An Improved Model of Ba Current through L-type Ca Channels Including Voltage- and Ion-Dependent Inactivation

S Morotti[1,2], E Grandi[2], A Summa[1], KS Ginsburg[2], DM Bers[2], S Severi[1]

[1]Biomedical Engineering Laboratory, University of Bologna, Cesena, Italy
[2]Department of Pharmacology, University of California, Davis, CA, U.S.A.

Abstract

The substitution of Ba ions for Ca has been widely used to separate voltage-dependent inactivation (VDI) from Ca-dependent inactivation (CDI) of the Ca current (I_{Ca}) through L-type Ca channels (LTCCs). However, a modest ion-dependent inactivation of Ba current (I_{Ba}) has been shown experimentally. We have incorporated the Mahajan et al. Markov model of LTCC, which describes I_{Ba} inactivation as VDI only, into the Shannon et al. excitation-contraction coupling model. We extended the LTCC model to assess whether and how experimental I_{Ba} inactivation could be recapitulated by modifying CDI. Simulation results show that I_{Ba} inactivation measured in rabbit myocytes at physiological temperature can be recapitulated when making the Ca-dependent transition rates 10-fold less sensitive to Ba vs. Ca. Our extended LTCC model provides a more faithful representation of purely VDI during I_{Ca}.

1. Introduction

The L-type Ca current (I_{Ca}) contributes to the action potential plateau and initiates excitation-contraction coupling (ECC) in cardiac myocytes [1]. L-type Ca channels (LTCCs) inactivate via both Ca- and voltage-dependent processes (CDI and VDI). CDI is due to binding of Ca to calmodulin (CaM), which causes a channel conformational change that accelerates inactivation (see [2] for review). During ECC, CDI predominates over VDI, especially with normal sarcoplasmic reticulum Ca release and Ca transients that amplify the Ca influx (Figure 1A, top I_{Ca} trace, $t_{1/2}$=17 ms). When Ca transients are abolished, I_{Ca} inactivation is slower (Figure 1A, $t_{1/2}$=37 ms), reflecting a smaller rise in local $[Ca]_i$ near the mouths of Ca channels due to Ca entering via the channels themselves.

To differentiate VDI from CDI, several studies have used the slower inactivation of Ba current via LTCC (I_{Ba}) as a measure of VDI [2, 3]. However, there is evidence

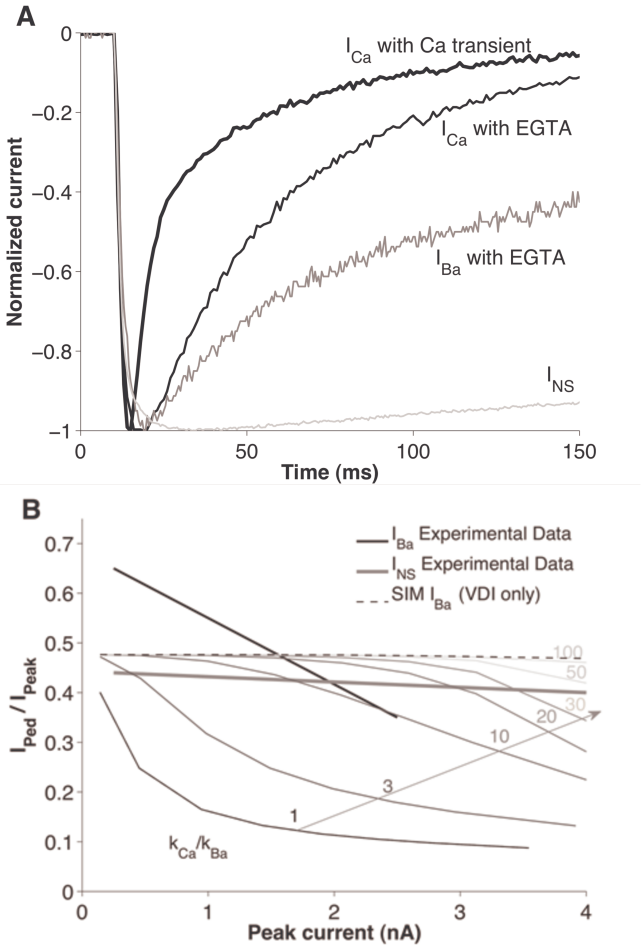

Figure 1. LTCC Inactivation. A. Normalized I_{Ca}, I_{Ba} and I_{NS} evoked by a voltage step to 0 mV (-30 mV for I_{NS}). Modified from [1]. B. Ratio between pedestal and peak current (plotted against peak current) in response to a voltage step to 0 mV (330 ms step for simulation data at 37°C, 1000 ms step for experimental data from [5] at room temperature) from a holding potential of -80 mV. Simulation results were obtained with reduced affinities of Ba (vs. Ca) for CaM (different traces corresponding to various k_{Ca}/k_{Ba}) and varying extracellular [Ba] (along every trace).

that Ba can bind to CaM and weakly mimic Ca [4], such that I_{Ba} inactivation (Figure 1A, $t_{1/2}$=161 ms) consists of both VDI and a moderate current-dependent component, like CDI (Figure 1B, *thick black line*), so that both Ca and Ba produce ion-dependent inactivation (IDI). To avoid this complication, some have used the monovalent cation current through LTCC (I_{NS}), whose inactivation rate is independent of current amplitude (Figure 1B, *thick grey line*) and hence could reflect pure VDI [5]. Inward I_{NS} has been reported to inactivate very slowly (Figure 1A, $t_{1/2}$>500 ms) and incompletely. However, we found that I_{NS} inactivation at physiological temperature is faster than VDI occurring during I_{Ba} [6] and suggested that VDI that occurs during I_{Ba} (and I_{Ca}) must differ intrinsically from VDI during I_{NS}.

In this study, we extended the LTCC mathematical model of VDI and CDI developed by the Weiss group [3], incorporated it into a well validated description of rabbit ECC developed by the Bers group [7] and modified the CDI component to account for Ba-dependent inactivation. I_{Ba} inactivation was recapitulated by mimicking a reduced affinity of Ba (vs. Ca) for CaM. Our model is an improved description of I_{Ba} inactivation, which will serve as a basis for the development of an improved model of VDI and CDI during I_{Ca}.

2. Methods

We used a minimal Markovian model for I_{Ca} (Figure 2) that was developed previously by Mahajan et al. [3] based on I_{Ca} and I_{Ba} measurements in rabbit ventricular myocytes at physiological temperature. Inactivation of I_{Ca} is described as occurring via both a voltage- (VDI) and an ion-dependent pathway (IDI). Mahajan et al. used I_{Ba} data to identify the VDI pathway (i.e., a reduced Markovian model without the two upper states of Figure 2). We used both their 5-state I_{Ba} model and an alternative 7-state I_{Ba} model, which includes the IDI pathway. To introduce a reduced Ba-dependent inactivation (vs. CDI), we simulated a weaker apparent affinity of Ba for CaM (k_{Ba}<k_{Ca}) by making IDI transitions less sensitive to Ba vs. Ca by a factor k_{Ca}/k_{Ba}. k_{Ca}/k_{Ba} varied from 1 to 100.

The Markovian model was incorporated into the ventricular myocyte action potential model developed by Shannon et al. [7]. We scaled the permeability of LTCCs for Ba as reported in [1] and in the 5-state model we tuned two parameters to fit the experimental I_{Ba}-voltage relation [3]. With respect to the original formulation, only the following equation was modified, affecting four transition rates (α, β, k_4, k_4' in Figure 2):

$$p_o^{\infty} = \frac{1}{1+e^{-(Vm+5)/4.4}}.$$

Model differential equations were implemented in Matlab and Simulink (Mathworks Inc., Natick, MA, U.S.A.) and solved numerically using a variable order solver (ode15s). The digital cell was stimulated with voltage steps replicating the experimental protocol.

3. Results

Since Ba-dependent inactivation is unlikely to appreciably affect the current peak, we first used the 5-state model (only voltage-dependent) to fit the I_{Ba}-V relation measured by Mahajan et al. [3]. Figure 3 shows that our model (*solid line*) well replicates the experimental data (*circles*).

Next, I_{Ba} inactivation was simulated using the whole 7-state model (VDI and IDI) in the condition of reduced affinity of Ba (vs. Ca) for CaM. In Figure 4A normalized I_{Ba} in response to a 300 ms depolarizing step to 0 mV

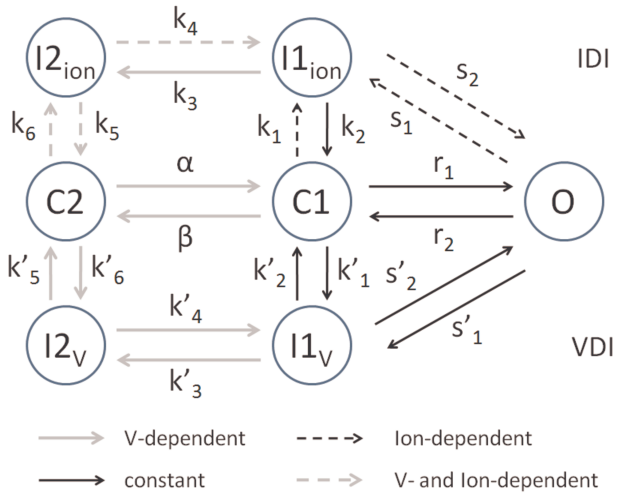

Figure 2. 7-state Markovian model of LTCC with voltage- and ion-dependent inactivation (VDI and IDI) from [3].

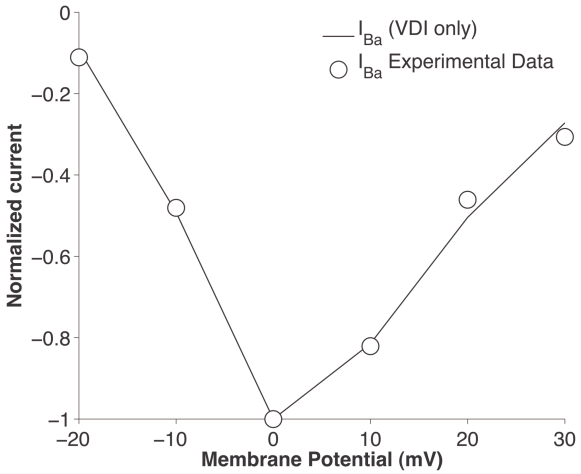

Figure 3. Comparison between experimental [3] and simulated I_{Ba} IV relation. Simulation results were obtained with only the 5-state model of I_{Ba} inactivation.

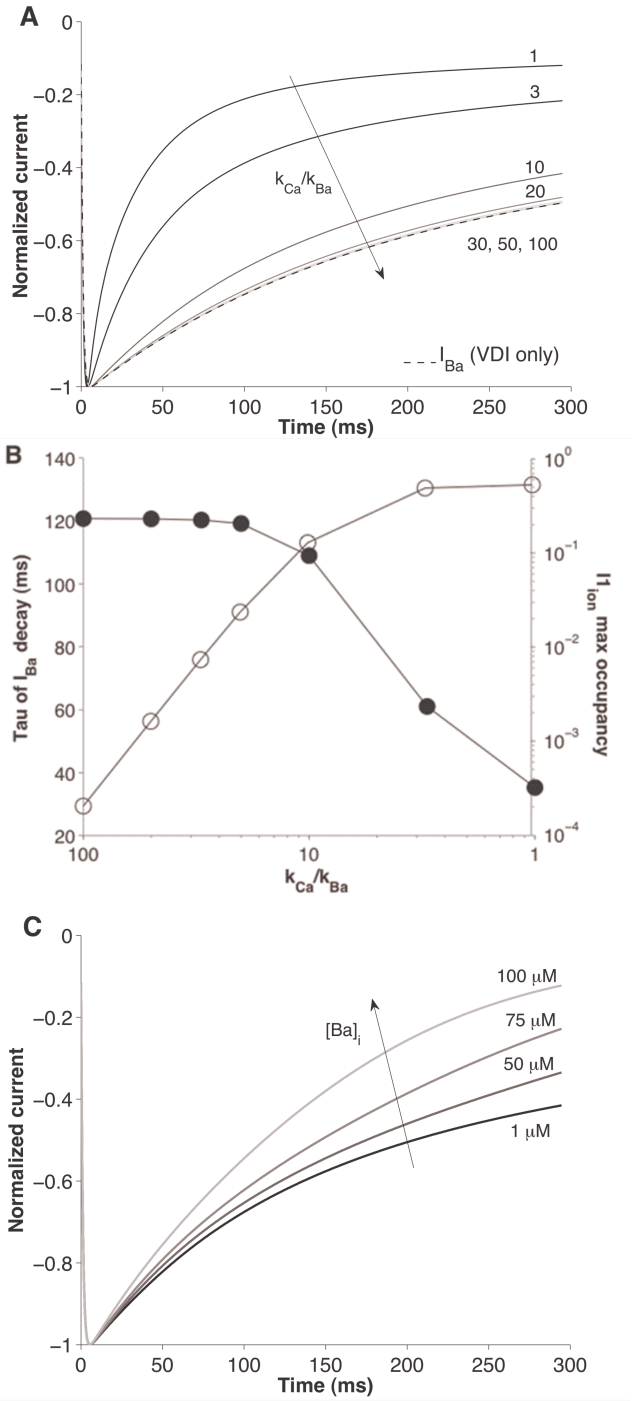

Figure 4. I_{Ba} simulated inactivation in response to a 300 ms voltage step to 0 mV. A. Normalized currents were obtained by reducing Ba affinity for CaM. I_{Ba} was also simulated as independent of Ba ion influx (*dashed line*). B. Time constant of I_{Ba} inactivation (left ordinate, *black circles*) and probability of occupancy of the Ba-dependent inactivation state $I1_{ion}$ (right ordinate, *white circles*) at varying k_{Ca}/k_{Ba}. C. Effect of intracellular Ba accumulation on current inactivation. Simulations were done adopting k_{Ca}/k_{Ba}=10.

(from a holding potential of -80 mV) are depicted for various k_{Ca}/k_{Ba}. As the apparent affinity of Ba for CaM increases (approaching that of Ca, k_{Ca}/k_{Ba} decreases), I_{Ba} exhibits faster inactivation. I_{Ba} simulated with the 5-state model (*dashed line*) approaches the currents simulated with the 7-state model and high k_{Ca}/k_{Ba}. The time constant of I_{Ba} decay is ~120 ms when the current inactivation is modelled as Ba-independent (i.e., only VDI). When Ba affinity is much smaller than that of Ca (20-100 times), the predicted effect of Ba-dependent inactivation is negligible and τ does not change compared to the 5-state VDI model (Figure 4B). Only when k_{Ca}/k_{Ba} is smaller than 10 does Ba-dependent inactivation become appreciable. Figure 4B also shows the maximal probability of occupancy of the Ba-dependent inactivation state $I1_{ion}$ (the maximal probability of occupancy of $I2_{ion}$ is about 10 times lower, data not shown). The channel never visits these states by design when only VDI is implemented and state occupancies are infrequent when Ba affinity for CaM is low. Ion-dependent inactivated states are more likely to be occupied when Ba affinity increases (e.g., when k_{Ca}/k_{Ba} equals 10 and τ is reduced by about one fifth). When Ba affinity equals that of Ca, τ of I_{Ba} inactivation is the same as I_{Ca} (without Ca transient) and the probabilities of occupancy of $I1_{ion}$ and $I2_{ion}$ are maximal.

When Ba is used instead of Ca in experiments, the normal myocyte Ca extrusion pathways do not remove Ba well, so that intracellular Ba would accumulate over several beats. Simulations show that the time constant of I_{Ba} decay decreases as intracellular [Ba] increases (Figure 4C), whereas it is unchanged when using the 5-state VDI only I_{Ba} model (not shown).

We tested whether our model could reproduce the dependence of the extent of I_{Ba} inactivation on the current amplitude, as has been shown experimentally (see Figure 1B, *thick black line*). We varied the external [Ba] from 0.2 to 3.6 mM to obtain increasing current peaks. If the 5-state model is used, as expected, I_{Ba} inactivation is independent of current amplitude (*dashed line*). So inactivation sufficient to bring the pedestal current to about 50% of the peak current is voltage-dependent in this model. For any given value of k_{Ca}/k_{Ba}, as the peak current (i.e., Ba influx) increases, I_{Ba} inactivation increases. As k_{Ca}/k_{Ba} decreases, less Ba current is needed to produce the additional Ba-dependent inactivation.

The effect of IDI on time constant of inactivation was also investigated over a wide range of test potentials (Figure 5). The trace obtained when running the 5-state model overlapped the simulated traces obtained with the 7-state model when k_{Ca}/k_{Ba}>20. In this range of k_{Ca}/k_{Ba} values, the experimental U-shaped dependence of τ on membrane potential [3] is not reproduced. Choosing k_{Ca}/k_{Ba}=10 allows a better reproduction of the experimentally voltage-dependent time constant of inactivation. This value also represents a threshold for

appreciable Ba-dependent inactivation. Notably, with this value we verified that the I_{Ba}-V relation is not altered compared to the simulation results obtained with the VDI only model plotted in Figure 3 (not shown).

4. Discussion and conclusions

The substitution of Ba ions for Ca has been widely used to separate VDI from CDI of the macroscopic LTCC currents and this is pragmatic because Ba produces only modest ion-dependent inactivation [4, 5] and probably has similar (but not identical) binding properties to Ca in the pore. However, I_{Ba} inactivation depends on Ba flux and it seems clear now that Ba can recapitulate a weak version of CDI. To account for the moderate Ba-dependent inactivation, we have incorporated the LTCC mathematical model of VDI and CDI developed by Mahajan et al. [3] into the Shannon et al. model of rabbit ECC [7] and modified CDI to account for Ba-dependent inactivation. We found that making the Ca-dependent transition rates (in Figure 2) 10-fold less sensitive to Ba resulted in appropriate Ba-dependent I_{Ba} inactivation. We previously obtained analogous results when incorporating the I_{Ba} model into the Mahajan model [3] of rabbit action potential and Ca transient [6]. Thus, our findings hold true independent of the known differences between the two ECC models with respect to cell geometry, compartmentalization, and description of the processes governing intracellular ion handling.

Ba is known to complex with CaM and activate interaction with CaM targets, but its apparent affinity in other systems has typically been 100-fold lower than for Ca. While our change in rate constants that produces appropriate I_{Ba} CDI could be construed to represent only a ~10-fold reduction in affinity, this lower value should not be taken to imply an actual affinity difference in the classical biochemical sense. Reasons for the relatively modest 10-fold reduction in Ba- vs. Ca-dependent inactivation (vs. ≥100-fold expected for CaM binding) could have to do with a) the way CaM is pre-bound to the LTCC, b) potentially weaker local Ba vs. Ca buffering, c) higher single channel conductance for Ba, d) dynamic considerations during current flow (Ba may equilibrate faster than Ca). In any event, the model derived here, by accounting for inactivation with Ba as current carrier, should provide a more faithful representation of purely VDI during I_{Ca}. An accurate model for VDI may be important when one seeks to dissect the relative roles of Ca and voltage in normal function and pathophysiology (e.g., arrhythmogenesis).

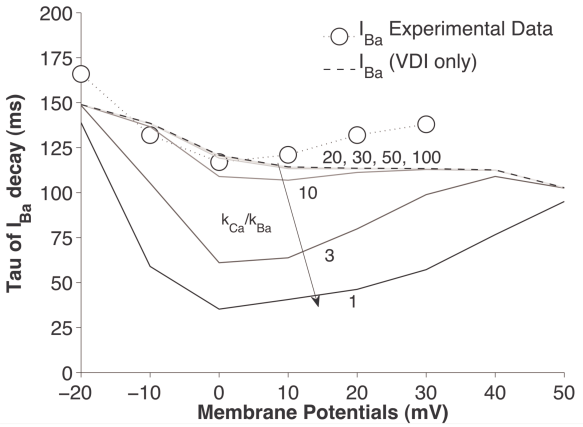

Figure 5. Time constants of I_{Ba} decay obtained in response to a voltage step to the indicate membrane potential, from a holding potential of -80 mV. Experimental data from [3]. I_{Ba} was simulated as independent of Ba ion influx (VDI only) and with reduced affinity of Ba for CaM.

Acknowledgements

This work was supported by NIH grants HL30077 and HL80101 to DMB and the Italian Ministry of University and Research (Italian National Project, PRIN2007) to SS.

References

[1] Bers DM. Excitation-Contraction Coupling and Cardiac Contractile Force. 2nd edn. ed: Kluwer Academic Press Dordrecht, The Netherlands; 2001.

[2] Cens T, Rousset M, Leyris JP, Fesquet P, Charnet P. Voltage- and calcium-dependent inactivation in high voltage-gated Ca^{2+} channels. Prog Biophys Mol Biol. 2006; 90(1-3):104-17.

[3] Mahajan A, Shiferaw Y, Sato D, Baher A, Olcese R, Xie L-H, et al. A Rabbit Ventricular Action Potential Model Replicating Cardiac Dynamics at Rapid Heart Rates. Biophys J. 2008; 94(2):392.

[4] Ferreira G, Yi J, Rios E, Shirokov R. Ion-dependent Inactivation of Barium Current through L-type Calcium Channels. J Gen Physiol. 1997; 109(4):449-61.

[5] Brunet S, Scheuer T, Catterall WA. Cooperative regulation of Cav1.2 channels by intracellular Mg^{2+}, the proximal C-terminal EF-hand, and the distal C-terminal domain. J Gen Physiol. 2009; 134(2):81-94.

[6] Grandi E, Morotti S, Ginsburg KS, Severi S, Bers DM. Interplay of voltage and Ca-dependent inactivation of L-type Ca current. Prog Biophys Mol Biol. 2010; 103:44-50.

[7] Shannon TR, Wang F, Puglisi J, Weber C, Bers DM. A mathematical treatment of integrated Ca dynamics within the ventricular myocyte. Biophys J. 2004; 87(5):3351-71.

Address for correspondence.

Eleonora Grandi
Department of Pharmacology
University of California, Davis
451 Health Sciences Drive, GBSF 3506
Davis, CA 95616, U.S.A.
E-mail: egrandi@ucdavis.edu