NEURONAL CALCIUM CHANNELS: KINETICS, BLOCKADE AND MODULATION

E. CARBONE* and D. SWANDULLA†

*Department of Anatomy and Physiology, University of Torino, I-10125 Torino, Italy
†Department of Membrane Biophysics, Max-Planck-lnstitut for Biophysical Chemistry, D-3400 Gottingen, F.R.G.

CONTENTS

I. INTRODUCTION 31

II. Ca CHANNEL TYPES 32

III. Ca CHANNEL KINETICS 32
   1. The Low-Threshold Channel 32
      (a) Activation kinetics 32
      (b) Inactivation kinetics 33
      (c) Voltage-dependent recovery from inactivation 34
      (d) Single low-threshold Ca channels 34
      (e) A kinetic model for the LVA channel 35
   2. The High-Threshold Channel 35
      (a) Activation kinetics 35
      (b) Inactivation kinetics 37
      (c) Voltage- and Ca2+-mediated inactivation 38
      (d) Voltage- and Ca2+-dependent recovery from inactivation 39
      (e) Single high-threshold Ca channels 41

IV. Ca CHANNEL LIGANDS AND BLOCKERS 41
   1. Blocking Ions 41
   2. Animal Toxins 43
      (a) α-CgTx-sensitive Ca channels 43
      (b) Spider toxins 44
   3. 1,4-Dihydropyridines as Ca-Antagonists and Ca-Agonists 45
   4. Kinetic vs Pharmacological Criteria for Identifying Ca Channel Subtypes 47

V. Ca CHANNEL MODULATION 47
   1. An Historical Overview 47
   2. G-Proteins as Regulators of Ca Channel Gating 49
   3. A Model for Voltage-Dependent Drug-Induced Inhibition 50
   4. Neurotransmitter Action on High-Threshold Ca Channel Subtypes 51
   5. Modulation of Ca Channel Inactivation 52

VI. CONCLUSIONS 53

ACKNOWLEDGEMENTS 53
REFERENCES 53

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 α1-subunit forms the ion-conducting pore and is expressed with a variable number of associated subunits (α2, 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
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, Cd²⁺-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).
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 Ca2+), 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 (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).

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 Ca2+ 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 Ca2+ is replaced by Na+ and the size of corresponding Na currents is reduced by micromolar additions of external Ca2+ or Mg2+ (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 $\tau$ of 1.4 sec. At -120 mV, the recovery is faster and $\tau$ 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
$\text{Ca}^{2+}$. 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 $\text{Ca}^{2+}$ (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 $\text{Ca}^{2+}$ or $\text{Ba}^{2+}$ 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 $\text{Ca}^{2+}$ 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 $\text{Ca}^{2+}$, 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.
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 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):
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 \(C_1 \rightarrow C_2\).

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.
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 \( \tau \): 170 \mu\text{s} in Ca^{2+} and 235 \mu\text{s} 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 currents 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).
(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. 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.)

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 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 Stanely, 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; Akaiake 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 \([\text{Ca}^{2+}]\), 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 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 \([\text{Ca}^{2+}]\)\textsuperscript{40} (Yatani \textit{et al.}, 1983; Gutnick \textit{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 \([\text{Ca}^{2+}]\), low. Under these conditions the speed and the effectiveness of repriming is considerably increased. A plot of maximal amplitude of reprimed currents vs \([\text{Ca}^{2+}]\), shows that the ability of the hyperpolarizing interval increases steeply when \([\text{Ca}^{2+}]\) is less than \(1 \times 10^{-7}\) M (Gutnick \textit{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.

![Graph](image-url)
(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; Cavalié et al., 1983) 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 \(\beta\)-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 \(\alpha\)-conotoxin (\(\alpha\)-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 \(\mu\)M) where their different affinity (\(K_P\)) for LVA and HVA channels allows dissection of the two currents. In most neurons 20 \(\mu\)M 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 \(\mu\)M 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 \(\mu\)M 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 currents are blocked effectively by 20 $\mu$M Cd$^{2+}$ during step depolarizations to $+10$ mV. 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 $\mu$M 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; Borttrain 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; Usovicz 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
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).

![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 application. (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 ω-CgTx (6.4 μM) (filled triangle), and after application of 1 μM Bay K 8644. External Ba$^{2+}$ 10 mM. (From Carbone et al., 1990b.)](image-url)
Neuronal calcium channels

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, $\omega$-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 $\omega$-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 $\mu$m), Cd$^{2+}$ and Co$^{2+}$ but were insensitive to $\omega$-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 disorders, 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 $\mu$m 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 $-50 \text{ mV}$. 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 ($+\text{-}(S)$)-202-791 and ($+\text{-}$)-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 ($K_D \sim 10 \text{ nM}$) and their action is maximal at very negative membrane potentials ($-90 \text{ mV}$) (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 $-20 \text{ mV}$) (see Fig. 11a). The current increase is less pronounced above $0 \text{ mV}$. (2) In the 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 $-50 \text{ mV}$ (Sanguinetti et al., 1986; Hoshi and Smith, 1987). (4) Ca channel openings are prolonged by nearly one order of magnitude with $1 \mu\text{M} \text{ Bay K 8644}$ (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 $0 \text{ mV}$ (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 $1 \mu\text{M} \text{ Bay K 8644}$ 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.

---

Fig. 11. Action of Bay K 8644 on $\alpha$-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 $\mu\text{M} \text{ Bay K 8644}$. Note the Ba current increase and the prolongation of tail currents at $-50 \text{ mV}$ in the presence of Bay K 8644. $V_h = -90 \text{ mV}$. (From Carbone et al., 1989.)
available in these neurons. Pretreatment of adult rat sensory neurons with \( \omega \)-CgTx (3 to 6 \( \mu \)M) 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 \( \omega \)-CgTx. There is also evidence for fast inactivating high-threshold Ca channels in hippocampal neurons that are DHP-sensitive (Toselli and Taglietti, 1990). In \( \omega \)-CgTx-treated rat DRG neurons, Bay K 8644 (1 \( \mu \)M) induces transient HVA Ca currents that are blocked by Cd\(^{2+}\) (40 \( \mu \)M) and are insensitive to amiloride (500 \( \mu \)M) (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 Polio, 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 \( \omega \)-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 \( \omega \)-CgTx (3-6 \( \mu \)M) 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), \( \omega \)-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 \( \omega \)-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 \( \omega \)-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), \( \gamma \)-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 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

---

**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.
Neuronal calcium channels

Table 1. Neurotransmitter-, Peptide- and GTP-γ-S-Induced Inhibition of High-Threshold Ca Channels

<table>
<thead>
<tr>
<th>Transmitter, drug</th>
<th>Action</th>
<th>Neuron type</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopamine</td>
<td>+ + +</td>
<td>Chick DRG</td>
<td>Marchetti et al., 1986</td>
</tr>
<tr>
<td>GABA&lt;sub&gt;b&lt;/sub&gt;</td>
<td>+ + +</td>
<td>Chick DRG</td>
<td>Deisz and Lux, 1985</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gras and Lux, 1989</td>
<td></td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>+ +</td>
<td>Chick DRG</td>
<td>Marchetti et al., 1986</td>
</tr>
<tr>
<td></td>
<td>+ +</td>
<td>Bullfrog DRG</td>
<td>Bean, 1989b</td>
</tr>
<tr>
<td></td>
<td>+ +</td>
<td>Frog DG</td>
<td>Lipscombe et al., 1989</td>
</tr>
<tr>
<td></td>
<td>+ +</td>
<td>NG108-15</td>
<td>McFadzean et al., 1989</td>
</tr>
<tr>
<td>Adrenaline</td>
<td>+ + +</td>
<td>Frog DG</td>
<td>Bley and Tson, 1990</td>
</tr>
<tr>
<td>Somatostatin</td>
<td>+ + +</td>
<td>AtT-20</td>
<td>Luini et al., 1986</td>
</tr>
<tr>
<td></td>
<td>+ + +</td>
<td>NG108-15</td>
<td>Tsunoo et al., 1986</td>
</tr>
<tr>
<td></td>
<td>+ + +</td>
<td>Rat SCG</td>
<td>Ikeda et al., 1987</td>
</tr>
<tr>
<td></td>
<td>+ +</td>
<td>Rat SC</td>
<td>Sah, 1990</td>
</tr>
<tr>
<td>Adenosine</td>
<td>+ + +</td>
<td>Rat DRG</td>
<td>Dolphin et al., 1986</td>
</tr>
<tr>
<td></td>
<td>+ + +</td>
<td>Chick DRG</td>
<td>Kasai and Aosaki, 1989</td>
</tr>
<tr>
<td>Ach</td>
<td>+ +</td>
<td>Rat SCG</td>
<td>Wanke et al., 1987</td>
</tr>
<tr>
<td></td>
<td>+ +</td>
<td>Rat Hippoc</td>
<td>Galwiler and Brown, 1987</td>
</tr>
<tr>
<td></td>
<td>+ +</td>
<td>Tolli and Lux, 1989</td>
<td></td>
</tr>
<tr>
<td>Leu-enkephalin</td>
<td>+ + +</td>
<td>NG108-15</td>
<td>Tsunoo et al., 1986</td>
</tr>
<tr>
<td>DADLE</td>
<td>+ +</td>
<td>NtG 5</td>
<td>Hescheler et al., 1987</td>
</tr>
<tr>
<td></td>
<td>+ +</td>
<td>NG108-15</td>
<td>McFadzean and Docherty, 1989</td>
</tr>
<tr>
<td>Dynorphin A</td>
<td>+ +</td>
<td>Mouse DRG</td>
<td>Gross and McDonald, 1987</td>
</tr>
<tr>
<td></td>
<td>+ + +</td>
<td>Bullfrog DRG</td>
<td>Bean, 1989b</td>
</tr>
<tr>
<td></td>
<td>+ +</td>
<td>Rat SC</td>
<td>Sah, 1990</td>
</tr>
<tr>
<td>Serotonin</td>
<td>+ +</td>
<td>Rat SC</td>
<td>Sah, 1990</td>
</tr>
<tr>
<td>LHRH</td>
<td>+ + +</td>
<td>Rat DRN</td>
<td>Pennington and Kelly, 1990</td>
</tr>
<tr>
<td></td>
<td>+ + +</td>
<td>Bullfrog DG</td>
<td>Elmslie et al., 1990</td>
</tr>
<tr>
<td></td>
<td>+ + +</td>
<td>Frog SG</td>
<td>Bley and Tson, 1990</td>
</tr>
<tr>
<td></td>
<td>+ + +</td>
<td>AtT-20</td>
<td>Lewis et al., 1986</td>
</tr>
<tr>
<td></td>
<td>+ +</td>
<td>Rat DRG</td>
<td>Dolphin and Scott, 1987</td>
</tr>
<tr>
<td></td>
<td>+ +</td>
<td>Rat SCG</td>
<td>Ikeda and Schofield, 1989</td>
</tr>
<tr>
<td></td>
<td>+ +</td>
<td>Chick DRG</td>
<td>Kasai and Aosaki, 1989; Gras and Lux, 1989; Marchetti and Robello, 1989</td>
</tr>
</tbody>
</table>

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, Polio 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 γ(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):

\[ C^* \xrightarrow{\gamma(V)} C \xrightarrow{\beta(V)} O \] (2)

In this scheme O represents the open state of the channel; γ(V) and β(V) are the forward and backward activation rates of the channel. For GABA and dopamine in chick DRG neurons, γ(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*⇒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

\[
\begin{align*}
\text{Resting [closed]} & \quad + \quad \text{Ca}^{++} \\
\text{Depol [activated]} & \quad + \quad \text{Ca}^{++}
\end{align*}
\]

---

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* \(\rightarrow\) 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.)

---

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-\(\gamma\)-S on transient N-like Ca channels (see above). Several

---

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-\(\gamma\)-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, Polio 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_A 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
Neuronal calcium channels

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 currents 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 $\omega$-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.

ACKNOWLEDGEMENTS

We wish to thank Drs. H. D. Lux, A. Pollo, A. Formenti and E. Sher for stimulating discussions. This work was partly supported by NATO (grant no. 0576/87).

REFERENCES


AOSAKI, T. and KASAI, H. (1989) Characterization of two kinds of high-voltage-activated Ca-channel currents in
AKAIKE, N., KOSTYUK, P. G. and OSIPCHUK, Y. V. (1989b) Dihydropyridine-sensitive low-threshold calcium
AKAIKE, N., KOSTYUK, P. G. and OSIPCHUK, Y. V. (1989a) Nitrendipine blockade of cardiac calcium channels. High-affinity binding to the inactivated state.
BEAN, B. (1989b) Neurotransmitter inhibition of neuronal calcium currents by changes in channel voltage
BEZANILLA, F. and ARMSTRONG, C. M. (1977) Inactivation of the sodium channel. I. Sodium current experiments. J.
gen. Physiol. 70, 549–566.
BINDER, V. P. and ADAMS, M. E. (1989) α-Aga-I: a presynaptic calcium channel antagonist from venom of the
BOLAND, L. M. and DINGLEDINE, R. (1990) Multiple components of both transient and sustained barium currents in
of the voltage dependent Ca2+ channel from skeletal muscle transverse tubules using [3H]-PN200–100. 100.
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.
311, 570–572.
4379–4393.
channels of snail neurons. J. gen. Physiol. 85, 491–518.
CARBONE, E. and LUH, H. D. (1984b) A low voltage-activated, fully inactivating Ca channel in vertebrate sensory
CARBONE, E. and LUH, H. D. (1986) Low- and high-voltage activated Ca channels in vertebrate cultured neurons:
CARBONE, E. and LUH, H. D. (1987a) Kinetics and selectivity of a low-voltage-activated calcium current in chick and
rat sensory neurons. J. Physiol. 386, 547–570.
CARBONE, E. and LUH, H. D. (1988) α-Conotoxin blockade distinguishes Ca from Na permeable states in neuronal
calcium channels. Pflügers Arch. 413, 14–22.
Functions, Regulation and Clinical Relevance, (eds L. HURWITZ, L. D. PARTRIDGE and J. K. LEACH), The
chick sensory neurones. Pflügers Arch. 408, 860.
CARBONE, E., FORMENTI, A. and POLLO, A. (1990a) Multiple actions of Bay K 8644 on high-threshold Ca channels in
CARBONE, E., SHER, E. and CLEMENTI, F. (1990b) Ca currents in human neuroblastoma IMR32 cells: kinetics,
Neuronal calcium channels


Cruz, L. J. and Oli


Feldman, D. H., Oli


Gray, W. R., Oli


Neuronal calcium channels

57


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


