Effects of Free Radicals on Na(+)-Ca(2+) Exchanger: A Computer Modeling

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Abstract

The aim of this work was to analyze the contribution of the Na+-Ca2+ exchanger in the generation of afterdepolarizations in a single cell by simulating the effects of free radicals in the context of ischemia-reperfusion, using a modified version of Luo and Rudy II action potential model (LRd model).

To simulate the effects of ischemia three conditions were considered: hyperkalemia, acidosis and hypoxia. Simulations were run as long as steady state of all parameters of the model were reached, and then the availability of the Na+-Ca2+ exchanger current (INaCa) was varied. The generation of Delayed After-Depolarizations(DADs) depends on the extra cellular potassium concentration, [K+]o, and on the availability of INaCa. DADs observed during reperfusion period appeared when just a percent of INaCa was used.

1. Introduction

During the ischemic period free oxygen radicals are produced. Oxygen radicals are chemical species with an unpaired electron, which makes them extremely reactive and capable of inducing oxidative modification of other molecules affecting the mechanisms of ionic transport. One of the affected mechanism is the Na^+-Ca^{2+} exchanger (NCX). NXC [1] is a transsarcolemmal protein that plays an important role in controlling levels of intra cellular calcium. NCX can operate in both a forward mode (Ca^{2+} out, Na^+ in) and reverse mode (Na^+ out, Ca^{2+} in) and it does so with a stoichiometry of 3:1 (ie, it exchanges 3 Na^+ ions for every 1 Ca^{2+} ion). As a result, NCX is electrogenic, producing an inward current (I_{NaCa}) when Ca^{2+} is extruded from the cell.

Myocardial Ca^{2+} overload is characterized by waves of spontaneous SR Ca^{2+} release that propagate through the cytoplasm generating inward I_{NaCa} and delayed afterdepolarizations (DADs). This mechanism is thought to underlie arrhythmias associated with myocardial ischemia-reperfusion [2].

Some studies report a decrease [3,4,5] and others an increase [6,7,8] of the Na^+-Ca^{2+} exchanger activity during reperfusion.

A computer model was used in order to analyze the free radicals effects on the Na^+-Ca^{2+} exchanger during ischemia-reperfusion and its contribution in generating afterdepolarizations. Simulations were carried out using a modified version of the Luo-Rudy II model [9].

2. Methods

To simulate acute ischemia three conditions were considered [10,11,12]: hyperkalemia, acidosis and hypoxia. Hyperkalemia was simulated elevating $[K^+]_o$ in the range of 5.5 – 12.5 mmol/L , Na^+ and L-type Ca^{2+} conductances were decrease by a factor from 100% of their total availability ($[K^+]_o$=4.5 mmol/L) to 50% ($[K^+]_o$=12.5 mmol/L). Na^+/K^+ current was decrease to simulate the [ATP]i depressing. The direct electrophysiological effects of anoxia are modeled by introducing $I_{K(ATP)}$ into LRd model. The formulation of this current is based on the following equation [13]:

$$I_{K(ATP)} = G_{K(ATP)} \cdot \left(\frac{[K]_o}{[K]_{o,normal}} \right)^n \cdot \frac{1}{1 + \left(\frac{[ATP]_i}{k_{1/2}} \right)^H}$$

where $G_{K(ATP)}$ is the channel conductance per cm^2 ($39x10^{-3}$ nS/cm^2), n is the power of $[K^+]_o$ dependence (n = 0.24), and $[ATP]_i$ ($[ATP]_i$=3 mmol/L) follows Hill-type formalism with $k_{1/2}$=250 μmol/L and H=2.

The $[ATP]_i$ dependence of L-type Ca^{2+} current (I_{CaL}) is simulating by the following equation:

$$P_{Ca(L),ATP} = \frac{1}{1 + \left(\frac{k_{1/2}}{[ATP]_i} \right)^H}$$

where $P_{Ca(L),ATP}$ is a fraction applied to total $I_{Ca(L)}$. The next values were used: $k_{1/2}$=1.4 mmol/L and H=2.6.

For the reperfusion process, I_{NaCa} current was depressed until an afterdepolarization was observed, while Na and L-type Ca conductances were back to their original values.

The cell was paced at 2 Hz. Both processes (ischemia and reperfusion) were simulated for 10 s to reach the steady state [14].

The Euler method was used to integrate the ordinary differential equation describing action potential, with a

time step fixed at 0.01 ms.

3. Results

During reperfusion an accumulation of $[Ca^{2+}]_i$ was observed causing a release of Ca^{2+} from the sarcoplasmic reticulum (SR) which generated an inward I_{NaCa} current meaning the NCX works in the forward mode due to the overload of Ca^{2+}. I_{NaCa} generated a depolarization during the resting potential, before the next stimulus cause a new action potential, so that I_{NaCa} induce a DAD.

The complete simulation was carried out during $4x10^4$ ms, the half of the time corresponds to the ischemic process while the last part correspond to reperfusion.

Figure 1 shows the spontaneous Ca^{2+} release from SR to myoplasm induced by overload Ca^{2+}. For this simulation we used a $[K^+]_o$ = 9.5 mmol/L, the 68.75% of I_{Na}, 80% of I_{NaK} and $[ATP]_i$ = 3 mmol/L during ischemia. During reperfusion only the 50% of I_{NaCa} was used.

Figure 2 shows the rise in $[Ca^{2+}]_i$ because of the release of Ca^{2+} from SR. The rise of $[Ca^{2+}]_i$ marked with filled-arrows is due to the release of the SR on overload Ca^{2+} conditions while the dashed –line arrows indicate the normal Ca^{2+} release (Ca^{2+}-induced Ca^{2+} release, CICR, process) from SR. These releases induced an inward I_{NaCa} current which works in the forward mode (Figure 3) trying to extrude the accumulation of Ca^{2+}.

Na^+-Ca^{2+} exchanger inward current produced a depolarization of the membrane before the stimulus did it, as figure 4 shows. So that, a delayed afterdepolarization is generated.

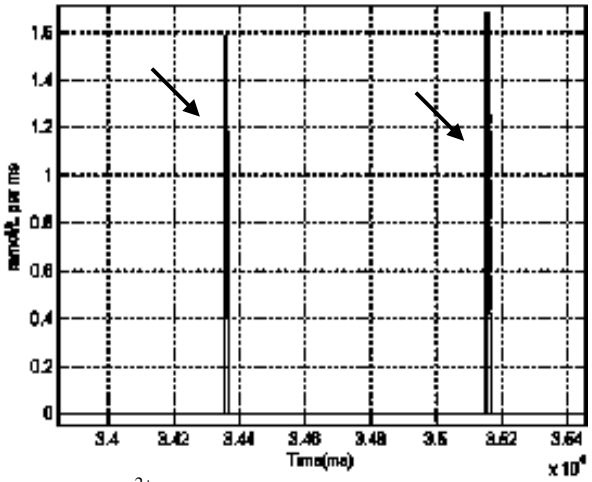

Figure 1. Ca^{2+} release from the sarcoplasmic reticulum to myoplasm due to overload Ca^{2+} conditions.

In figure 4 it is observed that a depolarization occurs when the membrane is in the resting potential (despite of the transients due to the high $[K^+]_o$ concentration). The cell was paced every 400 ms, if we take the reference of the beginning of the action potential generated in $3.4x10^4$ ms, the next action potential should be initiated in $3.44x10^4$ ms, however an afterdepolarization appeared before that time, and the initiation of this depolarization is, clearly (see figure 3), generated by the inward I_{NaCa}.

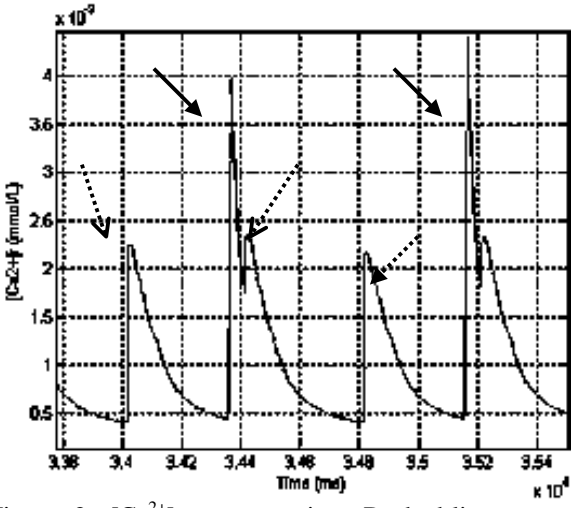

Figure 2. $[Ca^{2+}]_i$ concentration. Dashed-line arrows indicate the release of Ca^{2+} from SR in the CICR process and the full-line arrows indicate the release of SR under overload Ca^{2+} conditions.

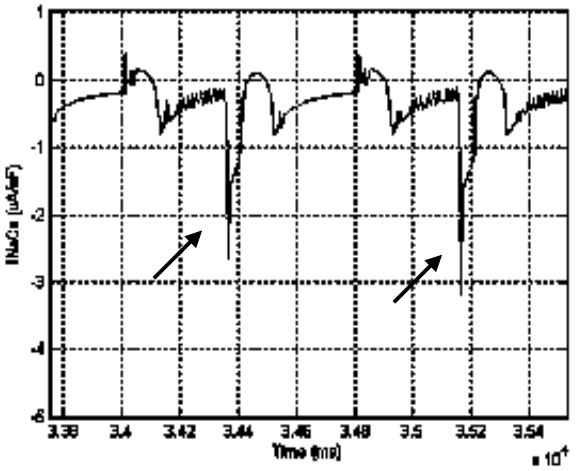

Figure 3. NCX current. Both arrows indicate the forward mode of NCX extruding Ca^{2+} and inserting Na^+, so an inward current is generated.

The both action potentials after DAD are not elicited by the Na^+ current (Figure 5) but by L-type Ca^{2+} current (Figure 6), this is because the depolarization of membrane produces an inactivation of Na^+ channels, so that, there is not enough opened Na^+ channels to induce a the inward current to produce the upstroke. The peak of action

potential induced by I_{Na} is smaller that the peak of action potential elicited by I_{CaL}.

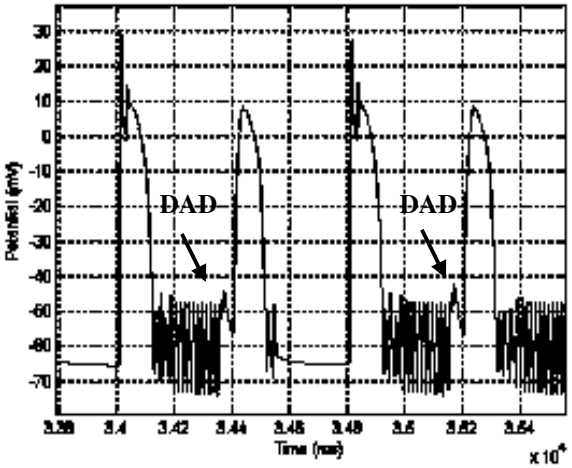

Figure 4. Action potentials during reperfusion using the 50% of I_{NaCa}. The delayed afterdepolarizations marked with the arrows are generated because of the inward I_{NaCa} working in forward mode, the exchanger try to reduce overload Ca^{2+} induced by the release from SR, extruding Ca^{2+} from cell.

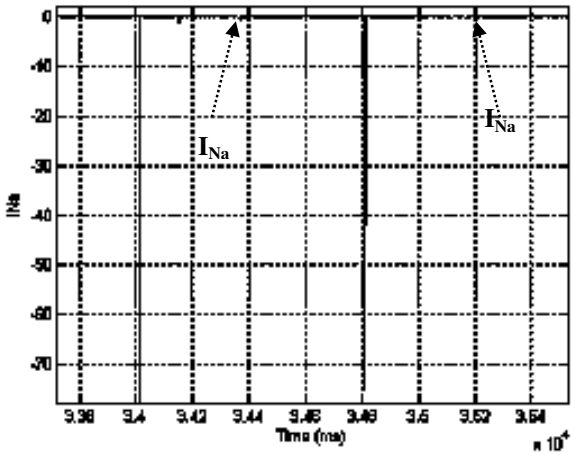

Figure 5. I_{Na} is inhibited due to the depolarization of membrane induces an inactivation of Na^+ channels, so action potentials after DADs, in figure 4, are not elicited by this current.

When I_{NaCa} was not changed, during reperfusion process, the $[Ca^{2+}]_i$ levels remained normal and no releases from SR were observed. So no overload conditions were presented and Na^+-Ca^{2+} exchanger did not produce an inward current that could depolarize the cell membrane, then it worked in right way and there were not DADs as can see it in figure 7.

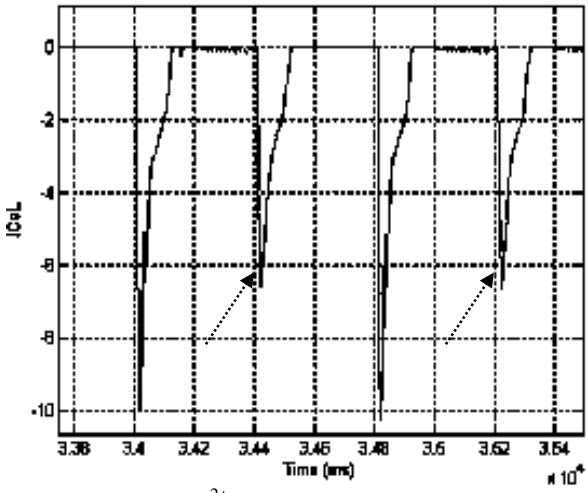

Figure 6. L-type Ca^{2+} current (marked with dashed-line arrows) triggered action potentials observed after DADs in figure 4.

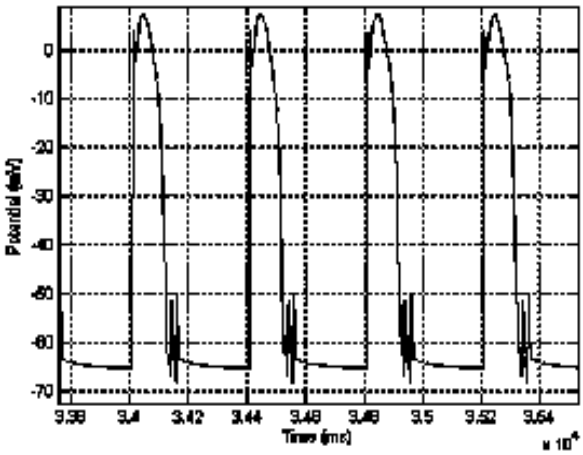

Figure 7. Action potentials during reperfusion process with I_{NaCa} unchanged. No delayed afterdepolarizations were observed.

Delayed afterdepolarizations appeared when $[K^+]_o$ concentrations were between 9.5 and 11.5 mmol/L and they started at 60% of NCX current. At concentrations below 9.5 mmol/L there were not afterdepolarizations. This could be interpreted as no much oxygen free radicals are produced during the ischemia episode that can affect the ionic transport. With $[K^+]_o$ > 11.5 mmol/L DADs were not observed neither with the I_{NaCa} decrease nor its increase. When $[K^+]_o$ was increased at 20.5 mmol/L and with 40% of NCX current (see Figure 8) no action potentials were elicited. The same behavior was observed when I_{NaCa} was unchanged and when was increased. At that levels of $[K^+]_o$ there is not conduction of action potential suggesting the electrical behavior is null.

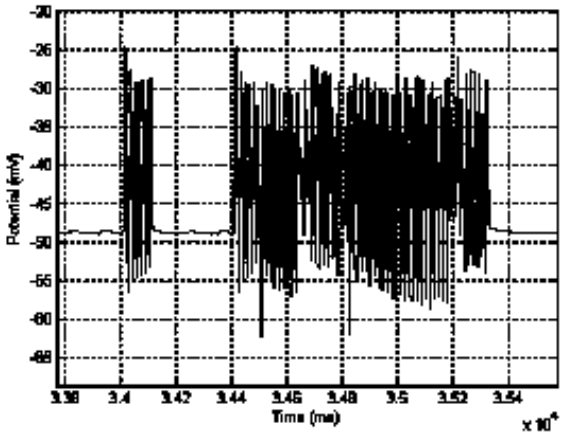

Figure 8. Electrical behavior of membrane during reperfusion process with a decrease of I_{NaCa} about 40% and $[K^+]_o$ = 20.5mmol/L. Action potential are not observed.

Releases from the SR under overload Ca^{2+} conditions could be observed during reperfusion, however just some of these induced the forward mode of NCX producing DADs.

4. Discussion and conclusions

This work allows to simulate the effects of ischemia-reperfusion on the Na^+-Ca^{2+} exchanger current.

The generation of delayed afterdepolarizations is due to an accumulation of intracellular Ca^{2+} causing a release of Ca^{2+} from SR which induces an inward I_{NaCa} current that depolarizes the membrane when it is in resting potential.

Delayed afterdepolarizations occur when I_{NaCa} is inhibited. On the other hand, if the current of NCX is unchanged or increased no overload Ca^{2+} conditions appeared and so, there is not inward current to depolarize the cell membrane.

This work support the idea of the decrement of the NCX activity and not its increment.

References

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