kanaporis and Blatterto were implemented in the O'Hara-Rudy model of human ventricular action potentials [5]. In this stage, APs generated by previous simulations were recorded. Alternative APs were described as AP_{CaT_Large} and AP_{CaT_Small} with large and small CaT amplitude respectively. Then they were applied to clamp the

O'Hara-Rudy model as a series of AP-waveforms using: (1) exclusively AP_{CaT_Large} - AP_{CaT_Large} protocol; (2) exclusively AP_{CaT_Small} - AP_{CaT_Small} protocol; (3) alternating AP_{CaT_Large} - AP_{CaT_Small} protocol.

In order to evaluate the impacts of intracellular Ca^{2+} dynamics on genesis of cardiac alternans, Ca^{2+} release current was inhibited in the model. Furthermore, during the time courses of cardiac alternans, I_{CaL} and the SR content were analysed to investigate their role on the formation of CaT alternans. We used the voltage clamp protocol and SR content measurement as used in [5,6] to test which one was more important in generating alternans in the human ventricular model. For voltage clamp simulations, I_{CaL} was activated by 100ms voltage step from -50 to 4mV since I_{CaL} has its peak value at 4mV in the O'Hara-Rudy model.

3. Results

3.1. Alternans generated by fast pacing

By increasing the pacing rate, the O'Hara-Rudy model began to generate the coincident beginning of beat-to-beat alternations in both APD and CaT amplitude at BCL=295ms. The differences between big and small APDs and the systolic levels of CaT increased with an increased pacing rate.

Specifically, AP and CaT alternans at BCL=250ms were recorded and compared in Figure 1. It was clearly manifested that AP and CaT had the same onset time, indicating these two alternans were strongly correlated with each other. Unlike those seen in the rabbit atrial and ventricular cells [5], the simulation with the human ventricular model showed insignificant difference of AP morphologies between AP_{CaT_Large} and AP_{CaT_Small}, with a small notch presented in AP_{CaT_Large} but not manifested in AP_{CaT_Small}. The measured APD of AP_{CaT_Large} was 215ms and that of AP_{CaT_Small} was 203ms, with a ratio of 1.06. However, the ratio of peak Ca^{2+} transient amplitude was about 2.48, which was much more significant.

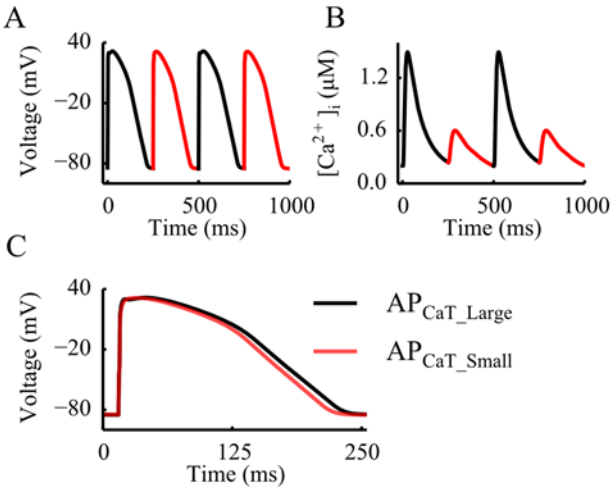

Figure 1. Simultaneously recorded APs (A) and CaTs (B) at BCL=250ms. (C): Superimposed AP traces recorded during large and small amplitude CaTs.

3.2. Primary mechanism underlying cardiac alternans

By using AP clamp protocols described in Section 2, we firstly clamped the cell membrane with continuous identical AP waveforms recorded previously (AP_{CaT_Large} - AP_{CaT_Large} and AP_{CaT_Small} - AP_{CaT_Small} pacing protocols) at fast and slow pacing rates respectively.

Figure 2 shows that both of the simulation data manifested the presence of CaT alternans at the fast pacing rate (4Hz, A-C) and absence of CaT alternans at

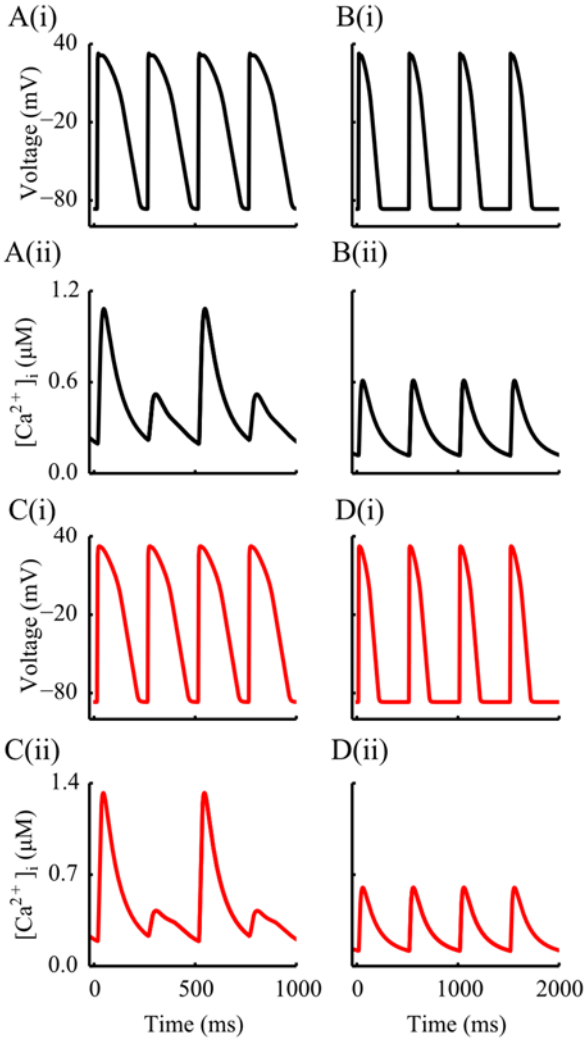

Figure 2. Results of identical AP-clamp of AP_{CaT_Large} - AP_{CaT_Large} protocol (A-B) and AP_{CaT_Small} - AP_{CaT_Small} protocol (C-D) at BCL=250ms (A-C) and BCL=500ms (B-D).

the slow pacing rate (2Hz, B-D) without any changes of AP properties. These results demonstrated that APD variations were not the requirements for the alternation in the intracellular CaT. That implied that CaT alternans could occur when AP alternans were absent.

Further simulations were conducted with the AP_{CaT_Large} - AP_{CaT_Small} clamp protocol being applied to the O'Hara Rudy model. Figure 3 shows the results of altered AP-clamp simulations at different BCLs. At higher pacing rate (4Hz, A(i-ii)), CaT alternans was induced reliably and CaT amplitude showed small – large alternans in response to AP_{CaT_Large} - AP_{CaT_Small} protocol. As the pacing rate decreased, the systolic CaT declined and the ratios of large and small CaT reduced, despite the alternating AP voltage commands in ventricular myocytes. At a lower pacing rate (1Hz, C(i-ii)), beat-to-beat change in the peak CaT almost disappeared, reproducing the result of the experimental study [5].

Experimentally both of in-phase (AP_{CaT_Large} and

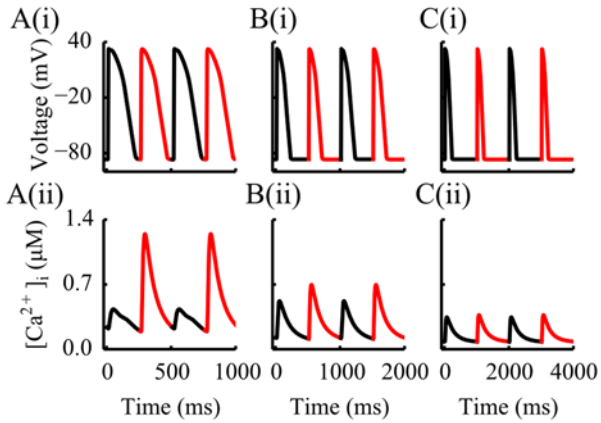

Figure 3. Results of alternans AP-clamp simulation at BCL = 250(A(i-ii)), 500(B(i-ii)), 1000ms(C(i-ii)).

AP_{CaT_Small} are consistent with the large and small CaT respectively) and out-of-phase (AP_{CaT_Large} triggered a small amplitude CaT and vice versa) alternans conditions were observed in rabbit atrial and ventricular myocytes [5]. However, in our simulations, only out-of-phase alternans were observed.

In summary, simulation results of AP-clamp strongly support the $[Ca^{2+}]_i \rightarrow V_m$ hypothesis because CaT alternans could be elicited irrespective APD alternans whereas APD alternans could not induce CaT oscillation at slow pacing rates.

3.3. Influence of Ca handling processes

For further demonstrating $[Ca^{2+}]_i \rightarrow V_m$ hypothesis, Ca^{2+} release current from the SR was inhibited in the O'Hara-Rudy model. Results showed that inhibiting the SR release current by 100% completely eliminated both APD alternans and CaT alternans, suggesting that APD

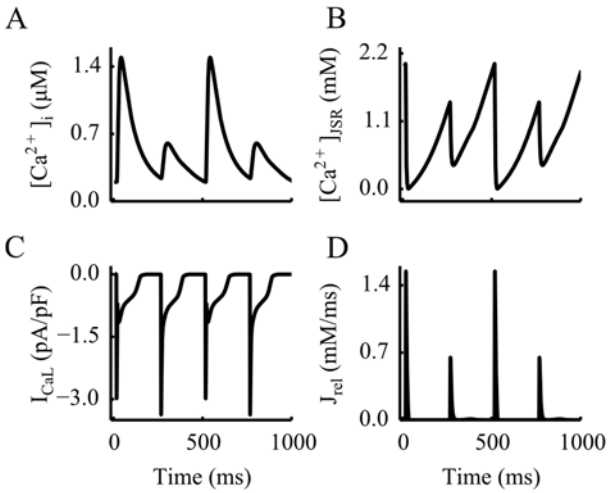

Figure 4. Time traces of CaT (A), the SR content (B), I_{CaL} (C) and Ca^{2+} release from the SR (D) during the time course of AP alternans.

alternans was driven by CaT alternans.

In order to understand the functional impact of Ca^{2+} handling on genesis of CaTs, in Figure 4, the time traces of CaT (Figure 4A), the SR content (Figure 4B), I_{CaL} (Figure 4C) and Ca^{2+} release from the SR (Figure 4D) were plotted during the time course of AP alternans. Corresponding to beat-to-beat alternans in CaT, significant alterations in the SR content and SR release were observed. However, I_{CaL} (Figure 4C) manifested slightly changes in the amplitude and even showed an opposite alternative trend to the CaT's, in which a small CaT correlated to a large I_{CaL}.

The role of I_{CaL} in the genesis of the alternans was further analysed by the voltage clamp protocol applied to O'Hara-Rudy model as described in Section 2. In Figure 5 A(i-ii), the recorded two consecutive I_{CaL} current traces and $[Ca^{2+}]_i$ when cytosolic CaT started to alter were superimposed together. It was shown that apparent CaT oscillations were not correlated with obvious I_{CaL} alternans. This result suggested that even square pulsed voltage could induce CaT alternans and I_{CaL} played a relatively small role in the genesis of CaT variations. Although the simulated CaT oscillation was not stable and disappeared in long run, the results provided proof-of-concept to the hypothesis that CaT alternans is not a consequence of I_{CaL} and APD alternans.

In contrast, CaT alternans showed a strong correlation with the SR content and release alternans as shown in Figure 4 B and D. It was shown that a higher SR load produced a greater SR release, resulting in a larger CaT transient and vice versa. To further illustrate the importance of SR content in generating cardiac alternans, we plotted the systolic CaT as a function of SR content. Results shown in Figure 5B(i-ii) showed that the CaT alternans did not occur until the SR content was above 3.3mM (blue), and during the alternans (red) CaT-SR

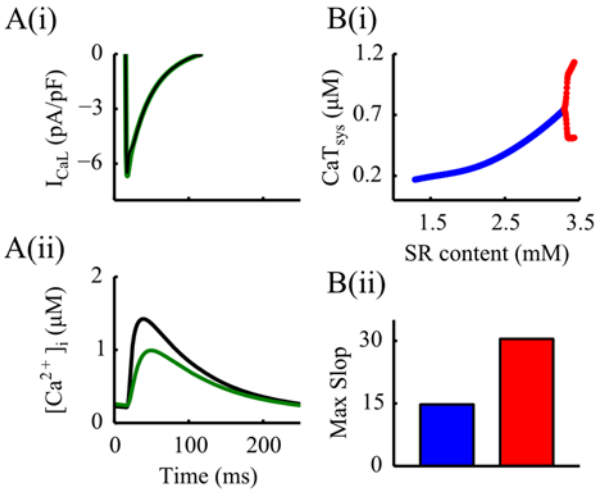

Figure 5. Superimposed two consecutive I_{CaL} current trace and $[Ca^{2+}]_i$ when cytosolic CaT started to alter (A(i-ii)). B(i): the dependence on SR content and systolic CaT; B(ii): the maximum slop of CaT-SR content curve before (blue) and after (red) CaT alternans.

content showed a stiffer relationship as the maximum slop of the curve was much more steeper than that in non-alternans condition. This suggested that after the SR content reached a threshold, a small variation in the SR content produced a big difference in CaT amplitude. When myocytes were fast paced, the balance between the SR release and uptake was disturbed. Due to the stiffer relationship between CaT-SR content, a small variation in the SR content gave rise to significant CaT alternans eventually.

4. Discussion and conclusion

In this study, we used O'Hara-Rudy model of human ventricular cells to investigate the primary mechanism underlying cardiac alternans. Simulation results showed that APD alternans and CaT alternans were correlated strongly to each other. The use of the AP-clamp approach enabled us to dissect CaT changes from AP variations, so that V_m could be treated as an independent variable and $[Ca^{2+}]_i$ was considered as a dependent function of V_m. The series of AP-clamp simulations manifested two key results: CaT alternans developed at fast pacing condition in the absence of APD alternans; CaT alternans was markedly reduced at slow pacing rates, regardless of the existence of AP alternans. These data demonstrated that only APD changes were insufficient to generate CaT alternans, thus strongly suggesting a $[Ca^{2+}]_i \rightarrow V_m$ coupling theory for the mechanism of electromechanical alternans.

Furthermore, our SR Ca^{2+} release inhibition simulation showed that suppression SR Ca^{2+} release current eliminated beat-to-beat oscillation in both APD and CaT amplitude, indicating a crucial role of intrinsic properties of intracellular Ca^{2+} handling dynamics in genesis of cardiac alternans. In contrast, analysis of I_{CaL} during control alternating time and voltage clamp simulation suggested that I_{CaL} had limited effect on the genesis of CaT alternans, but the SR content fluctuation was strongly linked to CaT oscillations.

In conclusion, our simulation data indicates that it is the CaT alternans that forms the major cause of cardiac alternans in the human ventricle, whereas AP alternans is the secondary result. These simulation findings agree with experimental study of Kanaporis and Blatterto [5], adding a theoretical basis for understanding the mechanism of cardiac alternans.

Acknowledgements

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