Ca\(^{2+}\) current potentiation by conditioning depolarization is a general mechanism by which excitable cells can control the level of Ca\(^{2+}\) entry during repetitive depolarizations. Several types of Ca\(^{2+}\) channels are sensitive to conditioning depolarization, however, using clearly distinguishable mechanisms. In the case of L-type Ca\(^{2+}\) channels, prepulse-induced current facilitation can only be recorded when the pore-forming \(\alpha\) subunit is coexpressed with the auxiliary \(\beta_1\), \(\beta_2\), or \(\beta_3\) but not \(\beta_4\) subunit. These \(\beta\) subunits are composed of two conserved domains surrounded by central, N-terminal, and C-terminal variable regions. Using different deleted and chimeric forms of the \(\beta_1\) and \(\beta_2\) subunits, we have mapped essential sequences for L-type Ca\(^{2+}\) channel facilitation. A first sequence, located in the second conserved domain of all \(\beta\) subunits, is responsible for the promotion of current facilitation by the \(\beta\) subunit. A second sequence of 16 amino acids, located on the N-terminal tail of the \(\beta_2\) subunit, induces a transferable block of L-type current facilitation. Site-specific mutations reveal the essential inhibitory role played by three positive charges on this segment. The lack of prepulse-induced current facilitation recorded with some truncated forms of the \(\beta_2\) subunit suggests the existence of an additional inhibitory sequence in the \(\beta_2\) subunit.

Beyond their role in membrane excitability, voltage-gated Ca\(^{2+}\) channels are of particular interest due to the fundamental importance of the biological processes regulated by incoming Ca\(^{2+}\) ions (for review, see Ref. 1). Several different types of Ca\(^{2+}\) channel have been identified in neurons, where they are crucial for secretion, synaptic transmission, or gene expression (2–4). Ca\(^{2+}\) entry through L-, N-, T-, or P-type channels is primarily regulated by the level of membrane depolarization. However, the frequency of these depolarizations is also an important regulatory factor that has been implicated in the regulation of synaptic transmission and that may be of importance for long-term potentiation. Accordingly, repetitive stimuli or strong pre-depolarization can up-regulate the activity of different types of Ca\(^{2+}\) channel, giving rise to the so-called Ca\(^{2+}\) current facilitation (for review, see Ref. 5). Although a similar increase is recorded for the various Ca\(^{2+}\) channels, different mechanisms, however, have been proposed. Experimental evidence firmly correlates a given mechanism with a particular Ca\(^{2+}\) channel type, as is the case for N-type Ca\(^{2+}\) channels, which are sensitive to a direct, voltage-dependent G-protein block.

L-type Ca\(^{2+}\) channel facilitation has been described using various preparations, and different mechanisms leading to current potentiation have been proposed. Strong pre-depolarizations can induce a change in gating mode in cardiac L-type Ca\(^{2+}\) channels, whereas the same protocol appears to lead, in skeletal and vascular muscle, to a voltage-dependent phosphorylation of the channel by protein kinase A and calmodulin kinase II, respectively (6–8). At the molecular level, these effects seem to be related to different isoforms of the L-type Ca\(^{2+}\) channel (\(\alpha_{1C}, \alpha_{1D}\), or \(\alpha_{1S}\)), different splice variants, and/or different cellular preparations. Current facilitation of the neuronal L-type Ca\(^{2+}\) channel (9) has been recorded, using the appropriate protocol, in isolated neurons (10, 11) or after expression of the \(\alpha_{1C}\) subunit in Xenopus oocytes (12, 13). This type of facilitation can be distinguished from cardiac, skeletal, or vascular L-type Ca\(^{2+}\) channel facilitation (6–8, 14–18) by its insensitivity to phosphatase inhibitors and permeating ions (12), suggesting a specific mechanism for neuronal facilitation. As opposed to N-type Ca\(^{2+}\) channels, coexpression of specific \(\beta\) subunits (\(\beta_1\), \(\beta_2\), or \(\beta_3\)) is absolutely necessary for the development of \(\alpha_{1C}\) subunit facilitation (12, 19). The inhibitory effect of the \(\beta_2\) subunit (13) and the insensitivity to G-protein stimulation or inhibition (12) are also distinctive features. Altogether, these data suggest that the molecular mechanisms of L- and N-type Ca\(^{2+}\) channel facilitation are different processes. In this work, using deleted and chimeric subunits constructed from permissive and nonpermissive \(\beta\) subunits, we demonstrate the existence of a conserved sequence in the \(\beta\) subunits necessary for L-type Ca\(^{2+}\) channel facilitation. We also identify a short autoinhibitory segment on the N-terminal tail of the \(\beta_2\) subunit partly responsible for the insensitivity of the \(\alpha_{1C} + \beta_2\) subunit combination to prepulse facilitation. Expression of individual \(\beta\) subunits could therefore reveal latent properties that are central for neuronal plasticity.

**EXPERIMENTAL PROCEDURES**

**Preparation of Truncated, Chimeric, and Mutated Forms of the \(\beta\) Subunits and Xenopus Oocyte Injection—**The following calcium channel subunits were used: \(\alpha_{1C}\) and \(\alpha_{1D}\) (9), \(\beta_1\) (20), and \(\beta_2\) (21). All these subunit cDNAs were inserted into the pMT2 expression vector under the control of an SV40 promoter (9). Deleted Mutants of the \(\beta\) Subunit—\(\beta_1\)-TF1–4 and \(\beta_2\)-TF1–4 were constructed by PCR. The sense primer was engineered to possess an

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1 The abbreviations used are: PCR, polymerase chain reaction; BAPTA, 1,2-bis(2-aminophenoxy)ethane-N,N,N’,N’-tetraacetic acid; ATPγS, adenosine 5’-O-(thiotriphosphate); AMP-PCP, adenosine 5’/(β,γ-methylenetriphosphate).
ECORI site and a start codon (boldface) when necessary. In the reverse primer, an XbaI site (preceded by a stop codon in the case of the TF4 subunit) was added at the 5'-end. Numbers in parentheses correspond to the position of the primer in the sequence. GenBank accession numbers were X61394 for the β1 subunit and M80545 for the β2 subunit. The primers used were as follows: β1-TF1: sense, EcoRI-ATG-(235–257 β1h), and antisense, XbaI-(1837–1857 β1h); β2-TF2: sense, EcoRI-ATG-(559–579 β2h), and antisense, identical to β1-TF1; β1-TF3: sense, EcoRI-ATG-(706–726 β2h), and antisense, identical to β1-TF1; β2-TF4: sense, EcoRI-(64–84 β2h), and antisense, identical to β1-TF1; β1-TF6: sense, EcoRI-ATG-(1009–1031 β2h), and antisense, identical to β1-TF1; β2-TF7: sense, EcoRI-ATG-(748–769 β2h), and antisense, identical to β1-TF1; β1-TF8: sense, EcoRI-ATG-(1298–1317 β2h), and antisense, identical to β1-TF1; β2-TF9: sense, EcoRI-ATG-(424–444 β2h), and antisense, EcoRI-TCA-(2168–2190 β2h); β2-TF10: sense, EcoRI-ATG-(748–769 β2h), and antisense, identical to β1-TF1; β2-TF11: sense, EcoRI-TCA-(1009–1031 β2h), and antisense, identical to β1-TF1; and β2-TF12: sense, EcoRI-ATG-(115–133 β2h), and antisense, XbaI-TCA-(1600–1623 β2h). The PCR product was checked and purified on agarose gel.

β Subunit Chimeras—The chimeras were obtained by a PCR strategy as described (22) using the Expand High Fidelity PCR system (Boehringer Mannheim). The N-terminal fragments were amplified using the following primers: βCH1: sense, identical to β2-TF4, and antisense, (559–576 β1h + 731–747 β2h); βCH2: sense, identical to β2-TF4, and antisense, (706–723 β1h + 878–894 β2h); βCH3: sense, identical to β2-TF4, and antisense, (706–724 β1h + 993–1008 β2h); and βCH4: sense, identical to β2-TF4, and antisense, (235–249 β1h + 409–423 β2h). The C-terminal fragments were amplified using the following primers: βCH1: sense, (731–747 β1h + 559–576 β2h), and antisense, identical to β1-TF1; βCH2: sense, (878–900 β2h + 706–723 β1h), and antisense, identical to β1-TF1; βCH3: sense, (407–423 β2h + 235–249 β1h), and antisense, identical to β1-TF1.

All PCR products were separated on 1% agarose gel, cut out, and purified. A second PCR was performed using these products to produce the final chimeras. β-Subunit truncated forms and chimeras were finally digested using EcoRI and XbaI, subcloned into pBluescript (Stratagene) for sequencing (Dideoxy Terminator technology, Applied Biosystems) and in vitro translation (TNT® coupled reticulocyte lysate systems, Promega), and subsequently into pMT2 for injection and expression.

βM123 and β1/β2-CH4M123 Constructions—The mutants βM123 and β1/β2-CH4M123 were obtained by a PCR strategy as described (22) using the Expand High Fidelity PCR system. The N-terminal fragments were amplified using the following primers: βM123 and β1/β2-CH4M123: sense, identical to β2-TF4, and antisense, 5’-GGGCTGGTACATGCCGCGGCAGTACGGGTGTCC-3’. The C-terminal fragments were amplified using the following primers: βM123: sense, 5’-GGGGTGTACATGCCGCGGCAGTACGGGTGTCC-3’ (388–420 β2h, excepted mutated sequence underlined), and antisense, identical to β2-TF1, using β2h as a matrix; and β1/β2-CH4M123: sense, identical to...
**Fig. 3.** L-type current facilitation recorded with truncated forms of the \( \beta_1 \) subunit. \( \text{Ba}^{2+} \) currents were recorded during a depolarization to +10 mV from a holding potential of -80 mV in *Xenopus* oocytes injected with the \( \alpha_{1C} \) subunit and different truncated forms of the \( \beta_1 \) subunit. Current facilitation was recorded, without significant differences upon coexpression of each of these truncated forms, when the appropriate conditioning depolarization was applied (200 ms in duration from -80 to +100 mV; traces labeled *). Left panel, constructions used; middle panel, current traces (scale bars = 1 nA, except for \( \beta_1 \)-TF4, 200 nA); right panel, averaged facilitation, quantified as the ratio of the current amplitude after pre-depolarization divided by the control current amplitude.

**Fig. 4.** L-type current facilitation recorded with truncated forms of the \( \beta_2 \) subunit. The same recording conditions were used as those described in the legend to Fig. 3, but using truncated \( \beta_2 \) subunits. Left panel, constructions used; middle panel, current traces (scale bars = 1 nA, except for \( \beta_2 \)-TF3, 500 nA); right panel, averaged facilitation, quantified as the ratio of the current amplitude with pre-depolarization divided by the control current amplitude. *, significantly different from the \( \beta_2 \) subunit.
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\(\beta_{\text{M123}}\), and antisense, identical to \(\beta_{-}\text{TF1}\), using \(\beta_{1}\beta_{2}\cdot\text{CH4}\) as a matrix. The complete sequence was obtained as described for chimeras. All the constructions of the \(\beta\) subunits used in this paper were checked for correct translation and molecular weight by in vitro translation using the T7 or T3 TNT coupled reticulocyte lysate system (Promega). All these constructions were able to increase Ba\(^{2+}\) current amplitude when co-injected with the \(\alpha_{1}\) subunit, demonstrating association with the \(\alpha_{1}\) subunit (respective current amplitudes for \(\alpha_{1}\) alone, \(-368 \pm 48\) nA (n = 5); for \(\beta_{1}\), \(\beta_{1}\text{-TF1}, \beta_{1}\text{-TF2, }\beta_{1}\text{-TF3, and }\beta_{1}\text{-TF4, }-513 \pm 306\) nA (n = 20), \(-702 \pm 235\) nA (n = 6), \(-687 \pm 273\) nA (n = 14), \(-757 \pm 414\) nA (n = 10), and \(-411 \pm 100\) nA (n = 5), respectively; for \(\beta_{1}\), \(\beta_{1}\text{-TF1, }\beta_{1}\text{-TF2, }\beta_{1}\text{-TF3, and }\beta_{1}\text{-TF4, }-815 \pm 403\) nA (n = 9), \(-1220 \pm 440\) nA (n = 11), \(-1035 \pm 399\) nA (n = 17), \(-527 \pm 200\) nA (n = 5), and \(-1447 \pm 678\) nA (n = 6), respectively; and for \(\beta_{2}/\beta_{\text{CH1}}, \beta_{2}/\beta_{2}\cdot\text{CH2, }\beta_{2}/\beta_{2}\cdot\text{CH3, and }\beta_{2}/\beta_{2}\cdot\text{CH4, }-930 \pm 424\) nA (n = 8), \(-1200 \pm 540\) nA (n = 9), \(-1633 \pm 823\) nA (n = 5), and \(-1650 \pm 644\) nA (n = 18), respectively).

**Deletion Mutants of the \(\alpha_{1}\) Subunit—\(\alpha_{1}\text{-DN and }\alpha_{1}\text{-DC were generated by PCR using the Expand Long Template PCR system (Boehringer Mannheim). An N-terminal fragment was generated to introduce the untranslated avian myeloblastosis virus sequence just before the sequence of the \(\alpha_{1}\) subunit (the antisense primers used were 5'-CCTCTGTCTTTCGACCATGGTTGACATTATTGGAGAAA-3' for \(\alpha_{1}\text{-DC (nucleotides 290–510, GenBank accession number M67515)), and 5'-CAGGTTGCTGACATAGGACC-3' for \(\alpha_{1}\text{-DN (nucleotides 377–396)). This fragment was then used as a megaprimer to generate the final mutated forms of }\alpha_{1}\text{-DC with an antisense primer (5'-GGTTCCTTTTCCCCGCGCTGACG-3') for }\alpha_{1}\text{-DC (No1, stop codon in boldface, nucleotides 5378–5404) and 5'-ATACTTATGCGCGCCTA-CAGGTTGCTGAGAACG-3' for }\alpha_{1}\text{-DN (No1, nucleotides 6699–6721)). The mutated forms were subcloned into the pMT2 expression vector.**

**Xenopus oocyte preparation and injection** (5–10 nl of \(\alpha_{1} + \alpha_{2}\delta + \beta\) subunit cDNAs at \(-0.3\) ng/nl) were performed as described elsewhere (13). Oocytes were then incubated for 2–7 days at 19 °C under gentle agitation before recording.

**Electrophysiological Recordings**—Whole cell Ba\(^{2+}\) currents were recorded under two-electrode voltage clamp using the GeneClamp 500 amplifier (Axon Instruments, Inc., Foster City, CA). Current and voltage electrodes (<1 megohms) were filled with 2.8 M CsCl and 10 mM Ba\(^{2+}\) (BAPTA, pH 7.2, with CsOH). Ba\(^{2+}\) current recordings were performed after injection of BAPTA (one or two 70-ns injections at 1 bar of 100 mM BAPTA-free acid (Sigma), 10 mM CsOH, and 10 mM HEPES, pH 7.2), according to the following composition: 10 mM BaOH, 20 mM tetrathydrofuran, 50 mM Na-methyl-d-glucamine, 2 mM CsOH, and 10 mM HEPES, pH 7.2, with methanesulfonic acid. Only oocytes expressing Ba\(^{2+}\) currents with amplitudes in the range of 0.5–5 μA were analyzed to ensure sufficient resolution and to avoid voltage-clamp problems. Currents were filtered and digitized using a DNA-Tecmar Labmaster and subsequently stored on an IPC 486 personal computer using pClamp software (Version 6.02, Axon Instruments, Inc.). Ba\(^{2+}\) currents were recorded during a typical test pulse from \(-80\) to +10 mV of 0.4-s duration. Current facilitation was elicited by applying a 200- or 400-ms depolarization (pre-depolarization) to+60 to +140 mV (the protocol is shown on top of the figure). A, oocytes were injected with the \(\alpha_{1}\) and \(\alpha_{2}\delta\) subunits and \(\beta_{1}, \beta_{1}\text{-TF1, }\beta_{1}\text{-TF2, or }\beta_{1}\text{-TF3, B, oocytes were injected with the }\alpha_{1}\text{ and }\alpha_{2}\delta\text{ subunits and }\beta_{2}, \beta_{2}\text{-TF2, or }\beta_{2}\text{-TF4. Note that facilitation of increasing amplitude was recorded with expression of }\beta_{1}, \beta_{1}\text{-TF1, }\beta_{1}\text{-TF2, or }\beta_{1}\text{-TF3, whereas pre-pulses of various amplitude were without effect on Ba\(^{2+}\) currents recorded upon coexpression of }\alpha_{1}\text{, }\alpha_{2}\delta,\text{ and }\beta_{1} \text{ or }\beta_{1}\text{-TF4.**

**RESULTS**

We have previously shown that the activity of the \(\alpha_{1}\) subunit L-type Ca\(^{2+}\) channel, recorded during a single voltage step at +10 mV, could be up-regulated by applying strong positive pre-depolarizations (12). This type of current facilitation was sensitive to the expression of a \(\beta\) subunit (12), but was not affected by the expression of the \(\alpha_{2}\delta\) subunit. We have further identified the existence of permissive and nonpermissive \(\beta\) subunits for this facilitation (13).

Fig. 1 shows that application of a +120-mV pre-depolarization to an oocyte expressing the \(\alpha_{1}\) and \(\beta_{1}\) subunits (see protocol shown on top of the figure) increases the Ba\(^{2+}\) current recorded during a subsequent depolarization (in the range of 20 to +30 mV). The same pre-depolarization was without effect when applied to oocytes expressing the \(\alpha_{1}\) and \(\beta_{2}\) subunits (Fig. 1A). This lack of facilitation was recorded independently of the amplitude of the test pulse, as exemplified for voltage steps from −20 to +30 mV (Fig. 1, A and B). It was not due to a \(\beta_{2}\) subunit-induced shift in the voltage dependence of facilitation since stronger (or longer) pre-depolarizations were also inefficient in producing \(\alpha_{1}\) and \(\beta_{2}\) subunit current potenti-

![Fig. 5. Voltage dependence of \(\alpha_{1}\) subunit current facilitation recorded with truncated \(\beta_{1}\) and \(\beta_{2}\) subunits. Current facilitation, quantified as described in the legend to Fig. 3, was recorded with prepulse (PP) amplitude varying from +60 to +140 mV (the protocol is shown on top of the figure). A, oocytes were injected with the \(\alpha_{1}\) and \(\alpha_{2}\delta\) subunits and \(\beta_{1}, \beta_{1}\text{-TF1, }\beta_{1}\text{-TF2, or }\beta_{1}\text{-TF3. B, oocytes were injected with the }\alpha_{1}\text{ and }\alpha_{2}\delta\text{ subunits and }\beta_{2}, \beta_{2}\text{-TF2, or }\beta_{2}\text{-TF4. Note that facilitation of increasing amplitude was recorded with expression of }\beta_{1}, \beta_{1}\text{-TF1, }\beta_{1}\text{-TF2, or }\beta_{1}\text{-TF3, whereas pre-pulses of various amplitude were without effect on Ba\(^{2+}\) currents recorded upon coexpression of }\alpha_{1}\text{, }\alpha_{2}\delta,\text{ and }\beta_{1} \text{ or }\beta_{1}\text{-TF4.**

**Future work** should focus on the identification of \(\alpha_{1}\) and \(\alpha_{2}\delta\) subunit interaction domains and the role of the \(\beta\) subunits in the facilitation process.
channel function (24). Keeping these results in mind, we constructed and expressed a series of N- and C-terminal deletions of the \( \beta_1 \) and \( \beta_2 \) subunits in which this \( \beta \) subunit interaction domain was preserved and four chimeric \( \beta \) subunits, between the permissive \( \beta_1 \) and nonpermissive \( \beta_2 \) subunits, in which each of the five domains was exchanged (Fig. 2).

Fig. 3 shows the effects of N- and C-terminal deletions of the \( \beta_1 \) subunit on current facilitation. Prepulse facilitation was elicited using a 200-ms-long prepulse at +100 mV. Under these conditions, the average current potentiation recorded by expressing the full-length \( \beta_1 \) subunit with the \( \alpha_{1c} \) subunit was 47 ± 18% (\( n = 17 \)) larger than the control current, recorded without pre-depolarization. Deletion of the first variable region (V1, first 58 amino acids, \( \beta_1\)-TF1) of the \( \beta_1 \) subunit did not affect its capacity to induce facilitation since the increase in current recorded after the pre-depolarization was 54 ± 16% (\( n = 5 \)) (Fig. 3). Subsequent deletions of the N-terminal region of the \( \beta_1 \) subunit including the first conserved domain and the second variable region (first 166 and 215 amino acids, respectively; \( \beta_1\)-TF2 and \( \beta_1\)-TF3) were also without effect on facilitation (respective increases of 66 ± 6% (\( n = 7 \)) and 48 ± 9% (\( n = 9 \)) (Fig. 3). In all cases, the increase in current following pre-depolarization was accompanied by an acceleration of inactivation, as already noted for class C L-type Ca\(^{2+} \) channels sensitive to pre-depolarization (43 ± 13% (\( n = 5 \)) of current increase; see trace labeled \( \beta_1\)-TF4 in Fig. 3). Altogether, these data suggested that the sequences necessary to induce L-type current facilitation are located in the second conserved domain of the \( \beta_1 \) subunit (C2, amino acids 215–418). Since this domain is highly conserved between the \( \beta_1 \) and \( \beta_2 \) subunits (92% homology), the \( \beta_2 \) subunit should also be able to produce facilitation. Under these conditions, the lack of facilitation recorded with the \( \beta_2 \) subunit may be due to some inhibitory processes instead of a missing facilitatory sequence.

We have tested this idea by constructing a series of four truncated \( \beta_2 \) subunits. In \( \beta_2\)-TF1, the first 16 amino acids of the \( \beta_2 \) subunit were removed. As shown in Fig. 4, Ba\(^{2+} \) currents recorded from oocytes expressing this subunit with the \( \alpha_{1c} \) subunit were not sensitive to pre-depolarization since their amplitude was not increased by the conditioning voltage step (5 ± 2%; \( n = 11 \)). The subsequent deletion of the first conserved domain (C1) gave rise to \( \beta_2\)-TF2 (Fig. 4). Surprisingly, application of pre-depolarization to oocytes injected with this subunit, in conjunction with the \( \alpha_{1c} \) subunit, significantly increased the Ba\(^{2+} \) current recorded during a subsequent test pulse to +10 mV (38 ± 6% (\( n = 8 \)) compared with 5 ± 6% (\( n = 9 \)) for the full-length \( \beta_2 \) subunit). The facilitated current displayed the typical inactivating phase usually recorded with this paradigm (compare traces labeled \( \beta_2\)-TF2 in Fig. 4 and \( \beta_1 \) in Fig. 1). A larger deletion including the central variable region V3 (amino acids 1–212, \( \beta_2\)-TF3 in Fig. 4) had the same effect and transformed this subunit into a permissive subunit for facilitation (average increase of 42 ± 13%, \( n = 3 \)). Deletion of V3, the C-terminal variable sequence of the \( \beta_2 \) subunit (amino acids 416–604, \( \beta_2\)-TF4 in Fig. 4), however, had no effect, and currents recorded with or without pre-depolarization were almost indistinguishable (7 ± 3%, \( n = 6 \)).

The voltage dependence of facilitation of these truncated subunits is shown in Fig. 5. Prepulse facilitation was observed at depolarizations up to 150 mV (Fig. 5A, E) and was more pronounced at +100 mV (Fig. 5E, F) at which the control current was maximal. The facilitation was not observed with truncated subunits, with the exception of \( \beta_2\)-TF2, which showed a significant (\( p < 0.05 \)) facilitation of 12% (\( n = 6 \; (n=6) \; \# \)) at +100 mV (Fig. 5E). The facilitation of the truncated subunits was not significantly different from that of the full-length \( \beta_2 \) subunit (Fig. 5E, F). The voltage dependence of facilitation at +100 mV was similar for the different subunits (Fig. 5E, F). The facilitation index (ratio of the current amplitude with pre-depolarization divided by the control current) increased with voltage for all subunits, with a steeper slope for the full-length \( \beta_2 \) subunit (Fig. 5E, F). The facilitation index at +100 mV was similar for the full-length \( \beta_2 \) subunit (Fig. 5E, F) and the truncated subunits (Fig. 5E, F). The facilitation index at +100 mV was similar for the full-length \( \beta_2 \) subunit (Fig. 5E, F) and the truncated subunits (Fig. 5E, F).
subunits was characterized by using depolarization in the range of +60 to +140 mV. Such depolarizations induced an increase in Ba\(^{2+}\) currents recorded during a subsequent test pulse for \(\alpha_{1C}\) subunit Ca\(^{2+}\) channels containing \(\beta_1, \beta_1\text{-TF1}, \beta_1\text{-TF3}, \text{or } \beta_2\text{-TF2}.\) As shown in Fig. 5 (A and B) for typical recordings, this increase rose with the amplitude of the conditioning depolarization, reaching a plateau for amplitude greater than +100 mV. The voltage dependence of this increase was similar for all these constructions (e fold changes for \(\beta_1, \beta_1\text{-TF1}, \beta_1\text{-TF3}, \text{and } \beta_2\text{-TF2 were } 45 \pm 12 \text{ mV } (n = 3), 23 \pm 15 \text{ mV } (n = 4), 52 \pm 26 \text{ mV } (n = 5), \text{and } 47 \pm 27 (n = 3), \text{respectively}.).\) Depolarization of the same amplitude was, however, without effect when the \(\alpha_{1C}\) subunit was expressed with either \(\beta_2\) or \(\beta_2\text{-TF4} \) (Fig. 5B). The induction of facilitation by deletions of the N-terminal tail (V1 + C1 domains) of the \(\beta_2\) subunit strongly suggests (i) the existence of a conserved facilitatory sequence in the C2 domain of the \(\beta\) subunit, able to induce facilitation by both the \(\beta_1\) and \(\beta_2\) subunits and (ii) the presence, in the full-length \(\beta_2\) subunit, of an inhibitory sequence located in the first 128 amino acids. To test this hypothesis more directly, we constructed four chimeras based on the N-terminal truncated \(\beta_1\) subunits \(\beta_1\text{-TF1}, \beta_1\text{-TF2, and } \beta_1\text{-TF3, which were all able to induce facilitation} \) (Fig. 3). The results are shown in Fig. 6. The average facilitation, recorded using the standard protocol, was 47 and 5% for the \(\beta_1\) and \(\beta_2\) subunits, respectively (Figs. 3, 4, and 6). In the first construct, the missing sequence of \(\beta_1\text{-TF3} \) (V1 + C1 + V2 domains) was replaced with the corresponding sequence of the \(\beta_2\) subunit (amino acids 1–212) (Fig. 2). Expression of this \(\beta_2/\beta_1\text{-CH3} \) chimera with the \(\alpha_{1C}\) subunit prevented the promotion of facilitation normally recorded with \(\beta_1\text{-TF3} \) (9 ± 1%, \(n = 3\) ) (Fig. 6). The lack of facilitation recorded with the \(\beta_2\) subunit can therefore be transferred to the \(\beta_1\) subunit by addition of these amino acids, which confirmed the existence of an inhibitory sequence. In \(\beta_2/\beta_1\text{-CH2, } \beta_2/\beta_1\text{-CH1, and } \beta_2/\beta_1\text{-CH4, the contribution of the } \beta_2 \text{ subunit to the total sequence of the chimera was further reduced. } \beta_2/\beta_1\text{-CH2 was deleted of an insert specifically found in the V2 domain of the } \beta_1 \text{ subunit (amino acids 170–212, black box in Fig. 6). } \beta_2/\beta_1\text{-CH1 and } \beta_2/\beta_1\text{-CH4 kept only the V1 + C1 and V1 sequences, respectively, from the } \beta_2 \text{ subunit, with the remaining sequence coming from the } \beta_1 \text{ subunit (Fig. 2). As shown in Fig. 6, none of these constructs was able to promote current facilitation in response to pre-depolarization. The extreme case is the } \beta_2/\beta_1\text{-CH4 chimera, where addition of the first 16 amino acids of } \beta_2 \text{ to } \beta_1 \text{ was able to completely block current facilitation } (4 \pm 5%, n = 10) \text{; not significantly different from the } \beta_2 \text{ subunit; see bottom of Fig. 6), suggesting that an inhibitory sequence was indeed present in these few amino acids.}

The primary sequence of this inhibitory segment of the \(\beta_2\) subunit is shown in Fig. 7 (top). The sequence is characterized by the presence of a short stretch of positively charged amino acids (boldface letters). To test the possible involvement of these charges in the inhibition of facilitation, we mutated these three arginines that are mutated to alanine in \(\beta_2/\beta_1\text{-CH4M123} \) in boldface. Middle, current traces recorded when the non-mutated (left) and mutated (right) \(\beta_2/\beta_1\text{-CH4 subunits were coexpressed with the } \alpha_{1C} \text{ subunit. Note that the replacement of the arginines allowed prepulse-induced facilitation. Facilitation is normally not observed with this chimera (scale bars } = 1 \mu\text{A). Bottom, average current facilitation recorded with } \beta_2/\beta_1\text{-CH4 and } \beta_2/\beta_1\text{-CH4M123. The prepulse lasted } 200 \text{ ms and had an amplitude of } +100 \text{ mV (test pulse of } 400 \text{ ms at } +10 \text{ mV).} \# \text{ significantly different from } \beta_2/\beta_1\text{-CH4.} \)
N-terminal nor C-terminal tail affected the response of these subunits to pre-depolarizations with the $\beta_1$ or $\beta_2$ subunit. Respective increases in Ba$^{2+}$ current recorded with the $\beta_1$ and $\beta_2$ subunits were 80 ± 30% ($n = 9$) and 11 ± 11% ($n = 11$) for $\alpha_{1C}\Delta N$, and 11 ± 3% ($n = 2$) for $\alpha_{1C}\Delta C$. We therefore conclude that these two sequences, despite their role in modulating current amplitude, were not directly involved in the regulation of $\alpha_1C$ subunit voltage-dependent facilitation.

**DISCUSSION**

Functional Diversity of Facilitation—Our results show that neuronal L-type Ca$^{2+}$ channel facilitation can be promoted or blocked by distinct short sequences of the ancillary $\beta$ subunit. The lack of effect of acute injection of okadaic acid, the insensitivity to incoming Ca$^{2+}$ ions (12), and the requirement for permissive $\beta_1\beta_3$, and $\beta_4$ subunits (13) clearly distinguished this type of facilitation from L-type facilitations previously characterized in cardiac, skeletal, and vascular cells (6–8, 14–18) and suggest that different underlying mechanisms are involved. The presence or absence of these different types of facilitation with related L-type Ca$^{2+}$ channels, such as, for example, the cardiac L-type Ca$^{2+}$ channel, may depend not only on the type of auxiliary subunit associated with the $\alpha$ subunit in a particular cell type, but also on specific kinase activities (6, 8, 17, 27) or expression of other associated proteins such as ryanodine receptors (for the L-type Ca$^{2+}$ channel) (28, 29) or syntaxine (for the N-type Ca$^{2+}$ channel) (30). All of these elements have been shown to be crucial for the normal development of specific forms of facilitation. Our results, however,
emphasize the importance of the auxiliary β subunits for neuronal L-type facilitation and can explain the diversity of the response of the same α1C subunit to conditioning depolarizations when recorded in cardiac and neuronal cells. Differentially spliced variants of the β2 subunit have been described in rat, rabbit, and human (23). At least three display important variations in their N-terminal V1 domains, suggesting that functional differences regarding L-type Ca\(^{2+}\) channel facilitation may exist. One consequence of the differential expression of these splicing variants could therefore be to finely tune the level of Ca\(^{2+}\) entry during sustained or repetitive depolarizations.

**Molecular Mechanism of Neuronal α1C Subunit Facilitation**—Although we have previously noted the importance of normal protein kinase A activity for facilitation (12), a mechanism involving voltage-dependent phosphorylation of the channel as a key step for the promotion of facilitation can be disregarded for the following reasons. First, we have demonstrated that ATP\(_S\), AMP-PCP, and okadaic acid cannot stabilize or prevent L-type Ca\(^{2+}\) channel facilitation (12). Second, facilitation can be recorded on expression of the β subunit, but not the β/β1-CH4 chimera, which retains the putative protein kinase A and C phosphorylation sites present in the β1 subunit (and of course in the α1C subunit). Third, cardiac L-type Ca\(^{2+}\) channels, which are highly sensitive to protein kinase A phosphorylation (31), are completely resistant to this type of voltage-dependent facilitation (13). Altogether, these data suggest that facilitation requires a phosphorylated α1C subunit, but do not support the existence of a voltage-dependent phosphorylation step, as described in skeletal muscle (7), in the pathway leading to current potentiation.

We have mapped two distinct regions in the sequence of the Ca\(^{2+}\) channel β subunit as critical for the promotion and inhibition of L-type Ca\(^{2+}\) channel facilitation (Fig. 9). The promoting sequence, localized in the C2 domain of β1, appears to be conserved on the four β subunits, as shown by the capability of β1 and β1 (12, 13) and β2-TF2 (this work) to promote facilitation. This domain contains the β subunit interaction domain identified by De Waard et al. (32) and shown to be responsible for the β subunit-induced regulation of Ca\(^{2+}\) channel properties. Facilitation is, however, the first property demonstrated to be carried out by the β subunit, although it appears to be specific to the α1C subunit. This suggests that the molecular consequences of β subunit binding are specific among the α1C subunits. Among possible molecular mechanisms of facilitation, we have excluded the involvement of a voltage-dependent block of the channel by the amino- and carboxyl-terminal tails of the α1C subunit, although their deletions have been shown to increase the L-type current amplitude (25). Another mechanism could be a prepulse-induced reactivation of inactivated channels since the same sequence that is shown here to block facilitation has already been shown to block voltage-dependent inactivation (33). Whether or not the three arginine residues that we have mutated and the chimeras used in this work also affect inactivation remains to be determined. Further experiments to test the effects of these on channel inactivation will be useful in clarifying this issue.

The inhibitory sequence is found only in the β2 subunit. The lack of current facilitation produced by coexpression of the β2/β1-CH4 chimera suggests that specific interactions with the first few amino acids of the β2 subunit are important for the mechanism by which facilitation is blocked. Furthermore, the abolition of the effect by the mutation of the three arginines located in this short sequence suggests that electrostatic inter-

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