Regulation of Ca-sensitive inactivation of a l-type Ca^{2+} channel by specific domains of β subunits

Thierry Cens, Sophie Restituito, Pierre Charnet*

CRBM, CNRS UPR 1086, IFR 24, 1919 Route de Mende, 34293 Montpellier Cedex 5, France

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Abstract **Ca^{2+} channel auxiliary β subunits have been shown to modulate voltage-dependent inactivation of various types of Ca^{2+} channels. The β_1 and β_2 subunits, that are differentially expressed with the L-type α_1 Ca^{2+} channel subunit in heart, muscle and brain, can specifically modulate the Ca^{2+}-dependent inactivation kinetics. Their expression in *Xenopus* oocytes with the α_{1C} subunit leads, in both cases, to biphasic Ca^{2+} current decays, the second phase being markedly slowed by expression of the β_2 subunit. Using a series of β subunit deletion mutants and chimeric constructs of β_1 and β_2 subunits, we show that the inhibitory site located on the amino-terminal region of the β_{2a} subunit is the major element of this regulation. These results thus suggest that different splice variants of the β_2 subunit can modulate, in a specific way, the Ca^{2+} entry through L-type Ca^{2+} channels in different brain or heart regions.**

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Key words: Voltage clamp; β_2/β_1 chimera; Heterologous expression; *Xenopus* oocyte

1. Introduction

L-type dihydropyridine (DHP)-sensitive Ca^{2+} channels represent the major pathway for voltage-gated Ca^{2+} entry into cardiac and smooth muscle cells [1]. In these cells, a rise in intracellular Ca^{2+} inactivates the L-type channel, thus providing a control for the Ca^{2+} influx through a negative feedback mechanism [2]. This so-called Ca-dependent inactivation [3–5] is only recorded on L-type DHP-sensitive Ca^{2+} channels and is an important regulatory factor for Ca^{2+}-activated processes such as excitation-contraction coupling [6]. Other types of Ca^{2+} channels are inactivated through different, voltage-dependent, mechanisms. Cardiac and vascular L-type Ca^{2+} channels consist of a main pore-forming subunit encoded by different isoforms of the same α_{1C} gene [7,8]. This α_{1C} subunit also contains the DHP binding site [9–11] and is associated with at least two other regulatory subunits: α_2-δ and β. Whereas three α_2-δ genes are known, four different β subunits exist of which β_1, β_2 and β_3 have been shown to be expressed in heart and smooth muscle [12,13]. Biochemical and functional studies have shown that association between the α_{1C} and the β subunits occurs on specific domains of these subunits [14,15]. This association, as is the case for other α_1 subunits, induced a profound functional alteration of the L-type channel in a β subunit isoform-specific manner. Therefore, specific combinations of the same α_{1C} subunit with different β subunits are expected to have important consequences on the kinetics and the voltage-dependency of Ca^{2+} entry in vivo.

When expressed in an heterologous system such as the *Xenopus laevis* oocyte, the α_{1C} subunit gives rise to Ca^{2+} current kinetics with a pronounced Ca^{2+}-dependent inactivation [16,17]. The molecular structures responsible for this type of inactivation were suspected to reside on the α_{1C} subunit itself [16]. Accordingly, several recent studies have located, on the carboxy-terminal tail of the α_{1C} subunit, a putative Ca^{2+} binding site and other sequences important for this type of inactivation [18,19]. The influence of the auxiliary β subunit on the Ca^{2+}-dependent inactivation has only recently been tested [20] and it has been suggested that the same molecular determinants affect both voltage- and Ca^{2+}-dependent inactivation. We have recently shown that the amino-terminal tail of the rat β_{2a} subunit is directly involved in the slowing effect of this subunit on both types of inactivation [20]. Since different splice variants of the β_{2a} subunit exist, not only on this tail but also on the central region of the subunit [8], we have tested the involvement of other sequences of the β subunits in the regulation of Ca^{2+}-dependent inactivation. 10 deletions and five chimeras of the β_1 and β_2 subunits have been used.

Here, we report that rapidly or slowly decaying Ca^{2+} currents can be recorded by co-expression of the α_{1C} subunit with the β_1 or β_2 subunit, respectively. Slowing of inactivation is exclusively due to the presence of the short stretch of amino acids located on the amino-terminal tail of the β_{2a} subunit, the central or carboxy-terminal sequence of the subunit being of minor importance. Our data suggest that the specific splice variants of the β subunit that composed the L-type Ca^{2+} channels are one of the critical factors regulating Ca^{2+} entry into muscle cells and neurons [8].

2. Materials and methods

2.1. Construction of deleted and chimeric β subunits

The following calcium channel subunits were used: α_{1C} [21] and α_2-δ [21], β_{1b} [22], β_{2a} [23]. All these subunit cDNAs were inserted into the pMT2 expression vector [21].

2.1.1. Deleted mutants of the β subunit. β_1-TF1-4 and β_2-TF1-4 were constructed by PCR. The sense primer was engineered to possess an *Eco*RI site (italic) and a start codon (bold) when necessary. In the reverse primer, an *Xba*I site (preceded by a stop codon in the case of the TF4 subunits) was added at the 5′ end. Numbers in parentheses correspond to the position of the primer in the sequence. GenBank accession numbers: β_{1b}, X61394 and β_{2a}, M80545. All sequences are given in the 5′-3′ orientation.

*Corresponding author. Fax: (33) (67) 521559.
E-mail: charnet@.crbm.cnrs-mop.fr

Abbreviations: AID, alpha interaction domain; BAPTA, 1,2-bis(2-aminophenoxy)-ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid; BID, β interaction domain; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid); NMDG, *N*-methyl-D-glucamine; TEAOH, tetra-ethyl ammonium hydroxide

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β_1-TF1: sense primer *Eco*RI-**ATG**-(235–257), antisense primer *Xba*I-(1837–1857).
β_1-TF2: sense primer *Eco*RI-**ATG**-(559–579), antisense primer identical to β_1-TF1.
β_1-TF3: sense primer *Eco*RI-**ATG**-(706–726), antisense primer identical to β_1-TF1.
β_1-TF4: sense primer *Eco*RI-(64–84), antisense primer *Xba*I-**TCA**-(1317–1298).
β_2-TF1: sense primer *Eco*RI-**ATG**-(424–444), antisense primer *Xba*I-(2168–2190).
β_2-TF2: sense primer *Eco*RI-**ATG**-(748–769), antisense primer identical to β_2-TF1.
β_2-TF3: sense primer *Eco*RI-**ATG**-(1009–1031), antisense primer identical to β_2-TF1.
β_2-TF4: sense primer *Eco*RI-(115–133), antisense primer *Xba*I-**TCA**-(1623–1600).
The PCR product was checked and purified on an agarose gel.

2.1.2. β Chimera. The chimera were obtained by a PCR strategy as described [24] using the Expand High Fidelity PCR System from Boehringer Mannheim.

The N-terminal fragments were amplified using the following primers:

β-CH1: sense primer identical to β_2-TF4; reverse primer: (559–576 β_{1b}+731–747 β_{2a}).
β-CH2: sense primer identical to β_2-TF4; reverse primer: (706–723 β_{1b}+878–894 β_{2a}).
β-CH3: sense primer identical to β_2-TF4; reverse primer: (706–724 β_{1b}+993–1008 β_{2a}).
β-CH4: sense primer identical to β_2-TF4; reverse primer: (235–249 β_{1b}+409–423 β_{2a}).

The C-terminal fragments were amplified using the following primers:

β-CH1: sense primer: (731–747 β_{2a}+559–576 β_{1b}); reverse primer identical to β_1-TF1.
β-CH2: sense primer: (878–894 β_{2a}+706–723 β_{1b}); reverse primer identical to β_1-TF1.
β-CH3: sense primer: (993–1008 β_{2a}+706–724 β_{1b}); reverse primer identical to β_1-TF1.
β-CH4: sense primer: (407–423 β_{2a}+235–249 β_{1b}); reverse primer identical to β_1-TF1.

All PCR products were separated on a 1% agarose gel, cut out and purified. A second PCR was performed using these products to produce the final chimeras. β-truncated forms and β chimera were finally digested using *Eco*RI and *Xba*I, sub-cloned into pBluescript (Stratagene) for sequencing (DiDeoxy Terminator technology, Applied Biosystem) and subsequently into pMT2 for injection and expression (see [17,25] for details on *Xenopus* oocytes preparation and injection). 10–20 nl of a mixture of α_{1C} and β subunits cDNA (concentration 1 μg/μl) was injected in oocytes. In Fig. 2 and 3, the α_2-δ subunit was co-injected. No significant changes in inactivation kinetics were noticed between α_{1C}+β+α_2-δ and α_{1C}+β.

2.2. Currents recording and analysis

Whole cell Ba^{2+} currents were recorded under a two electrodes voltage clamp using the GeneClamp 500 amplifier (Axon, Foster city, CA, USA). The current and voltage electrode (less than 1 MΩ) were filled with CsCl, 2.8 M; 1,2-bis(2-aminophenoxy)-ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA), 10 mM, pH = 7.2, with CsOH. Ba^{2+} and Ca^{2+} current recordings were performed after injection of BAPTA (one or two 40–70 ms injections at 1 bar of (in mM): BAPTA-free acid (Sigma), 100; CsOH, 10; *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid) (HEPES), 10, pH 7.2, see [17]). The recording solution had the following composition (in mM): BaOH/CaOH, 10; tetra-ethyl ammonium hydroxide (TEAOH), 20; *N*-methyl-D-glucamine (NMDG), 50; CsOH, 2; HEPES, 10, pH 7.2, with methanesulfonic acid. Currents were filtered and digitized using a DMA-Tecmar labmaster and subsequently stored on a IPC 486 personal computer by using the version 6.02 of the pClamp software (Axon). Under these conditions, the Ca^{2+} currents can be recorded without contamination by the endogenous Ca^{2+}-activated Cl^- current (see [14]). Currents were recorded during a typical test pulse from −80 mV to +10 mV of 2.5 s duration. All currents analyzed in this work had an amplitude in the range of 0.2–3 μA, when recorded 1–4 days after injections. Such currents could be repeatedly recorded with all the combinations of subunits tested. Measurements of the current amplitude and determination of inactivation time constants were performed using Clampfit (pClamp version 6.02, Axon). Inactivation was quantified by dividing the current measured at the end of a typical test pulse of +10 mV (2.5 s duration) by the peak amplitude (residual current in Fig. 2 and 3). All values are presented as mean ± S.E.M. (number of experminents).

3. Results

Fig. 1A shows typical current traces recorded during voltage steps from −80 mV to +10 mV (2.5 s duration), applied to a voltage-clamped oocyte expressing the rat L-type α_{1C} Ca^{2+} channel subunit in combination with either the β_1 or the β_2 subunits. In the presence of Ba^{2+} (traces labelled Ba), the L-type α_{1C} Ca^{2+} channels inactivated with a mono-exponential time course, whether the β_1 or the β_2 subunit was expressed (with respective time constants of 1780 ± 744 ms, $n = 15$ and 3723 ± 873 ms, $n = 3$). Therefore, very little voltage-dependent inactivation was observed with both subunit combinations under these conditions. Replacing the permeant ion Ba^{2+} by Ca^{2+} induced, with both subunit combinations, a marked acceleration of the decaying phase of the current (Fig. 1A, traces labelled Ca), suggesting that both β subunits can interact with the α_{1C} subunit and regulate the Ca^{2+}-dependent inactivation. Kinetic analysis of these currents show that, in the presence of Ca^{2+}, the decaying phase of the currents required two exponential phases to be well-described. For the two combinations, the fast and slow time constants (τ1 and τ2) were both different from the slow time constant recorded in Ba^{2+}, thus suggesting that they both reflect a Ca^{2+}-dependent process. Interestingly, Ca^{2+} currents flowing through the α_{1C}+β_1 combination inactivated significantly faster than the corresponding currents flowing through α_{1C}+β_2 channels (Fig. 1A, top right panel), leading to a global decrease in the amount of Ca^{2+} entering the cells of about 50%.

Comparison of the traces recorded with the two β subunits at different voltages showed that expression of the β_2 subunit induced a delayed inactivation, with the initial phase of current decay being almost unchanged (see Fig. 1B). At all voltages, τ1, the fast time constant of inactivation, was not modified when the α_{1C} subunit was expressed either with the β_1 or the β_2 subunit, while τ2, the slow time constant of inactivation, was significantly increased by expression of the β_2 subunit (Fig. 1B). This change in τ2 was not due to a modification in the level of expression since currents of similar amplitudes were recorded on oocytes expressing either α_{1C}+β_1 or α_{1C}+β_2 subunits combinations (−358 ± 245 nA and −324 ± 188 nA, respectively). Interestingly, τ1 and τ2 were only poorly dependent on the voltage (right panel of Fig. 1B) and the β_2 subunit-induced increase of τ2 was present at all voltages tested. We concluded that the Ca^{2+}-dependent inactivation of the α_{1C} subunit, and therefore the level of Ca^{2+} entry, can be regulated by expression of different β subunits.

To understand the mechanisms underlying this regulation and to identify their molecular determinant, we have constructed a series of deleted and chimeric forms of the β_1 and β_2 subunits and analyzed their effect on Ca^{2+}-dependent

inactivation after expression in oocytes with the α_{1C} subunit. The four β subunit genes are constructed around two conserved domains (called C1 and C2, white boxes in Fig. 2), surrounded with variable amino (V1), central (V2) and carboxy domains (V3, see shaded boxes in Fig. 2), where alternative splicing occurred ([8]). Our first deletions (TF1, see Fig. 2) removed the variable amino-terminal domains of the two β subunits. When made on β_1, this deletion did not affect the kinetics of the currents recorded during a typical test pulse of +10 mV from a holding potential of −80 mV (compare traces marked β_1 and β_1TF1 in part A and I2/I1 in part B of Fig. 2), suggesting that these amino acids are not crucial in setting the rate at which the α_{1C}+β_1 Ca^{2+} channels inactivate in the presence of Ca^{2+}. Expression of larger deletions in the amino (see traces labelled β_1-TF2, β_1-TF3 in Fig. 2) or carboxy (β_1-TF4) tails of the β_1 subunit, which removed domains V1+C1, V1+C1+V2 or V3 of the β subunit, respectively, with the α_{1C} subunit, were also without an effect on the kinetics of the corresponding Ca^{2+} currents, suggesting that the second conserved domain, C2, where association with the α_{1C} subunit occurred, is the only element regulating the effects of the β_1 subunit on L-type Ca^{2+} channel inactivation. This was confirmed by expression of this C2 domain alone (β_1-TF7) with the α_{1C} subunit. This subunit combination did not show any significant difference in the rate of Ca^{2+}-dependent inactivation (as measured by the I2/I1 ratio) when compared to the full length β_1 subunit (see Fig. 2).

Different results were obtained, however, when these deletions were made on the β_2 subunit. Co-expression of the first deletions, β_2-TF1, with the α_{1C} subunit produced functional Ca^{2+} channels that displayed a typical Ca^{2+}-dependent inactivation. However, the kinetics of these currents were significantly faster than those observed when the full length β_2 subunit was expressed (Fig. 2). In this case, current traces recorded with β_2-TF1 were very similar to those recorded

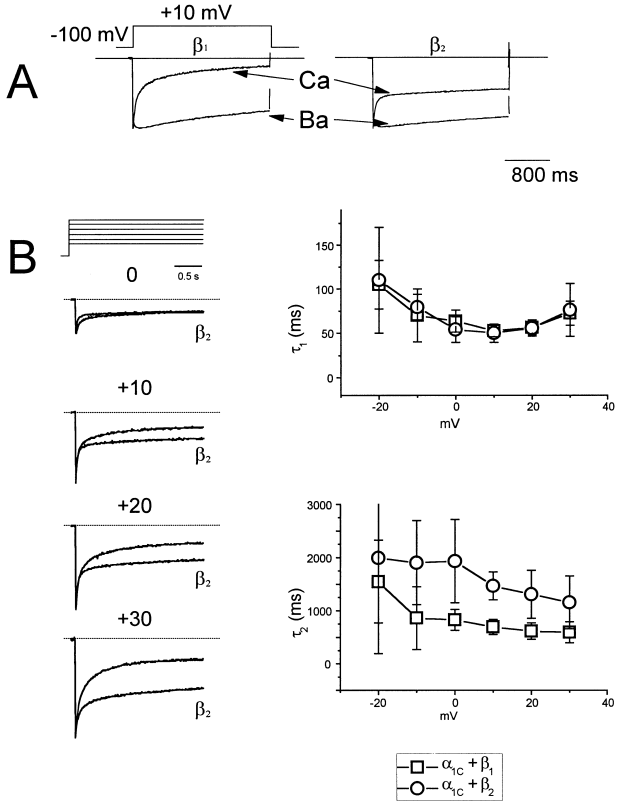

Fig. 1. α_{1C}+β_1 or α_{1C}+β_2 Ca^{2+} currents display different inactivation kinetics. A: Ba^{2+} (left) and Ca^{2+} (right) currents recorded from oocytes expressing the α_{1C} and α_2-δ Ca^{2+} channel subunits with either the β_1 or the β_2 subunit. Currents were recorded during voltage steps of 2.5 s with a duration from −100 mV to +10 mV. Traces are scaled at the same size for comparison (Ca^{2+} currents were about 1/3 of the Ba^{2+} currents [25]). B: (left) Ca^{2+} currents recorded from oocytes injected with either the α_{1C}+β_1 or the α_{1C}+β_2 Ca^{2+} channel subunits. Current traces from the two combinations recorded during depolarisations of the same amplitude were superimposed and scaled at the same size for the purpose of comparison. Depolarisations were applied from a holding potential of −80 mV to 0, 10, 20 or 30 mV. In all cases, the inactivation process can be described by a bi-exponential decay. B: (right) Voltage-dependency of the fast (τ1) and the slow (τ2) time constant of inactivation. Note that only τ2 is changed by expression of the β_2 subunit (n = 7 and 8 for β_1 and β_2, respectively)

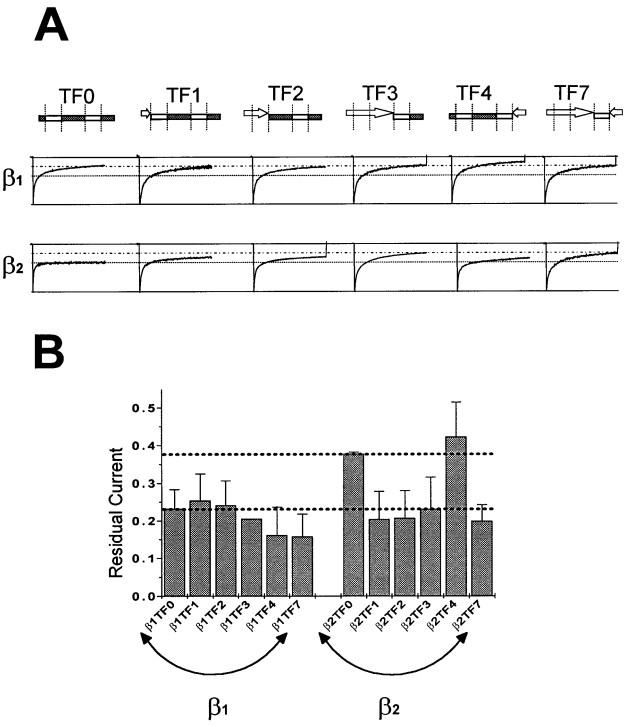

Fig. 2. Current traces and Ca^{2+}-dependent inactivation, recorded for different truncated β_1 and β_2 subunits. A: Schematic representation of different truncated forms of the β_1 and β_2 subunits and associated current traces recorded after their expression in oocytes with the α_{1C} subunit. Sizes of the deletions are marked by an arrow. TF0, full length β subunit; TF1, deletion of the first variable domain; TF2, deletion of the first variable and the first conserved domains; TF3, deletions up to the second conserved domain; TF4, deletion of the carboxy variable domain. Currents were recorded during depolarisations to +10 mV from −80 mV (duration of 2.5 s). B: Average inactivation calculated from traces similar to those shown in A for the different truncated β subunits. Inactivation was quantified by dividing the current recorded at the end of the depolarizing pulse by the peak current (residual current). n = 12, 5, 5, 3, 3 and 9 for β_1-TF0, -TF1, -TF2, -TF3, -TF4 and -TF7 and n = 4, 8, 5, 7, 4, 4 and 2 for β_2-TF0, -TF1, -TF2, -TF3, -TF4 and -TF7, respectively.

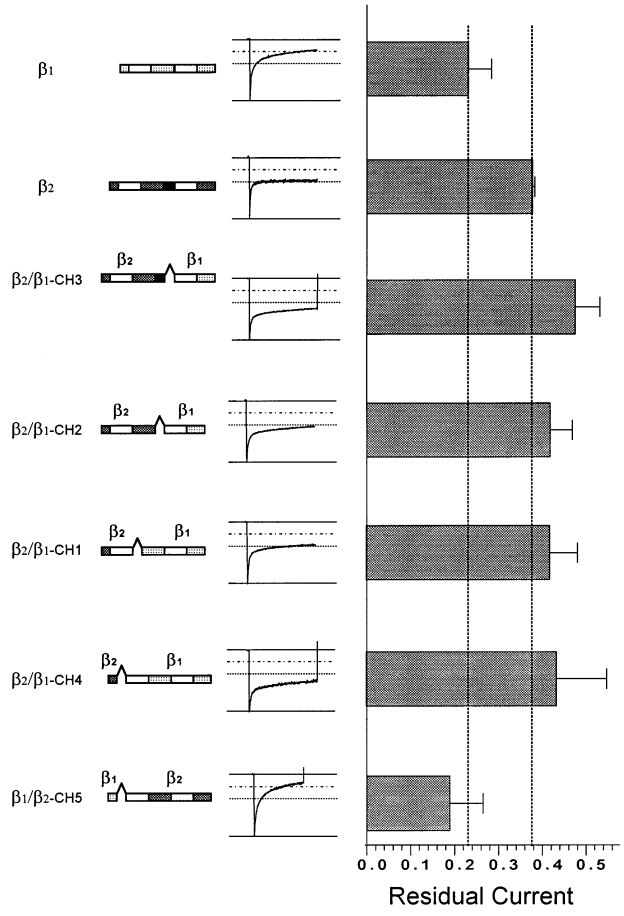

Fig. 3. Current traces and Ca^{2+}-dependent inactivation recorded for different chimeric β subunits. Left: schematic representation of different chimeric β subunits used in this work. In a first construct, the missing sequence of the β_1-TF3 subunit was replaced by the corresponding sequence of the β_2 subunit (amino acids 1–212). In β_2/β_1-CH2, β_2/β_1-CH1 and β_2/β_1-CH4, the participation of the β_2 subunit to the total sequence of the chimera was further reduced. β_2/β_1-CH2 was deleted for an insert specifically found in the second variable domain of the β_2 subunit (black box in the figure, amino acids 170–212). β_2/β_1-CH4 kept only the first 16 amino acids from β_2, the remaining sequence coming from β_1. Middle: associated current traces recorded after expression of the β_1, β_2 or the different chimera in oocytes with the α_{1C} subunit. Same recording conditions as in Fig. 2. Right: average inactivation calculated for the different chimeric β subunits. Inactivation was quantified as described in Fig. 2 (residual current). $n = 12, 4, 4, 8, 7, 4, 6$ for β_1, β_2, β_2/β_1-CH3, β_2/β_1-CH2, β_2/β_1-CH1, β_2/β_1-CH4 and β_2/β_1-CH5, respectively.

with the β_1 subunit (around 20% of the residual current after a depolarization of 2.5 s, see Fig. 2). All subsequent amino-terminal deletions of the β_2 subunit induced also a similar acceleration of inactivation (see traces β_2-TF2 and β_2-TF3 and bar graph of Fig. 2), while the carboxy deletion (β_2-TF4) was without an effect. Altogether, these data suggest that a short sequence on the β_2 subunit (V1, corresponding to the lost segment of the β_2-TF1 subunit) is fully responsible for the slow Ca^{2+}-dependent inactivation recorded with the β_2 subunit.

We have tested this idea by inserting different sequences of the β_2 subunit (corresponding to the deleted sequences of the β_2-TF1, -TF2, -TF3 subunits) in a N-terminal position of the β_1 subunit and tested their effect on Ca^{2+}-dependent inactivation. Four chimeras were thus constructed, where V1, V1+C1, V1+C1+V2 and V1+C1+V2+an insertion, only found in V2 from β_2, replaced the homologous sequences in the β_1 subunit (chimera β_2/β_1-CH4, β_2/β_1-CH1, β_2/β_1-CH2, β_2/β_1-CH3, respectively). A fifth chimera, β_1/β_2-CH5, was also constructed by replacing the V1 sequence of the β_2 subunit by the corresponding sequence of β_1 (see Fig. 3 for constructions). All these chimeras were co-expressed with the α_{1C} subunits in *Xenopus* oocytes and their effects on Ca^{2+}-dependent inactivation were tested. The results are displayed in Fig. 3. Interestingly, all these chimera (CH3, CH2, CH1 and CH4) displayed a slow Ca^{2+}-dependent inactivation, confirming that the acceleration of inactivation observed with the deleted forms of the β_2 subunit was indeed due to the removal of an inhibitory sequence present in β_2. β_2/β_1-CH4, the chimera with the smallest sequence of β_2 (the first 16 amino acids of β_2), has a slow Ca^{2+}-dependent inactivation (around 40% of the residual current after a 2.5 s long depolarization to +10 mV) and defined the minimum sequence for this effect. The reverse chimera, β_1/β_2-CH5 (V1 sequence of β_1 replacing V1 of β_2) has a fast inactivation comparable to β_2-TF1, β_1-TF1 or β_1 and confirms the lack of other regulating regions in the amino-terminal end of β_1.

4. Discussion

We have recorded Ba^{2+} and Ca^{2+} currents after expression of α_{1C} L-type Ca^{2+} channels in *Xenopus* oocytes. While inactivation kinetics of Ba^{2+} currents could be described by a single exponential component, a fast and a slow component of inactivation were necessary to account for the current kinetics recorded in the presence of Ca^{2+}. These two components were clearly Ca^{2+}-dependent since they were absent in the presence of Ba^{2+} and clearly faster (at least by a factor two at +10 mV) than the inactivation time constant of the Ba^{2+} currents. We show here that different Ca^{2+} current kinetics can be recorded by assembly of the L-type α_{1C} Ca^{2+} channel subunit with various ancillary β subunits. Kinetic modifications mainly reflect an increase of the slow time constant of the Ca^{2+}-dependent inactivation. We have previously shown that a short stretch of amino acids, located at the amino-terminal end of the β_2 subunit, was able to slow inactivation when present [20]. In this work, using extended deletions of the β_1 and β_2 subunits, we show that this sequence is the only site responsible for the β_2-specific regulation of the Ca^{2+}-dependent inactivation.

Up to now, Ca^{2+}-dependent inactivation of the L-type Ca^{2+} channel was considered as a property of the α_{1C} subunit, with little or no influence of the β subunit [16,26]. Accordingly, different sequences of the α_{1C} subunit, including a putative EF-hand Ca^{2+} binding site, have been shown to be essential for Ca^{2+}-dependent inactivation [18,19,27]. These sequences are located at the carboxy-terminal end of the α_{1C} subunit and their transposition to other α_1 subunits transferred Ca^{2+}-dependent inactivation to channels usually resistant to this type of inactivation. The fact that Ca^{2+}-dependent inactivation can be specifically modulated by the auxiliary β subunit constitutes therefore an important finding that could partially explain the variability of the L-type Ca^{2+} current kinetics recorded on different muscular preparations. It is

worth noting that alternative splice variants of the α_{1C} subunit have been isolated and are specifically expressed in these tissues [28–32]. However, although these isoforms can potentially participate in the observed variability of the channel properties, none of the functional studies performed yet evidenced any changes in the kinetics of the resulting Ca^{2+} current [26].

Interestingly, the N-terminal amino acid sequence responsible for the slow inactivation of the β_2 containing channels has recently been shown to affect the voltage-dependent inactivation of the class E and class A Ca^{2+} channels in a similar manner [20,33], arguing for a general regulatory mechanism of inactivation by the β_2 subunit. However, our results on Ca^{2+}-dependent inactivation, as opposed to those of Olcese et al. [33], de-emphasize the role of the amino-terminal region of the β_1 subunit in setting the rate of inactivation. Rather, they suggest that association of the sole β interacting domain (BID) of the β subunit is sufficient to speed the inactivation while in the β_2 subunit, a unique additional sequence is able to slow inactivation. Whether this unique sequence acted directly by functional interaction with the α_1 subunit at a site similar or distinct of the α interaction domain (AID) (α_1 interacting site [15]) or indirectly, by inducing a conformational rearrangement of the β subunit, remains to be determined. Identification of amino acids involved in this effect, together with functional analysis of the intracellular perfusion of this sequence (by peptide injection) on inactivation, will be critical to test these two hypothesis. The two cysteines (at position 2 and 3 of the β_2 subunit) involved in the regulation of the voltage-dependent inactivation [34], constitute undoubtedly a prime target for future experiments. Recent work carried out by Walker et al. [35] identified secondary sites of interaction between the α_1 and the β_4 subunit that plays a role in the regulation of channel inactivation. These sites were located on the carboxy-terminal regions of the β_4 subunit and the α_{1A} subunit. Whether such second sites exist on the α_{1C} subunit and affects Ca^{2+}-dependent inactivation is yet not known, but could also potentially participate in the broad panel of Ca^{2+}-dependent inactivation kinetics displayed by the L-type Ca^{2+} channels in different tissues.

Our β_2 regulatory sequence is located at the amino-terminal end of the protein and can be alternatively spliced to give rise to at least three different β_2 subunits (β_{2a}, β_{2b} and β_{2e}) in rat, rabbit or human [8]. Whether or not these different splice variants have the same effect on the kinetics of inactivation of the L-type Ca^{2+} current is not known, but one can predict that they do not, since (i) homology between the β_{2b} and the β_{2a} subunit is lower than with the β_1 subunit in this particular region and (ii) β_{2a} and β_{2b} have different effects on voltage-dependent inactivation [33]. Our results thus suggest that one of the immediate functional impacts of these different splice variants would be to finally tune the level and kinetics of Ca^{2+} entry into muscle cells. Regulation of inactivation by β subunits appears therefore to be a complex process involving multiple sites and mechanisms.

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