

Depolarization-Induced Calcium Channel Facilitation in Rod Photoreceptors Is Independent of G Proteins and Phosphorylation

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Kourennyi, Dmitri E. and Steven Barnes. Depolarization-induced calcium channel facilitation in rod photoreceptors is independent of G proteins and phosphorylation. *J Neurophysiol* 84: 133–138, 2000. Depolarization-induced facilitation of L-type Ca channels in rod photoreceptors was investigated with nystatin-perforated and ruptured whole cell patch-clamp techniques in cells isolated from tiger salamander retina. Induction of facilitation was voltage dependent with a half-maximal effect seen at prepulse potentials near +31 mV. Reversal of facilitation was time dependent with fast ($\tau \approx 20$ ms) and slow ($\tau \approx 1$ s) components at –60 mV. Incubation of cells with pertussis toxin or intracellular administration of guanosine 5'-O-(3-thiotriphosphate) or guanosine 5'-O-(2-thiodiphosphate) had no effect on the degree to which facilitation could be evoked, implying the absence of a significant role for G proteins. Application of the phosphatase inhibitor okadaic acid or inclusion of ATP, to boost levels of phosphorylation, or inclusion of 5'adenylylimidophosphate or inhibitors of protein kinase in the pipette, to reduce levels of phosphorylation, had no effect on the development of facilitation, suggesting that phosphorylation has little or no role in this phenomenon. These results show that the L-type Ca channels in rod photoreceptors, which appear to be composed of α_{1F} -like subunits, undergo voltage-dependent facilitation in a manner that differs from some other L-type Ca channels which undergo facilitation via phosphorylation or through G-protein-mediated inhibition.

INTRODUCTION

Vertebrate rod photoreceptors express noninactivating, high-voltage-activated (HVA) calcium channels that are considered to be of the L-type due to their dihydropyridine sensitivity (Kourennyi et al. 1994). In cones, the L-type Ca channels have a distinct pharmacological profile (Wilkinson and Barnes 1996), most closely resembling that of α_{1D} -subunit-containing Ca channels (Williams et al. 1992), but this characterization has not been made as completely for rods. Antibodies directed against α_{1D} subunits labeled cones, not rods, in mammalian retina (Morgans 1999; Taylor and Morgans 1998), and it is probable that rod Ca channels are composed of the newly identified α_{1F} subunits (Bech-Hansen et al. 1998; Strom et al. 1998). Calcium entry into rod photoreceptor terminals via noninactivating L-type Ca channels provides for the continuous release of the neurotransmitter glutamate in darkness, and this release is graded by light over a narrow range of hyperpolarization.

Depolarization-induced facilitation of L-type calcium chan-

nels was first described by Fenwick et al. (1982) in bovine chromaffin cells. Numerous studies since then have indicated, albeit not without controversy, that it is the voltage-dependent phosphorylation of the channels by protein kinase A (PKA) that accounts for the phenomenon in chromaffin cells (Artalejo et al. 1990, 1992; but see Albillos et al. 1996; Doupnik and Pun 1994), skeletal muscle (Sculptoreanu et al. 1993b), cardiac muscle cells (Schouten and Morad 1989; Sculptoreanu et al. 1993a; Tiaho et al. 1994; but see Foley and Pelzer 1994), and neurons (Bourinet et al. 1994; but see Parri and Lansman 1996) but not in cloned smooth muscle cells (Kleppisch et al. 1994). In many cases, the increase in peak Ca channel current is accompanied by a negative shift in channel activation. For some of these L channels, it remains an open question as to whether the channel itself or an intermediate G protein is a target for the protein kinase (see, for example, Dolphin 1996a,b; García and Carbone 1996). It has also been suggested that intracellular calcium participates in facilitation either in a phosphorylation-independent manner (Bates and Gurney 1993) or via a calcium-calmodulin-dependent kinase pathway (Anderson et al. 1994; Gurney et al. 1989).

Some neuronal Ca channels, in particular the N and P/Q subtypes, undergo voltage-dependent facilitation that is mediated by direct interactions between $\beta\gamma$ -subunits of GTP-binding proteins (G proteins) and the Ca channel (see Hille 1994; Zamponi and Snutch 1998). G-protein subunits inhibit these Ca channels and may be driven off by large depolarizations, accounting for the facilitation. Cone photoreceptor Ca channels undergo reversible block by ω -conotoxin GVIA, invoking properties of N-type Ca channels, but considered as well to be a property of the incompletely characterized α_{1D} -containing Ca channel. In addition to conotoxin sensitivity, do photoreceptor L-type Ca channels also share features of G-protein-mediated facilitation with N-type channels?

Recently Kammermeier and Jones (1998) described facilitation in L-type channels of thalamic neurons that is independent of G proteins and phosphorylation. Similar to suggestions that direct conformational changes in the Ca channel protein underlie the mechanism for the voltage-dependent facilitation in smooth muscle L-type Ca channels (Kleppisch et al. 1994), these authors concluded that no chemical modification was necessary to produce the facilitated state of the channels.

Since the rod photoreceptor Ca channel has not been phar-

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macologically characterized and may be composed of novel α_{1F} subunits, we sought to characterize features of the depolarization-induced facilitation of this L-type Ca channel. The results presented below show that it is unlikely that the facilitation mechanism involves phosphorylation or G proteins, suggesting that facilitation is an intrinsic property of this Ca channel subtype.

METHODS

Larval tiger salamanders, *Ambystoma tigrinum*, were purchased from Kons Scientific (Germantown, WI). The animals were stored at 4°C and then decapitated and the head hemisected or pithed. Both eyes were removed and placed in Ringer solution containing (in mM): 90 NaCl, 2.5 KCl, 3 CaCl₂, 15 HEPES 15, and 10 D-glucose (pH 7.6). After dissection of the cornea, iris, and lens, the retina was peeled gently from the eyecup and mechanically triturated. The dissociated cells were allowed to settle in a 0.25-ml recording chamber. Whole cell recordings were obtained from the inner segment of intact rod photoreceptors using both ruptured- and perforated-patch techniques. All experiments were performed under room light and microscope illumination (1–2 Wm⁻²) and at room temperature (20–23°C).

Silicone-elastomer (Sylgard)-coated fire-polished patch pipettes were pulled on a Kopf puller (model 730, Tujunga, CA) from hematocrit glass tubes (VWR, West Chester, PA) and filled with a solution containing (in mM): 95 CsCl, 3 MgCl₂, 10 HEPES, and 1 EGTA (pH 7.2). Drugs were included in the pipette solution, during rupture-patch recording, or added to the superfusing solution. For perforated-patch recordings, nystatin was dissolved in DMSO and included in the pipette solution (150 µg/ml). Three superfusing solutions were modified from the Ringer solution described in the preceding text to contain (in mM): 5 BaCl₂, 50 TEACl, and 15 CsCl; 10 BaCl₂, 50 TEACl, or 10 CsCl; or 10 BaCl₂, 25 TEACl, and 10 CsCl; where for each solution, BaCl₂ replaced CaCl₂ in an equimolar manner and TEACl and CsCl replaced equimolar NaCl. The concentration of HEPES was increased to 20 mM. In each series of experiments used for statistical analysis, the ionic conditions were identical. 1-(5-isoquinolinesulfonyl)-2-methylpiperazine (Iso-H-7), 5'-adenylylimidophosphate (AMP-PNP), guanosine 5'-O-(3-thiotriphosphate) (GTP-γ-S), guanosine 5'-O-(2-thiodiphosphate) (GDP-β-S), adenosine 5'-O-(2-thiodiphosphate) (ADP-β-S), and ATP were obtained from Sigma (St. Louis, MO). Okadaic acid was obtained from LC Services Corp. (Woburn, MA) and RBI (Natick, MA). Bay K 8644 was obtained from ICN Biochemicals (Cleveland, OH). PKI 5–24 was obtained from Peninsula Laboratories, (Belmont, CA).

Currents were recorded using an Axopatch-1B amplifier, TL-1 interface, and BASIC-FASTLAB software running on a 386 computer. Facilitation was induced by a depolarizing prepulse to 100 or 120 mV for 50 ms. Membrane currents were averaged over the last 2–5 ms of 25-ms voltage steps from a holding potential of -60 or -70 mV. We measured currents near the end of the test pulse to minimize possible current artifacts induced by the capacitive discharge following the large facilitation-inducing steps to +100 or +120 mV. However, since facilitation is removed at negative potentials with a bi-exponential time course having fast ($\tau \approx 20$ ms) and slow ($\tau \approx 1$ s) components, it is likely that such measurement near the end of the test pulse underestimates the degree to which facilitation occurred since some of the effect would have decayed during the first 20–23 ms of the test pulse. Activation curves were constructed from series resistance and leak corrected *I-V* relationships after division by the driving force. Leak correction was performed by subtracting a line fit to the *I-V* relation near -60 mV (typically from -70 to -50 mV). Activation curves were then constructed by dividing the *I-V* relation by a line fit to the relatively linear portion of the *I-V* relation just positive to 0 mV. This approach treats the voltage range over which

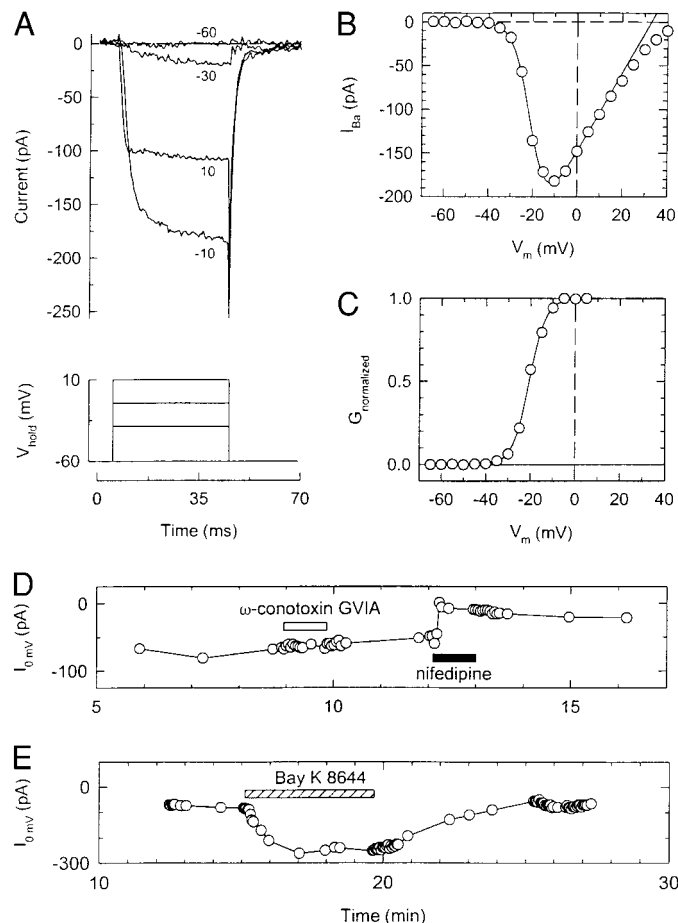


FIG. 1. Rod photoreceptor L-type calcium channel current. A: Ca channel current in a rod photoreceptor recorded using nystatin perforated-patch whole cell technique with Ba²⁺ as a charge carrier. Test step potentials are shown in millivolts near each trace. Holding potential was -60 mV. Leak and capacitance currents were digitally subtracted from the records. Leak current was estimated from linear fitting to the *I-V* relation between -65 and -45 mV. B: *I-V* relationship for the cell shown in A. —, product of the Boltzmann fit of the activation curve (shown in C) and a linear driving force reversing at +32 mV with $g_{\max} = 4.51$ pS. C: activation curve for Ca channel currents recorded in the rod shown in A with $V_{0.5} = -20.6$ mV and $s = 3.7$ mV. D: effect of calcium channel blockers. In a different cell, the N-channel blocker ω -conotoxin GVIA (0.5 µM, □) exerted little effect on Ca channel current elicited by steps to 0 mV, while subsequent application of the L-channel blocker nifedipine (10 µM, solid box) suppressed the current substantially. E: Ca channel current at 0 mV was increased by the application of Bay K 8644 (1 µM, ▨) in another cell. In these experiments, the superfusing solution contained (in mM) 5 BaCl₂, 50 TEACl, and 15 CsCl.

Ca channels activate (usually between -30 and 0 mV) as if there were a linear driving force, a prudent approximation that yields a valid description of channel activation, but one that assigns an artifactual reversal potential substantially more negative than the values observed or expected for nonlinear, fully-activated Ca channel currents (see Fig. 1B for example). The Boltzmann equation ($g = g_{\max} / [1 + \exp[(V_{0.5} - V)/s]]$) was used to fit activation curves, where g is a conductance due to calcium channels estimated from the slope of the fully activated, linear part of the *I-V* relationship, $V_{0.5}$ is the half-activation potential, and s is the slope factor. Statistical data are presented as means \pm SE. The absence of error bars in some graphs indicates that either only one cell was viable at the time of recording or that the error bars are smaller than the symbols. Statistical significance of facilitation was estimated using paired one-tailed Student's *t*-test unless otherwise indicated.

RESULTS

L-type calcium channels are present in photoreceptors

Figure 1 shows typical rod photoreceptor Ca channel barium currents recorded using perforated-patch whole cell techniques. Ca channel currents were activated at potentials positive to -40 mV and reached a maximum amplitude at about -10 mV. Application of nifedipine, a blocker of L-type channels, suppressed the Ca channel current (Fig. 1D), while Bay K 8644, an L-type channel agonist, caused a dramatic increase in current magnitude (Fig. 1E). The blocker of N-type calcium channels, ω -conotoxin GVIA, generally produced a slight decrease in Ca channel current, although in some cells no effect was seen (Fig. 1D). These data, taken together with the non-inactivating nature and high activation threshold of Ca channel current, indicate that rods express an L-type calcium channel. It should be noted that these properties of calcium channels in rod photoreceptors appear generally similar to those in cone photoreceptors of this same species, although the block by ω -conotoxin GVIA is greater in cones (Wilkinson and Barnes 1996).

Facilitation of Ca channel current

Ca channel current could be increased, or facilitated, by application of a strong depolarizing voltage prepulse (Fig. 2). The average increase in the maximum conductance after a prepulse to 100 mV was $22.7 \pm 5.9\%$ ($n = 16$, $0.001 < P < 0.01$) in perforated-patch recordings and $21.0 \pm 4.6\%$ ($n = 13$, $P < 0.001$) in ruptured-patch recordings. No significant change in the half activation potential was observed in either case: $V_{0.5}$ was 0.60 ± 0.46 mV ($n = 16$, $0.2 < P < 0.3$) in perforated-patch recordings and 0.61 ± 0.66 mV ($n = 13$, $0.3 < P < 0.4$) in ruptured-patch recordings. The slope factor also did not change significantly (increase of $2.3 \pm 1.9\%$, $0.2 < P < 0.3$ and $7.0 \pm 2.5\%$, $0.01 < P < 0.02$, respectively). We also calculated the percentage increase in peak Ca channel current, measured at a potential usually between -20 and 0 mV, to be

$35.4 \pm 5.6\%$ ($n = 30$, $P < 0.0005$) in perforated-patch and $41.5 \pm 13.8\%$ ($n = 7$, $0.01 < P < 0.025$) in ruptured-patch recordings.

Facilitation is voltage dependent

The extent to which Ca channel current was facilitated depended on conditioning depolarization. Such a dependence could be described well by a Boltzmann function (Fig. 3A). In three cells recorded from with the permeabilized patch technique, the prepulse amplitude causing 50% of the maximum facilitation amount was $+30.5 \pm 16.3$ mV and the slope factor was $+26.5 \pm 6.0$ mV.

Time-dependent removal of facilitation

The facilitation of Ca channel current was gradually eliminated with an increase in the gap period between a prepulse and a test pulse (Fig. 3B). In the same cells, described in the preceding text, in which the voltage-dependence of facilitation was established, the time-dependent process appeared to be double exponential with time constants of 22 ± 6 and 835 ± 400 ms ($n = 3$). Note the large standard error in some of these values.

L-type calcium channels are facilitated

Application of the nonselective calcium channel blocker, cadmium, eliminated Ca channel current and the facilitated component to an extent that quantitative analysis was impossible (Fig. 4A). The L-type channel blocker nifedipine ($1 \mu\text{M}$) suppressed Ca channel current dramatically and made facilitation less significant than in control (Ca channel current was increased after the prepulse by $34.9 \pm 15.5\%$, $n = 5$, $0.05 < P < 0.1$, see Fig. 4B). The L-type channel agonist Bay K 8644 ($0.5\text{--}1 \mu\text{M}$) increased Ca channel current substantially and did not affect the degree of facilitation ($21.9 \pm 4.2\%$, $n = 8$, $0.001 < P < 0.005$, see Fig. 4C). The effects of ω -conotoxin

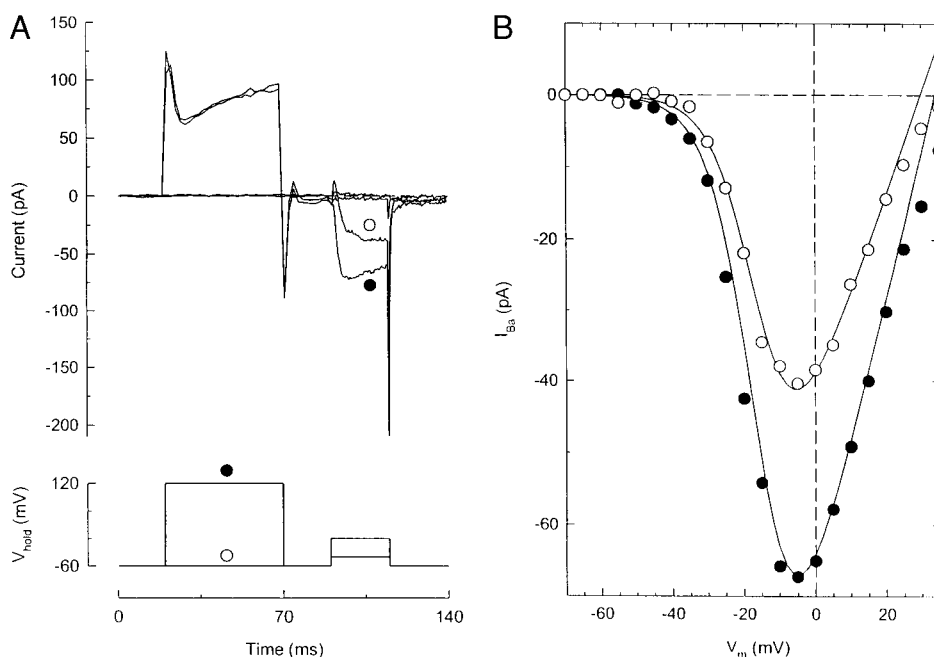


FIG. 2. Depolarization-induced facilitation of rod calcium channels. A: example of Ca channel current recorded using the perforated-patch configuration at -40 and at 0 mV. Ca channel current increased in amplitude when a voltage prepulse to $+120$ mV preceded the test step. B: I - V relations of Ca channel current without (\circ , $g_{\max} = 1.41$ pS, $V_{0.5} = 14.9$ mV, $s = 6.1$ mV) and with (\bullet , $g_{\max} = 2.05$ pS, $V_{0.5} = 15.2$ mV, $s = 6.1$ mV) the prepulse. Leak current was subtracted after estimation as a linear fit to the I - V relation between -70 and -50 mV. —, fits to the I - V relations for the control and facilitated currents, which were calculated as the product of the Boltzmann fit of the activation curve (not shown) and a linear driving force reversing at $+30$ and $+34$ mV, respectively. The cell shown was superfused with the 10 mM BaCl_2 , 25 mM TEACl, 10 CsCl containing Ringer solution.

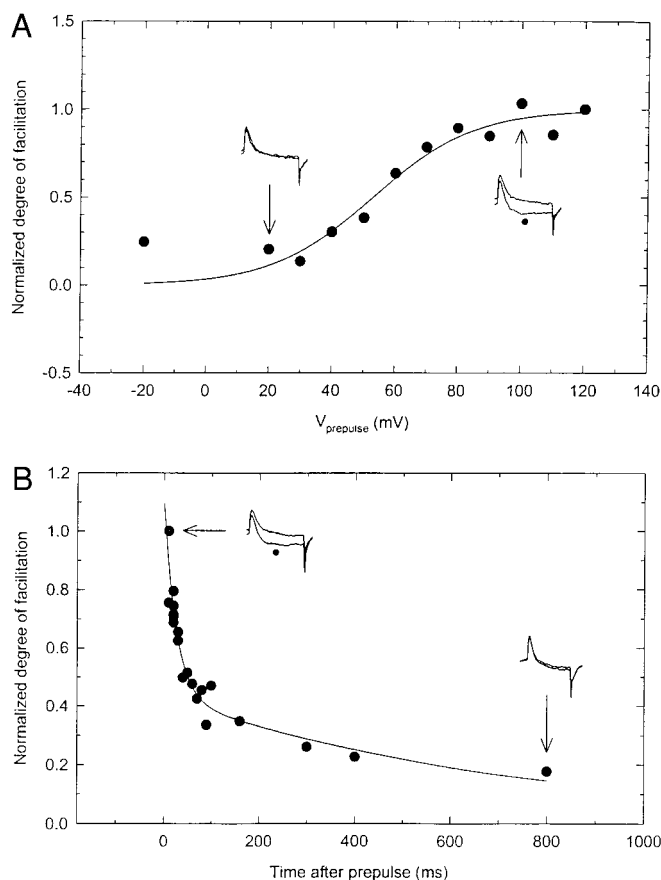


FIG. 3. Voltage and time dependence of Ca channel facilitation. *A*: the degree of facilitation produced in a rod as a function of the prepulse voltage. The data were fit to a Boltzmann function having a voltage for half-maximal effect at +51 mV and a slope factor of 12 mV (—). *B*: the degree of facilitation as a function of gap duration between the prepulse and the test pulse in the same rod. The data were fit to a double-exponential function with time constants of 61 and 1,024 ms having relative contributions of 0.62 and 0.38, respectively (—). In both *A* and *B*, the degree of facilitation was evaluated as $(I_f - I_c)/I_c$, where I_f and I_c are the amplitudes of Ca channel current after leak subtraction with and without the prepulse, respectively. This value was then normalized to the maximum value obtained in the series. *Insets*: examples of the raw current recorded at the voltages or times indicated (\downarrow , \leftarrow). The recordings shown were performed with perforated-patch techniques, and the rod was superfused with the 10 mM BaCl₂, 25 mM TEACl, 10 CsCl containing Ringer solution.

GVIA were minor on both the Ca channel current and the degree of facilitation ($39.1 \pm 12.2\%$, $n = 4$, $0.02 < P < 0.05$, see Fig. 4*D*). These results show that it was L-type calcium channels that were facilitated in rod photoreceptors by depolarization.

G proteins are not involved

One well-defined intracellular mechanisms by which calcium channels can be facilitated in a voltage-dependent manner is through the unbinding of G proteins from the channels. While this mechanism has been demonstrated most clearly in the case of N-type Ca channels (for reviews, see Hille 1994; Zamponi and Snutch 1998), we sought to test a role for G proteins in the facilitation of rod L channels.

As described in the preceding text, facilitation occurred equally with perforated- and ruptured-patch recordings, casting doubt on whether any soluble messengers have a role in this

phenomenon (Fig. 5). However, many intracellular processes, in particular some involving G proteins and phosphorylation, are membrane delimited. Therefore we used pertussis toxin (PTX) to block membrane delimited PTX-sensitive G proteins. Figure 5 shows that facilitation still occurred in rods dissociated from retinas pretreated with 5 μ g/ml PTX for 4 h. On average, with perforated-patch recordings from cells treated with 0.3 μ g/ml PTX for 1–2 days, 3 μ g/ml PTX overnight, or 5 μ g/ml PTX for 4 h, Ca channel current was increased after the prepulse by $41.2 \pm 8.0\%$ ($n = 12$, $P < 0.0005$).

The failure of PTX to block facilitation suggested that this effect was independent of PTX-sensitive G proteins. To further address the issue of the G-protein involvement in facilitation, we activated G proteins with 1 mM GTP- γ -S or locked G proteins in their inactive form using 2 mM GDP- β -S in the pipette solution. In both cases, the facilitation persisted, Ca channel current being increased by $23.8 \pm 1.5\%$ ($n = 2$, $0.01 < P < 0.025$) and $37.8 \pm 14.6\%$ ($n = 6$, $0.01 < P < 0.025$), respectively, suggesting a G-protein-independent mechanism (Fig. 5). The amount of facilitation seen with GTP- γ -S in the pipette was not significantly different from control ($0.8 < P < 0.9$; Student's unpaired *t*-test).

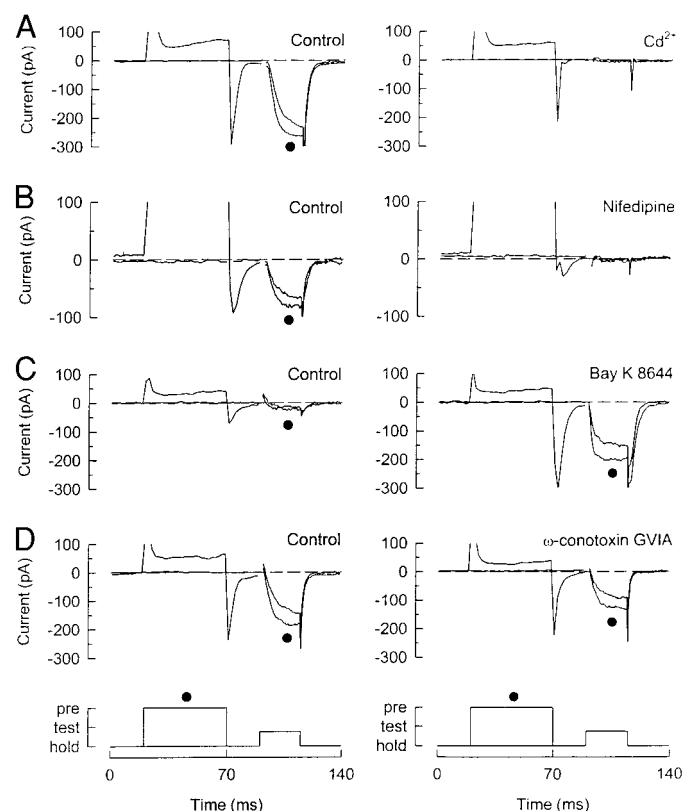


FIG. 4. L-type calcium channels are facilitated. *A*: Cd²⁺ (20 μ M) reduced Ca channel current and the facilitation effect. *B*: L-type channel blocker nifedipine (1 μ M) reduced Ca channel current and the facilitation effect. *C*: L-type channel activator Bay K 8644 (3.5 μ M) increased Ca channel current and revealed facilitation as seen from Ca channel current amplitude and tail current amplitude and duration. *D*: N-channel blocker ω -conotoxin GVIA (0.5 μ M) slightly suppressed Ca channel current and did not affect its facilitation. All data are from perforated-patch recordings. $V_{\text{hold}} = 60$ mV, $V_{\text{prepulse}} = 100$ mV, $V_{\text{test}} = -20$ mV (*A*, *C*, and *D*) or 0 mV (*B*). The rods shown in this figure were recorded from in the 10 mM BaCl₂, 25 mM TEACl, 10 CsCl containing Ringer solution.

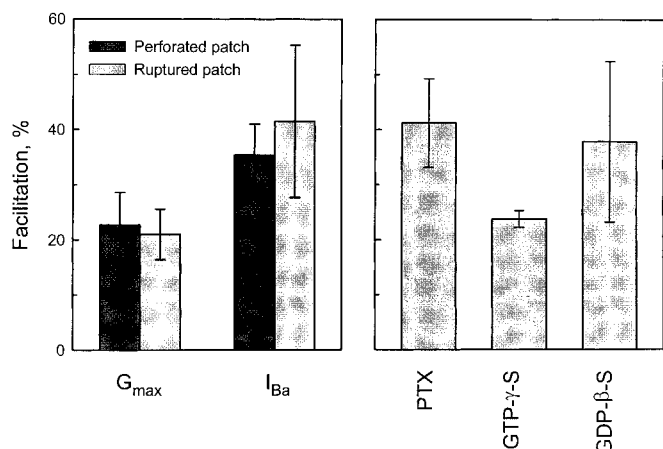


FIG. 5. Facilitation persists in the absence of G-protein modulation. *Left*: control levels of facilitation for ruptured- ($n = 16$) and perforated-patch ($n = 13$) recording conditions, expressed in terms of a change in maximum conductance (G_{max}) and peak Ca channel current (I_{Ba}). Average values and SE are given in the text. *Right*: Ca channel current still facilitates after treatment with pertussis toxin (PTX; $0.3\text{--}5\text{ }\mu\text{g/ml}$, $4\text{--}48\text{ h}$, $n = 12$, see text for details), 1 mM guanosine 5'-O-(3-thiotriphosphate) (GTP- γ -S; $n = 2$), or 2 mM guanosine 5'-O-(2-thiodiphosphate) (GDP- β -S; $n = 6$). In this series of experiments, the 5 mM BaCl_2 , 50 mM TEACl, 15 CsCl superfusion solution was used.

Phosphorylation is not involved

Phosphorylation-dependent facilitation has been shown for L-type calcium channels in several preparations (Artalejo et al. 1990, 1992; but see Albillos et al. 1996; Doupnik and Pun 1994), skeletal muscle (Sculptoreanu et al. 1993b), cardiac muscle cells (Schouten and Morad 1989; Sculptoreanu et al. 1993a; Tiaho et al. 1994; but see Foley and Pelzer 1994), and neurons (Bourinet et al. 1994). We checked this possibility in rod photoreceptors with special interest since earlier work had shown that another type of rod ion channel, a voltage-gated potassium channel, was regulated by phosphorylation (Kurenyi and Barnes 1997).

Figure 6 summarizes the results of a battery of tests designed to define the role of phosphorylation. When phosphorylation was favored and dephosphorylation suppressed by extracellular application of $0.5\text{--}1\text{ }\mu\text{M}$ okadaic acid in perforated-patch recordings, depolarizations still increased the maximum conductance reversibly by $33.6 \pm 11.4\%$ ($n = 8$, $0.02 < P < 0.025$), an amount statistically indistinguishable from that in control ($0.3 < P < 0.4$, unpaired t -test). There was no significant shift in the half activation potential ($V_{0.5} = 0.11 \pm 0.70\text{ mV}$, $0.8 < P < 0.9$). In ruptured-patch mode, the inclusion of $1\text{ }\mu\text{M}$ okadaic acid together with 1.8 mM ATP in the pipette ($n = 3$) also resulted in facilitation similar to that seen during control ruptured-patch experiments (maximum conductance increase of $27.7 \pm 5.6\%$, $0.02 < P < 0.05$; $V_{0.5} = 0.21 \pm 0.28\text{ mV}$, $0.5 < P < 0.6$).

An additional approach taken to address the problem was to suppress phosphorylation. We included in the patch pipette either $100\text{ }\mu\text{M}$ H-7 (a concentration that would likely block both cyclic nucleotide dependent kinases and PKC, $n = 2$), $1\text{ }\mu\text{M}$ PKI 5-24 (PKA inhibitor) with ($n = 4$) and without 1 mM ATP ($n = 3$), 0.5 mM AMP-PNP (nonmetabolizable analog of ATP, $n = 14$), or 2 mM ADP- β -S (to suppress adenylyl cyclase, $n = 2$) in ruptured-patch experiments. In all cases, facilitation persisted at the control level (the maximum con-

ductance increased by 47.1 ± 39.5 , 24.7 ± 9.2 , 25.6 ± 5.4 , 43.7 ± 9.8 , and $28.9 \pm 13.8\%$, respectively).

DISCUSSION

We interpret these findings to indicate that neither G-protein-coupled nor phosphorylation-dependent mechanisms are involved in voltage-dependent facilitation of L-type calcium channels in rod photoreceptors. It remains a possibility that G-protein- or phosphorylation-dependent mechanisms, unaffected by the drugs used presently, mediate aspects of the process of facilitation or its removal. Since none of our manipulations of the intracellular milieu affected facilitation, we further doubt a role for a soluble messenger system. We suggest that the likely mechanism responsible for facilitation may be via a direct voltage-dependent conformational change of the channels, similar to that suggested for L-channels in smooth muscle and thalamic neurons (Kammermeier and Jones 1998; Kleppisch et al. 1994).

L-type calcium channels are represented by a number of different α_1 subunits: α_{1S} (skeletal muscle), α_{1C} (cardiac, smooth muscle, brain), α_{1D} (kidney, brain) (reviewed in Dunlap et al. 1995), and α_{1F} (rod photoreceptors) (Bech-Hansen et al. 1998; Strom et al. 1998). Calcium channels in both salamander cones (Wilkinson and Barnes 1996) and rods (present study) share a similar pharmacological profile, e.g., sensitivity to both dihydropyridines and ω -conotoxin GVIA, with α_{1D} L-type Ca channels, suggesting that this may be characteristic of α_{1F} Ca channels. The voltage-dependent facilitation of putative α_{1D} channels in cerebellar granule cells (Parri and Lansman 1996) has properties consistent with those of rod photoreceptors.

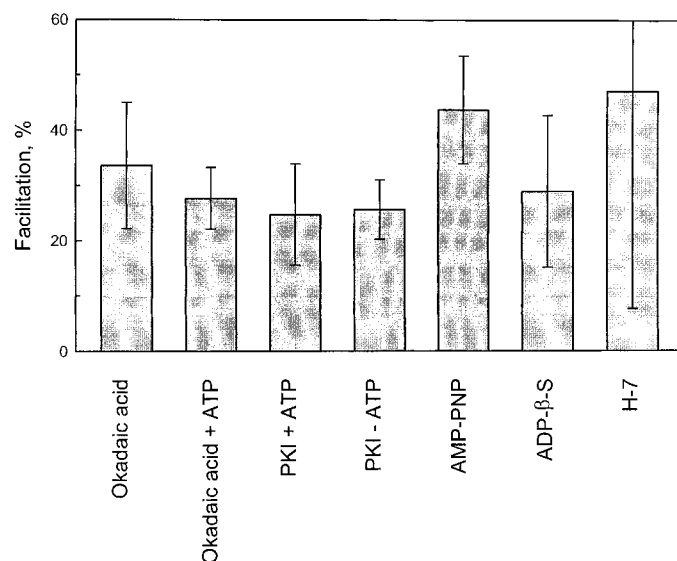


FIG. 6. Favoring or suppressing conditions suitable for protein phosphorylation do not affect facilitation. Okadaic acid ($0.5\text{--}1\text{ }\mu\text{M}$; $n = 8$) by itself or okadaic acid ($1\text{ }\mu\text{M}$) together with 1.8 mM ATP in the pipette ($n = 3$) led to no change in the level of facilitation of maximum Ca channel conductance. The specific protein kinase A (PKA) inhibitor, PKI 5-24 ($1\text{ }\mu\text{M}$), applied with ($n = 4$) and without 1 mM ATP ($n = 3$) did not change facilitation. 1-(5-isoquinolylsulfonyl)-2-methylpiperazine (Iso-H-7), 5'-adenylylimidophosphate (AMP-PNP; 0.5 mM ; $n = 14$), adenosine 5'-O-(2-thiodiphosphate) (ADP- β -S; 2 mM ; $n = 2$), and the broad spectrum protein kinase inhibitor 1-(5-isoquinolylsulfonyl)-2-methylpiperazine (H-7) ($100\text{ }\mu\text{M}$; $n = 2$) also failed to alter facilitation. In this series of experiments, the 10 mM BaCl_2 , 25 mM TEACl, 10 mM CsCl superfusion solution was used.

The molecular structures responsible for G-protein-mediated, voltage-dependent facilitation of α_{1A} and α_{1B} channels (P/Q and N types, respectively), as well as some α_{1E} channels (possibly R type) offer speculations into the problem of G-protein-independent facilitation. For these channel types, the domain I-II cytoplasmic linker has been identified as the protein region responsible for binding G-protein subunits (reviewed in Zamponi and Snutch 1998). Furthermore binding of syntaxin to synaptic N-type channels modulates G-protein-dependent facilitation (Stanley and Mirotznik 1997). It may be expected that analogous cytoplasmic regions of the G-protein-independent channels, such as those in rods, impart equivalent channel modulatory function in the absence of cytoplasmic molecules such as G proteins or syntaxin, in a manner independent of protein phosphorylation.

The molecular mechanisms responsible for L-channel facilitation in rod photoreceptors remain unclear, and a solution to this problem will likely require combined electrophysiological, biochemical, and molecular biological approaches. The present results offer evidence that the L-type Ca channels in photoreceptors are unique with respect to the facilitatory properties of α_{1A} -, α_{1B} -, α_{1C} -, and α_{1E} -like Ca channels. This finding furthers the functional distinctions being drawn between the classes of L-type Ca channels (e.g., α_{1F} L-type Ca channels as compared with α_{1C}).

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