Facilitation of L-type Calcium Current in Thalamic Neurons

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Kammermeier, Paul J. and Stephen W. Jones. Facilitation of L-type calcium current in thalamic neurons. J. Neurophysiol. 79: 410–417, 1998. We have studied facilitation of the L-type calcium current in neurons acutely isolated from the ventrobasal nucleus of the rat thalamus. Currents were recorded after pretreatment with 1 μM ω-conotoxin GVIA and 5 μM ω-conotoxin MVIIIC, to better isolate L-current. Long, strong depolarizations induced slow tail currents at negative voltages, but did not affect currents at voltages where channels were strongly activated. The initial peak tail current was not measurably increased. The time course of recovery from facilitation paralleled the time course of the tail current, indicating that facilitation does not outlast channel closing. The kinase inhibitors staurosporine and H-7 and the phosphatase inhibitor okadaic acid had no significant effect on L-current facilitation compared with control, but facilitation was greater with H-7 than with okadaic acid. The guanosine 5′-triphosphate (GTP) analogs GTP-γ-S and GDP-β-S did not affect facilitation. We conclude that L-current facilitation in thalamic neurons does not result from Ser/Thr phosphorylation, although phosphorylation may modulate facilitation. This form of facilitation differs kinetically and pharmacologically from facilitation induced by activation of G protein-coupled receptors.

INTRODUCTION

Ventralbasal thalamic relay neurons possess both low voltage-activated (LVA) calcium channels, thought to play an important role in bursting, and high-voltage-activated (HVA) channels (Hernandez-Cruz and Pape 1989). The HVA component is made up of about one-third L-type current and at least three other components (Kammermeier and Jones 1997). In the course of that study, we observed that long-lasting depolarizations to very positive voltages elicit a slow component of the subsequent tail current. The slow tail current results from activity of L-type calcium channels, as it is sensitive to Co2+ and to 5 μM nimodipine (Kammermeier and Jones 1997). Maintained calcium channel activity at negative voltages could lead to significant calcium influx, which is of particular interest given the proposed role of L-type calcium channels in neuronal signaling (Deisseroth et al. 1996; Tsien et al. 1988).

Many studies have reported facilitation of calcium currents by strong depolarization, but the underlying mechanisms remain controversial (Dolphin 1996a,b). One common mechanism, typically observed for N- and P/Q-type currents, involves a shift from the normal “willing” mode of gating, to a “reluctant” mode where the channels can still open and close, but longer or stronger depolarization is required to open a channel (Bean 1989; Jones and Elmslie 1997). That form of facilitation is observed during G protein activation and is thought to involve binding of G protein βγ subunits (Herlitze et al. 1996; Ikeda 1996), with facilitation reflecting dissociation of the βγ subunits from the channel.

Strong depolarization can also facilitate L-type calcium currents, generally by mechanisms not involving G protein activation (Artalejo et al. 1990; Pietrobon and Hess 1990; Sculptoreanu et al. 1993a,b). In some cases, the time course of facilitation and recovery was proposed to reflect a kinase-phosphatase cycle, with voltage- or state-dependent kinase activity (Artalejo et al. 1992; Sculptoreanu et al. 1993a,b). Because development of facilitation can be rapid (τ = 26 ms at 120 mV) (Artalejo et al. 1992), involvement of protein phosphorylation would be surprising, but close association of kinase and channel may allow unusually rapid phosphorylation (Johnson et al. 1994).

We describe here facilitation of L-current by strong depolarization in thalamic relay neurons, and characterize the voltage dependence, kinetics, and pharmacology of the effect. This form of facilitation does not increase the maximal number of open channels and requires neither G protein activation nor a kinase-phosphatase cycle.

METHODS

Cell isolation

Cells were acutely isolated from the ventrobasal thalamic nucleus of 7–14 day old rats (Kammermeier and Jones 1997) by a procedure based on Swartz and Bean (1992).

Solutions

The extracellular recording solution contained (in mM) 142 tetraethylammonium (TEA) Cl, 10 BaCl2, and 10 N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES), adjusted to pH 7.4 with TEA OH. The intracellular (pipette) solution contained 110 TEA Cl, 9 HEPES, 9 ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA), 4.5 MgCl2, 0.3 Li GTP, 14 creatine phosphate, and 4 tris(hydroxymethyl)aminomethane (Tris) ATP.

Drugs

ω-Conotoxin GVIA, was from Sigma (St. Louis, MO) and ω-conotoxin MVIIIC was from Research Biochemicals (Natick, MA). Stauroporine, H-7, and okadaic acid were from Calbiochem (San Diego, CA). Guanosine 5'-O-(3-thiotriphosphate) (GTP-γ-S) and guanosine 5'-O-(2-thiodiphosphate) (GDP-β-S) were from Boehringer Mannheim (Indianapolis, IN). Stauroporine and okadaic acid were dissolved in dimethyl sulfoxide (DMSO) as 1 mM stock solutions. H-7, GTP-γ-S, and GDP-β-S were dissolved in...
water and stored as 100 mM, 1 M, and 1 M stock solutions, respectively. For experiments with staurosporine, H-7, or okadaic acid, slices were preincubated in each drug for ≥1 h. The drug was also included in both extracellular and pipette solutions throughout the recording. For experiments with GTP-γ-S or GDP-β-S, these compounds were added to the pipette solution and only data recorded after ≥5 min were used, to allow time for the drugs to dialyze into the cell.

Electrophysiological recording and data analysis

We recorded 298 cells in the whole cell patch-clamp configuration, at room temperature (≈22°C). Pipettes were made from Corning 7052 glass (Garner Glass, Claremont, CA). Series resistances were 3–10 MΩ and were compensated 80% in all recordings. Data were recorded with an Axopatch 200 patch clamp amplifier (Axon Instruments, Foster City, CA), by using pClamp software and a TL-1 A/D converter (Axon Instruments). The holding potential for all experiments was −80 mV. Data were sampled at 1–50 kHz after Bessel filtering at one-fifth the sampling rate. All data were leak subtracted during analysis (P/4). Microsoft Excel was also used for data analysis and, where noted, curve fitting.

Isolation of L-current

About one-third of the total high-voltage–activated calcium current in isolated ventrobasal thalamic neurons is L-current, blocked by 5 μM nimodipine (Kammermeier and Jones 1997). However, 5 μM nimodipine blocked nearly all of the depolarization-induced slow tail current (88 ± 5%, mean ± SE) (Kammermeier and Jones 1997). To improve isolation of L-current during depolarizing steps, cells were preincubated in 2 μM ω-conotoxin GVIA and 10 μM ω-conotoxin MVIIC for ≥5 min before recording. After that preincubation and 14 ± 3 min of recording in the absence of toxins, 5 μM nimodipine blocked 76 ± 7% (n = 7) of the steady-state current at 0 mV (data not shown). All subsequent experiments were performed after preincubation with ω-conotoxin GVIA and ω-conotoxin MVIIC. Under our recording conditions (10 mM Ba2+), binding of ω-conotoxin GVIA should be nearly irreversible on the ~30 min time scale of our experiments (Boland et al. 1994). Reversibility of ω-conotoxin MVIIC is more difficult to evaluate, as its affinity for P and Q channels can vary (Hillyard et al. 1992; McDonough et al. 1996; Mintz et al. 1992; Randall and Tsien 1995). However, in these thalamic neurons, we found no detectable recovery from the combination of ω-conotoxin MVIIC and ω-conotoxin GVIA, at least in 2 mM Ba2+ (Kammermeier and Jones 1997).

RESULTS

Time and voltage dependence of facilitation

Long steps to positive voltages induce a slow L-type tail current in thalamic relay neurons (Kammermeier and Jones 1997). To characterize the kinetics of this effect, we first

![FIG. 1. Time and voltage dependence of facilitation. A: time course of development of facilitation at 4 voltages. Tail currents were recorded at −40 mV, after steps to indicated voltages. Because of variability in slow tail current amplitude from cell to cell, amplitude of slow component was normalized to that measured after a 300-ms step to 70 mV in each cell. In this and subsequent figures, error bars are ±SE, shown only when larger than symbol. At 0 mV, n = 5 for all points; at 40 mV, n = 18 at 2 ms and n = 9 at other times; at 70 mV, n = 18 at 2 ms, n = 10 from 202 to 802 ms, and n = 5 at other times; at 90 mV, n = 11 at 2 ms and n = 3 at other times. B: sample currents from one cell, recorded after steps lasting 2, 602, and 1,402 ms to 70 mV (top), 40 mV (middle), and 0 mV (bottom).]
examined the time course of development of the slow tail current at four voltages (90, 70, 40, and 0 mV) (Fig. 1). Each tail current was fitted to the sum of two exponentials and the amplitude of the slow component was normalized to the value for a 300-ms step to 70 mV. Averaged values are plotted versus duration of the depolarizing step in Fig. 1A and sample currents after steps to 70, 40, and 0 mV are shown in Fig. 1B. Facilitation was not observed at 0 mV, where currents during the depolarization inactivated slightly (14 ± 3% at 400 ms, n = 5; 34 ± 3% at 1,600 ms, n = 4). At more positive voltages, facilitation developed slowly, without obviously reaching a steady-state level even at 1.6 s. Longer or stronger depolarizations could not be used, as they tended to produce unacceptable increases in leakage current. However, it is clear that facilitation is stronger for larger depolarizations, at least up to 90 mV.

**Voltage dependence of facilitated tail currents**

To examine the effect of facilitation on tail current kinetics, we compared tail currents evoked by brief (18 ms) and long (416 ms) depolarizations to 70 mV (Fig. 2). At voltages where channels were normally closed in the steady state (−80 to −40 mV), the slow tail current decayed almost completely within 30 ms (Fig. 2A). At −20 mV, the facilitated current declined little over 30 ms, but tail currents evoked by the 18 ms and 416 ms depolarizations did eventually converge after hundreds of milliseconds (Fig. 2B). The time constant of the slow tail current showed a strong, nonexponential voltage dependence (Fig. 2C). When currents were measured during the slow tail, the effect of facilitation was visible as a shift of the current-voltage relationship to more negative voltages (Fig. 2D).

Above 0 mV, there was no evidence of facilitation (Fig. 2, A and D). This suggested that the effect of the facilitating step was to slow channel closing, because currents were unaffected at voltages where most channels did not open (shown by the lack of decay in the tail current at 0 and 20 mV, Fig. 2A). In other words, the facilitating step did not appear to increase the maximal open probability of the channels. Indeed close examination of tail currents confirmed this result (Fig. 3). Tail currents recorded at −40 mV, after 7-ms steps to 70 mV, were predominantly fast. After 307 ms steps to 70 mV however, there was considerable facilitation. When these tail currents were superimposed (Fig. 3), it can be seen that the peak tail current did not change, despite a considerable increase in the slow component of the tail. The ratio of facilitated to nonfacilitated tail current was 1.04 ± 0.03 at peak, and 2.41 ± 0.19 at 2–3 ms (n = 13).

**Test for persistence of facilitation**

Several mechanisms could produce maintained calcium channel activity at negative voltages. Facilitation might be
induction of a gating mode where channels open and close with altered kinetics, as proposed for G protein modulation (Bean 1989). A shift of channel activation to more negative voltages could explain the observed slower closing rate for facilitated channels (Fig. 2). That mechanism predicts that facilitation should outlast channel closing, if transitions between the normal and facilitated modes are slower than closed-open gating within a mode. An alternative hypothesis is that facilitation merely slows channel closing. If so, the channel should return to normal gating as soon as it closes.

The protocol illustrated in Fig. 4A was designed to test these alternative mechanisms. First, a prepulse to −20 mV was given, to inactivate LVA current and to measure L-current as the steady-state current at the end of the 100 ms step. Next a long (416 ms) step to 70 mV was given to induce facilitation. The cell was then partially repolarized to −60 mV for a variable duration, to measure the time course of channel closing at −60 mV. Finally, a test depolarization (postpulse) to −20 mV was given, to test for the presence of facilitation. The pre- and postpulse voltage of −20 mV was chosen because facilitation is clearly visible at that voltage, but decays only slowly (Fig. 2B), so if facilitation was present, it would be reflected in an increased peak current at −20 mV. Tail currents were examined at −60 mV rather than −40 mV, because there was a small steady-state current at −40 mV in some cells.

The decay of facilitation closely matched the time course of the tail current (Fig. 4B). After brief repolarization, where channels did not close completely, substantial facilitation was still visible at −20 mV (Postpulse/Pre-pulse = 1.7 ± 0.1, n = 4, after 2 ms at −60 mV). Facilitation was essentially absent, however, after 20 ms, in good agreement with the time course of the slow tail current at −60 mV (τ = 9.4 ± 0.7 ms, n = 8). We conclude that the time course of the slow tail current reflects recovery from facilitation.

**Phosphorylation**

We examined the effects of drugs that perturb phosphorylation and dephosphorylation on L-current facilitation in the thalamus. If facilitation were dependent on phosphorylation, as reported for L-channels in chromaffin cells and muscle, drugs that inhibit phosphorylation would be expected to disrupt facilitation. Cells were preincubated for ±1 h and were recorded in the continued presence of intracellular and extracellular staurosporine (1 μM), H-7 (100 μM), or okadaic acid (1 μM); at the concentrations used, staurosporine and H-7 should inhibit most serine/threonine protein kinases (Ruegg and Burgess 1989) and okadaic acid should inhibit protein phosphatases types 1 and 2A (Cohen et al. 1990). None of these treatments significantly affected the amplitude or decay rate of the slow tail current (Fig. 5; Table 1). There were also no effects on the absolute current level (or current density) during depolarizations to 0 mV (Table 1).

However there was a significant difference between slow tail currents in cells treated with H-7 and those exposed to okadaic acid, when the slow tail current was measured as the difference between tails after steps to 0 mV and 70 mV (Table 1). The direction of the effect was opposite from that expected if facilitation resulted from protein phosphorylation, with larger tail currents in H-7 than with okadaic acid. These results indicate that phosphorylation may affect facilitation, but demonstrate that facilitation is not likely to result from Ser/Thr phosphorylation.

**G proteins**

G protein activation inhibits N- and P/Q-type calcium currents in many neurons and the inhibition is partially reversed by strong depolarization (Hille 1994; Jones and Elmslie 1997; Kammermeier and Jones 1997). To test whether a similar mechanism was occurring in thalamic L-channels, or whether G protein activation was involved in any other way, we examined the ability of GTP analogs to influence L-current facilitation. When GTP was replaced in the pipet with either 100 μM GTP-γ-S or 1 mM GDP-β-S, neither current levels nor facilitation were affected (Table 1). This indicates that L-current facilitation is not dependent on G protein activation and is consistent with the notion that L-channels are not inhibited by activation of G proteins in thalamic neurons.

**DISCUSSION**

We have examined the kinetics and pharmacology of depolarization-induced facilitation of the L-type calcium current. In thalamic neurons, L-current facilitation does not increase the maximal number of open channels and does not produce a long-lived facilitated mode of gating, but simply slows channel closing. That could reflect either prolongation of the channel open time (i.e., slowing the microscopic channel closing rate), or channel reopenings.

![Figure 3](https://example.com/figure3.png)

**Fig. 3.** Facilitation does not change initial tail current amplitude. Currents were recorded after steps to 70 mV lasting 7 and 307 ms (*). Currents were sampled at 50 kHz after 10 kHz analog filtering, from a 9 pF cell, with a series resistance (before 80% compensation) of 6 MΩ.
Facilitation requires strong depolarization and develops slowly (>1 s). The resulting slow tail currents last several milliseconds even at voltages near the resting potential, potentially allowing maintained calcium influx after a period of maintained electrical activity. This form of calcium channel facilitation does not require G protein activation or protein phosphorylation.

There have been many previous reports of calcium channel facilitation (reviewed by Dolphin 1996a). We will consider several possible defining features of facilitation, including the biochemical mechanisms (e.g., involvement of phosphorylation or G proteins) and the kinetic mechanisms. Kinetic issues include the time and voltage dependence for facilitation on depolarization and for reversal of facilitation on repolarization. One key question is whether or not the facilitation outlasts channel closing. That determines whether or not facilitation is reflected solely in slower channel closing or in an increased current during a subsequent depolarization.

Our results differ in virtually all respects from the facilitation resulting from relief of G protein-mediated inhibition. Kinetically, the G protein-mediated effect develops much more rapidly (τ = ~5 ms at strongly depolarized voltages), occurs even for weak depolarizations (e.g., near 0 mV), and reverses more slowly (over tens of ms) (reviewed by Jones and Elmslie 1997). Reversal of G protein-mediated inhibition can slow tail currents, but the effect is small (Boland and Bean 1993; Elmslie et al. 1990). Instead, that form of facilitation is most clearly seen as an increase in current amplitude during a test pulse, after a strong but brief depolarization (Grassi and Lux 1989; Jones and Elmslie 1997). G protein-related
facilitation survives repolarization to −80 mV for several milliseconds, more than long enough for the channels to close. Pharmacologically, L-current facilitation in thalamic neurons is unaffected by GTP/GDP analogs and does not require activity of protein kinases or phosphatases (Fig. 5; Table 1). In contrast, effects mediated by G proteins are mimicked by GTP-γ-S, prevented by GDP-β-S (Schultz et al. 1990), and in some systems are inhibited by protein kinase activation (Swartz 1993; Zhu and Ikeda 1994). Finally the facilitation reported here is of L-current (Kammermeier and Jones 1997), whereas the G protein-mediated effects are usually specific for N and P/Q channels over L-channels (although L-channels can also be affected in some cases) (Dolphin 1996a). These results challenge the idea that different forms of calcium-channel facilitation share underlying similarities (Dolphin 1996a,b).

Our results also differ substantially from facilitation of calcium current in chromaffin cells. Artailejo et al. (1992) reported that facilitation results from voltage-dependent phosphorylation, because nonspecific kinase inhibitors or a protein phosphatase prevent facilitation and ATP-γ-S or okadaic acid makes facilitation irreversible. Similar results were reported in parasympathetic neurons (Scultoreanu et al. 1995). In many respects, the kinetics resemble G protein-related facilitation, as facilitation develops rapidly at strongly depolarized voltages (Artailejo et al. 1992), but repetitive depolarization to 0 mV is also effective (Artailejo et al. 1990, 1991) and reversal of facilitation is slow [stated as ~60 s (Artailejo et al. 1992), although the time course of reversal does not appear to have been examined in detail]. Indeed, activation of G protein-coupled receptors can inhibit N and/or P/Q channels in chromaffin cells, allowing depolarization-induced facilitation (Albillos et al. 1996; Currie and Fox 1996; Doupnik and Pun 1994). Thus it is not clear whether the original reports of facilitation in chromaffin cells (Fenwick et al. 1982; Hoshi et al. 1984) resulted from relief of tonic G protein activation or from “facilitation” L-current. The coexistence of these two pathways for facilitation, so similar in kinetics but so dramatically different in pharmacology, remains puzzling and controversial (Garcia and Carbone 1996).

L-current facilitation in thalamic neurons clearly differs from some reports of facilitation in cloned α1C L-channels (Bourinet et al. 1994; Cens et al. 1996; Eisfeld et al. 1996; Kleppisch et al. 1994), where the onset of facilitation is considerably faster, tail current slowing is not obvious and facilitation long outlasts channel closing. Test pulses can be facilitated even after 150 ms at −70 mV (Eisfeld et al. 1996). Bourinet et al. (1994) found that this form of facilitation is inhibited (60–80%) by protein kinase inhibitors, but the effect did not necessarily involve state-dependent phosphorylation, because ATP-γ-S and okadaic acid did not make facilitation irreversible. Others have questioned the role of phosphorylation in this form of facilitation (Eisfeld et al. 1996; Kleppisch et al. 1994).

In cerebellar granule cells (Forti and Petrobon 1993) and hippocampal pyramidal cells (Kavalali and Plummer 1994, 1996; see also Thibault et al. 1993), some L-channels facilitate in a manner that seems to differ significantly from “normal” L-channels. Those “anomalous gating” or “Lp” channels show prolonged activity even after relatively weak depolarizations, in contrast to the slow tails reported here for thalamic neurons. Kavalali et al. (1997) found that facilitation was not prevented by kinase inhibitors (including H7), although cAMP did enhance activity of both normal and Lp-type L-channels.

Several previous reports of L-channel facilitation found slow development of facilitation, requiring strong depolarization (greater than ±20 mV) and rapid loss of facilitation at negative voltages, similar to the results reported here. In some cases, facilitation did not clearly reach a maximal level, either with increasing duration or with increasing voltage (see Fig. 1A). Such facilitation was reported for cardiac (Petrobon and Hess 1990), skeletal (Fleig and Penner 1995, 1996; Johnson et al. 1997; Scultoreanu et al. 1993b), and smooth muscle (Nakayama and Brading 1993), neurons (Kavalali and Plummer 1996; Slesinger and Lansman 1996) and (in one study) cloned α1C L-channels (Scultoreanu et al. 1993a). There is strong evidence for the involvement of protein kinase A in some reports (Johnson et al. 1997; Scultoreanu et al. 1993a,b), but Fleig and Penner (1996) found only weak inhibition (~30%) by activators or inhibitors of protein kinase A.

We found that facilitation produced a transient left shift in the current-voltage relation, without affecting currents at more positive voltages (Fig. 2D). That agrees with

### Table 1. Pharmacology of L-current facilitation

<table>
<thead>
<tr>
<th>n</th>
<th>Current at 0 mV, nA</th>
<th>Current Density at 0 mV, pA/pF</th>
<th>Slow Tail Current Density, pA/pF</th>
<th>Slow Tail Current Amplitude, nA</th>
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<tbody>
<tr>
<td>11</td>
<td>−0.39 ± 0.05</td>
<td>19 ± 2</td>
<td>−0.25 ± 0.05</td>
<td>11 ± 2</td>
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<tr>
<td>9</td>
<td>−0.36 ± 0.04</td>
<td>20 ± 2</td>
<td>−0.34 ± 0.09</td>
<td>16 ± 3</td>
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<tr>
<td>8</td>
<td>−0.50 ± 0.05</td>
<td>28 ± 4</td>
<td>−0.36 ± 0.09*</td>
<td>18 ± 3*</td>
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<tr>
<td>7</td>
<td>−0.32 ± 0.03</td>
<td>17 ± 1</td>
<td>−0.22 ± 0.06</td>
<td>8 ± 1</td>
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<tr>
<td>7</td>
<td>−0.34 ± 0.03</td>
<td>17 ± 3</td>
<td>−0.21 ± 0.03</td>
<td>11 ± 2</td>
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<tr>
<td>100 μM staurospine</td>
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<tr>
<td>1 μM H-7</td>
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<tr>
<td>1 μM okadaic acid 6–7</td>
<td>−0.49 ± 0.04</td>
<td>25 ± 2</td>
<td>−0.14 ± 0.02</td>
<td>37 ± 8</td>
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<td>100 μM GTP-γ-S</td>
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<td>1 mM GDP-β-S</td>
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All values are mean ± SE; n is number of cells. Significance was defined as P = 0.05, by using a one-way analysis of variance (ANOVA or one-way ANOVA on ranks) and a multiple comparison test to determine significance (Sigma Stat.). Slow tail current was the current after a 250-ms step to 70 mV, minus the tail current after a 150-ms step to 0 mV, measured at −40 mV, 10–20 ms after repolarization. Slow tail current τ and slow tail amplitude were from 2 exponential fits to tail currents at −40 mV, after 250 ms steps to 70 mV. GTP-γ-S, guanosine 5′-O-(3-thiotriphosphate); GDP-β-S, guanosine 5′-O-(2-thiodiphosphate); *Significantly different from value in okadaic acid (one-way ANOVA, multiple comparison test, P = 0.05).
several previous studies (Fleig and Penner 1996; Nakayama and Brading 1993; Parri and Lansman 1996; Sculptoreanu et al. 1993a). One interpretation is that facilitation slows channel closing, without an increase in the maximal P(open) at positive voltages. That hypothesis predicts that the initial tail current amplitude, which reflects the channel open probability at the end of the preceding depolarization, should remain constant. Most previous studies were not able to address that issue directly, as fast tail currents were not resolved. One exception was Fleig and Penner (1995, 1996), who found that the tail currents did increase, in contrast to our result (Fig. 4), suggesting different mechanisms of facilitation.

McFarlane (1997) reported that long depolarizations slow tail currents, without an increase in initial tail current amplitude, in neurons of the squid giant fiber lobe. The degree of tail current slowing was less than in thalamic neurons and the onset of facilitation was somewhat faster, but overall the kinetics appear similar. Interestingly, the calcium current in squid neurons seems not to be an L-current, as it was blocked by @-agatoxin IVA but not nifedipine (McFarlane 1997). McDonough et al. (1997) recently reported that strong depolarizations also slow deactivation of P-current in cerebellar Purkinje neurons.

Our pharmacological results suggest that Ser/Thr protein kinase activity is not required for L-current facilitation in thalamic neurons. Perhaps facilitation reflects a conformational change in the L-channel, rather than a chemical modification. However the difference between cells treated with H-7 and okadac acid (Table 1, Fig. 5) suggests that phosphorylation may modulate facilitation. In summary it is clear that multiple mechanisms underlie facilitation in different systems. Our results in thalamic neurons illustrate one mechanism, where protein phosphorylation plays at most a secondary role in facilitation of the L-type calcium current.

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