Effect of Calcium Activated Chloride Current on Characteristics of DAD

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Abstract

High intracellular calcium concentration causes a transient inward current (I_{ti}) that can provoke delayed afterdepolarizations (DAD). I_{ti} comprises of sodium-calcium exchange current (I_{NaCa}) and calcium-activated chloride current (I_{ClCa}). We have developed a mathematical model of I_{ClCa} that was included into the atrium AP model by Linblad, Murphey, Clark and Giles (LMCG Model). I_{ClCa} appears as an important component (30-40%) of I_{ti}. The plots of DAD amplitudes and DAD higher slope in function of extracellular Ca^{2+} concentration show an interval between 5 mM/l to 6 mM/l in which I_{ClCa} blockade could to bring DAD amplitude to subthresholds level for triggered action potentials. Our results suggest that, in high intracellular Ca^{2+} concentration, that provokes DAD generating conditions, I_{ClCa} blockade could be potentially antiarrythmogenic

1. Introduction

Delayed afterdepolarizations (DAD) are oscillations in the membrane potential occurring after completion of the action potential. DAD could provoke triggered activity if DAD amplitude reach the threshold for triggering of action potentials. DAD are provoked by high heart rates under conditions in which $[Ca^{2+}]_i$ is elevated.

The mechanism behind DAD is described as transient inward current (I_{ti}), activated by spontaneous Ca^{2+} release from sarcoplasmic reticulum. The ionic nature of I_{ti} is subject of debate. Several authors have proposed, in rabbit atrial cells, two mechanisms to contribute to I_{ti}: an electrogenic Na-Ca exchange and a Cl current [1,2].

The Cl current is a calcium-activated chloride current (I_{ClCa}). It appears as an important component (30-40%) of I_{ti}[1,2]. Contribution of this current may be of special importance for treatment and prevention of arrhythmia due to DAD. Blockade of Na-Ca exchange current is very effective in reducing DAD amplitude. However, this would elevate $[Ca^{2+}]_i$, resulting in cell death. The effect of calcium-activated chloride current blockade on reduce DAD amplitude to prevent triggered activity is unknown.

Evermore propagation of DAD as ectopic beats depends on the amplitude and slope of DAD.

In order to investigate the effect of I_{ClCa} on characteristics of DAD, we provoke calcium overload, at high rates, by increasing of $[Ca^{2+}]_o$. We studied the DAD amplitudes and slope at different $[Ca^{2+}]_o$ in presence and blockade of $I_{Cl,Ca}$.

2. Materials and methods

The theoretical Linblad, Murphey, Clark and Giles (LMCG) model of a mammalian atrium action potential [11] provides the basis for the simulations in this study. The model is based on rabbit experimental data; it includes membrane ionic channel currents that are formulated mathematically using the Hodgkin-Huxley approach and ionic pumps and exchangers. The model also accounts for processes that regulate intracellular concentration changes of Na^+, K^+, and Ca^{2+}. Intracellular processes represented in the model include Ca^{2+} uptake and Ca^{2+} release by the SR and Ca^{2+} buffering by calmodulin and troponin (in the myoplasm) and calsequestrin (in the SR). For the purpose of this study, the formulation of $I_{Cl,Ca}$ is incorporated in the model.

It has been suggested that $I_{Cl,Ca}$ may play a significant

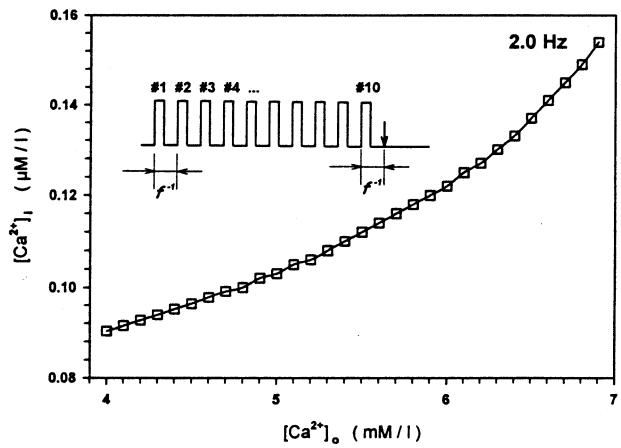

Fig. 1. $[Ca^{2+}]_i$ vs $[Ca^{2+}]_o$ after ten AP at 2.0 Hz. The value of $[Ca^{2+}]_i$ is plot after 500 ms from the last stimulus.

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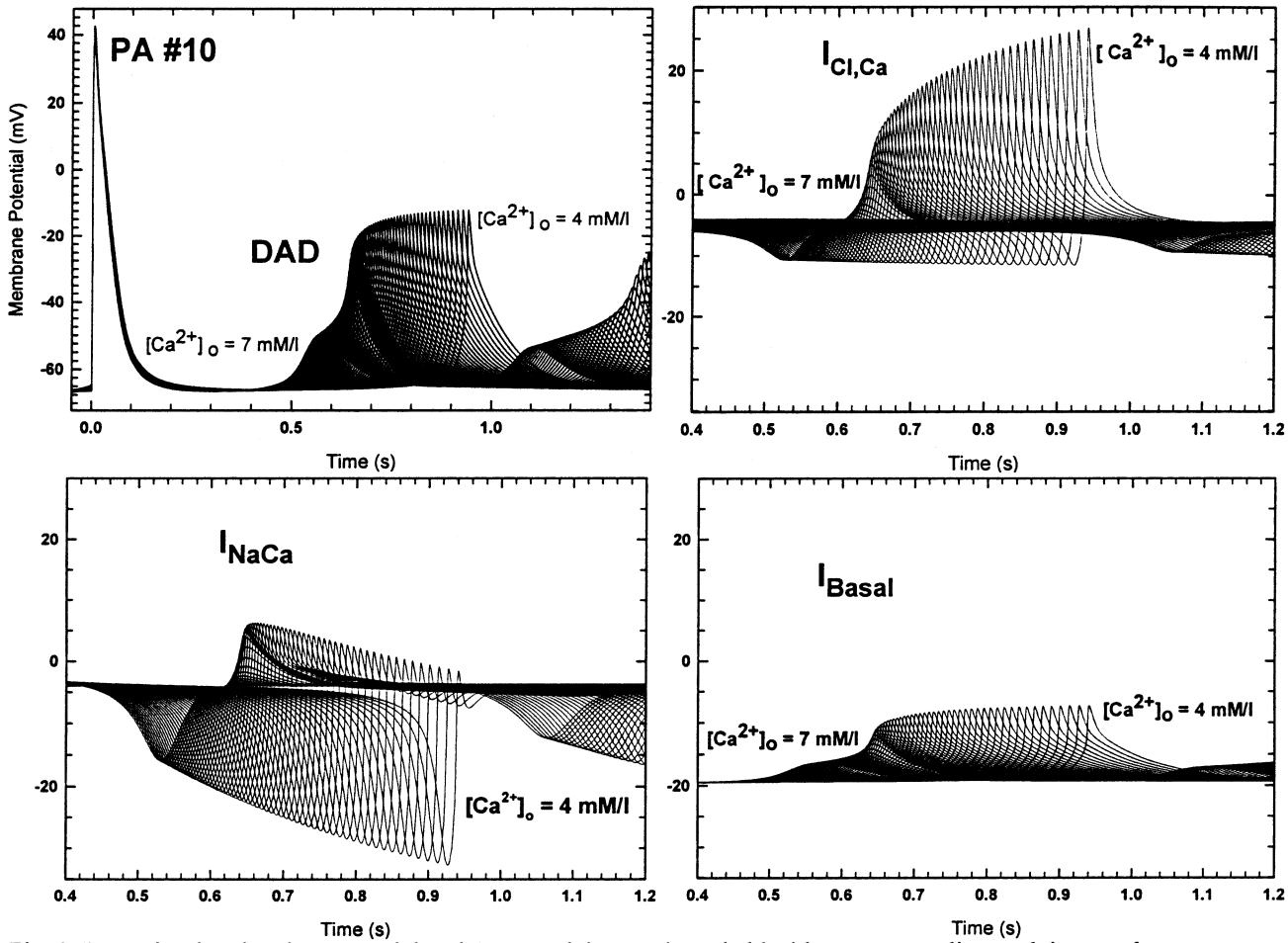

Fig. 2. Last stimulated action potential and DAD, calcium activated chloride current, sodium-calcium exchanger current and basal current are plotted for $[Ca^{2+}]_o$ from 4 to 7 mM/l (steps of 0.05 mM/l) after ten AP at 2.0 Hz.

role in DAD generation during conditions of elevated $[Ca^{2+}]_i$. To investigate this possibility, a formulation of $I_{Cl,Ca}$, developed by our group [16], is incorporated into LMCG model. $I_{Cl,Ca}$ is modeled as an outwardly rectifying, time-independent current with $[Ca^{2+}]_i$ dependence, that follows GHK law [3-10,12-15]. Formulation of this current is provided in the Appendix. The current-voltage (I-V) relationship of $I_{Cl,Ca}$ and other characteristics appear in previous works.

DAD conditions were simulated by $[Ca^{2+}]_i$ overload. In order to obtain an $[Ca^{2+}]_i$ elevated we increase the initial value of $[Ca^{2+}]_o$ and stimulate the cell with a train of ten impulses at 2.0 Hz. After tenth impulse the stimulation cease and the last AP and DAD are recorded, with and without $I_{Cl,Ca}$.

3. Results and discussion

An important step in investigating the effect of elevated $[Ca^{2+}]_i$ on DAD generation is to characterize the increasing of $[Ca^{2+}]_i$ in function of a initial value of $[Ca^{2+}]_o$ after a train of ten impulses as it was described in Methods. In Fig. 1, $[Ca^{2+}]_i$-$[Ca^{2+}]_{o,initial\ value}$ relationship is shown after ten impulses at 2.0 Hz rate.

The ionic nature of the transient inward current that generates DAD is still subject to debate. Several studies have proposed three mechanisms to contribute to I_{ti}: an electrogenic Na-Ca exchange, a Cl-Ca current and a background current. In order to determine the nature of I_{ti} we simulate the DAD generating conditions at initial value of $[Ca^{2+}]_o$ from 4 mM/l to 7 mM/l recording I_{NaCa}, $I_{Cl,Ca}$ and I_{Basal} currents provided by LMCG model, Fig.2. This figure show that I_{NaCa} and $I_{Cl,Ca}$ were activated before to DAD generation but I_{Basal} was activated after the DAD. It indicates that I_{NaCa} and $I_{Cl,Ca}$ will be the main cause of DAD.

Fig.2. show a decreasing relationship between DAD amplitude and the initial value of $[Ca^{2+}]_o$ (that reflex the $[Ca^{2+}]_i$). It is a paradoxical result (higher $[Ca^{2+}]_i$ but lower DAD amplitude). It could be explained by I_{NaCa} current that it decrease at higher initial values of $[Ca^{2+}]_o$.

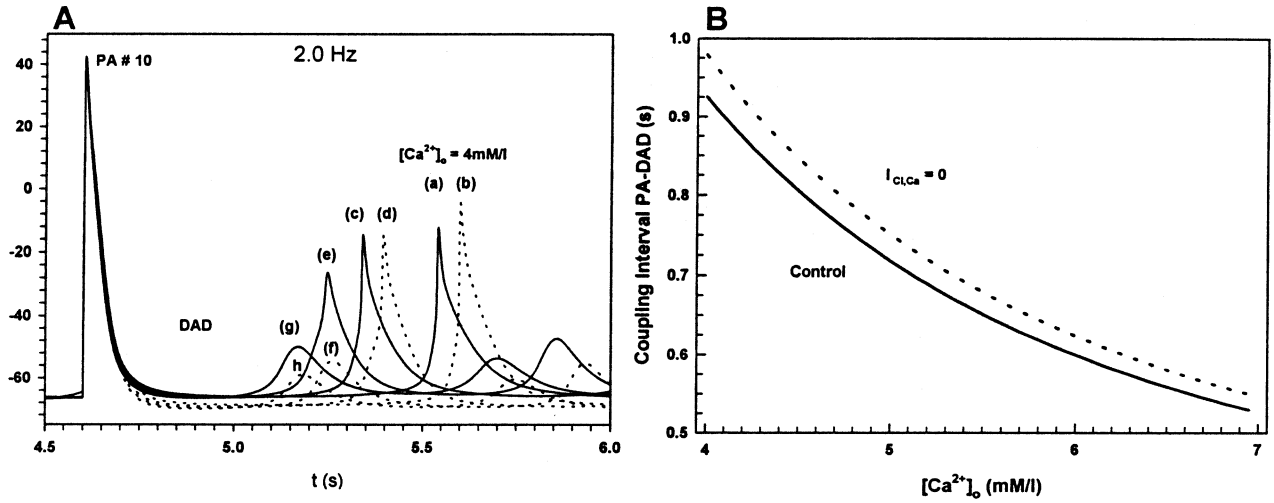

Fig. 3. Last stimulated action potential and DAD (A) and coupling interval (B) are plotted for $[Ca^{2+}]_o$ from 4 to 7 mM/l (steps of A: 1 mM/l and B: 0.05 mM/l) with (solid lines) and without $I_{Cl,Ca}$ (dotted lines) after ten AP at 2.0 Hz.

By other hand, it is curious to observe the I_{ClCa} current that to remain constant with slightly variations during all range. The coupling interval between last AP and DAD is also lower at higher initial values of $[Ca^{2+}]_o$.

In order to characterize the importance of $I_{Cl,Ca}$ on DAD generation we blockaded the current. Fig 3 show the same protocol in presence (solid lines) and absence (dotted lines) of I_{ClCa} at several initial values of $[Ca^{2+}]_o$. Traces a, c, e and g show control situation (presence of $I_{Cl,Ca}$) at 7, 6, 5 and 4 mM/l $[Ca^{2+}]_o$; traces b, d, f and h show blockade situation (absence of $I_{Cl,Ca}$). In both situations, DAD amplitude and DAD slope decrease at higher concentrations, but when I_{ClCa} is blockaded the reduction appear before. Fig. 3 B show the coupling interval between DAD and the last stimulated AP with (circles) and without (squares) I_{ClCa}. When I_{ClCa} is blockaded the value of coupling interval increases.

Fig. 4 A summarizes the results obtained when I_{ClCa} is present (circles) or absent (squares) on the DAD amplitude. In both cases the DAD amplitude decreases at higher initial values of $[Ca^{2+}]_o$ but when I_{ClCa} is blockaded DAD amplitude decreases before than control.

Fig. 4 B show the results in DAD slope; similar results are obtained, when I_{ClCa} is present (circles) or absent (squares) the slope decreases at higher initial values of $[Ca^{2+}]_o$ but when I_{ClCa} is blockaded DAD slope decreases before than control.

Figures 3 and 4 show that, in the range of $[Ca^{2+}]_o$ from 5 to 6 mM/l, amplitude and slope of DAD is significantly reduce when I_{ClCa} is blocked.

The thesis that proposes I_{ClCa} blockade to bring DAD amplitude (and slope) to values witch not to reach the threshold for triggering of action potentials is useful only in the study interval of $[Ca^{2+}]_o$ from 5 to 6 mM/l. At lower or higher values the blockade could not to have effect.

4. Conclusion

Our results show that under intracellular calcium overload conditions the $I_{Cl,Ca}$ blockade could to reduce the amplitude and slope of DAD decreasing the likelihood of DAD propagation as ectopic beat generation.

Consecuently, $I_{Cl,Ca}$ blockade could be potentially antiarritmogenic.

Appendix

Ca^{2+}-activated Cl^- current, $I_{Cl,Ca}$

$$I_{Cl,Ca} = pcl \cdot f_{Cl,Ca} \cdot Rc \cdot \frac{v \cdot F^2}{R \cdot T} \cdot \frac{[Cl^-]_o \cdot e^{v \cdot F/R \cdot T} - [Cl^-]_i}{e^{v \cdot F/R \cdot T} - 1}$$

$$f_{Cl,Ca} = \frac{1}{1 + (K_{mCl,Ca} / [Ca^{2+}]_i)} \qquad Rc = \frac{1}{1 + e^{(v-44.4)/17.2}}$$

Model Parameters:

$\mathbf{K_{mCl,Ca}} = 150{,}2 \cdot 10^{-3}$ mM/l **pcl** $= 1{,}1712 \cdot 10^{-3}$ nS

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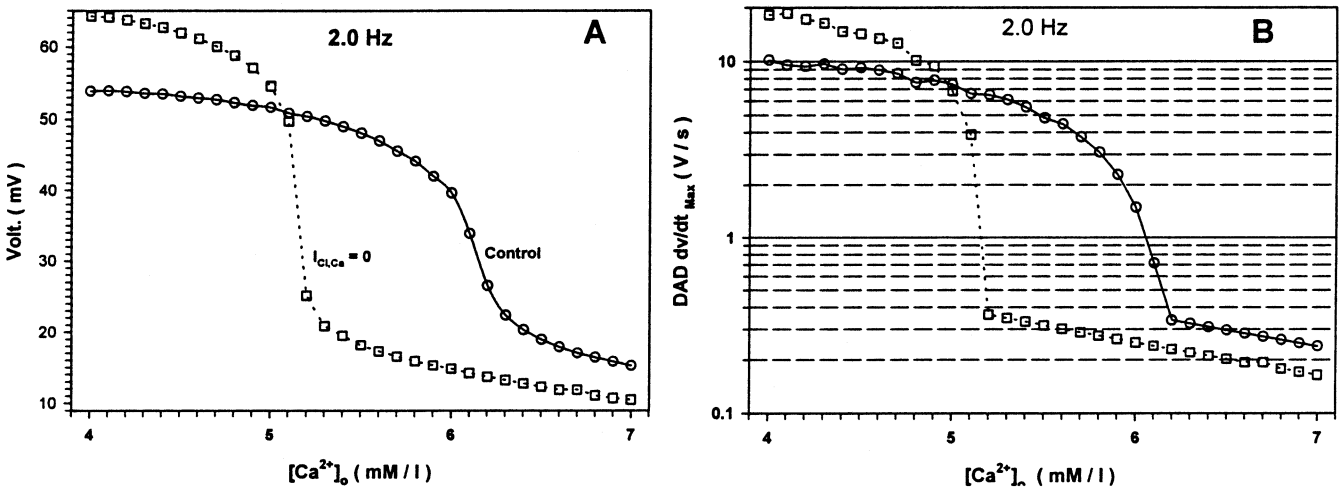

Fig. 4. DAD amplitude (A) and slope (B) for $[Ca^{2+}]_o$ from 4 to 7 mM/l (steps of 0.1 mM/l) with (circles) and without $I_{Cl,Ca}$ (squares) after ten AP at 2.0 Hz.

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