M i n i r e v i e w

Inactivation of L-type Calcium Channels in Cardiomyocytes. Experimental and Theoretical Approaches

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Abstract. The L-type calcium current (I_{Ca}) plays an important role in excitation-contraction coupling of heart cells. It is critical for forming the major trigger for Ca^{2+}-induced Ca^{2+} release from the sarcoplasmic reticulum and hence its feedback regulation is of fundamental biological significance. The channel inactivation sharpens the kinetics and temporal precision of the Ca^{2+} signals so that it prevents longer-term increases in free intracellular Ca^{2+} concentration. Cardiac L-type Ca^{2+} channels are known to inactivate through voltage- and Ca^{2+}-dependent mechanisms. Pure voltage-dependent inactivation has a much slower time course of development than Ca^{2+}-dependent inactivation and plays minor role in inhibition of Ca^{2+} influx into the cell. The major determinant of the inactivation kinetics of Ca^{2+} current during depolarization is Ca^{2+}-dependent mechanisms. Furthermore, it is possible to distinguish two phases in Ca^{2+}-dependent inactivation of calcium current: a slow phase that depends on Ca^{2+} flow through the channels (Ca^{2+} current-dependent inactivation) and a fast one that depends on Ca^{2+} released from the sarcoplasmic reticulum (Ca^{2+} release-dependent inactivation). Although both Ca^{2+} released from the SR and Ca^{2+} permeating channels play a role, SR-released Ca^{2+} is the most effective inactivation mechanism in inhibition of Ca^{2+} entry through the channel.

Key words: Ca^{2+} channels — Inactivation — Cardiac excitation-contraction coupling

Introduction

In heart muscle cells, excitation-contraction coupling involves a series of steps. It starts with voltage-dependent activation of sarcolemmal calcium channels that allows influx of Ca^{2+} ions into the tubulo-reticular junction. The tubulo-reticular junction arises from a close contact between the plasma membrane T-tubules, con-

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taining L-type calcium channels and the cisternae of sarcoplasmic reticulum, containing ryanodine receptors (Sun et al. 1995). Initial entry of Ca^{2+} ions through the activated calcium channels then triggers much larger Ca^{2+} release from sarcoplasmatic reticulum (SR) through adjacent ryanodine receptors (RyRs). Before activating contraction, calcium current flowing through calcium channels inactivates and at the same time, the Ca^{2+} release terminates.

Calcium channel inactivation transpires as a decay of current during the prolonged depolarization. Cardiac L-type Ca^{2+} channels are known to inactivate through voltage- and Ca^{2+}-dependent mechanisms (Hadley and Hume 1987; Shirokov et al. 1993). It is now well established that the major determinant of the inactivation kinetics of Ca^{2+} current during depolarization is the Ca^{2+}-dependent mechanism (Pelzer et al. 1990; Rose et al. 1992).

Different approaches have been used to understand the mechanisms of L-type channel inactivation. Progress in understanding has been greatly facilitated by the development of efficient measurement techniques (voltage clamp, confocal microscopy). Nevertheless, the resolution of this technique is insufficient to understand what is happening on the level of individual excitation-contraction coupling units as well as on the level of Ca^{2+} channels. Information about these processes can be obtained through modeling. This theoretical approach can reveal otherwise hidden processes and clarifies our understanding of the phenomena. And finally, the molecular biology also constitutes an important complement to mentioned approaches. The goal of this mini-review is to summarize the major characteristics of L-type Ca^{2+} channel inactivation in experimental and theoretical approaches.

Voltage-dependent inactivation

Two major molecular mechanisms have been previously implicated in voltage-dependent inactivation of voltage-gated ion channels (Yellen 1998). The "ball and chain" mechanism, which was described in the tetrameric Shaker K^+ channel, is responsible for fast N-type inactivation (Hoshi et al. 1990). This type of inactivation involves the occlusion of the inner channel mouth by a "ball region" at the amino (N-) terminus. Somewhat similarly, the "hinged-lid" mechanism in the Na^+ channel is mediated by the IFM motif of the cytoplasmic linker between repeats III and IV of the α_1-subunit (West et al. 1992). In both Na^+ and K^+ channels receptor sites for different inactivation gates are located in the S4-S5 intracellular loops (Isacoff et al. 1991). The second, C-type mechanism of slow K^+ channel inactivation (Choi et al. 1991) was found to involve a constriction of the pore by the S6 segments lining the intracellular side of the pore that are arranged in an inverted teepee structure (Doyle et al. 1998).

The molecular mechanism underlying the voltage-dependent inactivation process in the L-type Ca^{2+} channels appears to be more complex (Hering et al. 2000). Experimental examination of chimeras between the α_{1C} and the faster inactivating Ca^{2+} channel showed that multiple regions are involved in inactivation, including transmembrane segments, IS6 (Zhang et al. 1994), IIIS6 (Hering et al. 1997), IVS5

(Motoike et al. 1999) IVS6 (Berjukow et al. 2000), repeats I-II linker (Stotz et al. 2000), as well as the C-terminal determinants, E1537 of EF-hand motif and the Ca^{2+}-sensing 80-amino acid domain 1572–1651 (Soldatov et al. 1997). In these regions, multiple amino acids were shown to be critical for the rate of inactivation of Ba^{2+} current (Hering et al. 2000). Recently, Shi and Soldatov (2002) have shown that the slow inactivation is mediated by an annular determinant composed of hydrophobic amino acids located near the cytoplasmic ends of transmembrane segments S6, in repeats I-IV. Thus, inactivation of Ca^{2+} channels has features of the C-type inactivation.

Electrophysiological evidence for voltage-dependent inactivation is based on results obtained on the level of macroscopic currents as well as on the level of single-channel recordings. First, independent of the divalent cation charge carrier (Ba^{2+}, Sr^{2+}), macroscopic Ca^{2+} channel currents activated by a step depolarization decline with time during maintained depolarization (Kass and Sanguinetti 1984; Lee et al. 1985). Similarly, inward as well as outward directed L-type currents carried by Na^+, Cs^+, or K^+ undergo time dependent inactivation (Lee and Tsien 1982; Hess and Tsien 1984; Hadley and Hume 1987). In addition, prepulses that fail to trigger measurable I_{Ca} nevertheless produce inactivation (Hadley and Hume 1987). Correspondingly, in cell-attached patches of cardiac membrane, single channel L-type currents carried by Ba^{2+} or Na^+ inactivate with time during depolarization (Cavalié et al. 1983; McDonald et al. 1986) as do currents through cardiac channels incorporated into lipid bilayers (Rosenberg et al. 1986).

Voltage-dependent inactivation of calcium current is very slow and incomplete (the half time for nonspecific current carried by monovalent ions to reach steady-state is $t_{1/2} > 500$ ms at -30 mV) and becomes more prominent at more positive potentials (Hadley and Hume 1987; Bers and Perez-Reyes 1999). Interestingly, the time constant of inactivation carried by Ba^{2+} current (I_{Ba}) is shorter ($t_{1/2} = 161$ ms) than that of nonspecific current carried by monovalent ions and this may reflect a modest ability of Ba^{2+} to mimic Ca^{2+}-dependent inactivation (Ferreira et al. 1997).

The mechanism of the voltage-dependent inactivation has been presented in a mathematical model by Cavalié's group (Cavalié et al. 1986). They examined the Ca^{2+}-gating behavior during steady and stepwise depolarization with Ba^{2+} as the charge carrier in recordings of single Ca^{2+}-channel activity from cell-attached membrane patches of single guinea-pig ventricular cells. Upon step depolarization, they observed two types of current records: 1. traces with Ca^{2+} channel activity (in the form of closely-spaced brief pulses of inward current with unitary amplitude) of various length (fast bursting behavior), and 2. blank sweeps without any detectable single-channel opening (slow gating). They concluded that the blank sweeps reflect conditions where the channel is unavailable for opening (inactivated). The channel gating transitions were described by model containing two closed (C_1, C_2) and one open state (O), with gradual entry into a long lived shut state (inactivated state, I) (Cavalié et al. 1986) (Figure 1). In other words, the voltage-dependent inactivation was described as the transition of the channel into the absorbing inactivated state.

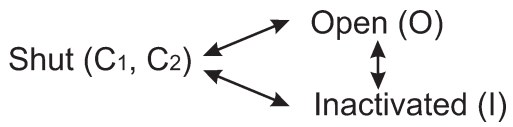

Figure 1. Model of the voltage-dependent inactivation of the calcium channel (modified from Cavalié et al. 1986). The states are lumped into two subsets. The upper block includes the short-lived shut states (C_1, C_2) and the open state (O). The lower block comprises the long-lived shut state (I). The transitions within the upper subset are related to the fast bursting behavior. The slow gating (i.e. blank sweeps without any detectable single-channel opening) is represented by the transitions between upper and lower subset.

Calcium-dependent inactivation

Elevated intracellular Ca^{2+} concentration near the mouth of the L-type channel triggers channel inactivation, providing negative feedback to Ca^{2+} influx. Critical molecular determinants for Ca^{2+}-dependent inactivation of the L-type calcium channel have been attributed to a putative Ca^{2+} binding EF-hand motif (DeLeon et al. 1995) and an IQ-like motif (Peterson et al. 1999) that are located close to each other near the inner channel mount at the cytoplasmic carboxyl-terminal tail of the $\alpha 1$ subunit. Recently it was shown that binding of calmodulin (CaM) to the IQ motif of the Ca^{2+} channel is a necessary prerequisite of I_{Ca} inactivation. Peterson et al. (1999) found that a mutant CaM which cannot bind Ca^{2+} at any of the four Ca^{2+} binding sites blocks the effects of Ca^{2+}-bound form of CaM (Ca^{2+}-CaM) on the L-type channel, suggesting that both the Ca^{2+}-free and Ca^{2+}-CaM bind to the calcium channel. However, only the Ca^{2+}-bound form can induce inactivation (Peterson et al. 1999). In particular, it appears that Ca^{2+} binding to only the C-terminal lobe of CaM (with two Ca^{2+} binding sites) appears to be important for inactivation (Peterson et al. 1999). Then, according to these authors, binding of Ca^{2+} to the C-terminal lobe of CaM allows binding of Ca-CaM to the IQ motif and in this way causes rapid inactivation of the channel. On the other hand, the EF-hand may support the transduction of the conformational changes induced by the Ca^{2+}-CaM-binding step onto the $\alpha 1$ subunit of the channel (Peterson et al. 2000).

Calcium ions that cause inactivation of the calcium channel can originate from several sources. In mammalian cardiac myocytes, the increase in cytoplasmic Ca^{2+} concentration is due to the inflow of Ca^{2+} through the L-type channel pore during depolarization (causing current-dependent inactivation), and due to the release of Ca^{2+} from the sarcoplasmic reticulum (causing release-dependent inactivation).

Current-dependent inactivation

Calcium current-dependent inactivation of calcium channel results from the increasing intracellular Ca^{2+} concentration near the mouth of the channel due to Ca^{2+} influx through the L-type channel pore during depolarization. The existence

of this type of inactivation was concluded from the findings observed in the ciliate Paramecium (Brehm and Eckert 1978) and in frog heart cells (Mentrard et al. 1984), which lack a well-developed T-tubular system. The current traces recorded from these preparations do not display fast component of inactivation dependent on Ca^{2+} release from SR, indicating that Ca^{2+} influx through the L-type channels is the major determinant of the inactivation kinetics of calcium current. In these types of cells inactivation of calcium current is faster with Ca^{2+} as the charge carrier than it is with Ba^{2+} or other divalent or monovalent ions. The next line of evidence that supports existence of current-dependent inactivation is that inactivation of I_{Ca} depends on the external Ca^{2+} concentration. Elevating external Ca^{2+} enhances the peak current and accelerates its decline (Mentrard et al. 1984).

In the frog heart cells, the U-shaped voltage dependence of the inactivation rates was reported (Mentrard et al. 1984). The time constant of I_{Ca} inactivation in cardiac myocytes, under conditions when release of Ca^{2+} from SR was suppressed (modified of ryanodine, caffeine), ranged between 20–50 ms at 0 mV, i.e., at the potential corresponding to the peak of I-V relationship (Sham et al. 1995).

To explain the mechanism of Ca^{2+} current-dependent inactivation, several models have been proposed. In the shell model (Figure 2A), the Ca^{2+} flowing through the channels accumulates into a macroscopic shell underneath the plasma membrane (Standen and Stanfield 1982; Chad and Eckert 1984). The shell is filled by Ca^{2+} entering through open channels, inactivating both open and closed channels with the elevated Ca^{2+} concentration at a rate, determined by how fast the

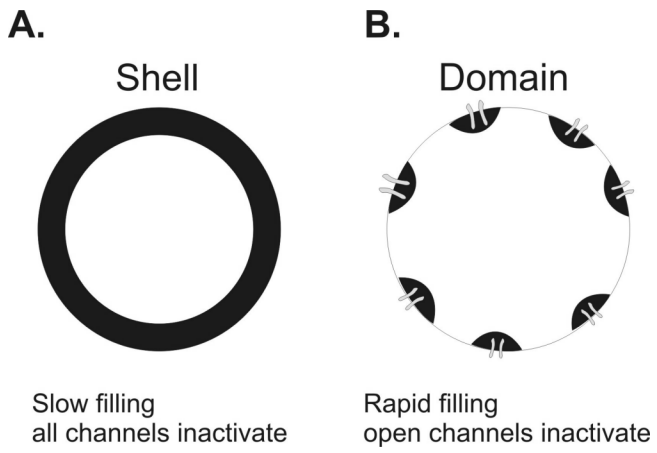

Figure 2. Shell and domain model for Ca^{2+} induced inactivation (modified from Sherman et al. 1990). **A.** In cells with high density domains may overlap to form a continuous submembrane compartment "shell" of elevated Ca^{2+}. **B.** In the cell with low channel density high Ca^{2+} will be found only in the "domains" underneath the inner mouths of open channels.

shell is filled by Ca^{2+}. To summarize, the key mechanism for Ca^{2+} sensitive inactivation according to this model is that neighboring transmembranous Ca^{2+} channels selectively inhibit each other, i.e., Ca^{2+} influx through one channel contributes to the inactivation of other adjacent channels. This model thus represents an extreme case of localized interdomain coupling of the channels in which the web of connectivity spans the entire cell membrane (i.e. model predicts a high density of the calcium channels).

However, for the cell with low channel density the idea for a "shell" is not appropriate. The high concentration Ca^{2+} "shell", in this case, fragments into a collection of non-overlapping "domains" localized near the open channels. These domains form rapidly when channels open and disappear rapidly when channels close. Therefore, in the local domain model (Sherman et al. 1990) (Figure 2B) inactivation results from the binding of "domain Ca^{2+}" to open channel, and the time scale of inactivation is determined by the kinetics of Ca^{2+} binding to and blocking the channel. The Ca^{2+}-sensing site is located very close to the channel mouth, making it less accessible to chelators (Sherman et al. 1990; Shirokov et al. 1993).

To summarize, the shell model predicts that neighboring transmembranous Ca^{2+} channels selectively inhibit each other since local domain model suggests that the existence of interactions among Ca^{2+} channel is not required to describe Ca^{2+} channel gating and inactivation of the open channel occurs by the binding of Ca^{2+} to it.

Noceti et al. (1998) have proposed a kinetic model for Ca^{2+}-current dependent inactivation of calcium channel based on the whole cell experiments on *Xenopus oocytes* expressing the cloned α_{1C} subunit of the rabbit cardiac Ca^{2+} channel. They observed that increasing the single channel amplitude increased the Ca^{2+}-dependent inactivation rates and left-shifted their voltage dependence. They also showed that the rates of Ca^{2+}-dependent inactivation were dependent on open probability (increasing the open probability with dihidropiridine agonist (−)Bay K 8644 produced an increase in the rate of inactivation). Summarizing they found the rates of Ca^{2+}-dependent inactivation sensitive to both Ca^{2+} flux through the channel (single channel amplitude) and open probability. The suggested kinetic model is shown in Figure 3. The local domain model proposed by Sherman et al. (1990) was used to account for the voltage shift, observed between the voltage dependence of inactivation rate of calcium current and the voltage dependence of Ca^{2+} flux. The internal concentration near the pore of the channel was expressed as linear function of the Ca^{2+} flux. Thus, with assumptions that Ca^{2+} binds instantaneously to a single site and at a fixed distance from the site, the rate of Ca^{2+}-dependent inactivation depends linearly on the flux (i.e. on the single channel amplitude). The dependence of inactivation process on open probability of the channel was achieved by a sequential connection of the inactivated state to the open state. The proposed kinetic scheme reproduced well the mentioned kinetic features of the experimental data.

Rose et al. (1992) used the model of the voltage-dependent inactivation of

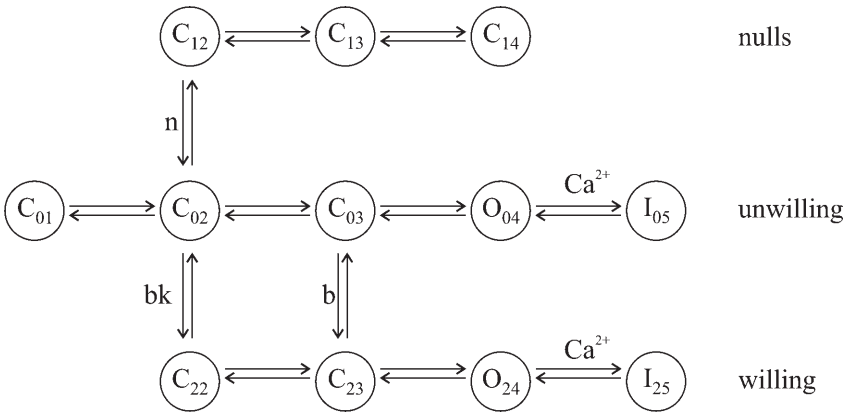

Figure 3. Schematic diagram for the transitions between the states of the L-type Ca^{2+} channel (Noceti et al. 1998). Three modes are originated from a single deep closed state (C_{01}). In the top line, the channel never opens, although it displaces all the charge of the voltage sensor (channel gates without opening). The middle (unwilling) and button (willing) lines end with open states and subsequent Ca^{2+}-dependent inactivated states. The parallel transitions carry the same amount of charge. The vertical transitions between the lines are voltage independent and can be (–)Bay K 8644 sensitive. The transition rates between all states in the kinetic model follow the Eyring rate theory (i.e., they are exponential functions of the energies required for the transition to the given state). The rates to the Ca^{2+}-inactivated state are voltage-independent.

Cavalié et al. (1986) (Figure 1) to fit the ensemble average current when Ca^{2+} is the charge carrier. They showed that this model does not adequately describe the ensemble average current. Model fails to reproduce the maintained component of I_{Ca} (Rose et al. 1992), suggesting that current decay does not reflect the progressive entry of channels into an absorbing inactivated state. Yue et al. (1990) obtained the evidence for Ca^{2+}-specific changes in gating at the unitary level by analysis of the conditional probability that a channel is open at any given time after it first opens. They showed that the chief effect of Ca^{2+} entry is not to accelerate inactivation to the absorbing state but rather to favor occupancy of a closed state from which the channel can still reopen. Reopenings continue to occur for hundreds of milliseconds. Later observations demonstrated that Ca^{2+} inactivation transpired as a discrete shift in the pattern of gating from a mode characterized by dense bursts of activity (mode normal) to one typified by infrequent openings (mode Ca), thus producing Ca^{2+}-mediated inactivation of the channel (Imredy and Yue 1994). The difference in gating arises primarily from a 100-fold reduction in the final opening rate in "mode Ca". Voltage sensitive inactivation is characterized as a kinetically separate effect (as a reduction in the overall availability of a channel to open at all (Cavalié et al. 1986)). Moreover, Imredy and Yue (1994) consider these two types of inactivation as independent processes. This conclusion

is further supported by the work of Shirokov et al. (1993). By parallel recording of prepulse-induced inactivation of I_{Ca} and of changes of charge movement evoked by inactivation, they showed that extracellular divalent cations modulated voltage-dependent inactivation of the Ca^{2+} channel. Increased intracellular Ca^{2+} concentration appears to cause inactivation of the channel without affecting its voltage sensor.

To summarize, in contrast to the model proposed by Noceti et al. (1998), the calcium channel according to the model described by Imredy and Yue (1994) cannot inactivate from the open state. The Ca^{2+}-dependent inactivation, in last case, transpires as a discrete shift in the pattern of gating from the mode characterized by dense bursts of activity to the mode characterized by infrequent openings.

The original Imredy-Yue model (Imredy and Yue 1994) described two-mode behaviour with five states (three in the normal mode and two in mode Ca) and served as the starting point of an improved L-type channel model described by Jafri et al. (1998). They added additional states, improved Ca^{2+}-dependent inactivation and voltage-dependent activation (Figure 4). Furthermore, they improved voltage-dependent inactivation, described as parallel process, and open-channel ion permeation. With elevation of free Ca^{2+} concentration in the vicinity of the channel, the channel transition from the normal mode to mode Ca is controlled by a Ca^{2+}-dependent rate constant (γ). The channel cannot inactivate from the open state. Voltage-dependent inactivation was modeled as a Hodgkin-Huxley-type gate that inactivates the channel independently of the Ca^{2+}-dependent states. The model produced Ca^{2+} inactivation with time course in good agreement with experimental results (Imredy and Yue 1994; Jafri et al. 1998).

Patch clamp experiments in cell-attached configuration revealed that calcium channel inactivation is promoted in multi-channel patches (Imredy and Yue 1992). Investigators compared the time course of decay of ensemble average currents de-

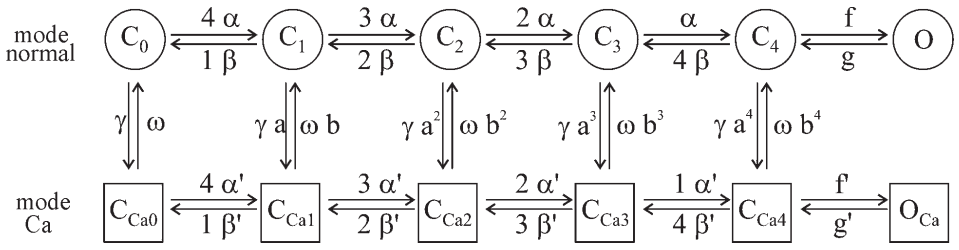

Figure 4. Schematic diagram for the transitions between the states of the L-type Ca^{2+} channel (Jafri et al. 1998). The upper row of states comprises the "mode normal", and the lower row comprises "mode Ca". The channel is composed of four independent subunits, each of which can close the channel. The corresponding states are C_n, where n is the number of permissive subunits. With depolarization, the channels undergo transitions from left to the right. With four permissive subunits, there is a voltage-independent transition to the conducting state O. With elevation of free Ca^{2+} concentration, the channel transition occurs from the mode normal to mode Ca.

rived from patches containing two versus one channel. The prominent enhancement of decay rate with two channels was observed, indicating the existence of appreciable interactions between the channels. It suggests that Ca^{2+} influx through one channel can selectively contribute to the inactivation of another, adjacent channel. These findings emphasize that Ca^{2+} currents are controlled not only by intrinsic channel properties but also by local diffusive interactions among neighboring channels (Imredy and Yue 1992).

Zahradníková et al. (2001) examined the extent of the effect on the geometry of the channel cluster by simulation of currents in the absence of the release dependent inactivation using the gating model of Jafri et al. (1998). Authors used a topology of L-type channel clusters that corresponds to the topology of the L-type channel in the dyad (Franzini-Armstrong et al. 1999). They found that a decrease in the distance between the channels, increase in the number of channels and increase in the channel conductance led to a progressive increase in the rate of inactivation in accordance with experiments (Imredy and Yue 1992). It should be emphasized that all the effects of varying cluster parameters arised from the variable magnitude and duration of the free Ca^{2+} elevations at the cytosolic side of the channels, resulting from the influx of calcium ions through the channels themselves. These results represent one of example of a widely proposed design theme, in which the spatial distribution of channels, not only their inherent kinetic properties, contribute importantly to overall system performance.

Release-dependent inactivation

Calcium ions released from SR significantly contribute to the inactivation of Ca^{2+} channels, as well. This is because SR Ca^{2+} is released into the same restricted junctional space where most of the L-type Ca^{2+} channels probably reside. This type of inactivation has faster kinetics (τ = 6–15 ms) compared with both the voltage-dependent and Ca^{2+}-current dependent inactivation (Sham et al. 1995; Sham 1997).

The extent of contribution of Ca^{2+} release from the SR on the inactivation of Ca^{2+} channel has been probed either by manipulating the Ca^{2+} content of the SR or by altering the diffusion distance of Ca^{2+} by dialysis with high concentration of Ca^{2+} buffers. Depletion of SR Ca^{2+} stores with caffeine or abolition of Ca^{2+} release with ryanodine caused a marked prolongation of inactivation (Balke and Wier 1991; Sham et al. 1995). On the contrary, enhancement of Ca^{2+} release by increasing Ca^{2+} loading of SR resulted in an acceleration of inactivation (Sipido et al. 1995). The inactivation of L-type Ca^{2+} channel was shown to depend linearly on the rate and magnitude of Ca^{2+} release from the RyRs (Adachi-Akahane et al. 1996).

Intracellular dialysis of myocytes with very high concentration of Ca^{2+} buffers (2 mmol/l Fura-2 plus 14 mmol/l EGTA or 10 mmol/l BAPTA, Adachi-Akahane et al. 1996; 10 mmol/l EGTA, Sham 1997) revealed that even through the transient rise in global myoplasmic Ca^{2+} concentration and contraction were strongly

suppressed, neither the ability of the SR to re-accumulate and release Ca^{2+}, nor the precise Ca^{2+} mediated cross-signaling between the Ca^{2+} channel and the ryanodine receptors were significantly altered. These results suggest that Ca^{2+}-dependent inactivation of I_{Ca} is independent of the global myoplasmic Ca^{2+} concentrations.

Quantitative analysis of the results from experiments with square pulses, where the release of Ca^{2+} from SR was modulated by using caffeine, thapsigargin and conditioning pulses indicate that Ca^{2+}-dependent inactivation of I_{Ca} is mediated primarily by Ca^{2+} released from SR (65–75 %) and is less dependent on its own Ca^{2+} influx (25–35 %) (Adachi-Akahane et al. 1996). Similarly, Puglisi et al. (1999) have shown that the overall integrated Ca^{2+} influx during the action potential (AP) *via* I_{Ca} is reduced by 50 % when normal SR Ca^{2+} release occurs. Based on experiments in ferret ventricular myocytes, Trafford et al. (1997) demonstrated that after caffeine-induced SR Ca^{2+} depletion, Ca^{2+} entry into the cell declined from 14.8 to 6.7 μmol/l cytosol. The fact that the 50–75 % of Ca^{2+} induced inactivation of Ca^{2+} channel was controlled by Ca^{2+} released from SR emphasizes proximity between Ca^{2+} channel and the ryanodine receptors.

Sham (1997) showed in rat ventricular myocytes that the inactivation of I_{Ca} by Ca^{2+} release was most prominent at −20 mV and diminished with increasing positive potentials (the potential corresponding to the peak of I-V relationship is at 0 mV). He also found the correlation between the Ca^{2+} release-induced inactivation and the amplitude of the single-channel current. It suggests that at negative potentials, the likelihood of successful activation of ryanodine receptors by Ca^{2+} influx *via* Ca^{2+} channels is higher, presumably due to the larger single calcium channel current. The higher rate of successful activation of ryanodine receptors then leads to the inactivation of a greater fraction of functionally coupled Ca^{2+} channels.

For simulation of calcium currents in the presence of the release-dependent inactivation the role of spatial distribution of channels is crucial. Release-dependent inactivation, which reflects tight Ca^{2+} coupling between L-type calcium channels and RyRs takes place at the narrow site of the tubulo-reticular junction where each L-type channel is closely associated with a group of RyRs (Sun et al. 1995). These junctions form a restricted subspace, in which concentration of free Ca^{2+} can build up very rapidly (Soeller and Cannell 1997). In such case, it is necessary to consider geometry of junction (Franzini-Armstrong et al. 1999), formation and dissipation of calcium gradients around an open channel pore, the time course of Ca^{2+} binding to calcium buffers present in junction (Soeller and Cannell 1997) and gating properties of ryanodine channels (Cheng et al. 1995; Schiefer et al. 1995; Keizer and Levine 1996; Zahradníková and Zahradník 1996; Cannel and Soeller 1997; Ondrias and Mojzisova 2002). The detailed description of mathematical modeling of the release-dependent inactivation is outside the scope of this minireview and therefore will not be discussed in detail.

Conclusions

Pure voltage-dependent inactivation is much slower than Ca^{2+}-dependent inactivation of I_{Ca} and may act as a form of longer-term regulation. Ca^{2+}-dependent inactivation accounts for a significant proportion of I_{Ca} inactivation. It can be concluded that Ca^{2+}-mediated process may play a more important role than the voltage-dependent mechanism in inactivating Ca^{2+} channels in cardiomyocytes. In addition, the release-dependent component of Ca^{2+}-dependent inactivation of Ca^{2+} channel may provide a mechanism for optimizing the probability of triggering Ca^{2+} release by calcium current (Sham 1997) and thus terminating explosive Ca^{2+} induced Ca^{2+} release process occurring in each dyadic junction.

References

Adachi-Akahane S., Cleemann L., Morad M. (1996): Cross-signaling between L-type Ca^{2+} Channels and Ryanodine Receptors in Rat Ventricular Myocytes. J. Gen. Physiol. **108**, 435—454

Balke C. W., Wier W. G. (1991): Ryanodine does not affect calcium current in guinea pig ventricular myocytes in which Ca^{2+} is buffered. Circ. Res. **68**, 897—902

Berjukow S., Marksteiner R., Gapp F., Sinnegger M. J., Hering S. (2000): Molecular mechanism of calcium channel block by isradipine. Role of a drug-induced inactivated channel conformation. J. Biol. Chem. **275**, 22114—22120

Bers D. M., Perez-Reyes E. (1999): Ca channels in cardiac myocytes: structure and function in Ca influx and intracellular Ca release. Cardiovascular Research **42**, 339—360

Brehm P., Eckert R. (1978): Calcium entry leads to inactivation of calcium channel in Paramecium. Science (Washington D. C.) **202**, 1203—1206

Cannell M. B., Soeller C. (1997): Numerical analysis of ryanodine receptor activation by L-type channel activity in the cardiac muscle diad. Biophys. J. **73**, 112—122

Cavalié A., Ochi R., Pelzer D. and Trautwein W. (1983): Elementary currents through Ca^{2+} channels in guinea pig myocytes. Pflügers Arch. **398**, 284—297

Cavalié A., Pelzer D., Trautwein W. (1986): Fast and slow gating behaviour of single calcium channels in cardiac cells. Relation to activation and inactivation of calcium-channel current. Pflügers Arch. **406**, 241—258

De Leon M., Wang Y., Jones L., Perez-Reyes E., Wei X., Soong T. W., Snutch T. P., Yue D. T. (1995): Essential Ca^{2+} binding motif for Ca^{2+} sensitive inactivation of L-typ Ca^{2+} channels. Sience **270**, 1506—1509

Doyle D. A., Morais Cabral J., Pfuetzner R. A., Kuo A., Gulbis J. M., Cohen S. L., Chait B. T., MacKinnon R. (1998): The structure of the potassium channel: molecular basis of K^+ conduction and selectivity. Science (Washington D. C.) **280**, 69—77

Ferreira G., Yi J. X., Rìos E., Shirokov R. (1997): Ion-dependent inactivation of barium current through L-type calcium channels. J. Gen. Physiol. **109**, 449—461

Franzini-Armstrong C., Protasi F., Ramesh V. (1999): Shape, size, and distribution of Ca^{2+} release units and couplons in skeletal and cardiac muscles Biophys. J. **77**, 1528—1539

Hadley R. W., Hume J. R. (1987): An intrinsic potential-dependent activation mechanism associated with calcium channels in guinea-pig myocytes. J. Physiol. (London) **389**, 205—222

Hering S., Aczel S., Kraus R. L., Berjukow S., Striessnig J., Timin E. N. (1997): Molecular mechanism of use-dependent calcium channel block by phenylalkylamines: role of inactivation. Proc. Natl. Acad. Sci. U.S.A. **94**, 13323—13328

Hering S., Berjukow S., Sokolov S., Marksteiner R., Weiss R. G., Kraus R., Timin E. N. (2000): Molecular determinants of inactivation in voltage-gated Ca^{2+} channels. J. Physiol. (London) **528**: 237—249

Hess P. and Tsien R. W. (1984): Mechanism of ion permeation through calcium channels. Nature **309**, 453—456

Hoshi T., Zagotta W. N., Aldrich R. W. (1990): Biophysical and molecular mechanisms of Shaker potassium channel inactivation. Science **250**, 533—538

Cheng H., Fill M., Valdivia H., Lederer W. J. (1995): Models of Ca^{2+} release channel adaptation. Science **267**, 2009—2010

Choi K. L., Aldrich R. W., Yellen G. (1991): Tetraethylammonium blockade distinguishes two inactivation mechanisms in voltage-activated K^+ channels. Proc. Natl. Acad. Sci. U.S.A. **88**: 5092—5095

Imredy J. P., Yue D. T. (1992): Submicroscopic Ca^{2+} diffusion mediates inhibitory coupling between individual Ca^{2+} channels. Neuron **9**, 197—207

Imredy, J. P., Yue, D. T. (1994): Mechanism of Ca^{2+}-sensitive inactivation of L-type Ca^{2+} channels. Neuron **12**, 1301—1318

Isacoff E. Y., Jan Y. N., Jan L. Y. (1991): Putative receptor for the cytoplasmic inactivation gate in the Shaker K^+ channel. Nature **353**, 86—90

Jafri M. S., Rice J. J., Winslow R. L. (1998): Cardiac Ca^{2+} dynamics: The roles of ryanodine receptor adaptation and sarcoplasmic reticulum load. Biophys. J. **74**, 1149—1168

Kass R. S., Sanguinetti M. C. (1984): Inactivation of calcium channel current in the calf cardiac Purkinje fiber. J. Gen. Physiol. **84**, 705—726

Keizer J., Levine L. (1996): Ryanodine receptor adaptation and Ca^{2+}-induced Ca^{2+} release dependent Ca^{2+} oscilations. Biophys. J. **71**, 3477—3487

Lee K. S., Tsien R. W. (1982): Reversal of current through calcium channels in dialysed single heart cells. Nature **297**, 489—501

Lee K. S., Marban E., Tsien R. W. (1985): Inactivation of calcium channels in mammalian heart cells: joint dependence on membrane potential and intracellular calcium. J. Physiol. **364**, 395—411

McDonald T. F., Cavalié A., Trautwein W., Pelzer D. (1986): Voltage-dependent properties of macroscopic and elementary calcium channel currents in guinea pig ventricular myocytes. Pflügers Arch. **406**, 437—448

Mentrard D., Vassort G., Fischmeister R. (1984): Calcium-mediated inactivation of the calcium conductance in cesium-loaded frog heart cells. J. Gen. Physiol. **83**, 105—131

Motoike H. K., Bodi I., Nakayama H., Schwartz A., Varadi G. (1999): A region in IVS5 of the human cardiac L-type calcium channel is required for the use-dependent block by phenylalkylamines and benzothiazepines. J. Biol. Chem. **274**, 9409—9420

Noceti F., Olcese R., Qin N., Zhou J., Stefani E. (1998): Effect of Bay K 8644(-) and the beta 2a subunit on Ca^{2+}-dependent inactivation in alpha 1C Ca^{2+} channels. J. Gen. Physiol. **111**, 463—475

Ondrias K., Mojzisova A. (2002): Coupled gating between individual cardiac ryanodine calcium release channels. Gen. Physiol. Biophys. **21**, 73—84

Pelzer D., Pelzer S., McDonald T. F. (1990): Properties and regulation of calcium channels in muscle cells. Kinetics of L-type calcium channels. Rev. Physiol. Biochem. Pharmacol. **114**, 111—131

Peterson B. Z., DeMaria C. D., Yue D. T. (1999): Calmodulin is the Ca^{2+} sensor for Ca^{2+}-dependent inactivation of L-type calcium channels. Neuron **22**, 549—558

Peterson B. Z., Lee J. S., Mulle J. G., Wang Y., de Leon M., Yue D. T. (2000): Critical determinants of Ca^{2+}-dependent inactivation within an EF-hand motif of L-type Ca^{2+} channels. Biophys. J. **78**, 1906—1920

Puglisi J. L., Yuan W., Bassani W. M., Bers D. M. (1999): Ca^{2+} influx through Ca^{2+} channels in rabbit ventricular myocytes during action potential clamp: influence of temperature. Circ. Res. **85**, e7—16

Rose W. C., Balke C. W., Wier W. G., Marban E. (1992): Macroscopic and unitary properties of physiological ion flux through L-type Ca^{2+} channels in guinea-pig heart cells. J. Physiol. **456**, 277—284

Rosenberg R. L., Hess P., Reeves J. P., Smilowitz H., Tsien R. W. (1986): Calcium channels in planar lipid bilayers: insights into mechanisms of ion permeation and gating. Science Wash. DC **231**, 1564—1566

Sham J. S. (1997): Ca^{2+} release-induced inactivation of Ca^{2+} current in rat ventricular myocytes: Evidence for local Ca^{2+} signaling. J. Physiol. (London) **500**, 285—295

Sham J. S., Cleemann L., Morad M. (1995): Functional coupling of Ca^{2+} channels and ryanodine receptors in cardiac myocytes. Proc. Natl. Acad. Sci. U.S.A. **92**, 121—125

Sherman A., Keizer J., Rinzel J. (1990): Domain model for Ca^{2+}-inactivation of Ca^{2+} channels at low channel density. Biophys. J. **58**, 985—995

Shi Ch., Soldatov N. M. (2002): Molecular determinants of voltage dependent slow inactivation of the Ca^{2+} channel. J. Biol. Chem. **277**, 6813—6821

Shirokov R., Levis R., Shirokova N., Riós E. (1993): Ca^{2+}-dependent inactivation of cardiac L-type Ca^{2+} channels does not affect their voltage sensor. J. Gen. Physiol. **102**, 1005—1030

Schiefer A., Meissner G., Isenberg G. (1995): Ca^{2+} activation and Ca^{2+} inactivation of canine reconstituted cardiac sarcoplasmic reticulum Ca^{2+} release channels. J. Physiol. (London) **489**, 337—348

Sipido K. R., Callewaert G., Carmeliet E. (1995): Inhibition and rapid recovery of Ca^{2+} current during Ca^{2+} release from sarcoplasmic reticulum in guinea pig ventricular myocytes. Circulation Research **76**, 102—109

Soeller C., Cannell M. B. (1997): Numerical simulation of local calcium movements during L-type calcium channel gating in the cardiac diad. Biophys. J. **73**, 97—111

Soldatov N. M., Zuhlke R. D., Bouron A., Reuter H. (1997): Molecular structures involved in L-type calcium channel inactivation. Role of the carboxyl-terminalregion encoded by exons 40–42 in alpha1C subunit in the kinetics and Ca^{2+} dependence of inactivation. J. Biol. Chem. **272**, 3560—3566

Standen N. B., Stanfield P. R. (1982): A binding-site model for calcium channel inactivation that depends on calcium entry. Proc. R. Soc. London, Ser. B **217**, 101—110

Stotz S. C., Hamid J., Spaetgens R. L., Jarvis S. E., Zamponi G. W. (2000): Fast inactivation of voltage-dependent calcium channels. A hinged-lid mechanism? J. Biol. Chem. **275**, 24575—24582

Sun X. H., Protasi F., Takahashi M., Takeshima H., Ferguson D. G., Franzini-Armstrong C. (1995): Molecular architecture of membranes involved in excitation-contraction coupling of cardiac muscle. J. Cell Biol. **129**, 659—671

Trafford A. W., Diaz M. E., Negretti N., Eisner D. A. (1997): Enhanced Ca^{2+} current and decreased Ca^{2+} efflux restore sarcoplasmic reticulum Ca^{2+} content after depletion. Circ. Res. **81**, 477—484

West J. W., Patton D. E., Scheuer T., Wang Y., Goldin A. L., Catterall W. A. (1992): A

cluster of hydrophobic amino acid residues required for fast Na^+ channel inactivation. Proc. Natl. Acad. Sci. U.S.A. **89**, 10910—10914

Yellen G. (1998): The moving parts of voltage-gated ion channels. Q. Rev. Biophys. **31**, 239—295

Yue D. T., Backx P. H., Imredy J. P. (1990): Calcium-sensitive inactivation in the gating of single calcium channels. Science **21**, 1735—1738

Zahradníková A., Zahradník I. (1996): A minimal gating model for the cardiac calcium release channel. Biophys. J. **71**, 2996—3012

Zahradníková A., Pavelková J., Kubalová Z., Zahradník I. (2001): Stimulation of calcium cross-signaling in cardiac cells at the single-channel level. In: Calcium Signaling and Cross-talk in Excitable Tissue (Eds. M. Morad and P. G. Kostyuk), NATO Asi Series, Series H, Cell Biology, pp. 167—176

Zhang J. F., Ellinor P. T., Aldrich R. W., Tsien R. W. (1994): Molecular determinants of voltage-dependent inactivation in calcium channels. Nature **372**, 97—100

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