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Overview of Voltage-Dependent Calcium Channels

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Voltage-dependent calcium channels couple electrical signals to cellular responses in excitable cells. Calcium channels are crucial for excitation–secretion coupling in neurons and endocrine cells, and excitation–contraction coupling in muscle. Several pharmacologically and kinetically distinct calcium channel types have been identified at the electrophysiological and molecular levels. This review summarizes the basic properties of voltage-dependent calcium channels, including mechanisms of ion permeation and block, gating kinetics, and modulation by G proteins and second messengers.

KEY WORDS: Activation; inactivation; modulation; permeation; gating; secretion; contraction; neurotransmitter; G protein; second messenger.

INTRODUCTION

Among the extended family of voltage-dependent ion channels, calcium channels are of particular functional importance, as they are involved both in electrical signaling itself, and in coupling electrical signals to changes in cytoplasmic calcium (Hille, 1992). Calcium then triggers a myriad of cellular processes, on a wide range of time and distance scales. Calcium channels can respond rapidly to changes in membrane potential, activating within milliseconds of a depolarization, but are also regulated on a slower time scale by a variety of mechanisms.

The goal of this review is to describe the fundamentals of calcium channel structure and function. Although some classical work is included, the emphasis is on recent progress, and aspects that may not be generally known.

CALCIUM CHANNEL TYPES

Physiological and molecular studies have identified several different voltage-dependent calcium channels (Table I). The primary distinction is between rapidly inactivating channels that are activated by weak depolarization (low voltage-activated channels, LVA), and channels with variable inactivation that require stronger depolarization (HVA) (Carbone and Lux, 1984). LVA channels are involved in generation of repetitive electrical activity, for example in cardiac cells and in thalamic neurons (Huguenard, 1996). Functional diversity among LVA channels exists, but has received relatively little attention, partially because no LVA channels had been cloned until very recently (Perez-Reyes, 1998; Perez-Reyes *et al.*, 1998). HVA

Table I. Classification of Voltage-Dependent Calcium Channels

α_1 Subunit	Physiologically defined current	Selective blocker
A	P- and/or Q-type?	ω-Aga IVA[a]
B	N-type	ω-conotoxin GVIA
E	R-type?	
C	L-type (cardiac, smooth muscle)	DHP antagonists
D	L-type (endocrine, neurons)	DHP antagonists
S	L-type (skeletal muscle)	DHP antagonists
G	T-type (many neurons)	
H	T-type	

[a] Faster and/or higher affinity block for P than Q. See text for effects of ω-conotoxin MVIIC.

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channels are primarily responsible for most of the other functions of calcium channels, including excitation–secretion and excitation–contraction coupling.

Functional Classification of HVA Channels

Much work has been devoted to identification of functional differences among HVA calcium channels, primarily at the level of whole-cell recording of macroscopic calcium-channel currents from neurons. The initial distinction was between L channels, named in part for their large single-channel conductance, and N channels (Nowycky *et al.*, 1985). The HVA channels of muscle are L-type, while both L and N channels are expressed in many neurons. In retrospect, the original definition of N channels as rapidly inactivating channels of intermediate single-channel conductance (Fox *et al.*, 1987) seems to have resulted from inclusion of a variety of non-L HVA channels with diverse properties (Elmslie *et al.*, 1994; Elmslie, 1997). The contemporary definition of L and N channels depends primarily on pharmacology. L channels are highly sensitive to dihydropyridines (DHPs), and N channels are blocked potently by ω-conotoxin GVIA (CgTx), a peptide isolated from the venom of a cone snail (Plummer *et al.*, 1989). This distinction has received strong support in a variety of experimental systems, but the First Law of Pharmacology still applies: "All drugs have two effects, the one you know, and the one you don't know." At high concentrations (> 1 μM), DHPs inhibit a variety of channels, including voltage-dependent sodium and potassium channels (Yatani and Brown, 1985; Nerbonne and Gurney, 1987; Jones and Jacobs, 1990). Actually, potentiation by DHP agonists is much more diagnostic for L current than is inhibition by DHP antagonists (Nowycky *et al.*, 1985; Plummer *et al.*, 1989). CgTx inhibits mammalian N channels almost irreversibly (the block is slowly reversible in frog; Boland *et al.*, 1994), but also produces a rapidly reversible block of some but not all L channels (Williams *et al.*, 1992).

While the distinction between L and N channels was still being debated, neuronal HVA channels were discovered that were blocked neither by DHPs nor by CgTx, thus fitting into neither category (Regan *et al.*, 1991). P channels, originally characterized in Purkinje neurons of the cerebellum, are now defined by rapid block by the spider toxin ω-Aga IVA at < 100 nM (Mintz *et al.*, 1992). Soon after, DHP- and CgTx-resistant channels were found that were blocked less rapidly and/or potently by ω-Aga IVA, and were named Q channels (Zhang *et al.*, 1993). The distinction between P and Q channels has been difficult to establish in many neurons, so most studies refer to P/Q channels unless high-affinity block by ω-Aga IVA is unequivocal. P/Q channels are also blocked in a nearly irreversible manner by ω-conotoxin MVIIC (Zhang *et al.*, 1993; McDonough *et al.*, 1996). That toxin also blocks N channels reversibly, but spares other HVA and LVA channels. Channels blocked potently by ω-conotoxin MVIIC are sometimes classified as P/Q even if they appear totally resistant to ω-Aga IVA (Mackie *et al.*, 1995).

Many neurons have a component of HVA current that is resistant to all of the above blockers (DHPs, CgTx, ω-Aga IVA, and ω-conotoxin MVIIC). This R (resistant) current often inactivates more rapidly than the other HVA currents, and may activate at somewhat more negative voltages (Zhang *et al.*, 1993; Elmslie *et al.*, 1994). It is likely that the R component of HVA current will be fractionated further (Tottene *et al.*, 1996).

Studies at the single-channel level have also identified a variety of HVA channels. Correlation with the channel types identified in whole-cell recordings has not been trivial, in part because of differences in the conditions used. Since the currents through single calcium channels are small, and channel opening and closing is rapid, nearly all single-channel studies have used high concentrations of Ba^{2+} (near 100 mM) to maximize current amplitudes. Most whole-cell recordings use 2–10 mM Ba^{2+} or Ca^{2+}, somewhat closer to physiological conditions. But divalent cations have a strong screening effect on fixed negative charges that may be present either in the lipid bilayer or on the channel protein itself. For N channels, that charge screening can shift the apparent voltage dependence of channel gating by as much as 40 mV between 2 mM and 100 mM Ba^{2+} (Elmslie *et al.*, 1994; Zhou and Jones, 1995). Comparision of results from whole-cell and single-channel recording must allow for this effect.

In isotonic Ba^{2+}, L channels have a relatively high single-channel conductance, ~25 pS (Fox *et al.*, 1987; Plummer *et al.*, 1989). (Since it is generally not possible to demonstrate a reversal potential in that condition, reported calcium channel conductances are not true chord conductances, calculated from $G = I / (V - V_r)$, where G = conductance, I = single channel current, V = voltage, and V_r = reversal potential. Instead, slope conductances are used, calculated from

the slope of the single-channel current–voltage relation.) N channels have only slightly lower conductances, ~20 pS (Plummer *et al.*, 1989; Elmslie, 1997). In the absence of DHPs, L and N channels are difficult to distinguish at the single-channel level. Some early reports of N channels with substantially lower conductances may be attributed to recording from R channels (Elmslie *et al.*, 1994; Elmslie, 1997). Few studies have attempted to determine the pharmacological profiles of single calcium channels, since the channels are generally recorded in cell-attached patches where exchange of the extracellular solution is difficult (but see Elmslie, 1997). Furthermore, binding of some of the selective toxins is inhibited by divalent cations (Boland *et al.*, 1994; McDonough *et al.*, 1996).

Molecular Basis of Calcium Channel Diversity

For both LVA and HVA calcium channels, one primary subunit (α_1) contains the domains necessary for basic channel function, including the calcium-selective pore, the voltage sensors, and many pharmacological determinants (Catterall, 1996; Mitterdorfer *et al.*, 1998) (Fig. 1). That subunit shares a transmembrane architecture, and some homology, with voltage-dependent sodium channels. There are four internally homologous domains, each containing six putative transmembrane segments (numbered S1–S6). The S4 segment is highly unusual among transmembrane regions in that it contains several positively charged amino acids, generally present in every third position in an otherwise highly hydrophobic region. Primarily from experiments on sodium and potassium channels, there is now good evidence that the S4 segment is the voltage sensor that couples changes in membrane potential to channel opening (Yang and Horn, 1995; Mannuzzu *et al.*, 1996). Many of the positively charged amino acids in S4 are exposed to the extra- or intracellular solution, rather than to other protein domains or membrane lipids (Yang *et al.*, 1996), demonstrating the danger of literal interpretation of cartoons such as Fig. 1B. An extracellular loop between S5 and S6 is part of the the ion conduction pathway, and plays a major role in calcium selectivity (Kim *et al.*, 1993; Yang *et al.*, 1993).

The functionally defined calcium channel types map reasonably well onto the different cloned α_1 subunits (Table I). The first α_1 subunit to be cloned was the L channel of skeletal muscle (Tanabe *et al.*, 1987). Soon after, five other α_1 subunits were cloned by

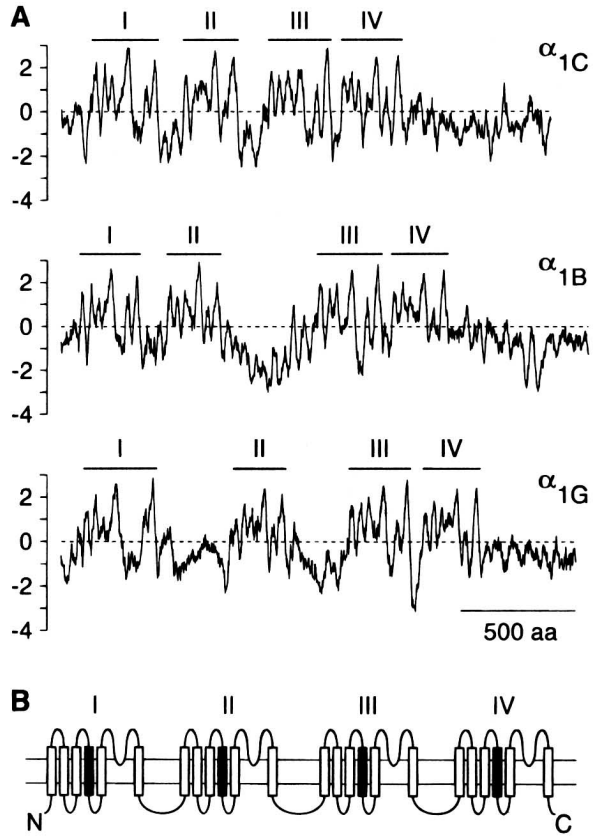

Fig. 1. Domain structure of α_1 subunits of voltage-dependent calcium channels. (A) Hydropathy plots for three representative calcium channels, using the algorithm of Kyte and Doolittle (1982) with a window size of 21 amino acids. Positive values indicate hydrophobic regions. GenBank accession numbers are X15539 for rabbit α_{1C}, M92905 for rat α_{1B}, and AF027984 for rat α_{1G}. (B) Schematic diagram of the predicted transmembrane topology of α_1 subunits. Within each domain, the transmembrane regions are denoted S1–S6 (left to right). The loop between S5 and S6 is the P (pore) region. The S4 regions, which contain several positively charged amino acids, are shown as filled boxes. The extra- and intracellular loops are not drawn to scale.

homology, including two additional L channels, and three other HVA channels (Mikami *et al.*, 1989; Snutch *et al.*, 1990). After a long wait, the first members of the LVA family have been cloned (Perez-Reyes, 1998; Perez-Reyes *et al.*, 1998). As expected from the functional differences, the LVA channels are only distantly related to the HVA α_1 subunits, but crucial functional domains (notably, the voltage sensors and the pore loops) show strong conservation.

Most work has concentrated on calcium channels of vertebrate animals. The main classes of calcium channel (L, non-L HVA, and LVA) also exist in the nematode *C. elegans* (Schafer and Kenyon, 1995; Gen-

Bank #U37548, #U61951), and L and non-L HVA channels are known in *Drosophila* (Zheng *et al.*, 1995; Smith *et al.*, 1996). Specific family members (e.g., α_{1B} and α_{1E}) are identifiable in fish (Horne *et al.*, 1993), but apparently not invertebrates. Yeasts have a 4-domain protein that is tantalizingly close to both sodium and calcium channels (Tettelin *et al.*, 1997).

Table I emphasizes the role of the α_1 subunit in channel diversity. But calcium channels are multisubunit proteins, including β and α_2/δ subunits (Catterall, 1996). (The α_2 and δ subunits come from a single protein precursor, and are linked by a disulfide bond). Four β subunits are known, and a second putative α_2/δ subunit has been cloned recently (GenBank #AF042792). A γ subunit appears to be unique to skeletal muscle. It is not yet known whether LVA channels have these accessory subunits. For HVA channels, the β and α_2/δ subunits increase expression levels in heterologous systems such as *Xenopus* oocytes and mammalian cell lines, and also influence the kinetic and pharmacological properties of the channel (Singer *et al.*, 1991). Genetic disorders of calcium channels can result from defects in either α_1 or β subunits (Fletcher *et al.*, 1998). The expression of calcium channel subunits is highly regulated developmentally, and among different cell types (McEnery, 1998; Sher *et al.*, 1998).

Some functional differences between native calcium channels and cloned channels in expression systems may result from differences in the accessory subunits. However, properties of native P channels, for example, differ from heterologously expressed α_{1A}, even with different β subunits (Stea *et al.*, 1994). Additional sources of channel diversity include alternative splicing and posttranslational modifications of the α_1 or other subunits.

It is not entirely clear whether the β subunit is an invariable stoichiometric subunit of the calcium channel, or whether it is reversibly associated. Some α_1 subunits can form calcium channels in the absence of added β subunits, but this may reflect the presence of endogenous β subunits in certain expression systems, as antisense to β can prevent expression of α_1 alone in *Xenopus* oocytes (Tareilus *et al.*, 1997). On the other hand, the effects of β subunits on channel expression levels may be separable from the effects on channel kinetics, implying multiple interactions. The role of β subunits is reviewed in more detail by Birnbaumer *et al.* (1998) and Chien and Hosey (1998).

PERMEATION

Selectivity

Calcium channels have the difficult task of selecting for Ca^{2+} in the presence of monovalent cations, which are present at ~ 100-fold higher concentrations (Tsien *et al.*, 1987). Under physiological ionic conditions, they do this extraordinarily well, with permeability ratios > 1000 (Ca^{2+}/K^+ and Ca^{2+}/Na^+). But the crucial clue to the mechanism of Ca^{2+} selectivity was the observation that calcium channels become nonselective cation channels in the absence of Ca^{2+}, permeable to monovalent cations as large as tetramethylammonium (McCleskey and Almers, 1985). Furthermore, currents carried by monovalents are blocked by Ca^{2+} with apparent K_i ~ 1 μM, with Ca^{2+} permeation occurring at millimolar concentrations (Almers and McCleskey, 1984; Hess and Tsien, 1984). These observations implied that calcium channels do not exclude monovalents using a "selectivity filter" tuned for Ca^{2+} ions. Instead, some form of competition between permeant ions seems to be necessary. This has generally been described by models based on Eyring rate theory, involving multiple binding sites within the pore (energy wells), separated by barriers (Fig. 2). The initial models postulated two binding sites with high intrinsic affinity for Ca^{2+} (Fig. 2A), which explains the high-affinity block of current carried by monovalent

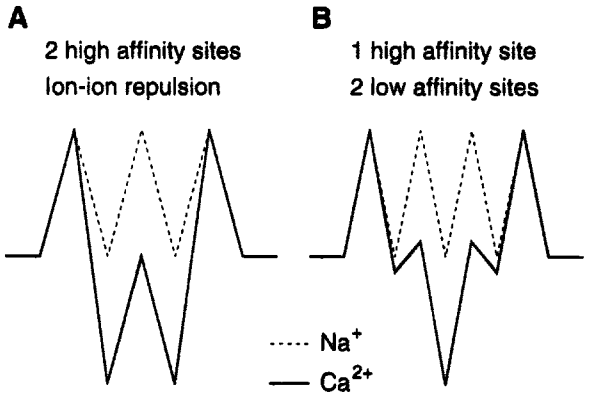

Fig. 2. Two models for permeation in calcium channels. Schematic energy level diagrams are shown for permeant divalent and monovalent cations (not drawn to scale). (A) The 2-site 3-barrier model of Almers and McCleskey (1984) and Hess and Tsien (1984). The two binding sites have a high intrinsic affinity for Ca^{2+}, but when both sites are occupied, ion–ion repulsion raises the energy wells closer to those shown for Na^+, allowing Ca^{2+} to permeate. (B) The 3-site 4-barrier model of Dang and McCleskey (1998). No ion–ion repulsion is necessary.

cations. To allow Ca^{2+} permeation at mM concentrations, it was assumed that ions at the two sites electrostatically repel each other, lowering the affinity (Almers and McCleskey, 1984; Hess and Tsien, 1984). Such models could explain the basic features of Ca^{2+} channel selectivity, and remained standard for over a decade.

The first indication of the molecular basis for calcium selectivity actually came from mutagenesis of cloned sodium channels (Heinemann *et al.*, 1992). Alignment of sodium and calcium channel sequences in the putative pore region revealed one site that is a glutamate in all four domains of HVA calcium channels, but not sodium channels. Mutation of some of the corresponding sites in sodium channels to glutamate produced a channel with many of the features of a calcium channel, including nonspecific cation currents in the absence of Ca^{2+}, block by Ca^{2+} (albeit with lower affinity than a real calcium channel), and permeation by Ca^{2+} and Ba^{2+} at millimolar concentrations (Heinemann *et al.*, 1992). That remarkable result implied that the basic mechanism of selectivity in calcium channels lies dormant in sodium channels as well.

The next step was to mutate the glutamate residues in the calcium channel pore. The most striking result was that mutations, even of a single glutamate, could eliminate high-affinity binding of Ca^{2+} (Yang *et al.*, 1993). That challenged the models for permeation, which assumed the existence of two distinct high-affinity binding sites. One suggestion was that the four glutamates could change their conformation depending on the number of Ca^{2+} ions in the pore, coordinating one Ca^{2+} tightly, or two Ca^{2+} ions less well (Yang *et al.*, 1993). The idea that residues within the pore are so flexible that they dynamically interact with permeant ions on the ~1 μs time scale of permeation has been called the "car wash" model of ion selectivity.

Recently, a model has been proposed where there is a single high-affinity binding site in the pore, with two flanking low-affinity sites (Fig. 2B). To exit the pore, the Ca^{2+} ion takes two small steps out of the center well, not one huge leap. This model also predicts block at micromolar Ca^{2+} and permeation at millimolar, even if there is no electrostatic repulsion between binding sites (Dang and McCleskey, 1998). The channel selects by competition, in the strict sense of competition as mutually exclusive binding. The major difference in the energy profiles experienced by Ca^{2+} and Na^+ is the absence of a high-affinity central binding site for Na^+ (or other monovalent cations). Strikingly, the same model can also explain many features of potassium channel permeation, with a single central high-affinity binding site selective for K^+ over Na^+ (Kiss *et al.*, 1998). This favors the idea that the same fundamental mechanism is responsible for ion selectivity in voltage-dependent sodium, calcium, and potassium channels.

Block

A number of cations block current through calcium channels (Lansman *et al.*, 1986). In terms of Fig. 2, the blocking ion binds to the high-affinity site(s) in the pore with even higher affinity than does Ca^{2+}. The kinetics of block provides additional information about the interactions of ions with the calcium channel pore.

If the high-affinity site is within the membrane electrical field, block will depend on voltage. That is observed for several calcium channel blockers. For example, Cd^{2+} can be forced through the channel at strong negative voltages (Swandulla and Armstrong, 1989), or forced back out to the extracellular side at strong positive voltages (Thévenod and Jones, 1992). Thus, the affinity of Cd^{2+} for the open channel is the highest at intermediate voltages, near 0 mV. Block by Ca^{2+} of current carried by monovalents has a similar voltage dependence (Carbone *et al.*, 1997).

For HVA channels of vertebrates, the closed state of the channel can also be blocked (Swandulla and Armstrong, 1989). Cd^{2+} equilibrates with the channel much more slowly when it is closed, suggesting that the channel can close around a Cd^{2+} ion (Thévenod and Jones, 1992). At strongly negative voltages, the closed channel is blocked more potently than the open channel, suggesting that the closed channel is closed at both ends.

As expected for a competitive interaction, the apparent affinity of blockers depends on the nature and concentration of the permeant ion. Currents carried by monovalent cations are blocked much more potently. For these reasons, it is difficult to compare the potency of block measured under different experimental conditions. For example, the apparent K_i for La^{3+} block of L channels was 14 μM (in 110 mM Ba^{2+}; Lansman, 1990), vs. 20 nM for N channels (in 2 mM Ba^{2+}; Block *et al.*, 1998). Despite these technical issues, there are true differences in block among different calcium channels. On most HVA channels, Cd^{2+} is much more potent than Ni^{2+}, but Ni^{2+} is comparable to Cd^{2+} or even more effective on many LVA channels, the α_{1E} cloned channel, and some R channels (Nara-

hashi *et al.*, 1987; Huguenard and Prince, 1992; Zhang *et al.*, 1993; Elmslie *et al.*, 1994; Todorovic and Lingle, 1998).

Calcium channels are also blocked by H^+. Block is affected by mutation of the crucial glutamate residues in the pore, suggesting that H^+ acts at the site involved in calcium selectivity (Klöckner *et al.*, 1996; Chen *et al.*, 1996). The pK depends on the permeant ion, with pK ~7.5 for L channels conducting Na^+ (Pietrobon *et al.*, 1989), but pK ~6 in 10 mM Ba^{2+} (Klöckner *et al.*, 1996). For N channels, pK = 5.1 with 2 mM Ba^{2+} (Zhou and Jones, 1996). The difference between these pK values and that for glutamate itself (pK = 4.3) may be attributed to environmental effects, and/or involvement of side chains of multiple glutamates in the protonation site (Klöckner *et al.*, 1996; Chen *et al.*, 1996). It is striking that protonation does not completely block the pore, but reduces the conductance, inducing a subconductance state (Pietrobon *et al.*, 1989). This implies that the high-affinity site can simultaneously hold a (blocking) H^+ ion and a second (permeating) monovalent cation.

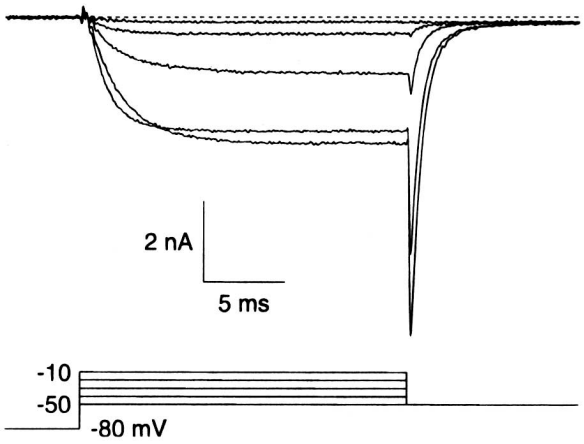

Fig. 3. Activation kinetics of calcium currents in frog sympathetic neurons. Whole-cell currents were recorded with 2 mM extracellular Ba^{2+}, in response to the voltage protocol shown below. The largest inward current during the depolarizing steps was at −20 mV. Note the large, rapidly deactivating tail currents recorded at −50 mV. These records were obtained by students in the 1995 Woods Hole Neurobiology Course. For general methods, see Jones and Marks (1989a) and Werz *et al.* (1993). Under these conditions, these currents are ~85% N-type.

GATING

Activation of HVA Channels

HVA calcium channels activate rapidly upon depolarization, although not as quickly as typical voltage-dependent sodium channels. For N channels of frog sympathetic neurons (Fig. 3), the time constant for activation is ~2 ms in the middle of the voltage range, and is faster at other voltages (Jones and Marks, 1989a; Sala, 1991). There is a slight delay before channel opening, reflected in a sigmoidal time course for the development of inward current upon depolarization. This suggests that the channel must pass through multiple closed states before opening. Empirically, activation is often described by an exponential raised to the power of 2 (Sala, 1991), which taken literally would mean that activation of two identical and independent voltage sensors results in channel opening. If the channel's four S4 regions were identical and independent voltage sensors, a power of 4 would be expected.

A channel opened by depolarization is closed by hyperpolarization. Channel closing upon hyperpolarization is called deactivation, and produces transient currents called tail currents (Fig. 3). HVA calcium channel tail currents are rapid, usually described by a single exponential. This kinetic feature may be critically important for calcium channel function. Rapid channel closing upon hyperpolarization would severely limit the time course of calcium entry following an action potential.

But closing of HVA calcium channels is delayed under certain circumstances, notably following strong depolarization (Pietrobon and Hess, 1990). In one report, delayed calcium channel openings occur for several seconds following a train of action potential-like depolarizations in hippocampal pyramidal neurons (Cloues *et al.*, 1997). This could have significant consequences for cellular signaling processes affected by intracellular calcium. Although strong depolarization can slow closing of P channels (McFarlane, 1997; McDonough *et al.*, 1997), L channels exhibit the most dramatic examples of delayed activity following repolarization. This may involve voltage-dependent phosphorylation of L channels (Sculptoreanu *et al.*, 1993; Dolphin, 1996; but see Kleppisch *et al.*, 1994; Kavalali *et al.*, 1997; Kammermeier and Jones, 1998).

Ca^{2+}-Dependent Inactivation of HVA Channels

During a maintained depolarization (tens of milliseconds to seconds), the initial activation of calcium

channnels is followed by a loss of activity, called inactivation. Early experiments with calcium channels of invertebrates found that the extent of inactivation depended on Ca^{2+} entry, rather than depending monotonically on depolarization (Eckert and Chad, 1984).

Ca^{2+}-dependent inactivation has been most thoroughly characterized for L channels of cardiac and smooth muscle, where it is responsible for a rapid ($\tau \sim 20$ ms) component of inactivation. Ca^{2+}-dependent inactivation has been observed at the single-channel level, suggesting that Ca^{2+} can inactivate the channel through which it enters (Yue *et al.*, 1990). Strong, rapid Ca^{2+} buffering by intracellular BAPTA reduces but does not eliminate Ca^{2+}-dependent inactivation of L channels, further evidence that Ca^{2+} acts in a highly localized fashion (Giannattasio *et al.*, 1991). BAPTA should reduce Ca^{2+} to basal levels within a few nanometers of the mouth of a Ca^{2+} channel, within a few microseconds (Ríos and Stern, 1997). But Ca^{2+} from nearby channels (Yue *et al.*, 1990), and even Ca^{2+} released from intracellular stores via Ca^{2+}-induced Ca^{2+} release (Mitchell *et al.*, 1984), can also contribute to Ca^{2+}-dependent inactivation.

The localized effect suggests that Ca^{2+} might bind directly to the channel. Indeed, Ca^{2+}-dependent inactivation is observed for the cloned α_{1C} channel (but not some other HVA channels) in heterologous expression systems. This involves the C-terminal region of the channel (de Leon *et al.*, 1995), but probably not a putative E–F hand motif (Zhou *et al.*, 1997).

Ca^{2+}-dependent inactivation may depend on cellular factors as well as on the channel type. In invertebrate neurons, even the slow Ca^{2+} buffer EGTA can interfere with Ca^{2+}-dependent inactivation (Eckert and Chad, 1984), suggesting a more diffuse effect. This may result from differences in Ca^{2+} metabolism, as the time course of a transient rise in Ca^{2+} is much slower in neurons than in muscle. Ca^{2+}-dependent inactivation may be fairly rapid (Kohr and Mody, 1991) or very slow (Heidelberger and Matthews, 1992) for L channels of vertebrate neurons. Non-L-type HVA channels do not exhibit a fast Ca^{2+}-dependent inactivation (Jones and Marks, 1989b; but see Cox and Dunlap, 1994), although accumulation of Ca^{2+} or Ba^{2+} in the cell can inhibit the current (Taylor, 1988).

Voltage-Dependent Inactivation of HVA Channels

Calcium channels also inactivate by pathways independent of Ca^{2+} influx. For L channels of muscle, this "voltage-dependent" inactivation is ~ 10-fold slower than Ca^{2+}-dependent inactivation (Kass and Sanguinetti, 1984; Giannattasio *et al.*, 1991). Other HVA channels exhibit varying rates of voltage-dependent inactivation. The α_{1E} and R type channels tend to inactivate rapidly, over tens of milliseconds (Zhang *et al.*, 1993). N channel inactivation is complex and variable. In frog sympathetic neurons, three components have been described, with time constants ~ 15, ~ 150, and ~ 1500 ms, plus a substantial apparently noninactivating component; the fastest component is negligible under basal conditions but is enhanced by phosphorylation (Werz *et al.*, 1993). Some N channels show no detectable inactivation (Artalejo *et al.*, 1992), while others interconvert between inactivating and noninactivating modes (Plummer and Hess, 1991). The P current of Purkinje neurons inactivates slowly, compared to P/Q channels or the α_{1A} clone (Zhang *et al.*, 1993). Inactivation rates can be modulated by coexpression of different β subunits (Singer *et al.*, 1991). For these reasons, the inactivation rate has proven to be treacherous as a criterion for distinguishing HVA channel types (Plummer *et al.*, 1989).

The relatively slow inactivation of HVA calcium channels might suggest that little inactivation would occur in response to typical brief neuronal action potentials. However, the maintained depolarizations typically used to study inactivation are not very physiological. Recently, it has been reported that the α_{1B} channel can inactivate rapidly in response to a train of brief depolarizations (Patil *et al.*, 1998). Such cumulative inactivation is well known for delayed rectifier potassium channels, which also inactivate slowly during a single maintained depolarization (Aldrich *et al.*, 1979). One attractive explanation is preferential inactivation from "partially activated" closed states; that is, states of the channel where some of the voltage sensors are in the activated position, but the channel is not open (Klemic *et al.*, 1998; Patil *et al.*, 1998). Repetitive pulses would optimize occupancy of the intermediate states, while long depolarizations would drive the channels all the way to the open state. This mechanism can also explain the puzzling observation that N channel inactivation is maximal at voltages suspiciously close to those producing peak inward current, even under conditions where Ca^{2+}-dependent inactivation can be excluded (Jones and Marks, 1989b). Occupancy of partially activated states would also be high at intermediate voltages. These results raise the possibility that cumulative inactivation of HVA calcium channels

could occur during a train of action potentials (Patil *et al.*, 1998).

In addition to the inactivation processes described above, which occur on a time scale of a few seconds or less, prolonged depolarization produces a slow inactivation over several seconds or minutes (Schouten and Morad, 1989; Jones and Marks, 1989b). This form of inactivation is little studied, but it can occur at relatively negative voltages, so it may be an important regulator of the resting availability of calcium channels. In *Xenopus* oocytes, slow inactivation of the α_{1B} channel is modulated by coexpression with syntaxin (Bezprozvanny *et al.*, 1995).

Gating of LVA Channels

LVA (T) channels differ considerably from HVA channels in both activation and inactivation kinetics (Huguenard, 1996). Both activation and inactivation of LVA channels occurs at more negative voltages. Relatively small changes in resting potential can significantly affect the availability of LVA channels, and relatively small depolarizations can open them.

The rates of activation and inactivation also differ from HVA channels. Most obviously, LVA channels inactivate rapidly (τ = 10–50 ms), although that can overlap to some extent with α_{1E}/R channels. LVA channels are somewhat slower to activate upon depolarization, although the rapid inactivation makes this difficult to measure, and are ~10-fold slower to deactivate upon hyperpolarization. This slow deactivation is a good diagnostic feature for LVA channels, leading to yet another synonym for them, "SD" or slowly deactivating channels (Armstrong and Matteson, 1985).

MODULATION

Calcium channels are both regulating and regulated. Influx of Ca^{2+} through calcium channels modulates cellular activity; cellular activity modulates calcium channels. This includes long-term modulation of calcium channel expression. But this review will emphasize short-term (seconds to minutes) regulation of the activity of preexisting channels.

A general rule is that L channels have a relatively low level of basal activity, which can be upregulated by a variety of mechanisms. In contrast, N and P/Q channels normally have a high probability of opening upon depolarization, which can be downregulated by a variety of mechanisms.

G protein-Mediated Inhibition of N and P/Q Channels

A variety of neurotransmitters can inhibit neuronal calcium channels (Dunlap and Fischbach, 1981). The most thoroughly characterized pathway involves binding of the $\beta\gamma$ subunits of the activated G protein to N or P/Q channels (Ikeda, 1996; Herlitze *et al.*, 1996). This is associated with several changes in channel gating, which can be summarized by saying that the channel is "reluctant" to open (Bean, 1989). The most robust test for this form of modulation is reversal of inhibition by strong depolarization (Grassi and Lux, 1989; Elmslie *et al.*, 1990) (Fig. 4). One intriguing possibility is that $\beta\gamma$ binding is state dependent: if the $\beta\gamma$ subunits have a higher affinity for the closed state of the channel, strong depolarization would favor dissociation, temporarily reversing the inhibition. Several

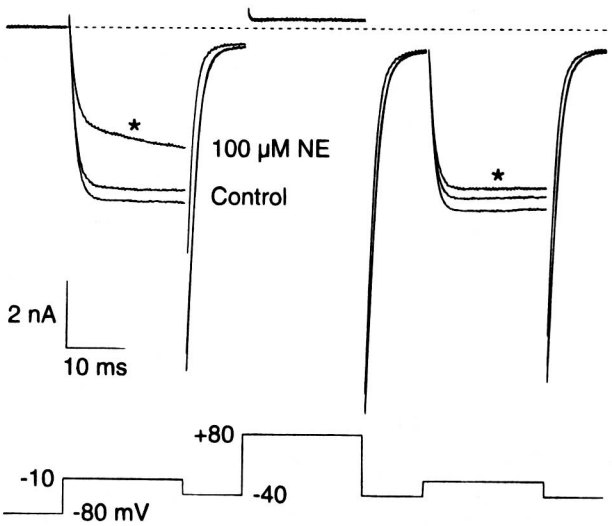

Fig. 4. Modulation of calcium currents by norepinephrine (NE) in frog sympathetic neurons. Three records are superimposed. The two largest inward currents (labeled "Control") were recorded before NE, and after recovery. The third record was recorded during application of 100 μM NE, using a flow tube system (Thévenod and Jones, 1992). Inhibition by NE is apparent during the direct depolarization from −80 to −10 mV (at the left). Note also the slow activation kinetics of the NE-modulated current. In control conditions, a brief depolarization to +80 mV had little effect on the current evoked a few milliseconds later at −10 mV. However, the current in NE was facilitated (with respect to that evoked by the previous step to −10 mV; asterisks), reflecting partial reversal of the effect of NE.

recent reviews can be consulted for more information on the kinetic and biochemical mechanisms of this modulation (Hille, 1994; Jones and Elmslie, 1997; Dolphin, 1998).

In heterologous expression systems, voltage-dependent modulation by G proteins is clearly seen for α_{1A} and α_{1B}, but not α_{1C} (Bourinet *et al.*, 1996). Results with α_{1E} are variable, depending in part upon whether a β subunit is coexpressed (Shekter *et al.*, 1997; Dolphin, 1998). Even for α_{1A} and α_{1B}, G protein-mediated inhibition is reduced by calcium channel β subunits, possibly resulting from an interaction between the binding sites for the G protein βγ and the calcium channel β (De Waard *et al.*, 1997). Possible sites have been found on the I–II loop and on the C-terminus of α_1 subunits (Zamponi *et al.*, 1997; DeWaard *et al.*, 1997; Qin *et al.*, 1997).

In most cases, inhibition is only partially relieved by strong depolarization (Fig. 4). Sometimes, the "voltage-independent" component of inhibition is interpreted as evidence for a distinct voltage-independent pathway, but it could also reflect a low level of steady-state binding of βγ subunits to the open state of the channel (Jones and Elmslie, 1997). On the other hand, it is clear that G proteins can modulate calcium channels by voltage-independent pathways, which may involve second messengers rather than direct binding of βγ to the channel (Hille, 1994; Dolphin, 1998).

Modulation of L Channels

β-Adrenergic agonists, acting via cAMP-dependent protein kinase, increase the activity of L-type calcium channels in the heart (Trautwein and Hescheler, 1990). The effect of β agonists is inhibited by activation of muscarinic acetylcholine receptors, with little effect on activity of L channels under basal conditions (Giles and Noble, 1976; Hartzell, 1988). These effects seem to be less common on L channels of smooth muscle, neurons, and endocrine cells.

FUNCTION

Contribution of Calcium Channels to Electrical Activity

An inward current activated by depolarization can produce an action potential. Classically, this involves sodium channels, but opening of calcium channels also produces an inward current. If the inward current exceeds the outward current through, for example, potassium channels, it will depolarize the cell, opening more calcium channels and producing still more depolarization, that is, a regenerative action potential.

LVA (T-type) calcium channels play an important role in generation of repetitive activity in several cell types, including cardiac pacemaker cells and thalamic neurons (Huguenard, 1996). In the thalamus, a hyperpolarization can remove resting inactivation from LVA channels. Following the hyperpolarization, the LVA channels activate, producing a low-threshold spike, which can trigger a burst of Na-dependent action potentials. The burst is then terminated by rapid inactivation of the LVA channels (and by activation of various potassium channels). If the afterhyperpolarization following a burst is sufficient to remove inactivation from LVA channels, the cell can burst repetitively.

Even when an action potential is triggered by opening of voltage-dependent sodium channels, subsequent activation of HVA calcium channels can increase the action potential duration. This is most clear in cardiac ventricular muscle, and may also occur in neurons. However, in some sensory neurons, action potential broadening results from a sodium channel that is TTX-resistant and Cd^{2+}-sensitive (Schild and Kunze, 1997).

In other neurons, blockade of calcium channels actually increases the duration of the action potential. This results from activation of a large conductance (BK) potassium channel that is activated synergistically by intracellular Ca^{2+} and depolarization (Adams *et al.*, 1982). BK channels are often colocalized with calcium channels, allowing rapid interactions (Roberts *et al.*, 1990). In many neurons, Ca^{2+} influx during action potentials also triggers a slow afterhyperpolarization (AHP), which can last several seconds (Storm, 1990). Slow AHPs result from low-conductance (SK) potassium channels that are voltage-independent but highly sensitive to intracellular Ca^{2+} (Kohler *et al.*, 1996).

Excitation-Secretion Coupling

Ca^{2+} entering a cell through calcium channels can act on a wide range of time and distance scales. Measured with the fluorescent dye fura-2, $[Ca^{2+}]_i$ increases rapidly to ~1 μM following a strong depolarization, and then decays slowly, over several seconds (Hernandez-Cruz *et al.*, 1990). However, Ca^{2+} can

reach much higher concentrations locally. Near the inner mouth of an open calcium channel, $[Ca^{2+}]_i$ can approach millimolar levels (Roberts *et al.*, 1990). Such "nanodomains" extend only a few nanometers from the channel, and the steep concentration gradient dissipates in a few microseconds following channel closure. Considered in "microdomains" near the membrane but away from the channel, $[Ca^{2+}]_i$ is at micromolar levels. These highly localized Ca^{2+} gradients are nearly impossible to measure, but can be estimated from simulations of Ca^{2+} diffusion and binding (Ríos and Stern, 1997). The involvement of nano- or microdomains in a Ca^{2+}-dependent physiological process can be tested using exogenous Ca^{2+} buffers (e.g., EGTA and BAPTA) that bind Ca^{2+} at different rates and/or with different affinity (Adler *et al.*, 1991).

Rapid, localized action of Ca^{2+} is especially important for the release of neurotransmitters (Matthews and von Gersdorff, 1996). There is less than 1 msec of synaptic delay between arrival of an action potential at a nerve terminal and transmitter release (Katz and Miledi, 1965). Termination of release following repolarization is almost as fast. This suggests that Ca^{2+} acts in nanodomains, implying that the machinery involved in transmitter release is molecularly close to the calcium channel. Indeed, such interactions have been detected biochemically (Seagar and Takahashi, 1998; Sheng *et al.*, 1998).

For a voltage-dependent calcium channel to mediate action potential-evoked transmitter release, it must activate and deactivate rapidly. This is true for all HVA channels (except α_{1S}). Evidence is strongest for involvement of N- and P/Q-type channels in transmitter release (Dunlap *et al.*, 1995), although some specialized synapses use L channels (Heidelberger and Matthews, 1992).

Many neurotransmitters act presynaptically to reduce transmitter release, producing presynaptic inhibition. One plausible mechanism is inhibition of voltage-dependent calcium channels (Yawo and Chuhma, 1993). It is tempting to speculate that the time- and voltage-dependence of G protein-mediated calcium-channel inhibition could allow relief of presynaptic inhibition during high-frequency stimulation (Elmslie *et al.*, 1990).

Excitation-Contraction Coupling

α_{1S} plays a unique role in skeletal muscle. Although it can function as a true L-type calcium channel, it primarily acts as a voltage sensor, coupling membrane depolarization to release of Ca^{2+} from the sarcoplasmic reticulum (SR). This appears to involve a direct physical interaction between α_{1S} and the Ca^{2+} release channel of the SR (Franzini-Armstrong and Protasi, 1997). In contrast, in cardiac cells, α_{1C} acts as a calcium channel to produce highly localized Ca^{2+} influx, which triggers Ca^{2+}-induced Ca^{2+} release from the cardiac SR (Cheng *et al.*, 1996; Ríos and Stern, 1997). The intracellular loop between domains II and III is a major determinant of the functional differences between α_{1S} and α_{1C} (Tanabe *et al.*, 1990).

Metabolic Consequences of Calcium Channel Activity

In addition to the rapid effects discussed above, increases in $[Ca^{2+}]_i$ can trigger many enzymatic processes. Influx of Ca^{2+} through voltage-dependent calcium channels has been implicated in synaptic plasticity, and in regulation of gene expression (reviewed by Ghosh and Greenberg, 1995; Bito *et al.*, 1997). Ca^{2+} entry is an attractive mechanism for coupling electrical activity to long-term changes in cell function, including such vital processes as neuronal development, and learning and memory.

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