**EFFECT OF CALCIUM-ACTIVATED CHLORIDE CURRENT BLOCKADE ON THE DELAYED AFTERDEPOLARIZATIONS. SIMULATION STUDY.**

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**Abstract** - High intracellular calcium conditions cause a calcium-activated transient inward current (\(I_{\text{Cl}}\)) that can provoke oscillations in membrane potential called delayed afterdepolarizations (DADs). The current \(I_{\text{Cl}}\) comprises the sodium-calcium exchange current \(I_{\text{NaCa}}\) and the calcium-activated chloride current \(I_{\text{ClCa}}\). Lindblad, Murphey, Clark and Giles developed a mathematical model (LMCG model) of the rabbit atrial AP. In this study, a modified AP LMCG model that includes \(I_{\text{ClCa}}\) is used to evaluate the contribution of \(I_{\text{ClCa}}\) to develop DADs. Our results suggest that although \(I_{\text{NaCa}}\) is the main component of \(I_{\text{Cl}}\), \(I_{\text{ClCa}}\) may play a significant role in DAD generation. Even more, the \(I_{\text{ClCa}}\) blockade could inhibit the DAD propagation and trigger activity associated to high \([\text{Ca}^{2+}]_{\text{i}}\) condition, in atrial tissue.

**Keywords** - \(I_{\text{ClCa}}\), chloride current, DAD, modelling

I. INTRODUCTION

Delayed afterdepolarizations (DADs) are oscillations in membrane potential occurring after completion of the action potential (AP). DADs are an important mechanism for cardiac arrhythmias [1, 2]. They usually occur under conditions in which \([\text{Ca}^{2+}]_{\text{i}}\) appears to be high. The mechanism underlying DAD is a transient inward current \(I_{\text{Cl}}\) activated by spontaneous \(\text{Ca}^{2+}\) release from sarcoplasmic reticulum.

Although the ionic nature of \(I_{\text{Cl}}\) is still subject to debate, recent experiments in ventricular and purkinje myocytes indicate that \(I_{\text{Cl}}\) is composed by an electrogenic sodium-calcium exchange \(I_{\text{NaCa}}\) and a calcium-activated chloride current \(I_{\text{ClCa}}\) [1]. These studies suggest that the main component of \(I_{\text{Cl}}\) is \(I_{\text{NaCa}}\) (about 60-80%) but the role of \(I_{\text{ClCa}}\) could be important.

\(I_{\text{ClCa}}\) is also known as the second component (\(I_{\text{so2}}\)) of a transient outward current (\(I_{\text{so}}\)) that is present in depolarization. \(I_{\text{ClCa}}\) magnitude is lower than \(I_{\text{so1}}, a potassium current, at normal conditions and slow rates. It is dependent on \([\text{Ca}^{2+}]_{\text{i}}\), time-independent and it presents a voltage-dependent outward rectification [4, 5, 6, 7]. \(I_{\text{so2}}\) is responsible for the maintenance of AP duration at high rates [5].

We developed a comprehensive model for the \(I_{\text{ClCa}}\), that was presented in a previous communication [8].

This model has been introduced in the Lindblad, Murphey, Clark and Giles mathematical model (LMCG model) [3] of the rabbit atrial myocyte that reproduces the AP in a variety of conditions.

The present paper pretends to clarify the role of \(I_{\text{ClCa}}\) in DAD generation and in the induction of triggered activity.

II. METHODOLOGY

A set of mathematical equations describes the dependence of \(I_{\text{ClCa}}\) on intracellular \(\text{Ca}^{2+}\), as well as on ionic concentrations. The current through Cl-Ca channels is described as a product of three terms: first term is expressed using the Goldmann-Hodgkin-Katz (GHK) equation, the second term (\(f_{\text{ClCa}}\)) is the dependence on calcium and follows a Hill-type equation, and the last term (\(R_{\text{c}}\)) is the rectification exhibited by Cl-Ca channels, which is due to a voltage-dependent blockade caused by intracellular cations (Table 1).

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<th>Table I</th>
<th>CALCIUM-DEPENDENT CHLORIDE CURRENT EQUATIONS</th>
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<td>(I_{\text{ClCa}} = p_{\text{cl}} \cdot f_{\text{ClCa}} \cdot \text{RC} \cdot \frac{v \cdot F^2 \cdot [\text{Cl}^-]<em>{\text{o}} \cdot e^{v \cdot F / R \cdot T} - [\text{Cl}^-]</em>{\text{i}}}{R \cdot T} - 1} )</td>
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<tr>
<td>(f_{\text{ClCa}} = \frac{1}{1 + (K_{m_{\text{ClCa}}} / [\text{Ca}^{2+}]_{\text{i}})} )</td>
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<tr>
<td>(R_{\text{c}} = \frac{1}{1 + e^{(v-(-44.4))/17.2}} )</td>
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**Model Parameters:**

| \(K_{m_{\text{ClCa}}} = 150, 2 \cdot 10^{-3} \text{mM/l} \) | \(p_{\text{cl}} = 1, 1712 \cdot 10^{-3} \text{nS} \) |

The mathematical description of \(I_{\text{ClCa}}\) was included into the atrial AP LMCG model. Software programs were written in ACSL language using Gear stiff algorithm to solve the nonlinear system of differential equations that results from the AP myocyte model. For studying propagation, the tissue is modeled as a one-dimensional 1-D segment (500 cells).

![Figure 1. Effect of varying \(I_{\text{ClCa}}\) conductance (10, 25, 50, 75, 100, 150, 200, 250, 300 % of nominal) on model action potential morphology during stimulation at 2 Hz.](image-url)
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The 1-D model was programmed in C++ language, the cable equation was solved using a central difference scheme in space and Crank-Nicholson method in time. Both, isolated myocyte and multicellular models, were implemented in a PC machine.

Protocol stimulation follows the nominal values of LMCG model for stimulation current ($I_{st}$) at 2 Hz rate [3].

III. RESULTS

Figure 1 shows the effect of varying the magnitude of the nominal $I_{Cl,Ca}$ conductance on action potential. Blockade of $I_{Cl,Ca}$ increase the action potential duration.

Figure 2 shows the time course of different membrane currents during the AP. Under normal conditions, $I_{Cl,Ca}$ is present mainly in depolarization.

Figure 3 shows the parameters $f_{Ca}$ and $Rc$ used to model $I_{Cl,Ca}$. In order to compare the model $I_{Cl,Ca}$ I-V plots with experimental data [5, 6], a train of voltage steps of 100 ms from – 70 to V was simulated at 0.1, 1 and 2 Hz. Plot C represents the normalized model results of peak current vs. experimental normalized data.

In order to study $I_t$ we considered DAD-generating conditions as intracellular calcium-overload. Calcium-overload is induced by an initial $[Ca^{2+}]_{i}$ higher than nominal.

In figures 4, 5 and 6, a train of 10 AP was stimulated but only the last AP was represented.

Figure 4 shows the membrane potential at different initial $[Ca^{2+}]_{i}$ (A: 4, B: 5, C: 6 and D: 7 mM/l) in control simulation (a) and when $I_{Cl,Ca}$ (b), $I_{NaCa}$ (c) and both (d) are forced to zero at 4.8 s.

It can be observed that at higher calcium-overload conditions the delay between AP and DAD is lower ($t_4 < t_1$).

For $[Ca^{2+}]_{i}$ of 4 and 5 mM/l, the inhibition of $I_{Cl,Ca}$ has not significant effect on DAD amplitude (differences are below than 11 %). However, for $[Ca^{2+}]_{i}$ of 6 mM/l the inhibition of $I_{Cl,Ca}$ provokes a reduction of 58 % in DAD amplitude.

Figure 5 shows the case of an initial $[Ca^{2+}]_{i}$= 6 mM/l. In plot A can be observed the effect of $I_{Cl,Ca}$ blockade at 5.2 s.
(b) vs. control (a) on DAD. In plot B can be observed that the contribution of $I_{\text{Cl}-\text{Ca}}$ and $I_{\text{Na}-\text{Ca}}$ is 35% and 65% respectively.

To study DAD propagation we use the 1-D model in a segment of 500 cells.

Figure 6 shows the membrane potential in the cell number 1, 125, 250, 375 and 500. As it can be observed, in plot A the DAD that appears in cell #1 is propagated like an AP in the cell #500. However, when $I_{\text{Cl}-\text{Ca}}$ is blocked, plot B, the oscillation in the membrane potential of cell #1 is not propagated like an AP in cell #500.

IV. DISCUSSION

The main goal of the present study is to find out the role of $I_{\text{Cl}-\text{Ca}}$ as a component of $I_{\text{ti}}$ on DAD formation and propagation as AP in the atrial tissue.

A mathematical model of the $I_{\text{Cl}-\text{Ca}}$ has been developed. Whenever possible, we use data directly measured on rabbit atrial cells to derive model parameters [5, 7]. When rabbit atrial data were not available to completely characterize the current we use reliable data of dog and rabbit ventricular cells [4, 6].

The ionic nature of $I_{\text{ti}}$ is still subject to debate. Several authors propose two ionic currents to contribute to $I_{\text{ti}}$: electrogentic $I_{\text{Na}-\text{Ca}}$ and $I_{\text{Cl}-\text{Ca}}$, but it is not clear in which percentage.

Recent data from Zygmunt et al. [1] indicate that $I_{\text{Na}-\text{Ca}}$ component is the main responsible, representing about 60% of the total calcium-activated current at the resting potentials ($I_{\text{i}}$). Other authors like Verker et al. [2] give values near to 80%.

Our results show that $I_{\text{Cl}-\text{Ca}}$ takes values near to 35% and $I_{\text{Na}-\text{Ca}}$ takes values near to 65% of the total calcium-activated current at the resting potentials ($I_{\text{i}}$).
V. CONCLUSION

Our results suggest that $I_{\text{Cl-Ca}}$ could play an important role in DAD generation mechanisms. The blockade of $I_{\text{Cl-Ca}}$ can reduce DAD amplitude to prevent DADs to reach the trigger threshold of action potentials.

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REFERENCES


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