

Effects of CaMKII Regulation on Atrial Action Potential under Oxidative Stress Condition

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Abstract

Previous studies have demonstrated that oxidative stress is closely associated with cardiac arrhythmias via altering electrical activity and intracellular calcium dynamics of cardiac myocytes. The present study developed a human atrial cell model including effects of oxidative stress by incorporating reactive oxygen species (ROS)-induced CaMKII activation and its downstream effects on different ionic channels. The CaMKII dynamics was mimicked by a novel 6-state Markov chain model, including both autophosphorylation and oxidation pathways of CaMKII activation. The effects of activated CaMKII on L-type Ca^{2+} channels (LCCs), sodium channels and other channels was simulated based on previous approaches developed by O'hara et al. and incorporated ryanodine receptors (RyRs) and phospholamban (PLB) from Soltis et al.

Simulation results show that highly activated CaMKII by massive ROS phosphorylated L-type Ca^{2+} current (I_{CaL}) and elevated intracellular calcium concentration, which eventually resulted in calcium overload in sarcoplasmic reticulum in the condition of oxidative stress. Meanwhile, calcium overload resulted in increase of calcium release and cytoplasmic calcium concentration, which triggered afterdepolarizations (DADs) by increasing the calcium extrusion via the Na^+ - Ca^{2+} exchanger (NCX) current. This study revealed effects of massive ROS on calcium cycling in atrial myocytes and shed lights on mechanisms of cardiac arrhythmias induced by oxidative stress.

1. Introduction

Several experiments have revealed that Calcium-

calmodulin dependent kinase II (CaMKII) is a critical player in promotion of cardiovascular disease and arrhythmia phenotypes [1]. CaMKII can regulate membrane ion channels and Ca^{2+} -handling proteins in cardiac myocytes and is the central mediator of excitation-contraction coupling. CaMKII is activated by binding of Ca^{2+} -bound calmodulin and can keep persistent activity through autophosphorylation and oxidation. CaMKII activation under oxidation has become a biomarker and proarrhythmic signal for connecting ROS and arrhythmia.

Experiment has shown that increased CaMKII expression results in hyperphosphorylation of RyR [2] that can promote diastolic sarcoplasmic reticulum Ca^{2+} release and leak with the potential to trigger after-depolarization (DAD). However, the effect of CaMKII on atrial action potential under oxidative stress condition is not completely understood. In this study, we developed a computational model of human atrial cell including CaMKII oxidative activation to investigate influences of CaMKII on human atrial electrophysiology.

2. Methods

CaMKII activation model developed by Zhang et al [3] was incorporated into a human atrial model developed by Grandi et al [4] to simulate CaMKII activation by autophosphorylation and oxidation. The parameters of the CaMKII autophosphorylation pathway were based on the model developed by Chiba et al [5]. The parameters of oxidation pathway were fitted based on the experimental data measured by Erickson et al [6]. To simulate the effects of CaMKII on ion channels, we modified the equations of corresponding membrane currents using the method proposed by O'hara et al [7]. In this approach, the effect of CaMKII on a single current was simulated using Hill

equation and, therefore, the amplitude and/or time constant of ion current were modified based on experiments.

RyR and PLB are very important regulatory protein on the SR to control calcium cycling. RyR can be phosphorylated by activated CaMKII, increasing RyR opening probability and SR leak. PLB phosphorylated by activated CaMKII can reduce SERCA inhibition, increasing SR pump calcium sensitivity during diastole. Thus, we incorporated RyR and PLB model developed Soltis et al [8]. The rate constants which controlling RyR opening probability and SR leak was modified under CaMKII activation. The altered rate constant increased calcium sensitivity of RyR and amplified RyR opening. Also, the equation of SR leak, which is associated with RyR opening at the diastolic phase, was modified under CaMKII activation. The Thr17 of PLB can be phosphorylated by CaMKII to increase SERCA pump calcium sensitivity which means decreasing the forward pump rate. This phenomenon was mimicked by modifying half maximal saturation constant of SERCA.

To investigate the effect oxidative CaMKII activation on the electrophysiology of cells, a dynamic stimulation process was used in the simulation. The stimulation frequency was selected as 0.5Hz, 1Hz and 2Hz to investigate the frequency dependence of CaMKII activation. Action potential were stimulated by a stimulus current of -12.5pA/pF for 0.5ms. The time step of simulation was 0.1ms. Under normal conditions, the level of H_2O_2 in human blood is about $\sim 35\mu\text{M}$. When ischemic reperfusion injury or heart failure occurred, the level of ROS could be increased by 100 times. Therefore, the oxidative stress was simulated using $200\mu\text{M}$ H_2O_2 in the model.

3. Results

3.1. Frequency dependent CaMKII activation

Previous experiment has shown that the activation rate and maximum activation level of CaMKII increase as frequency increases [9]. The frequency-dependent characteristics of CaMKII were investigated in the model without ROS. Fig. 1 shows the simulation results of activation level of CaMKII at 0.5Hz, 1Hz and 2Hz (Fig. 1A). Meanwhile, the activation level of RyR2 in the same condition (Fig. 1B) was recorded. The results showed that the slope of CaMKII activation curves increased with enlarging frequency. The RyR activation level showed a similar behaviour with increasing frequency, indicating a positive correlation between CaMKII activation and RyR opening probability. These results agreed with previous RyR phosphorylation model developed [10], demonstrating that CaMKII activation has a significantly influence on SR Ca^{2+} release and leak.

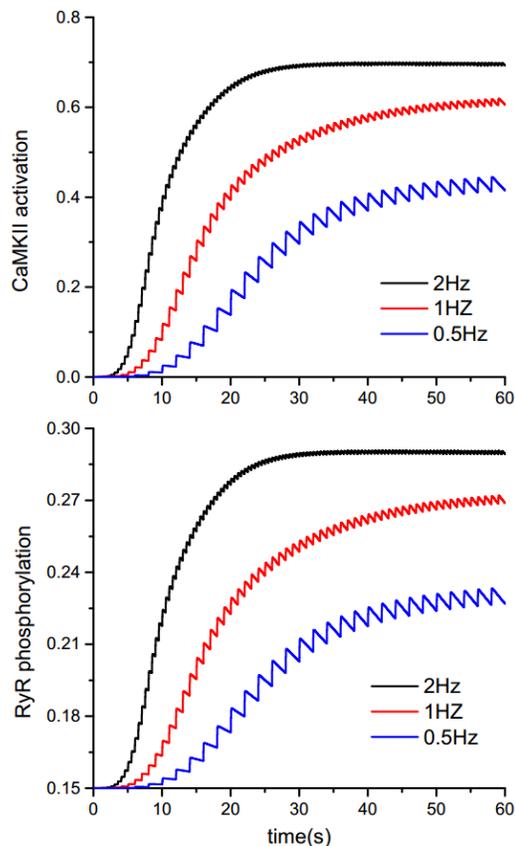


Figure 1. Simulation results of CaMKII activation and RyR phosphorylation from initial condition to stable state at 0.5Hz, 1Hz and 2Hz in the 60s.

3.2. H_2O_2 induced DADs

According to simulation results, a DAD was triggered in the action potential in conditions of elevated oxidative stress (Fig. 2). Due to the frequency-dependent CaMKII activation, H_2O_2 -induced DADs showed the rate dependence as well, which indicated more significant DADs were induced at higher frequency of stimulation. In this study, we chose to intercept part of the periodic data in the case of 0.5Hz to analysis the generation of DAD.

We inhibited NCX current at 0.5Hz with the presence H_2O_2 and to examine its contribution to H_2O_2 induced DAD (Fig. 3). The result shows that DAD did not occur with inhibiting NCX current, which is consistent with the experimental findings [11]. Since the mode of NCX current is determined by the concentration gradient on both sides of membrane, the increase of intracellular calcium promoted the forward transport of NCX. Meanwhile, CaMKII did not directly change NCX. Therefore, the release of calcium from SR to the cytoplasm (Fig. 2F-H) during phase 4 of AP induced the inflow of NCX, which leads to depolarization of the membrane potential and production of DAD.

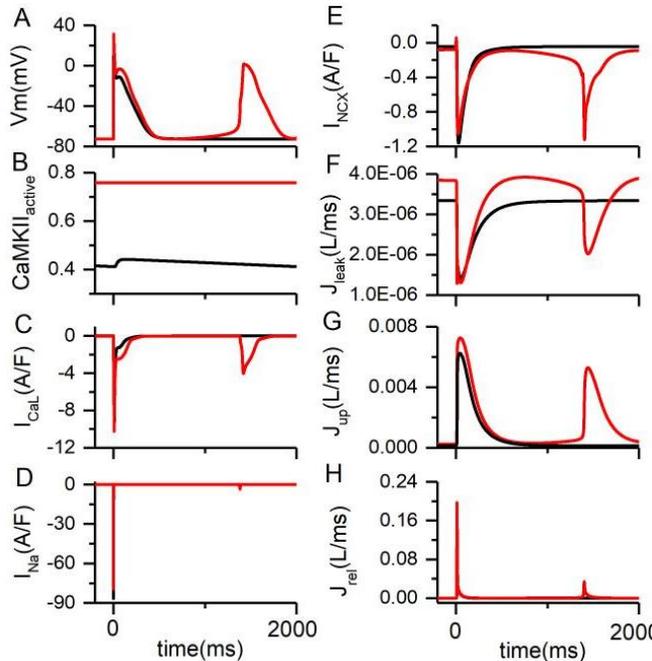


Figure 2. Simulated AP (A), CaMKII activation fraction (B) and CaMKII regulated current (C, D, E) and Ca^{2+} flux (F, G, H) under both control (Black curves) and oxidative stress condition (red curves).

CaMKII can regulate numerous ion channels and proteins in the membrane and intracellular, including I_{CaL} , I_{Na} and Ca^{2+} cycling related proteins. The simulation results showed that oxidative stress increased CaMKII activation, increased I_{CaL} and altered Ca^{2+} cycling (Fig. 2). Therefore, we simulated the action potential under inhibition of CaMKII effect on single ion channel. In Fig. 4A, DAD occurrence has been greatly suppressed when inhibiting CaMKII effect on I_{CaL} . In addition, the DAD amplitude is reduced after inhibiting CaMKII effect on SR calcium release or pump (Fig. 4B and C). But inhibiting CaMKII effect on SR calcium leak makes the occurrence of repetitive DADs (Fig. 4D). This case indirectly indicated that SR Ca^{2+} overload causes the generation of DADs. CaMKII activation has been shown to increase RyR opening probability and SR leakage. Inhibiting CaMKII effect on SR leak reduced the amount of leaked calcium which means it is easier for accumulate of calcium concentration in SR to overload, and leading to repetitive DAD phenomenon. CaMKII indirectly regulates SR calcium release, pump and leak in the Ca^{2+} cycling by phosphorylating of RyR. It was shown that DAD was eliminated when the level of RyR phosphorylation was clamped at normal level (Fig. 4 E). The simulation results showed that DAD was suppressed obviously. From the above results, it indicated that CaMKII regulated I_{CaL} and RyR could be important targets for eliminating the abnormal calcium cycling and DAD.

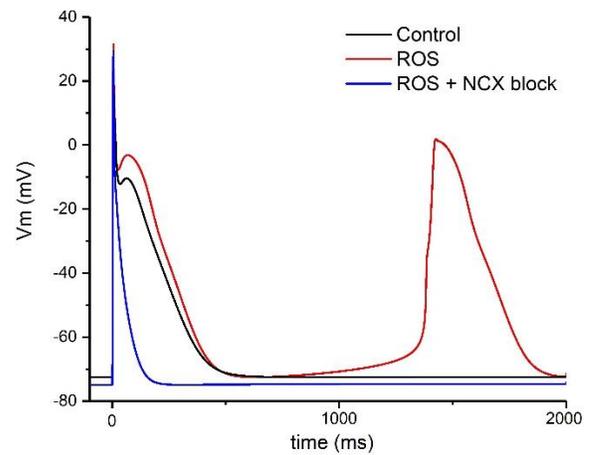


Figure 3. In oxidative stress condition, simulated AP in the 0.5Hz with NCX block. Note that no DAD occur in the NCX block condition.

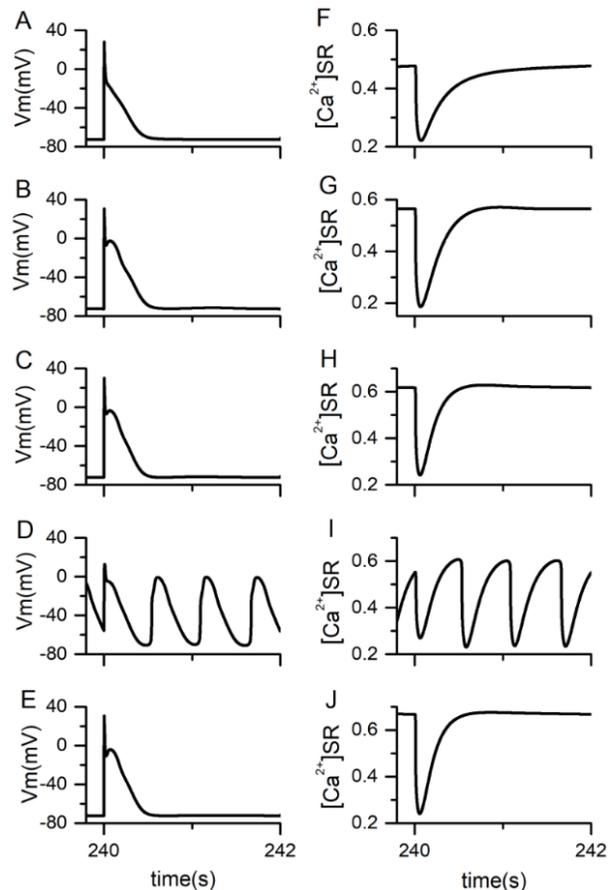


Figure 4. Simulated AP and SR Ca^{2+} concentration with inhibition of CaMKII effect on I_{CaL} (A, F), SR uptake (B, G), SR release (C, H), and SR leak (D, I) as well as with clamped RyR phosphorylation (E, J) in oxidative stress condition.

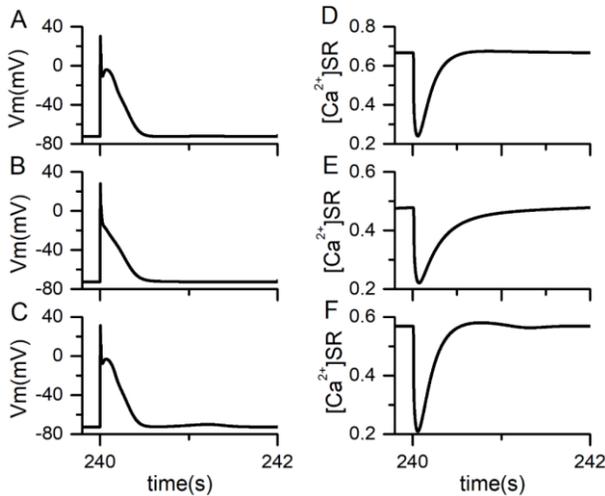


Figure 5. In oxidative stress condition, simulated AP and SR Ca^{2+} concentration under the conditions of CaMKII affecting I_{CaL} (A), RyR (B) only and both (C) respectively.

In Fig. 5, with CaMKII regulating I_{CaL} and RyR alone, there was no obvious DAD in action potential. However, when CaMKII regulated both I_{CaL} and RyR, there was an obvious DAD abnormality in action potential. I_{CaL} was not able to sufficiently increase the concentration of calcium in cytoplasm to induce DAD. The combined effect of I_{CaL} and RyR contributed to the elevated calcium concentration in cytoplasm and caused DAD generation.

In conclusion, the oxidative pathway of CaMKII significantly increased CaMKII activation and the RyR opening probability in oxidative stress condition. PLB phosphorylated by activated CaMKII reduced the inhibitory effect on SR pump, which in turn increased calcium uptake capacity of SR pump. These effects increased the calcium concentration of SR and led to SR calcium overload finally. Calcium overload produced a spontaneous calcium in phase 4, stimulating the inflow of NCX current on the cell membrane and leading to the occurrence of DAD.

4. Conclusion

In this study, we developed a human atrial computational model including CaMKII oxidation and revealed the mechanism of DAD induced under oxidative stress condition. The simulation results indicated that I_{CaL} and RyR regulated by CaMKII in oxidative stress condition primarily contributed to DAD generation, as either of them regulated by CaMKII is insufficient to cause H_2O_2 -induced DAD.

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