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Topical Review

Transmitter Modulation of Neuronal Calcium Channels

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Introduction

Calcium channels play many roles in neurons, but their most crucial function is in excitation-secretion coupling (Hille, 1992). An action potential arriving at a nerve terminal triggers release of neurotransmitter within a millisecond, depending critically on highly localized influx of Ca^{2+} through voltage-dependent calcium channels. The release of neurotransmitter depends on Ca^{2+} in a cooperative manner, so small changes in Ca^{2+} entry can have large effects on release. Thus, anything that affects the activity of calcium channels will powerfully affect synaptic transmission.

Rapid synaptic potentials are produced by neurotransmitters that directly open ligand-gated ion channels, but most neurotransmitters act more slowly, via G protein-coupled receptors, producing a variety of effects in the postsynaptic cell. Electrophysiological effects may be mediated by second messenger systems and protein phosphorylation (Levitan, 1994), or by direct effects of G proteins on ion channels. It is now well established that neurotransmitters can modulate the activity of voltage-dependent channels, but that was a new idea when early studies reported inhibition of neuronal calcium channels by neurotransmitters (Dunlap & Fischbach, 1981; Galvan & Adams, 1982). That effect immediately became a candidate mechanism for presynaptic inhibition, the action of neurotransmitters to inhibit further neurotransmitter release at the same (or other) synapses.

The predominant effect of neurotransmitters on neuronal calcium channels is inhibitory (for general reviews, *see* Anwyl, 1991; Dolphin, 1991, 1995; Hille, 1994). In most but not all cases, the effect is associated with slower rates of channel activation, and a shift of activation to more positive voltages (Bean, 1989; Grassi & Lux, 1989; Elmslie et al., 1990). It is striking that the inhibition is incomplete, with effects at maximal transmitter concentration varying greatly, typically 20% to 90% inhibition. Initially, that was explained by selective block of one type of calcium channel, specifically a rapidly inactivating N-type calcium channel (Wanke et al., 1987; Gross & Macdonald, 1987). That could explain both partial inhibition, and the apparently slow activation. Although the effect is indeed selective among calcium channel types, it is now clear that selective block of N-current cannot explain the kinetic effects. First, N-channels often do not inactivate rapidly, especially in sympathetic neurons (Jones & Marks, 1989; Plummer et al., 1989; Plummer & Hess, 1991). Second, only part of the pharmacologically defined N-current can be inhibited by transmitters (Plummer et al., 1991; Elmslie et al., 1992). In part for these reasons, we argue that the fundamental effect is not simple inhibition, but modulation of the manner in which changes in voltage are coupled to calcium channel opening.

The change in voltage dependence is reflected in facilitation of channel opening by strong depolarization (Fig. 1). Usually, a brief (~20 msec) depolarization to +80 mV has little effect on the ability of calcium channels to open in response to a moderate depolarization. But in the presence of neurotransmitters that inhibit calcium current, brief strong depolarization does considerably increase the subsequently evoked current. This provides a kinetic signature for the transmitter effect, which can be exploited in many ways, as discussed below.

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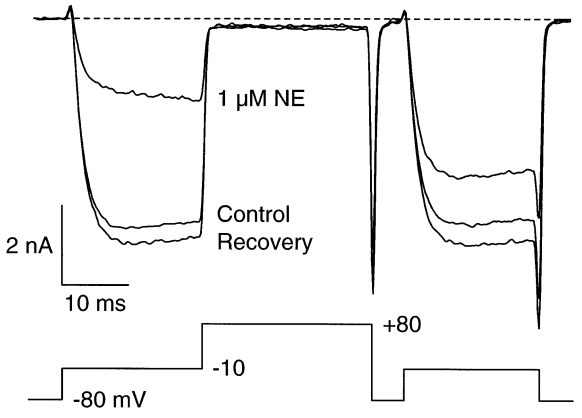

Fig. 1. Inhibition of calcium channel currents in a rat sympathetic neuron by norepinephrine (NE), and reversal of inhibition by strong depolarization. Previously unpublished data; *see* Ehrlich & Elmslie (1995). Three records are superimposed, in control conditions, during application of NE (smallest inward currents), and after recovery (largest inward currents). For this and related protocols, we will refer to the test depolarizations to near 0 mV as the ''prepulse'' and ''postpulse'' (respectively, left to right), and the step to a strongly depolarized voltage as the ''conditioning pulse.''

There is now convincing evidence of multiple pathways for receptor-mediated inhibition of calcium channels, some of which do not involve changes in the voltage dependence of channel gating (Hille, 1994). In this review, we will first discuss in detail the voltage-dependent mechanism, which seems to be the most widespread, and then briefly note other mechanisms and how they can be distinguished.

Neurotransmitters Modify Calcium Channel Gating

Several reports have examined the voltage- and time-dependence of calcium channel modulation, with the goal of using kinetic models to bridge the gap between descriptive data and the underlying mechanism (Grassi & Lux, 1989; Marchetti & Robello, 1989; Elmslie et al., 1990; Lopez & Brown, 1991; Kasai, 1992; Boland & Bean, 1993; Golard & Siegelbaum, 1993). Most of this work has been done using whole cell recording from sympathetic neurons from frog, rat and chick, with generally similar results. The data revealed several crucial qualitative properties. As mentioned above, inhibition is never complete. Strong depolarization restores normal fast activation kinetics, and increases the current amplitude, but not to the control level. At some voltages, the time course of activation is clearly biphasic. Channel activation is shifted to more positive voltages, and the activation curve is less steep.

The time course of facilitation, and reversal of facilitation, can be examined using multiple pulse protocols (Fig. 2). Facilitation is voltage-dependent, accelerating with depolarization, but at least in frog sympathetic neurons reaches a limited time constant ~5 msec above +40 mV (Fig. 2*B*). That probably indicates a voltage-independent step in the kinetic scheme, which becomes rate-limiting at extreme voltages. Reversal of facilitation (i.e., reinhibition) shows some voltage-dependence, but the time constant changes little in the voltage region where the channels are predominantly closed (Fig. 2*D*). The limiting rate for facilitation is ~10-fold faster than for reinhibition.

One explanation of this behavior is that modulation does not depend directly on voltage, but on the state of the channel. A popular idea is that the channel can exhibit either normal (''willing'') gating, or ''reluctant'' gating where the channel requires longer or stronger depolarization in order to open (Bean, 1989). In one simple form of this model (Elmslie et al., 1990), normal gating is approximated as a voltage-dependent C–O transition, paralleled by RC–RO gating for the modulated channel (Fig. 3). When the channel is closed, the equilibrium is toward the reluctant state; when it is open, toward the willing state. This scheme can account for the qualitative features of calcium channel modulation. Inhibition is never complete, even for maximal receptor activation, for two reasons: First, the reluctant channel can still open. Second, although the RC–C equilibrium favors the RC state, the reaction is reversible, so a fraction of the channels will always be in the normal gating states. Slow activation occurs because the RC $\rightarrow$ RO transition is intrinsically slower than C $\rightarrow$ O, and because the RO–O and C–O equilibria (which favor O) slowly pull channels out of the RC state. Since the willing–reluctant transitions are independent of voltage, the time constants for facilitation (at strong depolarization) and reinhibition (at strongly negative voltages) reach limiting values. Since the willing–reluctant transitions are equilibria, there are always some channels that gate normally, so transmitters never completely inhibit the current. Conversely, some channels remain in the reluctant state even at strong depolarization, so facilitation is incomplete. The shifted activation of reluctant channels moves the activation curve to more positive voltages. Interestingly, the model of Fig. 3 predicts no change in the steepness of the activation curve if it is measured at steady-state, after slow activation is complete, but a slight reduction in slope if activation is measured after ~5 msec.

In physical terms, what could the willing–reluctant transition be? As discussed below, modulation requires activation of G proteins, and may involve direct binding of G protein subunit(s) to the calcium channel. Thus, an attractive hypothesis is that the willing–reluctant transition is G protein binding. (We will use here G* to indicate the form of the G protein that acts on the channel, allowing also for the possibility that the active species is not the G protein itself but a downstream second mes-

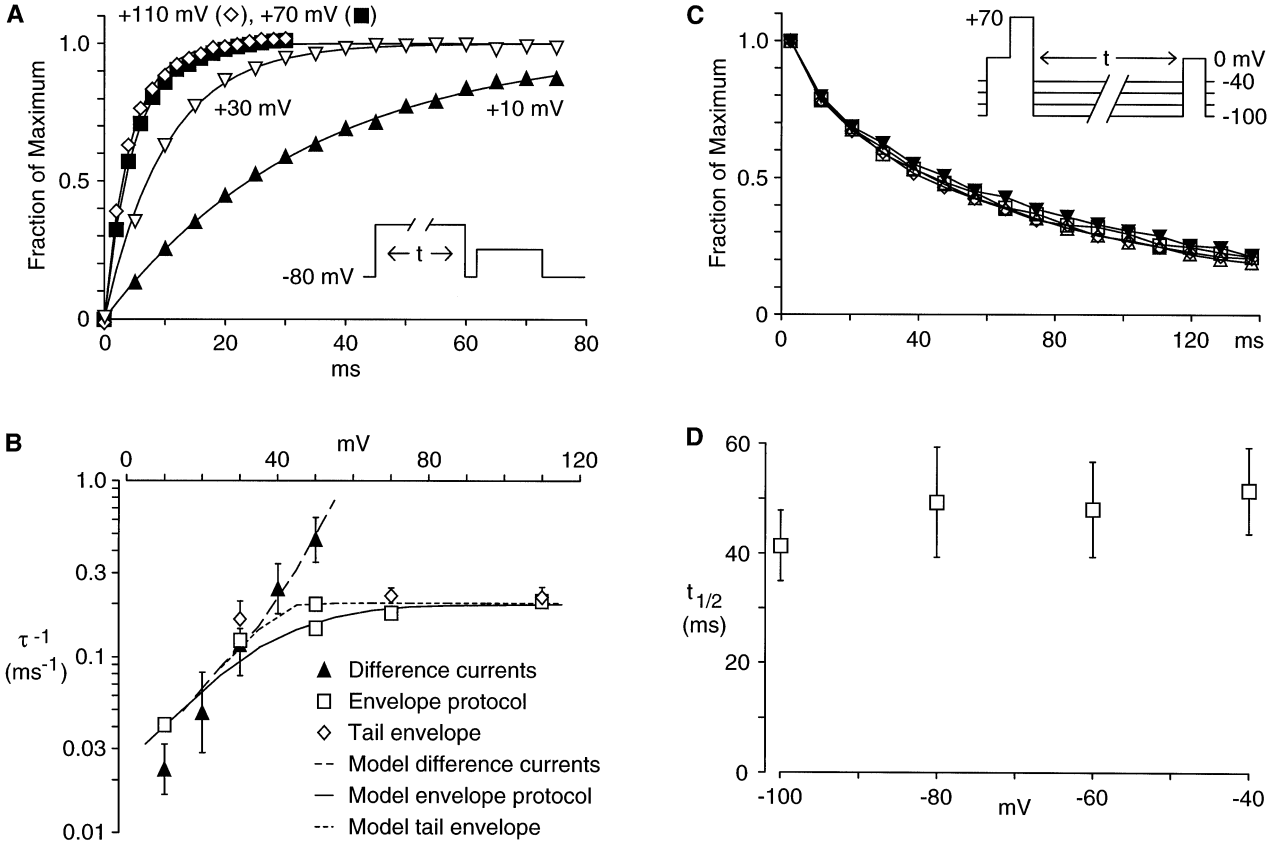

Fig. 2. Time course of facilitation in frog sympathetic neurons dialyzed with 100 μM GTP-γ-S. Previously unpublished data; *see* Elmslie et al., 1990, 1992. (*A*) Development of facilitation at positive voltages. Currents were measured during postpulses to 0 mV or +10 mV, following conditioning pulses of varying duration to the voltages indicated. The inset diagrams the voltage protocol. Currents were normalized, with the basal current (no conditioning pulse) defined as zero, and the maximally facilitated current at 1.0. The interval between the conditioning pulse and postpulse was 2 msec. The curves drawn are from single exponential fits with time constants of 4.3 msec (+110 mV), 5.0 msec (+70 mV), 10.2 msec (+30 mV), and 34.5 msec (+10 mV). (*B*) Comparison of different measures of the time course of modulation. Slow activation was measured from ''difference currents,'' the current during the postpulse minus the current during the prepulse, using the protocol of Fig. 1. Two protocols measured the time course of facilitation. The ''envelope protocol'' is illustrated in (*A*). The ''tail envelope'' protocol was similar, except that tail currents were measured upon repolarization to −40 mV directly after the conditioning pulse. The number of cells tested with each protocol ranges from 2–9; values are mean ± SD except that the individual points are shown when $n = 2$. The curves are from the model of Fig. 3, with voltage dependence shifted by +15 mV, reflecting the more positive activation observed with GTP-γ-S, compared to currents modulated by neurotransmitters (Elmslie et al., 1990). Note that slow activation and facilitation have essentially the same time course at +10 to +30 mV, but slow activation continues to accelerate at +50 mV, while facilitation reaches a limiting time constant at +50 to +100 mV. The difference in time course between slow activation and facilitation at +50 mV implies that channels can open without becoming facilitated; that is, channels can open while still in a ''reluctant'' state. (*C*) Decay of facilitation, in GTP-γ-S. The protocol illustrated in the inset was used to measure the time course of reinhibition at negative voltages, following facilitation produced by a step to +70 mV. Values were normalized to the current measured with a 2.5 msec interval between the steps to +70 mV and 0 mV. (*D*) The time to half reinhibition as a function of voltage. Values are mean ± SD ($n = 8$).

senger.) That implies that the willing–reluctant transitions, the vertical steps in Fig. 3, are actually bimolecular reactions, involving binding of G* to the channel. In this interpretation, the willing–reluctant model is essentially the same as the classical modulated receptor model for drug action (Hille, 1992).

If the willing–reluctant transition reflects binding of G* to the channel, some of the rate constants in the model depend on the concentration of G*, so the kinetics of modulation will be concentration-dependent (Lopez & Brown, 1991). That would seem to be a clear qualitative test for such a scheme. However, the time constants observable in recordings of macroscopic current depend in a complex way on the microscopic rate constants in a model, so whether the concentration-dependence is actually detectable depends on the details (Boland & Bean, 1993). Thus, absence of concentration-dependence (Kasai & Aosaki, 1989; Kasai, 1992) cannot conclusively rule out a dynamic involvement of G*. Qualitatively, concentration dependence should be stronger for reinhibition than for facilitation, as the equilibrium is toward the reluctant state when the channel is closed, so the kinetics are dominated by the faster rate, which is binding of G* (Golard & Siegelbaum, 1993). Several studies

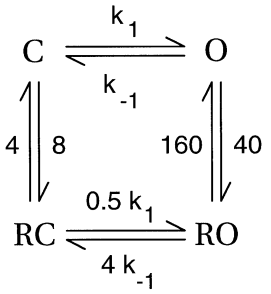

Fig. 3. A model for neuronal calcium currents during modulation by neurotransmitters. Redrawn from Elmslie et al. (1990). The rate constants in sec^{-1} for normal channel opening and closing (respectively) are $k_1 = 200\ e^{0.06(V+5)}$ and $k_{-1} = 200\ e^{-0.06(V+5)}$. Currents simulated from the model are shown in Fig. 5*C* below.

have now found concentration-dependent reinhibition (Golard & Siegelbaum, 1993; Elmslie & Jones, 1994; Ehrlich & Elmslie, 1995). That provides strong evidence that slow activation and facilitation truly reflect loss of modulation (unbinding of G*), and the slow decay of facilitation reflects concentration-dependent reinhibition (binding of G*). It is noteworthy that reinhibition is faster than the initial onset of inhibition upon application of neurotransmitter (roughly, 0.1 sec *vs.* 1 sec), suggesting that binding of G* is not rate limiting for the initial development of the response.

Although the basic concept of 'willing' and 'reluctant' states seems valid, it is clear that the 4-state model (Fig. 3) is oversimplified. For example, normal channel gating is not fully described by a C–O scheme, as there is a brief delay before channels open (suggesting multiple closed states), and channels also inactivate slowly (barely detectable on the time scale of Fig. 1). More complex models for channel modulation have been proposed (Boland & Bean, 1993; Golard & Siegelbaum, 1993), but have not yet been thoroughly tested experimentally. It seems likely that data from single channel recording will be crucial for discriminating kinetic models, but such studies have been hampered by problems with identifying the single channel basis of the whole-cell N-current (Elmslie et al., 1994). In recent studies, modulation by neurotransmitters produces a dramatic increase in the first latency, i.e., the time to first channel opening upon depolarization (Carabelli et al., 1996; Elmslie & Kelly, 1995; Patil et al., 1996). That may correspond to the slow transition from the reluctant to the willing state. However, willing–reluctant models predict that the reluctant channel can also open, although briefly (Fig. 3). A detailed kinetic analysis will be necessary to determine whether such ''RO'' openings actually occur.

G Proteins Mediate Calcium Channel Modulation

One clue to the biochemical mechanism of calcium channel modulation is the time course of the effect. The classical, direct action of neurotransmitters to open ligand-gated ion channels can be quite rapid, requiring less than 1 msec for channel opening and 1–10 msec for closing (Hille, 1992). The time course of modulatory effects on calcium channels is very different, with both onset and recovery of transmitter action requiring ~1 second (Bernheim et al., 1991; Jones, 1991). That, however, is considerably faster than typical responses involving second messengers and protein phosphorylation. β-adrenergic regulation of calcium channels in the heart has an absolute latency of several seconds, and lasts for about 1 min after removal of agonist (Hill-Smith & Purves, 1978; Frace et al., 1993).

Voltage-dependent modulation of neuronal calcium channels is mediated via activation of G proteins (Schultz et al., 1990). Intracellular dialysis with GTP-γ-S, which irreversibly activates G proteins, mimics the effect of neurotransmitters (Holz et al., 1986; Grassi & Lux, 1989; Marchetti & Robello, 1989; Kasai & Aosaki, 1989; Toselli et al., 1989). But there is a long tradition of evidence against the involvement of a water-soluble, diffusible second messenger (Forscher & Oxford, 1985; Forscher et al., 1986; Bley & Tsien, 1990; Bernheim et al., 1991; Plummer et al., 1991; Elmslie et al., 1994; Wilding et al., 1995). When recording from calcium channels in a cell-attached patch, application of transmitter in the bath is ineffective, even though plasma membrane receptors should be activated everywhere except in the patch itself (Forscher et al., 1986; Bernheim et al., 1991). In contrast, channels can be inhibited in a cell-attached patch by transmitter applied via the electrode (Elmslie et al., 1994), or in an outside-out patch by rapid application of transmitter to the patch (Wilding et al., 1995). That demonstrates that the effect is membrane-delimited: all of the molecular machinery needed to couple the receptor to the channel is present in the patch. Receptor-channel coupling still occurs when ATP is replaced by the nonhydrolyzable analogue AMP-PNP, additional evidence against the involvement of protein phosphorylation (Elmslie et al., 1993).

The original example of membrane-delimited receptor-channel coupling is muscarinic activation of an inwardly rectifying potassium channel in the cardiac atrium (reviewed by Szabo & Otero, 1990; Kurachi, 1995). That effect is rapid, by G protein-coupled receptor standards, and mimicked by intracellular GTP-γ-S or by application of preactivated G proteins. G protein activation is well known to cause dissociation of the heterotrimeric G protein, releasing the α subunit plus the βγ subunits. There was a prolonged controversy over which subunits mediate the action of muscarinic agonists on the potassium channel, which seems to have been conclusively resolved in favor of βγ (Reuveny et al., 1994). Although there is ample precedent now for actions of βγ, that is still a surprising result, as the specificity of G

protein signaling is thought to primarily reflect actions of α subunits. Several distinct β and γ subunits are known to exist, but there is little precedent for functional differences among them (*but see* Kleuss et al., 1992; Kalkbrenner et al., 1995).

In many cells, calcium channels can be inhibited by activation of several different receptors (Hille, 1994; Hille et al., 1995). To some extent, that is expected, as convergent signaling is characteristic of G protein-coupled receptors, where multiple receptors can activate a single type of G protein. Considerable effort has been devoted to demonstrating involvement of specific G proteins in receptor-calcium channel coupling (Hescheler et al., 1987; Ewald et al., 1988, 1989; McFadzean et al., 1989; Kleuss et al., 1991; Taussig et al., 1992; Menon-Johansson et al., 1993; Caulfield et al., 1994; Moises et al., 1994; Wilk-Blaszczak et al., 1994; Degtiar et al., 1996). In many cases, voltage-dependent modulation involves activation of G_o. However, it is now clear that G proteins containing several different α subunits can produce the same final effect on the channel. Both pertussis toxin-sensitive and -resistant G proteins can have kinetically indistinguishable effects on calcium channel gating (Elmslie, 1992; Ehrlich & Elmslie, 1995). In rat sympathetic neurons, activation of G_o, G_s, and a $G_?$ (resistant to both pertussis and cholera toxins) can modulate calcium channels (Hille, 1994). One interesting question is whether any receptor that activates any G protein can avoid modulating calcium channels, in cells where the appropriate channels are present.

There are at least three possible explanations for this apparent nonspecificity. (i) Several different G protein α subunits could bind directly to the calcium channel, producing kinetically identical effects. That would require that the calcium channel binds the α subunits with exquisite nonspecificity, as the time course of facilitation is the same whether the G protein involved is sensitive to pertussis toxin, cholera toxin, or neither (Elmslie, 1992; Ehrlich & Elmslie, 1995). (ii) Several different G proteins could converge upon a common second messenger, which then affects calcium channel gating. That could be mediated by either α or βγ subunits. The second messenger would have to act in a membrane-delimited manner, and not by protein phosphorylation. There is little precedent for such an effect, but lipid messengers such as arachidonic acid metabolites could conceivably act in that fashion. (iii) The βγ subunits produced by activation of different G proteins could bind to the calcium channel.

Recent evidence indicates that βγ subunits of G proteins do mediate membrane-delimited coupling of receptors to calcium channels (Ikeda, 1996; Herlitze et al., 1996). Expression of exogenous βγ subunits in rat sympathetic neurons induces slow activation and facilitation by strong depolarization, mimicking and at least partially occluding the effect of norepinephrine on the calcium current (Ikeda, 1996). In contrast, exogenous G_o α subunits have little effect by themselves, but reduce the effect of norepinephrine, possibly by binding βγ. Similar results are observed in cells transiently transfected with both calcium channel and G protein subunits (Herlitze et al., 1996).

Multiple Calcium Channels are Regulated via Multiple Pathways

So far, this review has concentrated on modulation of calcium channels in sympathetic and sensory neurons, where the N-type calcium channel is predominant. In these cells, voltage-dependent inhibition is selective for the ω-conotoxin GVIA-sensitive N-current, with little or no effect on the dihydropyridine-sensitive L-current (Kasai & Aosaki, 1989; Plummer et al., 1989, 1991; Elmslie et al., 1992). However, especially in rat sympathetic neurons, there is considerable evidence for other mechanisms of calcium channel inhibition (Hille, 1994; Hille et al., 1995). One pathway involves a diffusible second messenger, and targets both N- and L-currents (Mathie et al., 1992). Another is membrane-delimited, but shows no clear voltage dependence (Shapiro & Hille, 1993). In chick sensory neurons, calcium currents can be inhibited by voltage-dependent or voltage-independent mechanisms, with the latter apparently involving protein kinase C (Rane & Dunlap, 1986; Diversé-Pierluissi & Dunlap, 1993; Luebke & Dunlap, 1994). A pathway involving cGMP-dependent protein kinase has been reported in chick ciliary ganglia (Meriney et al., 1994).

The voltage-dependent pathway is also observed in many neurons in the central nervous system. In many cases, as in peripheral neurons, the modulation spares L-current but targets other high voltage-activated calcium channels, including N- and P-channels (Mintz & Bean, 1993; Rhim & Miller, 1994; Bayliss et al., 1995; Ishibashi & Akaike, 1995; Cardozo & Bean, 1995; for a review of neuronal calcium channel classification, *see* Olivera et al., 1994). However, multiple mechanisms for calcium current inhibition also exist in the central nervous system (Toselli & Taglietti, 1995; Guyon & Leresche, 1995), including pathways leading to inhibition of L-current (Heidelberger & Matthews, 1991; Matthews et al., 1994; Chavis et al., 1994). In general, calcium channels can be regulated by many of the converging and diverging pathways characteristic of second messenger systems. Although the voltage-dependent mechanism emphasized in this review is widespread, it is not the only way to regulate the activity of neuronal calcium channels.

Inhibition of calcium current need not be associated with facilitation; conversely, facilitation need not reflect G protein-mediated inhibition. In particular, L-current is often facilitated by strong depolarization (Dolphin,

1996), but the underlying mechanisms seem very different from modulation of N-current: typically, L-current facilitation is much slower, and (in some cases) may involve protein phosphorylation but not G protein activation.

Recent evidence suggests that the specificity of modulation among calcium channel types may involve not only the main (α_1) subunit, but also the associated β subunit. Inhibition by neurotransmitters and G proteins is weaker when channels are coexpressed with exogenous β subunits (Roche et al., 1995; Bourinet et al., 1996), and stronger when β subunits are depleted by antisense oligonucleotides (Campbell et al., 1995). This could reflect competition between G* and the calcium channel β subunit for binding to α_1 (Bourinet et al., 1996), but the results could also be explained by an allosteric effect of β on G* binding.

Measurement of Voltage-dependent Inhibition is Complicated

When a neurotransmitter inhibits a channel in a voltage-independent manner, without affecting the time course of channel gating, analysis of the effect is not very difficult. Simply measure the current at some convenient voltage and time under control conditions, in the presence of the transmitter, and (an important control often neglected) following recovery from the response. The fractional inhibition, measured as the current in the presence of the transmitter divided by the average of values before and after, is a robust measure of the effect.

The situation is different when the transmitter acts by modulating the kinetics of channel activation. Consider the standard protocol used for studying voltage-dependent channels, depolarizations of fixed length to various voltages from a fixed holding potential. How is the effect of a neurotransmitter to be measured? Since the effect of G* is maximal at negative voltages, and reversal of the effect is slow with respect to normal activation kinetics of the channel ($\tau = 3$ msec or faster), measurement at early times (e.g., 3–5 msec) comes close to reflecting the modulation of the channel in its resting state. Measurement at steady-state would include the slowly activating ''reluctant'' current, and would yield lower % inhibition values.

One approach is to use the slow activation kinetics as a defining characteristic of the voltage-dependent pathway (Luebke & Dunlap, 1994; Diversé-Pierluissi et al., 1995). Unfortunately, slow activation is not always clearly observed, even when the inhibition is voltage-dependent by other criteria. One problem is inactivation. In many cells, slow activation occurs on a time scale comparable to the inactivation seen in the absence of neurotransmitter. The interaction of the two effects can produce complex kinetics. Channels can inactivate in the presence of neurotransmitter (or GTP-γ-S; Fig. 4). However, it is not clear whether the affinity of the inactivated state for G* is like that of open channels, closed channels, or neither, so the combined effect of slow activation and inactivation is difficult to predict. But it should be remembered that some early studies described the effect of transmitters as reducing an inactivating current, rather than as production of slow activation (Wanke et al., 1987; Gross & Macdonald, 1987). That indeed appears

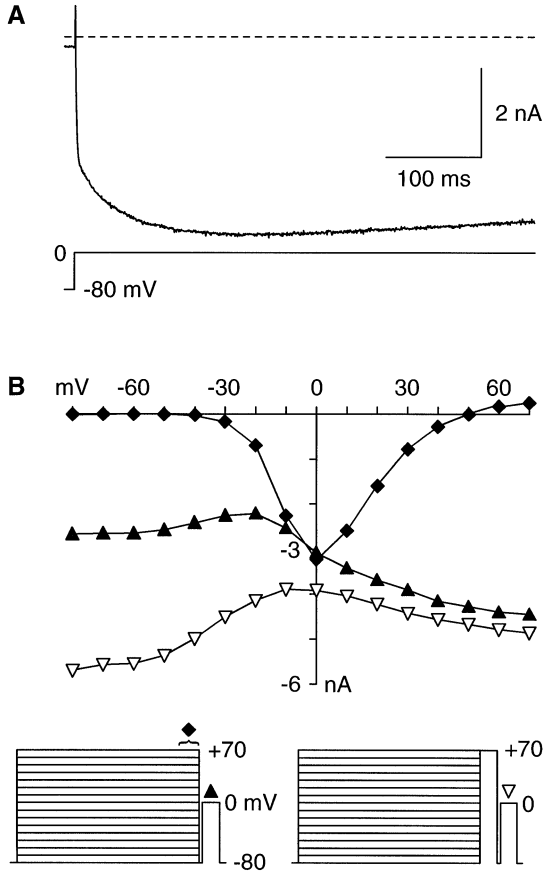

Fig. 4. Inactivation of current in frog sympathetic neurons in a cell dialyzed with GTP-γ-S. Previously unpublished data; *see* Elmslie et al., 1990, 1992. (*A*) Current recorded during a 500 msec step to 0 mV shows a rapid phase of activation (an essentially vertical line on this time scale), followed by slow activation, and then net inactivation. The current is not leak subtracted. (*B*) Current-voltage relations for the combination of facilitation and inactivation observed with long pulses in GTP-γ-S. The protocols are illustrated below. Currents were measured as the average between 450–500 msec during the prepulse, and 2.5–5 msec in the postpulse to 0 mV. Data are the average of two runs, with the prepulses given in ascending and descending order, to correct for rundown and/or slow inactivation. With the two-pulse protocol often used to examine inactivation (e.g., Jones & Marks, 1989), there was a net inactivation near −20 mV, but net facilitation at positive voltages. When the prepulses were followed by a 30 msec conditioning pulse to +70 mV, inactivation had a ∩-shaped voltage-dependence, as observed for normal inactivation without GTP-γ-S (Jones & Marks, 1989; Werz et al., 1993).

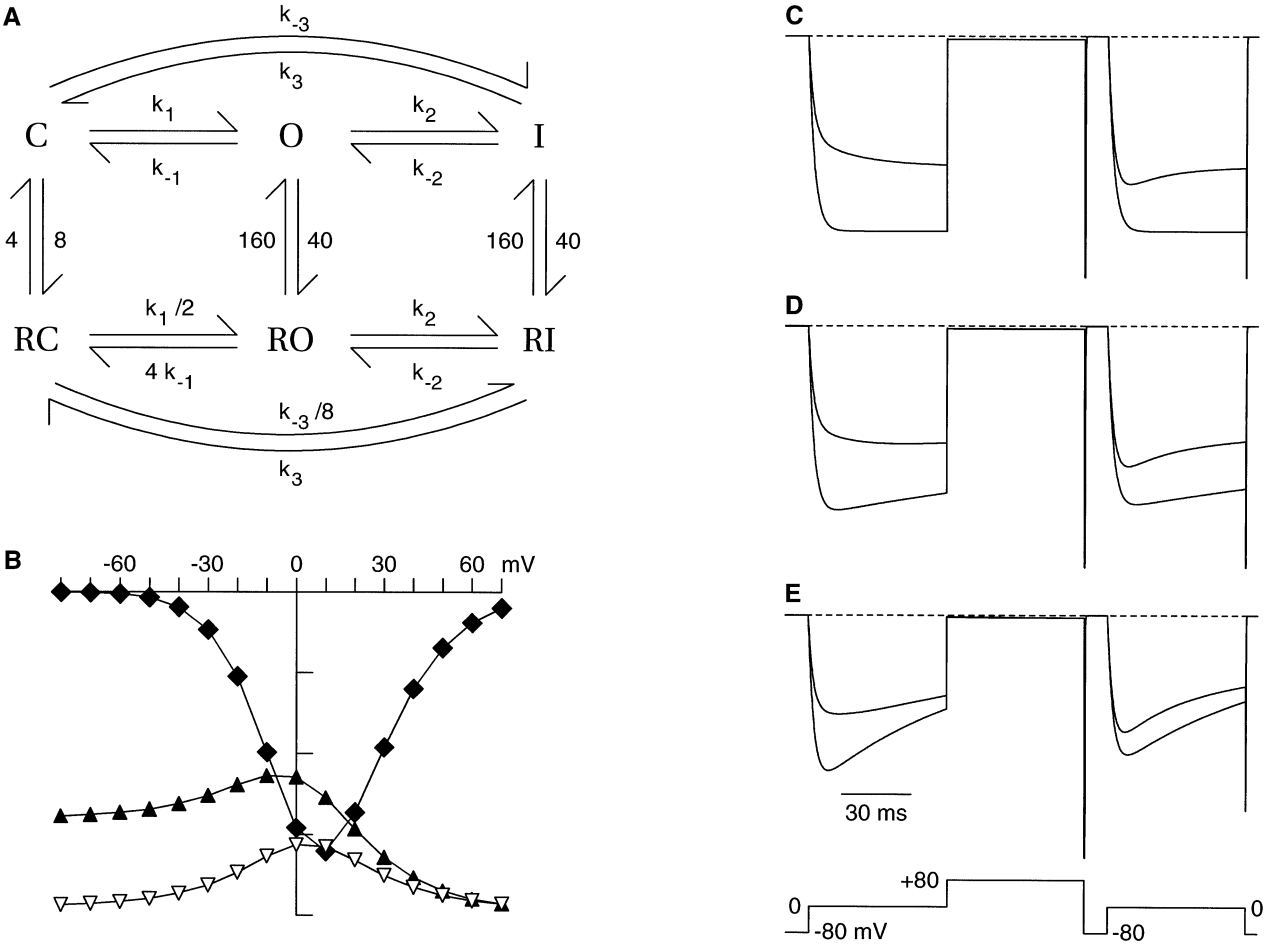

Fig. 5. Simulation of the interaction of facilitation and inactivation. (*A*) The kinetic scheme is based on a 3-state cyclic model for voltage-dependent inactivation (Jones & Marks, 1989), with willing–reluctant transitions as in the model of Elmslie et al. (1990). The inactivated state was assumed to be susceptible to modulation to the same degree as the open state. (*B*) Simulation of the voltage-dependence of combined facilitation and inactivation, using the protocol of Fig. 4*B*. k_1 and k_{-1} are as in Fig. 3, $k_2 = 2e^{-0.03(V-5)}$. $k_{-2} = 2e^{0.03(V-5)}$, $k_3 = e^{-0.02(V-5)}$, and k_{-3} is defined by microscopic reversibility (Jones & Marks, 1989). Simulations from the model are shown for three conditions: (*C*) no inactivation, where the model reduces to Fig. 3; (*D*) moderately slow and incomplete inactivation, as normally observed for N-current in frog sympathetic neurons; and (*E*) relatively fast inactivation (with k_2 5 × faster than in *B* and *D*). Similar inactivation is observed in conditions favoring phosphorylation (Werz et al., 1993), or under normal conditions in some other cell types. Note absence of slow activation, and reduced steady-state inhibition, in (*E*).

to happen if inactivation is relatively rapid and complete, as simulated in Fig. 5. Using the same basic model for voltage-dependent modulation described above (Fig. 3), but incorporating inactivation (Jones & Marks, 1989), slow activation can disappear. Thus, absence of slow activation cannot be used as evidence that modulation is voltage-independent. That is especially true when the inhibition produced by the transmitter is relatively small, where the resulting change in kinetics is subtle and can easily be obscured by inactivation.

Facilitation by strong depolarization is a much more robust measure of voltage-dependent inhibition. We standardly measure currents early in steps to voltages that produce large inward currents, recorded before and after a facilitating prepulse, and take the ratio of the postpulse/prepulse currents as an index of modulation (Jones, 1991; Elmslie, 1992). That reveals the existence of basal modulation, observed in the absence of known G protein activators, which is small in frog sympathetic ganglia (postpulse/prepulse ratio ~1.1; Elmslie, 1992), but substantial in rat (ratios 1.1–1.6; Ikeda, 1991; Ehrlich & Elmslie, 1995).

In both frog and rat sympathetic neurons, we consistently observe that facilitation does not fully restore the current to the level recorded in the absence of neurotransmitter (Elmslie et al., 1990; Ehrlich & Elmslie, 1995; but *see* Kasai, 1992) (Fig. 1). That might reflect a small amount of modulation occurring via an intrinsically voltage-independent pathway. However, it could also be a consequence of the mechanism of voltage-dependent inhibition. Models (e.g., Fig. 3) predict incomplete facilitation, as G* can still bind to the open

state of the channel, albeit with lower affinity. Thus, it is dangerous to assume that inhibition not reversed by strong depolarization must occur by a fundamentally different mechanism. As a corollary, measuring the fraction of the total inhibition that is reversed by facilitation (Luebke & Dunlap, 1994; Diversé-Pierluissi et al., 1995) could seriously underestimate the contribution of the voltage-dependent mechanism.

Calcium Channel Modulation Contributes to Presynaptic Inhibition

Calcium channels have many functions, so inhibition of calcium channel activity can have many consequences. Since the most crucial role of neuronal calcium channels is in the release of neurotransmitter, much attention has been given to the possibility that calcium channel modulation is responsible for presynaptic inhibition of neurotransmitter release. Unfortunately, most studies of calcium channel modulation have been conducted on the cell body, the most electrophysiologically accessible portion of the neuron, since direct studies of presynaptic calcium channels are difficult or impossible in most preparations. One exception is the chick ciliary ganglion, where adenosine has been shown to reduce presynaptic calcium current, $[Ca^{2+}]_i$, and transmitter release (Yawo & Chuma, 1993). In other systems, there is considerable indirect evidence that calcium channel modulation contributes to presynaptic inhibition (Wu & Saggau, 1994, 1995; Doze et al., 1995). In several cases, partial inhibition of transmitter release by the N-channel blocker ω-conotoxin GVIA partially occludes presynaptic inhibition, as expected if presynaptic inhibition involved a reduction in N-channel activity (Toth et al., 1993; Yawo & Chuma, 1993; Stefani et al., 1994; Wu & Saggau, 1995). However, some transmitters also inhibit release at a stage subsequent to calcium entry (Scholz & Miller, 1992).

We have speculated that the voltage-dependence of modulation could have physiological relevance (Elmslie et al., 1990). In frog sympathetic neurons, facilitation occurs in the voltage range normally encountered during an action potential (Fig. 2*A* and *B*), and the time constants for facilitation and reinhibition suggest that a train of action potentials could lead to a maintained facilitation. If that occurs in a nerve terminal, a modulatory neurotransmitter would effectively reduce the amplitude of calcium current evoked by a single brief action potential, but a train of action potentials might facilitate the current and reduce presynaptic inhibition. Tests of that hypothesis did find strong inhibition of calcium current in response to a single action potential-like waveform (Penington et al., 1992; Toth & Miller, 1995), but little or no facilitation during trains (Penington et al., 1991; Toth & Miller, 1995; Womack & McCleskey, 1995). However, since reinhibition is faster at higher G*, reversal of facilitation during a train should be strongest for submaximal transmitter effects, which have not yet been examined. It also needs to be determined whether presynaptic inhibition is frequency-dependent under physiological conditions.

In summary, voltage-dependent modulation of calcium currents is a common phenomenon in the nervous system. It is likely to play a role in presynaptic inhibition, and to affect other cellular functions regulated by neuronal calcium currents.

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