

Invited Review

Genetics and pathology of voltage-gated Ca²⁺ channels

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Summary. Neurotransmitter release, neuronal excitation, and a whole variety of other neuronal functions are controlled by the intra/extra cellular Ca²⁺ gradient. The major pathway for entry of Ca²⁺ into the excitable cells is mediated by voltage-gated Ca²⁺ channels. Several functional subclasses of voltage-dependent Ca²⁺ channels have been identified, based on their pharmacological, biophysical properties, and molecular cloning. Recently, three human diseases (familial hemiplegic migraine, episodic ataxia type 2, and spinocerebellar ataxia 6) were added to the growing list of ion-channel disorders, all caused by different mutations in the P/Q-type Ca²⁺ channel α_1 subunit. Molecular analysis of the Ca²⁺ channelopathies will provide new insights into the role, function and pathology of these voltage-gated Ca²⁺ channels.

Key words: Calcium, Channel, Subunit, Disorders, Review

Introduction

Neurotransmitter release, neuronal excitation, and a whole variety of other neuronal functions are controlled by the intra/extra cellular Ca²⁺ gradient. The major pathway for entry of Ca²⁺ into the excitable cells is mediated by voltage-gated Ca²⁺ channels. Recent advances in understanding the structural, biophysical and pharmacological properties of voltage-gated Ca²⁺ channels has highlighted their heterogeneity and diversity (De Waard et al., 1996). In the last five years a growing number of human disorders have been described in which mutations within voltage-dependent ion-channels form the underlying molecular defect. Mutations in a sodium channel result in hyperkalemic periodic paralysis, paramyotonia congenita and myotonia fluctuans (reviewed by Hudson et al., 1995); hypokalemic periodic paralysis is caused by mutations in the α_1 subunit of the skeletal muscle calcium channel (Ptacek et al., 1994) and episodic ataxia type 1 (with

myokymia) is produced by point mutations in a potassium channel (Browne et al., 1994). Recently, three other human diseases were added to the list of ion-channel disorders, all caused by mutations in the P/Q-type Ca²⁺ channel α_{1A} subunit gene (Ophoff et al., 1996; Zhuchenco et al., 1997). Missense mutations were found in patients with familial hemiplegic migraine, truncating mutations were associated with episodic ataxia type-2 (with nystagmus), and expanded trinucleotide repeats were observed in patients with spinocerebellar ataxia 6. Molecular studies of all of these so-called channelopathies will provide new insights into the world of voltage-gated ion-channels, cell membrane excitability, the patho-physiological mechanisms underlying these disorders, and form the basis for the development of new prophylactic therapy. This review surveys the diversity of Ca²⁺ channels and provides an overview of the growing list of Ca²⁺ channelopathies.

Basic properties of voltage-gated Ca²⁺ channels

Voltage-gated Ca²⁺ channels regulate Ca²⁺ entry in a potential-dependent manner and thereby contribute to Ca²⁺-signaling in a wide variety of cell types, including nerve, endocrine and muscle cells. These Ca²⁺ channels play a key role in signal transduction by acting as links between the realms of electric signaling and intracellular biochemical messengers (Hille, 1992). Similar to voltage-gated Na⁺ and K⁺ channels, activation of Ca²⁺ channels is highly voltage-dependent. The channels are more quickly activated within one or a few milliseconds and opened with larger membrane depolarizations. Inactivation, the closing of Ca²⁺ channels, occurs during maintained depolarization. The speed of inactivation varies widely, ranging from very slow (requiring second long depolarizations) to relatively rapid (tens of milliseconds).

The basic insight into the structural features of calcium channels has mainly emerged from the biochemical characterization of the skeletal muscle dihydropyridine (DHP) receptor, the skeletal L-type Ca²⁺ channel (Campbell et al., 1988; Catterall et al., 1988). These studies revealed that voltage-gated Ca²⁺ channels are comprised of multiple components, forming a large macromolecular complex of about 500 kDa. The

generic structure contains three subunits which include a major transmembrane α_1 protein, the intracellularly located β subunit and the disulfide-linked $\alpha_2\delta$ subunit anchored in the cell membrane with an extracellular domain (Fig. 1). The large α_1 subunit is functionally the most important component, forming the ion conducting pore with the voltage sensor and the gating machinery, modified by the other ancillary subunits (De Waard et al., 1996). Depending on the tissue of origin, a fourth subunit such as the skeletal muscle γ or the neuronal p95, may also form part of the channel complex; additional subunits may still be discovered.

Molecular diversity of Ca^{2+} channels

Recent advances from molecular cloning of Ca^{2+} channel genes have highlighted and increased their heterogeneity and diversity. Molecular cloning has revealed six classes of Ca^{2+} channel α_1 (A,B,C,D,E,S), and four β (1,2,3,4) genes, both representing a gene family; the $\alpha_2\delta$ subunit is encoded by a single gene. The molecular heterogeneity is further enlarged by alternative splicing of α_1 , β and $\alpha_2\delta$ transcripts (Perez-Reyes and Schneider, 1995). The characteristics of the different Ca^{2+} channel types are primarily related to the different α_1 isoforms (Table 1).

Classification

The traditional classification of calcium channels is based on the functional characteristics (Miller, 1992). Pharmacological properties, or sensitivity of the different channels to specific drugs, have been used more recently

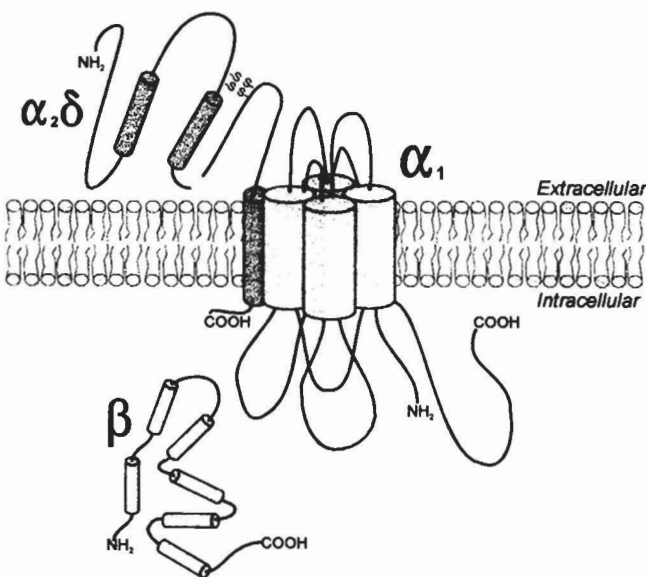


Fig. 1. Membrane topology and structural organization of the three main subunits of voltage-gated calcium channels: α_1 , β and $\alpha_2\delta$ (from Gurnett et al., 1996).

to define different channel subtypes (Olivera et al., 1994; De Waard et al., 1996). Two main categories of calcium channels can be distinguished in excitable cells: low voltage-activating (LVA), rapidly inactivating Ca^{2+} channels with smaller conductances, and a group of high voltage-activating (HVA) calcium channels. LVA calcium channels have been referred as T-type (for *transient* or *tiny*) and are identified in several tissues including neuronal, smooth muscle and cardiac muscle (Bean, 1985; Nowycky et al., 1985; Bean et al., 1986; Loirand et al., 1989). The very negative potentials needed for activation and the rapid inactivation most likely reflect the putative role of LVA T-type calcium channels in generation of rhythmic pacemaker activity in cardiac muscle and neurons (Hagiwara et al., 1988). Biophysical and pharmacological analyses of the HVA calcium channels have revealed a complexity of subtypes of which at least five main classes have been described. They have been divided into L, N, P, Q, and R classes, each with characteristic biophysical and drug sensitivity properties.

L-type channels

The L-type Ca^{2+} channels probably represent the most heterogeneous group of HVA Ca^{2+} channels, as several subtypes have been identified in a wide range of tissues from skeletal muscle to neuroendocrine cells (Hofmann et al., 1994). L-type channels (for long lasting) can be distinguished by their sensitivity to dihydropyridines (DHP), phenylalkylamines (PAA), and benzothiazepines (BTZ) (Tanabe et al., 1987; Tsien and Tsien, 1990; Spedding and Paoletti, 1992). The existence of at least three independent L-type related α_1 subunits (α_{1S} , α_{1C} , α_{1D}) have been reported, widely distributed in muscle, nerve and endocrine cells. In skeletal, cardiac and smooth muscles, L-type channels are essential for excitation-contraction coupling, a process whereby electrical signals generated by action potentials at the muscle cell surface are transduced into intracellular release of calcium and ultimately into muscle fiber

Table 1. Calcium channel subunits: nomenclature and genetic localization (from Lory et al., 1997).

SUBUNIT	CHANNEL TYPE	NOMENCLATURE	ALIAS	LOCALIZATION
α_{1A}	P/Q-type	CACNA1A	CACNL1A4	19p13.1
α_{1B}	N-type	CACNA1B	CACNL1A5	9q34
α_{1C}	L-type	CACNA1C	CACNL1A1	12p13.3
α_{1D}	L-type	CACNA1D	CACNL1A2	3p14.3
α_{1E}	R/T-type	CACNA1E	CACNL1A6	1q25-q31
α_{1S}	L-type	CACN1S	CACNL1A3	1q31-q32
β_1		CACNB1	CACNLB1	17q11.2-q22
β_2		CACNB2	CACNLB2	10p12
β_3		CACNB3	CACNLB3	12q13
β_4		CACNB4	CACNLB4	2q22-q23
$\alpha_2\delta$		CACNA2	CACNL2A	7q21-q22
γ		CACNG	CACNLG	17q24

contraction (Miller, 1992). L-type channels also seem to play an important role in mediating hormone release from endocrine cells (excitation-secretion coupling), e.g. insulin secretion from pancreatic β cells (Bokvist et al., 1995), and are thought to be involved in regulation of gene transcription (excitation-transcription coupling) (Morgan and Curran, 1989).

The presence of at least three different DHP-sensitive Ca^{2+} channels in rat cerebellar granule neurons as described by Forti and Pietrobon (1993) indicates that functionally different L-type channels can co-exist in the same cell type. Recently, Thibault and Landfield (1996) observed an increase of total neuronal L-type Ca^{2+} channels with aging, primarily because of an increase in the density of functional channels. They suggested that such an increase of functional L-type Ca^{2+} channels with aging could underlie the vulnerability of neurons to age-associated neurodegenerative conditions. The consensus of numerous investigations is that L-type Ca^{2+} channels are not involved in neurotransmitter release, although the precise role of L-type channels in neurons is yet not well understood.

N-type channels

The N-type calcium channel is characterized by intermediate inactivation kinetics, being neither transient (T-type) nor long-lasting (L-type), originally distinguished in chick sensory neurons (Nowycky et al., 1985). The diagnostic feature of N-type channels is their virtually irreversible blockade by ω -conotoxin GVIA from cone snail (McEnery et al., 1991, Witcher et al., 1993) combined with their DHP-insensitivity. The α_{1B} subunits, encoding the N-type channels, are restricted almost entirely to neurons in both peripheral and central nervous systems. Their major role is thought to be the mediation of neurotransmitter release at (pre-)synaptic terminals (Burke et al., 1993; Sugiura et al., 1995). In response to local membrane depolarization, the voltage-dependent channel regulates the influx of extracellular Ca^{2+} ions into the cytoplasm of the excitable cell, resulting in release of neurotransmitters at the nerve terminals.

P/Q-type channels

Another DHP-insensitive Ca^{2+} channel is the P-type channel, originally identified in cerebellar Purkinje cells (Llinas et al., 1989; Regan et al., 1991). This type of Ca^{2+} channel is specifically blocked by ω -agatoxin-IVA and the funnel web spider toxin (FTX). The P-type currents have been shown to be widespread in mammalian neurons and have been implicated in neurotransmitter release at some synapses (Hillman et al., 1991; Mintz et al., 1992; Usowicz et al., 1992; Takahashi and Momiyama, 1993; Volsen et al., 1995; Wheeler et al., 1995; Takahashi et al., 1996). A Ca^{2+} channel present in cerebral granule cells appears to be pharmacologically and electrically distinct from the P-

type channel, and has been designated Q-type (Sather et al., 1993; Zhang et al., 1993). Q-type currents are also shown to be involved in neurotransmitter release (Wheeler et al., 1994). The nature of the pore-forming subunit α_{1A} remains the subject of debate as to whether this subunit is the major component of the P-type, Q-type or both type of Ca^{2+} channels (Mori et al., 1991; Sather et al., 1993; Zhang et al., 1993; Stea et al., 1994; Randall and Tsien, 1995; Wheeler et al., 1995; Liu et al., 1996). A clearer distinction between these classes of channels apparently requires more functional studies and may include the identification of the exact subunit composition of both P- and Q-type Ca^{2+} channels.

R-type channel

When all known Ca^{2+} channel types are specifically blocked, a residual calcium current with distinct properties remains (Mintz et al., 1992). The residual class of Ca^{2+} channels is designated R-type (Ellinor et al. 1993; Zhang et al., 1993; Randall and Tsien, 1995). The R-type channel or also called B-type (for brain), resembles the characteristics of the T-type channels e.g. with a very fast time-dependent inactivation (Niidome et al., 1992; Soong et al., 1993). The α_{1E} subunits, presumably encoding the R-type channels, are identified in a broad range of central neurons, supporting an important role in pre- and postsynaptic neuronal Ca^{2+} influx (Yokoyama et al., 1995). The pharmacological identity of this class of Ca^{2+} channel remains undetermined.

The poreforming α_{1E} subunit

The cDNA sequences of the different α_1 genes are homologous to each other and encode proteins with molecular masses of 200 to 275 kDa. Topologically, an α_1 subunit consists of four internal homologous repeats (I-IV), each containing six putative α -helical membrane spanning segments (S1-S6) and one pore-forming (P) segment between S5-S6 that spans only the outer part of the transmembrane region (Figs. 1, 2) (Guy and Durell, 1996). This suggests the same general topological structure as described for Na^+ and K^+ channels (Catterall, 1995). The short N-terminal and the long C-terminal domains of the protein are located in the cytoplasm. The S4 transmembrane segment in each repeat has a conserved unusual pattern of positively charged arginine residue at every third or fourth position, separated by hydrophobic residues. In analogy to Na^+ and K^+ channels, it is likely that the S4 domains function as voltage sensors (Catterall, 1995).

Functional analyses have demonstrated that specific properties of the Ca^{2+} channel can be assigned to distinct parts of the ion conducting pore (i.e. Fig. 2). Expression of chimeric proteins of different types of α_1 Ca^{2+} subunits has revealed that the third segment of the first transmembrane repeat (IS3) and the extracellular IS3-S4 linker, are involved in the activation time of the

channel (Nakai et al., 1994), whereas domain IS6 and its surrounding region are critically important for inactivation characteristics (Zhang et al., 1994). The cytoplasmic loop between domain II and III regulates the type of excitation-contraction or excitation-secretion coupling (Sheng et al., 1994). The II-III cytoplasmic linker of the skeletal muscle α_{1S} subunit is believed to interact with the ryanodine receptor, directly or indirectly, to produce the extracellular Ca²⁺-independent type of E-C found in skeletal muscle (Catterall, 1991). In the N-type α_{1B} -subunit, the II-III loop is identified to bind to syntaxin, a protein required for transmitter release at the synapse (Leveque et al., 1994; Sheng et al., 1994). The corresponding loop in the α_{1A} protein, the α_1 subunit for the P/Q-type channel, also contains specific sites for interaction with presynaptic plasma membrane proteins syntaxin and SNAP-25 (synaptosome-associated protein of 25 kDa) (Rettig et al., 1996). It has been suggested that alternative splicing of α_{1A} leading to different isoforms of the α_{1A} subunit can modulate these binding sites in the II-III loop, implying that a neuron could adjust efficiency of synaptic transmission (synaptic vesicle fusion) by regulating the expression of different isoforms of the Ca²⁺ channel gene (Sheng et al., 1994; Rettig et al., 1996).

The α_1 subunit of the L-type channel contains the binding site for dihydropyridine formed by two extra-

cellular sequences just upstream from the S6 helices of repeats III and IV and the loop between S5 and S6 of the third repeat (Tang et al., 1993). A highly conserved motif in the cytoplasmic linker between repeats I and II, which is present in all α_1 , is involved in the interaction with the cytoplasmic β subunit, the constitutive component of the Ca²⁺ channels (Pragnell et al., 1994). Recent studies indicate that this I-II loop of the α_{1A} and α_{1B} subunits is involved in G-protein dependent modulation, most likely via interaction of subunits (Herlitze et al., 1996; Ikeda, 1996; Page et al., 1997). The C-terminal domain of α_1 subunits seems to contain important structural elements for the regulation of Ca²⁺ channel activity. The extreme C-terminal part of the α_{1C} subunit is involved in tonic inhibition of channel opening probability (Wei et al., 1994), and the intracellular sequence downstream of domain IVS6 is required for Ca²⁺ dependent inactivation of the α_{1C} subunit (De Leon et al., 1995).

Skeletal muscle Ca²⁺ channel disorders

Hypokalemic periodic paralysis in man and muscular dysgenesis in mouse

Hypokalemic periodic paralysis (HypoPP) is a skeletal muscle disorder manifested by episodic

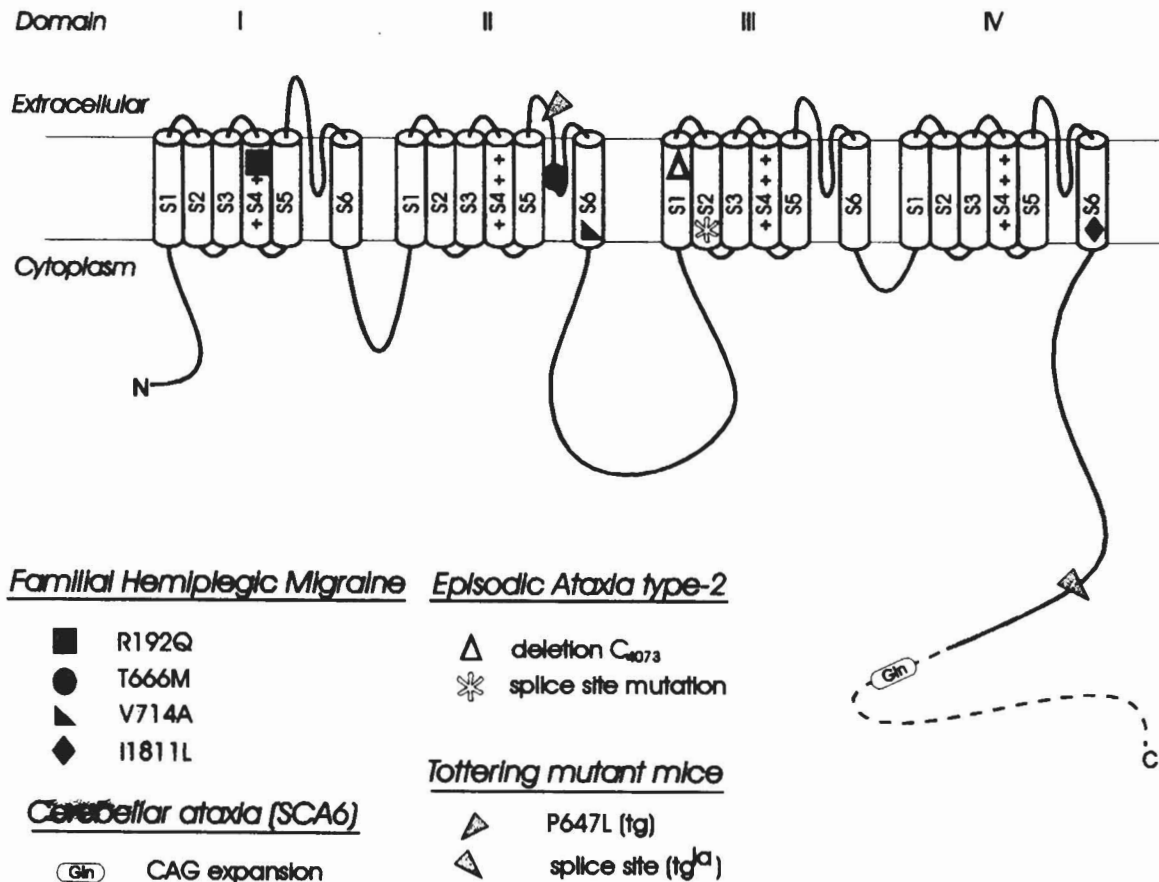


Fig. 2. Detailed membrane topology of the α_1 subunit of voltage-gated calcium channels. The location and amino acid substitutions are indicated for mutations in the P/Q-type calcium channel α_{1A} subunit that cause familial hemiplegic migraine (FHM), episodic ataxia type-2 (EA-2), spinocerebellar ataxia type-6 (SCA6) in human, and tottering (tg) and leaner (tgla) in mouse.

Voltage-gated Ca^{2+} channel disorders

weakness associated with decreased serum potassium levels. HypoPP is inherited autosomal dominantly with a reduced penetrance in females, which might suggest a role for hormonal modulation of disease expression. In several HypoPP families and a sporadic case, distinct mutations have been identified in the gene CACNA1S on chromosome 1q31-32, but locus heterogeneity has been observed (Plassart et al., 1994; Ptacek et al., 1994). This gene encodes for pore-forming subunit (α_{1S}) of the L-type skeletal muscle calcium channel. Interestingly, each mutation causes a substitution of the outermost arginine in segment S4 of domains II or IV. The S4 segments, present in all voltage-dependent ion channels, contains a repeating motif consisting of a positively charged amino acid (e.g. arginine) at every third position, which is thought to play a central role in voltage sensitivity in all voltage-gated ion channels (Fig. 2) (Catterall, 1995).

In the skeletal muscles, L-type Ca^{2+} channels are essential for excitation-contraction (E-C) coupling (Miller, 1992). Additional evidence for the role of this Ca^{2+} channel in the E-C process in skeletal muscle comes from analysis of the muscular dysgenesis (*mdg*) mutant mouse (Beam et al., 1986; Tanabe et al., 1988). The *mdg* mice carry a single deletion in the skeletal muscle-specific Ca^{2+} channel α_1 subunit, leading to a premature stop and a predicted truncated protein (Chaudhari, 1992). The skeletal muscle of homozygous *mdg* mice is completely paralyzed. The absence of functional skeletal muscle Ca^{2+} channel in homozygotes (*mdg/mdg*) is lethal and causes loss of L-type Ca^{2+} currents and E-C coupling (Beam et al., 1986; Tanabe et al., 1988). The heterozygous mice (*mdg/+*) appear normal whereas the missense mutations in the human α_{1S} subunit result in a dominant condition HypoPP. Chaudhari (1992) already demonstrated that the premature stop of the Ca^{2+} channel gene in *mdg* mice lead to instability of its transcript. The missense mutations in orthologous human gene, however, led to expression of functional L-type Ca^{2+} channels *in vitro*, though with reduced L-type Ca^{2+} currents (Lehmann-Horn et al., 1995; Sipos et al., 1995; Lapie et al., 1996). The recessive inheritance of muscular dysgenesis in mice can be explained by a mechanism in which the frameshift mutation causes a loss of function of the truncated allele (haplo-insufficiency). Conversely, the presence of missense mutations causing the dominant trait HypoPP strongly suggest a molecular mechanism in which the mutated α_{1S} subunits have gained an altered function with dominant negative effect on E-C coupling and Ca^{2+} currents in skeletal muscles. The periodic paralysis must therefore be caused by reduction of electrical excitability of the muscle cells; but how? Obviously, more studies are needed to characterize the functional defects of the L-type Ca^{2+} channel in skeletal muscles of HypoPP patients and to understand the mechanism of pathogenesis and the relation between extracellular K^+ and the paralysis. The identification of the second HypoPP gene in a yet genetically unlinked French family (Plassart et al., 1994) may also contribute

to the elucidation of the mechanism of this muscle disease.

Malignant hyperthermia: the ryanodine receptor and the skeletal Ca^{2+} L-type channel

Malignant hyperthermia is a potentially lethal human skeletal muscle disorder characterized by sustained contraction of muscles and hyperthermia, triggered by inhalation of some general anesthetics. Molecular studies of the recessive malignant hyperthermia in pigs had revealed a missense mutation in the ryanodine receptor (Fujii et al., 1991). Accordingly, missense mutations were found in the human ryanodine receptor gene on chromosome 19q in some families with malignant hyperthermia (Hogan et al., 1992). This receptor functions as the Ca^{2+} release channel in skeletal sarcoplasmic reticulum and is tightly linked to the same pathway of the skeletal muscle L-type Ca^{2+} channel (Catterall, 1991). It is not yet understood how missense mutations in the ryanodine receptor result in large efflux of Ca^{2+} from the sarcoplasmic reticulum. Furthermore, a number of other families with hyperthermia seems to be linked to chromosome 7q where the CACNA2 gene is located (Iles et al., 1994). This gene encodes the Ca^{2+} channel $\alpha_{2\delta}$ subunit, one of the constitutive components of all Ca^{2+} channels including the skeletal L-type channel (De Waard et al., 1995). Recently, Monnier et al. (1997) reported a missense mutation in the α_{1S} subunit associated with malignant hyperthermia in a large family, suggesting a direct interaction between the skeletal muscle L-type Ca^{2+} channel and the ryanodine receptor.

The acquired Lambert-Eaton syndrome

Lambert-Eaton myasthenic syndrome (LEMS) is a neurological acquired autoimmune disease most often associated with small-cell lung carcinoma (SCLC). LEMS is identified as a disorder of neuromuscular transmission caused by antibodies that impair the presynaptic release of acetylcholine, producing proximal muscle weakness (Fukunaga et al., 1983). There is substantial evidence that LEMS (and related paraneo-

Table 2. Genetic disorders involving different Ca^{2+} channel subunits

DISORDER	CALCIUM CHANNEL		SPECIES
	Subunit	type	
Hypokalemic periodic paralysis (HypoPP)	α_{1S}	L-type	human
Muscular dysgenesis (<i>mdg</i>)			mouse
Malignant hyperthermia	$\alpha_{2\delta}$	-	human
	α_{1S}	L-type	human
Familial hemiplegic migraine (FHM)			human
Episodic ataxia type-2 (EA-2)			human
spinocerebellar ataxia type-6 (SCA6)	α_{1A}	P/Q-type	human
Tottering (tg)			mouse
Leaner (tgla)			mouse
Lethargic (lh)	β_4	-	mouse

plastic syndromes) arise from immunologic reactivity against neuronal counterparts of components of neoplastic tumors (Lennon, 1995). The SCLC cells contain mRNAs encoding α_{1A} , α_{1B} , and α_{1C} subunits, implying that P/Q-, N-, and L-type Ca^{2+} channels are expressed (Oguro-Okana et al., 1992; Codignola et al., 1993). The P/Q-type Ca^{2+} channel is thought to be the predominant mediator of neuromuscular transmission (Sugiura et al., 1995; Protti et al., 1996). It has been demonstrated that almost all the patients with Lambert-Eaton syndrome, had serum antibodies to the P/Q-type Ca^{2+} channel, while only about half the patients also had antibodies against the N-type channel (Lennon et al., 1995). Furthermore, IgG from LEMS patients has been shown to selectively inhibit Q-like Ca^{2+} channels in rat insulinoma cells (Magnelli et al., 1996). Consequently, the pathogenesis of LEMS most likely involves the formation of antibodies to P/Q-type Ca^{2+} channels on paraneoplastic tumor cells, which impair the presynaptic Ca^{2+} currents that support neuromuscular transmission.

Neuronal P/Q-type channel disorders

Missense mutations in Familial Hemiplegic Migraine (FHM)

Familial hemiplegic migraine (FHM) is a rare autosomal dominant subtype of migraine with aura. FHM is characterized by migraine attacks associated with a transient hemiparesis in addition to other aura symptoms (Haan et al., 1994). Migraine attacks typically last one to three days, and present with severe, incapacitating, unilateral, pulsating headache, associated with nausea, vomiting, photophobia and phonophobia (migraine without aura). In about 15% of patients attacks are preceded by transient focal neurological aura symptoms, which are usually visual, but sometimes consist of hemiparesis, hemisensory symptoms, or aphasia (migraine with aura). The one-year prevalence of the common types of migraine with and without aura in the general population is very high, affecting up to 6% of males and 15% of females in Caucasian population (Russell et al., 1995). The disease onset of FHM usually is before 30 years, with a mean age of 10 years; attacks occur spontaneously or can be triggered by mild head trauma, or cerebral angiography (Haan et al., 1994). Some families have been reported with FHM associated with cerebellar symptoms including nystagmus, cerebellar ataxia, and cerebellar atrophy.

Deleterious mutations in the calcium channel α_{1A} subunit have been found in individuals with FHM (Ophoff et al., 1996). Thus far, four different mutations in the CACNA1A gene were found, including mutations in the S4 membrane-spanning helix of repeat I (R192Q), in the P region of repeat II (T666M), and in the S6 regions of repeat I (V714) and of repeat IV (I1811L) (Fig. 2). In each case, the point mutation produces a missense mutation that codes for a substitution that is highly conserved among the α_1 subunits of Ca^{2+} channels. These mutations most likely affect key

functional properties like permeation and gating. The missense mutations in the α_{1A} Ca^{2+} channel gene suggest a molecular mechanism for FHM similar to the situation described in other human channelopathies. It is likely that both alleles of the α_{1A} subunit are expressed with the allele harboring the missense mutation resulting in gain-of-function variants of the P/Q-type Ca^{2+} channels. Such mutations have been described e.g. in the α subunit of the skeletal muscle sodium channel producing hyperkalemic periodic paralysis, paramyotonia congenita, and sodium channel myotonias (Hudson et al., 1995). Each of these missense mutations causes a substitution at highly conserved residues, leading to impaired inactivation of the Na^+ channel.

Truncating mutations in Episodic Ataxia type-2

Episodic ataxia (EA) is a neurological disorder in which affected individuals suffer from recurrent attacks of generalized ataxia and other signs of cerebellar dysfunction (Ganchar and Nutt, 1986). The disease is heterogeneous and includes at least two clinically distinct syndromes. In one type, the paroxysmal cerebellar ataxic syndrome is associated with interictal neuromyotonia or myokymia (twitching of small muscles). Attacks are characterized by brief episodes of ataxia and dysarthria lasting seconds to minutes. This episodic ataxia with myokymia, also called episodic ataxia type-1 (EA-1), is reported due to missense mutations in a potassium channel gene (KCNA1) on chromosome 12p14 (Browne et al., 1994). The second type of episodic ataxia (EA-2) is characterized by recurrent attacks of dysmetric limb movements, severe truncal and gait ataxia, sometimes accompanied by dysarthria, vertigo, nausea, and headache or migraine (Ganchar and Nutt, 1986; Von Brederlow et al., 1995). In most EA-2 affected patients, treatment with acetazolamide is dramatically effective in preventing attacks.

Sofar two different truncating mutations were identified in EA-2 families (Ophoff et al., 1996). One mutation is a single nucleotide deletion, the other is a splice site mutation affecting the invariant first G nucleotide of the intron consensus sequence. Both mutations most likely result in shift of the translational reading frame, resulting in aberrant α_{1A} subunits predicted to contain the first two domains and segment S1 of the third transmembrane domain (Fig. 2). The truncated peptides are unlikely to form functional Ca^{2+} channels and may either negatively influence channel assembly in the membrane or the aberrant transcripts are unstable and degraded resulting in haploinsufficiency.

Small trinucleotide expansion in chronic cerebellar ataxia

A polymorphic CAG repeat was identified in the 3' untranslated region of the Ca^{2+} channel α_{1A} gene ranging from 4 to 14 units (Ophoff et al., 1996). Zhuchenko et al. (1997), however, reported six different cDNA isoforms of the human α_{1A} subunit of which three contained a 5-nucleotide insertion prior to the

previously described stop codon, resulting in a shift of the deduced open reading frame in which the CAG repeat encodes a polyglutamine stretch (Fig. 2). Small triplet expansions at this locus, ranging from 21 to 27 repeat units, were identified in patients with cerebellar ataxia (SCA6) (Zhuchenko et al., 1997). The patients are characterized by mild but progressive cerebellar ataxia of the limbs and gait, dysarthria, nystagmus, and cerebellar atrophy. The disease onset ranges from 28 years to late forties, and progresses over 20-30 years leading to impairment of gait and sometimes causing death. It is the subject of debate whether CAG repeat expansion in the same gene causes episodic ataxia first and develops later in life into a progressive cerebellar ataxia. Although the first report of repeat expansion associated with SCA6 suggested a progressive phenotype only, recently, an Italian family characterized by episodic and progressive cerebellar ataxia exhibited a small CAG repeat expansion ($n=23$) (Mantuano et al., 1997). This may suggest a clinical continuum between EA-2 and SCA6. However, further identification of mutations in other EA-2 and SCA6 families is required to confirm the existence of a spectrum comprising both disorders.

The pathogenic effect of the small expansion of the polyglutamine in α_{1A} Ca^{2+} channel subunit remains obscure. The expanded mutant alleles in SCA6 patients (21-27) are remarkably smaller than the expanded alleles observed in any other neurodegenerative diseases (36-121) and are well within the normal range of polyglutamine tracts observed (Zoghbi and Caskey, 1997). Furthermore, alternative splicing of the CACNA1A gene results in different mRNA isoforms, in which the CAG repeat is either part of the coding (polyglutamine) or non-coding region (Zhuchenko et al., 1997). The ratio of these distinct transcripts is unknown and may vary at different neuronal cell types. The question is how each of these mRNA isoforms contribute to the cerebellar ataxia (SCA6). Is a small expansion of a CAG array in the 3' untranslated region responsible for mRNA instability causing haploinsufficiency, or does such an expansion affect the splicing efficiency of the mRNA? Alternatively, SCA6 is caused by small polyglutamine expansions within the intracellular C-terminal that directly interferes with the normal function of the P/Q-type Ca^{2+} channel.

Tottering (*tg*) mutant mice

Mutations in the α_{1A} gene have recently been identified in tottering mutant mice, with an inherited neurological disease including ataxia and seizures (Fletcher et al., 1996). The recessive tottering mouse mutant is known as a model for absence epilepsy (Noebels and Sidman, 1979). The tottering mutation results in spike and wave discharges, mild ataxia, and intermittent convulsions. Homozygous tottering mice are characterized by a wobbly gait (ataxia) which begins at about 3 to 4 weeks, and by intermittent spontaneous attacks similar to human epileptic absence seizures and

infrequently spontaneously motor seizures. Two additional tottering mutations have been identified including leaner (*tg^{la}*) and rolling Nagoya (*tg^{rol}*). The leaner phenotype is sublethal and develops within 2 weeks displaying ataxia, stiffness, retarded motor activity, and signs of absence seizures (Heckroth and Abbot, 1994). The third recessive neurological mouse mutant, rolling Nagoya, manifest with poor motor coordination leading to falling and rolling, and sometimes stiffness of the hindlimbs and tail, but no motor seizures (Oda, 1981). In comparison to tottering mice, the rolling symptoms are somewhat more severe with an earlier onset.

The mutation recognized in tottering (*tg*) mouse is a missense mutation (proline-to-leucine amino acid substitution) very close to the pore-forming P loop of the second transmembrane domain, whereas the more severe allele leaner is associated with a splice site mutation producing an aberrant intracellular carboxyterminus (Fig. 2) (Fletcher et al., 1996). Either splice product of the leaner transcript contains a frameshift in the deduced reading frame and is therefore predicted to produce α_{1A} subunits with an aberrant C-terminal domain. However, detailed study of mouse brains demonstrated α_{1A} transcripts detectable as early as 10.5 days of prenatal development in normal mice and equal amounts in tottering mutant animals, whereas transcript were present in significantly reduced quantities in brains of leaner mice (Doyle et al., 1997). This study strongly suggest haploinsufficiency (loss-of-function) to be the underlying molecular mechanism in leaner mice and a gain-of-function model in tottering mutant animals. The nature of the leaner mutation resembles the two mutations described in EA-2 patients, which may suggest a similar molecular mechanism if this type of mutations also causes mRNA instability and therefore reduction of P/Q-type Ca^{2+} channels. The third tottering allele, rolling, has not been identified yet, but it is highly likely that this mouse mutants with an intermediate phenotype exhibit a mutation in the same Ca^{2+} channel gene.

β_4 mutations in lethargic (*lh*) mouse

Recently, a mutated Ca^{2+} channel β_4 subunit gene was identified in the recessive lethargic (*lh*) mouse mutant (Burgess et al., 1997). The lethargic phenotype share many features of the tottering mouse, including absence epilepsy, spontaneous focal motor seizures and ataxia. Since α_{1A} and β_4 subunits were demonstrated to be expressed together in the same cells at similar or identical locations, Ludwig et al. (1997) suggested that the α_{1A} and β_4 are part of the P/Q-type Ca^{2+} channel complex in almost all cells. The similar phenotypes of tottering and lethargic mice support the hypothesis that both subunits are involved in the same neuronal pathway of P/Q-type Ca^{2+} currents.

Concluding remarks

Considerable advances have been made in recent

years in the elucidation of the molecular physiology of many ion channels including Ca^{2+} channels. Pharmacological and electrophysiological studies have established the existence of multiple types of voltage-gated Ca^{2+} channels. Molecular biology has disclosed an even greater heterogeneity. Ca^{2+} channels are comprised of multiple components which include α_1 , $\alpha_{2\delta}$, and β . The specificity of Ca^{2+} channels is primarily related to the different isoforms of the pore-forming α_1 subunit. Molecular cloning has revealed six of Ca^{2+} channel α_1 subunit genes, four β genes and a single $\alpha_{2\delta}$ gene. Additional heterogeneity is introduced by differential splicing. Progress has in part been stimulated by the search for the molecular mechanisms responsible for inherited (human) diseases caused by abnormal Ca^{2+} channel genes. The naturally occurring mutations underlying these channelopathies provide a useful tool to unravel the structural components involved in normal Ca^{2+} channel functions. The growing list of Ca^{2+} channelopathies particularly involves abnormalities in the neuronal P/Q-type channel showing a spectrum comprising migraine, epilepsy, and episodic to progressive ataxia including neuro-degenerative changes. These findings have opened avenues that will lead to new rationales for diagnosis and development of prophylactic therapy.

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