SYMPOSIUM REPORT

Physiological modulation of inactivation in L-type Ca^{2+} channels: one switch

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The relative contributions of voltage- and Ca^{2+}-dependent mechanisms of inactivation to the decay of L-type Ca^{2+} channel currents (I_{CaL}) is an old story to which recent results have given an unexpected twist. In cardiac myocytes voltage-dependent inactivation (VDI) was thought to be slow and Ca^{2+}-dependent inactivation (CDI) resulting from Ca^{2+} influx and Ca^{2+}-induced Ca^{2+}-release (CICR) from the sarcoplasmic reticulum provided an automatic negative feedback mechanism to limit Ca^{2+} entry and the contribution of I_{CaL} to the cardiac action potential. Physiological modulation of I_{CaL} by β-adrenergic and muscarinic agonists then involved essentially more or less of the same by enhancing or reducing Ca^{2+} channel activity, Ca^{2+} influx, sarcoplasmic reticulum load and thus CDI. Recent results on the other hand place VDI at the centre of the regulation of I_{CaL}. Under basal conditions it has been found that depolarization increases the probability that an ion channel will show rapid VDI. This is prevented by β-adrenergic stimulation. Evidence also suggests that a channel which shows rapid VDI inactivates before CDI can become effective. Therefore the contributions of VDI and CDI to the decay of I_{CaL} are determined by the turning on, by depolarization, and the turning off, by phosphorylation, of the mechanism of rapid VDI. The physiological implications of these ideas are that under basal conditions the contribution of I_{CaL} to the action potential will be determined largely by voltage and by Ca^{2+} following β-adrenergic stimulation.

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Introduction

Mutagenesis, chimera constructions and differential subunit expression have provided original insight into the processes of activation, permeation and inactivation of L-type Ca^{2+} channels (ICa_L). The fundamental role of calmodulin mediating the process of Ca^{2+}-dependent inactivation has been defined (Qin *et al.* 1999; Anderson, 2001) along with the action of calcium-calmodulin-dependent kinase II (Maier & Bers, 2002). The ensemble of this data accounts for variation in the behaviour of ICa_L due to the expression in different tissues of splice variants, long and short forms of the α_{1c} subunit and combinations with different β subunits. On the other hand, physiological modulation of the behaviour of ICa_L by the means of phosphorylation via protein kinase A (PKA) which underlies sympathetic, parasympathetic and purinergic regulation of the Ca^{2+} current (McDonald *et al.* 1994; Kamp & Hell, 2000) has been less amenable to explanation by the methods of *in vitro* reconstruction. Few have succeeded in the reconstitution of the response of ICa_L to stimulation via PKA (Gao *et al.* 1997; Naguro *et al.* 2001). Part of this problem has been found to be the assemblage of all of the elements required to affirm this reaction (Gao *et al.* 1997). The rest has been in determining what actually is or are the reactions of ICa_L to agonist stimulation.

It is commonly held that the effect of β-adrenergic stimulation of ICa_L is the increase in amplitude of the whole-cell current arising from an increase in the availability of the channels as well as an increase in their probability of opening; the voltage dependence of activation and inactivation of ICa_L are shifted to more negative voltages (McDonald *et al.* 1994; Bers & Perez-Reyes, 1999). Inactivation of ICa_L occurs via

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processes associated with membrane voltage and the increase in intracellular Ca^{2+}. Initially, the relative extents of these two mechanisms was disputed (Kass & Sanguinetti, 1984; Mentrard *et al.* 1984; Lee *et al.* 1985; Hadley & Hume, 1987; Argibay *et al.* 1988). Today it is generally assumed that the dominant character determining the rapid decay of I_{CaL} is inactivation consequent to the influx of Ca^{2+} (Linz & Meyer, 1998; Sun *et al.* 2000). The increase of inactivation following β-adrenergic stimulation can then readily be understood as a consequence of enhanced Ca^{2+} influx upon increased channel activity.

The physical mechanism of inactivation of ICa_L is not known. In Ca^{2+} channels there is no obvious equivalent of the 'ball-and-chain' or rearrangement of the pore-lining segments which produce inactivation of voltage-gated Na^+ or K^+ channels (Antz & Fakler, 1998; Cantrell & Catterall, 2001). It is not known whether the processes of voltage-dependent inactivation and Ca^{2+}-dependent inactivation have the same physical endpoint or whether they represent different mechanisms?

Recently a series of studies of ICa_L in native cardiac myocytes have opened new perspectives upon the old problem of voltage- and Ca^{2+}-dependent inactivation (Mitarai *et al.* 2000; Findlay, 2002*a*,*b*,*c*,*d*). This short review will summarize these novel ideas and attempt to integrate them into a simple framework. This framework is the switching on and the switching off of rapid voltage-dependent inactivation of the individual Ca^{2+} channel.

Voltage-dependent inactivation

The classical view of voltage-dependent inactivation (VDI) of I_{CaL} is that it is slow and accelerates only slightly with depolarization (Bechem & Pott, 1985; Fukushima & Hagiwara, 1985; Matsuda, 1986). This vision resulted from experiments that were conducted under conditions that were the equivalent of β-adrenergic stimulation (see below). When attention was paid to record I_{CaL} under basal conditions VDI strongly increased with progressive depolarization (Fig. 1) and was rapid at positive membrane potentials (Mitarai *et al.* 2000; Findlay, 2002*a*,*d*).

A detailed analysis of the time course of development and voltage dependence of VDI (Fig. 2*A* and *B*) (Findlay, 2002*d*) showed that at membrane potentials of –30 and –20 mV the whole-cell current could be divided into two components, a part which showed slow time-dependent decay and a part which showed essentially no decay. At –10 mV a third and rapidly decaying time-dependent component appeared and with further depolarization the amplitude of this rapid time-dependent component increased. At the same time, the amplitudes of slow time-dependent and time-independent components of the whole-cell current declined. Maximal VDI followed a biphasic time course ($\tau_f \sim$30 ms and $\tau_s \sim$300 ms) which was dominated by the rapid phase of inactivation.

Biphasic decay of I_{CaL} in cardiac myocytes has been traditionally assigned to the separate processes of Ca^{2+}- (fast decay) and voltage- (slow decay) dependent inactivation (Adams & Tanabe, 1997; Ferreira *et al.* 1997; Sun *et al.* 1997). It was not expected that VDI alone would show a biphasic time course (Findlay, 2002*d*), though this can be explained by individual Ca^{2+} channel 'modal' behaviour (Hess *et al.* 1984; Tsien *et al.* 1986; Pietrobon & Hess, 1990). Thus an individual ion channel could either rapidly inactivate after the onset of opening or not (Plummer & Hess, 1991; Rose *et al.* 1992). This simple switch would convert an ion channel which did not show VDI into an ion channel which did show VDI. In mechanistic terms all that would be required is for VDI to be turned on by the voltage step and with an increasing probability of this occurring with depolarization. The stochastic nature of this mechanism would then account for the different kinetics of decay of I_{CaL} and the graded development of rapid inactivation at the expense of slow

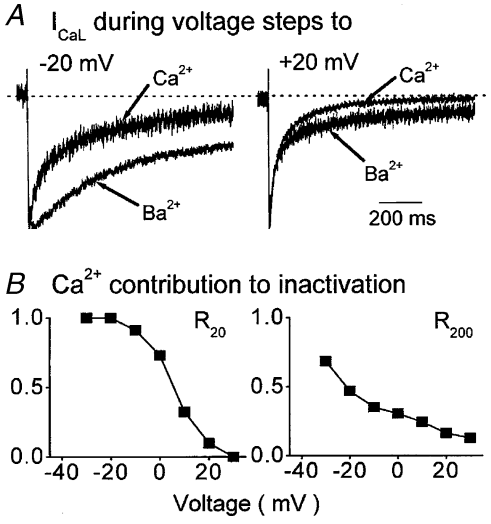

Figure 1. Inactivation of *I*CaL under basal conditions
A, normalized I_{CaL} carried by Ca^{2+} and Ba^{2+} in one ventricular myocyte. At negative voltages (left) Ca^{2+} current inactivates more rapidly than Ba^{2+} current. At positive voltages (right) the initial decay of Ba^{2+} current is as fast as that of Ca^{2+} current, only later does Ca^{2+} current show more inactivation. *B*, the contribution of Ca^{2+} to the inactivation of I_{CaL} evaluated by the ratio of decay recorded with Ca^{2+} relative to that recorded with Ba^{2+}, 20 ms (R20, left) and 200 ms (R200, right) after activation. At both times the contribution of Ca^{2+} declines with depolarization though there is clearly more of an effect of Ca^{2+} at positive voltages after 200 ms than after 20 ms. The figure has been redrawn from Findlay (2002*a*).

or no inactivation in the whole-cell population of ion channels (Findlay, 2002*d*).

Sympathetic and parasympathetic regulation of ICa_L in cardiac muscle follows activation of adenylate cyclase and PKA (Kamp & Hell, 2000). There are several prospective sites for the action of PKA upon α_{1C} and β_2 subunits (Bunemann *et al.* 1999) and the close association of PKA with the Ca^{2+} channel in a supramolecular complex is required for *in vitro* reconstitution of the β-adrenergic response (Gao *et al.* 1997). The isolation of VDI following β-adrenergic stimulation has not been easy. The effect of β-adrenergic stimulation upon the apparently voltage-dependent inactivation of I_{CaL} carried by Ba^{2+} or Sr^{2+} has given rather mixed results with clear indications of the contribution of an ion-dependent process (Tiaho *et al.* 1991; Ferreira *et al.* 1997; Findlay, 2002*a*). On the other hand, following β-adrenergic stimulation I_{CaL} carried by Na^+ clearly retains the character of VDI without ion-dependent inactivation (Mitarai *et al.* 2000; Findlay, 2002*a*). In these circumstances VDI is slow and shows little development with depolarization. These recent results therefore correspond to the classical view of VDI (Bechem & Pott, 1985; Fukushima & Hagiwara, 1985; Matsuda, 1986) which for diverse reasons were conducted in fact under experimental conditions that were likely to lead to activation of PKA.

Analysis of the effects of agonists upon VDI (Mitarai *et al.* 2000; Findlay, 2002*c*) revealed that the number of ion channels which showed rapid VDI was reduced by the β-adrenergic agonist isoprenaline in a dose-dependent manner (Fig. 4). At the same time the number of ion channels which did not show VDI was increased. At each dose of isoprenaline, the addition of the muscarinic agonist carbachol increased the number of channels which showed rapid VDI and reduced the number of channels which did not show VDI (Findlay, 2002*c*). There was clearly a reciprocal relationship between the number of channels which did show rapid VDI and the number of channels which did not show rapid VDI. Since these β-adrenergic and muscarinic agonists are considered to act upon the Ca^{2+} channels by, respectively, increasing and decreasing their phosphorylation via PKA, this would suggest that phosphorylation turns off or otherwise prevents the mechanism of rapid VDI. This simple switch would convert ion channels which did show rapid VDI into channels which did not show VDI.

Thus a single and simple mechanism can be proposed to account for the development of VDI with depolarization and the modulation of VDI by agonists. The stochastic operation of this on–off switch for VDI in the single channel would account for the multiphasic nature of the behaviour of the whole-cell population of ion channels.

In historical terms, it is not the drastic reduction of VDI of I_{CaL} in cardiac myocytes which is evoked by β-adrenergic stimulation that would be considered to be an unusual result. Though it should be mentioned that this effect

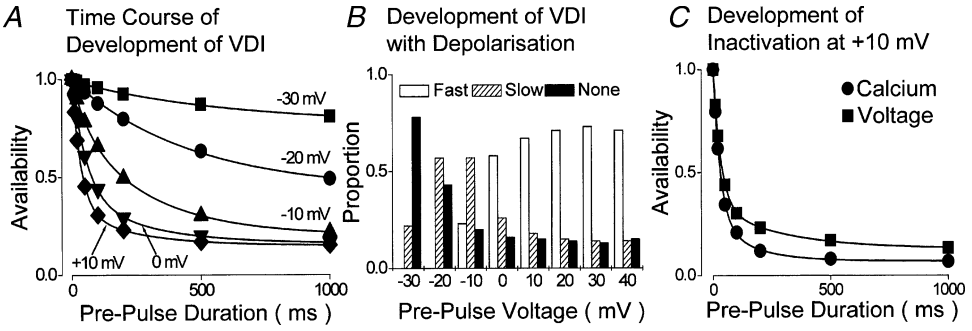

Figure 2. The development of rapid VDI under basal conditions

A, the time course of the development of VDI was recorded in the absence of Ca^{2+}, and in the absence of ion flux through the channels, between voltages which evoke minimal (–30 mV) and maximal (+10 mV) inactivation. The lines connecting data points represent the fitting of either a single (–30 and –20 mV) or a double (–10, 0 and +10 mV) exponential function to the data. *B*, the proportions of fast (open columns), slow (hatched columns) and no (filled columns) VDI which were recorded at different membrane potentials show that as the amount of current which showed fast VDI increased, that which showed no VDI declined. *C*, the relative contributions of Ca^{2+} and voltage to the decay of I_{CaL} were assessed by measuring the time course of development of inactivation at +10 mV in the presence (circles) and the absence (squares) of extracellular Ca^{2+}. The first recorded total inactivation, being the sum of VDI and CDI. The second recorded only VDI since no ion flux through I_{CaL} occurred at this voltage under these conditions (see Findlay, 2002*b* for further details). It is clear that Ca^{2+} influx does little to increase the initial rapid phase of the development of inactivation. The results shown here were conducted in the presence of ryanodine. *A* and *B* have been redrawn from Findlay (2002*d*) and *C* from Findlay (2002*b*).

of agonist modulation of a voltage-gated ion channel is unique to ICa_L. Agonist modulation of voltage-gated Na^+ and K^+ channel currents is confined to shifting activation and inactivation curves along the voltage axis (Cantrell & Catterall, 2001). They do not result in the reduction of the minimum of the inactivation curve (Fig. 4*A*). The novel aspect of this analysis of VDI of I_{CaL} in cardiac myocytes is the discovery of rapid VDI under basal conditions in the absence of agonists (Findlay, 2002*a*,*b*,*c*,*d*). The decay of I_{CaL} carried by Ca^{2+} in cardiac myocytes is rapid and when it was thought that VDI was slow, it was inferred that Ca^{2+}-dependent inactivation was the dominant physiological mechanism of inactivation (Linz & Meyer, 1998; Sun *et al.* 2000). This conclusion is called into question with the discovery of rapid VDI.

Ca^{2+}-dependent Inactivation

Ca^{2+}-dependent inactivation (CDI) of I_{CaL} is mediated by the interaction of the Ca^{2+}-binding protein calmodulin with the proximal region of the cytoplasmic C-terminus of the α_{1C} subunit of the Ca^{2+} channel (Anderson, 2001; Maier & Bers, 2002). Calmodulin is attached to the Ca^{2+} channel in the presence of basal intracellular Ca^{2+} concentrations (Erickson *et al.* 2001; Pitt *et al.* 2001). An increase in intracellular Ca^{2+} activates calmodulin which reorientates its attachments and in consequence is thought to alter the 3D structure of the C-terminus (Petersen *et al.* 2000). In an unknown manner, this procedure provokes inactivation of ICa_L. The implication of calmodulin with the process of CDI of ICa_L has several interesting consequences. The first of these is that calmodulin is not selective and there is no obligation for Ca^{2+} which has entered via a Ca^{2+} channel to provoke inactivation. Any increase of Ca^{2+} in the vicinity of the internal surface of the channel will suffice to trigger CDI irrespective of whether the channel has opened or not. Thus Ca^{2+}-induced Ca^{2+}-release (CICR) from the cardiac sarcoplasmic reticulum can cause CDI (Adachi-Akahane *et al.* 1996; Sham, 1997). The opening of a neighbouring Ca^{2+} channel can cause CDI (Imredy & Yue, 1992). The triggering of CICR by chemical means can cause CDI (Lipp *et al.* 1987; Adachi-Akahane *et al.* 1996). The photolytic release of Ca^{2+} from intracellular caged compounds can cause CDI (Hadley & Lederer, 1991; Bates & Gurney, 1993). In cardiac myocytes it is not clear to what extent Ca^{2+} which enters via a given Ca^{2+} channel causes CDI of that channel. Otherwise it would be difficult to account for the significant effects of CICR upon CDI within the local-control domain (Stern, 1992; Wier *et al.* 1994) which suggests that while sufficient Ca^{2+} can enter via the surface Ca^{2+} channel to provoke CICR, this is insufficient to locally provoke CDI. Also, in cells which lack the geometry of close association between surface membrane and junctional sarcoplasmic reticulum, CDI is related more closely to the overall cell current density of I_{CaL} rather than the driving force for Ca^{2+} entry via the single channel (Argibay *et al.* 1988). The second point which arises from the association of calmodulin with the process of CDI is that the interaction between Ca^{2+} and calmodulin is readily reversible and entirely dependent upon the local Ca^{2+} concentration. But it is dogma that inactivation of voltage-gated ion channels is an absorptive state that requires a period at a hyperpolarized potential to recover (Imredy & Yue, 1994) while several studies have shown that CDI might be reversible without repolarization (Sipido *et al.* 1995; Brette *et al.* 2003). It was therefore in the context of this complexity of the kinetics and sources of CDI that recent studies which wished to evaluate the relative contributions of CDI and VDI to the decay of I_{CaL} in native cardiac myocytes chose to block CICR with ryanodine (Findlay, 2002*a*,*c*). In this manner, CDI where it occurred would result entirely from the influx of Ca^{2+} through sarcolemmal Ca^{2+} channels.

Two methods were used to assess the relative contributions of CDI and VDI in isolated cardiac myocytes. The first compared the decay of I_{CaL} carried by Ca^{2+} with that of I_{CaL} carried by Ba^{2+}, Sr^{2+} and Na^+ (Findlay, 2002*a*). The second utilized the technique developed by Hadley & Hume (1987) which enables the recording of I_{CaL} in the absence of cation influx when ICa_L is blocked by extracellular Mg^{2+} (Findlay, 2002*b*). In basal conditions at positive membrane potentials the development of inactivation followed a biphasic time course and there was little or no difference between the initial rapid phase of inactivation of I_{CaL} when this resulted from the influx of Ca^{2+}, Ba^{2+}, Sr^{2+}, Na^+ or no ion flux (Figs 1*A* and 2*C*). The second, slow phase of inactivation was less when I_{CaL} was carried by Ca^{2+} (Fig. 1*A*). These results quite clearly showed that the initial rapid phase of inactivation of I_{CaL} was independent of CDI and therefore due to VDI. This represents the reverse of the traditional description of fast decay of I_{CaL} due to CDI and slow decay of I_{CaL} due to VDI (Adams & Tanabe, 1997; Ferreira *et al.* 1997; Sun *et al.* 1997). At negative membrane potentials the distinction between the decay of I_{CaL} carried by Ca^{2+} and the other cations was larger (Fig. 1*A*) and it was clear that CDI did play an important role in determining the decay of I_{CaL} (Fig. 1*B*). When these experiments were repeated following maximal β-adrenergic stimulation with isoprenaline I_{CaL} carried by Ca^{2+} decayed more rapidly than I_{CaL} carried by the other

cations at all voltages (Fig. 3*A*). In these circumstances CDI dominated the decay of I_{CaL} (Figs 3*B* and 4*C*).

The results which had been obtained by directly comparing total inactivation recorded during Ca^{2+} influx with isolated VDI show an interesting relation between these two phenomena. Under basal conditions the contribution of CDI to the decay of I_{CaL} progressively declined with depolarization (Fig. 1*B*). VDI increased with depolarization and in particular the number of channels which show fast VDI increased (Fig. 2*B*). CDI dominated decay of I_{CaL} at all voltages following β-adrenergic stimulation (Fig. 3*B*) when rapid VDI had been suppressed (Fig. 4*B* and *C*).

Conclusions and perspectives

It is not necessary to look for complicated explanations for the variation of the contributions of VDI and CDI to the decay of I_{CaL} under a range of physiological conditions. Physiological modulation of inactivation of I_{CaL} is proposed to act at a single point, the triggering of rapid voltage-dependent inactivation. This is turned on with depolarization, and turned off by phosphorylation. When it is on, CDI is ineffective; when it is off, CDI is effective. The graded influence of VDI and CDI and the effects of β-adrenergic and muscarinic agonists are then a consequence of the variation in the number of individual ion channels which show VDI. It is proposed that at the level of the single L-type Ca^{2+} channel rapid VDI will either occur or not. That single Ca^{2+} channels are capable of switching between rapid VDI and no inactivation has already been shown for a neuronal N-type Ca^{2+} channel (Plummer & Hess, 1991). The probability of this occurring in the L-type Ca^{2+} channel would increase with increasing depolarization. Since a channel which has already been inactivated by voltage cannot show inactivation which is caused by Ca^{2+} it is clear that the on–off switch for the occurrence of fast VDI determines the visible contribution of Ca^{2+} to the overall time course of decay of I_{CaL}. Thus, as the number of channels which adopt rapid VDI increases, the number of channels which are inactivated by Ca^{2+} decreases. The prevention of fast VDI by phosphorylation of the channel either at the α_{1C} or at the β_2 subunit will then permit CDI.

It seems likely that CDI is a process that has a certain latency. This does not necessarily arise from the interaction of calmodulin with the C-terminus of the channel, in particular since calmodulin can be expected to be tethered to the channel under all physiological conditions (Erickson *et al.* 2001; Pitt *et al.* 2001). But the rise in 'fuzzy space' Ca^{2+} to the level required to trigger the transformation of calmodulin might require a certain time, in particular since Ca^{2+} from several sources is known to provoke CDI and may be required to provoke CDI (Imredy & Yue, 1994; Adachi-Akahane *et al.* 1996).

The physiological consequences of these observations reside in the observation of the dominance of VDI at positive membrane potentials under basal conditions which is replaced by the dominance of CDI following β-adrenergic stimulation (Findlay, 2002*b*). These results predict that should there be an error in the process of CDI this would have little effect upon I_{CaL} and its contribution to the action potential under basal conditions. On the other hand, this would cause a drastic slowing of the decay of I_{CaL} and probably massive elongation of the action potential following β-adrenergic stimulation. Alseikhan *et al.* (2002) have expressed mutant Ca^{2+}-insensitive calmodulin in isolated cardiac myocytes. The action potentials recorded from these cells were drastically increased. This clearly illustrates the importance that CDI could have to limit the contribution of I_{CaL} to the electrophysiology of cardiac myocytes. These experiments were conducted in the absence of agonists and thus presumably under basal conditions, which would contradict the importance of VDI which is suggested here. But it was noted that the adenovirus infection technique by itself evoked an increase in the density of I_{CaL} (Alseikhan *et al.* 2002). It is therefore possible that PKA was activated

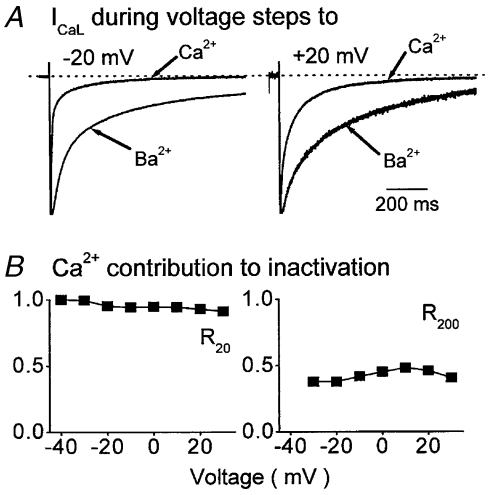

Figure 3. Inactivation of *I*CaL following β-adrenergic stimulation

A, normalized I_{CaL} carried by Ca^{2+} and Ba^{2+} in one ventricular myocyte. At negative (left) and positive (right) voltages Ca^{2+} current inactivates more rapidly than Ba^{2+} current. *B*, the contribution of Ca^{2+} to the inactivation of I_{CaL} 20 ms (R20, left) and 200 ms (R200, right) after activation is clearly sustained at all membrane voltages. The figure has been redrawn from Findlay (2002*a*).

in these cells. It remains to be seen whether, with a normal basal density of I_{CaL}, the duration of the cardiac action potential would have been elongated by the expression of mutated calmodulin.

It is interesting also to consider that abolition of the process of VDI of I_{CaL} might be without obvious consequence upon the electrophysiology of the cardiac myocyte. VDI is already essentially abolished by β-adrenergic stimulation under which circumstances rapid decay of I_{CaL} is undertaken by CDI (Fig. 4*C*: Findlay, 2002*b*). There is therefore no reason to suppose that this would not also occur should VDI be impeded under basal conditions. In any case, CDI already contributes to the decay of I_{CaL} under basal conditions at negative membrane potentials where VDI has little effect (Findlay, 2002*a*).

Notwithstanding that chimera constructions of the α_1 subunit and the expression of different β subunits of voltage-gated Ca^{2+} channels have been able to indicate regions of the channel that can be involved in VDI (Stotz & Zamponi, 2001*b*), none have had to account for the physiological modulation of this process. Three sites upon the L-type Ca^{2+} channel are known to be phosphorylated by PKA: two sites upon the β_2 subunit and one site distal to the calmodulin binding regions of the C-terminus of the α_{1C} subunit (Puri *et al.* 1997). Although it is now clear that the proximal region of the C-terminus of the α_{1C} subunit is responsible for CDI (Erickson *et al.* 2001; Pitt *et al.* 2001), it is not known how this is achieved and it is not known what effect PKA-induced phosphorylation of this region (Puri *et al.* 1997) has upon either CDI or VDI. None of the experiments conducted by either Mitarai *et al.* (2000) or Findlay (2002*a*,*b*,*c*,*d*) suggest that CDI is a process that could be influenced directly by phosphorylation of the Ca^{2+} channel.

β_2 subunits which influence, amongst other things, the rate of inactivation of Ca^{2+} channels (Birnbaumer *et al.* 1998) are attached to the α_{1C} subunit at the I–II intracellular linker (Pragnell *et al.* 1994). This linker has been suggested to be responsible for VDI via a 'hinged-lid' mechanism which would occlude the internal vestibule of the channel pore (Stotz & Zamponi, 2001*a*). If movement of the I–II linker and attached β_2 subunit was responsible for the mechanism of VDI it would not be difficult to imagine that phosphorylation of the β_2 subunit might physically affect this process. There may be analogy to be drawn with the effect of phosphorylation of the N-terminal inactivation particle of voltage-gated K^+ channels altering its configuration and affinity for the vestibule receptor (Covarrubias *et al.* 1994; Antz *et al.* 1999). Alternatively, phosphorylation or dephosphorylation of the β_2 subunit might be responsible for dissociation of the β_2 subunit from the I–II linker (Bichet *et al.* 2000; Restituito *et al.* 2001). Finally, it is not to be excluded that the distal region of the C-terminus of α_{1C} which is truncated by post-translational proteolysis, but which can remain attached to the channel complex (Gerhardstein *et al.* 2000; Gao *et al.* 2001) may play a role which could be influenced by phosphorylation of Ser ^{1928} (Gao *et al.* 1997).

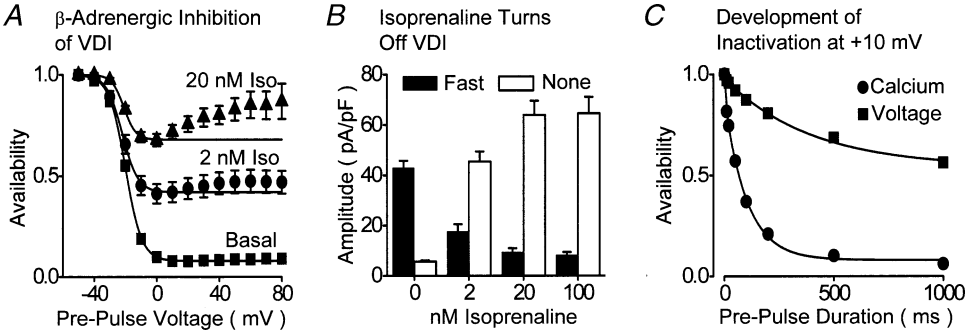

Figure 4. Inhibition of rapid VDI by β-adrenergic stimulation
A, dose-dependent reduction of the availability–voltage relationship of I_{CaL} by Isoprenaline (Iso). Experiments were conducted in the absence of extracellular Ca^{2+} with a double-pulse voltage-clamp protocol with 1000 ms duration prepulse voltage steps. *B*, the effects of isoprenaline upon the amount of I_{CaL} which showed fast VDI (filled columns) and no inactivation (open columns). These experiments were conducted in the absence of extracellular Ca^{2+} and Findlay (2002*c*) should be consulted for further details. *C*, the relative contributions of Ca^{2+} and voltage to the decay of I_{CaL} were assessed as described in Fig. 2*C* in the presence (circles) and the absence (squares) of extracellular Ca^{2+} in the presence of 100 nM isoprenaline. It is clear that Ca^{2+} influx dramatically increases the rate of development of inactivation, and comparison with Fig. 2*C* reveals that this is due to the suppression of fast VDI. These experiments were conducted in the presence of ryanodine. *A* and *B* have been redrawn from Findlay (2002*c*) and *C* from Findlay (2002*b*).

In conclusion, it is clear that the concept of physiological modulation of VDI of ICa_L ($Ca_V1.2$) which has arisen from studies of the native channel in its physiological context poses some direct questions that remain to be answered with the tools of molecular biology and mutagenesis. The description of a very loose, open and flexible structure of a voltage-gated K^+ channel (Jiang *et al.* 2003*a*,*b*) may provide impetus towards the decryption of the processes of activation and inactivation of the more complex supramolecular structure of the mammalian Ca^{2+} channel. This review has concentrated attention upon the behaviour of ICa_L in cardiac myocytes in the absence of CICR. The influence of CICR upon the inactivation of ICa_L in native cardiac myocytes is an important component of the physiology of the regulation of the cardiac action potential. But this geometric arrangement is particular to cardiac myocytes. By concentrating attention upon the influence of Ca^{2+} influx upon inactivation of I_{CaL} it is hoped that the concepts which have been developed here from work upon cardiac myocytes may find application to a wider range of tissues.

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