Calcium channel diversity
(updated 2003)

Annette C Dolphin
Department of Pharmacology
University College London
Gower St. London WC1E6BT, UK

tel. +44 207 679 3276
fax +44 207 813 2808
a.dolphin@ucl.ac.uk
I Calcium channel diversity

III Contents list
Introduction
1. Low threshold and high threshold voltage-gated calcium channels
2. Structure-function studies of voltage-gated calcium channels
3. The CaV1 or SCDF family of calcium channels
4. The CaV2 or ABE family of calcium channels
5. The CaV3 or GHI family of calcium channels
6. Calcium channel subunits are localised to different parts of the neuron
7. Intracellular calcium channels

IV Article definition
Calcium channels, when open, allow Ca\(^{2+}\) ions to pass down their electrochemical gradient. Here I describe the subtypes of Ca\(^{2+}\) channel and their functions

V Introduction
Voltage-dependent calcium channels (VDCCs) were first identified in crustacean muscle by Paul Fatt and Bernard Katz (1953) and subsequently studied extensively by Hagiwara and colleagues. These muscles still showed action potentials in the absence of external Na\(^{+}\), that were dependent on calcium (Ca\(^{2+}\)) entry. Ca\(^{2+}\) is generally present at a concentration of a few mM in the extracellular space, but inside the cell, the cytoplasmic concentration is about 0.1\(\mu\)M, kept low by a number of different pumps and buffering systems, as well as the general impermeability of the plasma membrane to the entry of Ca\(^{2+}\). VDCCs have subsequently been found in all types of excitable cell in vertebrates and invertebrates, and even plants. They fulfil numerous functions depending on the tissue, and it is thus not surprising that a number of subclasses of VDCC have been identified.

Examination of the biophysical properties of VDCCs required the advent of voltage-clamp and subsequently patch clamp technology. VDCCs are normally closed at resting membrane potentials, and open upon depolarisation, because part of the channel structure senses the change in transmembrane voltage (see below). The resultant current through the cell membrane can be characterised by a number of properties, including the membrane potential range over which the channel reaches its maximum open probability, and as well as the kinetics or time-dependent properties of the current. Different single channel currents can also be identified, with varying properties (see Figure 1 for examples of cloned N type (CaV2.2) single channel currents). The task of matching these single channel types with the currents observed in entire cells is a difficult one, but has been made easier by the cloning of the cDNAs for a number of VDCCs and the use of selective drugs and toxins to identify specific current components, that correspond to particular channel types.

VI Main text
1. Low threshold and high threshold voltage-gated calcium channels
In a number of tissues, including certain cardiac muscle cells and subsequently also in neurons and other excitable cells, it became apparent that there are two types of calcium current. One is activated by small depolarisations and shows rapid voltage-dependent inactivation; this is termed low voltage-activated (LVA), or T for transient. The second is activated by large depolarisations and is termed high voltage-activated (HVA). The single calcium channels underlying these currents are also clearly distinct, T type channels being of small conductance (5-9 pS in 110 mM Ba\(^{2+}\)) and showing rapid inactivation during a voltage step, whereas HVA channels are of larger conductance (13-24 pS) (Carbone & Lux 1984).

HVA currents have been further subdivided. In skeletal and cardiac muscle, the HVA current was termed L for long lasting, and was found to be sensitive to a number of calcium channel antagonist drugs including the 1,4-dihydropyridines (DHPs), such as nifedipine. Furthermore, L type current could be enhanced by another drug in the DHP class, called BayK8644, which has proved very useful as a diagnostic tool for the presence of L type channels. Subsequent studies by Tsien and colleagues (Nowycky et al. 1985) in sensory neurons showed the presence not only of L-type currents, but also of a second HVA component of current that was termed N (for neuronal). This was found to have an intermediate single channel conductance (13-18 pS) and was not sensitive to DHPs but was irreversibly inhibited by \(\omega\)-conotoxin GVIA (\(\omega\)-CTX GVIA), a peptide toxin from the cone shell mollusc _Conus geographus._

Another sub-group of calcium currents, insensitive to both \(\omega\)-CTX GVIA and DHPs, has now been reported in many tissues, indicating the presence of further current components. An extreme example is the cerebellar Purkinje cell where only a small proportion of the calcium current corresponds to N and L current, and the major calcium current in these cells has been termed P type. A selective blocker for the Purkinje cell calcium current has been found in a peptide toxin from the venom of the American funnel web spider _Agelenopsis aperta_, called \(\omega\)-Agatoxin IVA (\(\omega\)-Aga IVA). At higher concentrations this toxin also blocks a current component that has been termed Q (the letter after P), although the distinction between P and Q current is not always clear. In many neurons, despite the application of all three blockers, there often remains a substantial proportion that cannot be classified as L, N or P/Q. This residual current has been termed R (for resistant). Thus in native neurons and other cell types, biophysical properties and selective drugs and toxins allow the identification of 5 distinct current components T, L, N, P/Q and R. The more recent challenge has been to marry these components with the cloned VDCC classes (Figure 2).

2. Structure-function studies of voltage-gated calcium channels
The first VDCC to be cloned was \(\alpha_1\)S (for skeletal) now renamed Ca\(\alpha_{1.1}\), following purification of the DHP receptor from skeletal muscle, where it is concentrated in the T tubules, providing a rich source of starting material (Tanabe et al. 1987). The purified oligomeric complex from muscle consisted of 5 proteins, \(\alpha_1\) (~200 kD), \(\alpha_2\) (~140 kD), \(\beta\) (~50 kD), \(\delta\) (~20 kD) and \(\gamma\) (~30 kD). cDNA clones were obtained using primers obtained from the amino acid sequence of proteolytic fragments of the individual proteins.

2.1 The \(\alpha_1\) subunit
The deduced sequence of the skeletal muscle $\alpha_1$ subunit contained 1873 amino acids, and hydropathy plots indicated a structure consisting of 24 $\alpha$-helical transmembrane segments arranged as four repeated domains (I-IV) (Tanabe et al. 1987). The proposed model for the $\alpha_1$ subunit is presented in Figure 3A, and all the other cloned calcium channel $\alpha_1$ subunits, whose cDNAs were obtained by homology cloning, follow the same general pattern. The $\alpha_1$ subunit is thought to form a cylindrical structure encompassing the pore of the channel (Figure 3B). Specific structural elements include the positively charged arginine and lysine residues in transmembrane segment S4 of each domain (Figure 3A), also found in voltage-dependent Na$^+$ and K$^+$ channels. These amino acids are thought to form the voltage sensor, which may physically move in the membrane, in response to depolarisation. Such a movement would then be coupled to opening of the channel pore. The re-entrant P (pore) loops, between transmembrane segments 5 and 6 of each domain, are thought to form the external mouth and lining of the pore, by analogy with certain K$^+$ channels, the structure of which has been elucidated. Within each P loop there is a specific negatively charged glutamate residue (or aspartate in some positions in LVA channels), which appears to provide the selectivity filter of the channel. These glutamate residues are thought to co-ordinate Ca$^{2+}$ during its passage through the pore (Ellinor et al. 1995), and also provide a mechanism for channel block by heavy metal ions. Permeation through Ca$^{2+}$ channels can be blocked by a number of heavy metals including Cd$^{2+}$ and Co$^{2+}$, which associate very strongly with the Ca$^{2+}$ binding sites within the pore.

2.2 The $\beta$ subunit

This is termed an accessory or auxiliary subunit, as it does not form part of the channel pore. The skeletal muscle $\beta$ subunit was the first to be cloned, and the deduced molecular weight is 58 kDa. It contains no possible transmembrane segments (Figure 3B). Its domain structure has been elucidated, and one of them bears strong resemblance to an SH3 domain, that binds proline-rich regions, reinforcing the idea that the $\beta$ subunit also serves to anchor the $\alpha_1$ subunit to other proteins (Hanlon et al. 1999). Four $\beta$ subunit genes have been cloned, and a number of tissue-specific splice variants found. The nomenclature (with most prevalent distribution in parentheses) is $\beta_{1a}$ (skeletal muscle), $\beta_{1b}$ (brain), $\beta_2$ (heart, lung, brain), $\beta_3$ (brain, smooth muscle) and $\beta_4$ (brain, particularly cerebellum). The $\beta$ subunits bind to $\alpha_1$ subunits via a specific sequence on the intracellular loop between domains I and II of the $\alpha_1$ subunit, called the $\alpha$ interaction domain (AID) (Pragnell et al. 1994) (Figure 3B). The corresponding domain in $\beta$ is called the $\beta$ interaction domain or BID. In the lethargic mutant mouse, there is a mutation in $\beta_4$ before the BID, which results in a truncated mRNA, and loss of functional protein. This has been found to be responsible for the neurological defects in this mutant, which include absence epilepsy and ataxia (Burgess et al. 1997).

$\beta$ subunits have several roles, firstly they are important for the membrane expression of the $\alpha_1$ subunits, which otherwise are trapped in the endoplasmic reticulum (Brice et al. 1997). Secondly they have effects on the biophysical properties of the $\alpha_1$ subunits, resulting in their activation at more hyperpolarised potentials, and effects on inactivation that are specific to the different $\beta$ subunits.
2.3 The $\alpha_2$-$\delta$ subunit

The two auxiliary proteins $\alpha_2$ and $\delta$ are products of the same gene, and arise by post-translational proteolytic cleavage (Figure 3B). The cloned $\alpha_2$-$\delta$ subunit from skeletal muscle ($\alpha_2\delta$-1) has a deduced molecular weight of 125 kD (Ellis et al. 1988), but is highly glycosylated. The $\alpha_2$ subunit is now accepted to be located completely extracellularly and is linked by a disulfide bridge to the transmembrane $\delta$ subunit (Brickley et al. 1995). Most excitable cells contain $\alpha_2\delta$-1 protein, although a three further genes have been identified. Both $\alpha_2\delta$-2 and $\alpha_2\delta$-3 are largely localized to neuronal tissue, and a mutation in $\alpha_2\delta$-2 is responsible for spike-wave seizures and ataxia in the spontaneous mouse mutant *ducky* (Barclay et al. 2001).

2.4 The $\gamma$ subunit

The deduced primary sequence of the skeletal muscle $\gamma_1$ subunit indicates that it is a protein of 25 kD with four putative transmembrane domains (Figure 3B). Its function in skeletal muscle calcium channels remains obscure. A second $\gamma$ subunit gene ($\gamma_2$ or stargazin) has been cloned from brain tissue, by virtue of containing the mutation in another genetic mouse model of absence epilepsy *stargazer* (Letts et al. 1998). The $\gamma_2$ has about 25% homology with skeletal muscle $\gamma_1$. Subsequently 7 further putative $\gamma$ subunits have been cloned, but whether $\gamma_2$-$\gamma_7$ are calcium channel subunits remains to be determined.

3. The CaV1 (SCDF) family of calcium channels

The skeletal muscle CaV1.1 ($\alpha_1S$) subunit is unique in its properties, being very slowly activating, with relatively large gating charge movements. These are transient outward currents, occurring before channel opening, that are thought to represent movement of the S4 voltage sensors outwards in the membrane in response to depolarisation. As the channels activate so slowly, little or no inward Ca$^{2+}$ current passes during a single skeletal muscle action potential. Excitation-contraction coupling is thought to occur by direct coupling of the skeletal muscle channel to the ryanodine receptor on the sarcoplasmic reticulum, such that depolarisation and gating charge movement in the former results in opening of the ryanodine receptor and release of intracellular Ca$^{2+}$.

In addition to the skeletal muscle CaV1.1 ($\alpha_1S$) subunit, three further subtypes of L type calcium channel $\alpha_1$ subunit have been identified, termed $\alpha_1C$, $D$ and $F$ or CaV1.2, 1.3, and 1.4 (Figure 2). The CaV1.2 is present in cardiac tissue, and also in many other excitable tissues including brain. Distinct short and long isoforms of CaV1.2 occur as a result of alternative splicing from a single gene. The CaV1.3 class shows over 70% homology with CaV1.2, but its localisation shows particular prevalence in brain, neuroendocrine tissue and pancreatic islets. It is also found in hair cells of the ear, and cardiac pace-maker cells (Platzer et al. 2000), and has the distinctive property of being activated at lower potentials than CaV1.2 (Koschak et al. 2001). A novel VDCC gene, termed CaV1.4 ($\alpha_1F$) has recently been identified whose expression is restricted to retina. It also encodes an L type channel, and a mutation in this gene has been identified to be responsible for one type of congenital night blindness (Bech-Hansen et al. 1998).

Several sites at which alternative splicing occurs have been identified in a number of the $\alpha_1$ subunits. Studies have also identified the regions of the channel to which the DHP calcium channel ligands bind. As expected, these drugs are not
simple pore blockers, and their binding seems to be restricted to specific transmembrane residues in domains III and IV (Grabner et al. 1996).

4. The CaV2 (ABE) family of calcium channels

The neuron-specific CaV2.2 (α1B) codes for the N type calcium channel. When it is expressed in a number of different systems, the ensuing current can be completely and irreversibly blocked by ω-CTX GVIA (Dubel et al. 1992).

Expression of CaV2.1 (α1A) results in a current that can be inhibited by ω-Agatoxin-IVA, and also by ω-CTX MVIIIC. Thus CaV2.1 is thought to represent both P and Q type current first identified in cerebellar Purkinje and granule cells respectively. The variable sensitivity to toxins may be a result of expression of different splice variants in different tissues. The CaV2.1 channel is highly expressed in both Purkinje and granule cells (Mori et al. 1991), but is not restricted to neurons. It has about 33% homology with the L type channels CaV1.2 and CaV1.3 and much greater homology (54%) with CaV2.2 (Figure 2). One interesting feature of CaV2.1 is the number of different disease-related mutations that have been associated with this gene which result in ataxias and cerebellar degeneration, both in man and in spontaneously arising mutant strains of mice (Zhuchenko et al. 1997; Lorenzon & Beam 2000).

CaV2.3 or α1E gives rise to a current showing greater inactivation than the other CaV2 family members. When expressed in Xenopus oocytes and mammalian expression systems, the current induced by CaV2.3 is not inhibited by ω-conotoxin GVIA. It is also fairly sensitive to Ni2+, and shows activation at quite hyperpolarised potentials. It was initially suggested that CaV2.3 codes for a subtype of LVA channel (Soong et al. 1993), but more recently it has been suggested to constitute the residual toxin-insensitive HVA R type current (Randall & Tsien 1995) (Figure 2). The CaV2.3 channel is found in most regions of the central nervous system, and within the cerebellum is found in both Purkinje and granule cell layers.

4.1 Modulation by G proteins of CaV2 calcium channels

Activation of a number of G-protein-coupled receptors, associated with pertussis toxin sensitive G proteins, results in an inhibition of calcium currents in neurons and neurosecretory cells (Dolphin 1998). This is characterised by a slowing of the activation phase of the current and a reduction of current amplitude, both of which can be reversed by large depolarisations, or more physiologically by trains of action potentials. Thus the inhibition is said to be voltage-dependent. Part of the physiological importance of this inhibitory process lies in the fact that these channels are situated at presynaptic terminals, where they supply the Ca2+ for transmitter release. This mechanism thus provides a basis for presynaptic inhibition (Toth et al. 1993), a very important physiological process in the regulation of the excitability of neuronal circuits.

This inhibition can be reconstituted with cloned and expressed calcium channels, and is shown by all three of this subfamily of channels, with CaV2.2 showing the greatest ability to be modulated by G proteins, and CaV2.3 the least. The activated G protein species involved is Gβγ (Ikeda 1996), and its mechanism of action appears to involve the calcium channel β subunit (Meir et al. 2000). Gβγ and calcium channel β subunits both bind to the I-II linker of all CaV2 channels (Walker & De Waard 1998), but the N terminus of these channels is essential for their G
protein modulation (Page et al. 1998; Canti et al. 1999). Research is now in progress to understand further the mechanism by which these channels are modulated.

5. The CaV3 (GHI) family of calcium channels

Recently a new sub-family of voltage-dependent calcium channel α1 subunits has been cloned, whose structure is superficially very similar to the CaV1 and CaV2 α1 subunits (Perez-Reyes et al. 1998) (Figure 2). When expressed, CaV3.1, CaV3.2 and CaV3.3 (α1G, H and I) form low voltage-activated currents that also have a small single channel conductance, like native T type channels. However, unlike the HVA channels, these α1 subunits do not contain the same consensus binding site (AID) for a β subunit on the I-II loop, that is present in all the HVA channels. It is unclear whether, and if so, how, they are influenced by any accessory subunits. They show differential distributions with all being present in various brain regions and CaV3.2 being prevalent in cardiac tissue.

6. Calcium channel subunits are localised to different parts of the neuron

Different neuronal cell types have widely varying complements of calcium channels, and these are not located uniformly within the neuron. There are numerous studies that show N-type channels to be involved in the entry of Ca\(^{2+}\) for transmitter release particularly in the peripheral, but also the central, nervous system, and P/Q type channels to be essential for transmitter release in the CNS. In many pathways, it appears that both types of channel are present and necessary for transmitter release (Dunlap et al. 1995). The CaV2.1 and CaV2.2 channels are also localised to presynaptic terminals by immunohistochemistry, although they are not exclusively targeted to this location (Figure 4). Both these channels bind proteins involved in synaptic transmission via the cytoplasmic linker between domains II and III.

In contrast, L type channels are largely present in cell bodies and dendrites and are involved in the depolarisation-induced entry of Ca\(^{2+}\) controlling gene transcription and other Ca\(^{2+}\)-dependent processes (Figure 4). There is much evidence that native T type channels are situated in the dendrites in a number of cell types. The mechanisms whereby different VDCCs are targeted to specific regions of neurons is unknown.

7. Intracellular calcium channels

The inositol trisphosphate and ryanodine receptors are present in the membranes of the endoplasmic and sarcoplasmic reticulum, and are involved in the release of Ca\(^{2+}\) into the cytoplasm from these intracellular stores (Mignery et al. 1989). They have a very similar structure, each consisting of 4 subunits, with an estimated 12 transmembrane segments at the C terminal end, and a very large cytoplasmic N terminal domain. This forms a vestibule for drug binding and allosteric effects associated with Ca\(^{2+}\)-dependent Ca\(^{2+}\) release.

7.1 The inositol trisphosphate receptor

The trigger that opens this channel is IP\(_3\), generated by the activation of receptors that stimulate phospholipase C (PLC). The IP\(_3\) receptor is thus an integral part of a number of pathways involving G protein coupled receptors, linked to the
Gq/11 subclass of GTP binding protein and stimulating PLCβ, or growth factor receptors coupled to PLCγ. Their effect is to increase cytoplasmic Ca\(^{2+}\) from intracellular stores via elevation of IP3, rather than by direct entry across the plasma membrane. At least three IP3 receptor isoforms are known. The N terminal portion of the channel binds IP3 and other allosteric effectors. Up to four molecules of IP3 are able to bind, one to each subunit, and positive co-operativity is shown. There are also phosphorylation sites and ATP binding sites in this domain (Mikoshiba 1993). IP3 receptors have recently been shown to be physically linked to certain G protein coupled receptors via the synaptic protein Homer, which appears to be a means of physically co-localising these functionally-coupled proteins (Tu et al. 1998).

7.2 The ryanodine receptor calcium channel
The skeletal muscle ryanodine receptor (RyR1) is one of the largest cloned proteins. Each monomer has over 5000 amino acids, and thus the tetrameric channel has a molecular weight of over 2 million. There are also two other ryanodine receptor isoforms (RyR2 and 3) in cardiac muscle, brain and other tissues. In skeletal muscle, ryanodine receptors are activated by direct mechanical coupling to skeletal muscle L type Ca\(^{2+}\) channels brought about by juxtaposition of the T tubules and the sarcoplasmic reticulum, via interaction sites on the calcium channel II-III linker of Cav1.1 (Dirksen & Beam 1999). This causes Ca\(^{2+}\) release from the sarcoplasmic reticulum without prior Ca\(^{2+}\) entry through the L type channels (see section 3). In contrast, in cardiac muscle, the activation of the ryanodine receptors is thought to occur via initial Ca\(^{2+}\) entry across the plasma membrane, opening the ryanodine receptor channels by the co-operative process of Ca\(^{2+}\)-induced Ca\(^{2+}\) release. The drug ryanodine, which is a plant alkaloid, locks the channels in a low conductance open state, and depletes the internal stores of Ca\(^{2+}\). Caffeine also activates ryanodine receptors.

8. Summary
This article has outlined a number of ways in which Ca\(^{2+}\) levels may be elevated inside cells, and also examined in detail the different functions of voltage-dependent calcium channels. An outline has been given of their subunit composition and how information from structure-function studies, suggests the mechanism of action of these channels.

VII References


incomplete X-linked congenital stationary night blindness. Nature Genetics 19, 264-267.


encoding the $\alpha_1$ and $\alpha_2$ subunits of a DHP-sensitive calcium channel. Science 241, 1661-1664.


MEIR, A., BELL, D. C., STEPHENS, G. J., PAGE, K. M. & DOLPHIN, A. C. 2000: Calcium channel $\beta$ subunit promotes voltage-dependent modulation of $\alpha1B$ by G$\beta\gamma$. Biophysical Journal 79, 731-746.


PAGE, K. M., CANTI, C., STEPHENS, G. J., BERROW, N. S. & DOLPHIN, A. C. 1998: Identification of the amino terminus of neuronal Ca$^{2+}$ channel $\alpha1$ subunits $\alpha1B$
and α1E as an essential determinant of G protein modulation. Journal of Neuroscience 18, 4815-4824.


VIII Bibliography of recent reviews and books
IX Glossary

*Voltage-dependent calcium channel*: An ion channel, situated in the plasma membrane, that is selectively permeable to calcium ions, and opens in response to membrane depolarisation. Also termed voltage-gated calcium channel.

*Non-selective cation channel*: an ion channel that is selective for cations (positively charged ions) over anions (negatively charged ions), but shows little selectivity between Ca$^{2+}$, Na$^+$ and K$^+$.  

*Receptor channel*: a channel in which the receptor site for a transmitter forms an integral part of the channel itself, and binding of the receptor agonist is responsible for channel opening, for example the nicotinic acetylcholine receptor channel.
X Illustrations

Figure 1
Examples of expressed $\alpha_{1B}$ VDCC single channel and ensemble average currents. The cDNA for the calcium channel subunits was transfected into a cell line which contains no endogenous calcium channels. In this way it properties may be investigated in isolation. The experiment was performed as described in Meir et al. (2000)

Figure 2
LVA and HVA channel types
Figure 3
Model of the Ca$^{2+}$ channel oligomeric complex
A) the topology of the $\alpha_1$ subunit, showing the S4 transmembrane segments, containing a motif of positively charged amino acid residues, and the P regions between S5 and S6.
B) the putative structure of the oligomeric VDCC complex. The region of association of the $\gamma$ subunit is not yet known (reprinted with modification from *Trends in Neurosciences* 21, WALKER, D. & DE WAARD, M. Subunit interaction sites in voltage-dependent Ca2+ channels. 148-154 (1998), with permission from Elsevier Science.)
Figure 4
Idealised neuron with most prevalent distribution of VDCCs
Different neuronal cell types have widely varying complements of VDCCs, for example they may not express T-type currents. The bold letters indicate the predominant distributions of the different VDCC subtypes, when they are present.