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Interplay of Voltage and Ca-dependent inactivation of L-type Ca current

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Abstract

Inactivation of L-type Ca channels (LTCC) is regulated by both Ca and voltage-dependent processes (CDI and VDI). 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 mimic Ca, such that I_{Ba} inactivation is still a mixture of CDI and VDI. To avoid this complication, some have used the monovalent cation current through LTCC (I_{NS}), which can be measured when divalent cation concentrations are very low. Notably, I_{NS} inactivation rate does not depend on current amplitude, and hence may reflect purely VDI. However, based on analysis of existent and new data, and modeling, we find that I_{NS} can inactivate more rapidly and completely than I_{Ba} , especially at physiological temperature. Thus VDI that occurs during I_{Ba} (or I_{Ca}) must differ intrinsically from VDI during I_{NS} . To account for this, we have extended a previously published LTCC mathematical model of VDI and CDI into an excitation-contraction coupling model, and assessed whether and how experimental I_{Ba} inactivation results (traditionally used in VDI experiments and models) could be recapitulated by modifying CDI to account for Ba-dependent inactivation. Thus, the view of a slow and incomplete I_{NS} inactivation should be revised, and I_{NS} inactivation is a poor measure of VDI during I_{Ca} or I_{Ba} . This complicates VDI analysis experimentally, but raises intriguing new questions about how the molecular mechanisms of VDI differ for divalent and monovalent currents through LTCCs.

Keywords

L-type calcium current; Voltage-dependent inactivation; Calcium-dependent inactivation Barium; Non specific current

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1. INTRODUCTION

The L-type Ca current (I_{Ca}) contributes to the action potential plateau and initiates excitation-contraction coupling (ECC) in cardiac myocytes (Bers, 2001). It is conducted by $Ca_v1.2$ channels consisting of a pore-forming α_{1C} subunit, with four homologous domains of six transmembrane segments, in association with β and $\alpha_2\text{-}\delta$ subunits (Catterall, 2000). The C terminal domain of the α_{1C} subunit contains several regulatory sites, such as a PKA phosphorylation site, an EF-hand region and an IQ motif at which calmodulin (CaM) can bind.

Cardiac I_{Ca} is rapidly activated by membrane depolarization, whereas inactivation of L-type Ca channels (LTCC) is regulated by both Ca- and voltage-dependent inactivation (CDI and VDI) (Lee et al., 1985). CDI is due to binding of Ca to CaM (Peterson et al., 1999; Zuhlke et al., 1999), which causes a channel conformational change that prevents the EF-hand in the C-terminus from interacting with the cytosolic I-II linker, which then occludes the channel pore, thus accelerating inactivation (Cens et al., 2006). CDI is an important feedback mechanism that limits the amount of Ca entry during each Ca transient, regulates sarcoplasmic reticulum (SR) Ca load and modulates action potential duration (Bers, 2001). Indeed, prevention of CDI by expression of a mutant Ca-insensitive CaM in adult cardiomyocytes induces a dramatic (4- to 5-fold) prolongation of the cardiac action potential (Alseikhan et al., 2002). Computational analysis also identified CDI as the key player in shortening the action potential when external $[Ca]$ is increased (Grandi et al., 2009). The physiological significance of VDI is illustrated by the dramatic consequences of Timothy syndrome (Brunet et al., 2009; Splawski et al., 2005; Splawski et al., 2004), which impairs VDI and causes severe ventricular arrhythmias (and dysfunction in other tissues, e.g. brain).

I_{Ca} inactivates with a biexponential time course when Ca is the charge carrier, and it has been widely assumed that the initial fast decay represents CDI, and the slower phase reflects VDI. During ECC, with normal SR Ca release and Ca transients, local $[Ca]_i$ near the L-type Ca channel is elevated and inactivation is rapid (Fig. 1A top I_{Ca} trace, $t_{1/2} = 17$ ms). When Ca transients are abolished (e.g. by ryanodine or very strong $[Ca]_i$ buffering), I_{Ca} inactivation is slower (Fig. 1A, $t_{1/2} = 37$ ms) and reflects a small rise in local $[Ca]_i$ near the mouths of Ca channels due to Ca entering via the channels themselves. In addition to Ca LTCC allow permeation by other ions including Na, K, Sr, Cs, Ba, depending on ionic conditions, but inactivation is slower than with Ca and monoexponential when Ba, Sr, or Na are the charge carrier.

To differentiate VDI from CDI, several experimental and theoretical studies have used inactivation of Ba current via LTCC (I_{Ba}) as a measure of VDI (Cens et al., 2006; Lee et al., 1985; Mahajan et al., 2008; Peterson et al., 2000; Thiel et al., 2008). However, there is evidence that Ba can weakly mimic Ca (Ferreira et al., 1997), such that I_{Ba} inactivation is still a mixture of CDI and VDI, and I_{Ba} inactivation depends on current amplitude (Fig. 1A, $t_{1/2} = 161$ ms and Fig. 1B). To avoid this complication, some have used the monovalent cation current through LTCC (I_{NS}), which can be measured only when divalent cation concentrations are very low (Brunet et al., 2009; Hadley and Hume, 1987; Hryshko and Bers, 1992; Sun et al., 2000; Yuan et al., 1996). That is, extracellular Ca and Mg block I_{NS} ($K_i \sim 1$ and $50 \mu M$ respectively). Notably, I_{NS} inactivation rate does not depend on current amplitude (Fig. 1B, grey line) and hence could reflect purely VDI (Brunet et al., 2009). Inward I_{NS} has been reported to inactivate very slowly (Fig. 1A, $t_{1/2} > 500$ ms at $E_m = -30$ mV). Even at more positive E_m where inactivation is faster it is still incomplete (Fig. 1C). Note that extracellular divalent cations screen external membrane surface charge, shifting the membrane field and channel gating to more positive E_m . Thus, the activation state of I_{NS}

at -30 mV is comparable to that of I_{Ca} or I_{Ba} at ~ 0 mV in Fig. 1A (Bers, 2001; Hadley and Hume, 1987; Linz and Meyer, 1998).

In the present study, we sought to determine whether I_{NS} or I_{Ba} is appropriate for the study of the VDI component of I_{Ca} . New and existing data, and modeling, suggest that VDI of I_{NS} is faster and more complete than I_{Ba} inactivation, and that I_{Ba} exhibits Ba-dependent inactivation. Thus, neither I_{Ba} nor I_{NS} are accurate measures of VDI for I_{Ca} , although I_{Ba} VDI is largely like I_{Ca} VDI. This complicates VDI analysis experimentally, but raises intriguing new questions about how the molecular mechanisms of VDI differ for divalent and monovalent currents through LTCCs.

2. METHODS

2.1 I_{NS} measurements

Ventricular myocytes were dissociated enzymatically from hearts of adult male New Zealand White rabbits using a standard Langendorff perfusion procedure, as has been described previously (Bassani et al., 1994). Isolated cells were allowed to adhere to laminin-treated coverslips and were used acutely.

Monovalent current via LTCCs was recorded in whole cell ruptured-patch mode as described previously (Yuan et al., 1996). Cells were held at -65 mV and stimulated with infrequent (<0.1 Hz) depolarizing steps. The bathing solution contained (mM): 10 NaCl, 6 CsCl, 5 EDTA, 120 TEA-Cl, 10 glucose and 10 H-Hepes, adjusted to pH 7.4 with TEA-OH. The pipette solution contained (mM): 3 NaCl, 120 CsCl, 10 EGTA, 5 MgATP, 0.3 GTP, buffered to pH 7.2 with 20 mM H-Hepes and CsOH. Importantly, in recording I_{NS} EDTA was used to ensure chelation of both Ca and Mg, to prevent Mg from blocking the current ($k_i \sim 50$ μ M (Hryshko and Bers, 1992; Matsuda, 1986)). A measured liquid junction potential of 5 mV was corrected during analysis. Experiments were conducted at room temperature (22°C); a few recordings were obtained at 25°C and 35°C as specified in the results. Current decay was fitted with a monoexponential time constant (τ), with a steady-state plateau at the end of the voltage step. Use of biexponential functions did not improve the fitting.

2.2 Model development

We used a minimal Markovian model for I_{Ca} (Figure 2) that was developed previously by the Weiss group (Mahajan et al., 2008) based on I_{Ca} and I_{Ba} measurements in rabbit ventricular myocytes at physiological temperature. Inactivation of I_{Ca} is described as occurring via a voltage-dependent pathway (VDI) and an ion-dependent pathway (IDI). In the Mahajan formulation I_{Ba} inactivation was considered to define the VDI pathway. Here, we used both their 5-state model of I_{Ba} and an alternative 7-state I_{Ba} model, which uses the IDI pathway with parameters tuned to mimic a reduced affinity of Ba vs. Ca for CaM (i.e. [Ba] was reduced by a factor 1, 3, 10, 20, 30, 50 100 in the expression of the ion-dependent transition rates, Fig.2). We scaled the permeability (and single channel conductance) of LTCCs for Ba as reported in (Bers, 2001). Models of I_{Ba} were inserted in a model of ECC (Mahajan et al., 2008).

Model differential equations were implemented in Matlab (Mathworks Inc., Natick, MA, U.S.A.) and solved numerically using a variable order solver (ode15s) (Shampine and Reichelt, 1997). The digital cell was stimulated with voltage steps replicating the experimental protocols.

3. RESULTS

3.1 Inactivation kinetics

Typical recordings of I_{NS} (with Na and Cs as charge carriers) are shown in Fig. 3A, elicited by depolarizing pulses described in inset. With increasing depolarization, the net current changes from inward to outward, due to reduction in the driving force for Na and an increased driving force for Cs (I_{NS} reversal potential is about -4.5 mV). We characterized I_{NS} kinetics by considering the time course of current decay throughout the duration of depolarization. Inactivation τ s are reported in Fig. 3B for each depolarization E_m . Current decay is slower at $E_m < -10$ mV, and is hastened as the voltage steps are more depolarized (and the current becomes outward). Nevertheless, I_{NS} inactivation τ s measured in rabbit ventricular myocytes at room temperature are relatively fast (especially compared to the I_{NS} trace in Fig. 1A). I_{NS} inactivation was assessed in the same cell at two different temperatures (25 and 35°C, Figure 3 C vs. D). Availability curves were recorded using various durations of the inactivating pre-pulses, and τ of inactivation was estimated as described in (Findlay, 2002b). At physiological temperature I_{NS} inactivated more completely and rapidly than at room temperature. From these measurements, we calculated an average Q_{10} of ~1.85 for I_{NS} inactivation kinetics over the full range of E_m (although Q_{10} was larger at more negative E_m ; ~2.3 at -20 mV). We compared our data (corrected at 37°C, Figure 4A, $\diamond$) with previously published inactivation τ and found concordance with several previous studies of I_{NS} inactivation.

I_{NS} τ values were averaged (broken curve in Fig. 4A) and plotted vs. E_m as inactivation rate constants ($k = 1/\tau$) in Figure 4B, along with rate constants of inactivation of I_{Ba} and I_{Ca} (bi-exponential for I_{Ca} from (Mahajan et al., 2008)). I_{Ca} inactivation is faster when SR Ca release is present (top curve). Notably, I_{NS} inactivation was much faster than I_{Ba} inactivation, which was comparable to the slow component of I_{Ca} inactivation. I_{NS} inactivation becomes faster with increasing depolarization consistent with VDI, and gets faster when the current is outward. Thus, I_{NS} inactivates more rapidly and completely than I_{Ba} at 35-37°C (even if one accounts for surface charge effects). Thus, these cannot both reflect the same VDI process. Moreover if I_{NS} exhibits pure VDI, then the rate of I_{Ba} inactivation (which may include some weak CDI) would have to be faster than I_{NS} inactivation (i.e. $k_{VDI} + f(k_{CDI})$). So I_{NS} inactivation is too fast to reflect the VDI that occurs during I_{Ba} . On the other hand, I_{Ba} is not purely VDI either, because the rate of inactivation has a bell-shaped E_m -dependence (Fig. 4B inset and Fig. 6, circles, (Mahajan et al., 2008)) and increases with larger peak Ba current amplitude, as shown in Fig. 1B (Brunet et al., 2009).

We suggest that I_{NS} inactivation is VDI, but that it is fundamentally (mechanistically) different from VDI that occurs during I_{Ba} or I_{Ca} , and may therefore not be useful to characterize the VDI component of I_{Ca} . Further, I_{Ba} inactivation includes a moderate component of CDI (where Ba substitutes for Ca) and also VDI that is the same as the VDI component of I_{Ca} . Indeed, with strong fast intracellular Ca buffering, I_{Ca} inactivation is reduced (Katzka and Morad, 1989; Linz and Meyer, 1998) and approaches that seen for I_{Ba} .

3.2 Model analysis

We sought to determine whether and how experimental I_{Ba} inactivation (traditionally used in VDI experiments and models) could be recapitulated by extending a previously published model of CDI (Mahajan et al., 2008) to account for Ba-dependent inactivation. To mimic a reduced affinity of Ba (vs. Ca) in CDI, the parameters describing the Ca-dependent transitions (Fig. 2, dashed arrows) were modified to make it 3-100 times less sensitive to [Ba] (vs. [Ca]). In Figure 5A normalized I_{Ba} simulated in response to a voltage step to 0 mV

are shown. As the apparent affinity of Ba for CaM increases (approaching that of Ca), I_{Ba} exhibits faster inactivation (Fig. 5B). The τ of I_{Ba} decay (at 0 mV) is ~125 ms when the current inactivation is modeled as Ba-independent (e.g. only including VDI). When Ba affinity is much smaller than that of Ca (20-100 times), the predicted Ba-dependent inactivation is negligible, still indistinguishable from only the 5-state VDI model. Only when the relative k_{Ca}/k_{Ba} affinity falls to 10 or below does Ba-dependent inactivation become appreciable.

Figure 5B also shows the maximal probability of occupancy of the two Ba-dependent inactivation states (filled and open squares). 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} is equal to 10, 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 SR Ca release), and the probabilities of occupancy of I_{1ion} and I_{2ion} 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 τ of I_{Ba} decay decreases as intracellular [Ba] increases (Fig. 5C), whereas it is unchanged when using the 5-state VDI only I_{Ba} model (not shown).

In Figure 6 the effect of Ba-dependent inactivation is shown over a wide range of voltages. Note that the τ for I_{Ca} (or I_{Ba} at $k_{Ca}/k_{Ba}=1$) reflects the strong CDI seen for I_{Ca} , whereas the curve for $k_{Ca}/k_{Ba}=10$ approaches the shape seen for I_{Ba} measurements. While the model is an imperfect match to the data, this simple version provides a useful framework for separating VDI and CDI for I_{Ca} and I_{Ba} .

4. DISCUSSION

I_{Ca} plays an essential role in cardiac electrophysiology, affecting action potential and arrhythmogenesis, and also is central to ECC, by controlling SR Ca release and contributing to the SR replenishment (Bers, 2001). Inactivation of I_{Ca} is both Ca- and E_m - dependent, and there is evidence that (1) the degree and the rate of inactivation depends on the amount of Ca current (Brehm and Eckert, 1978; Peterson et al., 2000); (2) SR Ca release increases the rate of I_{Ca} inactivation (Adachi-Akahane et al., 1996; Puglisi et al., 1999); (3) inactivation is reduced when Ba is the current carrier (Ferreira et al., 1997; Mahajan et al., 2008; Sun et al., 1997). In cardiac myocytes, whether there is true CDI when SR Ca release is prevented is debated. E_m -dependent inactivation is indicated by the current decay when monovalent cations are the charge carrier. In these conditions, the rate and the extent of current inactivation do not depend on current amplitude (Brunet et al., 2009).

Based on these observations, I_{NS} and I_{Ba} are commonly used in experiments and models as a surrogate of VDI. The aim of this study was to determine whether I_{NS} or I_{Ba} inactivation are a true measure of VDI for I_{Ca} , and if not to develop a practical model incorporating CDI and VDI.

4.1 Monovalent current

I_{NS} inactivates incompletely at room temperature (Hadley and Hume, 1987; Sun et al., 1997; Sun et al., 2000), but when the temperature was raised to 30°C the extent of inactivation was greater (Hryshko and Bers, 1992). Inactivation was even more complete at physiological temperature (compare Figure 3C to 3D). Mitarai et al. (Mitarai et al., 2000) also showed strong VDI of I_{NS} at physiological temperature (Figure 4, ►), and rescaling available experimental data to 37°C gave comparable results. Mitarai et al. (Mitarai et al., 2000)

explained some previous work reporting slower I_{NS} decay by suggesting that β -adrenergic stimulation could slow LTCC inactivation (as shown previously (Yuan and Bers, 1995)). Indeed, when measuring I_{NS} in the presence of extracellular divalent cations (which partially block I_{NS}), isoproterenol was added to compensate for I_{NS} reduction (e.g. (Linz and Meyer, 1998; Matsuda, 1986)). However, since divalent cations can strongly block I_{NS} , it is debatable whether intrinsic I_{NS} gating can be measured in the presence of divalent cations in the bathing solution.

There are two tacit assumptions usually made when using I_{NS} as an experimental model to study I_{Ca} VDI: 1) the absence of ion-dependent inactivation, which seems appropriate, and was recently confirmed by (Brunet et al., 2009); 2) VDI of I_{NS} , I_{Ba} and I_{Ca} are the same process, which we now think may not be the case (see 4.3 and 4.4 for discussion).

4.2 Ba current

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 (Brunet et al., 2009; Ferreira et al., 1997) 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. Ba is known to complex with CaM and activate interaction with CaM targets (Ozawa et al., 1999; Yamazaki et al., 1996), 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.

To account for the moderate Ba-dependent inactivation, we have extended a previously published LTCC mathematical model of VDI and CDI into an ECC model, and assessed whether and how experimental I_{Ba} inactivation results could be recapitulated by modifying CDI to account for Ba-dependent inactivation. We found that making 10-fold reductions in the Ca-dependent transition rates (in Fig 2) resulted in appropriate Ba-dependent I_{Ba} inactivation. Sun et al. (Sun et al., 2000) similarly fit their data of I_{Ba} by decreasing the Ba association constant to 10% of that of Ca. Thus their model suggested a smaller reduction of Ba affinity vs. Ca compared to Ferreira et al. (Ferreira et al., 1997), where the affinity for the inactivation site was reckoned to be ~ 100 times lower for Ba than Ca for Cav1.2 using a heterologous expression system. Reasons that 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 prebound 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, given the Ba-dependent inactivation implemented here, one could derive a slower VDI model, which should be a more faithful representation of purely VDI during I_{Ca} . This may be an important distinction when one seeks to dissect the relative roles of Ca and E_m in mediating normal function and pathophysiology (e.g. arrhythmogenesis).

4.3 Ba vs. Na current via LTCCs

Some studies in guinea pig ventricular myocytes showed similar inactivation of Ba, Sr, and Na currents (Findlay, 2002a), at least at room temperature. Analogously, Sun et al. (Sun et al., 2000) also reported similar inactivation τ for I_{NS} and I_{Ba} in rat cardiomyocytes (at room temperature), whereas I_{NS} (and I_{Sr}) inactivated more slowly than I_{Ba} in human atrial cells at 23°C (Sun et al., 1997). Mahajan et al. measured I_{Ba} (and I_{Ca}) in rabbit cardiomyocytes at physiological temperature and reported that I_{Ba} inactivates incompletely with a monoexponential decay with $\tau \sim 150$ ms (with little voltage-dependence) in the absence of β -adrenergic

stimulation. Here, we found that I_{NS} inactivation is accelerated as temperature increases (especially at negative E_m , where I_{NS} inactivation is slowest). Thus, we find that I_{NS} can inactivate more rapidly and completely than I_{Ba} , especially at physiological temperature (Figure 3), suggesting that VDI that occurs during I_{Ba} (or I_{Ca}) must differ intrinsically from VDI during I_{NS} . During I_{Ca} and I_{Ba} flow, it is thought that the channel is essentially always occupied by at least one divalent cation occupying the pore (Yang et al., 1993), and this binding prevents monovalent permeation. We suggest that in the absence of divalent cation occupancy (during I_{NS}), VDI of the LTCC is fundamentally different, exhibiting steeper E_m -dependence, which is faster than when divalent cations occupy the channel pore.

4.4 Ca vs. V-dependent inactivation

Some studies have suggested that VDI predominates over CDI in cardiac myocytes (Findlay, 2002a; Findlay, 2002b; Findlay et al., 2008), but most studies suggest that CDI predominates under physiological conditions, especially where local SR Ca release amplifies Ca influx (Adachi-Akahane et al., 1996; Linz and Meyer, 1998; Pelzer et al., 1990; Puglisi et al., 1999; Shannon et al., 2000; Sun et al., 1997; Yuan and Bers, 1995). As indicated in Figs. 1A and 4B, it is clear that I_{Ca} inactivates much faster than I_{Ba} , even when there is no SR Ca release. Indeed, the integrated Ca entry for I_{Ca} without SR Ca release in Fig. 1A (normalizing to peak current) is 61% of that for I_{Ba} , and with SR Ca release it drops to 32% of that for I_{Ba} . Similarly, during the cardiac action potential SR Ca release diminished integrated Ca influx via I_{Ca} by approximately 50% at both 25°C and 35°C (Puglisi et al., 1999) whereas peak I_{Ca} hardly changed. These results indicate that CDI is dominant in I_{Ca} inactivation in a physiological setting, but this also suggests that VDI is not negligible.

In most studies, including the present model of I_{Ca} inactivation, it is assumed that CDI and VDI are independent processes. However, based on single channel recordings, Josephson *et al.* concluded that Ca- and voltage- dependent mechanisms involved in the decay of the ensemble LTCC current were highly interdependent, so that both the slow and fast phase of I_{Ca} decay are related to CDI (Josephson et al., 2009). Thus, it is possible that the two processes interact, and VDI when monovalent ions serve as charge carrier is intrinsically different from I_{Ba} and I_{Ca} VDI.

4.5 Conclusions

Our work indicates that neither I_{Ba} nor I_{NS} reflect pure VDI for I_{Ca} . This raises new questions of whether fast inactivation of I_{NS} is due to the absence of divalent cations that bind in the channel pore, or whether inactivation of inward I_{NS} differs from outward I_{NS} (e.g. due to the intracellular milieu). The main limitation to the use of I_{Ba} as a measure of VDI is its ability to modestly mimic Ca-dependent inactivation. Modeling results predicted a detectable Ba-dependent inactivation (i.e., bell-shaped dependence on E_m) when apparent Ba affinity for CaM is one tenth than that of Ca. Nevertheless, if intracellular Ba accumulation is avoided (and if I_{Ba} amplitude is kept small), I_{Ba} is probably a better VDI model for I_{Ca} than is I_{NS} .

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Glossary

I_{Ca}	L-type Ca current
I_{Ba}	Ba current through LTCC
I_{NS}	Non specific (monovalent) current through LTCC
LTCC	L-type Ca channel
CDI	Ca-dependent inactivation
IDI	Ion-dependent inactivation
VDI	Voltage-dependent inactivation
ECC	Excitation-contraction coupling

SR	Sarcoplasmic reticulum
τ	time constant

Figure 1.

I_{Ca} inactivation: role of Ca released from the SR and of Ca permeating LTCC; I_{Ba} and I_{NS} inactivation. A. Normalized Ca, Ba and Na currents (I_{Ca} , I_{Ba} & I_{NS}) measured at 0 mV (except I_{NS} at -30 mV to obtain comparable activation state). I_{Ca} was recorded under conditions where normal SR Ca release and Ca transients were allowed to occur (perforated patch) or prevented (ruptured patch with cells dialyzed with 10 mM EGTA). I_{Ba} was also recorded in absence of Ca release and transient. I_{NS} was measured in divalent-free conditions (10 mM EDTA inside and out). B: Ratio between pedestal and peak current, in response to a 1000 ms- depolarizing step to 0 mV, is plotted against current amplitude of I_{Ba} and I_{NS} . The lines represent linear regressions. Data are from Brunet et al. (Brunet et al.,

2009). C. I_{NS} and I_{Ca} availability through Ca channels (at -10 mV) after 500 ms pulses to the indicated E_m in guinea-pig ventricular myocytes (redrawn from (Hadley and Hume, 1987)).

Figure 2.

Seven-state Markov model of I_{Ca} . Grey lines correspond to voltage-dependent transitions and dashed lines correspond to ion-dependent transitions. A 5-state model (5 lower states) without ion-dependent transitions was used for comparison in some simulations.

Figure 3.

I_{NS} inactivation. A: representative recordings of membrane currents in rabbit ventricular myocytes elicited by 200-ms steps (*inset*) from -65 mV to the indicated E_m . B: τ of I_{NS} decay. C, D: I_{NS} availability was recorded at 25 °C and 35 °C following pre-pulse steps of 362, 148, 59, 26, 6.5, 3.5, 1.75 ms duration. Data from C and D have been transposed onto a time scale (availability against prepulse interval) and τ s were calculated as in (Findlay, 2002b).

Figure 4.

A: Voltage dependence of the decay time of I_{NS} . Data from Mitarai et al. (▴) were collected at physiological temperature. All the other experimental data were obtained at room temperature and here rescaled at 37 °C ($Q_{10}=1.85$). The dashed line is obtained by interpolation of the averaged τ s. B: Rates of the fast (◇, □) and slow (◆, ■) components of a double exponential fit of I_{Ca} decay vs. E_m recorded by Mahajan et al. (Mahajan et al., 2008) with and without Ca transient (CaT). Rates of I_{Ba} (○ and *inset*) and I_{NS} (dashed lines) inactivation are obtained from monoexponential fits of current decay.

Figure 5.

I_{Ba} inactivation. A: Normalized I_{Ba} traces obtained when applying a voltage step to 0 mV (from a holding potential of -80 mV) and k_{Ca}/k_{Ba} is equal to 100, 50, 30, 20, 10, 3, 1 (VDI +f(CDI)). I_{Ba} was also simulated as independent of Ba ion flux (VDI only, dashed line). B: Left ordinate: τ of I_{Ba} decay decreases as the simulated Ba affinity for CaM is increased (i.e. k_{Ca}/k_{Ba} decreased). Right ordinate: Ion(Ba)-dependent state occupancy during I_{Ba} decay (in response to a voltage step to 0 mV) is enhanced with increasing affinity of Ba for CaM (i.e., decreasing k_{Ca}/k_{Ba}). C: Effect of intracellular Ba accumulation on current inactivation. Normalized I_{Ba} traces obtained in response to a voltage step to 0 mV are shown in correspondence of various levels of $[Ba]_i$ (from 1 nM to 150 μ M).

Figure 6.

τ s of I_{Ba} decay at the indicated E_m when I_{Ba} inactivation is simulated as independent of Ba ion flux (VDI only, dashed line) and when Ba affinity for CaM is varied ((VDI+ $f(CDI)$), k_{Ca}/k_{Ba} equal to 100, 50, 30, 20, 10, 3, 1). Experimental data are from (Mahajan et al., 2008).