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Ca^{2+} channels and epilepsy

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Abstract

The epilepsies encompass diverse seizure disorders afflicting as many as 50 million people worldwide. Many forms of epilepsy are intractable to current therapies and there is a pressing need to develop agents and strategies to not only suppress seizures, but also cure epilepsy. Recent insights from molecular genetics and pharmacology now point to an important role for voltage-dependent calcium channels in epilepsy. In this article, I first provide an introduction to the classification of the epilepsies and an overview of neuronal Ca^{2+} channels. Next, I attempt to review the evidence for a role of Ca^{2+} channels in epilepsy and the insights gained from genetics and pharmacology. Lastly, I describe new avenues for how such information might be exploited in the development of therapeutic reagents.

Keywords: Molecular genetics; Pharmacology; Voltage-dependent calcium channel

1. Introduction

Epilepsy is the most common primary neurological disorder known, affecting 0.4–0.8% of the population and up to 50 million people worldwide (Engel and Pedley, 1998; McNamara, 1999). Stemming from the Greek epilamvanein "to be grasped", the term epilepsy is to some degree a misnomer, referring more accurately to a group of disorders with diverse origins and manifestations, collectively known as the epilepsies. The essential feature of the epilepsies is the appearance of behavioural changes, termed seizures. Such seizures are thought to occur via an alteration in the behaviour of neuronal networks in the brain that induce the spontaneous expression of periods of synchronized burst firing interspersed by periods of normal electrical activity (Dichter, 1997). Seizures are classified according to their origins (Commission on Classification and Terminology of Epilepsy of the International League Against Epilepsy, 1989). Those that begin at a cortical focus are termed partial seizures, and their behavioural manifestation reflects disruption of the normal roles of the afflicted region, for example, the motor cortex. When consciousness is retained, the partial seizure is said to be simple; however, when consciousness is lost the partial seizure is termed complex. In contrast to partial seizures are the generalized seizures, which arise via widespread activity throughout both cortical hemispheres and typically last for a few seconds to a minute. The most common generalized seizures are myoclonic seizures involving short, sharp, periods of muscle contraction with intermittent relaxation in one or more body regions, and tonic seizures involving sustained contraction of muscle groups throughout the body and loss of consciousness. A third type of generalized seizure is absence epilepsy, characterized by the abrupt and transient cessation of an ongoing activity, for example, speech.

In spite of their prevalence, there is no drug-based *cure* for the epilepsies. In those patients where, for example, epilepsy arises through local vascular malformation or dysplasia, epileptic tissue may be removed surgically (Kemeny, 2001). However, such cases are relatively rare. Thus, current therapy is directed towards reducing seizure frequency. Unfortunately, as many as 30% of medicated patients have inadequate seizure control, many anti-epileptic drugs have serious side effects and lifelong medication may be required. Bearing these factors in mind, it has been proposed, recently, that the focus of epilepsy research should be shifted to curing epilepsy as defined by "no seizures and no side effects" in those patients at risk (Jacobs et al., 2001). To attain this goal requires a more detailed

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understanding of the mechanisms of epileptogenesis especially those revealed by emerging molecular and genetic data. One area where this approach is already shedding light is in the contribution of voltage-dependent Ca^{2+} channels (VDCCs) to epilepsy.

Voltage-dependent Ca^{2+} channels are critical for nerve function (Johnston et al., 1996; Catterall, 2000). By coupling changes in the membrane potential to the influx of the pivotal "second messenger" Ca^{2+}, VDCCs represent the primary route for translating electrical signals into the biochemical events underlying key processes such as neurotransmitter release, cell excitability and gene expression. Originally, VDCCs were classified according to their biophysical and pharmacological characteristics into P/Q, N, L, R (collectively termed high voltage activated (HVA)) and T VDCC (collectively termed low voltage activated (LVA)) subtypes (Catterall, 2000) (Table 1). However, molecular cloning, expression and biochemical studies now indicate this scheme is too simplistic and a more rigorous molecular/structural nomenclature (Table 1) has recently been introduced (Ertel et al., 2000). All VDCCs are large (>400 kDa) heteromers comprised minimally of three core subunits α_1, α_2/δ, and β found in a 1:1:1 stoichiometry (Catterall, 2000) (Fig. 1). Expression of VDCC gene products in *Xenopus* oocytes (Mori et al., 1991; Williams et al., 1992a), or transfected mammalian cells (Williams et al., 1992b; Fujita et al., 1993; Stea et al., 1993) shows that the α_1 subunits contain the ion channel pore while the auxiliary α_2/δ and β subunits confer optimal cell surface expression and channel kinetics. A major feature of VDCCs is their diverse composition: multiple genes (numbers in parentheses) encode the $\alpha_1(10)$, $\alpha_2/\delta(3)$ and $\beta(4)$ subunits and many of the RNA transcripts undergo alternative splicing (Catterall, 2000). Such diversity is highly significant since the precise nature of the α_1, α_2/d and β gene products defines the pharmacological and biophysical characteristics of the expressed VDCC heteromers (Catterall, 2000). In addition, specific VDCCs in vivo have unique, but often overlapping, patterns of expression in discrete brain regions and even within individual neurones (Jones et al., 1989; Westenbroek et al., 1990, 1992, 1995; Hell et al., 1993; Haydon et al., 1994; Mills et al., 1994; Elliott et al., 1995). Thus, VDCC diversity is thought to confer neurones with the ability to tailor voltage-dependent Ca^{2+} influx to the demands of discrete functional compartments (Elliott et al., 1995).

Until recently, the only exception to the above paradigm was the skeletal muscle VDCC, which, in addition to the α_1, α_2/δ, β core motif, also contains an additional tightly associated integral membrane subunit termed γ. However, very recent molecular cloning and expression studies (see Stargazer below) now indicate the existence of other mammalian γ subunits, (designated γ_2–γ_5), several of which (γ_2–γ_4) appear to be brain specific (Letts et al., 1998). While γ's$_{2-4}$ are closely related (>60% sequence similarity), this brain subgroup is only distantly related to γ_1 or γ_5 subunits (25% similarity) (Chu et al., 2001). Precisely what function these γ subunits serve is not fully resolved. Upon co-expression with the $\alpha_{1.1}$, α_2/d_1, β_{1a} subunits of the skeletal muscle VDCC, γ subunits alter the peak currents, and the kinetics of channel activation and inactivation with the overall effect being a normalisation of currents to those resembling the endogenous channel (Singer et al., 1991). However, similar studies with brain γ subunits reveal more complex effects (below).

Table 1
Classification and properties of voltage-dependent Ca^{2+} channel subunits

Subunit	Class	Current type	Protein size[a] (kDa)	Genbank[b]	Ligand
$Ca_v1.1$ (α_{1S})	L-type	HVA	212	X99897	Dihydropyridines
$Ca_v1.2$ (α_{1C})	L-type	HVA	240	L04569	Dihydropyridines
$Ca_v1.3$ (α_{1D})	L-type	HVA	187	M76558	Dihydropyridines
$Ca_v1.4$ (α_{1F})	L-type?	HVA	222	AF192497	Dihydropyridines
$Ca_v2.1$ (α_{1A})	P/Q-type	HVA	257	P54282	MVIIC; ω-AgatoxinIVA
$Ca_v2.2$ (α_{1B})	N-type	HVA	262	M94172	ω-conotoxin GVIA
$Ca_v2.3$ (α_{1E})	R-type	HVA	252	L27745	SNX-482
$Ca_v3.1$ (α_{1G})	T-type	LVA	250	AF027984	None
$Ca_v3.2$ (α_{1H})	T-type	LVA	261	AAG35187	None
$Ca_v3.3$ (α_{1I})	T-type	LVA	224	NP066919	None
α_2/δ-1			123	M76559	Gabapentin[c]
α_2/δ-2			129	AF042792	Gabapentin
α_2/δ-3			123	AJ010949	NA
β_1			65	X61934	NA
β_2			53	M80545	NA
β_3			55	M88751	NA
β_4			58	L02315	NA
γ_1			25	P99707	NA
γ_2			36	Q9Y698	NA
γ_3			36	NP006530	NA
γ_4			36	Q9UBN1	NA
γ_5			36	Q9UF02	NA

The revised and old (parentheses) nomenclature is indicated for the α_1 subunits together with whether they yield high voltage activated (HVA) or low voltage-activated (LVA) currents. Earlier classifications were based on the biophysical and pharmacological characteristics of the VDCCs. As this nomenclature is still in widespread use, classes and discriminating ligands are also shown. See text for further details.

[a] Protein sizes are those predicted from the DNA sequence and often differ from those found in immunoblots due to post-translational modification (see Catterall, 2000).

[b] Genbank accession numbers refer to rat, mouse or human sequences and are given as an aid to seeking related sequences.

[c] Gabapentin is probably not selective.

2. Evidence for a role of VDCCs in epilepsy

2.1. Background

The original evidence that VDCCs might play a role in epilepsy can be traced back to work that began over two decades ago showing that decreases in extracellular free Ca^{2+} concentrations might trigger seizure type activities in brain tissues (Traub and Llinas, 1979; Jefferys and Haas, 1982; Yaari et al., 1983) mirroring those seen in response to paroxysmal depolarising shifts (Heinemann and Hamon, 1986). Owing to the small volume of the extracellular space, such decreases in Ca^{2+} are now thought to reflect, at least in part, Ca^{2+} influx through neuronal VDCCs (Heinemann and Hamon, 1986). The magnitude of such Ca^{2+} sinks depends on specific cells and subcellular regions. Especially large sinks are noticeable in the somata and dendrites of hippocampal regions CA1 and CA3 and in specific layers of the cortex (Taylor and Dudek, 1982; Pumain et al., 1983; Heinemann and Hamon, 1986). Based on the finding that such signals are enhanced in several seizure models in vitro, (Pumain et al., 1983) numerous studies have attempted, subsequently, to identify both the routes of Ca^{2+} influx, focusing, in particular on VDCCs and the roles VDCCs might play in epilepsy. For simplicity, I have classified such evidence into that stemming from epileptic tissues in patients and experimental models, molecular genetics and pharmacological studies.

2.2. VDCCs in epileptic tissues

Acutely, Ca^{2+} currents are thought to contribute to epilepsy by enhancing postsynaptic responses in somatodendrites, bursting in pacemaker cells and re-excitation following periods of bursting (Dichter, 1997; McNamara, 1999). The observation that activation of VDCCs can trigger changes in gene expression (Dolmetsch et al., 2001) also suggests a chronic role in epilepsy. In part, these chronic changes may be structural (Ikegaya, 1999) but may also include cell death (McNamara, 1999). Owing to the difficulty of obtaining sufficient, especially control, samples, there is a paucity of studies documenting changes in VDCCs in human epileptic tissue. Moreover, any data generated must be viewed in the knowledge that tissues may have been obtained from patients on prolonged medication. Available data indicates that dentate granule neurones from patients with temporal lobe epilepsy display N, L and T currents characteristic of those that might be found in normal patients (Sayer et al., 1993; Beck et al., 1997). Nevertheless, there is some evidence for pronounced Ca^{2+}-dependent inactivation of HVA Ca^{2+} currents (Nagerl and Mody, 1998; Beck et al., 1999; Nagerl et al., 2000) in temporal lobe epilepsy and Ammon's horn sclerosis (Nagerl et al., 2000). One possible mechanism is via loss of the intracellular Ca^{2+} buffer, calbindin D-28k as reported for human granule cells during temporal lobe epilepsy (Magloczky et al., 1997). Although these authors suggested that depletion of calbindin might induce hyperexcitability in the dentate gyrus (Magloczky et al., 1997), an equally plausible outcome would be a facilitation of VDCC inactivation as part of a negative feedback mechanism designed to serve a neuroprotective role by limiting Ca^{2+} entry during prolonged seizure activity.

While few studies have been conducted at the molecular level in human tissues, an enhancement of β_1 and β_2 immunoreactivity in neuronal cell bodies, and a transloca-

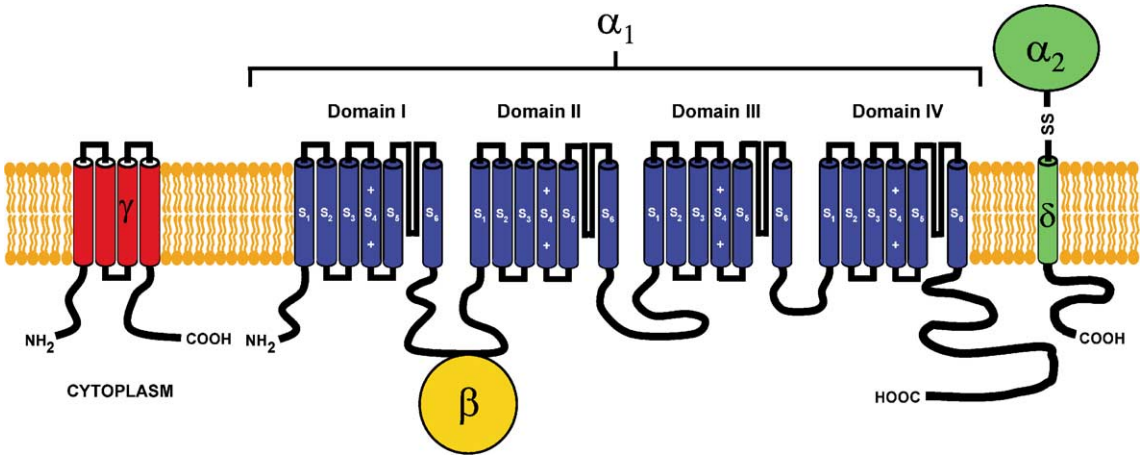


Fig. 1. Current model for the structure of the voltage-dependent Ca^{2+} channel complex. The central element of each complex is the pore-forming α_1 subunit consisting of a single polypeptide folded into four homologous domains each with six transmembrane spanning regions (S_1–S_6). The pseudo-tetrameric arrangement of the domains allows each to contribute a pore-lining element that lies between S_5 and S_6. Voltage sensing is achieved through translocation of positively charged S_4 regions. Faithful expression of the VDCCs depends on the auxiliary subunits, especially the cytoplasmic β subunit and the α_2/δ subunit. This latter subunit is synthesised as a single polypeptide but is then processed to yield a highly glycosylated, extracellular, α_2 subunit tethered via a disulphide bridge to the trans-membranous δ subunit. Recent studies suggest that a fourth subunit, δ, also exists in brain as well as skeletal muscle VDCCs. The site of interaction on α_1 is only known for the β subunit. See text for details.

tion of these subunits into the dendrites of severely sclerotic hippocampal regions, has been detected in Ammon's horn sclerosis (Lie et al., 1999). Such changes may lead to increased VDCC currents and to enhanced synaptic excitability and are especially interesting since they suggest that VDCC-trafficking mechanisms may be altered in epilepsy.

Some of the best-documented evidence for a role for VDCCs in epilepsy has come from the kindling animal seizure model (Goddard et al., 1969; McNamara et al., 1993). Kindling is a process where repeated focal application of initially sub-convulsive electrical stimulation, typically to the amygdala, ultimately generates intense limbic and clonic motor seizures. Initially, each bout of stimulation induces electrographic seizures or after-discharges within the stimulated structure. However, with each further stimulus the seizures grow longer and propagate into more widespread brain regions whereupon they induce behavioral responses of increasing intensity (Goddard et al., 1969; Racine 1972) until full blown, "stage 5" seizures (Racine, 1972) are elicited. Important features of the kindling model, especially compared to other seizure models, are its reproducibility and its ability to render the brain permanently hyper-excitable (McNamara et al., 1993). Moreover, kindling permits the discrimination of transient effects arising simply in response to seizure from longer-lasting plastic effects that are thought to underlie a permanent increase in seizure disposition (typically measured 24 h and 1 month after stimulation, respectively) (Kelly, 1998). Most important, kindling shares many behavioral features with complex partial seizures (Kelly, 1998; McNamara et al., 1993) in humans.

By applying patch clamp techniques to brain slices from kindled animals Vreugdenhil and Wadman (1992) and Faas et al. (1996) found that both LVA- and HVA Ca^{2+} currents were enhanced in the hippocampal CA1 region after kindling (by 50% and 80%, respectively). Unfortunately, a pharmacological or biophysical dissection of the underlying HVA currents was not, and has not been, forthcoming, presumably due to the technical demands of such experiments. Some contribution of L-VDCCs was proposed. In an attempt to pinpoint the molecular basis for such changes, Hendriksen et al. (1997) used in situ hybridisation techniques to measure the expression of α_1 subunit mRNA in the hippocampi of similarly kindled animals. Although increases in α_{1A}, α_{1D}, and α_{1E} and a decrease in α_{1B} were noted throughout the hippocampus during the early stages of *epileptogenesis*, the only change seen in fully kindled animals was an enhancement of α_{1B} 24 h after the last full blown (stage 5) seizure. No changes were found in the brains of kindled versus control animals 6 weeks after the last such seizure.

In spite of the above results, strong evidence that changes in VDCCs at the molecular level do accompany kindling has come recently (Blalock et al., 2001). Here, the expression of the $Ca_v1.3$ VDCC subunit was assayed by semi-quantitative reverse transcription PCR of mRNA harvested from partially dissociated CA1 neurones from entorhinally kindled rats. This strategy is especially powerful as the same cells are amenable to prior electrophysiological analysis by cell-attached patch recording techniques, thus, providing a degree of resolution impossible by in situ hybridisation techniques. The salient finding of this study was a significant >50% reduction in L-VDCC activity and a concomitant specific reduction in $Ca_v1.3$ mRNA evident 1.5–3 months after kindling. These results provide the first direct evidence, at the gene expression level, that chronic expression of a discrete VDCC is persistently altered by seizure activity. The authors argue that a decrease in L-VDCC expression may contribute to the long-term maintenance of epileptiform activity, possibly via a reduction in the Ca^{2+}-dependent after hyperpolarisation. While there are always concerns regarding the extent to which mRNA and protein expression are correlated, the possibility that similar approaches could be extended to other VDCC subunits and tissues, especially those from patients, is very exciting.

Additional support for long-term changes in VDCC proteins during kindling has also been forthcoming. Using selective fluorescent labels and detailed confocal image analysis, Bernstein et al. (1999a,b) found an enhancement in the surface density of N-VDCCs in the dendritic fields of CA1 and CA3 hippocampal neurones from amygdala-kindled rat brain. The changes in CA3 neurones were transient and presumed to reflect an acute response to seizure. However, the increase in N-VDCC expression in CA1 was still present 28 days after the last seizure, suggesting kindling caused some persistent changes at the molecular and cellular level. Surprisingly, this long-term enhancement in N-VDCC expression was localised to the stratum radiatum rather than throughout CA1. Interestingly, these regions are precisely those where large decreases in extracellular Ca^{2+} were first noted (Heinemann and Hamon, 1986). Together, the above data suggest that kindling may promote the trafficking of N-VDCCs, either to the pre-synaptic axon (Schaeffer collateral) terminals of CA3 neurones or to the CA1 dendrites, or both. In addition to rationalising the lack of change in α_{1B} mRNA expression, such a proposal is consistent with the large pools of intracellular VDCCs thought to reside in neurones (Passafaro et al., 1994) and the observation that other channels may be translocated to the nerve surface in response to synaptic input (Lu et al., 2001). An alternative possibility is that VDCC activity increases N-VDCC expression by stabilising VDCC mRNA (Schorge et al., 1999) in discrete cells but any such changes are beyond the resolution of the tissue in situ hybridisation methods used.

2.3. Insights from genetics

Arguably the most compelling evidence for a role of VDCCs in epilepsy has come from genetics. While a genetic component for many common human paroxysmal disorders

of the central nervous system has long been recognised, the underlying bases for most forms of epilepsy await identification (Meisler et al., 2001). Nevertheless, important clues are now emerging from studies on animal models, especially those identified through neurological deficits observed in breeding rodent colonies. For the sake of convenience these can be divided into, first, those studies which have mapped epileptic phenotypes to discrete VDCC gene products (Table 2) and second, those studies where VDCCs have been implicated in genetic models of epilepsy but where the mutation(s) remain(s) to be defined. Where possible I have attempted to link information from such models to relevant data from patient studies.

2.3.1. Mutations in VDCC gene products

2.3.1.1. The tottering locus. The first VDCC-associated epilepsy mutations to be identified were found at the tottering (*tg*) locus (Fletcher et al., 1996). The original *tg* mouse is a neurological mutant that exhibits *mild* ataxia, infrequent, involuntary spasms of limbs often progressing to trunk and face, reminiscent of tonic–clonic seizures as well as electrographic and behavioral hallmarks of absence epilepsy (Noebels and Sidman, 1979). Through positional cloning, the underlying defect in *tg* mice has been identified as a point mutation (P601L) in *cacna1a*, the mouse $Ca_v2.1$ (α_{1A}) gene (Fletcher et al., 1996). To address what effect the *tg* mutation might have on the channel, Wakamori et al. (1998) obtained whole cell patch clamp recordings from dissociated purkinje cells, where α_{1A} expression is very high. Their data reveal significant (40%) decreases in *tg*/*tg* versus wild type (*wt*) P-type (Ba^{2+}) currents and an increase in the proportion of non-inactivating current. To examine these changes in more detail, and resolve contributions from the P/Q-channels versus contaminating currents, the *tg* mutant α_{1A} subunit was co-expressed with α_2/δ and β_{1b} subunits in a simple cell line. Such transfection experiments confirmed a significant reduction in P-VDCC current density in mutant (48 pA/pF) compared to *wt* mice (109 pA/pF) but no change in the voltage dependence of activation or inactivation. Even though the mutation lies proximal to the pore forming region of the channel, specif-

Table 2
Molecular genetic features of voltage-dependent Ca^{2+} channels with special reference to epilepsy and other neurological disorders in man and mouse

Subunit	GDB/HUGO designation	Chromosomal localisation	Known human disorders	Class	Mouse designation	Epileptic mouse mutant	Epilepsy and knockouts
$Ca_v1.1$ (α_{1S})	CACNA1S	1q32		L-type	*Cacna1s*	–	Sk. muscle
$Ca_v1.2$ (α_{1C})	CACNA1C	12p13.3		L-type	*Cacna1c*	–	– [a]
$Ca_v1.3$ (α_{1D})	CACNA1D	3p14.3		L-type	*Cacna1d*	–	– [b]
$Ca_v1.4$ (α_{1F})	CACNA1F	Xp11.23		L-type?	*Cacna1f*	–	Retina
$Ca_v2.1$ (α_{1A})	CACNA1A	19p13.1	Familial Hemiplegic Migraine, Episodic Ataxia-2, Absence epilepsy, Spino-Cerebellar Ataxia-6	P-Type	*Cacna1a*	*tg*, tg^{la}, *rkr*, tg^{rol}	+ [c]
$Ca_v2.2$ (α_{1B})	CACNA1B	9q34		N-type	*Cacna1b*	–	– [d]
$Ca_v2.3$ (α_{1E})	CACNA1E	1q25–q31		R-type	*Cacna1e*	–	– [e]
$Ca_v3.1$ (α_{1G})	CACNA1G	17q22		T-type	*Cacna1g*	–	+ [f]
$Ca_v3.2$ (α_{1H})	CACNA1H	16p13.3		T-type	*Cacna1h*	–	NA
$Ca_v3.3$ (α_{1I})	CACNA1I	22q13.1	Incomplete congenital night blindness	T-type	*Cacna1I*	–	NA
α_2/δ-1	CACNA2D1	7q21–q22		–	*Cacna2d1*	–	NA
α_2/δ-2	CACNA2D2	3p21.3		–	*Cacna2d2*	*du*	NA
α_2/δ-3	CACNA2D3	3p21.1		–	*Cacna2d3*	–	NA
β_1	CACNB1	17q21–q22	Hypokalaemic Periodic paralysis	–	*Cacnb1*	–	– [g,h]
β_2	CACNB2	10p12		–	*Cacnb2*	–	– [h]
β_3	CACNB3	12q13		–	*Cacnb3*	–	– [i,h]
β_4	CACNB4	2q22–q23	Idiopathic generalized epilepsy	–	*Cacnb4*	*lh*	– [h]
γ_1	CACNG1	17q24		–	*Cacng1*	–	Sk. Muscle [j]
γ_2	CACNG2	22q13		–	*Cacng2*	*stg*, stg^{wag}	NA
γ_3	CACNG3	16p13.1–p12		–	*Cacng3*	–	NA
γ_4	CACNG4	17q24		–	*Cacng4*	–	NA
γ_5	CACNG5	17q24		–	*Cacng5*	–	NA

The human gene designation, chromosomal localisation and accompanying disorders are shown for each VDCC subunit. Mouse mutations or those induced through knockout approaches are also indicated. Knockout mice have not been reported for all brain VDCC subunits (indicated as NA), the presence of a + or – sign indicates that seizures (or seizure suppression) were, or were not, observed, respectively. Brain VDCC knockout references are: [a]Seisenberger et al. (2000), [b]Platzer et al. (2000), [c]Jun et al. (1999), [d]Kim et al. (2001a,b), [e]Saegusa et al. (2000), [f]Kim et al. (2001a,b), [g]Gregg et al. (1996), [h]Freise et al. (1999), [i]Namkung et al. (1998) and [j]Freise et al. (2000).

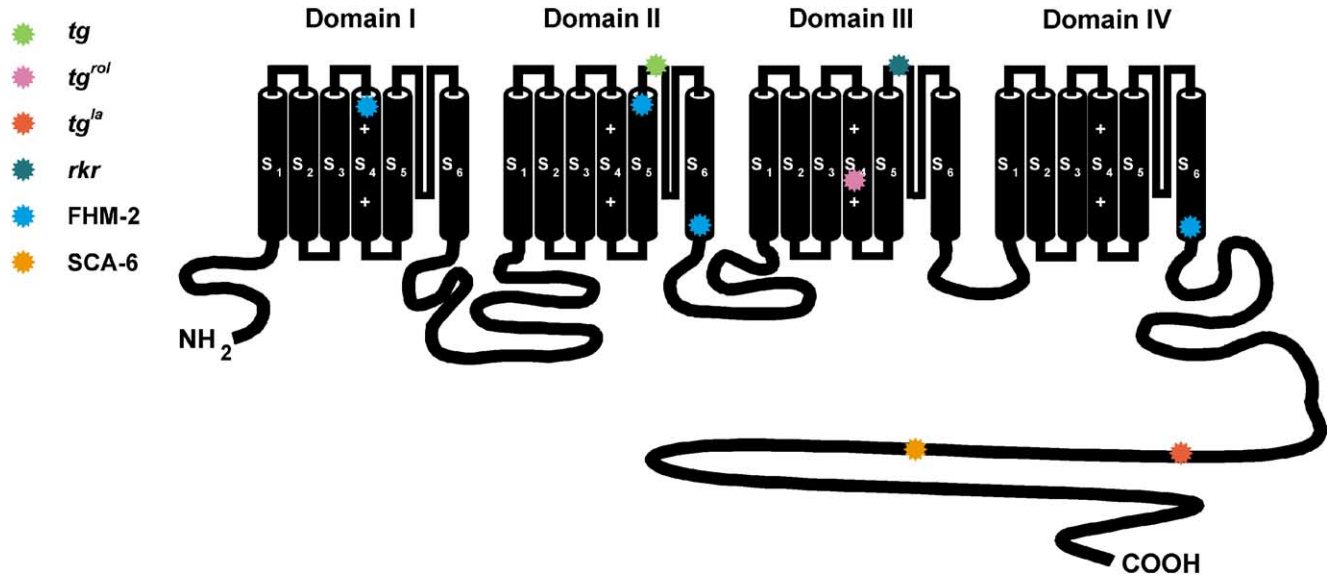


Fig. 2. Disposition of neurological mutations in the P/Q-type Ca^{2+} channel α_{1A} ($Ca_v2.1$) subunit. The locations of the mouse (*tg*, tg^{rol}, *rkr* and tg^{la}) and human mutations (Familial Hemiplegic Migraine type 2 (FHM-2) and Spinocerebellar ataxia type 6 (SCA-6)) in the α_{1A} subunit are colour coded as indicated at left.

ically the S5–S6 region of domain II (Fig. 2), the single channel conductance and apparent reversal potential appear to be unaffected, suggesting that the decreased current density is not due to impaired ion conductance.

Three additional mouse mutations, rocker (*rkr*), tottering leaner (tg^{la}) and rolling Nagoya (tg^{rol}) also map to the *cacna1a* locus (Zwingman et al., 2001; Fletcher et al., 1996; Mori et al, 2000). Perhaps what is most remarkable about all these *cacna1a* mutants is their phenotypic heterogeneity (Table 3). Such differences include the seizure characteristics, the degree of cerebellar degeneration and ataxia and the times of onset of these features. Thus tg^{la}, *rkr* and tg^{rol} but not *tg* mice show marked ataxia, while *tg*, tg^{la} and *rkr* but not tg^{rol} mice show seizure activity. (Oda, 1973; Noebels and Sidman 1979; Mori et al., 2000; Zwingman et al., 2001). Like *tg*, rocker arises through a point mutation (T1310K) that occurs in the extracellular S_5–S_6 region but in this instance that of domain III (Zwingman et al., 2001). The effect of the *rkr* mutation on P/Q currents awaits description but its effects are most likely to resemble those in *tg* mice. A separate mutation in domain III, that arises through substitution of an arginine for a glycine (R1262G), within the S_4 region, occurs in tg^{rol} mice (Mori et al., 2000). Not surprisingly, this mutation—lying within a voltage sensor—displays a marked reduction in the voltage sensitivity of channel activation.

Arguably, the most interesting but complex *cacna1a* mutation is that underlying the phenotype of the tottering leaner mouse. The tg^{la} mutant exhibits absence seizures and is notable for its severe ataxia and cerebellar damage, especially when compared to the original *tg* mouse (Tsuji and Meier, 1971). In tg^{la}, a point mutation at a splice/donor consensus sequence leads to aberrant RNA splicing in the region encoding the carboxy terminus of the α_{1A} subunit. As a result, two primary protein products are made, each representing a truncated α_{1A} subunit bearing a novel and distinct C-terminus. The first, tg^{la}_{long}, consists of an α_{1A} subunit with a distal carboxy terminus formed by translation of an intron (intron B, Fig. 3) and translation of the subsequent frameshifted exons up to a premature stop codon in exon 45. The second product, tg^{la}_{long}, consists of an α_{1A} subunit whose distal carboxy terminus is formed by skipping of exon 42 and translation of the subsequent frameshifted exons, again up to the premature stop codon in exon 45 (Fig. 3). Studies on purkinje cells indicate that the fundamental electrophysiological deficit caused by the tg^{la} mutation is a marked reduction of around 60% in whole cell P/Q-VDCC currents or current densities compared to wild

Table 3
Symptomology of mice bearing mutations in *cacna1a1*

Mutant	Designation	Age of onset	Viability	Ataxia	Dyskinesia	Absence seizures	Cerebellar cell death
Tottering	*tg*	P25	Normal	Mild	Present	Present	Absent
Leaner	tg^{la}	P8	Reduced	Severe	Absent	Present	Present
Rocker	*rkr*	P25	Normal	Mild	Absent	Present	Absent
Rolling Nagoya	tg^{rol}	P12	Normal	Medium	Absent	Absent	Modest

Neurological mutations in the $Ca_v2.1$ subunit cause extraordinary phenotypic heterogeneity in homozygous mice.

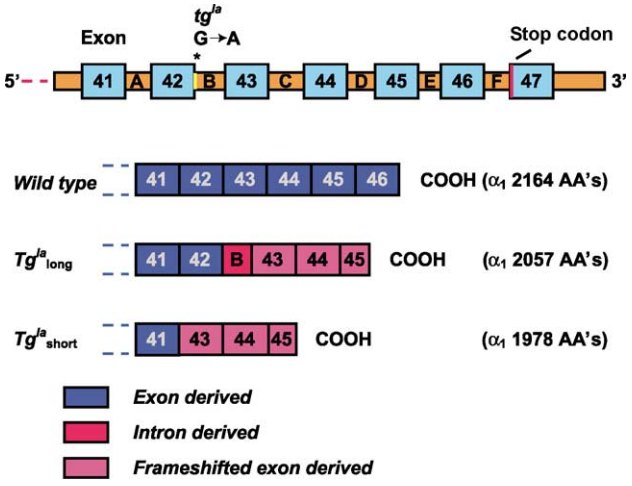


Fig. 3. Generation of aberrant carboxy termini by the tg^{la} mutation. The genomic organisation of the α_{1A} subunits is complex and consists of 47 exons of which those corresponding to the distal carboxy terminus are indicated (Top, orange and blue blue). Faithful splicing in the wild type mouse leads, ultimately, to the translation of the contiguous exons. However, in tg^{la} mice, a point mutation (G to A) at a critical splice donor consensus sequence at the end of exon 42 (top) causes aberrant splicing and the generation of two α_{1A} subunit products, that, as a result of frameshifting and intron translation (tg^{la}_{long}) or exon skipping (tg^{la}_{short}), contain novel, prematurely truncated, carboxy terminal sequences. See text for further details.

type (Lorenzon et al., 1998; Dove et al., 1998; Wakamori et al., 1998). These changes seem to be specific, as other HVA currents are unaffected (Lorenzon et al., 1998). Profound alterations in the voltage dependence of activation or inactivation are absent although a 10-mV shift in inactivation to more +ve potentials was noted by one group (Wakamori et al. 1998). At the single channel level, the decrease in current densities cannot be rationalised by effects on either the channel conductance or lifetimes, which are unaffected (Dove et al., 1998). Nevertheless, a three-fold reduction in mean patch open channel probability (NP_o) is evident, suggesting an effect on channel opening probability (P_o) or a decrease in the number of channels expressed at the surface (N). In transfected cells, where the contribution of tg^{la}_{long} and tg^{la}_{short} forms can be resolved separately, only the tg^{la}_{short} form showed a significant reduction in current density (Wakamori et al., 1998). In contrast, shifts in the voltage dependence of activation and inactivation were only observed for the tg^{la}_{long} channel.

At around the same time as the *tg* mutations were being mapped, genetic studies on families with a history of neurological disorders revealed numerous mutations in CACNA1A, the gene encoding human P/Q-VDCCs (Ophoff et al., 1996). Until recently, these mutations were associated with ataxia and Familial Hemiplegic Migraine but no seizure disorder (Chioza et al., 2002). However, a novel heterozygous point mutation (C5733T) has now been identified in an 11-year-old boy with primary generalised epilepsy and episodic and progressive ataxia (Jouvenceau et al., 2001). By introducing a premature stop codon (R1820X), this mutation results in complete loss of the C terminus of the α_{1A} subunit and impairment of channel function. The marked parallels between the phenotypes of this patient and mouse absence epilepsy models (especially tg^{la}) make this mutation especially interesting and underscore the significance of the C-terminus in VDCC function.

2.3.1.2. Lethargic mice. Epilepsy mutations are not restricted to the α_1 subunit. Mutation of the β_4 subunit has recently been shown to occur in the lethargic (*lh/lh*) epileptic mouse strain (Burgess et al., 1997). Homozygous lethargic mice exhibit convulsions and absence seizures (McEnery et al., 1998). In spite of their staggering gait, however, such mice show no structural cerebellar defects. While the *lh/lh* phenotype is thought to arise via a global decrease in β_4 mRNA expression (Lin et al., 1999) and premature truncation of the β_4 protein, neither full length nor truncated polypeptides have been detected in immunoblots of cerebellar or forebrain membranes (McEnery et al., 1998). Presumably, any translated product is rapidly degraded. At least one consequence of the *lh/lh* mutation seems to be impaired neurotransmitter release. Synaptosomes from *lh/lh* mice show a reduction in depolarization-induced [$^{45}Ca^{2+}$] uptake that has been attributed to aberrant P/Q-VDCC activity (Lin et al., 1999).

An important phenomenon exemplified by, but not restricted to the *lh/lh* mutant, is the compensatory changes seen in other β subunits (McEnery et al., 1998). While the total pool of β subunits is decreased in *lh/lh* versus *wt* mice, both the expression and incorporation of β_{1b}, but not β_2 or β_3, subunits into N-VDCC complexes, is increased. As β_1–β_3, (but not β_4) mRNA levels are reported to be unchanged in *lh/lh* versus wild type mouse brains, (Lin et al., 1999) the selective rise in β_{1b} may well reflect post-translational mechanisms. In any event, these studies argue that the neurological features induced in *lh/lh* mice do not reflect the absence of β_4 per se but, rather, the altered biophysical or targeting characteristics of the resultant β_{1b}-containing VDCC complexes. Such mechanisms presumably serve to ensure survival of the *lh/lh* mice albeit at the expense of normal brain function.

Very recent data (Escayg et al., 2000) have reported that related human disorders can also arise in humans bearing mutations in the β_4 gene (CACNB4). Screens of mutations in small pedigrees with familial epilepsy and ataxia have revealed a premature-termination mutation (R482X) in a patient with juvenile myoclonic epilepsy and a missense mutation (C104F) in a German family with generalized epilepsy and praxis-induced seizures. Such mutations were absent in control individuals.

2.3.1.3. Ducky. The ducky (*du*) mouse mutant displays ataxia and spike–wave seizures and behaviours characteristic of absence epilepsy (Meier and McPike, 1970). High-resolution genetic mapping of the ducky gene has identified

the underlying mutation as a lesion in *Cacna2d2*, the gene encoding the $\alpha_2\delta$-2 VDCC subunit (Barclay et al., 2001). Together with an additional mutation (*du(2J)*), ducky generates a prematurely truncated $\alpha_2\delta$-2 protein (Brodbeck et al., 2002) predicted to be comprised of the first three exons of *Cacna2d2*, followed by eight novel amino acids (Barclay et al., 2001). In cerebellar Purkinje cells, where $\alpha_2\delta$-2 is normally strongly expressed, electrophysiological analysis has revealed a marked reduction in VDCC currents in *du/du* versus *wt* mice (Barclay et al., 2001). Furthermore, in transfected cells, the enhancement of current density seen upon co-expression of $Ca_v2.1/\beta4$ with wild type $\alpha_2\delta$-2 subunits, is absent if the wild type $\alpha_2\delta$-2 subunit is substituted with the mutant $\alpha_2\delta$-2 subunit. Together, these data introduce the $\alpha_2\delta$-2 gene as a locus for ataxia and epilepsy in the mouse. It remains to be seen whether similar mutations occur in humans.

2.3.1.4. Stargazer. From the perspective of VDCC biology, stargazer (*stg*) represents one of the most interesting epileptic mouse mutants. Stargazer mice have spike–wave seizures characteristic of absence epilepsy as well as defects in the cerebellum and inner ear (Noebels et al., 1999). Analysis of the stargazer locus identified a mutation in a new protein—stargazin—which shows partial homology to the γ_1 subunit of the skeletal muscle VDCC (Letts et al., 1998). The identification of stargazin (designated as γ_2) is remarkable on three levels. First, it led to the disclosure of several additional putative γ subunits (γ's_{3-5}) that all had escaped detection as components of non-skeletal muscle VDCCs (Burgess et al., 1999; Klugbauer et al., 2000; Chu et al., 2001). Second, it indicated that mutation of a γ subunit could cause epilepsy. In stargazer, the mutation leads to loss of the γ_2 subunit with no compensatory change in the other brain γ subunits (Sharp et al., 2001). Third, and perhaps most intriguing, it seems that these putative VDCC γ subunits interact with, and modulate the function of, both AMPA-subtypes of glutamate receptors as well as VDCCs (Chen et al., 2000; Sharp et al., 2001; Kang et al., 2001). Exactly what this modulation includes is somewhat murky, at least for the VDCCs. In their original paper, Klugbauer et al. (2000) found that co-expression of the γ_2 and γ_4 subunits with $Ca_v2.1$ shifted the steady-state inactivation curve to more hyperpolarized potentials. In oocytes, expression of human γ subunits had little effect on $Ca_v2.1$ currents but upon co-expression with β subunits increased the appearance of unusual slow inactivating currents (Rousset et al., 2001). With α_1I, human γ_2, but not γ_3 or γ_4, subunits seem to slow the rate of deactivation in co-transfected HEK 293 cells (Green et al., 2001). The likely explanation for these diverse effects is the precise molecular composition of the VDCC complexes. In support of this, Kang et al. (2001), found that γ_2 induced a 40% decrease in the current amplitude of both $Ca_v2.1$ and $Ca_v2.2$ VDCCs in a manner that depended upon the co-expression of the $\alpha_2\delta$-2 subunit. In this study, both γ_1 and γ_2 reduced the activation kinetics of $Ca_v2.2$ VDCCs. Whatever the details, the preponderance of data so far indicate that γ_2 and perhaps the related brain γ subunits, are indeed components of VDCCs and down-regulate VDCC-mediated Ca^{2+} influx. Another interesting possibility, supported by their putative topologies (Green et al., 2001), synaptic distribution (Chen et al., 2000) and phosphorylation-regulated protein–protein interaction motifs (Choi et al., 2002), is that γ subunits also control the cell surface expression and distribution of VDCCs, AMPA receptors (Chen et al., 2000) and perhaps other membrane proteins. Mutations in human γ_{2-4} subunits have not been described, although they are excellent candidates for epilepsy. It is notable, however, that γ_3 is adjacent to a marker for a convulsive disorder (Black and Lennon, 1999).

2.3.2. Knockout mice

Additional insights into the role of VDCCs in epilepsy may be gleaned from mutations involving ablation of the expression of select gene products using knockout mouse technology. Knockout mice have been generated for several VDCC subunits (Table 2). Notwithstanding complications due to lethality and developmental compensation, two of these null mutants show features of enhanced seizure activity or diminished seizure susceptibility. Thus, ablation of the P/Q-VDCC results in mice that, in addition to severe falling and rolling ataxia, showed brief attacks of movement arrest—a hallmark of absence epilepsy (Jun et al., 1999). A detailed electrographic analysis of these mice certainly seems warranted. Increased or decreased seizure activity has not been reported for the mice lacking the α_{1B}, α_{1C}, α_{1D}, α_{1E} or auxiliary subunits that have been generated to date. However, the thalamo-cortical relay neurones of mice lacking the T-VDCC α_{1G} subunit lack the burst firing of action potentials seen in their wild type counterparts and are resistant to the generation of spike-and-wave discharges in response to $GABA_B$ receptor activation (Kim et al., 2001a,b). Together, these data confirm that T-VDCCs containing α_{1G} are key to the genesis of absence seizures in the thalamo-cortical pathway.

2.3.3. Studies on other inherited models of epilepsy

In contrast to the above models, where the underlying mutations are known, there are two genetic models of epilepsy where the inherited defect(s) are unclear but have provided additional information on the potential role of VDCCs—the GAERS rat and the audiogenic seizure-prone mouse DBA/2.

2.3.3.1. Genetic absence epilepsy rat from Strasbourg. Studies on a rat strain designated as the GAERS (Genetic Absence Epilepsy Rat from Strasbourg) rat have afforded compelling evidence for a role of T-VDCCs in epilepsy (Marescaux et al., 1992). Inherited in an autosomal dominant fashion, the phenotype of these rats includes recurrent generalized non-convulsive seizures, electrographic behav-

iour including bi-lateral synchronized spike–wave discharges and the abrupt behavioural arrest characteristic of absence epilepsy in humans. Although the frequency of spike–wave discharges is different in the GAERS rat and humans (3 versus 7–11 Hz, respectively), the effects are suppressed by drugs like ethosuximide, (below), used to control absence seizures, and involve an enhanced probability of burst firing and synchronization in thalamo-cortical structures. Since oscillatory firing of cortico-thalamic neurones requires the activity of low threshold Ca^{2+} spikes (Steriade and Llinas, 1988; Luthi and McCormick, 1998), a role for T-VDCCs has long been posited in absence epilepsy (Crunelli et al., 1989; Suzuki and Rogawski, 1989). To explore this further, Tsakiridou et al. (1995) examined the properties of VDCCs in acutely dissociated thalamo-cortical relay neurones of the nucleus ventrobasalis and neurones of the nucleus reticularis from GAERS rats. In nucleus reticularis but not ventrobasalis neurones, T-VDCC currents were increased, selectively, from a value of $-128\pm$ 14 pA found in non-epileptic rats to -198 ± 19 pA. Interestingly, the enhancement of T-current in the nucleus reticularis neurones attained significance after postnatal day 11 well before P30 when the expression of spike–wave discharges is reported to occur (Marescaux et al., 1992). These authors suggest that the enhanced T-current is likely to enhance the probability of recurrent intra-thalamic bursting and thalamo-cortical synchronisation. As the enhancement in nucleus reticularis T-currents seems to occur without changes in their kinetic properties and voltage dependence, it seems likely to reflect increased expression of T-VDCCs. Some support for this notion has come from quantitative in situ hybridization of α_{1G}, α_{1H}, α_{1I}, and α_{1E} subunit mRNAs from adult and juvenile GAERS rats (Talley et al., 2000). Higher levels of α_{1G} mRNA were found in neurones of the ventral posterior thalamic relay nuclei of adult GAERS versus control rats (64.8 ± 3.5 vs. 53.5 ± 1.7 grains/1000 pixels, respectively) as well as increased levels of α_{1H} mRNA in NT neurones in juvenile GAERS versus control rats (32.6 ± 0.8 vs. 28.2 ± 1.6 grains/1000 pixels, respectively). Although significant ($P<0.05$), these changes are modest (15–25%). However, it should be appreciated that larger increases in T-VDCC mRNAs may occur within discrete neurones that are difficult to resolve by in situ hybridisation. It would be interesting to re-examine this issue in the GAERS rat using both single cell semi-quantitative PCR (Blalock et al., 2001) and, equally important, by probing for changes at the protein level using subunit-specific antibodies.

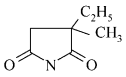
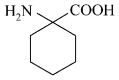
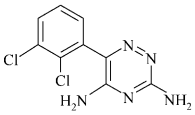
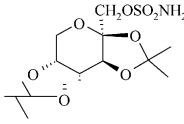
2.3.3.2. DBA/2 mice. Several inbred strains of mice exhibit seizures in response to physical and chemical stimuli. Of these, the DBA/2J mouse has found widespread use as an animal seizure model and in screens of anticonvulsant activity (Hu et al., 1999; Ryder et al., 2000). Mature DBA/2J mice show severe generalized convulsions in response to loud, high-frequency sound. The susceptibility of DBA/2J mice to audiogenic seizures seems to be a multi-factorial disorder involving several alleles (Neumann and Collins, 1991). The only reported alteration in VDCCs comes from binding studies on DBA/2J synaptosomal preparations. In their study, Esplin et al. (1994) found that the ontogeny of N-VDCCs was quite different in DBA/2J versus wild type mice and correlated well with the onset of seizure susceptibility. Some alteration in synaptic activity or synaptogenesis was suggested but this has not been pursued.

3. Pharmacological insights

In spite of the pivotal role of Ca^{2+} in epileptogenesis, significant interest in the anti-epileptic potential of drugs directed against VDCCs has only recently emerged. To some degree this situation reflects the importance of VDCCs in epilepsy highlighted by recent genetic studies. Equally salient contributions, however, have come from advances in the molecular pharmacology of VDCCs, without which it would have been impossible to identify and characterize the VDCC proteins (Triggle, 1999). It is not so long ago that dihydropyridines, which target just a subset (L-VDCCs) of the multiple types of VDCCs now known to exist in neurones, represented the only available ''selective'' ligands. Even so, it is now recognised that L-VDCCs include subtypes formed from α_{1S}, α_{1C}, α_{1D} and perhaps α_{1F} subunits (Catterall, 2000; Ertel et al., 2000). An additional problem has been the difficulty in unraveling the mechanisms of action of many existing anti-convulsant agents owing to their lack of selectivity. Nevertheless, several therapeutically significant anti-convulsant compounds (listed in Table 4) have now been identified which seem to act, at least in part via VDCCs.

The first such compound, ethosuximide, is unusual in its selective efficacy against absence but not partial or generalized tonic–clonic seizures (Bromfield, 1998). Based on its ability to block partially T-type currents in thalamic neurones at therapeutically relevant concentrations (Coulter et al., 1989), ethosuximide was proposed to work by inhibition of T-type channels (T-VDCCs). Further support for this notion was provided by the observation that methyl–phenyl suximide the active metabolite of a related compound methsuximide, but not its inactive analog, also caused T-VDCC blockade (Coulter et al., 1990). Nevertheless, several subsequent studies provided a contradictory picture of the blockade of T-VDCCs by ethosuximide at therapeutic doses (Leresche et al., 1998). In a further excellent example of the power of molecular approaches, Gomora et al. (2001) revisited this issue by testing the effects of methyl–phenyl suximide and ethoxysuximide on the cloned human T-VDCCs expressed in mammalian (Human Embryonic Kidney 293) cells. Here, both ethosuximide and methyl–phenyl suximide blocked the expressed T-VDCC currents in a state-dependent manner, with higher affinity for inactivated channels ($K_i \approx 0.4$ mM). In contrast, analogs that were

Table 4
Pharmacology of clinically useful anti-epileptic drugs thought to act on voltage-dependent Ca^{2+} channels

Drug	Structure	Target	Kd	Therapeutic concentration	Alternate specificity	Major use in seizures	Maintenance dose in monotherapy
Ethosuximide		$Ca_v3.1$-3.3) (α_{1G-I})	400 μM	500 μM	Na^+channels	Absence	1.5 g/day
Gabapentin		α_2/δ-1-2	150 nM	15 μM	GABA-B receptors?	Partial	1.2 g/day
Lamotrigine		HVA currents	10 μM	10 μM	Na^+channels?	Partial, tonic clonic	150 mg/day
Topiramate		HVA current	15 μM	10 μM	Multiple	Patial tonic-clonic	300 mg/day

Information was gathered from Engel and Pedley (1998), the British National Formulary and references within the text.

not anticonvulsive were poor blockers. Thus, ethosuximide and related antiepileptic drugs, clearly, block human T-type channels at clinical doses.

One of the most intriguing stories to emerge in VDCC pharmacology concerns GABApentin, an extremely safe anti-epileptic, primarily used in treating partial seizures (Chadwick and Brown, 1998). In spite of the structural similarity of GABApentin to GABA, this compound does not appear to interact directly with either $GABA_A$ or $GABA_B$ receptor subtypes and its mode of action has long been a mystery. There is now very strong evidence, however, that GABApentin exerts its actions through VDCCs. Purification of high affinity [^{3}H]GABApentin binding sites from porcine brain identified the target as the $\alpha_2\delta$ subunit protein—a ubiquitous VDCC component (Gee et al., 1996). Thus, GABApentin represents the first known ligand for this auxiliary subunit. Binding of GABApentin appears to involve a single class of sites and is subtype specific, occurring with a higher affinity to $\alpha_2\delta$-1 than $\alpha_2\delta$-2 subunits (Marais et al., 2001). The $\alpha_2\delta$-3 subtype seems unable to bind GABApentin. Precisely what effect GABApentin has on VDCCs is uncertain. Several reports indicate that GABApentin inhibits Ca^{2+} currents but the pathway is somewhat unclear. GABApentin has been shown to be a potent, voltage-dependent, blocker of Ca^{2+} currents (L, P/Q and predominantly N-type) in dorsal root ganglion neurones in culture (IC_{50} 157 nM) (Sutton et al., 2002). Such inhibition was unaffected by a $GABA_B$ receptor antagonist, thus, supporting a direct action of GABApentin on the VDCCs, consistent with the binding data. In contrast, in hippocampal and mIL cells, where GABApentin also reduces voltage-dependent Ca^{2+} responses, such inhibition does seem to be suppressed by $GABA_B$ receptor antagonists (Bertrand et al., 2001). Since presynaptic $GABA_B$ receptors are known to inhibit P/Q VDCCs through G-coupled pathways (Mintz and Bean, 1993), these workers argued that GABApentin-inhibits VDCCs indirectly by activating $GABA_B$ receptors. While it is notable that the EC_{50} for GABApentin's effects was 10-fold higher in the latter study, at the therapeutic concentrations seen in vivo, both mechanisms may occur. Nevertheless, direct studies of recombinant $GABA_B$ receptor-mediated G-coupling failed to detect any stimulation of G-coupled pathways by GABApentin (Lanneau et al., 2001). Consequently, the precise response to GABApentin is unresolved although a defined endpoint—suppression of excitatory transmitter release via inhibition of P/Q but not N-VDCCs—has been detected (Fink et al., 2000).

Of the other major anti-epileptic agents in clinical use, two in particular have aroused interest as potential modulators of VDCC currents—topiramate and lamotrigine. Topiramate is a recently introduced anticonvulsant that, owing to its low risk of adverse effects, is finding widespread use in treating childhood epilepsies (Shank et al., 2000). At present, there is no consensus about topiramate's mechanism of action. At therapeutically relevant concentrations, topiramate has been shown to block epileptiform activity induced experimentally in brain slices in a manner that has been attributed to a possible disruption of VDCC activity (DeLorenzo et al., 2000; Jahromi et al., 2000). This notion has been supported by whole cell patch clamp recordings indicating a partial reduction of the L-VDCC component of the HVA currents in dentate granule cells at 10 μM topiramate (Zhang et al., 2000). The reported biphasic concentration dependence of this effect suggests a potentially complex mode of action that may include modulation of channel phosphorylation (Shank et al., 2000). Lamotrigine is another clinically important new antiepileptic that is reported to inhibit VDCC currents. In rat cortical neurones, Lamotrigine has been found to cause a dose-dependent inhibition of N- and P- but not L-VDCC currents at therapeutic concentrations (Stefani et al., 1996). Similar effects on N-type currents have been obtained in neurones from rat amygdala (Wang et al., 1998).

Having discussed the growing evidence for VDCCs as targets for therapeutically relevant anti-epileptic agents, it is pertinent to consider the converse question, specifically what is the therapeutic potential of agents known to disrupt specific VDCC subtypes? It has long been known that the L-VDCC-selective dihydropyridine agonists, notably BAY-K8644 and antagonists can induce or suppress epileptiform activity, respectively, in experimental models of seizure, especially in vitro models (Walden et al., 1992, 1996). Although BAY-K8644 causes convulsions (Walden et al., 1992), the potential anti-convulsant actions of dihydropyridine antagonists have not been exploited except as add-on therapy in intractable cases of epilepsy (Larkin et al., 1991). Why dihydropyridine antagonists have not been used widely may reflect the greater efficacy of traditional anti-epileptic drugs, cardiovascular side effects, or enhanced metabolism when given in conjunction with such drugs. However, it may also reflect the possibility that, except for T-VDCCs and absence seizures, the most beneficial VDCC-directed antiepileptic agents may be those targeting VDCCs mediating neurotransmitter release—notably the P/Q and N-VDCCs. These channels are specific targets for naturally occurring polypeptide toxins (Olivera, 1999). Such toxins are known to suppress epileptiform activity in vitro, but, generally, do not cross the blood–brain barrier. Nevertheless, a variety of N, P/Q and R-VDCC-directed agents are being introduced which may show anti-convulsant activity (Hu et al., 1999; Ryder et al., 2000). One such drug is daurisoline, an alkaloid isolated from a Chinese medicinal herb used for the treatment of epilepsy, hypertension and asthma. While daurisoline was found to block currents through P/Q but not other VDCCs (Lu et al., 1994) and to reduce spontaneous activity in cerebellar neurones, it was unable to prevent electroshock-induced convulsions in rats or mice up to 30 mg/kg and at higher doses caused death (Lingenhohl et al., 1997). The effectiveness of 4-benzyloxyaniline derivatives in suppressing audiogenic seizures in DBA/2J mice (Hu et al., 1999) and their less pivotal role in orchestrating transmitter release compared to P/Q VDCCs suggests exploitation of these and other putative N-VDCC blockers may be a more useful avenue for the development of new anti-convulsants.

4. Conclusions and future perspectives

In this article I have attempted to review salient information on the role of VDCCs in epilepsy. Only a few years ago this task would have been largely confined to phenomenology. However, the application of molecular biology approaches has exposed key roles for VDCCs in epilepsy and begun to provide a mechanistic basis for the development of novel therapeutic agents. Much remains to be established. An enormous challenge will be to fill in the gaps between the molecular deficits observed in genetic models and the expression of specific epilepsy phenotypes. It is already clear that mutations in a single gene product, for example, the α_{1A} subunit, can lead to quite different seizure phenotypes (Table 3). Often mutant animals show considerable phenotypic pleiotropy—a phenomenon that emphasises the importance of genetic approaches in pinpointing the underlying mutations (Austin et al., 1992; Nakayama and Nagai, 1996). An additional problem will be to resolve the effects of a specific VDCC mutation, such as loss of channel function, from those introduced by compensatory expression of other VDCC subunits or proteins (McEnery et al., 1998; Campbell and Hess, 1999). The effects of such mutations will also require a considerably more detailed knowledge of the cell biological role of VDCCs than available at present. It has long been recognised that VDCC activity is required for neurite outgrowth (Doherty et al., 1993) and there is good evidence that VDCCs mediate cellular changes in response to epileptiform activity (Ikegaya, 1999) and anticonvulsants (Katoh-Semba et al., 2001).

Another important goal will be to identify novel targets and agents for suppressing seizure activity and ultimately curing epilepsy. The expression of defined VDCC complexes in simple transfection systems has already afforded confirmatory evidence for the mode of action of existing anti-convulsants such as ethosuximide (Gomora et al., 2001) and is clearly critical for screening potential new agents. However, many drugs, especially those for patients with inherited epileptic VDCC mutations, may need to be phenotype, rather than purely channel, specific (Hoffman and Gardner, 1997). It is also essential to realise that the contribution of any ion channel to nerve function depends not only on its biophysical characteristics but also on its distributions over the nerve surface. Thus, the development of agents that modulate the targeting of specific VDCCs, while requiring a much better knowledge of the underlying mechanisms is likely to represent a fruitful area of research.

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