

# Calcium Calmodulin Dependent Protein Kinase II (CaMKII) Contribute to Arrhythmias after Acidosis: Simulation Study

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## Abstract

*In this paper, to analyze the functional influence of acidosis on cardiac electrical activity and subsequently on ventricular arrhythmia, a human ventricular acidotic model with PH and CaM kinase II (CaMKII) regulation was developed. Dynamic changes of ionic currents and action potentials during the acidosis process were simulated, and the changes of action potential of different conditions (with CaMKII and without CaMKII) were compared during acidosis. In addition the acidosis-induced changes of electrocardiogram waveform were computed using the one-dimensional tissue model. The experimental results showed that in the process of acidosis, CaMKII was highly activated, the concentration of both sodium and calcium within the cell elevated. Especially, at the early stage of the post acidosis, delayed afterdepolarizations (DADs) were generated in the cellular membrane potential, but DADs would disappear when eliminating the effect of CaMKII on all ion currents. At last, the triggered activities induced in cells during post acidosis period caused ectopic depolarization and ectopic repolarization in the cardiac tissue. Meanwhile, the simulated electrocardiogram showed premature ventricular contractions.*

## 1. Introduction

In a number of pathological conditions, myocardial cells become acidic. Acidosis affects the response of  $\text{Ca}^{2+}$  to muscle filaments and the process of excitation-contraction coupling, including muscle membrane and sarcoplasmic reticulum ion flows [1]. These changes can reduce the contractility of myocardial cells, causing arrhythmias. Most importantly, at the early stage of the post acidosis, triggered activities induced in the cellular membrane potential could cause arrhythmias, such as ventricular tachycardia or ventricular fibrillation. However, the underlying mechanisms of arrhythmias are incompletely understood yet. This study aims to investigate mechanisms responsible for the genesis of post acidosis

arrhythmias.

Electrocardiogram (ECG) is one of the most powerful tools in the diagnosis of cardiac arrhythmias [2]. Therefore, it is of great importance to establish a relationship between electrophysiological changes of cells and ECG waveform during acidosis.

To solve the above problems, we take into account of pathophysiological consequences of acidosis: reduced PH and highly activated CaMKII, and develop a human ventricular acidotic model with PH and CaMKII regulation. And based on this model, we further established 1-dimensional tissue model. Using these models, we simulated the changes of ionic currents, action potentials and the ECG waveform during the acidosis process.

## 2. Methods

### 2.1. Acidotic model

In order to study the human arrhythmia during the process of acidosis, we developed a human ventricular acidotic model with PH and CaMKII regulation based on TNNP06 model [3]. The model can be described by a first-order differential equations:

$$C_m \frac{\partial V_m}{\partial t} = -(I_{\text{stim}} + I_{\text{ion}}) \quad (1)$$

$$I_{\text{ion}} = I_{\text{Ks}} + I_{\text{Kr}} + I_{\text{Kl}} + I_{\text{to}} + I_{\text{Na}} + I_{\text{bNa}} + I_{\text{CaL}} + I_{\text{bCa}} + I_{\text{NaK}} + I_{\text{NaCa}} + I_{\text{pCa}} + I_{\text{pK}} + I_{\text{NaL}} \quad (2)$$

where  $V_m$  is transmembrane potential,  $C_m$  is the membrane capacitance,  $I_{\text{ion}}$  is the sum of ionic currents,  $I_{\text{stim}}$  is the externally applied stimulus current.

In the case of acidosis, the electrophysiological changes in cells include PH decreased, and CaMKII activity increased [4]. Therefore, we added the regulation of PH and CaMKII on ionic currents. The ion currents effected by both PH and CaMKII can be expressed by the following equation:

$$I_{\text{sum}} = f_{\text{CaMK}} \cdot f_{\text{PH}} \cdot I_{\text{base}} \quad (3)$$

where  $I_{\text{sum}}$  is total ion current,  $I_{\text{base}}$  is fundamental current,  $f_{\text{CaMK}}$  is the regulatory factors for CaMKII on

ionic currents, and  $f_{PH}$  is the regulatory factors for PH on ionic currents.

For the regulation of CaMKII, we integrated the dynamic activation of CaMKII model <sup>[5]</sup>,  $f_{CaMK}$  is calculated as follows:

$$f_{CaMK} = \left( 1 + \frac{IF_{CaMKII}}{1 + \frac{K_{mCaMKII}}{CaMKII_{act}}} \right) \quad (4)$$

where  $CaMKII_{act}$  represents the proportion of activation of intracellular CaMKII,  $K_{mCaMKII}$  is the Michaelis constant, and the value is 0.0015 mM.  $IF_{CaMKII}$  represents the largest increased proportion of  $I_{base}$  in the case of CaMKII activation. CaMKII effected L-type  $Ca^{2+}$  channel ( $I_{CaL}$ ), RyR2 receptor ( $I_{rel}$ ), SERCA2a ( $I_{up}$ ), late  $Na^+$  current ( $I_{NaL}$ ), and transient Outward Current ( $I_{to}$ ) <sup>[5]</sup>. The values of  $IF_{CaMKII}$  are shown in table 1<sup>[5]</sup>, for the currents not affected by CaMKII,  $f_{CaMK} = 1$ .

**Table 1 Model parameters of CaMKII regulation**

| Targets   | $IF_{CaMKII}$ |
|-----------|---------------|
| $I_{CaL}$ | 0.25          |
| $I_{rel}$ | 0.05          |
| $I_{up}$  | 0.45          |
| $I_{to}$  | 0.08          |
| $I_{NaL}$ | 0.20          |

**Table 2 Model parameters of PH regulation (PH=6.7)**

| Target s  | $f_0$ | $n$    | $PK$  | $f_{PH}$ |
|-----------|-------|--------|-------|----------|
| $I_{CaL}$ | 1.110 | 1.530  | 6.520 | 0.720    |
| $I_{rel}$ | 1.110 | 1.870  | 6.640 | 0.627    |
| $I_{up}$  | 3.710 | 1.140  | 7.530 | 0.377    |
| $I_{NCX}$ | 2.650 | 0.990  | 7.370 | 0.472    |
| $I_{NaK}$ | 1.430 | -0.860 | 6.720 | 0.700    |
| $I_{K1}$  | 1.430 | -1.410 | 6.890 | 0.500    |
| $I_{to}$  | 1.430 | -0.860 | 6.720 | 0.700    |

For the regulation of PH, the  $f_{PH}$  can be described by the equation (5) [6].

$$f_{PH} = \frac{f_0}{1 + 10^{n(-PH+PK)}} \quad (5)$$

where  $f_0$  represents the largest increased proportion of

$I_{base}$  with PH regulation,  $n$  is Hill constant, and  $PK$  is dissociation constant. Under normal circumstances (PH=7.15),  $f_{PH}=1$ ; in the case of acidosis (PH=6.7), the values of  $f_0$ ,  $n$ ,  $PK$  are shown in table 2<sup>[7]</sup>. In addition, currents effected by PH include:  $I_{CaL}$ ,  $I_{to}$ ,  $I_{rel}$ ,  $I_{up}$ , inward rectifier potassium current ( $I_{K1}$ ), and sodium calcium exchange current ( $I_{NCX}$ ), sodium potassium exchange current ( $I_{NaK}$ )<sup>[7]</sup>.

## 2.2. Multicellular model

Electrical excitation conducted through the ventricular tissue by the electric coupling between the cells, which could be described as the following nonlinear reaction- diffusion equation:

$$\frac{\partial V_m}{\partial t} = -\frac{I_{ion} + I_{stim}}{C_m} + \nabla \cdot (D \nabla V_m) \quad (6)$$

where  $\nabla$  is Gradient operator,  $D$  is the diffusion tensor, and the other parameters are the same with equation (1).

In addition, the ECG (pseudo-ECG) formulation is described as follows:

$$\phi_e(x', y', z') = \frac{s^2 \sigma_i}{4\sigma_e} \int (-\nabla V_m) \cdot \left[ \nabla \frac{1}{r} \right] dr \quad (7)$$

where  $r = \sqrt{(x-x')^2 + (y-y')^2 + (z-z')^2}$ ,  $\nabla V_m$  is the

is spatial gradient of  $V_m$ , and  $\frac{s^2 \sigma_i}{4\sigma_e}$  is a constant,

reflecting the conductivity of tissue. In this paper, the virtual electrode is placed on the right of the 1D model, 2cm from the epicardial cells.

## 2.3. Experimental method

To simulate the process of cells and tissues from normal to acidosis then to post acidosis, firstly, we set PH to 7.15 (normal) and applied current stimulation to cells or tissues for 1 minute. Secondly, after action potential was stable, we changed the PH to 6.7 (acidosis) and continuously applying current stimulation for 6 minutes. Thirdly, we return PH to 7.15 for 6 minutes. Throughout the process, we used a fixed stimulation cycle (850 ms, about 70 times per minute, closely to human heart rate), and recorded the changes of electrophysiological activity of cells and tissue.

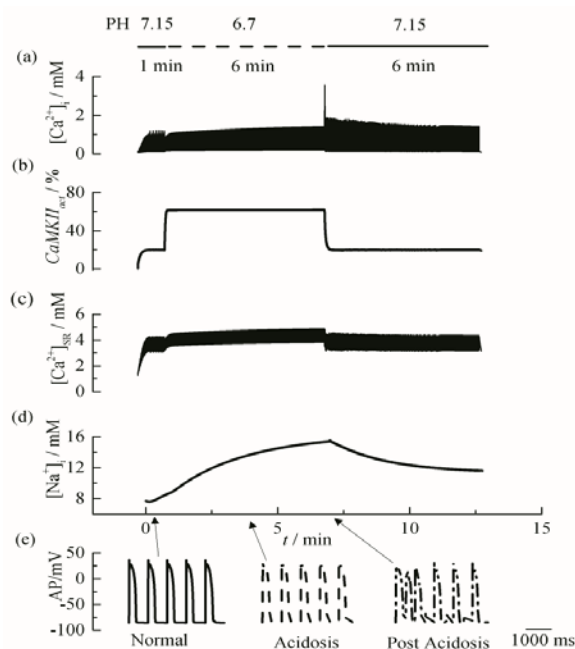
Finally, we Compared the APs,  $[Ca^{2+}]_{SR}$  and ECG between with CaMKII regulation and without CaMKII regulation at the early stage of the post acidosis.

### 3. Results

#### 3.1. Cellular simulation

The changes in action potential and ion concentration of EPI are shown in Figure 1, where, (a-d) represent the dynamic changes sequences of  $[Ca^{2+}]_i$ , fraction of activated CaMKII ( $CaMKII_{act}$ ),  $[Ca^{2+}]_{SR}$  and  $[Na^+]_i$  from normal, acidosis to post acidosis respectively, (e) represents action potentials under normal (solid), acidosis (dash) and post acidosis (dash dot).

Figure 1 shows that during the acidotic period, with elevated intracellular calcium and sodium concentration, CaMKII was highly activated. Due to the effects of CaMKII on L-type  $Ca^{2+}$  channel ( $I_{CaL}$ ) and sarcoplasmic reticulum (SR)  $Ca^{2+}$ -ATPase (SERCA2a), sarcoplasmic reticulum  $Ca^{2+}$  concentration ( $[Ca^{2+}]_{SR}$ ) continuously accumulated and reached a high level ( $\sim 4.9$  mM) at the end of acidosis. When PH returned to normal (PH=7.15) after acidosis, DADs was observed.



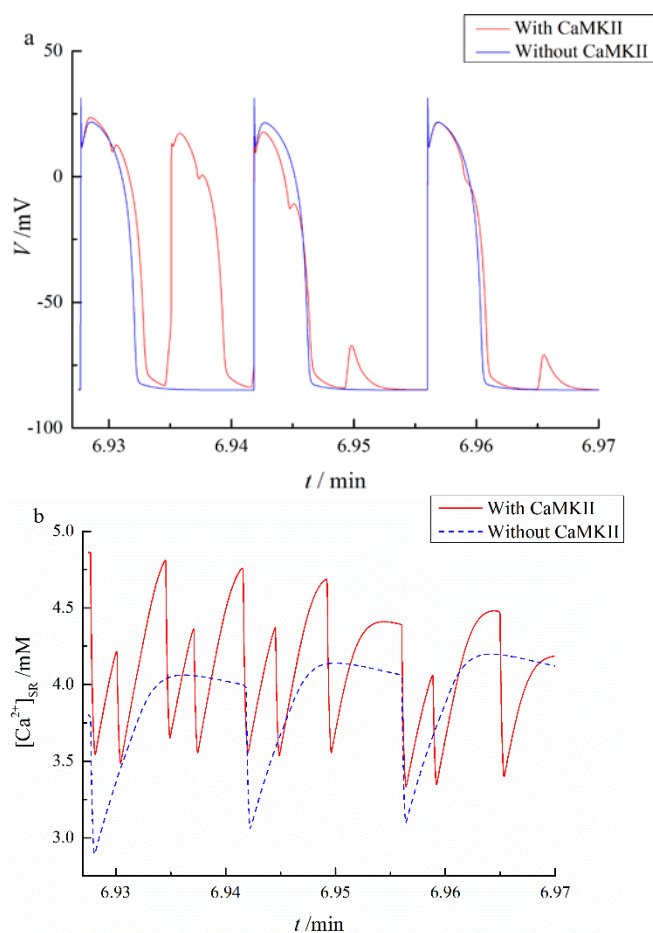
**Figure 1. Changes in intracellular ion concentration and action potentials during acidosis**

To study the effect of CaMKII on DADs induced in the cellular membrane potential at the early stage of the post acidosis, we further made a comparative experiment. During the experiment, we recorded APs of EPI and  $[Ca^{2+}]_{SR}$  at the early stage of the post acidosis (first three cycles) by keeping the regulation of CaMKII and removing the effects of CaMKII on all

ion currents. Results are shown in Figure 2.

Compared the situation with CaMKII regulation, there are no DADs induced in the cellular membrane potential when removed the effect of CaMKII on ion currents (Fig 2(a)). Similarly, consistent with the action potential, the sarcoplasmic reticulum also became to release  $Ca^{2+}$  regularly, and the  $[Ca^{2+}]_{SR}$  decreased significantly.

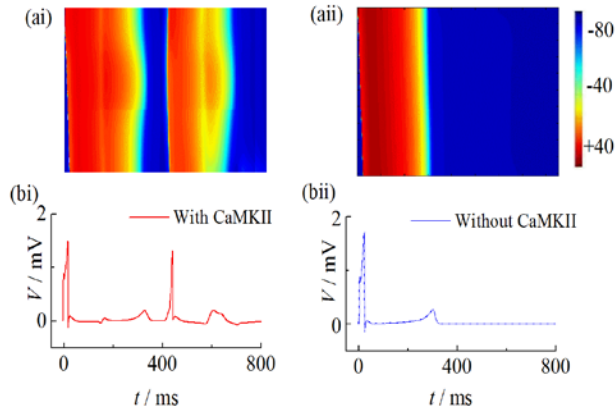
It is well known that DADs are associated with increased sarcoplasmic reticulum (SR)  $Ca^{2+}$  load [4,7]. However, CaMKII can regulate  $Ca^{2+}$  channels and  $Ca^{2+}$  receptors [4,7]. So, It can be deduced that CaMKII contribute to the  $[Ca^{2+}]_{SR}$  overload, leading to the triggered activities of cells. Therefore, CaMKII plays an important role in the generation of DADs.



**Figure 2. Changes of AP and  $[Ca^{2+}]_{SR}$  at the early stage of post acidosis.**

#### 3.2. Excitation waves and ECG

To further investigate the contact between cellular triggered activities and cardiac arrhythmias during the process of acidosis. We established a one-dimensional model to simulate the propagation of electrical waves, and calculate the Pseudo-ECG



**Figure 3. Propagation of electrical excitation wave in tissue and corresponding ECG**

Figure 3 shows that, at the early stage of post acidosis, triggered activities induced in the cellular membrane potential caused ectopic depolarization along the 1D tissue, resulting in the generation of premature ventricular contractions (PVC) and leading corresponding ECG disorders. However, the ectopic beats did not happen when the effects of CaMKII on all ion currents were removed. (Fig 3(aii, bii))

#### 4. Conclusion

In summary, during acidosis  $[Ca^{2+}]_i$  and  $[Ca^{2+}]_{SR}$  increased. Due to the increased calcium leak from sarcoplasmic reticulum, delayed afterdepolarizations in the cellular membrane potential were generated when PH return to normal. Further, the trigger activities can lead to PVC in tissue, resulting in arrhythmogenic pattern in ECG. However, all of the above phenomena disappeared when removed the regulation of CaMKII on related ion channels.

Therefore, We can conclude that the  $[Ca^{2+}]_{SR}$  overload is the main factor for cellular activity triggers. However, highly activated CaMKII is closely related to the  $[Ca^{2+}]_{SR}$  overload. Therefore, the results of this study suggest that CaMKII is one of the important targets for preventing the post acidosis arrhythmias.

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