CALCIUM as a CELLULAR REGULATOR

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Voltage-Gated Calcium Channels

Richard W. Tsien
David B. Wheeler

Generic Properties and Structure of Voltage-Gated Ca\textsuperscript{2+} Channels

Voltage-gated Ca\textsuperscript{2+} channels regulate Ca\textsuperscript{2+} entry in a potential-dependent manner and thereby contribute to Ca\textsuperscript{2+}-signaling in a wide variety of cell types, including nerve, endocrine, and muscle cells. As signal transduction molecules par excellence, these channels act as links between the realms of electrical signaling and intracellular messengers (Hille, 1992). A single opening of a Ca\textsuperscript{2+} channel can allow many hundreds or thousands of Ca\textsuperscript{2+} ions to flow into the cytoplasm, thus generating a rise in [Ca\textsuperscript{2+}]\textsubscript{i} that may control vital functions such as excitability, rhythmicity, transmitter or hormone release, contraction, metabolism, and gene expression (Tsien and Tsien, 1990). To initiate such events effectively, Ca\textsuperscript{2+} channels have evolved as very efficient and highly regulated enzymes to catalyze the downhill flow of Ca\textsuperscript{2+} across membranes (Tsien et al., 1987b). Some key features of this enzymatic activity are as follows. Activation of Ca\textsuperscript{2+} channels is steeply voltage-dependent. The opening of individual channels occurs more quickly and is more complete with larger depolarizations, similar to voltage-gated Na\textsuperscript{+} and K\textsuperscript{+} channels. Typically, Ca\textsuperscript{2+} channels open within one or a few milliseconds after the membrane is depolarized from rest, and close (deactivate) within a fraction of a millisecond following repolarization. Inactivation, the closing of channels during maintained depolarization, strongly influences the cytosolic Ca\textsuperscript{2+} signal that arises from cellular electrical activity. While inactivation is a general property of Ca\textsuperscript{2+} channels, the speed of inactivation varies widely, ranging from very slow (requiring second-long depolarizations) to relatively rapid (tens of milliseconds). Selectivity of voltage-gated Ca\textsuperscript{2+} channels for Ca\textsuperscript{2+} ions is remarkably high, so that Ca\textsuperscript{2+} is the main charge carrier even when Ca\textsuperscript{2+} is greatly outnumbered by other ions, as under normal physiological conditions. Permeation of Ca\textsuperscript{2+} through a single open Ca\textsuperscript{2+} channel can achieve rates of millions of ions per second when the electrochemical gradient is large. At driving forces reached physiologically, the flux rate is more modest, but sufficient to cause a large increase in [Ca\textsuperscript{2+}]\textsubscript{i} (>1 \textmu M) in a very localized domain (~1 \mu m) near the mouth of the open channel. The basic features of channel opening and closing (collectively referred to as “gating”) and Ca\textsuperscript{2+} ion selectivity and permeation are intrinsic properties of all voltage-gated Ca\textsuperscript{2+} channels, evidently highly conserved in evolution. Our newly increased understanding of how these physiological characteristics come about is reviewed at the end of the chapter.

The powerful functional capabilities of Ca\textsuperscript{2+} channels are rooted in their molecular architecture. As far as we know, all voltage-gated Ca\textsuperscript{2+} channels are comprised of multiple components, that come together to form a large macromolecular complex (~500 kDa). The generic structure contains four subunits called \( \alpha_1, \alpha_2, \delta, \) and \( \beta \) (Fig. 8.1). The first examples of each of these subunits were originally isolated from skeletal muscle transverse tubules by biochemical techniques more than a decade ago (Catterall and Curtis, 1987; Campbell et al., 1988; Catterall et al., 1988; Glossmann and Striessnig, 1990). Each subunit has been cloned in one or more forms within the last dozen years (Tanabe et al., 1987; Ellis et al., 1988; Ruth et al., 1989). The \( \alpha_1 \) subunit is a large (200–260 kDa) transmembrane protein that contains the channel pore, the voltage-sensor, and the gating machinery. Because the \( \alpha_1 \) subunit appears to be able to form a functional Ca\textsuperscript{2+} channel on its own, the other subunits are sometimes referred to as auxiliary or ancillary subunits. The \( \alpha_2\delta \)-subunit (175 kDa) is a dimer, consisting of glycosylated \( \alpha_2 \) and \( \delta \) proteins linked together by disulfide bonds, derived by post-translational processing of a single
parent polypeptide (Ellis et al., 1988; De Jongh et al., 1990; Williams et al., 1992b). In current models (Fig. 8.1), δ is pictured as the transmembrane protein anchor and α2 as entirely extracellular (Jay et al., 1991; Hofmann et al., 1994). In contrast, the β-subunit (55 kDa) is entirely intracellular in location. A fifth subunit, known as γ (25 kDa), has long been known to be part of Ca^{2+} channels in skeletal muscle (Bosse et al., 1990; Jay et al., 1990), and the neuronal homologue of this subunit has recently been found in mouse neurons where it increases steady-state inactivation of voltage-gated Ca^{2+} channels (Lett et al., 1998).

With this brief introduction to the general properties of Ca^{2+} channels, we will proceed to an overview of the multiple types of Ca^{2+} channels, their molecular composition, and their adaptation for specialized roles in cellular Ca^{2+} signaling. In this chapter, we will not touch on other fundamental Ca^{2+} entry mechanisms, such as Ca^{2+} channels not gated by depolarization (Lewis and Cahan, 1995), nor will we discuss in detail the modulation of Ca^{2+} channels by G-proteins (Hille, 1994).

### Ca^{2+} Channel Subtypes Differ in Biophysical and Pharmacological Properties

Multiple types of voltage-gated Ca^{2+} channels were first distinguished on the basis of their voltage- and time-dependence, single-channel conductance, and pharmacology (e.g., Carbone and Lux, 1984; Nowycky et al., 1985). These criteria have led to a widely accepted classification of Ca^{2+} channels as T-, L-, N-, P-, Q-, and R-type (Tsien et al., 1987a; Llinás et al., 1992; Randall and Tsien, 1995). While these categories make good sense in view of the varied functional roles of the channel types in different organ systems, their relationship to the detailed subunit composition of Ca^{2+} channels in native tissue remains incompletely understood.

One physiologically relevant characteristic which varies considerably among the different Ca^{2+} channel types is the degree of depolarization required to cause significant activation. Based on such requirements, voltage-gated Ca^{2+} channels are sometimes divided into two groups: low-voltage-activated (LVA) and high-voltage-activated (HVA).

#### T-type Ca^{2+} Channels

The LVA Ca^{2+} channels are exemplified by T-type channels, so-named because they carry tiny unitary Ba^{2+} currents (6–8 pS) that occur soon after the depolarization step, giving rise to a transient average current (Carbone and Lux, 1984; Nilius et al., 1985; Nowycky et al., 1985). Another defining characteristic of classical T-type channels is their slow deactivation following a sudden repolarization (Matselson and Armstrong, 1986). T-type channel current records also exhibit a distinctive kinetic fingerprint: the superimposed current responses cross over each
other in a pattern not found with other rapidly inactivating Ca\(^{2+}\) channels, such as the R-type (Randall and Tsien, 1997). The kinetic properties are dominated by a strikingly voltage-dependent delay between the depolarizing step and the channel's first opening (Droogmans and Nilius, 1989). In addition to these properties, T-type channels have a unique pharmacological profile, characterized by mild sensitivity to 1,4-dihydropyridines (DHPs), such as nifedipine or nimodipine (Cohen and McCarthy, 1987), but acute sensitivity to mibebradil and newer experimental drugs (Ertel and Ertel, 1997). Within the overall category of T-type Ca\(^{2+}\) channel, further diversity has been found, particularly with respect to kinetic characteristics and pharmacology (Akaike et al., 1989; Kostyuk and Shirokov, 1989; Huguenard and Prince, 1992). Various subtypes of T-type Ca\(^{2+}\) channel may coexist in the same cell type and show rates of inactivation differing by as much as 5-fold, while sharing similar voltage-dependence of inactivation (Huguenard and Prince, 1992). Within a given neuron T-type Ca\(^{2+}\) channels have been found to contribute a greater percentage of the Ca\(^{2+}\) current measured in dendrites than in soma, suggesting that these channels support specialized functional roles in different parts of the cell (Kavalali et al., 1997).

L-type Ca\(^{2+}\) Channels

The L-type channels are generally categorized with the HVA group of channels, along with N-, P-, Q- and R-type channels. However, it is important to note that L-type channels may exhibit LVA properties under certain circumstances (Avery and Johnston, 1996). L-type channels in vertebrate sensory neurons and heart cells were initially labeled as large Ba\(^{2+}\) conductance contributing to a long-lasting current, with characteristic sensitivity to DHPs such as nifedipine or Bay K 8444 (Bean, 1985; Nilius et al., 1985; Nowycky et al., 1985). Members of this group were subsequently identified in other excitable cells, such as vascular smooth muscle, uterus, and pancreatic \(\beta\)-cells. Later, the designation of L-type was extended to refer to all channels with strong sensitivity to DHPs, including those found in skeletal muscle (Hofmann et al., 1988), even though clear-cut biophysical distinctions between skeletal and cardiac L-type channels were already known (Rosenberg et al., 1986). Thus, the category of L-type channels contains individual subtypes of considerable diversity. For example, three subtypes of L-type channel appear to coexist in cerebellar granule neurons: two subtypes that resemble those found in heart and a third that shows prominent voltage-dependent potentiation (Forti and Pietrobon, 1993).

N-type Ca\(^{2+}\) Channels

The most extensively characterized non-L-type Ca\(^{2+}\) channel was named N-type since it appeared to be largely specific to neurons as opposed to muscle cells and was clearly neither T- or L-type (Nowycky et al., 1985). It requires relatively negative resting potentials to be available for opening, somewhat like T-type, but is high-voltage-activated, like L-type. This Ca\(^{2+}\) channel is insensitive to DHPs, but is potently and specifically blocked by a peptide toxin derived from the venom of the marine snail, Conus geographus, \(\omega\)-conotoxin GVIA (\(\omega\)-CTX-GVIA). The N-type channel is found primarily in presynaptic nerve terminals and neuronal dendrites, in addition to cell bodies (Westenbroek et al., 1992).

P-type Ca\(^{2+}\) Channels

Currents carried by P-type channels were originally recorded from cell bodies of cerebellar Purkinje cells, hence, the label “P-type” (Llinás et al., 1989, 1992). These channels are not blocked by DHPs or \(\omega\)-CTX-GVIA, but are exquisitely sensitive to block by \(\omega\)-Aga-IVA, a component of the venom of the funnel-web spider, Agelenopsis aperta (Mintz et al., 1992b), with an IC\(_{50}\) of ~1 nM (Mintz and Bean, 1993). P-type channels support a current that hardly inactivates during depolarizations lasting for several seconds. They are seen in virtual isolation in cerebellar Purkinje neuron cell bodies, but also contribute substantially to somatic currents in many other central neurons (Mintz et al., 1992a).

Q-type Ca\(^{2+}\) Channels

Currents supported by Q-type channels were initially characterized in cell bodies of cerebellar granule neurons (Zhang et al., 1993; Randall and Tsien, 1995). This component of current displays prominent inactivation during a ~0.1 s depolarization and is ~40–100-fold less sensitive to \(\omega\)-Aga-IVA than the P-type current. Thus, Q-type current differs from P-type current as classically defined in Purkinje neurons and found to coexist with Q-type current in granule neurons. Confidence in the distinct nature of Q-type current is based, in part, on its resemblance to currents generated by expression of cloned \(\alpha_{1A}\) subunits (Sather et al., 1993; Sten et al., 1993), as discussed further below. In some circumstances, the designation P/Q-type may serve to indicate \(\omega\)-Aga-IVA-sensitive current without further distinction between P- and Q-type Ca\(^{2+}\) channels.
R-type Ca\textsuperscript{2+} Channels

The R-type Ca\textsuperscript{2+} channel currents were identified in cerebellar granule cells as a current that remained in the presence of nimodipine, \(\omega\)-CTx-GIVIA, and \(\omega\)-Aga-IVA, inhibitors of the L-, N-, and P/Q-type channels, respectively (Ellinor et al., 1993; Zhang et al., 1993; Randall and Tsien, 1995). Figure 8.2 illustrates the pharmacological dissection of the various HVA Ca\textsuperscript{2+} currents, including R-type. This HVA current decays rapidly, and is unusually sensitive to block by Ni\textsuperscript{2+}. The biophysical and pharmacological properties of the macroscopic R-type current seem homogeneous enough to allow it to be treated as a single current component (Randall and Tsien, 1997), although it seems likely that further subdivision of this category will be appropriate (Tottene et al., 1996; Newcombe et al., 1998).

Molecular Basis of Ca\textsuperscript{2+} Channel Diversity

In recent years, findings from molecular cloning of Ca\textsuperscript{2+} channels have greatly increased our understanding of Ca\textsuperscript{2+} channel diversity. This has allowed (1) a new perspective on the familial relationships between various channel types, (2) the discovery of Ca\textsuperscript{2+} channels beyond those types uncovered by earlier biophysical and pharmacological analysis, and (3) a more precise description of the pharmacological properties of individual channel types.

Multiple Forms of \(\alpha\)-Subunit

Much of the diversity of Ca\textsuperscript{2+} channel types seems to arise from the expression of multiple forms of the \(\alpha\)-subunit, isolated by molecular cloning (e.g., Tanabe et al., 1987; Mikami et al., 1989; Mori et al., 1991; Starr et al., 1991; Dubel et al., 1992; Williams et al., 1992a, 1992b; Soong et al., 1993). Eight different Ca\textsuperscript{2+} channel \(\alpha\)-subunit genes have been distinguished in mammalian brain (Perez-Reyes et al., 1990; Snutch et al., 1990) and have been labeled as classes A, B, C, D, E (Snutch et al., 1990; Snutch and Reiner, 1992) G, H and I (Perez-Reyes et al., 1998; Cribbs et al., 1998; Talley et al., 1998). In a generally accepted nomenclature (Birnbaumer et al., 1994),
Figure 8.3 Phylogenetic relationships, based on amino acid sequence homology of membrane spanning regions, amongst \( \alpha_1 \)-subunits of voltage-gated \( Ca^{2+} \) channels. Dendrogram adapted from Zhang et al., (1993) Tsien (1998) and personal communication from E. Perez-Reyes and L. Cribbs. Chromosome locations according to Diriong et al., (1995), Perez-Reyes et al. (1998), and Cribbs et al. (1998).

The different \( \alpha_1 \) isoforms are designated as \( \alpha_{1A}, \alpha_{1B}, \alpha_{1C}, \alpha_{1D}, \alpha_{1E}, \alpha_{1G}, \alpha_{1H}, \alpha_{1I} \) and \( \alpha_{1S} \), where \( \alpha_{1S} \) refers to the original \( Ca^{2+} \) channel clone from skeletal muscle, first isolated by the group of the late Shosaku Numa (Tanabe et al., 1987). Based on sequence homology, the nine \( \alpha_1 \)-subunits can be assigned to various branches of a family tree as reviewed in Fig. 8.3. As indicated in this diagram, the nine \( \alpha_1 \) genes have been localized to at least seven different chromosomes (Diriong et al., 1995).

Three major subfamilies of \( \alpha_1 \) subunits clearly emerge on the basis of sequence homology. The first subfamily consists of \( \alpha_1 \)-subunits of classes S, C, and D. Along with the \( \alpha_1S \)-subunit from skeletal muscle, these include subunits first derived from heart muscle (\( \alpha_{1C} \) and neuroendocrine tissues (\( \alpha_{1D} \) but found in a variety of other organs, including brain and lung (Mikami et al., 1989; Williams et al., 1992b). These cDNAs encode channels classified as “L-type” because they are responsive to DHPS. The second \( \alpha_1 \) subfamily consists thus far of cDNAs derived from nervous tissue, including mammalian brain (A, B, and E) and marine ray electric lobe (doe-1 and doe-4, Ellinor et al., 1993). When expressed, they lack the characteristic DHP-response of L-type channels. Individual genes within this subfamily show > 60% identity with each other but only ~ 45% or less with members of the L-type subfamily. The third subfamily consists so far of \( \alpha_1 \) subunits supporting low voltage activated (T-type) channels, and were originally derived from brain (G, I) and heart (H).

The evolutionary divergence of the three channel subfamilies occurred relatively early, as would be expected from the fairly low sequence homology between various subfamily members (Fig. 8.3). This deduction can be corroborated by an examination of the distribution of the three \( Ca^{2+} \) channel types in organisms spread across many phyla, particularly L-type and N, P/Q, R-type channels. Both channel subfamilies are represented in species ranging from marine rays (Horne et al., 1993) through humans (Williams et al., 1992a. 1992b), and in many cases both families of channels are expressed within the same cells (e.g., Randall and Tsien, 1995). Amongst invertebrates, both channel subfamilies have been observed in molluscs (Edmonds et al., 1990), insects (Grabner et al., 1994; Smith et al., 1996), and nematodes (Schafer and Kenyon, 1995). Nematodes also express a relative of \( \alpha_{1C} \). Given the widespread distribution of the three \( Ca^{2+} \) channel subfamilies across the animal kingdom, their bifurcation must have occurred quite early during the speciation of Animalia.
Even greater diversity arises from alternative splicing of transcripts from the multiple Ca\(^{2+}\) channel genes (Tsien et al., 1991; Snutch and Reiner, 1992), as reviewed below.

Limited Variability of the \(\alpha_2\delta\) Complex

Similar or identical forms of the \(\alpha_2\delta\) complex are found in a variety of tissues, including skeletal muscle, heart, vascular and intestinal smooth muscle, and brain (Ellis et al., 1988; Biel et al., 1991). The limited variation that has been found has been attributed to alternative splicing of primary transcripts of a single gene (Kim et al., 1992; Williams et al., 1992b).

Multiple Forms of \(\beta\)-Subunit

All Ca\(^{2+}\) channels in their native state appear to contain \(\beta\)-subunits. The \(\beta\)-subunit was first identified in voltage-sensitive Ca\(^{2+}\) channels purified from skeletal muscle as an integral component with an apparent molecular weight of \(\sim 60\) kDa (Glossman et al., 1987; Takahashi et al., 1987). Four different types of \(\beta\)-subunit are known to exist in mammals and are now known as \(\beta_1\)–\(\beta_4\) (Birnbaumer et al., 1994) although they were called CaB1–CaB4 when first isolated (Hofmann et al., 1994). Diversity of these proteins is greatly increased by alternative splicing (designated by lower case letters, \(\beta_{2a}, \beta_{2b}, \ldots\)). This family of subunits has been extensively reviewed (Hofmann et al., 1994; Isom et al., 1994; De Waard et al., 1996).

cDNAs for the \(\beta_{1a}\)-subunit (L-type Ca\(^{2+}\) channel, rabbit skeletal muscle) were first isolated on the basis of peptide sequence and encoded a protein (524 amino acids), lacking homology to other known protein sequences (Ruth et al., 1989). Soon thereafter, cDNAs for two additional \(\beta\)-subunits, \(\beta_2\) and \(\beta_3\), were isolated by homology from rabbit heart (Hullin et al., 1992). A fourth \(\beta\)-subunit, \(\beta_4\), has been cloned from rat brain (Castellano et al., 1993).

In general, \(\beta\)-subunits are not found in one organ or tissue exclusively. Whereas \(\beta_1\) transcripts are expressed primarily in skeletal muscle, they also appear in brain, \(\beta_2\) is predominantly expressed in heart, aorta, and brain, while \(\beta_3\) is most abundant in brain but also present in aorta, trachea, lung, heart, and skeletal muscle, \(\beta_4\) mRNA is expressed exclusively in neuronal tissues, with the highest levels being found in the cerebellum.

The sequence of the various \(\beta\)-subunits supports biochemical evidence that they are peripheral membrane proteins associated with the cytoplasmic aspect of the surface membrane. Thus, the \(\beta\)-subunit of Ca\(^{2+}\) channel is not homologous to \(\beta_1\) and \(\beta_2\)-subunits of Na\(^{+}\) channels, which contain putative transmembrane spanning domains and are significantly glycosylated (Isom et al., 1994). Not only is the \(\beta\)-subunit not an integral membrane protein, but it is also "auxiliary" in the sense that it is not absolutely required for gating or permeation. In most but not all cases, Ca\(^{2+}\) channel function can be obtained with \(\alpha_1\), alone, even in the apparent absence of endogenous \(\beta\)-subunits. Nevertheless, \(\beta\)-subunits serve several important and intriguing functions: (1) they play a key role in the appropriate targeting of the complex of Ca\(^{2+}\)-channel subunits; (2) they act as modulators of the gating properties of \(\alpha_1\)-subunits, thereby contributing greatly to the functional diversity of Ca\(^{2+}\) channels; and (3) they are targets of regulation by protein kinases.

All of the \(\beta\)-subunits show the striking ability to increase functional \(\alpha_1\) activity. This has been measured in several ways, for example as increased DHP binding to membranes of mammalian cells transfected with appropriate \(\alpha_1\) subunits, or as increased Ca\(^{2+}\) channel current in Xenopus oocytes (e.g., Lacerra et al., 1991). Indeed, modulation of Ca\(^{2+}\) channel activity by the \(\beta\)-subunit has been observed on coexpression of all six \(\alpha_1\)-subunits with four \(\beta\)-subunits in all \(\alpha_1\)–\(\beta\) combinations tested (De Waard et al., 1995). It has been found by many groups that \(\beta\)-subunits tend to shift the voltage-dependence of activation and, if anything, to accelerate channel opening; inactivation rate was also increased (Lacerra et al., 1991; Singer et al., 1991; Varadi et al., 1991). \(\beta\)-subunits differ strikingly in their ability to speed inactivation, following the order \(\beta_3 > \beta_1 > \beta_4 > \beta_2\) (Hullin et al., 1992; Ellinor et al., 1993; Sather et al., 1993; De Waard et al., 1995). In addition to the multiplicity of \(\beta\)-subunit genes, alternative splicing of primary transcripts further increases the diversity of the \(\beta\)-subunit isoforms. Because each of the \(\beta\)-subunits appears able to partner with each of the \(\alpha_1\)-subunits, \(\beta\)-subunits heterogeneity may contribute to the diversity of Ca\(^{2+}\) channels in a multiplicative manner.

Analysis of the family of \(\beta\)-subunit sequences suggests an overall domain structure of VCCV, where V denotes highly variable regions located at the N- and C-termini and in a central region, and C indicates highly conserved regions (De Waard et al., 1995). Interestingly, the V regions are prone to splice variations, while the C regions contain the consensus sites for phosphorylation by protein kinases. The second C region also incorporates a stretch of amino acids critical for interaction with the \(\alpha_1\)-subunit (Witcher et al., 1995). The \(\beta\)-subunit binds to the cytoplasmic linker between repeats I and II of all the Ca\(^{2+}\) channel \(\alpha_1\) subunits tested. This linker contains a conserved motif (QQ-E–L-GY–WL–E), positioned 24 amino acids from the I86 transmembrane domain in each \(\alpha_1\)-subunit (De Waard et al., 1995). Mutations within this motif reduce the stimulation of
peak currents by the \( \beta \)-subunit and alter inactivation kinetics and voltage-dependence of activation (Pragnell et al., 1994). Glutathione S-transferase fusion proteins containing this motif interact with \(^{35}\)S-labeled \( \beta \)-subunits. The binding is fast and almost irreversible over an 8 h period.

Gene targeting has been used to knock out the \( \beta_1 \) gene (Gregg et al., 1996). The homozygous mice die at birth from asphyxia. In cultured myotubes, caffeine contractions are intact; action potentials are also normal, but fail to induce intracellular Ca\(^{2+} \) transients, as expected from a defect in excitation–contraction coupling. Most interesting is the finding that deletion of the \( \beta_1 \)-subunit reduces the level of \( \alpha_{1S} \) in the membrane to undetectable levels. This may be contrasted with the normal surface membrane localization of \( \beta_1 \) in \( \alpha_{1S} \)-null animals (mdg/mdg, Kndson et al., 1989).

The function of the \( \beta_3 \) subunit in neurons has also been investigated using targeted gene disruption (Namkung et al., 1998). Mice deficient in the \( \beta_3 \) subunit were indistinguishable from their wild type brethren by anatomical and gross behavioral criteria. However, examination of whole-cell Ca\(^{2+} \) currents in sympathetic neurons revealed a relative decrease in the fraction of L- and N-type current in the absence of \( \beta_3 \) subunit expression. Furthermore, without \( \beta_3 \) subunits, the voltage-dependence of P/Q-type Ca channel activation was altered, facilitating the opening of these channels with weaker depolarizations. This study emphasizes the diverse effects that \( \beta \) subunits may exert on various Ca\(^{2+} \) channel subtypes.

**New insights into the Molecular Basis of T-type Ca\(^{2+} \) Channels**

An understanding of the molecular basis of T-type channels has long been sought. Perez-Reyes and colleagues have recently succeeded in cloning two \( \alpha \) subunits—\( \alpha_{1G} \), \( \alpha_{1H} \)—each capable of supporting classical T-type calcium channel activity (Cribbs et al., 1998; Perez-Reyes et al., 1998). This goes a long way to closing the circle on Ca\(^{2+} \) channel diversity, which began with separation of high and low voltage-activated Ca\(^{2+} \) channels (Carbone and Lux, 1984), quickly developed into distinctions between T-, N-, and L-type channels (Nowacky et al., 1985), and eventually P-, Q- and R-type channels (Randall and Tsien, 1995; Linãs et al., 1992). Interestingly, the T-type channels provide the first example in which a brand new Ca\(^{2+} \) channel subunit has been isolated with the help of sequence information from the human genome. The evidence is convincing that \( \alpha_{1G} \) and \( \alpha_{1H} \) are both authentic T-type channel subunits (Cribbs et al., 1998; Perez-Reyes et al., 1998). Both subunits have the signature motifs one would expect for a Ca\(^{2+} \) channel, including pseudotetrameric transmembrane repeats, key acidic residues needed to support divalent cation selectivity and permeation, as well as positively charged residues in the S4 transmembrane region, key to voltage-dependent gating. Expression of these subunits in Xenopus oocytes or mammalian cell lines yielded currents with key earmarks of classical T-type channels, including a single-channel conductance of 6–8 pS, slow deactivation (shutting-off of conductance following a sudden repolarization), and a characteristic crossing pattern of current families evoked by a series of depolarizing voltage steps. Isolation of a T-type channel \( \alpha_1 \)-subunits opens up many promising avenues for future work. Localization of T-type channels and understanding their functional roles will be greatly facilitated by development of specific antibodies, knockout mice, and channel-Ca\(^{2+} \)-sensor hybrids. Renewed effort will be invested in finding agents to inhibit T-type channels selectively, following up present efforts with mibebradil, a T-type blocker, effective in the treatment of hypertension (Clozel et al., 1997), and curtoxin, a peptide toxin that blocks T-type channels but spares other voltage-gated Ca\(^{2+} \) channels (Chuang et al., 1998).
Many Possible Sources of Diversity of L-type Ca\(^{2+}\) Channels

The existence of three \(\alpha_1\)-subunits, classes S, C, and D, each capable of supporting L-type channel activity, provides an obvious starting point for attempts at understanding how L-type Ca\(^{2+}\) channel diversity might be generated from specific molecular structures. However, little information is yet available to link individual \(\alpha_1\) isoforms to functionally distinct forms of L-type channel activity (e.g., Forti and Pirotton, 1993; Kavalali and Plummer, 1994). While \(\alpha_{1D}\) subunit appears to be largely excluded from neurons according to Northern analysis and electrophysiological criteria, no sharp distinction has been made between currents generated by \(\alpha_{1C}\) (Mikami et al., 1989) and \(\alpha_{1D}\) (Williams et al., 1992b). Single-channel recordings of expressed \(\alpha_{1D}\) channels are lacking and analysis of the functional impact of various \(\beta\)-subunits on \(\alpha_{1C}\) and \(\alpha_{1D}\) is not extensive.

Most of the attention to date has been focused on splice variations of \(\alpha_{1C}\). These have a marked impact on channel behavior in several cases, producing (1) differences in sensitivity to DHPs in \(\alpha_{1C}\) variants found in cardiac or smooth muscle (Welling et al., 1993), (2) differences in the voltage-dependence of DHP binding (Soldatov et al., 1995), and (3) differences in susceptibility to cyclic AMP-dependent phosphorylation (Hell et al., 1993b). Further analysis will be greatly facilitated by knowledge of the genomic structure of the human \(\alpha_{1C}\) gene, which spans an estimated 150 kb of the human genome and is composed of 44 invariant and 6 alternative exons (Soldatov, 1994). The L-type channel in chick hair cells incorporates an \(\alpha_{1D}\)-subunit that differs from the \(\alpha_{1D}\)-subunit in brain due to expression of distinct exons at three locations (Kollmar et al. 1997b). It will be interesting to see if additional splice variations can account for L-type channel activity found at the resting potential of hippocampal neurons, possibly important for setting the resting [Ca\(^{2+}\)]\(_i\) (Avery and Johnson, 1996).

Advances in Understanding Diversity of N-type Ca\(^{2+}\) Channels

As discussed earlier, an important source of channel heterogeneity is the association of \(\alpha_1\)-subunits with different ancillary subunits. A good example of this is provided by the N-type Ca\(^{2+}\) channel in brain. Biochemical analysis has shown that the \(\alpha_{1B}\)-subunit associates with three different isoforms of \(\beta\)-subunit in rabbit brain (Scott et al., 1996). Antibodies against individual \(\beta\)-subunits were each able to immunoprecipitate \(\omega\)-CTX-GIVA binding activity (a marker of \(\alpha_{1B}\), while immunoprecipitation of \(\alpha_{1B}\) showed its association with \(\beta_{1B}, \beta_2, \) and \(\beta_4\).

Different isoforms of the N-type Ca\(^{2+}\)-channel subunit \(\alpha_{1B}\) have been isolated from rat sympathetic ganglia and brain by Lin et al. (1997). Alternative splicing determines the presence or absence of small inserts in the S3–S4 regions of domains III and IV (SFMG and ET, respectively). Different combinations of inserts in these putative extracellular loop regions are dominant in brain (+SFMG, δET) and ganglia (δSFMG, +ET). Most interestingly, the gating kinetics of the brain-dominant form are 2–4 fold faster than the ganglia-dominant form. This work provides one of the clearest examples of how alternative splicing contributes to a wide range of functional properties.

What Type(s) of Ca\(^{2+}\) Channel Currents Are Generated by \(\alpha_{1A}\)?

Initial observations about the class A \(\alpha_1\)-subunit suggested that it corresponds to the P-type channel (Mori et al., 1991; Linas et al., 1992). Closer comparison of the properties of \(\alpha_{1A}\)-subunits expressed in X. oocytes and those of P-type channels in cerebellar Purkinje cells, however, revealed clear differences. P-type channel currents activate at relatively negative potentials and support a sustained, non-inactivating current during depolarizing pulses longer than 1 s (Linas et al., 1992; Usowicz et al., 1992), whereas \(\alpha_{1A}\)-subunits expressed in X. oocytes activate at less negative potentials and exhibit marked inactivation within 100 ms (Sather et al., 1993). Furthermore, P-type current in cerebellar Purkinje neurons is half-blocked at \(\sim\)1 mM \(\omega\)-Aga-IVA, while the IC\(_{50}\) for blockade of class A channels expressed in oocytes is 100–200 nM (Sather et al., 1993; Stea et al., 1994). These biophysical and pharmacological differences cannot be attributed to a general peculiarity of the oocyte expression system since similar properties of expressed \(\alpha_{1A}\)-subunits have been observed in a study of \(\alpha_{1A}\) stably expressed in baby hamster kidney cells (Niidome et al., 1994). While it is possible that the kinetic differences between P-type channels and exogenously expressed Ca\(^{2+}\) channels might be attributed to alternative splicing of \(\alpha_{1A}\) (Skea et al., 1994; Sakurai et al., 1995) or its association with different \(\beta\)-subunits (Liu et al., 1996a; Moreno et al., 1997) there is no explanation yet for the difference in \(\omega\)-Aga-IVA sensitivity. An intriguing but speculative hypothesis is that the \(\omega\)-type channel arises from the 95 kDa protein often observed in the course of biochemical purification of non-L-type Ca\(^{2+}\) channels. Originally interpreted as a subunit of the N-type Ca\(^{2+}\) channel, the 95 kDa protein has now been shown to be a truncated version of \(\alpha_{1A}\) containing repeats I and II and the II–III...
loop (Scott et al., 1998). It is conceivable that this "hemi-α1-subunit" dimerizes to form a channel (cf., dimeric K⁺ channels, Ketchum et al., 1995). Systematic studies of the electrophysiological properties of the 95 kDa form of α₁A are a logical next step.

While characteristics of α₁A-subunits expressed in oocytes of mammalian cells do not match P-type, they do correspond well to a component of Ca²⁺ channel current in cultured cerebellar granule cells, designated Q-type to distinguish it from P-type current (Zhang et al., 1993; Randall and Tsien, 1995). In the granule neurons, about half of the total Ca²⁺ channel current was blocked by high concentrations of ω-Aga-IVA. Analysis of the overall ω-Aga-IVA-responsive current revealed two distinct components: a non-inactivating P-type current, blocked with an IC₅₀ of about 1 nM, and an inactivating Q-type current that was half-blocked by 90 nM ω-Aga-IVA. These components were present in a ~1:3 ratio, where the Q-type current represented the largest individual component in the cerebellar granule neurons. In all respects, Q-type current displayed a strong qualitative resemblance to α₁A channels heterologously expressed in oocytes (Sather et al., 1993; Stea et al., 1994) or baby hamster kidney cells (Niidome et al., 1994).

Functional Roles of Ca²⁺ Channels

The molecular diversity of voltage-gated Ca²⁺ channels may reflect the variety of functional roles that they are called upon to serve. With the exception of α₁S, which appears highly localized to skeletal muscle, α₁-subunits are broadly distributed across the spectrum of excytotic cells. At the level of individual cells, however, the different channel types often show distinct patterns of localization to different parts of the cell. Some channel types are mainly found at presynaptic release sites where they allow Ca²⁺ entry that triggers neurotransmitter release, while other tend to be found primarily on cell bodies where they may help to shape the action potentials or regulate excitability (Lemos and Nowycky, 1989; Fisher and Bourque, 1996; Reuter, 1996). Here, we provide an overview of the physiological actions of the voltage-gated Ca²⁺ channels.

T-type Ca²⁺ Channels

The T-type Ca²⁺ channels are of considerable importance for many different organ systems. In cardiac cells, T-type Ca²⁺ channels are generally present at much lower density than L-type channels, if at all. Consequently, L-type channels represent the predominant pathway for Ca²⁺ entry and excitation-contraction coupling. However, T-type channels supply a major fraction of the current recorded in cells from the sinoatrial node, the natural source of cardiac rhythms, and thus provide a significant contribution to the inward current that drives the last stages of the pacemaker depolarization (Lei et al., 1998; Hagiwaara et al., 1988). T-type channels also support oscillatory activity and repetitive activity in the thalamus (Jahnsen and Llinás, 1984), particularly in the nucleus reticularis, which acts as a major rhythm generator of cortical electrical poteniations. T-type Ca²⁺ channels play a prominent role in dendritic Ca²⁺ signaling in hippocampal and cortical neurons (Magee et al., 1995). Because of their ability to open at relatively negative membrane potentials, they can provide dendritic Ca²⁺ entry during synaptic depolarizations too weak to trigger a regenerative Na⁺-dependent action potential. Interestingly, expression of T-type channels in smooth muscle fluctuates in synchrony with the cell cycle (Kuga et al., 1996), and may be associated with cell proliferation (Schmitt et al., 1995).

L-type Ca²⁺ Channels

The L-type Ca²⁺ channels are widely distributed in muscle, nerve, and endocrine cells. Their unique biochemical properties and their subcellular localization put them in a good position to act as transducers that link membrane depolarization to intracellular signaling. In the brain, for example, L-type Ca²⁺ channels are found in the cell bodies and proximal dendrites of hippocampal pyramidal cells, as visualized with a monoclonal antibody (Westenbroek et al., 1990). Class C calcium channels were concentrated in clusters at the base of major dendrites, while class D calcium channels were most generally distributed across cell surface membrane of cell bodies and proximal dendrites (Hell et al., 1993a). The most prominent roles of L-type channels include initiation of skeletal muscle contraction, hormone or neurotransmitter release, and provision of a Ca²⁺ signal involved in the regulation of gene expression.

Excitation–Contraction Coupling

The L-type Ca²⁺ channels play a central role in excitation–contraction coupling in skeletal, cardiac, and smooth muscle. In skeletal muscle, L-type Ca²⁺ channels contain the ω₁β subunit and are largely localized to the transverse tubule system. Ca²⁺ through the L-type channel is not required for skeletal muscle contraction (reviewed in Miller and Freedman, 1984), in contrast to cardiac muscle, where Ca²⁺ entry through these channels is essential for contractility (Näbauer et al., 1989). Interestingly, blockade of L-type channels in skeletal muscle by organic Ca²⁺ antagonists completely inhibits contraction.
(Eisenberg et al., 1983). The explanation of these findings centered around gating charge movement in the T-tubule membrane, which was known to be essential for intracellular Ca$^{2+}$ release (Schneider and Chandler, 1973). The DHPs eliminate charge movement, thereby blocking skeletal muscle contraction (Rios and Brum, 1987). The implication of these findings was that the DHP-sensitive L-type Ca$^{2+}$ channels act as voltage sensors to link T-tubule depolarization to intracellular Ca$^{2+}$ release.

This hypothesis was tested in elegant experiments by Tanabe, Numa, Beam, and their colleagues. The cloning of the DHP-receptor protein from skeletal muscle led immediately to its identification as a voltage-gated channel (Tanabe et al., 1987). Later, expression of the cloned DHP receptor in dysgenic skeletal muscle myotubes showed that it could restore electrically evoked contractility in these formerly nonresponsive cells (Tanabe et al., 1988), along with L-type Ca$^{2+}$ current (Tanabe et al., 1988; Garcia et al., 1994) and gating charge movement (Adams et al., 1990). While the skeletal DHP receptor allowed contraction even in the absence of extracellular Ca$^{2+}$, the cardiac L-type Ca$^{2+}$ channel restored contractility only if Ca$^{2+}$ entry occurred (Tanabe et al., 1990). The structural basis of the skeletal-type excitation–contraction coupling was investigated with molecular chimeras. By inserting pieces of the α1S gene into an α1C background, Tanabe et al. (1990) showed that the key domain was the intracellular loop joining repeats II and III of α1S (Fig. 8.4). More recently, other groups have shown that purified II–III loop fragments can directly activate the ryanodine receptor (Lu et al., 1994; El-Hayek et al., 1995) and that this region may contain phosphorylation sites for the regulation of excitation–contraction coupling (Lu et al., 1995).

**Excitation–Secretion Coupling**

While the vast majority of studies of neurotransmitter release have failed to identify a role for L-type Ca$^{2+}$ channels, this subtype has been implicated in a few specialized forms of exocytosis. For example, activation of L-type channels is required for zona pellucida-induced exocytosis from the acrosome of mammalian sperm (Florman et al., 1992). L-type channels also seem to play an important role in mediating hormone release from endocrine cells. Insulin secretion from pancreatic β-cells (Ashcroft et al., 1994; Bokvist et al., 1995), luteinizing hormone-releasing hormone release from the bovine infundibulum (Dippel et al., 1995), and catecholamine release from adrenal chromaffin cells (Lopez et al., 1994) are all reduced by inhibition of L-type Ca$^{2+}$ channels. L-type channels also seem to play an important role in supporting release of GABA from retinal bipolar cells (Maguire et al., 1989; Duarte et al., 1992), glutamate release from cochlear hair cells (Kollmar et al., 1997a), as well as dynorphin release from dendritic domains of hippocampal neurons (Simmons et al., 1993).

**Excitation–Transcription Coupling**

A number of extracellular factors that influence cell growth and activity depolarize the membranes of their target cells (Hill and Triesman, 1995). Membrane depolarization open voltage-gated Ca$^{2+}$ channels and the resulting influx of Ca$^{2+}$ can trigger gene transcription (for a review, see Morgan and Curran, 1989). L-type Ca$^{2+}$ channels are thought to play a role in this cascade because agonists of these channels can induce expression of several protooncogenes in the absence of other stimuli (Morgan and Curran, 1988). Ca$^{2+}$ entry through L-type channels has also been shown to induce expression of acetylcholine receptors in skeletal muscle (Huang et al., 1994). Interestingly, it is only the Ca$^{2+}$ that enters the cell via the L-type channel, and not that released from the sarcoplasmic reticulum, that can trigger this response. These findings suggest that the mode and location of Ca$^{2+}$ entry may be important to how the Ca$^{2+}$ signal is interpreted by the cell (Ghosh et al., 1994; Rosen and Greenberg, 1994). Some recent studies have shed light on the cascade of events that follows influx of Ca$^{2+}$ through L-type channels.

An example of a signal-transduction cascade where Ca$^{2+}$ entry is important involves the cAMP and Ca$^{2+}$ response element (CRE), and its nuclear binding protein (CREB) (Montminy and Bilezikjian, 1987; Hoeffler et al., 1988). The interaction of CREB with the CRE is facilitated when CREB is phosphorylated on serine-133 (Gonzalez and Montminy, 1989). The phosphorylation of CREB is catalyzed by several kinases, including Ca$^{2+}$-calmodulin kinases II and IV, and cAMP-dependent protein kinase (Greenberg et al., 1992). Thus, rises in [Ca$^{2+}$] may act either directly, via Ca$^{2+}$-calmodulin and its dependent kinases, or indirectly, by stimulating Ca$^{2+}$-calmodulin-sensitive adenylyl cyclase which leads to increased cAMP levels. Recent work has shown that Ca$^{2+}$ entry through L-type channels can trigger CREB phosphorylation (Yoshida et al., 1995; Deisseroth et al., 1996), but that Ca$^{2+}$ entry through N- and P-type channels is surprisingly ineffective (Deisseroth et al., 1998). Localized actions of Ca$^{2+}$ within close proximity of the mouths of the effective Ca$^{2+}$ channels (Deisseroth et al., 1996) may help explain the channel specificity.

Among the other divalent ions, Zn$^{2+}$ is particularly interesting because it regulates a wide variety of enzymes and DNA-binding proteins, provides an important developmental signal, and may be
Figure 8.4 Injection of L-type cDNA into mouse dystrophic myotubes rescues contractility. Expression of α1S allows electrically evoked contractions even in the absence of Ca\(^{2+}\) influx. In contrast, expression of α1C cannot support contractions without the influx of extracellular Ca\(^{2+}\). Transfer of the cytoplasmic loop joining domains II and III of the skeletal Ca\(^{2+}\)-channel into the cardiac isoform re-establishes Ca\(^{2+}\)-independent contractility. (Adapted from Tanabe et al. 1990, copyright Macmillan Magazines Limited.)

Involved in excitotoxicity and responses to trauma (for a review, see Smart et al., 1994). Interestingly, L-type Ca\(^{2+}\) channels can support Zn\(^{2+}\) influx into heart cells, where it can induce transcription of genes driven by a metallothionein promoter (Atar et al., 1995). Morphological studies have revealed that Zn\(^{2+}\) is highly enriched in a number of nerve fiber pathways, especially in boutons where it appears to be contained within vesicles (Smart et al., 1994). Furthermore, Zn\(^{2+}\) can be released from brain tissue during electrical or chemical stimulation (Assaf and Chung, 1984; Howell et al., 1984; Charton et al., 1985). Given that Zn\(^{2+}\) can be released by synaptic activity, and can enter cells via voltage-dependent Ca\(^{2+}\) channels, it seems likely that Zn\(^{2+}\) may play an important role in excitation–transcription coupling.

N-, P/Q-, and R-type Ca\(^{2+}\) Channels

The non-L-type HVA Ca\(^{2+}\) channels are widely distributed both pre- and postsynaptically in the central and peripheral nervous systems (Fig. 8.5). In the brain, antibodies to the N-type Ca\(^{2+}\) channel α2-subunit bind primarily on dendrites and nerve terminals in most regions (Westenbroek et al., 1992). P/Q-type channels are concentrated in presynaptic terminals, making synapses on cell bodies and on dendritic shafts and spines of many classes of neurons, and are present at lower density in the surface membrane of dendrites of most major classes of neurons (Westenbroek et al., 1995). Ca\(^{2+}\) channels containing the α1E-subunit, presumably encoding the R-type channel, are found mostly on cell bodies, and in some cases in dendrites, of a broad range of central neurons (Yokoyama et al., 1995). Thus, these classes of Ca\(^{2+}\) channels are ideally positioned to allow the Ca\(^{2+}\) influx that triggers neurotransmitter release and shapes the postsynaptic response to that release.

**Generic Properties of Excitation–Secretion Coupling**

The most commonly studied role of Ca\(^{2+}\) is its ability to trigger neurotransmitter release. The importance of Ca\(^{2+}\) ions in the release of neurotransmitter has been appreciated for more than 60 years (Feng, 1936). Seminal work by Katz (1969) and his colleagues demonstrated that Ca\(^{2+}\) ions exert their influence at the nerve terminal where they control the amount of neurotransmitter that is released. The action of Ca\(^{2+}\) ions in the regulation of neurotransmission was shown to be cooperative, requiring about four Ca\(^{2+}\) ions to bind to their receptor in order to trigger release (Dodge and Rahamimoff, 1967). The centrality of Ca\(^{2+}\) action in the nerve terminal was further supported by the observation that injection of Ca\(^{2+}\) into the terminal triggered...
Figure 8.5 Differential subcellular localization of class A–E Ca\(^{2+}\) channel subtypes in cortical neurons. Sagittal sections through the dorsal cerebral cortex of adult rats were incubated with affinity-purified antipeptide antibodies against unique amino acid sequences in the II-III intracellular loop of the indicated Ca\(^{2+}\) channel α1-subunits. The various antibodies were detected using fluorescein-tagged secondary antibodies and then visualized using a confocal microscope as described previously (Westenbroek et al., 1995). Reproduced with permission from Catterall et al. (1998).

the release of transmitter at the squid giant synapse (Miledi, 1973). Subsequently, the Ca\(^{2+}\)-sensitive protein, aequorin, was used to show that presynaptic [Ca\(^{2+}\)]\(_i\) increases during neurotransmission (Llinás and Nicholson, 1975).

Studies using simultaneous voltage-clamp of the presynaptic terminal and postsynaptic axon of the squid giant synapse provided direct measurements of the Ca\(^{2+}\) currents in the presynaptic membrane that trigger the release of neurotransmitter (Llinás et al., 1981; Augustine et al., 1985). Ongoing issues include the identification of presynaptic Ca\(^{2+}\) channels and clarification of the functional consequences of their diversity (for other recent reviews, see Olivera et al., 1994; Dunlap et al., 1995; Reuter, 1996).

Transmitter Release at Peripheral Synapses

At the neuromuscular junction, the release of neurotransmitter is generally mediated by a single Ca\(^{2+}\) channel type, although which type predominates varies from species to species. Invertebrate motor end plates utilize primarily P/Q-type channels. In crayfish, for example, inhibitory and excitatory transmitter release onto the claw-opener muscle was completely abolished by α-Aga-IVA, while α-Ctx-GVIA and nifedipine were both ineffective (Araque et al., 1994). In locusts and houseflies, motor end plate potentials are blocked by type I and II agonists, which inhibit P/Q-type channels, but not by type III agonists, which potently block both L- and N-type channels (Bindokas et al., 1991). In nonmammalian vertebrates, unlike invertebrates, neurotransmitter release at the neuromuscular junction is completely blocked by α-Ctx-GIVA. This is true for frogs (Kerr and Yoshihama, 1984; Katz et al., 1995), lizards (Lindgren and Moore, 1989), and chicks (De Luca et al., 1991; Gray et al., 1992). In mammals, on the other hand, α-Ctx-GIVA does not seem to have any effect on the evoked release of acetylcholine at the neuromuscular junction (Sano et al., 1987; Wessler et al., 1990; De Luca et al., 1991; Protti et al., 1991; Bowersox et al., 1995). In contrast, blockade of P/Q-type Ca\(^{2+}\) channels by α-Ctx-MVIIC, α-Aga-IVA or FTX completely abolishes transmission in mice (Protti and Uchitel, 1993; Bowersox et al., 1995; Hong and Chang, 1995) and humans (Protti et al., 1996). In all of these species, neuromuscular trans-
mission seems to rely on a single type of channel, one or another of those not sensitive to DHPs.

Sympathetic neurons contain both L- and N-type Ca$^{2+}$ channels but not P/Q-type channels (Hirning et al., 1988; Mintz et al., 1992b; Zhu and Ikeda, 1993). However, only N-type Ca$^{2+}$ channels seem to be important for the release of norepinephrine, inasmuch as α-CTx-GVIA blocks NE secretion (Hirning et al., 1988; Fabi et al., 1993) but DHPs do not (Perney et al., 1986; Hirning et al., 1988; Koh and Hille, 1996). Along similar lines, N- but not L-type Ca$^{2+}$ channels in sympathetic nerve terminals are susceptible to modulation of Ca$^{2+}$ current via autoreceptors for NE or neuropeptide Y (Toth et al., 1993). Thus, sympathetic nerve endings are like motor nerve terminals in relying on a single predominant type of Ca$^{2+}$ channel, in this case N-type, despite the sizeable contribution of L-type channels to the global Ca$^{2+}$ current. Reliance on N-type channels cannot be generalized to all autonomic terminals since P/Q-type channels have been found to play a prominent role in transmitter release in rat urinary bladder (Frew and Lundy, 1995).

Transmitter Release at Central Synapses

At central synapses, unlike synapses in the periphery, neurotransmitter release often involves more than one Ca$^{2+}$ channel type. Central neurons appear to be richly endowed with Ca$^{2+}$ channels, with as many as five or six different types of channels in an individual nerve cell (Mintz et al., 1992a; Randall and Tsien, 1995). Several more recent papers have reported that neurotransmission at specific synapses in the CNS depends upon the concerted actions of more than one type of Ca$^{2+}$ channel (Luebke et al., 1993; Takahashi and Momiyama, 1993; Castillo et al., 1994; Regehr and Mintz, 1994; Wheeler et al., 1994; Mintz et al., 1995; Wu et al., 1998). In general, L-type channels have not been found to play a significant role in the regulation of neurotransmitter release in the brain (Dunlap et al., 1995), although at some synapses they may contribute when potentiated by the DHP agonist Bay K 8644 (e.g., see Sabaté et al., 1995). The relative importance of N-type as opposed to P/Q-type Ca$^{2+}$ channels can also vary from one synapse to another. Studies of synapses in hippocampal and cerebellar slices suggest that a large majority of single-release sites are in close proximity to a mixed population of Ca$^{2+}$ channels that jointly contribute to the local Ca$^{2+}$ transient that triggers vesicular fusion (e.g., Mintz et al., 1995; Reid et al., 1998; but see also Reuter, 1995). The synergistic effect of multiple Ca$^{2+}$ channels arises because of limitations on the Ca$^{2+}$ flux through individual channels under physiological conditions. Indeed, the reliance on multiple types of Ca$^{2+}$ channels was not absolute but was relieved by increasing the Ca$^{2+}$ influx per channel, either by prolonging the presynaptic action potential or increasing [Ca$^{2+}$]o (Wheeler et al., 1996).

Interactions between Ca$^{2+}$ Channels and Components of the Release Machinery

The close functional relationship between specific types of Ca$^{2+}$ channels and transmitter release raises interesting questions about a possible molecular basis. Over the last few years, several groups have provided evidence for structural interactions between N- and P/Q-type Ca$^{2+}$ channels and molecular components of transmitter-release machinery. The synaptic membrane protein syntaxin 1 (p35), a key player in the fusion process, binds strongly to N-type Ca$^{2+}$ channels. This was revealed by experiments where antibodies against syntaxin immunoprecipitated α-CTx-GVIA binding activity from synapticosomal membranes (for review, see Bennett and Scheller, 1994). The syntaxin-binding site on N-type Ca$^{2+}$ channels has been localized to the intracellular loop between repeats II and III, more specifically to an 87 amino acid segment (residues 773–859) which includes a helix-loop-helix-loop-helix structure (Sheng et al., 1994). The corresponding 87 amino acid syntaxin-binding peptide was capable of interfering with the interaction between syntaxin and the solubilized α1B-subunit. The interaction of syntaxin 1A with loop II–III was not seen with α1A (skeletal L-type Ca$^{2+}$ channel), although it has recently been found with α1A (Martin-Moutot et al., 1996; Rettig et al., 1996). The participation of the II–III loop is particularly intriguing because of the involvement of the corresponding region in excitation–contraction coupling.

A different approach to the interaction between syntaxin and various types of voltage-gated Ca$^{2+}$ channels has been provided by coexpression experiments in Xenopus oocytes. Syntaxin 1A sharply decreased the availability of N-type channels (Bezprozvanny et al., 1995). This functional effect was due to stabilization of channel inactivation rather than simple block or lack of channel expression, as it was overcome by strong hyperpolarization. It was also found with Q-type but not L-type Ca$^{2+}$ channels. Thus, the syntaxin effect is specific for Ca$^{2+}$ channel types that participate in fast transmitter release in the mammalian CNS. The shift in inactivation was abolished by deletion of syntaxin’s carboxy-terminal transmembrane domain in accord with what would be expected from binding studies (Sheng et al., 1994). These results raise the possibility that, in addition to acting as a vesicle docking site, syntaxin may influence presynaptic Ca$^{2+}$ channels, promoting their
inactivation in the aftermath of activity-dependent vesicular turnover.

**Postsynaptic Ca\(^{2+}\) Influx**

Much of the electrical and biochemical signal processing in central neurons takes place within their dendritic trees. Ca\(^{2+}\) entry through voltage-gated channels is critical for many of these events. The idea that voltage-gated Ca\(^{2+}\) channels may contribute to electrophysiology in dendrites first arose in the interpretation of intracellular recordings from hippocampal pyramidal neurons (Spencer and Kandel, 1961). Initial intradendritic voltage recordings were conducted on the dendritic arbors of cerebellar Purkinje neurons (Linás and Nicholson, 1971; Linás and Hess, 1976; Linás and Sugimori, 1980) and apical dendrites of hippocampal pyramidal neurons (Wong et al., 1979). The ability of dendrites to support Ca\(^{2+}\)-dependent action potential firing was reinforced by experiments where apical dendrites of pyramidal neurons were surgically isolated from their cell bodies in a hippocampal slice preparation (Benardo et al., 1982; Masukawa and Prince, 1984). These experiments revealed a variety of Ca\(^{2+}\)-dependent active responses in the dendrites of central neurons that could be elicited by excitatory postsynaptic potentials or injection of depolarizing current pulses. Postsynaptic Ca\(^{2+}\) channels, including N- and L-type, have also been implicated in the release of neuropeptides from dendritic domains (Simmons et al., 1995).

More recent studies of the electrical properties of dendrites have been facilitated by the ability to visualize dendrites in brain slices, thus rendering dendrites accessible to patch electrodes (Stuart et al., 1993). These studies revealed that back-propagating Na\(^{+}\)-dependent action potentials can activate dendritic Ca\(^{2+}\) channels, thereby causing substantial decreases in intradendritic free Ca\(^{2+}\) (Jaffe et al., 1992; Stuart and Sakmann, 1994; Markram et al., 1995; Schiller et al., 1995; Spruston et al., 1995). Subthreshold excitatory postsynaptic potentials can also open Ca\(^{2+}\) channels and result in more localized changes in intradendritic Ca\(^{2+}\) concentration (Markram and Sakmann, 1994; Yuste et al., 1994; Magee et al., 1995). The presence of multiple types of voltage-gated Ca\(^{2+}\) channels on dendrites has been demonstrated by several techniques, including Ca\(^{2+}\) imaging (Markram et al., 1995), dendrite-attached patch-clamp recordings (Usowicz et al., 1992; Magee and Johnston, 1995), and immunocytochemistry (Westenbroek et al., 1990, 1992, 1995; Hell et al., 1993a; Yokoyama et al., 1995). In recent results from our group, recordings were made from isolated dendritic segments obtained by acute hippocampal dissection, and indicated that N-, P/Q-, and R-type channels all contribute to the overall HVA current in dendrites (Kavalali et al., 1997).

**Key Functions of Calcium Channels and Their Molecular Basis**

There is great interest in understanding the molecular determinants of key properties of voltage-gated Ca\(^{2+}\) channels, including generic characteristics common to the entire family of channels, as well as those properties that give individual types of Ca\(^{2+}\) channels their distinct character. Structure-function studies have advanced considerably through the analysis of recombinant channels.

**Selectivity, Permeation, and Block**

As mentioned in the introduction, Ca\(^{2+}\) channels operate with great efficiency in allowing Ca\(^{2+}\) permeation with rapid turnover rates while also showing exquisite selectivity for Ca\(^{2+}\) over other more abundant extracellular ions like Na\(^{+}\) (Tsien et al., 1987b). Permeation through open Ca\(^{2+}\) channels has been studied with patch-clamp techniques, often with ~100 mM external Ba\(^{2+}\) to increase the unitary current size. Under these conditions, the Ca\(^{2+}\) channel type with the largest Ba\(^{2+}\) conductance (L-type, ~25 pS) shows a unitary current amplitude of approximately ~1.6 pA at 0 mV, corresponding to a transfer rate of 5 million Ba\(^{2+}\) ions/s (Hess et al., 1986). Recordings from L-type channels at physiological levels of external Ca\(^{2+}\) (2 mM) yield a much smaller unitary conductance, 2.4 pS (Church and Stanley, 1996). Selectivity of voltage-gated Ca\(^{2+}\) channels for Ca\(^{2+}\) ions over monovalent cations is ~1000-fold (Hess et al., 1986), so that Ca\(^{2+}\) is the main charge carrier even when it is greatly outnumbered by other ions, as under normal physiological conditions. Since the Ca\(^{2+}\) channel pore is relatively large (~6 Å diameter) (McCleskey and Almers, 1985), the selectivity cannot be explained by molecular sieving (Tsien et al., 1987b).

Recent mutagenesis studies have revealed that the Ca\(^{2+}\) channel pore contains a single locus of high-affinity binding within the pore that can either bind a single Ca\(^{2+}\) ion with high affinity (\(K_d \sim 1 \mu M\)) or multiple divalent cations with lower affinity (Ellinor et al., 1995). This locus comprises four highly conserved glutamate residues, one from each of the pore lining H5 regions of the \(\alpha_1\) subunit repeats (Kim et al., 1993; Tang et al., 1993; Yang et al., 1993; Ellinor et al., 1995) (Fig. 8.6). The localization of the Ca\(^{2+}\) interaction to a specific cluster of carboxylates fits in with earlier hypotheses about the mechanism of selectivity and permeation (Almers and McCleskey, 1984; Hess and Tsien, 1984; Armstrong and Neyton, 1992;
Kuo and Hess, 1993). Smaller cations such as Na⁺ and K⁺ are thought to bind weakly and permeate rapidly in the absence of Ca²⁺, but are rejected when Ca²⁺ occupies the high-affinity site. Ca²⁺ fluxes become appreciable at millimolar levels of [Ca²⁺]o, when mass action drives more than one Ca²⁺ ion into the pore. The flux rate for Ca²⁺ is then dependent on negative interactions between individual divalent cations with the pore (either through electrostatic repulsion or competition for a limited number of negatively charged oxygen groups). In the absence of Ca²⁺, larger divalent cations like Ba²⁺ and Sr²⁺ permeate the channel better than Ca²⁺, since they do not bind as tightly to the high-affinity sites. Ca²⁺ channels are generally impermeable to Mg²⁺, probably because of its slow rate of dehydration. At supraphysiological concentrations, Mg²⁺ is capable of blocking Ca²⁺ flow through the channel.

Blockade of Ca²⁺ channels can also be demonstrated with larger divalent cations, such as Cd²⁺ and Co²⁺, which potently inhibit Ca²⁺ influx by binding more tightly than Ca²⁺ to the high-affinity site (Lansman et al., 1986). The order of potency of the blockade depends somewhat on the subtype of Ca²⁺ channel. As with the binding of Ca²⁺, the blockade of L-type channels by other divalent cations is also strongly reduced in channels with changes in the conserved glutamates (Kim et al., 1993; Elinor et al., 1993). Open Ca²⁺ channels are also blocked by H⁺ ions, which titrate the glutamates that support Ca²⁺ selectivity. Mutagenesis experiments have demonstrated that the carboxylate side chains from repeats I, II, and III act together to form the proton-binding site (Chen et al., 1997). The coordinated action of the multiple oxygen groups creates a site with much higher affinity for protons than a single carboxylate alone. Acidification also decreases the degree of Ca²⁺ channel opening by shifting the voltage-dependence of gating toward more depolarized potentials (Klockner and Isenberg, 1994).

Activation

Membrane depolarization causes many kinds of ion channels to open, a process termed activation. As in the case of other voltage-gated channels, activation
of Ca\(^{2+}\) channels occurs more quickly and is more complete with larger depolarizations. A positively charged transmembrane segment (S4) has been found in each of the four homologous repeats of Ca\(^{2+}\) channels (Tanabe et al., 1987), and is very similar to S4 segments in Na\(^{+}\) and K\(^{+}\) channels (Jan and Jan, 1989) where S4 has been firmly established as part of the voltage-sensing mechanism (e.g., Stuhmer et al., 1989; Papazian et al., 1991; Yang and Horn, 1995; Larsson et al., 1996). Analysis of Ca\(^{2+}\) channel gating is less extensive than for the other ion channels, but Ca\(^{2+}\) channel chimeras have provided insights into specific contributions of individual repeats and motifs within them. The determinants of the rate of activation—slow, skeletal muscle-like activation as opposed to rapid, cardiac-like activation—were first traced to repeat 1 (Tanabe et al., 1991), then further localized to the membrane-spanning segment IS3 and the external linker between IS3 and IS4 (Nakai et al., 1994). Mutant DHP receptors with \(\alpha_{1S}\) sequence in this region activate relatively slowly (\(t_{\text{act}} > 5\) ms), whereas mutants that have the \(\alpha_{1C}\) sequence in the same region activate relatively rapidly (\(t_{\text{act}} < 5\) ms). More recent analysis has focused on how charge movements associated with Ca\(^{2+}\) channel gating are affected by mutations in \(\alpha_{1}\)-subunits.

Inactivation

Inactivation refers to the closing of channels during maintained depolarization, another fairly general property of Ca\(^{2+}\) channels. The speed of inactivation varies widely, ranging from very slow (hardly visible during second-long depolarizations), as in the inner segments of photoreceptors, to relatively rapid (complete within tens of milliseconds), as in the case of certain Ca\(^{2+}\) channels found on nerve terminals (Lemos and Nowycky, 1989). In most cases, the underlying mechanism of inactivation is dependent on depolarization per se. The rate of this voltage-dependent inactivation is strongly dependent on a region in the first repeat, including IS6 and extracellular and cytoplasmic residues on either side of it (Zhang et al., 1994) (Figure 8.6). Residues in the S6 transmembrane segments of other repeats may also be influential (Hering et al., 1996). The mechanism of channel closing may resemble a kind of inactivation in K\(^{+}\) channels known as “C-type” (Hoehi et al., 1991; Liu et al., 1996b).

Some Ca\(^{2+}\) channels, such as L-type channels, are subject to Ca\(^{2+}\)-dependent inactivation along with voltage-dependent inactivation. The Ca\(^{2+}\)-dependence is an important negative feedback property that helps limit voltage-gated Ca\(^{2+}\) entry as intracellular [Ca\(^{2+}\)]\(_i\) increases beyond a critical level (Chad and Eckert, 1986). Ca\(^{2+}\) influx through one channel can promote the inactivation of another adjacent channel, without a generalized elevation of bulk intracellular Ca\(^{2+}\) concentration, a specific example of localized Ca\(^{2+}\) signaling (Imredy and Yue, 1992). Intracellular application of the Ca\(^{2+}\) chelator BAPTA greatly diminishes such negative interactions within Ca\(^{2+}\) channel pairs. The Ca\(^{2+}\)-dependent inactivation transpires by a Ca\(^{2+}\)-induced shift of channel gating to a low open probability mode, distinguished by a more than 100-fold reduction of entry rate to the open state (Imredy and Yue, 1994). Both calmodulin activation and channel (de)phosphorylation were excluded as significant signaling events underlying Ca\(^{2+}\)-induced mode shifts, leaving direct binding of Ca\(^{2+}\) to the channel as the most likely initiating event for inactivation. Indeed, the L-type channel contains a consensus EF-hand Ca\(^{2+}\)-binding motif in the carboxy-terminal region downstream of repeat IV (Babitch, 1990). Involvement of this motif in Ca\(^{2+}\)-dependent inactivation was revealed by analysis of chimeric constructs between \(\alpha_{1C}\) and \(\alpha_{1L}\), which lack Ca\(^{2+}\)-dependent inactivation (de Leon et al., 1995). Donation of the \(\alpha_{1C}\) EF-hand region to the \(\alpha_{1L}\) channel conferred the phenotype of Ca\(^{2+}\)-dependent inactivation. These results strongly suggest that Ca\(^{2+}\)-dependent inactivation is initiated directly by Ca\(^{2+}\) binding to the \(\alpha_{1C}\)-subunit. Additional aspects of the C-terminal tail beyond the EF-hand region may also participate (Soldatov et al., 1995; Zuhlke and Reuter, 1998). Swapping of an \(\sim 80\) amino acid segment speeds inactivation by 8- to 10-fold in one particular splice variant, and also eliminates the Ca\(^{2+}\)-dependence. Figure 8.7 provides a summary of the structural components of the \(\alpha_{1}\)-subunit that are responsible for regulation of Ca\(^{2+}\) channel function as discussed above.

Responsiveness to Drugs and Toxins

Another interesting characteristic of voltage-gated Ca\(^{2+}\) channels is their ability to respond to drugs and toxins. Sensitivity to neurotoxins, many derived from the venoms of spiders and marine snails, is an important earmark of individual types of voltage-gated Ca\(^{2+}\) channels (Oli vera et al., 1994). In the case of \(\omega\)-CTX-GVIA, the potent and selective blocker of N-type channels, high-affinity binding seems to involve extracellular channel domain located in all four repeats of the \(\alpha_{1B}\)-subunit, but particularly in repeats I and III (Ellinor et al., 1994). Specific differences between \(\alpha_{1B}\) and \(\alpha_{1L}\) have been localized to individual amino acids it loops near H5. Thus, the available evidence is consistent with a scenario in which the toxin straddles the mouth of the pore. The precise mechanism of blockade is not known, but observations of competition with permeant cations (Boland et al., 1994)
McDonogh et al., 1996) suggest the possibility of direct occlusion of the permeation pathway. Further analysis may allow the rigid toxin to be used as a kind of molecular caliper to gain information about the outer aspects of the channel. In this context, it is noteworthy that blockade of N-type channels by ω-CTx-GVIA, ω-CTx-MVIIC, and related toxins is strongly voltage-dependent as a result of enhancement of block by channel inactivation (Stocker et al., 1996). The dependence of toxin blockade on channel gating is a familiar phenomenon in Ca²⁺ channels; for example, ω-Aga-IVA block of P/Q-type channels is strongly antagonized by channel activation (Mintz et al., 1992a).

The pore region has also been implicated in the blockade of L-type Ca²⁺ channels by DHPs and other small organic agents. This was suggested by early photoaffinity-labeling experiments in which photoactive DHPs labeled a putative extracellular loop between segments IIIS5 and IIIS6 and transmembrane segment IVS6 (Nakayama et al., 1991). General agreement has now been reached that the IVS6 segment is critical for the action of DHPs and phenylalkylamines (Schuster et al., 1996). Some of the most compelling evidence comes from experiments where DHP sensitivity is transferred to chimeras based on the α₁A subunit (Grabner et al., 1996). These reinforce the idea that the minimum sequence for DHP sensitivity includes segments IIIS5 and IIIS6 and the connecting linker, as well as the IVS5-IVS6 linker plus segment IVS6. Interestingly, the DHP-responsive α₁A chimera still retains sensitivity to ω-Aga-IVA and ω-CTx-MVIIC.

The idea that organic agents interact at or near the pore has been further reinforced by consideration of the Ca²⁺-dependence of drug binding (Mitterdorfer et al., 1995; Peterson and Catterall, 1995). The high-affinity Ca²⁺ site that regulates DHP binding can be eliminated by replacing the conserved pore glutamate in repeat III with a lysine residue. This finding and other data indicate that high-affinity DHP binding is dependent on Ca²⁺ coordination by the same glutamate residues which form the locus of selectivity (Yang et al., 1993). A three-state model has been proposed whereby affinity for DHPs is promoted by the presence of one bound Ca²⁺ ion within the pore as opposed to zero or two (Peterson and Catterall, 1995).

Ca²⁺ Channels and Disease States

Because voltage-gated Ca²⁺ channels play so many critical roles in cells, acting as initiators of regulated exocytosis and shaping the postsynaptic responsiveness to the secreted transmitters, it is not surprising that a number of pathological states can be attributed to a malfunction these proteins. Here, we provide a few examples of disorders involving Ca²⁺ channels that provide interesting perspectives on the relationship between Ca²⁺ channel structure and function.

Lambert–Eaton Syndrome as an Autoimmune Disease of Ca²⁺ Channels

Lambert–Eaton myasthenic syndrome (LEMS) is an acquired autoimmune disease associated with a
decreased probability of neurotransmitter release at the neuromuscular junction. There is substantial evidence that LEMS and related disorders arise from immunologic reactivity against neuronal counterparts of components of neoplastic tumors (for a review, see Lennon, 1994). Mice injected with IgG from patients with LEMS display physiological and morphological features of the syndrome, including fatiguable muscle weakness and selective depletion of particles from presynaptic active zones (Fukunaga et al., 1983). Later studies showed that K+-induced 45Ca2+ flux in human small-cell lung carcinoma (SCC) cells was significantly reduced by LEMS IgG, indicating the formation of autoantibodies to tumor cell Ca2+ channels (Roberts et al., 1985).

The SCC cells contain mRNAs encoding α1A-, α1B-, and α1D-subunits, suggesting that P/Q-, N-, and L-type Ca2+ channels might be expressed (Oguro-Okano et al., 1992; Codignola et al., 1993). However, blockers of P/Q-type Ca2+ channels are much more effective in inhibiting 45Ca2+ flux in SCC cells than inhibitors of N- or L-type Ca2+ channels (Lennon et al., 1995). Furthermore, patients with LEMS are much more likely to display immunoactivity to P/Q-type channels than to N-type channels (Lennon et al., 1995). P/Q-type Ca2+ channels are the dominant Ca2+ entry pathway at the human neuromuscular junction (Protti et al., 1996) and IgG from LEMS patients has been shown to selectively inhibit Q-like Ca2+ channels in a rat insulinoma cells (Magnelli et al., 1996). Thus, it seems likely that the pathogenesis of LEMS involves the formation of autoantibodies to P/Q-type Ca2+ channels on para-neoplastic tumor cells, which, in turn, inhibit the corresponding channels on presynaptic terminals that support neuromuscular transmission.

Hypokalemic Periodic Paralysis

Hypokalemic periodic paralysis (HypoPP) is an autosomal dominant muscle disease long believed to arise from an ion channel dysfunction. Expression of the disease often follows increased carbohydrate intake or unaccustomed exercise, and is characterized by transient attacks of muscle weakness of varying duration and severity, followed by a drop in serum K+ concentration. Linkage analysis in three affected families localized the HypoPP locus to chromosome 1q31–32 (Fontaine et al., 1994), the same region as the gene encoding the α1S-subunit (Gregg et al., 1993). The α1S-subunit cosegregated with the disease gene without recombination (Fontaine et al., 1994). Subsequent analysis demonstrated a guanine-to-adenine mutation caused an arginine-to-histidine substitution at residue 528 in transmembrane segment II54 of α1S (Boerman et al., 1995). Examination of myo-

tubes from individuals with HypoPP revealed transcription of both the normal and mutant genes but the maximum L-type current density was smaller than in myotubes from normal controls (Sipos et al., 1995). Furthermore, while the voltage-dependence of L-type channel activation appeared normal, the mutant myotubes showed a −40 mV shift in the voltage-dependence inactivation. Given the central role of L-type Ca2+ channels in skeletal muscle excitation–contraction coupling, it is not surprising that a mutation that facilitates inactivation should contribute to a paralytic disorder.

Mutational Defects in Migraine and Movement Disorders

Deleterious mutations in the α1A-subunit have been found in individuals who display certain forms of migraine and ataxia (Ophoff et al., 1996) (Fig. 8.8). Familial hemiplegic migraine (FHM) is a rare autosomal dominant form of migraine with aura that is associated with ictal hemipaesthesia. In FHM families, four different missense mutations were found, including mutations in the S4 membrane-spanning helix of repeat I (RR192Q), in the P region of repeat II (T66M), and in the S6 regions of repeat I (V714A) and of repeat IV (I811L). Each of these mutations would be expected to affect a key functional property, be it permeation, gating, or both, but this needs to be tested directly by expression of appropriately mutated α1A-subunits.

Episodic ataxia type 2 (EA-2) is another autosomal dominant disease, characterized by attacks of cerebellar ataxia and migraine-like symptoms. The Leiden group (Ophoff et al., 1996) found two mutations in individuals affected with EA-2: (1) a single nucleotide deletion in II51 (ΔC4073 in codon 1266), which caused a frameshift and a premature stop further downstream (at codon 1333), and (2) a mutation which was predicted to cause aberrant splicing at a splice site in II52. It is possible that both defects cause transcript instability, thereby sharply reducing the levels of α1A in heterozygotes.

A mutation in mice, at the ‘‘trotting’’ locus on chromosome 8, causes ataxia, seizures, and behavioral absence similar to petit mal epilepsy in humans (for a review, see Kostopoulos, 1992). Positional cloning has been used to demonstrate that the ‘‘trotting’’ locus lies within the mouse gene encoding the α1A subunit (Fletcher et al., 1996). The trotting mutants have a single cytosine-to-thymine change, leading to proline-to-leucine substitution in the pore-forming region of repeat II. A more severe of this allele, called feiner, was found to be the result of a mutation in a splice donor consensus sequence, leading to truncation of the C-terminus. The mechanisms by which these channel mutations could cause movement disorder
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Figure 8.8 Map of the \( \alpha_{IA} \)-subunit showing genetic defects underlying some forms of migraine and movement disorders. The location of mutations found in patients with familial hemiplegic migraine are shown by the open symbols and derangements underlying episodic ataxia type 2 are represented by the filled symbols. (Adapted from Ophoff et al., 1996, copyright Cell Press.)

or epileptiform activity remain unclear. However, all of these recent studies raise interesting questions for future investigations into the functional roles of P/Q-type Ca\(^{2+}\) channels in the central nervous system.

**Concluding Remarks**

Understanding of the workings of voltage-gated Ca\(^{2+}\) channels has benefited from many approaches over the last decade or so. The identification of multiple types of Ca\(^{2+}\) channels on the basis of biophysical and pharmacological criteria has been complemented by major advances in the biochemistry and molecular biology of their underlying subunit components. Considerable progress has been made in clarifying molecular mechanisms of generic properties such as selectivity, permeation, and gating, as well as structural features that allow individual types of Ca\(^{2+}\) channels to serve specialized functional roles or to respond to type-selective drugs. It is particularly intriguing to learn that molecular defects in diseases such as migraine and ataxia can not only be traced to a particular Ca\(^{2+}\) channel subunit, but also to the very domains that were previously shown to be important for key channel functions. While there have been major developments in understanding the basis of the functional channel classes, the Ca\(^{2+}\) channel’s three-dimensional structure is completely unknown.

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