Q J Med 1999; **92**:133–141

Mini-review

QJM

Calcium channelopathies

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Introduction

Calcium (Ca^{2+}) is an essential signalling molecule in many biological systems, and normal intracellular calcium levels at ~100 nM are 20 000-fold lower than the 2 mM concentration found extracellularly. Homoeostatic mechanisms are tightly controlled to enable Ca^{2+} to mediate a variety of cellular functions and to prevent sustained rises in intracellular calcium concentration which result in cell death.

Abnormalities of calcium homoeostasis are recognized to play an important role in the excitotoxic damage which occurs in the setting of cerebrovascular disease and various neurodegenerative diseases.[1] More recently, however, abnormalities of the voltage-gated calcium channel (VGCC), one of the key regulators of intracellular calcium concentration, have been implicated in a wide range of human diseases, including migraine,[2] episodic ataxia,[2] spinocerebellar degeneration,[3] the Lambert-Eaton myasthenic syndrome,[4] hypokalaemic periodic paralysis[5] and X-linked congenital stationary night blindness (xlCSNB).[6,7] It has been suggested that amyotrophic lateral sclerosis may be mediated by antibodies directed against the VGCC,[8] but the weight of evidence does not support this hypothesis.[9] Of further interest is the implication of the voltage-gated calcium channel in spontaneous mouse models of epilepsy[10–12] and the muscular dysgenesis mouse.[13]

Recently there have been many advances in the understanding of the molecular biology of the VGCC, and of the immunological and genetic basis for the disorders mentioned above, but our understanding of how such a diverse array of diseases may result from dysfunction of a single ion channel remains incomplete. In this article, I hope to summarize what is known about the VGCC with reference to these diseases and to speculate on the implications for other human diseases.

VGCC structure and function

VGCCs are transmembrane proteins that open in response to membrane depolarization and allow Ca^{2+} ions to enter the cell from the extracellular space. VGCCs are hetero-oligomeric proteins, comprising an α_1 subunit which forms the channel pore and voltage sensor as well as accessory β, γ and $\alpha_2\delta$ subunits. Until recently it was thought that an accessory γ-subunit was only present in skeletal muscle, but the existence of a neuronal γ-subunit, which associates with the α_{1B}-subunit to form an N-type VGCC, is now recognized.[14] These accessory subunits influence the voltage-dependence and kinetics of the α_1 subunit. The α_1 subunit comprises four domains (I–IV), each of which has six transmembrane-spanning helices (S1–S6). The fourth helix (S4) of each domain is highly charged and acts as a voltage sensor. The extracellular loop between the fifth (S5) and sixth (S6) helices dips into the membrane, and contributes to the channel pore (Figure 2). The whole structure is envisaged to fold around, with the amino and carboxy termini meeting, to form a barrel-like structure.[15,16] The β-subunits lack α-helices capable of spanning the membrane, suggesting that they are entirely intracellular in location. The α_2 and δ subunits are encoded by a single gene and then processed to form two disulphide-linked polypeptides. The δ-subunit traverses the plasma

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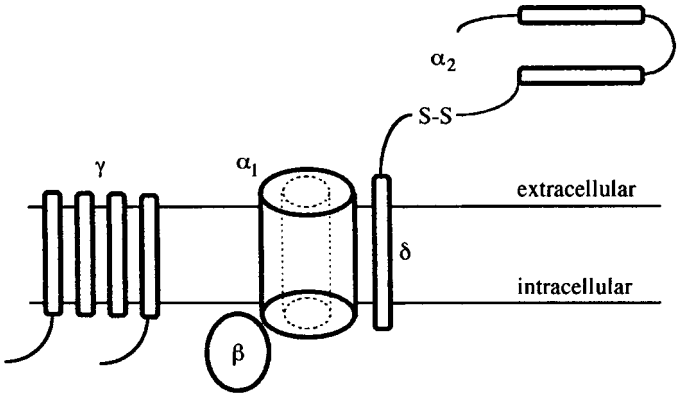

Figure 1. Structural organization of the voltage-gated calcium channel (VGCC). The α_1 subunit comprises four homologous domains, each composed of six transmembrane segments. It forms the central pore of the VGCC. The β-subunit interacts with the α_1-subunit via the L_{I-II} cytoplasmic loop of the α_1 subunit and a minimal binding sequence of 18 amino acids on the β-subunit. The $\alpha_2\delta$ subunit is anchored in the plasma membrane via the transmembrane segment of the δ-subunit. The α_2-subunit is entirely extracellular and connected to the δ-subunit via a disulphide bond. The γ-subunit comprises four transmembrane domains, but the nature of its interaction with the α_1 subunit is not clear.

membrane whilst the α_2 component is entirely extracellular.[17] The γ-subunit has four transmembrane domains (Figure 1).

VGCCs have been characterized genetically and pharmacologically (functionally), but these two approaches do not easily converge to yield a single coherent classification. Nine α_1 (Table 1), one $\alpha_2\delta$, one γ and four β genes have been identified and cloned. Different accessory subunits combine with the various α_1 subunits to form functionally distinct channels. Further channel diversity is generated by alternative splicing.

Dihydropyridine-sensitive L-type VGCCs are a group of high-voltage-activated calcium channels in

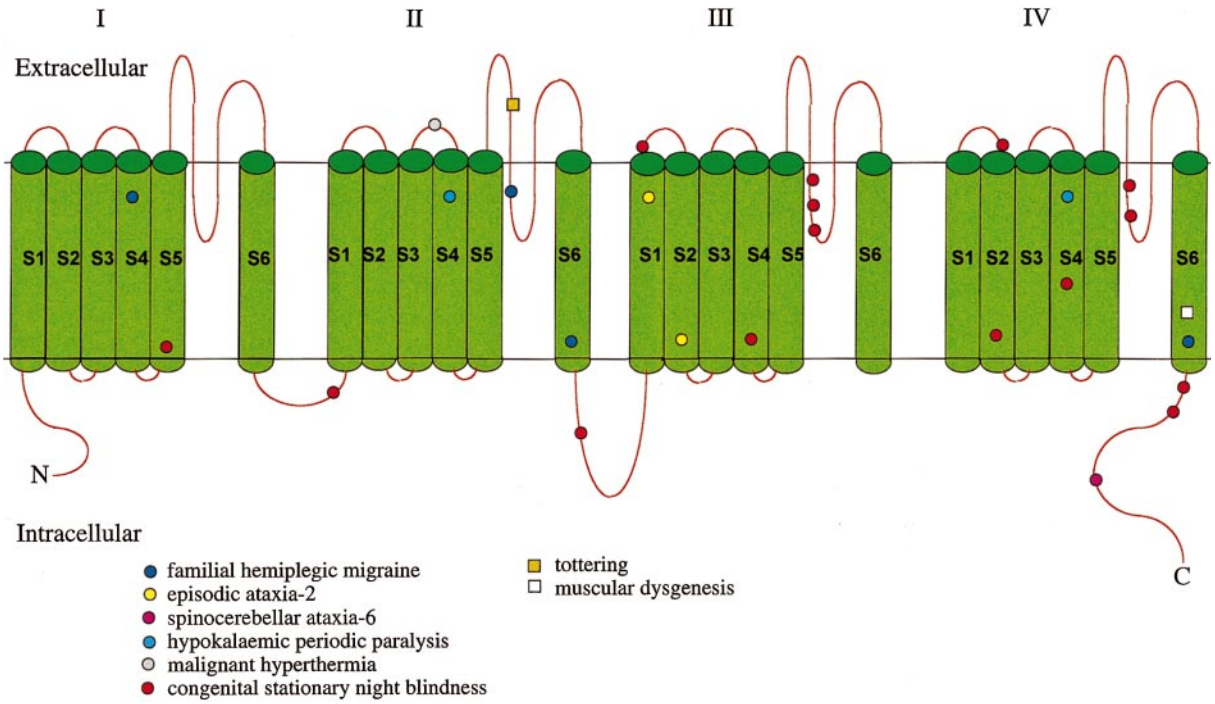

Figure 2. Membrane topology of the pore-forming α_1 subunit of the voltage-gated calcium channel. It comprises four domains (I–IV), each of which is made up of six transmembrane regions. The fourth transmembrane region (S4) is highly charged and serves as a voltage-sensor. The region between S5 and S6 of each domain dips into the plasma membrane and contributes to the channel pore. The β subunit interacts with the α_1 subunit via the cytoplasmic loop between domains I and II ($Loop_{I-II}$). The cytoplasmic loop between domains II and III is important for excitation-contraction coupling in skeletal muscle and for excitation-secretion coupling in neurons. The locations of mutations in human diseases and mouse models are indicated.

Table 1 Human α_1 VGCC subunits: pharmacological type, genetic designation, chromosomal localization, tissue distribution and function

Channel type	Pore-forming subunit	Tissue distribution	Function	Chromosomal location
P/Q	α_{1A} (CACNA1A)	Brain, neuromuscular junction, autonomic neurons	Neurotransmitter release	19p13
N	α_{1B} (CACNA1B)	Neurons, endocrine cells	Neurotransmitter and neuropeptide release	9q32–34
L	α_{1C} (CACNA1C)	Heart, smooth muscle, brain	Excitation-contraction coupling; intracellular calcium homoeostasis	12p14.3
L	α_{1D} (CACNA1D)	Endocrine, kidney, brain		3p14.3
L	α_{1S} (CACNA1S)	Skeletal muscle	Excitation-contraction coupling	1q31–32
L	α_{1F} (CACNA1F)	Retina	Neurotransmitter release from photoreceptor cells	Xp11.23
T	α_{1G} (CACNA1G)	Brain	Pacemaker activity, neuronal oscillations, rebound burst firing	17q22
T	α_{1H} (CACNA1H)	Heart, kidney, brain	?Pacemaker activity	16p13.3
? R	α_{1E} (CACNA1E)	Neuronal		1q25–31

which the pore-forming subunit may comprise the α_{1C}, α_{1D}, α_{1F} or α_{1S} subunit. Depolarization of the skeletal muscle membrane is sensed by the α_{1S}-containing L-type VGCC which, through a physical interaction with the ryanodine receptor (via the $L_{II–III}$ intracellular loop), leads to a release of Ca^{2+} from the sarcoplasmic reticulum (SR). Movement of Ca^{2+} from the SR to the cytosol leads to muscle contraction. In contrast, Ca^{2+} entry via the P/Q-type VGCC controls neurotransmitter release at most central synapses and at the neuromuscular junction (NMJ). N-type VGCCs may also play a role in exocytosis at certain central synapses (Table 1).[18]

The Lambert-Eaton myasthenic syndrome (LEMS)

LEMS is a disorder characterized by skeletal muscle weakness and autonomic dysfunction. In 60% of cases it is considered paraneoplastic and associated with an underlying small-cell lung carcinoma. Most evidence suggests that P/Q-type channels control acetylcholine release from motor nerve terminals at the NMJ[19] and antibodies directed against P/Q-type VGCCs have been identified in the majority of patients with LEMS.[20,21] Some patients with LEMS may also have antibodies directed against N- or L-type VGCCs or against the intracellular β-subunit.[22] The functional significance of these antibodies has been established by the demonstration of distorted active zones (comprising VGCCs and other proteins) in mice passively transferred with LEMS IgG[23] and the ability of LEMS antisera to passively transfer the disease to animals.[4] These anti-VGCC antibodies are thought to exert their effect by causing VGCC internalization and consequent reduction in the number of VGCCs available for coupling to neurotransmitter release. The specificity of these antibodies in reducing P/Q-type calcium currents has recently been demonstrated.[24] These same antibodies have recently been shown to be responsible for the autonomic disturbance which also characterizes the LEMS.[25]

The muscular dysgenesis mouse, hypokalaemic periodic paralysis and malignant hyperthermia

The mouse autosomal recessive *muscular dysgenesis* (*mdg*) mutation results in paralysis. This disorder results from a single nucleotide deletion in the gene encoding the α_{1S} skeletal muscle L-type VGCC. The consequence is a truncated protein with reduced levels of expression and defective excitation-contraction coupling[13] (Table 2).

Hypokalaemic periodic paralysis is an autosomal dominant skeletal muscle disorder characterized by episodic weakness associated with low serum potassium. A variety of mutations, leading to amino-acid substitutions in the voltage-sensor (S4) segments, have been identified in the human skeletal muscle α_{1S} gene (CACNA1S) on chromosome 1q31 in pedigrees with this disorder[5] (Table 2). These mutations lead to enhanced Ca^{2+} channel inactivation and an abnormal resting membrane potential, but it is not clear how this altered function produces the clinical phenotype.

Malignant hyperthermia is an autosomal dominant disorder of skeletal muscle in which crises of hyperthermia, skeletal muscle rigidity, tachyarrythmia and acidosis may be triggered by volatile anaesthetics and depolarising muscle relaxants. Whilst in most cases it is caused by mutations in the ryanodine receptor (muscle sarcoplasmic reticular efflux Ca^{2+} channel),[26] it has also been described in association with mutations in the α_{1S} skeletal muscle L-type VGCC on chromosome 1q31,[27] and linkage has been established to 7q21-22 which encompasses the CACNA2 gene for the $\alpha_2\delta$ accessory subunit[28] (Table 2). The α_{1S} mutations are in the $L_{II–III}$ domain of the VGCC which are involved in the interaction with the ryanodine receptor.

Familial hemiplegic migraine, episodic ataxia-2 and spinocerebellar ataxia-6

The gene encoding the human P/Q-type VGCC α_{1A} subunit has been identified on the long arm of chromosome 19 and designated CACNA1A.[29] Three apparently distinct neurological disorders have been shown to be associated with different mutations in this gene (Table 2).

Familial hemiplegic migraine (FHM) is a rare autosomal dominant condition characterized by migraine with aura, ictal hemiparesis and, in some families, progressive cerebellar atrophy. Approximately 50% of patients with FHM show linkage to chromosome 19p, and four different missense mutations have been identified in these pedigrees in conserved functional domains of the channel.[2] Moreover, this locus has been implicated in some patients with the more common forms of migraine with and without aura.[30]

Episodic ataxia type-2 (EA-2) is also an autosomal disorder and is characterized by paroxysmal attacks of cerebellar ataxia, vertigo, visual disturbance and dysarthria lasting from minutes to days, interictal nystagmus and cerebellar atrophy. Patients with EA-2 may show a dramatic response to acetazolamide, and it is speculated that this carbonic anhydrase inhibitor exerts its effect by altering neuronal pH.

Table 2 Human and mouse VGCC mutations

VGCC subunit	Gene	Disease	Mutation	Protein location
Human α_{1A}	CACNA1A	FHM	Arg 192 Gln	Domain I S4
			Thr 666 Met	Domain II S5-S6 loop
			Val 714 Ala	Domain II S6
			Ile 1811 Leu	Domain IV S6
		EA-2	4073 1bp del, fs 1294 stop	Domain III S1
			4270 1bp ins, aberrant splice	Splice site
		SCA-6	expanded CAG repeat	C-terminal cytoplasmic tail
Human α_{1F}	CACNA1F	X-linked congenital stationary night blindness	Asp 341 del C	$Loop_{II–III}$
			Gly 369 Asp	Domain I S6
			Arg 508 Gln	$Loop_{I–II}$
			Arg 830 X	Domain III S1/S2 loop
			Arg 958 X	Domain III S4
			Leu 991 ins C	Domain III S5/S6 loop
			3133 ins C	Domain III S5/S6 loop
			Arg 1049 Trp	Domain III S5/S6 loop
			Ile 1159 del C	Domain IV S2
			Arg 1234 X	Domain IV S4
			Gln 1348 X	Domain IV S5/S6 loop
			Leu 1364 His	Domain IV S5/S6 loop
			Trp 1386 X	C-terminal cytoplasmic tail
			del 3658-3669	Domain IV S2
			Lys 1591 X	C-terminal cytoplasmic tail
Human α_{1S}	CACNA1S	HypoK-PP	Arg 1239 His	Domain IV S4
			Arg 1239 Gly	Domain IV S4
			Arg 528 His	Domain II S4
		Malignant hyperthermia	Arg 1086 His	Domain II S3–S4 loop
Human $\alpha_2\delta$	CACNA2	Malignant hyperthermia	Unidentified	Unknown
Mouse α_{1A}	Cacnlla4	*Tottering*	Pro 1802 Thr	Domain II S5–S6 loop
		Leaner	5901 98bp insertion or 139bp deletion at nt5763–5901, aberrant splice	Abnormal C-terminal cytoplasmic tail
Mouse α_{1S}	Cehlla3	Muscular dysgenesis	16p del at nt4010	Absent domain IV S6 and abnormal cytoplasmic tail
Mouse β_4	Cchb4	*Lethargic*	4bp ins, aberrant splice	Loss of α_1 binding site of the β_4 subunit
Mouse γ_2	Cacng2	*Stargazer*	intron-1 transposon insertion	Transcriptional termination

FHM, familial hemiplegic migraine; EA-2, episodic ataxia type 2; SCA-6, spinocerebellar ataxia type 6; HypoK-PP, hypokalaemic periodic paralysis; Arg, arginine; Gln, glutamine; Thr, threonine; Met, methionine; Val, valine; Ala, alanine; Ile, isoleucine; Leu, leucine; Asp, aspartate; Gly, glycine; Trp, tryptophan; His, histidine; X, nonsense amino acid; del, deletion; ins, insertion; fs, frame shift; bp, base pair.

Two frame-shift mutations which result in premature stop-codons have been identified CACNA1A in four families with EA-2.[2]

The hereditary spinocerebellar ataxias are a genetically and neurologically heterogeneous group of conditions characterized by dysfunction of the cerebellum and its afferent and efferent connections. Spinocerebellar ataxia type-6 (SCA6) has been shown to be caused by a modest expansion of a polymorphic CAG repeat in the CACNA1A gene. This is translated into a polyglutamine repeat which is located within the C-terminal intracytoplasmic tail of the P/Q-type VGCC.[3]

X-linked congenital stationary night blindness

X-linked congenital stationary night blindness (xlCSNB) is a recessive non-progressive retinal disorder characterized by night blindness, decreased visual acuity, myopia, nystagmus and strabismus. This is a clinically and genetically heterogeneous condition with at least two different loci identified. The disorder is thought to result from impaired synaptic transmission between photoreceptors and second-order (bipolar) retinal neurons. A connection with calcium channels was first considered when it was established that L-type VGCCs were important in the release of glutamate from photoreceptor presynaptic terminals in the frog retina.[31] More recently, a novel retinal-specific gene has been mapped to Xp11.23 and characterized as an L-type VGCC α_1-subunit (which has been designated CACNA1F). Analysis of a number of different pedigrees with xlCSNB has demonstrated 15 different mutations which manifest as missense, nonsense, deletions or frameshifts with or without a premature stop codon.[6,7] These mutations are summarized in Figure 2 and Table 2. It is predicted that these represent loss-of-function mutations which would impair the influx of calcium required for the tonic release of glutamate from photoreceptor presynaptic terminals in darkness.[6]

Amyotrophic lateral sclerosis

It has been suggested that anti-VGCC antibodies may be present in a large proportion of patients with ALS.[8] Other investigators, however, have been unable to confirm these results.[32] Furthermore, the data which favour a pathogenic role of these antibodies in ALS include a number of apparent contradictions. Although it was initially shown that these antibodies enhance spontaneous neurotransmitter release from motor nerve terminals,[33] this being taken to imply an activation of calcium channels, subsequent data have demonstrated an inhibitory effect on calcium channel currents.[34] Moreover, any effect on the VGCC involved in neurotransmitter release at the NMJ implicates the P/Q-type VGCC, but (rabbit) skeletal muscle L-type VGCC were used in the seminal study claiming the presence of anti-VGCC antibodies in patients with ALS.[8] There is no ready explanation for how antibodies directed against a skeletal muscle isoform of the VGCC may mediate a functional effect on the motor nerve.

The *Tottering, Leaner, Lethargic* and *Stargazer* mice

The epilepsies are a heterogeneous group of paroxysmal disorders characterized by recurrent synchronized neuronal discharges. Many human epilepsy syndromes are likely to have an important genetic component, but efforts to identify these genetic factors are complicated by clinical and genetic heterogeneity. In the mouse, a number of single gene mutations that cause epilepsy have been identified and it is possible that investigation of these genes will provide insights into the human epilepsies.

The *tottering* mouse represents a recessive model of absence epilepsy characterized by ethosuxamide-responsive behavioural arrest accompanied by a generalized spike and wave discharge. In addition to absence seizures, the tottering mouse also develops motor seizures and ataxia. Point mutations have been identified in the mouse α_{1A} gene in this spontaneous mouse model of absence epilepsy and ataxia.[11] The *leaner* mouse model is characterized by severe progressive ataxia and absence seizures. It results from a mutation that causes a frame shift and premature stop codon in the α_{1A} subunit of the VGCC.[11]

Homozygous *lethargic* mice express a phenotype characterized by lethargic behaviour and ataxia, as well as focal motor and absence seizures. A mutation in the gene encoding the VGCC β_4 subunit (Cchb4) has recently been shown to be responsible for the *lethargic* mouse. The identified mutation comprises a four nucleotide insertion into a splice donor site which results in exon skipping, translational frameshift and protein truncation with loss of the α_1-binding site.[12] Whilst this mutation might be predicted to produce a truncated β_4 protein that does not possess the α_1-binding site, western analysis has demonstrated that there is complete loss of β_4 expression in both cerebellum and forebrain[14] and that this is accompanied by decreased expression of N-type VGCCs. Furthermore, there is increased fractional incorporation of the β_{1b} subunit into N-type VGCCs in the forebrain. This may alter N-type VGCC

function either by influencing its biophysical properties or by affecting their modulation by second-messenger systems. The effects of altered β_4 expression on the assembly and function of other types of VGCCs has not yet been examined.

Stargazer is a spontaneous mouse model of epilepsy characterized by absence seizures, a distinctive head movement presumed to be due to vestibular disturbance, and cerebellar dysfunction. The gene defect underlying the *stargazer* mutant has recently been identified as the insertion of a long transposon into intron-1 of a gene designated *Cacng2*, which manifests as (incomplete) transcriptional termination.[10] *Cacng2* has sequence and structural similarity to the skeletal muscle VGCC γ-subunit, and is expressed exclusively in the brain, where it is enriched in the synaptic plasma membrane. Regional expression is highest in the cerebellum, olfactory bulb, thalamus and hippocampus, similar to that of the α_{1A} subunit, and the *stargazer* phenotype is similar to that of the *tottering* and *lethargic* mice which harbour defects in the α_{1A} and β_4 subunits of the VGCC, respectively. Coexpression of α_{1A} and the protein product of the wild-type *Cacng2* gene (*stargazin*) in BHK cells leads to a shift in the voltage-dependence of α_{1A} VGCC inactivation towards more negative potentials, significantly reducing channel availability at the neuronal resting membrane potential of -70 mV.[10] This is consistent with the effect of the skeletal muscle VGCC γ-subunit on the function of the α_{1S} VGCC. Reduced expression of *stargazin*, as expected in the *stargazer* mouse, would result in increased or inappropriate Ca^{2+} entry into the presynaptic terminal. Together, these data suggest that *stargazin* is a novel VGCC γ-subunit.

The *stargazer* mutant, therefore, predicts an increase in presynaptic Ca^{2+} entry, whereas reduced Ca^{2+} entry is expected in the *tottering* mouse. How such apparently different effects may account for a similar phenotype is not well established, but may relate to the balance between the release of excitatory and inhibitory neurotransmitters or to the effects of downstream second-messenger systems. In this regard, it has been suggested that the effect of the *stargazer* mutation in reducing the expression of brain-derived neurotrophic factor (BDNF) in the cerebellar granular layer may be responsible for the ataxia manifest in this mouse.[10]

Discussion

Although VGCCs have now been implicated in a wide range of diseases, the details of our understanding of the precise role that these channels play in disease, is very variable. It is well established that LEMS is an autoimmune disease, and that the presence of anti-VGCC antibodies correlates with disease, and passive transfer studies in mice have established the pathogenic role of these antibodies. There is now good evidence that the disease-causing antibodies specifically target the α_{1A} P/Q-type VGCCs. In FHM, EA-2 and SCA-6, different types of mutations in the α_{1A} gene (CACNA1A) have been shown to segregate with disease. However, there is, as yet, no functional data to demonstrate the precise nature of the functional abnormality of the VGCC which results in the particular disease phenotype. The demonstration that FHM, EA-2 and SCA-6 are all allelic, and that they affect the same gene product which is the target of the immune response in LEMS, raises further questions. If dysfunction of the P/Q-type VGCC underlies all of these disorders, why do the autoantibodies in LEMS not produce migraine, ataxia or spinocerebellar degeneration? Could this simply result from failure of the autoantibodies to cross the blood brain barrier? Similarly, why are none of the symptoms of LEMS present in patients with FHM, EA-2 or SCA-6? The answers to these questions will hopefully follow from elucidation of the distinct functional abnormalities which result from the various mutations in CACNA1A.

The original descriptions of the genetics of EA-2 and SCA-6 suggested that they were phenotypically and genetically distinct disorders. SCA-6, characterized by permanent and progressive cerebellar atrophy and dysfunction, was found to be due to a small CAG repeat expansion. EA-2 results from a truncated protein and manifests as mild and intermittent cerebellar dysfunction. More recently, however, it has been suggested that small CAG expansions in the CACNA1A gene may also be responsible for intermittent cerebellar deficit, and this has raised doubts about the distinction between SCA-6 and EA-2.[35]

The elucidation of the molecular genetic basis for FHM, and the demonstration of linkage of common forms of migraine with and without aura to the same locus in certain families, raises the intriguing possibility that abnormalities of the same VGCC may underly the more common forms of the disease. Enthusiasm for such prospects, however, should be tempered by the recognition that FHM is genetically heterogeneous, with two further loci having been identified.[36,37] Even greater genetic heterogeneity is apparent in the spinocerebellar ataxias, with seven different loci established and further heterogeneity likely. These observations make it difficult to extrapolate from these rare Mendelian disorders to the more common complex traits of sporadic disease.

The observation that abnormalities of the VGCC complex underlie the absence epilepsy of the *tottering*, *leaner*, *lethargic* and *stargazer* mice raises the exciting prospect that similar dysfunction may

contribute to absence epilepsy in humans. This possibility, however, is perhaps at variance with prevailing views about the pathogenesis of absence epilepsy. Epileptic seizures are thought to result from an altered network property of neuronal circuits that result in intermittent, synchronized bursting of neurons separated by periods of normal function. The evidence suggests a central role for thalamocortical circuits in the genesis of bilateral cortical spike and wave discharges,[38] and low-threshold T-type VGCCs are thought to play a critical role in generating the thalamic activity which underlies these discharges.[39] The human T-type α_{1G} gene has recently been cloned, and its distribution in the brain is wide, with relatively high expression in the diencephalon.[40] These findings facilitate further investigation of the α_{1G} gene as a candidate in human absence epilepsy.

Conclusion

Dysfunction of a wide array of ion channels underlies a large number of neurological and non-neurological diseases. This article has focused on those disorders characterized by voltage-gated calcium channel dysfunction. Specifically, dysfunction of the P/Q-type VGCC is implicated in LEMS, FHM, EA2, SCA6 and in a spontaneous mouse model of epilepsy. Different L-type VGCC are dysfunctional in the muscular dysgenesis mouse, in hypokalaemic period paralysis, malignant hyperthermia and X-linked congenital stationary night blindness.

Specific ligands are available for studying many of the different types of VGCCs. L-type VGCC antagonists are already well-established agents in the treatment of ischaemic heart disease and hypertension. Specific agonists and antagonists are already available for the *in vitro* investigation of some of the other types of VGCCs. The development of such agents for clinical use may open up new therapeutic possibilities for the treatment of the rare diseases discussed above, but also for the more common neurological conditions such as migraine and epilepsy.

Acknowledgements

I am grateful for the helpful comments made by an anonymous referee.

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