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Davis, January 25th, 2011

European Society of Cardiology
The European Heart House
Councils Relations
2035 Route des Colles, Les Templiers
B.P. 179, 06903 Sophia Antipolis, France

Object: "ESC First Contact Initiative Grant" outcome report

Dear Sirs,

my name is Stefano Morotti and I have been awarded the "ESC First Contact Initiative Grant" after my application in last August. I'm writing to report the outcome of the initiative.

I am a PhD student in Bioengineering at University of Bologna, Department of Electronics, Computer Science and Systems. My studies focus on computational modeling of cardiac cells and tissues excitability in physiological and pathological conditions. I used the grant to visit the Bers Lab, in the Department of Pharmacology of the University of California at Davis, USA, from October 26th to November 18th, 2010.

During this period, we set the basis for collaboration on a study on the mechanisms of inactivation of L-type calcium channels in ventricular myocytes. This report describes briefly the guidelines of the research program we planned. Preliminary results are also shown.

The L-type Ca current (I_{Ca}) contributes to the action potential (AP) plateau and initiates excitation-contraction coupling (ECC). I_{Ca} is rapidly activated by membrane depolarization, whereas inactivation of L-type calcium channels (LTCCs) is regulated by both Ca- and voltage-dependent inactivation (CDI and VDI). CDI is due to binding of Ca to calmodulin (CaM) that is pre-bound to the channel protein on the internal side of the membrane. The binding between Ca and CaM causes a channel conformational change that accelerates inactivation. CDI is an important feedback mechanism that limits the amount of Ca entering the cell and dictates cardiac repolarisation duration and arrhythmogenic triggered activity.

To differentiate VDI and CDI, several experimental and theoretical studies have considered the inactivation of Ba current through LTCC (I_{Ba}) as a measure of VDI. However, there is evidence that Ba can weakly bind CaM, such that I_{Ba} inactivation is still a mixture of CDI and VDI. In these studies, CDI resulted underestimated, while the importance of VDI is overestimated.

Our aim is to develop a model that recapitulates realistically the relative importance of VDI and CDI. A more accurate model for VDI may be important when one seeks to dissect the relative roles of Ca and voltage in normal function and pathophysiology.

Our first effort was the assessment of how experimental I_{Ba} inactivation results could be recapitulated by modifying CDI to account for Ba-dependent inactivation.

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Henceforth we will refer to Ca- or Ba-dependent inactivation as the generic "ion-dependent" inactivation (IDI).
We used the Mahajan-Weiss model of I_{Ca} (Mahajan et al, 2008), in which VDI was identified to fit experimental I_{Ba} inactivation, and incorporated into the Shannon-Bers rabbit ECC model (Shannon et al, 2004). Due to geometric differences in sub-cellular compartments definition, parameter adjustments were made during implementation to preserve the Ca-handling characteristics of the Shannon-Bers model.
To reproduce the weaker apparent affinity of Ba (vs. Ca) for CaM ($k_{Ba}<k_{Ca}$), we made all ion-dependent transition rates less sensitive to the Ba concentration, introducing a scaling factor (k_{Ca}/k_{Ba}). We found that a 10-fold reduction resulted in appropriate IDI of I_{Ba}. Thus, adopting $k_{Ca}/k_{Ba}=10$, model transition rates were optimized to fit measured I_{Ba}, based on a large set of experimental data from several authors (Figure 1).

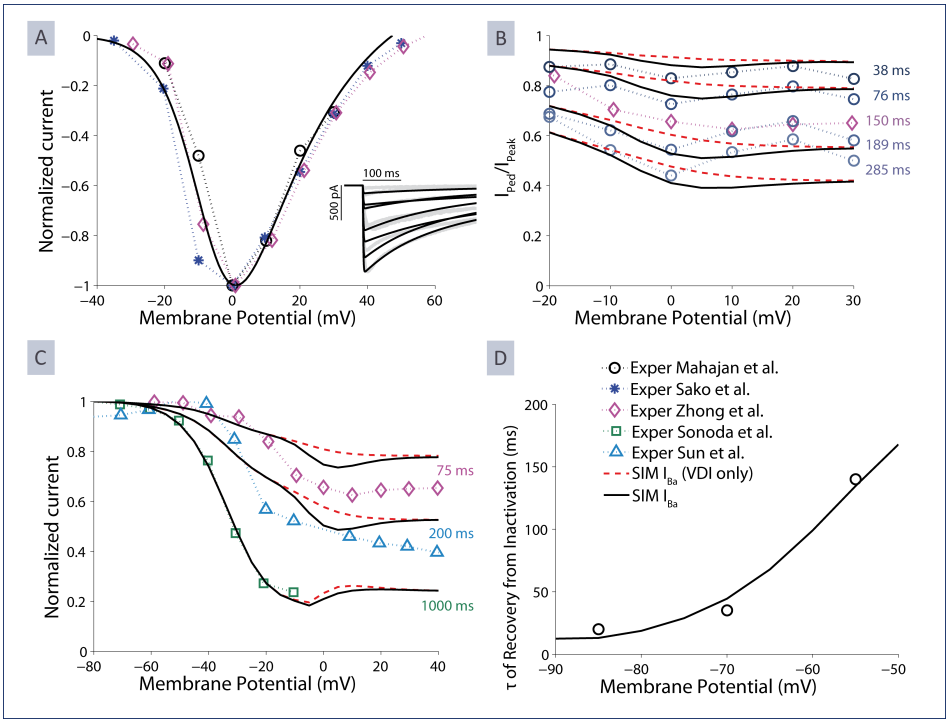

Figure 1. I_{Ba} model identification with $k_{Ca}/k_{Ba}=10$: a broad set of experimental data [including IV (panel A) and ratio of pedestral to peak current relations (B), quasi Steady-State Inactivation (C) and Recovery from Inactivation (D)] was used.

Then the effect of varying the scaling factor from 1 to 100 was analyzed (Figure 2): sensitivity analysis predicted a detectable Ba-dependent inactivation (i.e. bell-shaped dependence on membrane potential, mirroring the bell-shaped IV curve and suggesting that the more Ba enters the cell via LTCCs the faster the channels inactivate) when apparent Ba affinity for CaM is one tenth than that of Ca (or higher).
The model derived here, by accounting for IDI with Ba as charge carrier, should provide with a more faithful representation of purely VDI during I_{Ca} and serves as a basis for the ongoing identification of VDI and CDI models for I_{Ca}.

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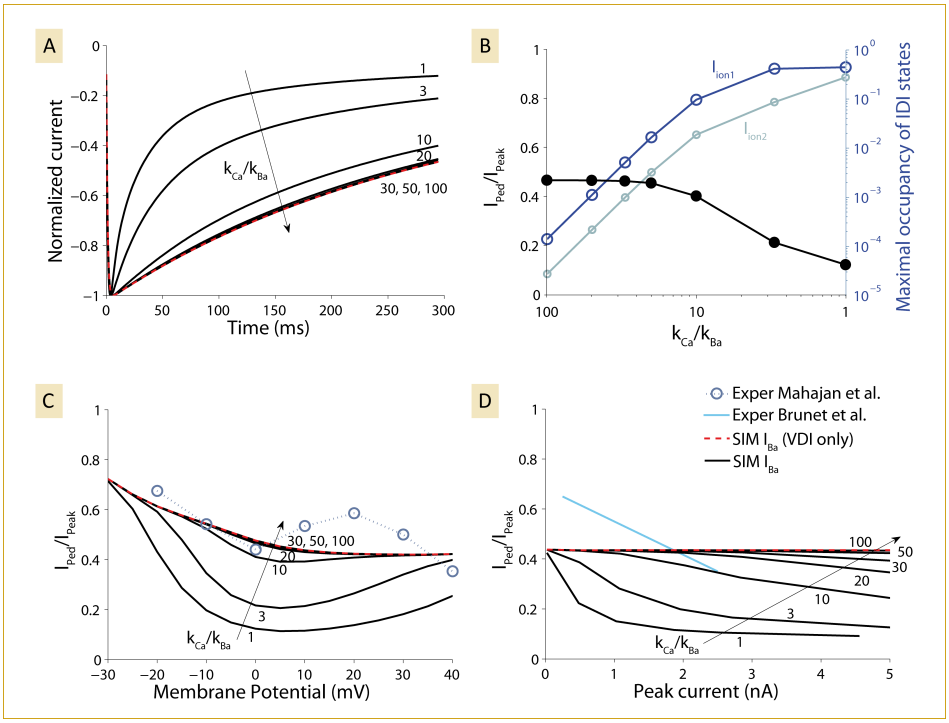

Figure 2. Sensitivity analysis on k_{Ca}/k_{Ba} variation from 1 to 100. Following features were studied: normalized I_{Ba} (panel A), ratio of pedestral to peak current (I_{Ped}/I_{Peak}) and maximal occupancy of IDI states (B) in response to a voltage step to 0 mV, I_{Ped}/I_{Peak} relations obtained in response of voltage step to various voltage (C) and I_{Ped}/I_{Peak} relations obtained (in response of voltage step to 0 mV) by varying extracellular Ba concentration.

I just returned to the Bers Lab a few days ago and we are currently tuning the ion-dependent transitions rates in order to reproduce experimental I_{Ca} data. We want to simulate current traces recorded when sarcoplasmic reticulum (SR) Ca release is abolished (by exogenous buffers or SR Ca depletion) or in physiological condition of Ca transients (when SR Ca release amplifies Ca influx). Next, we will validate the I_{Ca} model against action potential clamp data obtained with and without Ca transients. I am working in this Lab until the end of May, when, hopefully, all these targets will be achieved. Then we will use our model to predict the effects of Timothy Syndrome (which impairs VDI and causes severe ventricular arrhythmias) and to study mechanisms of triggered arrhythmias.

I want to thank you again for the award.
Yours sincerely,

Stefano Morotti

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