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NEURONAL CALCIUM CHANNELS: KINETICS, BLOCKADE AND MODULATION

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CONTENTS

I. INTRODUCTION

Voltage-sensitive calcium channels exist in most cell types studied so far. Like other ion pores, calcium channels are membrane spanning proteins consisting of several transmembrane subunits. A principal α_1-subunit forms the ion-conducting pore and is expressed with a variable number of associated subunits (α_2, β, γ) in different cells (Noda *et al.*, 1984; Campbell *et al.*, 1988; Catterall, 1988). The understanding of calcium channel properties has become of importance following the discovery of multiple types of voltage-operated calcium channels in most animal tissues (for a recent review see Tsien *et al.*, 1988; Bean, 1989a).

A major focus in neuronal Ca channel research has been the identification and

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classification of membrane Ca channels according to their biophysical and pharmacological properties and the determination of their role in the control of neuronal activities. Earlier classifications based on kinetic criteria, sensitivity of channel activity to holding potential and single channel properties (Nowycky *et al.*, 1985a) have proven to be insufficient in dealing with Ca channel diversity (Carbone and Lux, 1987a,b; Swandulla and Armstrong, 1988; Aosaki and Kasai, 1989; Plummer *et al.*, 1989). Alternatively, recent pharmacological data seem to provide a more adequate approach to this issue and have stimulated an increasing demand for new ligands of high selectivity for neuronal Ca channels.

In this review we describe the basic properties of neuronal Ca channels and some of the recent advances in the understanding of their diversity and modulation. The description of Ca channel modulation focuses on the action of inorganic compounds, neurotoxins and neurotransmitters, in particular on the modulatory pathways involved in Ca channel–receptor couplings. We have also attempted to critically evaluate existing channel classifications with regard to their usefulness in assigning specific functions to distinct neuronal channel types.

II. Ca CHANNEL TYPES

Neuronal calcium channels are commonly distinguished on the basis of their voltage range of activation. Channels that are activated above −50 mV are usually referred to as "low-threshold" or "low-voltage activated" (LVA, T) and those activated at potentials positive to −10 mV are named "high-threshold" or "high-voltage activated" (HVA, N and L). Channels that exhibit intermediate ranges of activation are usually assigned to either one of the two classes and may represent subtypes of closely related channels.

Calcium channels can be distinguished also on the basis of their sensitivity to drugs (DHP-sensitive, ω-conotoxin-sensitive, Cd^{2+}-blocked or amiloride-blocked), their single channel conductance and their inactivation time course (fully inactivating, rapidly or slowly inactivating). Multiple relaxations during inactivation, however, do not necessarily imply inactivation of more than one channel type. They may rather reflect complex inactivation of an homogeneous population of channels, as has been demonstrated for the high-threshold calcium channel of vertebrate and invertebrate neurons whose inactivation decay is often multiexponential and cannot be fully resolved during short depolarizations (see Carbone and Swandulla, 1991 and below).

III. Ca CHANNEL KINETICS

1. *The Low-Threshold Channel*

LVA (T-type) calcium channels are a unique class of calcium channels with stereotyped inactivation kinetics, single channel conductance and pharmacological properties. Besides in central and peripheral neurons, they exist in cardiac, skeletal and smooth muscle cells, fibroblasts, osteoblasts, oocytes and secretory cells. In excitable tissues the LVA channels are likely to be involved in pacemaking and rhythmic activity (Llinas and Yarom, 1981; Bean, 1985). Detailed lists of the many reports on this matter have been given in recent reviews and books (Morad *et al.*, 1988; Wray *et al.*, 1989).

(a) *Activation kinetics*

In most neurons low-threshold Ca channels activate at potentials near −60 mV (Carbone and Lux, 1984a; Bossu *et al.*, 1985; Fedulova *et al.*, 1985). Activation of these channels becomes faster and peak amplitude increases with increasing depolarization (Fig. 1a). A 15–20 mV potential variation is usually required to produce an e-fold change of the voltage-dependent probability of channel openings. In neurons possessing only LVA channels, the increasing number of open channels with increasing depolarization is revealed by a net increase of current noise at about −40 mV which decreases with further depolarization (Carbone and Lux, 1987a). The time course of activation is sigmoidal, suggesting two or more sequential closed states of the channel in equilibrium with a final open channel configuration (Fenwick *et al.*, 1982).

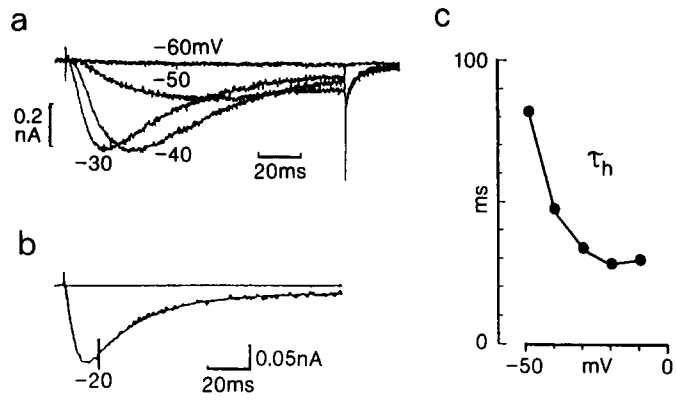

FIG. 1. (a) Whole-cell clamp LVA (T) calcium currents recorded from an embryonic rat DRG neuron at the potentials indicated in 5 mM Ca^{2+}. Holding potential (V_h) −80 mV. Step repolarization to V_h. T = 12°C. (b) An LVA current trace recorded at −20 mV in 5 mM Ca^{2+}. The inactivating phase of the current is fitted with a single exponential with a time constant of 27 msec (τ_h). The beginning of the fitting is indicated by the vertical bar. (c) Plot of τ_h vs voltage for the records of panel b. (From Carbone and Lux, 1986.)

Tail currents associated with LVA channel closing (or deactivation) can be fitted by single exponentials with time constants in the range of milliseconds that decrease with more negative repolarizing steps (Matteson and Armstrong, 1986; Carbone and Lux, 1987a). Deactivation time constants are independent of the duration of the preceding depolarization, of the degree of LVA channel inactivation and of the type of cations flowing through open LVA channels (Lux *et al.*, 1990). Taken together this indicates that, based on activation–deactivation kinetics, neuronal LVA channels form a rather homogeneous class of Ca channels which obey open–closed kinetic schemes similar to those described for other voltage-dependent channels, such as Na and K channels (Fenwick *et al.*, 1982; Hille, 1984).

(b) *Inactivation kinetics*

Inactivation of LVA channels develops monoexponentially, is complete within 100 msec and shows a strict dependence on voltage (Fig. 1b, c) (Carbone and Lux, 1984a; Bossu *et al.*, 1985; Fedulova *et al.*, 1985). In chick dorsal root ganglion (DRG) neurons (5 mM external Ca^{2+}), maximal voltage sensitivity of the inactivation rate occurs between −40 and −30 mV, changing e-fold with a 14 mV potential variation (Carbone and Lux, 1987a). In mammals, inactivation of LVA channels is nearly two times faster than in avian LVA channels suggesting species-specific variations in their kinetic properties (Bean, 1985; Bossu and Feltz, 1986; Carbone and Lux, 1987a; Droogmans and Nilius, 1989; Hirano *et al.*, 1989; Carbone *et al.*, 1990b).

Inactivation kinetics of LVA channels is independent of Ca-influx through the channel. This is best proven by comparing the time course of LVA currents recorded on step depolarizations from increasingly negative holding potentials (−60 to −110 mV; see Section III.1.(c)). The corresponding LVA currents of different size, and thus different Ca-entry, have nearly identical inactivation time courses (Carbone and Lux, 1984a). The independence on Ca-entry of LVA channel inactivation becomes particularly obvious when Na^+ is the main current-carrying ion. Replacing Ca^{2+} with Na^+ causes a nearly 4-fold increase of the low threshold current size with little effects on its activation–inactivation kinetics (see Fig. 1 in Carbone and Lux, 1988). Similar findings have been reported for the low-threshold calcium channel of mouse neoplastic B lymphocytes (Fukushima and Hagiwara, 1985). Activation and inactivation kinetics of this channel are unaffected when the external Ca^{2+} is replaced by Na^+ and the size of corresponding Na currents is reduced by micromolar additions of external Ca^{2+} or Mg^{2+} (see Lux *et al.*, 1990).

The similarity of LVA channel inactivation with that of other voltage-dependently

inactivating channels extends to its temperature sensitivity. In chick DRG neurons a temperature change from 20 to 30°C causes a 2.2-fold acceleration of the LVA inactivation time course (Q_{10} 2.2) and a comparable acceleration of the activation process (Nobile *et al.*, 1990). Somewhat larger values have been reported for the LVA channel in mouse neuroblastoma cells (Q_{10} 2.5, Narahashi *et al.*, 1987) and rat thalamic relay neurons (Q_{10} 2.8, Coulter *et al.*, 1989) for the same range of temperature. These values compare well with those of sodium channel inactivation (Q_{10} 2.3 to 3) in peripheral neurons (Chiu *et al.*, 1979; Kimura and Meves, 1979) and cardiac cells (Colatsky *et al.*, 1980), but are markedly smaller than those for HVA channel inactivation ($Q_{10} > 9$, Nobile *et al.*, 1990). Thus, temperature reveals differences between the inactivation gating of HVA and LVA channels, suggesting close activation energies for similarly voltage-dependent inactivation processes.

(c) *Voltage-dependent recovery from inactivation*

LVA currents inactivate completely after prolonged depolarizations to potentials above resting values (> -60 mV). LVA channels, however, recover from inactivation, as do HVA channels, provided that the membrane is maintained at negative membrane potentials (-80 to -100 mV) for sufficiently long periods of time. Hyperpolarizations of 200 msec to -100 mV following steady depolarizations to -30 mV are already sufficient to elicit again sizeable LVA currents. Maximal current amplitudes are reached with hyperpolarizations of about 6 sec duration (Carbone and Lux, 1987a).

As with sodium channels, recovery from inactivation of LVA channels in vertebrate neurons is time- and voltage-dependent. At -80 mV, Ca currents recover monoexponentially with a τ of 1.4 sec. At -120 mV, the recovery is faster and τ falls to 0.4 sec. Comparable values are reported for the low-threshold channels of rat hypothalamic and thalamocortical neurons (Akaike *et al.*, 1989a; Coulter *et al.*, 1989) and cells of lateral geniculate nucleus (Crunelli *et al.*, 1989). Detailed studies in rat cranial ganglia (Bossu and Feltz, 1986) have shown that recovery from inactivation may develop with one or two time constants depending on whether short (several hundreds of milliseconds) or long (tens of seconds) predepolarizations have been applied (see Akaike *et al.*, 1989b). Fast recovery occurs following large but brief depolarizations, whereas slow recovery develops after long inactivating prepulses of small amplitude. Bossu and Feltz (1986) concluded that the two components of recovery are independent processes and that the slow one may depend on Ca^{2+}. Recent studies on LVA channels in canine cardiac Purkinje cells, however, show that recovery from inactivation of LVA channels follows complex kinetics that depend strictly on voltage but not on Ca^{2+} (Hirano *et al.*, 1989).

(d) *Single low-threshold Ca channels*

Single LVA calcium channel openings can be resolved in excised as well as in cell-attached membrane patches of a variety of cells using high extracellular Ca^{2+} or Ba^{2+} concentrations (40 to 100 mM) (Carbone and Lux, 1984b; Nilius *et al.*, 1985). These ionic conditions are required to resolve the unitary LVA current events that are particularly small with Ca^{2+} as the current carrying ion (0.3 to 0.7 pA). Despite the low signal-to-noise ratio of the recordings at these favourable ionic conditions, single LVA channel events can be identified by their sensitivity to extracellular Ca^{2+}, voltage-dependent activation–inactivation kinetics and sensitivity to holding potential. Their prolonged activity in excised membrane patches suggests that, contrary to HVA channels, there is no particular metabolic requirement for their functioning.

LVA channel activity starts near resting potential showing discrete inward going current events with virtually no sign of channel inactivation during pulses of 200 msec duration (Fig. 2). At -40 mV, channel activity and the average number of openings per trace increases considerably. Events are separated by short- and long-lasting closures which indicate fast and slow bursting activity of the channel. Time-dependent inactivation becomes evident at higher membrane voltages (> -20 mV) where the probability of channel openings is higher shortly after the onset of the step than it is at later times. There is a close correlation between single channel currents and inactivation kinetics.

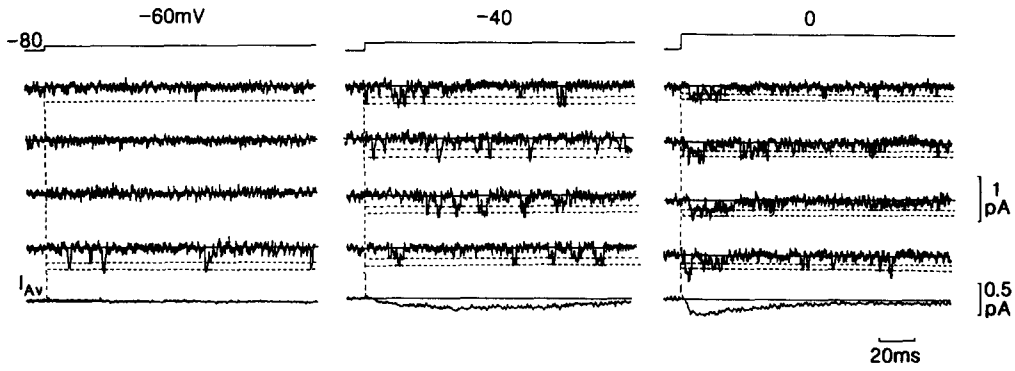

FIG. 2. Unitary LVA (T) calcium currents in an outside-out patch from a chick DRG neuron in 50 mM Ca^{2+} recorded at the potentials indicated. Horizontal dashed lines represent the mean amplitudes of channel openings. The last trace (I_{Av}) at −60, −40 and 0 mV repesents the average over 30, 68 and 41 samples, respectively. Note the voltage-dependent time course of current activation and inactivation. $V_h = -80$ mV. (From Carbone and Lux, 1987b.)

The activation and inactivation kinetics of LVA channels are preserved when the external free Ca^{2+} concentration is reduced to submicromolar values, and extracellular choline is replaced by Na^+ or Li^+ (Fig. 3). Unitary sodium currents are larger but show similar sensitivity to holding potentials compared to unitary LVA Ca currents. They are blocked by addition of 5 mM Ni^{2+} or 1 mM Cd^{2+} to the bath, and are unmeasurable in the absence of

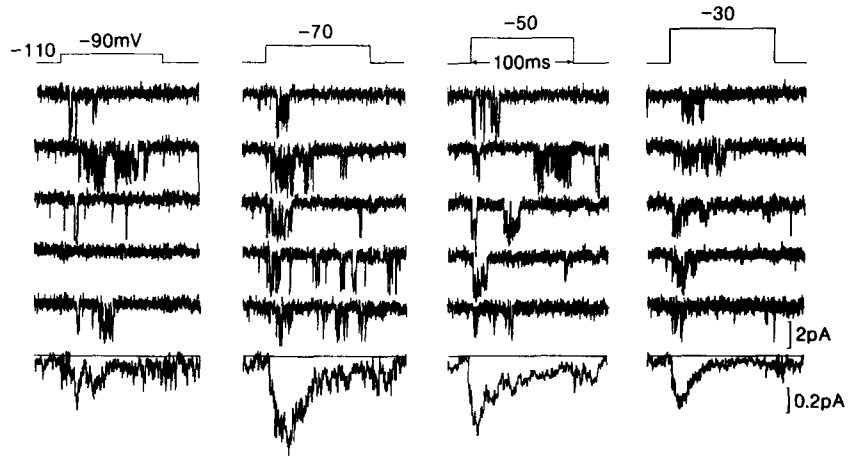

FIG. 3. Unitary LVA sodium currents in an outside-out patch from a chick DRG neuron recorded at the potential indicated. The bath contained 3×10^{-8} Ca^{2+}, 140 mM Na^+, 10 mM Na-HEPES and 3 μM TTX. The last trace of each panel represents the current average over 60 (−90 mV), 65 (−70 mV), 58 (−50 mV) and 30 (−30 mV) samples. 3 kHz low-pass filter. Sampling rate: 36 μsec per point. Note the increased unit sizes of the events compared to those of Fig. 2 and the rapid bursting kinetic during openings. (From Carbone and Lux, 1987b.)

alkali metal ions. These findings indicate that monovalent and divalent ions cross LVA channels without interfering with the voltage-sensitive activation–inactivation gates of the channel.

(e) *A kinetic model for the LVA channel*

As shown above, the activation and inactivation time courses of isolated LVA currents are strictly voltage dependent. Activation develops sigmoidally while inactivation appears to be monoexponential and much slower than activation. Single-channel and whole-cell LVA currents of chick DRG neurons can be well accounted for by the following kinetic scheme (Carbone and Lux, 1987b):

$$\begin{array}{ccccc} & & \mathrm{I} & & \\ & \nearrow & \uparrow & \nwarrow & \\ \mathrm{C_1} & \rightleftarrows & \mathrm{C_2} & \rightleftarrows & \mathrm{O} \end{array} \qquad (1)$$

The scheme is derived from "the basic kinetic model" adopted for the fast sodium channel of neurons (Bezanilla and Armstrong, 1977; Vandenberg and Horn, 1984; Kunze *et al.*, 1985). It includes an open state (O), two closed (C_1 and C_2) and one inactivated state that is assumed to be absorbing (Aldrich *et al.*, 1983) since LVA channels inactivation is nearly complete after sufficiently long depolarizations. The channels are assumed to stay in state C_1 at rest and the rate constants of all reactions are voltage dependent.

The open state O is the resultant of two distinct open states of the channel with comparable occupational probability. The two open states indicate the existence of subconductive levels that seem to be a common feature of calcium channels (Carbone and Lux, 1987b; Droogmans and Nilius, 1989; Lacerda and Brown, 1989; Plummer *et al.*, 1989), rather than the result of unresolved short openings due to limited frequency resolution. Maximal slope conductances of 5.2 and 3.7 pS are observed in outside-out patches of chick DRG with 40 mM Ca^{2+} (Carbone and Lux, 1987b), that compare well with those of the cardiac LVA channel in cell-attached patches: 6.8 and 3.4 pS with 110 mM Ca^{2+} (Droogmans and Nilius, 1989).

Neuronal (Carbone and Lux, 1984b, 1987b; Nowycky *et al.*, 1985a) and cardiac low-threshold calcium channels (Nilius *et al.*, 1985; Droogmans and Nilius, 1989) have closely related kinetic features that can be summarized as follows:

(1) The channels can enter two open states whose mean lifetimes (1 and 3 msec) are weakly dependent on voltage.

(2) Openings occur in bursts, i.e. with a high probability of reopenings, and the first latency of opening is long lasting and strongly voltage dependent. A consequence of this is that channel activation derives its voltage sensitivity mainly from the strong voltage dependency of transition $C_1 \rightleftarrows C_2$.

(3) The channel reopens several times before it inactivates, suggesting that macroscopic inactivation is not the result of appropriately delayed first openings but occurs independently of channel activation. Inactivation may also proceed from closed states attained before and after the open state. In other words, the channel can inactivate without opening.

(4) In chick DRG neurons, inactivation of LVA channels is weakly coupled to activation and derives its voltage sensitivity from its inherent voltage-dependent gate; this is very likely associated to some charged groups within the channel protein.

(5) Macroscopic inactivation and mean burst duration of LVA channels in avian neurons last longer than in mammalian DRG and cardiac cells, supporting the view that in mammals inactivation of LVA channels is at least partially controlled by microscopic activation and comparable to that of fast sodium channels (Aldrich *et al.*, 1983; Vandenberg and Horn, 1984).

2. *The High-Threshold Channel*

HVA channels are widely distributed in neuronal, muscle and secretory cells. Besides their high-threshold of activation, they can be identified by their unique type of inactivation kinetics that is voltage- and Ca^{2+}-dependent and by their single channel conductance that is two- to three-fold larger than that of the LVA channel. Although complex, the dual nature of inactivation provides the basis for the understanding of most of the regulatory actions of these channels in various cellular functions.

(a) *Activation kinetics*

At physiological Ca concentrations, high-threshold Ca channels turn on at potentials near −30 mV. During an activating voltage step that lasts several milliseconds the current reaches a peak and then declines as channels inactivate. On return to the initial membrane potential open channels close and the calcium current turns off or deactivates. Figure 4a and b shows the voltage-dependent inward currents carried by Ca^{2+} and Ba^{2+} ions through Ca channels

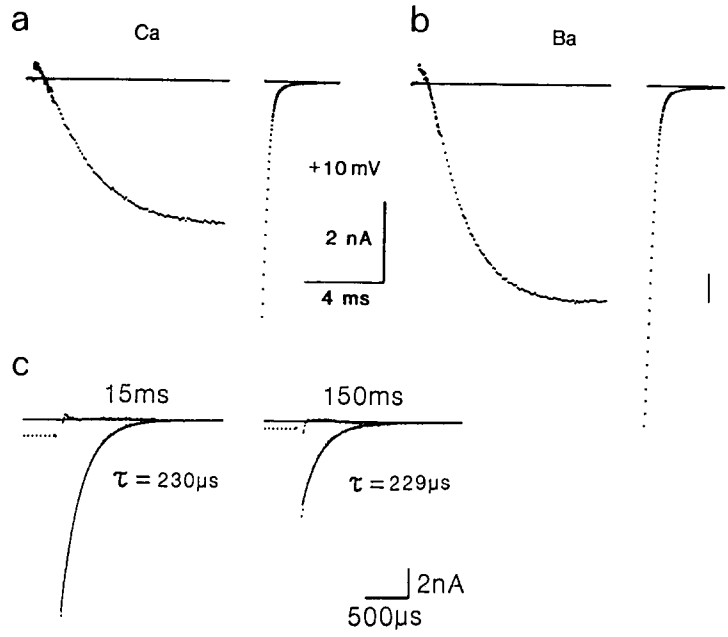

FIG. 4. Whole-cell calcium (a) and barium (b) currents recorded from a chick DRG neuron at +10 mV. Test pulses (10 msec) were from −80 mV. External solutions contained 10 mM Ca^{2+} and Ba^{2+} respectively. Note the different current scale for tail currents (indicated in b). $T = 20°C$. (c) Tail current kinetics during HVA current inactivation in 5 mM Ba^{2+}. Tail currents were recorded on return from +20 to −60 mV following activation for the duration indicated. Single exponentials were fitted to the tail current decay (solid lines) with τ as indicated. The upper traces are the residual currents left after subtraction of the fitted curves from the tail currents. (From Swandulla and Armstrong, 1988.)

in a chick DRG neuron. During depolarizations from rather negative holding potentials (−80 mV) at which nearly all channels are available for activation, i.e. not inactivated, whole-cell Ca currents activate with a sigmoidal time course and reach their maximal amplitude within several milliseconds. The time to peak (t_p) and peak current amplitude (I_p) are both steeply voltage dependent. t_p decreases monotonically with more positive potentials. I_p increases from −30 to 0 mV to decrease again at more positive potentials. Inward currents are preceded by a sizeable initial transient of outward current that may represent the calcium channel gating current (Kostyuk *et al.*, 1977; Adams and Gage, 1977) that results from the displacement of charged groups associated to the channel protein in response to step changes of membrane voltage.

Tail currents in Ca^{2+} as well as Ba^{2+} can be very well fitted by a single exponential function with slightly different τ: 170 μsec in Ca^{2+} and 235 μsec in Ba^{2+} (Swandulla and Armstrong, 1988). In chick sensory neurons, tail currents develop with the same kinetics when recorded early or late during inactivation of HVA currrents induced by a sustained (300 msec) depolarizing pulse (Fig. 4c). This suggests that the complex inactivation kinetics observed in these neurons are due most likely to the inactivation of one predominant class of HVA channels (Swandulla and Armstrong, 1988; see below).

(b) *Inactivation kinetics*

Inactivation kinetics of HVA channels differ sharply from those of LVA channels in most neurons (Fig. 5). Inactivation develops on a much slower time scale and is largely incomplete when Ba^{2+} is the main current-carrying ion or the cell is loaded with EGTA-Ca^{2+} buffers. This suggests that Ca^{2+} plays an important role in the control of Ca channel inactivation (Eckert and Chad, 1984; for a recent review see Carbone and Swandulla, 1991). In a variety of neurons, however, HVA channel inactivation has been shown to be dependent also on membrane voltage (see e.g. Brown *et al.*, 1981; Akaike *et al.*, 1988; Gutnick *et al.*, 1989).

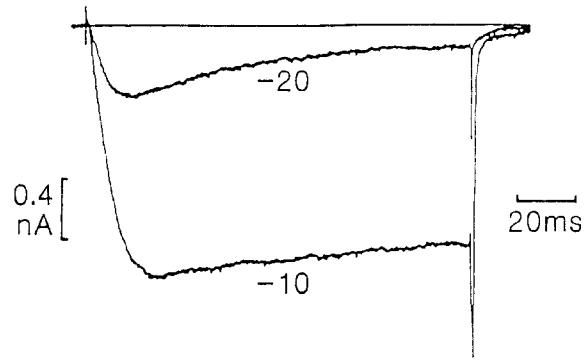

FIG. 5. Comparison of LVA and HVA calcium current inactivation in a chick DRG neuron in 5 mM Ca^{2+} at the potential indicated. $V_h = -80$ mV. (From Carbone and Lux, 1986.)

(c) *Voltage- and Ca^{2+}-mediated inactivation*

An example of voltage- as well as Ca^{2+}-dependent inactivation of HVA channels has been recently studied in detail in snail neurons where the size of the cells (100 μm diameter) allows better intracellular manipulations than in vertebrate neurons (Gutnick *et al.*, 1989). Inactivation of these channels has strong similarities to those of other neuronal Ca channels. The current relaxation exhibits an initial fast phase followed by a slower decline (Fig. 6a). Inactivation kinetics can be well approximated by a double exponential function with two time constants of about 30 and 300 msec respectively with activating pulses to +30mV at 30°C. Replacing Ca^{2+} with Ba^{2+} as the charge carrier results in a slowing of inactivation kinetics, which is clear indication for a Ca^{2+}-dependent inactivation process (Fig. 6b).

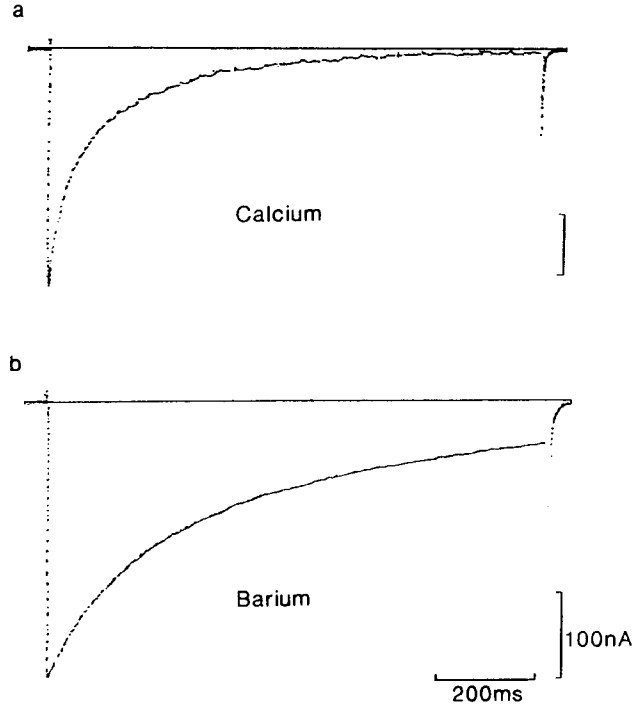

FIG. 6. Inactivation of whole-cell calcium current in an *Helix pomatia* neuron in 40 mM Ca^{2+}. Activating pulses were from V_h −50 mV to +30 (a) and +20 mV (b). The current in (b) was recorded 10 min after substituting Ca^{2+} with 40 mM Ba^{2+} isosmotically. $T = 30$°C. (From Gutnick *et al.*, 1989.)

Inactivation can also be visualized when comparing the currents activated with two sequential voltage pulses. If the interval between the pulses is short and does not allow for substantial recovery of inactivated channels, inactivation is evident as a decrease of the peak current amplitude during the second activating pulse. By changing the amplitude of the first activating pulse (prepulse, P_1), Ca^{2+} entry during this period can be varied and can be correlated to the peak current amplitude associated with the second pulse (test, P_2) (see inset in Fig. 7). As shown in Fig. 7, the degree of inactivation (filled triangles) increases to a

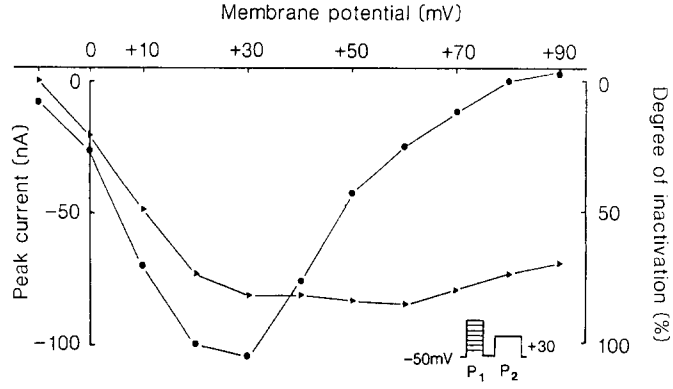

FIG. 7. *I–V* relationship (dots) for peak HVA calcium currents (40 mM Ca^{2+}) and degree of inactivation as a function of prepulse amplitude (triangles). Activating pulses were from V_h −50 mV. Prepulses (P_1, 60 msec) to various potentials were followed by test pulses (P_2) to −30 mV. Between the pulses the cell was repolarized for 10 msec to −50 mV (see inset). Peak currents recorded during the test pulses (I_2) were normalized to peak current of an unconditioned test pulse (I_1) and plotted as $(1-I_2/I_1)$ (% degree of inactivation) against prepulse voltage. (From Gutnick *et al.*, 1989.)

maximum at +60 mV, and then declines slightly at more positive potentials. At +90 mV, however, inactivation is still substantial (70%) even though Ca^{2+} entry is almost zero, as can be seen from the corresponding *I–V* relationship of the peak Ca current induced by P_1 (filled circles).

These findings deviate substantially from those reported in other neurons mainly with regard to the high degree of inactivation observed at very positive potentials (+50 to +90 mV) where calcium currents are small (Tillotson, 1979; Plant and Standen, 1981; Eckert and Chad, 1984). Such discrepancy could arise from the different pulse protocols used in these experiments. Prolonged interpulse intervals (100 to 400 msec) and short prepulses (50 to 300 msec) favour a substantial recovery of HVA channels between activating pulses that may result in a more U-shaped inactivation curve (Brown *et al.*, 1981; Gutnick *et al.*, 1989). Besides that, Ca^{2+}-sensitivities of HVA channel inactivation may vary in different neurons (Yatani *et al.*, 1983; Akaike *et al.*, 1988; Kasai and Aosaki, 1988; Jones and Marks, 1989). This variability includes also cardiac (Fischmeister *et al.*, 1981; Marban and Tsien, 1982; Kass and Sanguinetti, 1984; Lee *et al.*, 1985; Hadley and Hum, 1987; Argibay *et al.*, 1988; Campbell *et al.*, 1988) and secretory cells (Satin and Cook, 1989), where Ca channel inactivation has been reported to be Ca^{2+} and voltage dependent.

(d) *Voltage- and Ca^{2+}-dependent recovery from inactivation*

The most convincing evidence for the voltage dependence of the inactivation process comes from the finding that the recovery of inactivated channels (repriming) is sensitive to membrane potential (Yatani *et al.*, 1983; Gutnick *et al.*, 1989). Brief hyperpolarizations to −100 mV superimposed on a sustained depolarizing step to +30 mV evoke Ca currents that inactivate substantially less even though Ca^{2+} entry is about twice of that during the sustained pulse (Fig. 8a). From these results it is obvious that hyperpolarization enhances recovery from inactivation and that Ca^{2+} entry is not the sole mechanism affecting

inactivation kinetics. The role of Ca^{2+} in the inactivation process becomes evident when determining the relationship between the internal Ca^{2+} concentration and the time course of HVA channel inactivation. The relationship between free $[Ca^{2+}]_i$ and inactivation can be determined by introducing Ca^{2+} buffers such as EGTA, HEDTA and BAPTA intracellularly. As shown in Fig. 8b, injections of Ca^{2+} buffers with free levels of Ca^{2+} in the

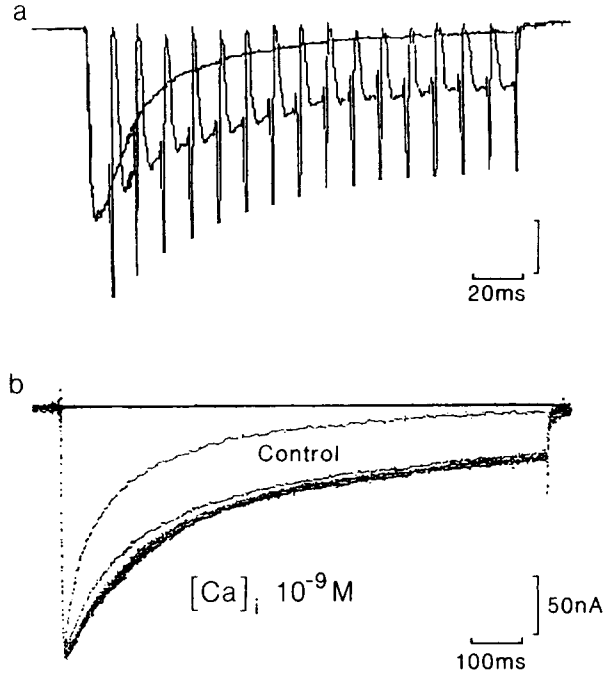

FIG. 8. (a) Recovery from HVA channel inactivation is voltage-dependent. The HVA current induced by a sustained depolarizing step from −50 to +30 mV was superimposed on the current generated by a depolarizing step that was frequently interrupted by 4 msec intervals of hyperpolarization to −110 mV. $T = 18°C$. (b) Effect of internal calcium buffering on inactivation. The cell was injected with EGTA-Ca^{2+} buffer to fix $[Ca^{2+}]_i$ at the level indicated. Inactivation was slowed with subsequent injections. $T = 20°C$. (From Gutnick *et al.*, 1989.)

nanomolar range slow down inactivation particularly by reducing the fast phase of Ca current decay. Inactivation appears also rather incomplete even with longer depolarizations.

Recovery from inactivation is also dependent on free $[Ca^{2+}]_i$ (Yatani *et al.*, 1983; Gutnick *et al.*, 1989). The ability of hyperpolarizing pulses to restore inactivated Ca currents is strongly enhanced in cells that had been injected with large amounts of Ca^{2+} buffers to keep free $[Ca^{2+}]_i$ low. Under these conditions the speed and the effectiveness of repriming is considerably increased. A plot of maximal amplitude of reprimed currents vs $[Ca^{2+}]_i$ shows that the ability of the hyperpolarizing interval increases steeply when $[Ca^{2+}]_i$ is less than 1×10^{-7} M (Gutnick *et al.*, 1989).

From the above it is obvious that HVA channel inactivation in snail neurons does not depend simply on the accumulation of Ca^{2+} near the inner membrane surface but is rather influenced by preexisting intracellular levels of Ca^{2+}. These findings are in line with observations on single calcium channels on the same preparation. Single Ca currents recordings have shown that inactivation of whole-cell Ca currents is associated with a decrease in the probability of channel opening and that inactivation of single channel currents is only weakly related to prior Ca^{2+}-entry through the channel (Lux and Brown, 1984a). In conclusion, these findings indicate that inactivation of HVA channels is not simply due to a build up of internal Ca^{2+} during channel activity but depends on Ca^{2+} and voltage in a rather complex manner.

(e) *Single high-threshold Ca channels*

Single HVA Ca channels were first resolved in cell attached patches of neuronal (Lux and Nagy, 1981; Brown *et al.*, 1982; Hagiwara and Ohmori, 1982), secretory (Fenwick *et al.*, 1982) and cardiac cells (Reuter *et al.*, 1982; Cavalié *et al.*, 1983). These early reports suggested the existence of a single calcium channel type with activation–inactivation kinetics and sensitivity to Ca^{2+} and Ba^{2+} similar to that of macroscopic HVA calcium currents previously described. Microscopic inactivation of this channel was never complete even after prolonged depolarizations and showed fast and slow phases that varied from cell to cell (Lux and Brown, 1984b; Cavalié *et al.*, 1983). There is also indication of a large single channel conductance in high $[Ca^{2+}]_o$ (9 to 12 pS in 50 mM Ca^{2+}) that nearly doubled with isotonic external Ba^{2+} (20 to 25 pS in 110 mM Ba^{2+}). The channel current is blocked by inorganic calcium channel blockers (Cd^{2+}, Ni^{2+}, La^{3+} etc. . . .) or, as in cardiac tissues, effectively prolonged by the 1,4-dihydropyridine Bay K 8644 (Hess *et al.*, 1984; Brown *et al.*, 1984) and modulated by β-adrenergic stimulation (Reuter, 1983; Bean *et al.*, 1984).

Subsequent reports in vertebrate neurons indicated the existence of a second type of HVA channel (N-type) that differs from the L-type mainly in its higher sensitivity to holding potentials, lower single channel conductance (13 to 15 pS) and faster and strictly voltage-dependent inactivation (Nowycky *et al.*, 1985a,b). There is, however, poor correlation between single channel and macroscopic calcium current recordings (Fox *et al.*, 1987a,b) that impedes a general acceptance of L- and N-type as distinct Ca^{2+} channel types (Carbone and Lux, 1987a; Swandulla and Armstrong, 1988). Besides that, the N-channel cannot be separated from the L-type channel (Tsien *et al.*, 1988). The two channels show equal sensitivity to ω-conotoxin (ω-CgTx) (McCleskey *et al.*, 1987; but see below) and Cd^{2+} (Fox *et al.*, 1987a), and display common resistance to LVA channel blockers (Carbone *et al.*, 1987; Tang *et al.*, 1988).

They also exhibit a similarly high instability in excised membrane patches (Carbone and Lux, 1987b; Fox *et al.*, 1987b). Recent studies have shown that their inactivation may be Ca^{2+} and voltage dependent (Hirning *et al.*, 1988). In some neurons N-channel inactivation is rather incomplete and hardly distinguishable from that of the L-type (DHP-sensitive) channel (Kongsamut *et al.*, 1989).

Other reports also argue against significantly different single channel conductances (Plummer *et al.*, 1989) and sensitivity to holding potential of the two HVA channels (Aosaki and Kasai, 1989; Carbone *et al.*, 1990a,b; Usowicz *et al.*, 1990). In PC12 cells, for instance, in the absence of DHP-agonists, N- and L-type channels are inseparable in terms of their mean open time, unitary conductance and inactivation time course (Plummer *et al.*, 1989). Indeed, it cannot be excluded that the higher conductance of L-type channels in Bay K 8644-treated cells (25 vs 22 pS) may be the consequence of better resolved prolonged openings and increased single channel conductance induced by the DHP (Lacerda and Brown, 1989) rather than a distinct biophysical property of the two channels.

IV. Ca CHANNEL LIGANDS AND BLOCKERS

1. *Blocking Ions*

Polyvalent cations such as Cd^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+} and La^{3+} are potent Ca channel blockers in a variety of preparations (Hagiwara and Byerly, 1982). In neurons, at millimolar concentrations, they cause an unselective block of Ca currents. Their selectivity increases at micromolar concentrations (2 to 20 μM) where their different affinity (K_D) for LVA and HVA channels allows dissection of the two currents. In most neurons 20 μM Cd^{2+} blocks selectively the high-threshold (L and N) Ca currents while leaving the LVA channel unaffected (Fox *et al.*, 1987a). Alternatively, in a number of neurons 40 μM Ni^{2+} depress LVA but not HVA currents (Carbone *et al.*, 1987; Fox *et al.*, 1987a; Crunelli *et al.*, 1989). There are, however, exceptions to this rule that argue against using Ni^{2+}-sensitivity as a criterion to identify transient current components as T-(Ni^{2+}-sensitive) or N-type (Ni^{2+}-insensitive) (Boland and Dingledine, 1989). For instance, LVA currents of adult rat DRG and human neuroblastoma IMR32 cells are only partially reduced by 100 μM Ni^{2+}, despite the fact that

they possess typical features of low-threshold Ca currents (Carbone *et al.*, 1990a, b). This suggests either that, depending on the cell type, functionally similar LVA channels display some variability in their sensitivity to Ca-antagonists or that multiple types of LVA channels coexist in neurons.

Although there are no obvious reasons for the selective action of Cd^{2+} and Ni^{2+} on neuronal Ca channel types, it is likely that both ions exhibit their blocking action by interfering with a binding site inside the pore with different energy profiles or locations for the two channel types. This is also probably the reason for the larger permeability of HVA vs LVA channels for Ca ions and for the different permeability ratio P_{Ca}/P_{Ba} of the two channels: 1.2 for the LVA and 0.4 for the HVA.

A binding site for Cd^{2+} inside the channel is suggested also by the strong voltage dependency of HVA current block by micromolar concentrations of Cd^{2+} (Byerly *et al.*, 1985; Lansman *et al.*, 1986; Swandulla and Armstrong, 1989). As shown in Fig. 9a and b, Ba

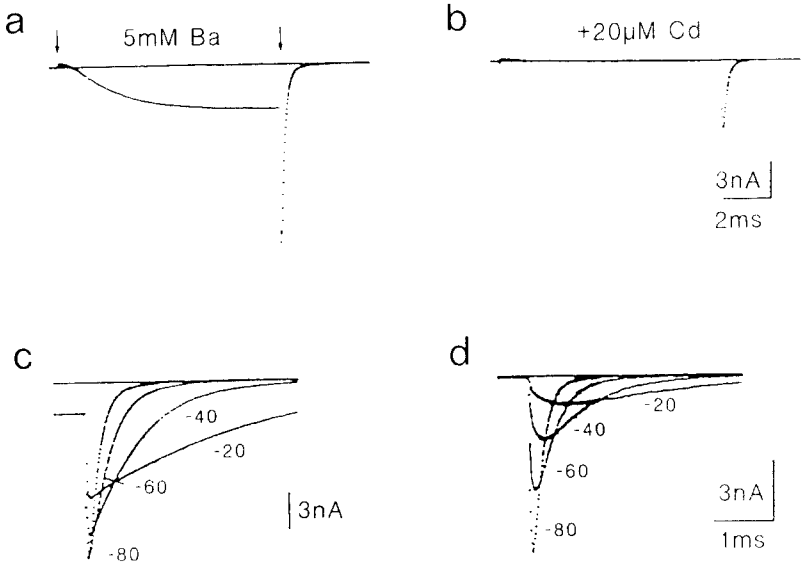

FIG. 9. (a, b) Cadmium does not effectively block tail current through calcium channels. Ba currents were activated by pulses to +10 mV (left arrow) with repolarization to −80 mV (right arrow). Traces were recorded before (a) and during (b) application of 20 μM Cd^{2+}. While pulse currents were almost completely blocked by Cd^{2+}, tail current is still clearly visible. $V_h = -80$ mV. $T = 20°C$. (c, d) Cd^{2+} leaves calcium channels at a detectable rate. Tail currents were recorded on repolarization to the potentials indicated following 10 msec activating pulses to +20 mV. Tail currents in Cd^{2+} (right traces) show hooks, which were particularly prominent at potentials positive to −80 mV. Note the difference in current scales. (From Swandulla and Armstrong, 1989.)

currents are blocked effectively by 20 μM Cd^{2+} during step depolarizations to +10mV. However, a significant tail current is recorded upon repolarization to −80 mV with similar time course to control (Fig. 9b). This suggests that a fraction of blocked channels is cleared from Cd^{2+} with negative voltages and that Cd^{2+} does not interfere with calcium channel closing (Swandulla and Armstrong, 1989). Tail current analysis also shows that clearing of channels is progressively faster and more complete as the potential is made more negative (Fig. 9c, d), indicating that Cd^{2+} lodges at a site inside the channel of high voltage sensitivity. Similar findings are reported for the cardiac Bay K 8644-treated L-type channel (Lansman *et al.*, 1986). During test pulses to 0 mV, 20 μM Cd^{2+} induces a substantial block of unitary Ba currents that is revealed by repeated short closures of the long-lasting openings promoted by Bay K 8644. Tail currents recorded at −70 mV, however, are less affected by Cd^{2+}. The duration of the blocking events is longer, and the degree of block is less pronounced in the presence of Cd^{2+} (Lansman *et al.*, 1986). Similar findings are reported for the block of Ca channels by Zn^{2+} (Winegar and Lansman, 1990). Compared to Cd^{2+}, however, Zn^{2+} causes

a fast flickering block of the open channel suggesting that the rate of Zn^{2+} exit from the channel pore is very rapid (6000 vs 1000 sec^{-1} for Cd^{2+}). This may account for the ability of Zn^{2+} to produce action potentials in snail neurons when present as the only divalent cation in the external solution (Kawa, 1979).

There are no detailed data on the blocking action of Ni^{2+} on LVA Ca currents. Preliminary experiments in adult rat DRG indicate that, at potentials below -40 mV, Ni^{2+} (100 μM) has a strong depressive action on LVA Ca currents (5 mM Ca^{2+}) and that the effect is partly relieved at higher potentials (Carbone *et al.*, 1989). Thus, like Cd^{2+}, block of calcium channels by Ni^{2+} may be also regulated by membrane voltage. This could also explain the selective action of Ni^{2+} on LVA Ca channels, i.e., LVA channels that activate at low potentials (-60 to -40 mV) are more potently blocked by the small but strongly hydrated Ni^{2+} ion that plugs the channel at very negative potentials. HVA channels that activate at more depolarized potentials are less effectively occluded.

Also the lanthanoid gadolinium (Gd^{3+}) is a potent Ca channel blocker. In clonal NG108-15 cells, Gd^{3+} blocks more effectively HVA than LVA Ca channels in a dose-dependent manner (Docherty, 1988). Above 10 μM, blockade of HVA currents is nearly complete and independent of membrane potential. This is at variance with recent findings on cardiac cells where, like Cd^{2+} and La^{3+}, 10 to 100 μM Gd^{3+} are shown to block L-type Ca channels in a voltage-dependent manner (Lansman, 1990). These findings may argue against the proposal of Gd^{3+} as a selective blocker of N-type Ca channels in neurons (Docherty, 1988). Thus, a clarification of Gd^{3+} action on neuronal Ca channels requires further experiments in different neuronal tissues.

2. *Animal toxins*

Animal toxins of high selectivity have proven to be crucial for the identification of structure and function of ion channels in excitable membranes (Hille, 1984). Among them, only few appear suitable to probe neuronal calcium channels (for a recent review see Porzig, 1990). The neuropeptide ω-conotoxin GVIA (ω-CgTx) purified from the venom of the marine snail *Conus geographus* belongs to this category and is at present the most widely used toxin to study neuronal calcium channels (Olivera *et al.*, 1984; Cruz and Olivera, 1986). Neurotoxins purified from spider venoms are now becoming available, which may also prove useful as selective Ca channel blockers in vertebrate neurons (Llinas *et al.*, 1989; Adams *et al.*, 1990; Leung *et al.*, 1990).

(a) *ω-CgTx-sensitive Ca channels*

ω-CgTx is a 27 amino acid peptide that binds specifically to neurons and weakly to heart, skeletal and smooth muscle cells (Kerr and Yoshikami, 1984; Feldman *et al.*, 1987; Bortrain *et al.*, 1988; for a recent review see Gray *et al.*, 1988). Earlier reports have shown that micromolar concentrations of ω-CgTx (3 to 10 μM) block persistently high-threshold Ca channels sparing most of the low-threshold channels (McCleskey *et al.*, 1987). From the observation that the toxin depresses the transient as well as the steady-state component of the HVA currents in chick DRG neurons, it was concluded that ω-CgTx inhibits both transient N-type and long lasting L-type Ca channels (Nowycky *et al.*, 1985a). Recent findings, however, put this interpretation into question and propose a selective action of the toxin on one class of Ca channels with activation–inactivation kinetics closer to the L- rather than to the N-type channel proposed by Nowycky *et al.* (1985a) (Kasai *et al.*, 1987; Kasai and Aosaki, 1989; Plummer *et al.*, 1989; Carbone *et al.*, 1990b; Usowicz *et al.*, 1990). In this view, ω-CgTx-sensitive HVA channels inactivate slowly, incompletely and more in a Ca^{2+} than in a voltage-dependent manner (Fig. 10a), i.e., with quite different kinetic properties than those attributed to the N-type channel. Nevertheless, the ω-CgTx-sensitive channel is often referred to as N-type, probably for no other reason than that of being *neither L nor T*.

Electrophysiological and binding experiments suggest that the density of ω-CgTx-sensitive Ca channels varies largely depending on the type of neuron under study. In peripheral neurons (Kasai and Aosaki, 1989; Plummer *et al.*, 1989; Jones and Marks, 1989; Carbone *et al.*, 1990a, b), saturating concentrations of ω-CgTx (3 to 6 μM) depress by 70 to

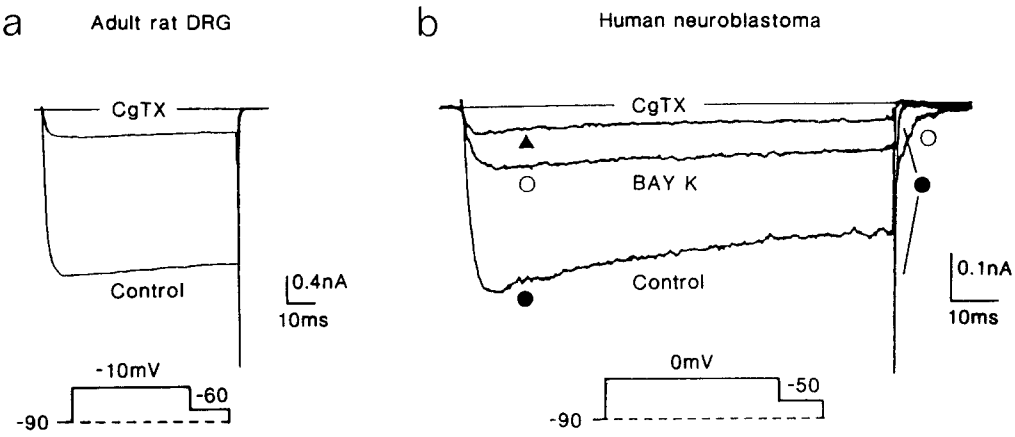

FIG. 10. (a) Incomplete block of HVA barium currents by ω-conotoxin (6.4 μM). The ω-CgTx trace was recorded at the potential indicated, two minutes after drug appplication. (From Carbone *et al.*, 1990a.) (b) Time course of ω-CgTx- and DHP-sensitive Ba currents obtained from a differentiated IMR32 human neuroblastoma cell. The currents were recorded before (filled circles), two minutes after application of ωCgTx (6.4 μM) (filled triangle), and after application of 1 μM Bay K 8644. External Ba^{2+} 10 mM. (From Carbone *et al.*, 1990b.)

90% the HVA current (Fig. 10a, b), while in central neurons the toxin seems to be less effective (40 to 50% HVA current inhibition) (Sah *et al.*, 1989). On the other hand, the distribution of ω-CgTx-binding sites in brain tissue differs significantly from that of dihydropyridines (DHP) (Wagner *et al.*, 1988) and the density of ω-CgTx-binding sites increases in clonal cell lines after cell differentiation (Sher *et al.*, 1988; Usowicz *et al.*, 1990; see also Porzig, 1990). Apart from this, ω-CgTx-sensitive channels are hardly distinguishable in terms of their activation–inactivation kinetics from other HVA channels (DHP-sensitive, see below). Threshold of activation and time course of activation and deactivation do not allow any distinction of HVA channel types (Swandulla and Armstrong, 1988; Kasai and Aosaki, 1989; Carbone *et al.*, 1990b). In vertebrate neurons, inactivation of HVA channels is slow, incomplete and largely Ca^{2+}-mediated. Thus, an apparently fast relaxation of Ca currents followed by a slower one may just reflect complex inactivation kinetics of one Ca channel type rather than different inactivation kinetics of two independent channels, i.e. a rapidly and a slowly inactivating one.

Another interesting property of ω-CgTx is the sensitivity of its binding affinity to external divalent cation concentration (Cruz and Olivera, 1986). With 2 mM external Ca^{2+}, 1 μM ω-CgTx causes nearly maximum inhibition of HVA Ca currents while current depression is fully prevented with 110 mM external Ca^{2+} or Ba^{2+} (Feldman *et al.*, 1987; McCleskey *et al.*, 1987). Blocking conditions of ω-CgTx can change markedly when Na^{+} is the main charge carrier in extracellular solutions containing low Ca^{2+} (Carbone and Lux, 1988). Block of Na currents through HVA channels by ω-CgTx (5 μM) can be reversed by washing and the persistent block of Ca currents in 5 mM Ca^{2+} can be partly relieved when the external Ca^{2+} concentration is maintained below submicromolar levels, even though ω-CgTx remains bound to the channel protein.

(b) *Spider toxins*

Neurotoxins purified from spider venoms are potent ligands of presynaptic and dendritic Ca channels and thus useful for neuronal Ca channel purification (Bowers *et al.*, 1987; Sugimori and Llinas, 1987; Bindokas and Adams, 1989; for a review see also Jackson and Usherwood, 1988). The polypeptide toxin ω-Agatoxin-1A (ω-Aga-1A) isolated from the venom of the funnel-web spider *Agelopsis aspersa* blocks neuromuscular transmission in both insect and frog (Bindokas and Adams, 1989). In rat sensory neurons, ω-Aga-1A (10 nM) inhibits mainly HVA and less effectively LVA Ca channels (Adams *et al.*, 1989). The

depressive action is reported to be fast and more complete when Ba^{2+} (2.5 mM) instead of Ca^{2+} (5 mM) is the current carrying ion. Thus, ω-Aga-1A appears to be a potent inhibitor of Ca channel activity in vertebrate neurons. Present data, however, do not allow to compare its action to that of ω-CgTx and dihydropyridines, with regard to its HVA channel selectivity.

Spider toxins also show some degree of selectivity for HVA Ca current in invertebrate neurons. In cultured neurons of Drosophila, Hololena toxin (HoTX, 50 nM) blocks preferentially a non-inactivating Ca current component, whereas Plectreurys toxin (PLTX) blocks both inactivating and non-inactivating currents (Leung *et al.*, 1989). However, the two pharmacologically identified Ca currents could not be separated by voltage protocols and their biophysical properties remain to be determined (Byerly and Leung, 1988).

A funnel-web spider toxin (FTX) of low molecular weight (200 to 400 Da) has been also reported to act selectively on Ca channels of the presynaptic terminal in squid giant synapse and in mammalian Purkinje cells (Llinas *et al.*, 1989). Both channels were blocked by FTX (0.5 μM), Cd^{2+} and Co^{2+} but were insensitive to ω-CgTx and dihydropyridines. From this it has been concluded that FTX identifies a new type of neuronal Ca channel (P-type) with properties that differ from those of the neuronal Ca channels already described. However, the lack of whole-cell and single channel recordings from the two preparations and of detailed pharmacological tests do not allow direct comparisons with previous data on vertebrate neurons. Although it is conceivable that multiple types of HVA Ca channels may coexist at the neuronal membrane and that new Ca channel types will emerge soon, the proposal of a novel HVA Ca channel subtype, the P-type channel, requires more substantiated studies.

3. *1,4-Dihydropyridines as Ca-Antagonists and Ca-Agonists*

Organic compounds of high affinity for Ca channels are widely used for therapeutic treatment of cardiovascular diseases and cerebral ischemia (for recent reviews see Janis *et al.*, 1987; Janis and Triggle, 1990; Porzig, 1990). More recently, evidence has been accumulating that Ca channel ligands have direct neuronal effects and may be useful in the treatment of some central nervous system disorder, including focal brain ischemia, epilepsy and brain aging (Scriabine *et al.*, 1989). Among Ca channel ligands, the 1,4-dihydropyridines (DHPs) are probably the most frequently used drugs to inhibit or potentiate Ca^{2+} fluxes across plasma membranes. Because of their selectivity and potency, the DHPs have been used as ligands to locate, isolate and purify the high-threshold L-type calcium channel from skeletal muscle (Glossmann *et al.*, 1983; Borsotto *et al.*, 1984; Curtis and Catterall, 1984; Tanabe *et al.*, 1987; Campbell *et al.*, 1988; Ahlijanian *et al.*, 1990).

DHPs identify also a class of high-threshold Ca channels in neurons with characteristics similar to those in muscle tissue (see Tsien *et al.*, 1988). Despite their extensive use in the analysis of stimulus–secretion coupling in neurons (see Morad *et al.*, 1988; Wray *et al.*, 1989), there is a surprisingly limited number of reports on their effects on neuronal Ca channels. One of the reasons is that inhibition of Ca channels by DHPs depends strongly on the cell membrane potential, V_m (Bean, 1984; Sanguinetti and Kass, 1984; Rane *et al.*, 1987). Lowering V_m from −80 to −10 mV in cardiac cells, decreases the apparent K_D of Ca channel inhibition by nitrendipine by about three orders of magnitude (from 0.76 μM to 0.36 nM). Similar findings have been reported for the Ca-antagonist nifedipine in chick sensory neurons (Rane *et al.*, 1987) and nimodipine in rat pituitary cells (Cohen and McCarthy, 1987). Nifedipine (100 nM) depresses by about 60% the high-threshold Ca currents at −30 mV and less than 10% at −90 mV, whereas nimodipine (10 nM) inhibits HVA Ca channels in a time- and voltage-dependent manner. Affinity of nimodipine for open Ca channels is below 1 nM, i.e. close to the low K_D values derived from ligand binding studies using tritiated DHPs (for a recent review see Janis and Triggle, 1990).

The voltage dependency of DHPs's action limits rather strictly the range of test potentials to levels where the drugs act potently on Ca channels (−30 to −50 mV). At these voltages, however, the "run-down" of Ca currents is accelerated by the increased probability of Ca channels to open. This makes it difficult to distinguish DHPs inhibition from an irreversible loss of functioning Ca channels due to run-down. Besides that, the depolarized potentials

required to achieve maximal inhibition by DHPs, confine the selectivity of these compounds to those channels that are available above resting potentials, i.e. the majority of high-threshold channels. Thus, there is no direct test whether DHPs act on LVA channels, that are already inactivated at potentials around −50 mV. There are, however, reports of a specific inhibition of LVA channels by DHPs in central neurons (see below).

Ca-agonists, such as the racemic Bay K 8644 and the pure enantiomers (+)-(S)-202-791 and (+)-PN200-110, overcome some of the above limitations and are often used to identify DHP-sensitive channels in animal tissues (see Reuter *et al.*, 1985). These compounds have high affinity for cardiac and neuronal cells (K_D 10 nM) and their action is maximal at very negative membrane potentials (−90 mV) (Brown *et al.*, 1984; Kokubun *et al.*, 1986; Sanguinetti *et al.*, 1986). The agonistic action of DHPs can be summarized as follows: (1) The drugs cause a marked increase of Ca current amplitude at low membrane potentials (−40 to −20 mV) (see Fig. 11a). The current increase is less pronounced above 0 mV. (2) In the

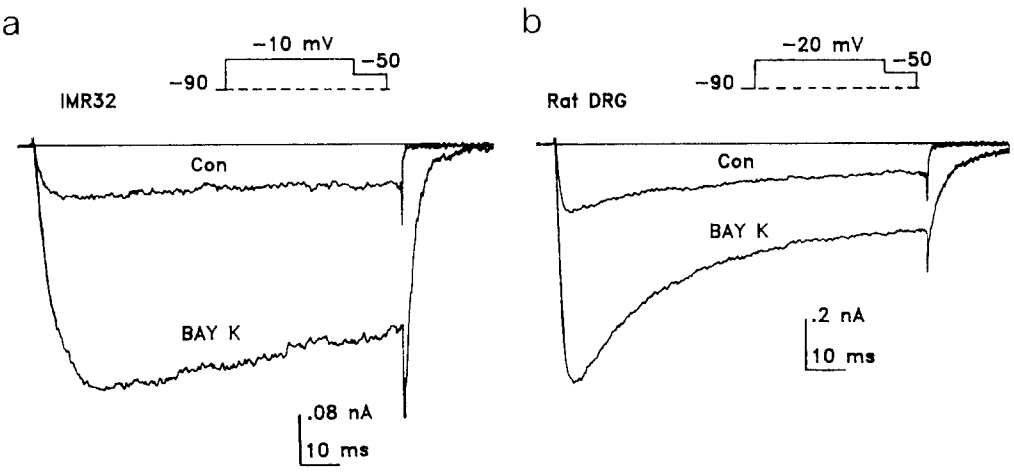

FIG. 11. Action of Bay K 8644 on ω-CgTx-resistant Ba currents in a human neuroblastoma IMR32 cell (a) and an adult rat DRG neuron (b) in 10 mM Ba^{2+}. Current traces were recorded before (Con) and during bath application of 1 μM Bay K 8644. Note the Ba current increase and the prolongation of tail currents at −50 mV in the presence of Bay K 8644. $V_h = -90$ mV. (From Carbone *et al.*, 1989.)

presence of DHPs, *I–V* and steady-state inactivation curves are shifted by 10 to 20 mV toward more negative potentials (Sanguinetti *et al.*, 1986). (3) Ca channel deactivation is prolonged by more than ten times at potentials near −50 mV (Sanguinetti *et al.*, 1986; Hoshi and Smith, 1987). (4) Ca channel openings are prolonged by nearly one order of magnitude with 1 μM Bay K 8644 (Hess *et al.*, 1984; Brown *et al.*, 1984). (5) In cardiac cells and some neurons the inactivation kinetics are accelerated (see Fig. 11b) (Sanguinetti *et al.*, 1986; Brown *et al.*, 1986; Markwardt and Nilius, 1988; Carbone *et al.*, 1990a). (6) Strong depolarizing prepulses increase the probability of prolonged channel openings during step depolarizations to around 0 mV (Hoshi and Smith, 1987; Lansman, 1990). All these effects may derive from a selective action of the Ca-agonists on the voltage-dependent closing rate of the channel (see Section III.2.(a)). A simple reduction or a negative voltage shift of this parameter might be sufficient to account for the above observations, but interpretations based on other kinetic models cannot be discarded (Hess *et al.*, 1984; Kokubun and Reuter, 1984; Brown *et al.*, 1986; Sanguinetti *et al.*, 1986; Lacerda and Brown, 1989).

In neurons the agonistic action of DHPs is more variable than in cardiac cells. In some cases 1 μM Bay K 8644 shows slight or no agonistic action on HVA Ca channels (Dolphin and Scott, 1988; Jones and Marks, 1989). In other cases Bay K 8644 acts as an antagonist when external Ca^{2+} is replaced by Ba^{2+} (Boll and Lux, 1985). As recently suggested, this variability in peripheral neurons may merely reflect the heterogeneity of neuronal Ca channel subtypes; the DHP-sensitive channel being a minority (10 to 30%) of the total Ca channels

available in these neurons. Pretreatment of adult rat sensory neurons with ω-CgTx (3 to 6 μM) reveals Ca currents of comparably smaller size than untreated neurons but with higher sensitivity to Bay K 8644 (Carbone *et al.*, 1990b). An interesting conclusion of these studies is that Ca-agonists could be more suitable than Ca-antagonists for identifying and characterizing DHP-sensitive Ca channels in neurons.

4. *Kinetics vs Pharmacological Criteria for Identifying Ca Channel Subtypes*

From the above arguments it is clear that Ca channel identification using earlier proposed criteria (Nowycky *et al.*, 1985a) may be misleading if not supported by pharmacological tests. The available pharmacological tools, however, require some caution when used to dissect Ca currents components in whole cell recordings (see below). The recurrent use of the holding potential to discriminate N- (transient) from L-type (long lasting) Ca currents may lead to substantially wrong conclusions with respect to the proportion of different channels that contribute to the total current. Lowering the holding potential to less negative values usually results in Ca currents of smaller amplitude and slower inactivation that may be the consequence of a reduced Ca^{2+} entry in the cell, rather than the steady inactivation of a transient Ca channel that is more sensitive to the holding potential. Also the rate of Ca channel inactivation may not be taken as a main criterion to classify Ca channel subtypes. Inactivation of Ca currents is fast or slow independently of the channel sensitivity to DHPs or ω-CgTx. There is also evidence for fast inactivating high-threshold Ca channels in hippocampal neurons that are DHP-sensitive (Toselli and Taglietti, 1990). In ω-CgTx-treated rat DRG neurons, Bay K 8644 (1 μM) induces transient HVA Ca currents that are blocked by Cd^{2+} (40 μM) and are insensitive to amiloride (500 μM) (see Fig. 11b; Carbone *et al.*, 1990a). On the other hand DHP-sensitivity does not seem to be confined to high-threshold Ca channels. Rat hypothalamic neurons are shown to possess DHP-sensitive low-threshold Ca channels (Akaike *et al.*, 1989) and we found that LVA currents of rat DRG neurons are partially potentiated by Bay K 8644 (Carbone, Formenti and Pollo, unpublished).

Despite these limitations, a pharmacological approach to identify Ca channel subtypes appears to be more reliable than criteria based on holding potential and inactivation kinetics. Various groups seem to agree that HVA currents can be divided in at least two classes: one ω-CgTx-sensitive and one DHP-sensitive (Kasai *et al.*, 1987; Sher *et al.*, 1988; Aosaki and Kasai, 1989, Plummer *et al.*, 1989; Carbone *et al.*, 1990b). A rationale for this comes from the following observations. First, saturating concentrations of ω-CgTx (3–6 μM) only partly depress the HVA calcium currents in a variety of neurons. In sympathetic neurons (Plummer *et al.*, 1989; Jones and Marks, 1989), sensory ganglia (Aosaki and Kasai, 1989; Carbone *et al.*, 1990a), and human neuroblastoma (Carbone *et al.*, 1990b), ω-CgTx blocks 70–90% of the total current (Fig. 10b), while in central neurons the toxin seems to spare a larger fraction of the current (50–60%) (Sah *et al.*, 1989). Second, DHP derivatives can either block or potentiate the HVA calcium currents. Their action, however, is more marked in ω-CgTx preincubated neurons, where the agonistic action of the 1,4-dihydropyridine, Bay K 8644, is particularly evident (Fig. 11). The DHP causes a two- to three-fold increase of HVA currents as well as a drastic prolongation of HVA channel deactivation in ω-CgTx-treated neurons but more reduced effects in normal cells (Carbone *et al.*, 1990a).

V. Ca CHANNEL MODULATION

1. *An Historical Overview*

Dunlap and Fischbach (1978, 1981) were first to report the inhibitory action of several neurotransmitters on neuronal Ca channels and to propose this effect as a possible mechanism for presynaptic inhibition. Micromolar concentrations of noradrenaline (NA), γ-aminobutyric acid (GABA) and serotonin (5-HT) decreased the duration of Ca^{2+} spikes and the corresponding Ca current's amplitude in chick sensory neurons. Subsequent findings confirmed and extended these observations to rat sympathetic neurons (Galvan and Adams, 1982) and suggested a marked selectivity for the high-threshold Ca channel of chick sensory neurons (Deisz and Lux, 1985) but no requirements of cAMP, cGMP or Ca^{2+} as second

messengers (Forscher and Oxford, 1985). A voltage-controlled inhibition of Ca currents became evident only after observing the effects of dopamine (DA) and noradrenaline (10 μM) on Ca channels of avian peripheral neurons (Marchetti *et al.*, 1986). DA caused a dramatic slow down of HVA channel activation at low membrane potentials that was accelerated and partly relieved at higher membrane depolarizations (Fig. 12a). Similar findings apply to

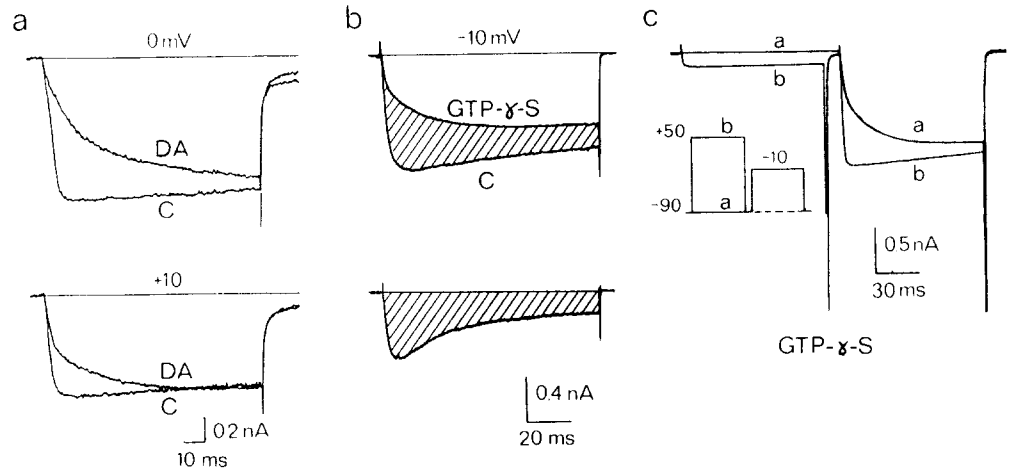

FIG. 12. (a) Effect of dopamine (10 μM) on HVA calcium currents in a chick DRG neuron. Currents were activated with pulses from −80 mV at the potentials indicated. External Ca^{2+} 5 mM. (From Marchetti *et al.*, 1986.) (b) The inhibitory action of GTP-γ-S (100 μM) on HVA calcium currents in adult rat DRG at −10 mV. On top are shown the control (C) and the GTP-modified current traces. Note the prolonged activation kinetics of HVA channels induced by the GTP-analogue. The transient current at the bottom panel represents the algebraic difference of the two above recordings. External Ca^{2+} 5 mM. $V_h = -60$ mV. (c) Voltage-dependent release of GTP-γ-S-induced inhibition of HVA calcium currents in adult rat DRG. The inhibition of calcium currents at −10 mV (trace a) is fully removed by preconditioning the cell with pulses to +50 mV for 100 msec followed by a 10 msec repolarization to −90 mV (V_h) (trace b). External Ca^{2+} 5 mM; GTP-γ-S 100 μM.

somatostatin (Luini *et al.*, 1986; Tsunoo *et al.*, 1986; Ikeda *et al.*, 1987; Sah, 1990), leu-enkephalin (Tsunoo *et al.*, 1986), Ach (Wanke *et al.*, 1987), $GABA_B$ (Grassi and Lux, 1989), adenosine (Kasai and Aosaki, 1989), dynorphin (Bean, 1989b; Sah, 1990), LHRH (Elmslie *et al.*, 1990) and other neurotransmitters and drugs (see Table 1).

Down-modulation of Ca channels by neurotransmitters is usually accompanied by a partial depression of Ca currents and often attributed to a receptor-mediated reaction modulated by G-proteins (Lewis *et al.*, 1986; Holz *et al.*, 1986; Dolphin and Scott, 1987; Hescheler *et al.*, 1987; Wanke *et al.*, 1987). Involvement of GTP-binding proteins in Ca channel modulation is usually supported by one or more of the following observations: (1) The non-hydrolyzable GTP analogue, GTP-γ-S, mimics the inhibitory action of external neurotransmitter (Dolphin and Scott, 1987), (2) Ca channel modulation by neurotransmitter and GTP-γ-S is prevented by cell incubation with pertussis toxin (PTX) (Lewis *et al.*, 1986), (3) intracellular application of purified G-proteins subunits restore the receptor mediated response to PTX-treated cells (Toselli and Lux, 1990), and (4) monoclonal antibodies against G-protein subunits prevent neurotransmitter-induced inhibition of HVA Ca currents (Harris-Warwick *et al.*, 1989; McFadzean *et al.*, 1989).

Alternative mechanisms of Ca channel modulation involve the activation of protein kinase C in a variety of neurons (Holz *et al.*, 1986; Marchetti and Brown, 1988; Lewis and Weight, 1988). Micromolar concentrations of diacylglycerol analogues and phorbol esters decrease the size of high-threshold Ca currents with little change to their activation kinetics. Recent reports, however, show that the depression of high-threshold Ca currents is rapid and unaffected by intracellular application of several protein kinase inhibitors and that the activators are ineffective when applied intracellularly (Hockberger *et al.*, 1989). Thus, the

TABLE 1. NEUROTRANSMITTER-, PEPTIDE- AND GTP-γ-S-INDUCED INHIBITION OF HIGH-THRESHOLD Ca CHANNELS

Transmitter, drug	Action	Neuron type	Refs
Dopamine	+ + +	Chick DRG	Marchetti *et al.*, 1986
$GABA_B$	+ + +	Chick DRG	Deisz and Lux, 1985 Grassi and Lux, 1989
	+ +	Rat DRG	Dolphin and Scott, 1987
Noradrenaline	+ +	Chick DRG	Marchetti *et al.*, 1986
	+ +	Bullfrog DRG	Bean, 1989b
	+ +	Frog SG	Lipscombe *et al.*, 1989
	+ +	NG108–15	McFadzean *et al.*, 1989
Adrenaline	+ + +	Frog SG	Bley and Tsien, 1990
Somatostatin	+ + +	AtT-20	Luini *et al.*, 1986
	+ + +	NG108-15	Tsunoo *et al.*, 1986
	+ + +	Rat SCG	Ikeda *et al.*, 1987
	+ + +	Rat SC	Sah, 1990
Adenosine	+ +	Rat DRG	Dolphin *et al.*, 1986
	+ + +	Chick DRG	Kasai and Aosaki, 1989
Ach	+ +	Rat SCG	Wanke *et al.*, 1987
	+ +	Rat Hippoc	Gahwiler and Brown, 1987 Toselli and Lux, 1989
Leu-enkephalin	+ + +	NG108-15	Tsunoo *et al.*, 1986
DADLE	+ +	NxG 5	Hescheler *et al.*, 1987
	+ +	NG108-15	McFadzean and Docherty, 1989
Dynorphin A	+ +	Mouse DRG	Gross and McDonald, 1987
	+ + +	Bullfrog DRG	Bean, 1989b
	+ + +	Rat SC	Sah, 1990
Serotonin	+ +	Rat SC	Sah, 1990
	+ + +	Rat DRN	Pennington and Kelly, 1990
LHRH	+ + +	Bullfrog SG	Elmslie *et al.*, 1990
	+ + +	Frog SG	Bley and Tsien, 1990
GTP-γ-S	+ + +	AtT-20	Lewis *et al.*, 1986
	+ + +	Rat DRG	Dolphin and Scott, 1987
	+ + +	Rat SCG	Ikeda and Schofield, 1989
	+ + +	Chick DRG	Kasai and Aosaki, 1989; Grassi and Lux, 1989; Marchetti and Robello, 1989

Abbreviations: DRG, dorsal root ganglion; DRN, dorsal raphe nucleus; SC, spinal cord; SG sympathetic ganglion; SCG, superior cervical ganglion.

question remains open whether Ca channel inhibition is due to the action of some protein kinase C activator or by a direct effect of the drug on the channel.

2. *G-Proteins as Regulators of Ca Channel Gating*

There is a striking analogy between some neurotransmitter-induced inhibition and the modulatory action of the intracellularly applied GTP-γ-S (Lewis *et al.*, 1986; Dolphin and Scott, 1987; Wanke *et al.*, 1987; Ikeda and Schofield, 1989; Kasai and Aosaki, 1989; Grassi and Lux, 1989; Marchetti and Robello, 1989; Toselli *et al.*, 1989; Elmslie *et al.*, 1990) (see Table 1). Like external neurotransmitters, intracellular GTP-γ-S (100 μM) causes a progressive prolongation of HVA current activation that appears as a marked depression in the first few msec of the current rise which then relaxes back to the control level with double exponential kinetics. The inhibitory action is concentration dependent and is rate limited by the time required to replace endogenous GDP by GTP. Figure 12b shows an example of down-modulation of HVA Ca channels induced by GTP-γ-S in adult rat DRG. The Ca current recorded at −10 mV after 4 min of intracellular perfusion with GTP-γ-S (100 μM) activates more slowly and reaches a lower level than the control record (C).

There seem to be at least two alternative interpretations to these findings. One is that GTP-γ-S inhibits a transient Ca current component (N-like) (trace at the bottom panel in Fig. 12b) that is maximal at about −10 mV and becomes progressively smaller at more positive membrane potentials (Wanke *et al.*, 1987; Dolphin and Scott, 1987; Lipscombe *et al.*, 1989). This hypothesis predicts that the inhibitory action of GTP-γ-S persists as long as the GTP analogue is internally applied and is insensitive to membrane potential.

Alternatively, GTP-γ-S is thought to delay the activation of HVA Ca channels in a voltage-dependent manner (Marchetti *et al.*, 1986; Tsunoo *et al.*, 1986; Kasai and Aosaki, 1989; Grassi and Lux, 1989; Bean, 1989b; Elmslie *et al.*, 1990). In this way, Ca channels reach steady-state conditions of activation only at the end of a 100 msec pulse at low membrane potentials (-20 mV) but within shorter times at larger depolarizations (10 msec at $+40$ mV). In other words, the slow rise of Ca currents reflects a slow recovery of Ca channels from drug-induced inhibition, that is favoured by very negative membrane potentials (-60 to -100 mV) and relieved by strong depolarizations. This idea is supported by two main lines of evidence: (1) The size of the modulated Ca currents recorded during step depolarizations near 0 mV increases markedly if the pulse is preceded by positive conditioning prepulses (Grassi and Lux, 1989; Elmslie *et al.*, 1990), and (2) the Ca current inhibition is largely relieved and the time course of activation is faster at high membrane potentials (Marchetti *et al.*, 1986; Tsunoo *et al.*, 1986; Kasai and Aosaki, 1989; Bean, 1989b).

Full recovery of Ca channel activation induced by conditioning prepulses in GTP-γ-S-dialyzed neurons is evident using the pulse protocol shown in Fig. 12c. The slowly activating Ca current recorded at -10 mV (V_t from -90 mV) (trace a) turns on more quickly and reaches a larger amplitude if a conditioning prepulse of 100 msec to $+50$ mV and a brief (10 msec) repolarization to -90 mV precede V_t (trace b). The rational for this is that resting HVA channels are allowed to recover from drugs inhibition during step depolarization to $+50$ mV and to close quickly with a short repolarization to -90 mV. Subsequent depolarizations to -10 mV will reopen Ca channels which are no longer inhibited by the drug and that display quick activation (Carbone, Pollo and Taglialatela, unpublished results).

3. *A Model for the Voltage-Dependent Drug-Induced Inhibition*

Recent reports indicate that the voltage-dependent inhibition of Ca currents by neurotransmitters and drugs may be accounted for by a kinetic model in which a G-protein subunit interacts in a voltage-dependent manner with a closed state (C) of the channel to give a closed-inhibited conformation (C*). State C* is assumed to equilibrate with state C through voltage-dependent rate constants $\gamma(V)$ and δ that rate limit the activation of Ca channels during membrane depolarizations (Grassi and Lux, 1989; Kasai and Aosaki, 1989; see also Bean, 1989b; Elmslie *et al.*, 1990):

$$C^* \underset{\delta}{\overset{\gamma(V)}{\rightleftarrows}} C \underset{\beta(V)}{\overset{\alpha(V)}{\rightleftarrows}} O \qquad (2)$$

In this scheme O represents the open state of the channel; $\alpha(V)$ and $\beta(V)$ are the forward and backward activation rates of the channel. For GABA and dopamine in chick DRG neurons, $\gamma(V)$ is about 0.1 msec^{-1} at 0 mV and increases e-fold with a $+40$ mV change of membrane potential while δ is nearly voltage insensitive (0.03 msec^{-1}) and determines the slow time course at which the inhibition is reestablished at rest (Grassi and Lux, 1989). The probability of HVA Ca channels to populate state C* increases with increasing the concentration of neurotransmitters or drugs and with the time of internal perfusion required to replace endogenous GDP by GTP analogues. The model assumes that the G-protein subunit interferes directly with channel gating when closed and that removal of the G-protein inhibition is a prerequisite for Ca channel opening during step depolarizations. Also, the model does not include transitions C*$\rightleftharpoons$O (Marchetti and Robello, 1989; Bean, 1989b) since open channels are shown to deactivate very quickly to state C on repolarizations to very negative potentials (see Fig. 12c). Some coupling between G-protein subunits and open Ca channels, however, cannot be excluded. Neither can be neglected a direct or indirect coupling of GTP-γ-S to Ca channel inactivation. Indeed, prolongation of Ca channel activation by neurotransmitters and drugs is expected to cause a diminished Ca-dependent Ca channel inactivation (see Fig. 2 in Marchetti *et al.*, 1986) that would be hardly distinguishable from a direct interaction of the drug with the inactivation gate of the channel. On the other hand, we

found that using the double-pulse protocols of Fig. 12c, the HVA currents inactivate faster and more completely than in the absence of the GTP analogue suggesting an interaction of the drug with open channels. Future experiments will probably allow a more detailed evaluation of the kinetic parameters.

Scheme (2) accounts nicely for the results of Fig. 12c. Without conditioning prepulses (trace a) HVA Ca channels are mostly in state C* at rest and open slowly during step depolarizations to −10 mV, due to the low value of $\gamma(V)$. During preconditioning pulses to +50 mV, $\gamma(V)$ increases steeply and Ca channels can open and deactivate rapidly during the brief hyperpolarization to −90 mV. Ca channels will then be closed, relieved from drug inhibition and ready to give rise to fast activating HVA currents during subsequent depolarizations to −10 mV (trace b).

A schematic drawing of scheme (2) is illustrated in Fig. 13 showing how drug inhibition can be hypothetically modulated by membrane voltage. The prerequisites for the model are: (1) In the absence of neurotransmitter or intracellular GTP the channel opens (O) and closes

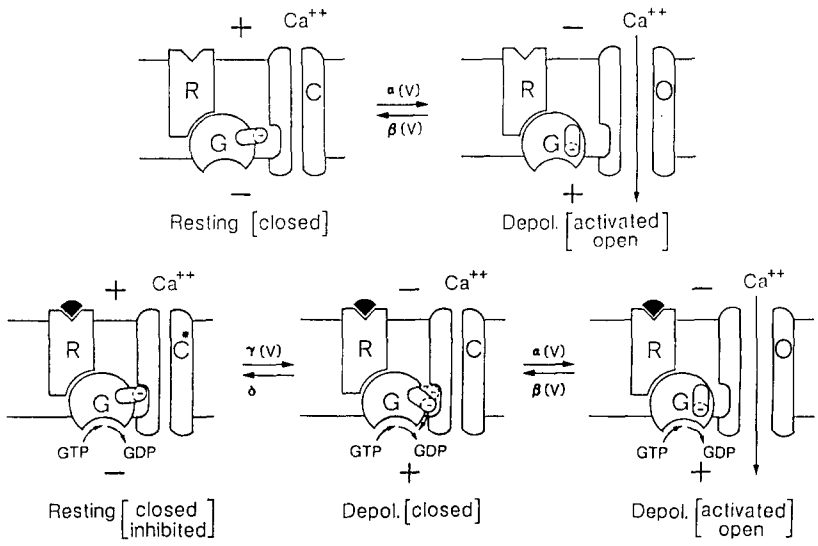

FIG. 13. Schematic representation of drug-induced, voltage-dependent inhibition of HVA calcium channels. Top panels illustrate opening (O) and closing (C) of the channel in response to voltage changes in the absence of drugs. Drug-induced coupling of G-protein to the channel inhibits Ca^{2+} flow (bottom left and central panels). Charged groups of the G-protein (arbitrarily assumed to be negative) reorient under the influence of strong depolarizations (transition C* → C). This leads to a relief of drug-induced inhibition (bottom central panel) and channels open with some delay (bottom right panel). (From Swandulla *et al.*, 1991.)

(C) regularly in a voltage-dependent manner (upper panels), (2) the neurotransmitter receptor (R) is coupled to a G-protein (G), (3) channel inhibition (C*) is triggered either by external neurotransmitters or by free intracellular GTP and is favoured by negative membrane potentials (left-lower panel), (4) charged groups of the G-protein (with net negative charge in Fig. 5) regulate the channel inhibition and can be quickly displaced from their inhibitory position by substained depolarizations (middle-lower panel), and (5) removal of the inhibition allows the channel (C) to open regularly (right-lower panel).

4. *Neurotransmitter Action on High-Threshold Ca Channel Subtypes*

The above findings argue against a simple scheme of selective actions of neurotransmitters, peptides and GTP-analogues on high-threshold Ca channel subtypes. As pointed out earlier (Section IV.4), some of the present ambiguities derive from the criteria used to distinguish Ca channel subtypes. Using kinetic criteria, all the present evidence argues against a specific effect of neurotransmitters and GTP-γ-S on transient N-like Ca channels (see above). Several

groups have shown that the neurotransmitter- and GTP-γ-S-induced inhibition is widely independent of the holding potential (Ikeda and Schofield, 1989; Kasai and Aosaki, 1989; Marchetti and Robello, 1989). At -50 mV where N-type Ca channels are mostly inactive (Nowycky *et al.*, 1985a), GTP-γ-S prolongs the Ca current rise as it does at more negative holding potentials where N-channel are expected to be fully reprimed.

On the other hand, using pharmacological criteria, ω-CgTx has been shown to depress with the same efficacy control as well as GTP-γ-S-prolonged currents in chick DRG (Kasai and Aosaki, 1989), suggesting that both currents and their difference are pharmacologically undistinguishable. In bull frog sympathetic neurons, which possess only slowly-inactivating ω-CgTx-sensitive Ca channels, dynorphin, noradrenaline and somatostatin inhibit these Ca channels in a voltage-dependent manner (Bean, 1989b). In a recent series of experiments we found that ω-CgTx-resistant and ω-CgTx-sensitive Ca channels are similarly inhibited by GTP-γ-S and that the presence of Ca antagonists does not produce any significant variation to the inhibitory action of GTP-γ-S, which remains intrinsically voltage dependent (Carbone, Pollo and Taglialatela, unpublished results). An unspecific action of GTP-γ-S on high-threshold Ca channels can also be inferred by the finding that this compound prolongs Ca channel activation in nearly all cells tested so far, including peripheral neurons that mostly contain a majority of ω-CgTx-sensitive channels (Kasai and Aosaki, 1989; Jones and Marks, 1989) and clonal insulin secreting cells (RINm5F) that possess mainly DHP-sensitive channels (Carbone, Pollo and Sher, unpublished results). Thus, although plausible, there is still weak support of the idea that neurotransmitters and drugs display a significant specificity to either type of high threshold Ca channels. Neurotransmitter-sensitive and -insensitive Ca currents may indeed flow through distinct populations of Ca channels, but to prove this more convincing pharmacological evidence is required. It is thus likely that the reported "dominant" action of neurotransmitters on one Ca channel type might just reflect the action of the drug on a channel that is "dominant" in a cell preparation rather than a drug specificity for one channel subtype that contributes only partially to the total current.

A further complication of Ca channel modulation arises from the alternative pathways by which Ca channels can be modulated (directly, through G-proteins or second messengers) and by the different response that the same neurotransmitter may induce through a common modulator in different neurons. Thus, in chick DRG the GABA-induced inhibition of high-threshold Ca channels can be fully removed by strong depolarizations (Grassi and Lux, 1989), while in rat sensory neurons the HVA current depression induced by the $GABA_B$ agonist (−)-baclofen could not be reversed by large depolarizing potentials (Dolphin and Scott, 1990). This suggests that voltage-dependent slowing down of Ca channel activation may not be the unique product of a G-protein-mediated Ca channel interaction. Indeed, G-proteins may also induce Ca channel inhibition through voltage-insensitive mechanisms (Dolphin and Scott, 1990; Formenti, Sansone and Carbone, unpublished results) or via PTX-insensitive G-protein subunits (Bley and Tsien, 1990).

5. *Modulation of Ca Channel Inactivation*

Recent studies have shown that menthol (a cyclic alcohol derived from peppermint oil) exerts a specific action on low- and high-threshold calcium channels in vertebrate neurons (Swandulla *et al.*, 1986, 1987). At 0.5 mM, menthol increases 3- to 6-fold the rate of HVA channel inactivation and depresses by about 70% the size of LVA currents with little change in their time course. The action of menthol on LVA and HVA currents is dose dependent, fully reversible and independent of voltage (Swandulla *et al.*, 1987).

Menthol action on HVA current inactivation has some interesting features. Menthol accelerates and enhances HVA current inactivation. The time constant of inactivation decreases from 120 to 40 msec and becomes rather insensitive to calcium current amplitude, current carrying ion, and internal buffering (Swandulla *et al.*, 1986, 1987). As previously shown (Swandulla *et al.*, 1986), menthol facilitates the inactivation gating of HVA channels, thereby most probably suppressing the inactivating action of intracellular Ca^{2+}. On the other hand, preliminary experiments have shown that menthol's action is strongly reduced

when Na^+ ions flow through open HVA channels (Carbone, Swandulla and Lux, unpublished observations). Taken together, these findings suggest that menthol action may depend on the permeability state of the channel being much less pronounced when the channel is in the Na^+-mode as compared to the Ca^{2+}-mode (see Section V).

In all cases, the action of menthol seems unrelated to the presence of the fast inactivating current observed in neurons (N-type). This was concluded from the observations that: (i) menthol also accelerates HVA current inactivation in cells with weakly inactivating calcium currents, (ii) the action of menthol on calcium current inactivation was identical if the holding potential was changed from -80 to -50 mV, and (iii) in menthol-treated cells (1 mM), HVA currrents showed no sign of voltage-dependent inactivation positive to $+10$ mV (see Figs 6 and 7 in Swandulla *et al.*, 1987).

Since in chick DRG neurons the majority of high-threshold Ca currents are carried through ω-CgTx sensitive channels it is most likely that menthol mainly accelerates the inactivation process of these channels without affecting their activation and deactivation kinetics.

VI. CONCLUSIONS

The discovery of new classes of voltage-activated Ca channels with distinct pharmacology has greatly stimulated the multidisciplinary studies of their properties, functions and modulatory responses. As Ca^{2+} is involved in the regulation of many cellular activities, the identification and characterization of Ca channels has become a prerequisite for understanding in detail the mechanisms underlying Ca^{2+}-mediated processes. As shown, a pharmacological approach to this problem may overcome the weaknesses of earlier classifications based on kinetic criteria, sensitivity to holding potential and ion permeability. This justifies the effort of various groups to develop new Ca channel ligands of high selectivity that can facilitate distinctions among Ca channel subtypes. Some clarification has also been achieved concerning the mechanism of Ca channel modulation by extracellular and intracellular agents, but a better understanding of this phenomenon is expected to bring essential information about Ca channel subtypes and their role. Along this line, some of the problems that remain to be solved are the identification of the main Ca channel modulators at the plasma membrane and within the cytosol, whether they are common or unique mechanisms and their degree of coupling to the channel.

Since voltage-activated calcium channels form a broad family of membrane proteins with close properties, it would not be surprising if strong analogies between their amino acid sequences and quaternary structures are soon discovered. Major molecular differences are expected between high- and low-threshold Ca channels. In this case, genetic mutations should account for the different range of activation, inactivation mechanisms, Ca^{2+}/Ba^{2+} selectivity, single channel conductance and drug sensitivity. Minor differences are expected among the high-threshold Ca channels whose heterogeneity seems to reflect a different pharmacological sensitivity to ligands rather than diverse biophysical properties.

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REFERENCES

ADAMS, D. J. and GAGE, P. W. (1977) Calcium channel in *Aplysia* nerve cell membrane: ionic and gating current kinetics. *Proc. Aust. Physiol. Pharmac. Soc.* **8**, 22P.

ADAMS, M. E., BINDOKAS, V. P., DOLPHIN, A. C. and SCOTT, H. (1989) The inhibition of Ca^{2+} channel currents in cultured neonatal rat dorsal root ganglion (DRG) neurones by ω-Agatoxin-1A (a funnel-web-spider toxin). *J. Physiol.* **418**, 34P.

AHLIJANIAN, M. K., WESTENBROEK, R. E. and CATTERALL, W. A. (1990) Subunit structure and localization of dihydropyridine-sensitive calcium channels in mammalian brain, spinal cord, and retina. *Neuron* **3**, 819–832.

ALDRICH, R. W., COREY, D. P. and STEVENS, C. F. (1983) A reinterpretation of mammalian sodium channel gating based on single channel recording. *Nature* **306**, 436–441.

AKAIKE, N., TSUDA, Y. and OYAMA, Y. (1988) Separation of current- and voltage-dependent inactivation of calcium current in frog sensory neuron. *Neurosci. Lett.* **84**, 46–50.

AKAIKE, N., KANAIDE, H., KUGA, T., NAKAMURA, M., SADOSHIMA, J. I. and TOMOIKE, H. (1989a) Low-voltage-activated calcium current in rat aorta smooth muscle cells in primary culture *J. Physiol.* **416,** 141–160.

AKAIKE, N., KOSTYUK, P. G. and OSIPCHUK, Y. V. (1989b) Dihydropyridine-sensitive low-threshold calcium channels in isolated rat hypothalamic neurones. *J. Physiol.* **412,** 181–195.

AOSAKI, T. and KASAI, H. (1989) Characterization of two kinds of high-voltage-activated Ca-channel currents in chick sensory neurons. Differential sensitivity to dihydropyridines and ω-Conotoxin GVIA. *Pflügers Arch.* **414,** 150–156.

ARGIBAY, J. A., FISCHMEISTER, R. and HARTZELL, H. C. (1988) Inactivation, reactivation and pacing dependence of calcium current in frog cardiocytes: correlation with current density. *J. Physiol.* **401,** 201–226.

BEAN, B. P. (1984) Nitrendipine block of cardiac calcium channels: High-affinity binding to the inactivated state. *Proc. natn. Acad. Sci. U.S.A.* **81,** 6388–6392.

BEAN, B. P. (1985) Two kinds of calcium channels in canine atrial cells. Differences in kinetics, selectivity, and pharmacology. *J. gen. Physiol.* **86,** 1–30.

BEAN, B. (1989a) Classes of calcium channels in vertebrate cells. *A. Rev. Physiol.* **51,** 367–384.

BEAN, B. (1989b) Neurotransmitter inhibition of neuronal calcium currents by changes in channel voltage dependence. *Nature* **340,** 153–156.

BEAN, B. P., NOWYCKY, M. C. and TSIEN, R. W. (1984) β-Adrenergic modulation of calcium channels in frog ventricular heart cells. *Nature* **307,** 371–375.

BEZANILLA, F. and ARMSTRONG, C. M. (1977) Inactivation of the sodium channel. I. Sodium current experiments. *J. gen. Physiol.* **70,** 549–566.

BINDOKAS, V. P. and ADAMS, M. E. (1989) ω-Aga-I: a presynaptic calcium channel antagonist from venom of the funnel web spider *Agelenopsis aperta. J. Neurobiol.* **20,** 171–188.

BLEY, K. R. and TSIEN, R. W. (1990) Inhibition of Ca^{2+} and K^+ channels in sympathetic neurons by neuropeptides and other ganglionic transmitters. *Neuron* **2,** 379–391.

BOLAND, L. M. and DINGLEDINE, R. (1990) Multiple components of both transient and sustained barium currents in a rat dorsal root ganglion cell line. *J. Physiol.* **420,** 223–245.

BOLL, W. and LUX, H. D. (1985) Action of organic antagonist on neuronal calcium currents. *Neurosci. Lett.* **56,** 35–39.

BOROSOTTO, M., BARHAMIN, J., NORMAN, R. and LAZDUNSKI, M. (1984) Purification of the dihydropyridine receptor of the voltage dependent Ca^{2+} channel from skeletal muscle transverse tubules using [^{3}H]-(+)-PN200 – 100. *Biochem. biophys. Res. Commun.* **122,** 1357–1366.

BOSSU, J. L. and FELTZ, A. (1986) Inactivation of the low-threshold transient calcium current in rat sensory neurones: evidence for a dual process. *J. Physiol.* **376,** 341–357.

BOSSU, J. L., FELTZ, A. and THOMANN, J. M. (1985) Depolarization elicits two distinct calcium currents in vertebrate sensory neurons. *Pflügers Arch.* **403,** 360–368.

BOWERS, C. W., PHILLIPS, H. S., LEE, P., JAN, Y. N. and JAN, L. J. (1987) Identification and purification of an irreversible presynaptic neurotoxin from the venom of the spider *Hololena curta. Proc. natn. Acad. Sci. U.S.A.* **84,** 3506–3510.

BROWN, A. M., MORIMOTO, K., TSUDA, Y. and WILSON, D. L. (1981) Calcium current-dependent and voltage-dependent inactivation of calcium channels in *Helix Aspersa. J. Physiol.* **320,** 193–218.

BROWN, A. M., CAMERER, H., KUNZE, D. L. and LUX, H. D. (1982) Similarity of unitary Ca^{2+} currents in three different species. *Nature* **299,** 156–158.

BROWN, A. M., KUNZE, D. L. and YATANI, A. (1984) The agonistic effect of dihydropyridines on Ca channels. *Nature* **311,** 570–572.

BYERLY, L. and LEUNG, H. T. (1988) Ionic currents of drosophila neurons in embryonic cultures. *J. Neurosci.* **8**(11), 4379–4393.

BYERLY, L., CHASE, P. B. and STIMERS, J. R. (1985) Permeation and interaction of divalent cations in calcium channels of snail neurons. *J. gen. Physiol.* **85,** 491–518.

CAMPBELL, K. P., LEUNG, A. T. and SHARP, A. H. (1988) The biochemistry and molecular biology of the dihydropyridine-sensitive calcium channel. *Trends Neurosci.* **11,** 425–430.

CARBONE, E. and LUX, H. D. (1984a) A low voltage-activated calcium conductance in embryonic chick sensory neurons. *Biophys. J.* **46,** 413–418.

CARBONE, E. and LUX, H. D. (1984b) A low voltage-activated, fully inactivating Ca channel in vertebrate sensory neurones. *Nature* **310,** 510–502.

CARBONE, E. and LUX, H. D. (1986) Low- and high-voltage activated Ca channels in vertebrate cultured neurons: properties and functions. *Expl. Brain Res.* **14** (Suppl.), 1–8.

CARBONE, E. and LUX, H. D. (1987a) Kinetics and selectivity of a low-voltage-activated calcium current in chick and rat sensory neurones. *J. Physiol.* **386,** 547–570.

CARBONE, E. and LUX, H. D. (1987b) Single low-voltage-activated calcium channels in chick and rat sensory neurones. *J. Physiol.* **386,** 571–601.

CARBONE, E. and LUX, H. D. (1988) ω-Conotoxin blockade distinguishes Ca from Na permeable states in neuronal calcium channels. *Pflügers Arch.* **413,** 14–22.

CARBONE, E. and SWANDULLA, D. (1991) Calcium channel inactivation. In *Calcium Channels: Their Properties, Functions, Regulation and Clinical Relevance*, (eds L. HURWITZ, L. D. PARTRIDGE and J. K. LEACH), The Telford Press, Caldwell, NJ, in press.

CARBONE, E., MORAD, M. and LUX, H. D. (1987) External Ni^{2+} selectively blocks the low-threshold Ca^{2+} current of chick sensory neurons. *Pflügers Arch.* **408,** R60.

CARBONE, E., CLEMENTI, F., FORMENTI, A., POLLO, A. and SHER, E. (1989) Action of Ca^{2+} agonists/antagonists in mammalian peripheral neurons. *Cell. Biol. Int. Rep.* **13,** 1155–1164.

CARBONE, E., FORMENTI, A. and POLLO, A. (1990a) Multiple actions of Bay K 8644 on high-threshold Ca channels in adult rat sensory neurons. *Neurosci. Lett.* **111,** 315–320.

CARBONE, E., SHER, E. and CLEMENTI, F. (1990b) Ca currents in human neuroblastoma IMR32 cells: kinetics, permeability and pharmacology. *Pflügers Arch.* **416,** 170–179.

CATTERALL, W. A. (1988) Structure and function of voltage-sensitive ion channels. *Science* **242,** 50–61.

CAVALIÉ, A., OCHI, R., PELZER, D. and TRAUTWEIN, W. (1983) Elementary currents through Ca^{2+} channels in guinea-pig myocytes. *Pflügers Arch.* **398,** 284–297.

CHIU, S. Y., MROSE, H. E. and RITCHIE, J. M. (1979) Anomalous temperature dependence of the sodium conductance in rabbit nerve compared with frog nerve. *Nature* **279,** 327–328.

COHEN, C. J. and MCCARTHY, R. T. (1987) Nimodipine block of calcium channels in rat anterior pituitary cells. *J. Physiol.* **387,** 195–225.

COLATSKY, T. J.(1980) Voltage clamp measurements of sodium channel properties in rabbit cardiac Purkinje fibres. *J. Physiol.* **305,** 215–234.

COULTER, D. A., HUGUENARD, J. R. and PRINCE, D. A. (1989) Calcium currents in rat thalamocortical relay neurones: kinetic properties of the transient, low-threshold current. *J. Physiol.* **414,** 587–604.

CRUNELLI, V., LIGHTOWLER, S. and POLLARD, C. E. (1989) A T-type Ca^{2+} current underlies low-threshold Ca^{2+} potentials in cells of the cat and rat lateral geniculate nucleus. *J. Physiol.* **413,** 543–561.

CRUZ, L. J. and OLIVERA, B. M. (1986) Calcium channel antagonists. *J. biol. Chem.* **261,** 6230–6233.

CURTIS, B. M. and CATTERALL, W. A. (1984) Purification of the calcium antagonist receptor of the voltage-sensitive calcium channel from skeletel muscle transverse tubules. *Biochemistry* **23,** 2113–2118.

DEISZ, R. A. and LUX, H. D. (1985) γ-aminobutyric acid-induced depression of calcium currents of chick sensory neurons. *Neurosci. Lett.* **56,** 205–210.

DOCHERTY, R. J. (1988) Gadolinium selectively blocks a component of calcium current in rodent neuroblastomaxglioma hybrid (NG108–15) cells. *J. Physiol.* **398,** 33–47.

DOLPHIN, A. C. and SCOTT, R. H. (1987) Calcium channel currents and their inhibition by (−)-baclofen in rat sensory neurones modulation by guanine nucleotides. *J. Physiol.* **386,** 670–672.

DOLPHIN, A. C. and SCOTT, R. H. (1989) Interaction between calcium channel ligands and guanine nucleotides in cultured rat sensory and sympathetic neurones. *J. Physiol.* **413,** 271–288.

DOLPHIN, A. C. and SCOTT, R. H. (1990) Activation of calcium channel currents in rat sensory neurons by large depolarizations: Effect of guanine nucleotides and (−)-baclofen. *Eur. J. Neurosci.* **2,** 104–108.

DOLPHIN, A. C., FORDA, S. R. and SCOTT, R. H. (1986) Calcium-dependent currents in cultured rat dorsal root ganglion neurones are inhibited by an adenosine analogue. *J. Physiol.* **373,** 47–61.

DROOGMANS, G. and NILIUS, B. (1989) Kinetic properties of the cardiac T-type calcium channel in the guinea-pig. *J. Physiol.* **419,** 627–650.

DUNLAP, K. and FISCHBACH, G. D. (1978) Neurotransmitters decrease the calcium component of sensory neurone action potentials. *Nature* **276,** 837–838.

DUNLAP, K. and FISCHBACH, G. D. (1981) Neurotransmitters decrease the calcium conductance activated by depolarization of embryonic chick sensory neurones. *J. Physiol.* **317,** 519–535.

DUNLAP, K., HOLZ, G. G. and RANE, S. G. (1987) G proteins as regulators of ion channel function. *Trends Neurosci.* **10,** 241–244.

ECKERT, R. and CHAD, J. E. (1984) Inactivation of Ca channels. *Prog. Biophys. molec. Biol.* **44,** 215–267.

ELMSLIE, K. S., ZHOU, W. and JONES, S. W. (1990) LHRH and GTP-γ-S modify calcium current activation in bullfrog sympathetic neurons. *Neuron* **5,** 75–80.

FEDULOVA, S. A., KOSTYUK, P. G. and VESELOVSKY, N. S. (1985) Two types of calcium channels in the somatic membrane of new-born rat dorsal root ganglion neurones. *J. Physiol.* **359,** 431–446.

FELDMAN, D. H., OLIVERA, B. M. and YOSHIKAMI, D. (1987) Omega *Conus geographus* toxin: a peptide that blocks calcium channels. *FEBS Lett.* **214,** 295–300.

FENWICK, E. M., MARTY, A. and NEHER, E. (1982) Sodium and calcium channels in bovine chromaffine cells. *J. Physiol.* **331,** 599–635.

FORSCHER, P. and OXFORD, G. S. (1985) Modulation of calcium channels by norepinephrine in internally dialyzed avian sensory neurons. *J. gen. Physiol.* **85,** 743–763.

FOX, A. P., NOWYCKY, M. C. and TSIEN, R. W. (1987a) Kinetic and pharmacological properties distinguishing three types of calcium currents in chick sensory neurones. *J. Physiol.* **394,** 149–172.

FOX, A. P., NOWYCKY, M. C. and TSIEN, R. W. (1987b) Single-channel recordings of three types of calcium channels in chick sensory neurones. *J. Physiol.* **394,** 173–200.

FUKUSHIMA, Y. and HAGIWARA, S. (1985) Current carried by monovalent cations through calcium channels in mouse neoplastic B lymphocytes. *J. Physiol.* **358,** 255–284.

GAHWILER, B. H. and BROWN, D. A. (1987) Muscarine affects calcium-currents in rat hippocampal pyramidal cells *in vitro*. *Neurosci. Lett.* **76,** 301–306.

GALVAN, M. and ADAMS, P. R. (1982) Control of calcium current in rat sympathetic neurons by norepinephrine. *Brain Res.* **244,** 135–144.

GLOSSMANN, H., FERRY, D. R. and BOSCHEK, C. B. (1983) Purification of the putative calcium channel from skeletal muscle with the aid of [^{3}H]-nimodipine binding. *Naunyn-Schmiedebergs Arch. Pharmac.* **323,** 1–11.

GRASSI, F. and LUX, H. D. (1989) Voltage-dependent GABA-induced modulation of calcium currents in chick sensory neurons. *Neurosci. Lett.* **105,** 113–119.

GRAY, W. R., OLIVERA, B. M. and CRUZ, L. J. (1988) Peptide toxins from venomous conus snails. *A. Rev. Biochem.* **57,** 665–700.

GROSS, R. A. and MACDONALD, R. L. (1987) Dynorphin A selectively reduces a large transient (N-type) calcium current of mouse dorsal root ganglion neurons in cell culture. *Proc. natn. Acad. Sci. U.S.A.* **84,** 5469–5473.

GUTNICK, M. J., LUX, H. D., SWANDULLA, D. and ZUCKER, H. (1989) Voltage-dependent and calcium-dependent inactivation of calcium channel current in identified snail neurones. *J. Physiol.* **412,** 197–220.

HAGIWARA, S. and BYERLY, L. (1981) Calcium channel. *A. Rev. Neurosci.* **4,** 69–125.

HAGIWARA, S. and OHMORI, H. (1982) Studies of single calcium channel currents in rat clonal pituitary cells. *J. Physiol.* **336,** 649–661.

HAMIL, O. P., MARTY, A., NEHER, E., SAKMANN, B. and SIGWORTH, F. J. (1981) Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Arch.* **391,** 85–100.

HADLEY, R. W. and HUME, J. R. (1987) An intrinsic potential-dependent inactivation mechanism associated with calcium channels in guinea-pig myocytes. *J. Physiol.* **389,** 205–222.

HARRIS-WARRICK, R. M., HAMMOND, C., PAUPARDIN-TRITSCH, D., HOMBURGER, V., ROUOT, B., BOCKAERT, J. and GERSCHENFELD, H. M. (1989) An α_{40} subunit of a GTP-binding protein immunologically related to G_o mediates a dopamine-induced decrease of Ca^{2+} current in snail neurons. *Neuron* **1,** 27–32.

HESCHELER, J., ROSENTHAL, W., TRAUTWEIN, W. and SCHULTZ, G. (1987) The GTP-binding protein, G_o, regulates neuronal calcium channels. *Nature* **325,** 445–447.

HESS, P., LANSMAN, J. B. and TSIEN, R. W. (1984) Different modes of Ca^{2+} channel gating behavior favored by dihydropyridine Ca^{2+} agonists and antagonists. *Nature* **311,** 538–544.

HILLE, B. (1984) *Ionic Channels of Excitable Membranes*, Sinauer, Sunderland, MA.

HIRANO, Y., FOZZARD, H. A. and JANUARY, C. T. (1989) Inactivation properties of T-type calcium current in canine cardiac Purkinje cells. *Biophys. J.* **56,** 1007–1016.

HIRNING, L. D., FOX, A. P., MCCLESKEY, E. W., OLIVERA, B. M., THAYER, S. A., MILLER, R. J. and TSIEN, R. W. (1988) Dominant role of N-type Ca^{2+} channels in evoked release of norepinephrine from sympathetic neurons. *Science* **239,** 57–61.

HOCKBERGER, P., TOSELLI, M., SWANDULLA, D. and LUX, H. D. (1989) A diacylglycerol analogue reduces neuronal calcium currents independently of protein kinase C activation. *Nature* **338,** 340–342.

HOLZ, G. G., RANE, S. G. and DUNLAP, K. (1986) GTP-binding proteins mediate transmitter inhibition of voltage-dependent calcium channels. *Nature* **319,** 670–672.

HOSHI, T. and SMITH, S. J., (1987) Large depolarization induces long openings of voltage-dependent calcium channels in adrenal chromaffine cells. *J. Neurosci.* **7,** 571–580.

IKEDA, S. R., SCHOFIELD, G. G. and WEIGHT, F. F. (1987) Somatostatin blocks a calcium current in acutely isolated adult rat superior cervical ganglion neurons. *Neurosci. Lett.* **81,** 123–128.

IKEDA, S. R. and SCHOFIELD, G. G. (1989) Somatostatin blocks a calcium current in rat sympathetic ganglion neurones. *J. Physiol.* **409,** 221–240.

JACKSON, H. and USHERWOOD, P. N. R. (1988) Spider toxins as tools for dissecting elements of excitatory amino acid transmission. *Trends Neurosci.* **11,** 278–283.

JANIS, R. A. and TRIGGLE, D. J. (1990) Drugs acting on calcium channels. In *Calcium Channels: Their Properties, Functions, Regulation and Clinical Relevance*, (eds. L. HURWITZ, L. D. PARTRIDGE and J. K. LEACH), The Telford Press, Caldwell, NJ, in press.

JANIS, R. A., SILVER, P. J. and TRIGGLE, D. J. (1987) Drug action and cellular calcium regulation. *Adv. Drug Res.* **16,** 309–589.

JONES, S. W. and MARKS, T. N. (1989) Calcium currents in bullfrog sympathetic neurons. I. Activation kinetics and pharmacology. *J. gen. Physiol.* **94,** 151–167.

KARSCHIN, A. and LIPTON, S. A. (1989) Calcium channels in solitary retinal ganglion cells from post-natal rat. *J. Physiol.* **418,** 379–396.

KASAI, H. and AOSAKI, T. (1988) Divalent cation dependent inactivation of the high-voltage-activated Ca channel current in chick sensory neurons. *Pflügers Arch.* **411,** 695–697.

KASAI, H. and AOSAKI, T. (1989) Modulation of Ca-channel current by an adenosine analog mediated by a GTP-binding protein in chick sensory neurons. *Pflügers Arch.* **414,** 145–149.

KASAI, H., AOSAKI, T. and FUKUDA, J. (1987) Presynaptic Ca-antagonist ω-Conotoxin irreversibly blocks N-type Ca channels in chick sensory neurons. *Neurosci. Res.* **4,** 228–235.

KASS, R. S. and SANGUINETTI, M. C. (1984) Inactivation of calcium channel current in the calf cardiac Purkinje fiber: evidence for voltage- and calcium-mediated mechanisms. *J. gen. Physiol.* **84,** 705–726.

KAWA, K. (1979) Zinc-dependent action potentials in giant neurons of the snail, *Euhadra quaestia*. *J. membr. Biol.* **49,** 325–344.

KERR, L. M. and YOSHIKAMI, D. (1984) A venom peptide with a novel presynaptic blocking action. *Nature* **308,** 282–284.

KIMURA, J. E. and MEEVES, H. (1979) The effect of temperature on the asymmetrical charge movement in squid giant axons. *J. Physiol.* **289,** 479–500.

KOKUBUN, S. and REUTER, H. (1984) Dihydropyridine derivatives prolong the open state of Ca channels in cultured cardiac cells. *Proc. natn. Acad. Sci. U.S.A.* **81,** 4824–4827.

KOKUBUN, S., PROD'HOM, B., BECKER, C., PORZIG, H. and REUTER, H. (1986) Studies on Ca channels in intact cardiac cells: voltage-dependent effects and cooperative interactions of dihydropyridine enantiomers. *Molec. Pharmac.* **30,** 571–584.

KONGSAMUT, S., LIPSCOMBE, D. and TSIEN, R. W. (1989) The N-type Ca channel in frog sympathetic neurons and its role in α-adrenergic modulation of transmitter release. *Ann. N. Y. Acad. Sci.* **560,** 312–333.

KOSTYUK, P. G., VESELOVSKY, N. S. and FEDULOVA, S. A. (1981) Ionic currents in the somatic membrane of rat dorsal root ganglion neurons. II. Calcium currents. *Neuroscience* **6,** 2431–2437.

KUNZE, D. L., LACERDA, A. E., WILSON, D. L. and BROWN, A. M. (1985) Cardiac Na^+ currents and the inactivating, reopening, and waiting properties of single cardiac Na^+ channels. *J. gen. Physiol.* **86,** 691–719.

LACERDA, A. E. and BROWN, A. M. (1984) Nonmodal gating of cardiac calcium channels as revealed by dihydropyridines. *J. gen. Physiol.* **93,** 1243–1273.

LANSMAN, J. B. (1990) Blockade of current through single calcium channels by trivalent lanthanide cations. *J. gen. Physiol.* **95,** 679–696.

LANSMAN, J. B., HESS, P. and TSIEN, R. W. (1986) Blockade of current through single calcium channels by Cd^{2+}, Mg^{2+}, and Ca^{2+}. *J. gen. Physiol.* **88,** 321–347.

LEE, K. S., MARBAN, E. and TSIEN, R. W. (1985) Inactivation of calcium channels in mammalian heart cells: joint dependence on membrane potential and intracellular calcium. *J. Physiol.* **364,** 395–411.

LEUNG, H. T., BRANTON, W. D., PHILIPS, H. S., JAN, L. and BYERLY, L. (1989) Spider toxins selectively block calcium currents in drosophila. *Neuron* **3,** 767–772.

LEWIS, D., WEIGHT, F. F. and LUINI, A. (1986) A guanine nucleotide-binding protein mediates the inhibition of

voltage-dependent calcium current by somatostatin in a pituitary cell line. *Proc. natn. Acad. Sci. U.S.A.* **83,** 9035–9039.

LEWIS, D. and WEIGHT, F. F. (1988) The protein kinase C activator 1-oleoyl-2-acetyl-glycerol (OAG) inhibits voltage-dependent Ca^{2+} current in the pituitary cell line, AtT20. *Neuroendoscience* **47,** 169–173.

LIPSCOMBE, D., KONGSAMUT, S. and TSIEN, R. W. (1989) α-Adrenergic inhibition of sympathetic neurotransmitter release mediated by modulation of N-type calcium-channel gating. *Nature* **340,** 639–642.

LLINAS, R. and YAROM, Y. (1981) Properties and distribution of ionic conductances generating electroresponsiveness of mammalian olivary neurones *in vitro*. *J. Physiol.* **315,** 569–584.

LLINAS, R., SUGIMORI, M., LIN, J. W. and CHERKSEY, B. (1989) Blocking and isolation of a calcium channel from neurons in mammals and cephalopods utilizing a toxin fraction (FTX) from funnel-web spider poison. *Proc. natn. Acad. Sci. U.S.A.* **86,** 1689–1693.

LUX, H. D. and BROWN, A. M. (1984a) Single channel studies on inactivation of calcium currents. *Science* **225,** 432–434.

LUX, H. D. and BROWN, A. M. (1984b) Patch and whole cell calcium currents recorded simultaneously in snail neurons. *J. gen. Physiol.* **83,** 727–750.

LUX, H. D. and NAGY, K. (1981) Single Ca^{2+} currents in *Helix pomatia* neurons. *Pflügers Arch.* **391,** 252–254.

LUX, H. D., CARBONE, E. and ZUCKER, H. (1989) Block of Na^+ ion permeation and selectivity of Ca channels. *Ann. N. Y. Acad. Sci.* **560,** 94–102.

LUX, H. D., CARBONE, E. and ZUCKER, H. (1990) Na^+ currents through low-voltage-activated Ca^{2+} channels of chick sensory neurones: block by external Ca^{2+} and Mg^{2+}. *J. Physiol.*, in press.

MARBAN, E. and TSIEN, R. W. (1982) Effects of nystatin-mediated intracellular ion substitution on membrane currents in calf Purkinje fibres. *J. Physiol.* **329,** 569–587.

MARCHETTI, C. and BROWN, A. M. (1988) The protein kinase activator, 1,2-oleoyl-acetyl-glycerol, inhibits two types of calcium currents in GH3 cells. *Am. J. Physiol.* **254,** c206–c210.

MARCHETTI, C. and ROBELLO, M. (1989) Guanosine-5′-*O*-(3-thiotriphosphate) modifies kinetics of voltage-dependent calcium current in chick sensory neurons. *Biophys. J.* **56,** 1267–1272.

MARCHETTI, C., CARBONE, E. and LUX, H. D. (1986) Effects of dopamine and noradrenaline on Ca^{2+} channels of cultured sensory and sympathetic neurons of chick. *Pflügers Arch.* **406,** 104–111.

MARKWARDT, F. and NILIUS, B. (1988) Modulation of calcium channel currents in guinea-pig single ventricular heart cells by the dihydropyridine Bay K 8644. *J. Physiol.* **399,** 559–575.

MATTESON, D. R. and ARMSTRONG, C. M. (1986) Properties of two types of calcium channels in clonal pituitary cells. *J. gen. Physiol.* **87,** 161–182.

MCCLESKEY, E. W., FOX, A. P., FELDMAN, D. H., CRUZ, L. J., OLIVERA, B. M., TSIEN, R. W. and YOSHIKAMI, D. (1987) ω-Conotoxin: Direct and persistent blockade of specific types of calcium channels in neurons but not in muscle. *Proc. natn. Acad. Sci. U.S.A.* **84,** 4327–4331.

MCFADZEAN, I. and DOCHERTY, R. J. (1989) On the mechanism by which enkephalin and noradrenaline inhibit voltage-dependent calcium currents recorded from NG108-15 hybrid cells. *Eur. J. Neurosci.* **1,** 141–147.

MCFADZEAN, I., MULLANEY, I., BROWN, D. A. and MILLIGAN, G. (1989) Antibodies to the GTP binding protein, G_o, antagonize noradrenaline-induced calcium current inhibition in NG108-15 hybrid cells. *Neuron* **3,** 177–182.

MILLER, R. J. (1987) Multiple calcium channels and neuronal function. *Science* **232,** 46–52.

MORAD, M., NAYLER, W., KAZDA, S. and SCHRAMM, M. (1988) *The Calcium Channel: Structure, Function and Implications*, Springer Verlag, Berlin.

NARAHASHI, T., TSUNOO, A. and YOSHII, M. (1987) Characterization of two types of calcium channels in mouse neuroblastoma cells. *J. Physiol.* **383,** 231–249.

NILIUS, B., HESS, P., LANSMAN, J. B. and TSIEN, R. W. (1985) A novel type of cardiac calcium channel in ventricular cells. *Nature* **316,** 443–446.

NOBILE, M., CARBONE, E., LUX, H. D. and ZUCKER, H. (1990) Temperature sensitivity of Ca currents in chick sensory neurons. *Pflügers Arch.* **415,** 658–663.

NODA, M., SHIMIZU, S., TANABE, T., TAKAI, T., KAYANO, T., IKEDA, T., TAKAHASHI, H., NAKAYAMA, H., KANAOKA, Y., MINAMINO, N., KANGAWA, K., MATSUO, H., RAFTERY, M. A., HIROSE, T., INAYAMA, S., HAYASHIDA, H., MIYATA, T. and NUMA, S. (1984) Primary structure of *Electrophorus Electricus* sodium channel deduced from cDNA sequence. *Nature* **312,** 121–127.

NOWYCKY, M. C., FOX, A. P. and TSIEN, R. W. (1985a) Three types of neuronal calcium channels with different calcium agonist sensitivity. *Nature* **316,** 440–443.

NOWYCKY, M. C., FOX, A. P. and TSIEN, R. W. (1985b) Long-opening mode of gating of neuronal calcium channels and its promotion by the dihydropyridine calcium agonist Bay K 8644. *Proc. natn. Acad. Sci. U.S.A.* **82,** 2178–2182.

OLIVERA, B. M., MCINTOSH, J. M., CRUZ, L. J., LUQUE, F. A. and GRAY, W. R. (1984) Purification and sequence of a presynaptic peptide toxin from *conus geographus* venom. *Biochemistry* **23,** 5087–5090.

PENNINGTON, N. J. and KELLY, J. S. (1990) Serotonin receptor activation reduces calcium current in an acutely dissociated adult central neuron. *Neuron* **4,** 751–758.

PLUMMER, M. R., LOGOTHETIS, D. E. and HESS, P. (1989) Elementary properties and pharmacological sensitivities of calcium channels in mammalian peripheral neurons. *Neuron* **2,** 1453–1463.

PORZIG, H. (1990) Pharmacological modulation of voltage-dependent calcium channels in intact cells. *Rev. Physiol. Biochem. Pharmac.* **114,** 209–262.

RANE, S. G., HOLZ, G. G. IV and DUNLAP, K. (1987) Dihydropyridine inhibition of neuronal calcium current and substance P release. *Pflügers Arch.* **409,** 361–366.

REUTER, H. (1983) Calcium channel modulation of neurotransmitters, enzymes and drugs. *Nature* **301,** 569–574.

REUTER, H., STEVENS, C. F., TSIEN, R. W. and YELLEN, G. (1982) Properties of single calcium channels in cardiac cell culture. *Nature* **297,** 501–504.

REUTER, H., PORZIG, H., KOKUBUN, S. and PROD'HOM, B. (1985) 1,4-Dihydropyridines as tools in the study of Ca^{2+} channels. *Trends Neurosci.* **8,** 396–400.

SAH, D. W. Y. (1990) Neurotransmitter modulation of calcium current in rat spinal cord neurons. *J. Neurosci.* **10,** 136–141.

SAH, D. W. Y., REGAN, L. J. and BEAN, B. P. (1989) Calcium channels in rat neurons: high threshold channels that are resistant to both ω-Conotoxin and dihydropyridine blockers. *Neurosci. Abstr.* **15,** 823.

SANGUINETTI, M. C., KRAFTE, D. S. and KASS, R. S. (1986) Voltage-dependent modulation of Ca channel current in heart cells by Bay K8644. *J. gen. Physiol.* **88,** 369–392.

SATIN, L. S. and COOK, D. L. (1989) Calcium current inactivation in insulin-secreting cells is mediated by calcium influx and membrane depolarization. *Pflügers Arch.* **414,** 1–10.

SCOTT, R. H. and DOLPHIN, A. C. (1988) The agonist effect of Bay K 8644 on neuronal calcium currents is promoted by G-protein. *Neurosci. Lett.* **89,** 170–175.

SCOTT, R. H. and DOLPHIN, A. C. (1989) G-protein regulation of neuronal voltage-activated calcium currents. *Gen. Pharmac.* **20,** 715–720.

SHER, E., PANDIELLA, A. and CLEMENTI, F. (1988) ω-Conotoxin binding and effects on calcium channel function in human neuroblastoma and rat pheochromocytoma cell lines. *FEBS Lett.* **235,** 178–182.

SWANDULLA, D. (1990) Activation and deactivation of neuronal high voltage-activated, fast deactivating calcium channels. In *Calcium Channels: Their Properties, Functions, Regulation and Clinical Relevance*, (eds L. HURWITZ, L. D. PARTRIDGE and J. K. LEACH), The Telford Press, Caldwell, NJ, in press.

SWANDULLA, D. and ARMSTRONG, C. M. (1988) Fast deactivating calcium channels in chick sensory neurons. *J. gen. Physiol.* **92,** 197–218.

SWANDULLA, D. and ARMSTRONG, C. M. (1989) Calcium channel block by cadmium in chicken sensory neurons. *Proc. natn. Acad. Sci. U.S.A.* **86,** 1736–1740.

SWANDULLA, D., SCHÄFER, K. and LUX, H. D. (1986) Ca channel current is selectively modulated by menthol. *Neurosci. Lett.* **68,** 23–28.

SWANDULLA, D., CARBONE, E., SCHÄFER, K. and LUX, H. D. (1987) Effect of menthol on two types of Ca currents in cultured sensory neurons of vertebrates. *Pflügers Arch.* **409,** 52–59.

SWANDULLA, D., CARBONE, E. and LUX, H. D. (1991) Do calcium channel classifications account for neuronal calcium channel diversity? *Trends Neurosci.*, in press.

TANABE, T., TAKESHIMA, H., MIKAMI, A., FLOCKERZI, TAKAHASHI, H., KANGAWA, K., KOJIMA, M., MATSUO, H., HIROSE, T. and NUMA, S. (1987) Primary structure of the receptor of calcium channel blockers from skeletal muscle. *Nature* **240,** 213–215.

TANG, C., PRESSER, F. and MORAD (1988) Amiloride selectively blocks the low threshold (T) calcium channel. *Science* **240,** 213–215.

TOSELLI, M. and LUX, H. D. (1989) GTP-binding proteins mediate acetylcholine inhibition of voltage dependent calcium channels in hippocampal neurons. *Pflügers Arch.* **413,** 319–321.

TOSELLI, M. and TAGLIETTI, V. (1990) Pharmacological characterization of voltage-dependent calcium currents in rat hippocampal neurons. *Neurosci Lett.* **112,** 70–75.

TOSELLI, M., LANG, J., COSTA, T. and LUX, H. D. (1989) Direct modulation of voltage-dependent calcium channels by muscarinic activation of a pertussis toxin-sensitive G-protein in hippocampal neurons. *Pflügers Arch.* **415,** 255–261.

TSEIN, R. W., LIPSCOMBE, D. V., MADISON, K. R., BLEY, K. R. and FOX, A. P. (1988) Multiple types of neuronal calcium channels and their selective modulation. *Trends Neurosci.* **11,** 431–438.

TSUNOO, A., YOSHII, M. and NARAHASHI, T. (1986) Block of calcium channels by enkephalin and somatostatin in neuroblastomaxglioma hybrid NG108–15 cells. *Proc. natn. Acad. Sci. U.S.A.* **83,** 9832–9836.

USOWICZ, M. M.,PORZIG, H., BECKER, C. and REUTER, H. (1990) Differential expression by nerve growth factor of two types of Ca^{2+} channels in rat phaeochromocytoma cell lines. *J. Physiol.* **426,** 95–116.

VANDENBERG, C. A. and HORN, R. (1984) Inactivation viewed through single sodium channels. *J. gen. Physiol.* **84,** 535–564.

WANGER, J. A., SNOWMAN, A. M., BISWAS, A., OLIVERA, B. M. and SNYDER, S. H. (1988) ω-Conotoxin GVIA binding to a high-affinity receptor in brain: characterization, calcium sensitivity and solubilization. *J. Neurosci.* **8,** 3354–3359.

WANKE, E., FERRONI, A., MALGAROLI, A., AMBROSINI, A., POZZAN, T. and MELDOLESI, J. (1987) Activation of a muscarinic receptor selectively inhibits a rapidly inactivated Ca^{2+} current in rat sympathetic neurons. *Proc. natn. Acad. Sci. U.S.A.* **84,** 4313–4317.

WINEGARD, B. D. and LANSMAN, J. B. (1990) Voltage-dependent block of zinc of single calcium channels in mouse myotubes. *J. Physiol.* **425,** 563–578.

WRAY, D. W., NORMAN, R. I. and HESS. P. (1989) Calcium channels: structure and function. *Ann. N.Y. Acad. Sci.* **560.**

YAARI, Y., HAMON, B. and LUX, H. D. (1987) Development of two types of calcium channels in cultured mammalian hippocampal neurons. *Science* **235,** 680–682.

YATANI, A., WILSON, D. L. and BROWN, A. M. (1983) Recovery of Ca currents from inactivation: the roles of Ca influx, membrane potential, and cellular metabolism. *Cell molec. Neurobiol.* **3,** 381–395.