INVITED REVIEW

The neuronal channelopathies

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Summary
This review addresses the molecular and cellular mechanisms of diseases caused by inherited mutations of ion channels in neurones. Among important recent advances is the elucidation of several dominantly inherited epilepsies caused by mutations of both voltage-gated and ligand-gated ion channels. The neuronal channelopathies show evidence of phenotypic convergence; notably, episodic ataxia can be caused by mutations of either calcium or potassium channels. The channelopathies also show evidence of phenotypic divergence; for instance, different mutations of the same calcium channel gene are associated with familial hemiplegic migraine, episodic or progressive ataxia, coma and epilepsy. Future developments are likely to include the discovery of other ion channel genes associated with inherited and sporadic CNS disorders. The full range of manifestations of inherited ion channel mutations remains to be established.

Keywords: channelopathies; episodic ataxia; hyperekplexia; inherited epilepsies; ion channels

Abbreviations: ADNFLE = autosomal dominant nocturnal frontal lobe epilepsy; BFNC = benign familial neonatal convulsions; EA = episodic ataxia; FH = familial hyperekplexia; FHM = familial hemiplegic migraine; GABA = γ-aminobutyric acid; GEFS+ = generalized epilepsy with febrile seizures plus; SCA = spinocerebellar ataxia; SMEI = severe myoclonic epilepsy of infancy

Introduction
One of the most exciting developments in clinical neurology over the last decade has been the identification of ion channel mutations as the cause of a wide variety of inherited disorders. This development started with the discovery that several disorders of muscle membrane excitability were linked to missense mutations of calcium, sodium and chloride channels and acetylcholine receptors (reviewed in Cannon, 1996; Ptacek, 1997; Davies and Hanna, 1999; Lehmann-Horn and Jurkat-Rott, 1999; Celesia, 2001). This group of diseases has been termed ‘channelopathies’ (Griggs and Nutt, 1995). Some rare inherited diseases, manifesting primarily as movement disorders, headache and epilepsy, have also been linked to ion channels. This review addresses the manifestations and mechanisms of channelopathies affecting neurones. The breakthroughs in understanding these diseases have raised expectations that the causes of far commoner idiopathic neurological diseases, in particular primary generalized epilepsy, will soon be understood. Although the molecular mechanisms underlying common disorders may not be just around the corner, the channelopathies offer an unprecedented insight into the diversity of cellular mechanisms underlying the abnormal function of neuronal circuits.

General features of ion channels
A detailed description of ion channel structure and function is beyond the scope of this review (Ashcroft, 2000; Hille, 2001). Nevertheless, a few important principles must be borne in mind while attempting to understand the consequences of mutations of individual channels. Conventionally, ion channels are divided into those that are voltage-gated and ligand-gated, although this distinction is not absolute, and other channel types exist (see below). Ion channels of both types are heavily glycosylated multimeric proteins. Although some are homomultimers, most are assembled from distinct subunits coded for by different genes (heteromultimers). Voltage-gated channels are made up of one or more pore-forming subunits (generally referred to as α subunits), and variable numbers of accessory subunits (often denoted β, γ,
etc.). The α subunits determine the ion selectivity and mediate the voltage-sensing functions of the channel. In other words, they are sufficient to form functional channels. However, the properties of native channels isolated from the brain are usually only accounted for by the additional presence of the accessory subunits.

The α subunits of the voltage-gated potassium, sodium and calcium channels affected in the known neuronal channelopathies are all evolutionarily related, and share a similar structure. Because potassium channels exhibit several important features common to the other channels, we will describe their properties first.

**Voltage-gated potassium channels**

Potassium channels set the resting membrane potential, repolarize neurones following action potentials, and also mediate some forms of subthreshold signalling. Different classes of potassium channels play these roles. Thus, voltage-gated channels of the Kv family tend to be closed at resting membrane potential, but open rapidly on depolarization. Some other potassium channels show inward rectification, meaning that they mediate inward potassium flux when the membrane potential is relatively negative than they mediate outward flux when it is relatively depolarized.

Voltage-gated potassium channels of the Kv family are made up from four homologous α subunits. Each subunit contains six transmembrane segments (S1–S6), linked by extracellular and intracellular loops (Fig. 1A). Both N- and C-termini are cytoplasmic. One of the transmembrane segments (S4) has several positively charged amino acids which cause a conformational change upon membrane depolarization. This movement underlies voltage-dependent channel activation (opening of the ion pore). Fast inactivation (or closure in the face of continued depolarization) proceeds through the occlusion of the ion pore by a cytoplasmic peptide, which, depending on the identity of the channel, is either an intrinsic part of the same protein (the extreme N-terminus of the α subunit), or is contributed by an accessory (β) subunit. Another critical part of the α subunit is the S5–S6 linker, which contains the selectivity filter that lines the ion pore itself. This part of the protein is in the extracellular side, but loops deep into the central part of the channel, and contains a highly conserved glycine-tyrosine-glycine sequence. The homologous regions of the four α subunits come together at this point, with the amino acid side-chains pointing away from the pore. The physical dimensions of the pore, together with the electrostatic properties of the peptide backbone, render the channel permeable to potassium ions and relatively impermeable to other ions. Among other important regions are the N- and C-termini, which play important roles in inactivation, channel assembly, targeting, and interactions with accessory proteins.

Although potassium channels with these general properties are known as voltage-gated, some members of this large family are actually primarily gated by intracellular messengers, such as G proteins and nucleotides. Other, more distantly related potassium channels have either fewer or more transmembrane segments, and respond to a wide variety of intra- and extracellular agents, including pH, permeant and non-permeant cations, polyamines and arachidonic acid. Although these potassium channels are not known to be mutated in the neuronal channelopathies, many of them play important roles in regulating cell firing and mediating the effects of neurotransmitters acting on G protein-coupled receptors. Several members of this extended family of potassium channels are implicated in channelopathies affecting muscle, kidney, pancreatic β-cells or heart. Because homologous channels also exist in the CNS they are candidates for yet-to-be-discovered neuronal channelopathies.

In addition to the four α subunits, voltage-gated potassium channels of the Kv family also contain four accessory cytoplasmic β subunits. Depending on their identity, the β-subunits can play important roles in modulation of channel function and inactivation. The α and β subunit composition shows considerable variability, such that different members of the subclasses can substitute for one another. For instance, Kvα1.1, the substrate of episodic ataxia type 1 (see below), coassembles with Kvα1.2 and Kvα1.4 (Wang et al., 1993, 1994; Coleman et al., 1999). How much rearrangement exists at the level of β subunits is unclear, but there is potentially room for an enormous range of channel stoichiometries.

**Sodium and calcium channels**

The amino acid sequences of α subunits of sodium and calcium channels contain four highly homologous domains in tandem, each of which resembles a voltage-gated potassium channel α subunit. They probably arose in evolution through two duplications of a potassium channel. Each domain contains six transmembrane segments in the same way as the potassium channels, and movement of the four S4 segments underlies activation. Only one α subunit is thus required to form a channel. There are, however, some important differences between potassium, sodium and calcium channels. Notably, the amino acid sequence at the selectivity filter is different. Four highly conserved glutamate residues line the selectivity filter of calcium channels, while the selectivity of sodium channels appears to be determined by different amino acids in the four domains. The mechanism of inactivation of sodium channels is also different, being principally determined by the movement of the cytoplasmic linker between the third and fourth domains.

Sodium channel α subunits are associated with two β subunits, one of which, β1, contains a single transmembrane segment and an extracellular loop held by a disulphide bond (Catterall, 2000a). This subunit accelerates the gating kinetics of the sodium channel. Calcium channels are associated with at least two distinct accessory subunits (Catterall, 2000b). The α2δ subunit also consists of a single transmembrane segment, as well as a second extracellular peptide chain held
Fig. 1 KCNA1 mutations impair potassium channel function. (A) Structure of the Kvα1.1 subunit, showing the six transmembrane domains, the voltage-sensing S4 domain (+), and the pore-lining loop between S5 and S6. Each circle represents a single amino acid, and filled circles indicate positions of known missense mutations. (B) Missense mutations can potentially affect several stages of ion channel expression and function, indicated schematically here. Wild-type Kvα1.1 subunits may assemble with mutant subunits (indicated by a star) in a variety of stoichiometries. (C) Example of K⁺ channel dysfunction caused by a mutation associated with severe, drug-resistant episodic ataxia type 1. The R417stop mutation truncates the C-terminus, and gives rise to a non-functional channel. However, the mutant subunit can assemble with the wild-type (WT) subunit, as shown by a significant (\(^*P < 0.05\)) reduction in potassium current obtained by coexpressing the two alleles together (a dominant negative effect; histogram on left), and by a change in the activation rate (current traces on right). In addition, the mutant shifted the activation threshold to more positive voltages, and accelerated deactivation (not shown). These effects are predicted to result in a profound reduction in potassium flux and impaired repolarization of neurones (Eunson et al., 2000).
by a disulphide bond. Both peptides are cleavage products of a single gene. The α2δ subunit enhances surface expression of the α subunit (in this case denoted α1). Calcium channels are also associated with an intracellular β subunit, which contributes to their targeting and/or anchoring, and the modulation of their biophysical properties by intracellular secondary messengers. Some controversy surrounds the existence and role of a third (γ) subunit in association with neuronal calcium channels in the brain (Letts et al., 1998; Chen et al., 2000).

Other voltage-gated channels
Chloride channels will only be mentioned briefly, because they are not known to be mutated in neuronal, as opposed to muscle, channelopathies. They are unrelated to potassium channels, have between eight and 12 transmembrane segments, and at least some subtypes exist as homodimers with two distinct conducting pores. In contrast to skeletal muscle, chloride channels play a relatively small role in setting the resting potential of neurones. However, they are involved in acidification of synaptic vesicles, and mutations may yet be found to underlie some disorders of neurotransmission (Stobrawa et al., 2001).

Gap junction proteins
Gap junctions mediate ion flux between electrically coupled cells. The connexins are a family of transmembrane proteins that assemble as hexamers. Two hexamers, one in each of the apposed membranes, make up the entire channel. These channels are relatively non-selective for permeant ions, are weakly voltage- and pH-dependent, and are thought to mediate electrical coupling among neurones and/or glial cells, as well as the movement of water, and possibly small messenger molecules down their (electro)chemical gradients. Connexin 32 is expressed in Schwann cells, and mutations are associated with X-linked hereditary neuropathy (Bergoffen et al., 1993). However, gap junction protein mutations have not been identified in neuronal channelopathies.

Ligand-gated channels
Ligand-gated channels (or ionotropic receptors) open in response to extracellular agonists, but they usually also desensitize if the agonist is not cleared. Desensitization is analogous to inactivation of voltage-sensitive channels.

Three families of ligand-gated channels are recognized: (i) the nicotinic receptor superfamily (including GABA<sub>A</sub>, glycine, serotonin, and nicotinic acetylcholine receptors); (ii) the glutamate receptor family; and (iii) the ionotropic ATP receptors. Channelopathies have only been identified in GABA<sub>A</sub>, glycine and nicotinic acetylcholine receptors, all members of the nicotinic superfamily. These channels are pentameric, with each subunit containing four transmembrane domains, the second of which (the M2 region) lines the pore and determines the ionic selectivity (Fig. 2A). Both N- and C-termini are extracellular, in contrast to voltage-gated potassium channels. Nicotinic and serotonergic receptors show relatively little selectivity among monovalent cations, and some are even permeable to calcium. Glycine and GABA<sub>A</sub> receptors, on the other hand, are selective for small anions, and allow both chloride and bicarbonate to permeate. Distinct subunits, denoted by Greek letters, coassemble to form functional pentamers. Several types of neuronal nicotinic receptors have been identified in the mammalian
brain; these fall into two general classes: heteropentamers, notably those composed of \( \alpha_4 \) and \( \beta_2 \) subunits, and homopentamers, in particular \( \alpha_7 \). Binding of two molecules of acetylcholine is required to open the channel. The residues that determine agonist binding are located in several parts of the N-terminus of the \( \alpha \) subunits. Paradoxically, the affinity of \( \alpha_4\beta_2 \) receptors for acetylcholine is actually higher than that of \( \alpha_7 \) homopentamers, even though they contain fewer \( \alpha \) subunits. This is explained by the fact that acetylcholine binds at the interface between the extracellular parts of neighbouring subunits, so that the \( \beta \) subunits also contribute to affinity.

The main type of glycine receptor in the brainstem and spinal cord is an \( \alpha_2\beta \) heteropentamer, although the foetal form is an \( \alpha_2 \) homopentamer. Channels are thought to be composed of three \( \alpha \) subunits and two \( \beta \) subunits (Fig. 2B). Agonist binding is principally determined by the \( \alpha \) subunits, in particular several parts of the N-terminus as well as the loop between M2 and M3. The main role of the \( \beta \) subunit appears to be to target and/or anchor the receptor to synapses via the scaffolding protein gephrin.

GABA\(_A\) receptors exist in a vast range of stoichiometries, because at least 15 subunits in seven different subfamilies (\( \alpha \), \( \beta \), \( \gamma \), \( \delta \), etc.) have been identified. The most abundant GABA\(_A\) receptor, at least in the rodent brain, is thought to contain \( \alpha_1 \), \( \beta_2 \) and \( \gamma_2 \) subunits in the stoichiometry \( (\alpha_1)_3(\beta_2)_2(\gamma_2)_1 \). The subunit composition affects not only the kinetics and pharmacological profile of the receptor, but also its membrane targeting. GABA binds mainly at the interface between \( \alpha \) and \( \beta \) subunits.

Channelopathies have not, as yet, been identified among 5HT\(_3\), glutamate or ATP receptors, although glutamate receptors, with their central role in excitatory transmission, are obvious candidates.

### Regional and temporal diversity of ion channels

The distinct genes coding for particular ion channel subunits generally show characteristic regional expression patterns. Even within the same cell, distinct compartments express different gene products; for instance, different sodium channels are expressed in axons and dendrites, with subtly different kinetic properties. This is complicated further by the fact that many channel genes undergo differential splicing, which again varies among brain regions and neuronal populations. Thus, some disease-associated mutations may only be expressed in a subset of the neurones expressing the gene product. Other mutations may occur at exon–intron boundaries, potentially giving rise to aberrant transcripts. Finally, some channels undergo age-dependent changes in expression. The result of this complexity is that some mutations that derange ion channel function may be expected to affect only very specific populations of neurones, and possibly only at certain ages.

### Understanding the channelopathies

The process of discovery in genetic diseases is characterized by an initial linkage of a specific syndrome to a locus, and then to an individual genetic defect. This is often followed by the realization that other mutations of the same gene can give rise to a wider spectrum of disorders. The neuronal channelopathies are, at present, probably only part of the way through this process. The ion channel mutations that have been discovered are generally those that are associated with characteristic phenotypes, especially when these are transmitted in an autosomal dominant manner with high penetrance. Here, we will only consider those diseases where mutations of neuronal ion channel genes have been identified (Table 1). These mutations have been argued to be pathogenic, and not polymorphisms, on the basis that they do not occur in a large number of control chromosomes in the background population, and that they co-segregate with the disease in affected families. However, the most direct test that they underlie the disease is usually to demonstrate that the mutations alter ion channel function.

### Voltage-gated channelopathies

#### Potassium channelopathies

**\( K_\text{V} \alpha 1 \)**

The product of the KCNA1 gene on chromosome 12 is \( K_\text{V}\alpha 1.1 \), a voltage-gated potassium channel \( \alpha \) subunit. \( K_\text{V}\alpha 1.1 \) and \( K_\text{V}1.1 \) are frequently used interchangeably, but here we will reserve \( K_\text{V}1.1 \) for the channel formed by assembly of four \( K_\text{V}\alpha 1.1 \) subunits. This is the mammalian orthologue of the Shaker channel, the first potassium channel to be cloned in *Drosophila*. \( K_\text{V}1.1 \) is a delayed rectifier channel, i.e. it opens upon depolarization, lagging after the opening of sodium channels, and it does not show rapid inactivation when expressed as a homotetrameric channel.

Missense mutations of KCNA1 were first identified in association with the autosomal dominant disorder episodic ataxia type 1 (EA1) (Browne *et al.*, 1994). Patients have short-lasting (seconds to minutes) attacks of cerebellar incoordination, often triggered by exertion, stress or startle. In addition, they exhibit persistent inter-ictal motor unit activity (myokymia or neuromyotonia). Close examination of some kindreds has revealed unexpected variability in the phenotype, including epilepsy, infantile contractions, and even isolated neuromyotonia (Browne *et al.*, 1994; Comu *et al.*, 1996; Scheffer *et al.*, 1998; Zuberi *et al.*, 1999; Eunson *et al.*, 2000). Given that some carriers report only cramps, the disease spectrum for KCNA1 mutations may be very wide indeed, and it remains to be determined whether the penetrance is always high. Many patients with EA1 experience a reduction in attack severity during treatment with acetazolamide or carbamazepine (Lubbers *et al.*, 1995), although the response is variable. The variability in phenotype and response to medication is more striking.
when different families carrying distinct mutations are compared, than within individual families.

Based on immunohistochemistry in rodents, Kv1.1 is enriched in the juxtaparanodal region of the axons of motor neurones (Zhou et al., 1998). In the brain, Kv1.1 is widely distributed, but especially prominent around the initial segments of many axons, including those of pyramidal neurones in the hippocampus and Purkinje cells of the cerebellum (Veh et al., 1995). They are therefore strategically positioned to regulate the firing rates of these neurones. However, they are also present in dendrites and presynaptic terminals, so they may play far more extensive roles in regulating signalling (Wang et al., 1994).

Over a dozen missense and one premature stop codon mutations have been identified in KCNA1 (Browne et al., 1994; Comu et al., 1996; Scheffer et al., 1998; Zuberi et al., 1999; Eunson et al., 2000; Knight et al., 2000). The mutations all affect highly conserved residues, and are scattered throughout the peptide sequence (Fig. 1A). However, none have been found either in the N-terminus (which is important for channel assembly) or in the selectivity filter. Several studies have examined the consequences of most of the known mutations by expressing the channels in oocytes of the clawed frog *Xenopus laevis* (Adelman et al., 1995; D’Adamo et al., 1998; Zerr et al., 1998a, b; Boland et al., 1999; Spauschus et al., 1999; Zuberi et al., 1999; Eunson et al., 2000) or in mammalian cell lines (Bretschneider et al., 1999; Spauschus et al., 1999; Zuberi et al., 1999; Eunson et al., 2000). When expressed alone, several Kv1.1 mutants show a reduction in peak current amplitude, evoked by large depolarizing steps, relative to wild-type channels (Fig. 1B and C). This reduction is, however, very variable among the different EA1 mutations. The peak current ranges from undetectable (implying that the channel subunits are non-functional) to an amplitude indistinguishable from wild type (Adelman et al., 1995; D’Adamo et al., 1998; Spauschus et al., 1999; Zuberi et al., 1999; Eunson et al., 2000). A wide variety of alterations in voltage threshold and gating kinetics have also been observed. Several mutations shift the activation threshold to more depolarized levels, while others increase or decrease the rate at which channels activate.

### Table 1 Neuronal ion channels associated with human neurological diseases

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosomal location</th>
<th>Protein</th>
<th>Function</th>
<th>Principal phenotype(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KCNA1</td>
<td>12p13</td>
<td>α subunit of Kv1.1</td>
<td>Repolarization in axons</td>
<td>EA1</td>
</tr>
<tr>
<td>KCNQ2</td>
<td>20q13.3</td>
<td>KCNQ2 α subunit</td>
<td>M current (low threshold current modulated by muscarinic receptors) Partner of KCNQ3</td>
<td>BFNC</td>
</tr>
<tr>
<td>KCNQ3</td>
<td>8q24</td>
<td>KCNQ3 α subunit</td>
<td>M current. Partner of KCNQ2</td>
<td>BFNC</td>
</tr>
<tr>
<td>CACNA1A</td>
<td>19p13</td>
<td>α1A subunit of CaV2.1</td>
<td>P/Q-type current in Purkinje and granule cells, and presynaptic terminals</td>
<td>FHM; EA2; SCA6</td>
</tr>
<tr>
<td>SCN1A</td>
<td>2q24</td>
<td>α subunit of NaV1.1</td>
<td>Somatodendritic sodium influx (dendritic integration)</td>
<td>GEFS+; SMEI</td>
</tr>
<tr>
<td>SCN2A</td>
<td>2q23–2q24.3</td>
<td>α subunit of NaV1.2</td>
<td>Axonal fast sodium influx (action potential initiation and propagation)</td>
<td>Febrile and afebrile seizures (one patient only, so status uncertain)</td>
</tr>
<tr>
<td>SCN1B</td>
<td>19q13.1</td>
<td>β1 subunit of brain sodium channels</td>
<td>Modulates function of α subunits</td>
<td>GEFS+</td>
</tr>
</tbody>
</table>

EA1 = episodic ataxia and neuromyotonia; BFNC = benign familial neonatal convulsions; FHM = familial hemiplegic migraine; EA2 = episodic ataxia type 2; SCA6 = spinocerebellar ataxia type 6; GEFS+ = generalized epilepsy with febrile seizures plus; SMEI = severe myoclonic epilepsy of infancy; ADNFLE = autosomal dominant nocturnal frontal lobe epilepsy; FH = familial hyperekplexia.
inactivate or deactivate. Overall, these alterations in current amplitude and kinetics are predicted to result in a decrease in potassium flux during depolarization, although the size of this effect varies extensively among the different mutations.

In order to understand the consequences for neuronal excitability, it is important to bear in mind that patients are heterozygous and, moreover, that wild-type \( KV_{\alpha 1.1} \) subunits can coassemble with some other members of this subfamily of potassium channels (in particular \( KV_{\alpha 1.2} \)) as well as with accessory \( \beta \) subunits (Ruppersberg et al., 1990; Coleman et al., 1999). Although the range of possible stoichiometries is potentially very large, a first step towards simulating the situation \textit{in vivo} can be taken by coexpressing mutant and wild-type \( KV_{\alpha 1.1} \) (Zerr et al., 1998b; Spauschus et al., 1999; Zuberi et al., 1999). In several cases, mutant subunits exert dominant negative effects on current amplitude (Fig. 2C). This implies that some mutant subunits are able to coassemble with wild-type subunits and suppress their function. When kinetic parameters have been measured, these have in general been found to fall between the values estimated for homomeric wild-type and mutant subunits. Nevertheless, in at least one case, the subtle alterations in kinetics observed when the mutant was expressed alone were completely corrected upon coexpression with wild-type channels (Eunson et al., 2000). It is not possible to explain the disease in this case, except by postulating that other functions of the subunit, not examined in this expression system, were affected, such as assembly with other subunits or interactions with targeting proteins. Interestingly, there appears to be a correlation between the severity of the phenotype and the predicted reduction in potassium flux (Eunson et al., 2000).

How can a reduction in potassium current account for neuromyotonia and ataxia? Neuromyotonia in association with \( KV_{\alpha 1.1} \) mutations is probably caused by failure of repolarization of motor axons, giving rise to repetitive discharges. Episodic ataxia is more difficult to explain. Indeed, although \( KV_{\alpha 1.1} \) is present in Purkinje cell axons, it is also present in the axons of cerebellar basket cells, which inhibit Purkinje cells (Wang et al., 1993, 1994; Veh et al., 1995; Laube et al., 1996; Zhou et al., 1998). Thus, it is not clear from the localization of the channel whether patients with EA1 have an enhanced or a decreased output from the cerebellar cortex. The broad distribution of \( KV_{\alpha 1.1} \) in the brain may account for the increased incidence of epilepsy in kindreds with EA1 (Browne et al., 1994; Comu et al., 1996; Scheffler et al., 1998; Zuberi et al., 1999; Eunson et al., 2000), although at present insufficient clinical information is available to know whether the seizures occurring in EA1 patients originate in mesial temporal structures. Although the channel has also been detected in the heart, skeletal muscle, retina and pancreatic islet cells, there is no evidence that mutations are associated with dysfunction of these organs.

An alternative insight into the consequences of diminished potassium fluxes carried by \( KV_{1.1} \) comes from mice that have had the KCNA1 gene deleted (Smart et al., 1998). Although heterozygotes do not have an obvious phenotype, homozygotes have seizures and shaking attacks at low temperatures caused by repetitive discharges originating in the preterminal region of motor axons (Zhou et al., 1998). Seizures are not a consistent feature of EA1 (Zuberi et al., 1999; Eunson et al., 2000), but the shaking attacks are reminiscent of neuromyotonia, although this is not noticeably temperature-dependent in affected patients. Interestingly, ataxia has not been reported in knockout mice. However, enhanced action potential invasion of cerebellar basket cell axons has been reported, implying that inhibition of Purkinje cells may be enhanced (Zhang et al., 1999). Since Purkinje cells are themselves inhibitory, this would be predicted to result in excessive firing of their target neurones in the deep cerebellar nuclei.

Even if this account of ataxia is correct, a major puzzle remains: why is cerebellar function affected only intermittently? Moreover, how can one explain the triggering of attacks by exertion and startle, and the therapeutic effects of carbamazepine and acetazolamide (Lubbers et al., 1995)? The answers to these questions may await the development of knock-in models of EA1 (i.e. mice where one or both wild-type alleles have been replaced by an allele containing a disease-associated mutation). However, it is worth noting that heterozygous \( KV_{\alpha 1.1} \)-null mice have so far failed to show any obvious abnormalities, implying that subtle derangements of potassium channel function may escape detection in murine models of the disease.

\textit{KCNQ2 and KCNQ3}

\( KCNQ2 \) and \( KCNQ3 \) are two closely related potassium channels that were actually discovered as a result of a search for mutations underlying a rare epilepsy syndrome, which also led to an unexpected breakthrough in understanding neuronal signalling mechanisms.

The autosomal dominant disorder benign familial neonatal convulsions (BFNC) is characterized by brief generalized seizures beginning in the first week of life, usually resolving by 6 weeks (reviewed in Leppert and Singh, 1999). Attacks are frequent, and are both partial and generalized, including tonic posturing, staring, blinking, automatisms and clonic seizures. The EEG is attenuated at the onset, followed by slow waves, spikes and a burst-suppression pattern. The ictal EEG, however, is generally normal. There is an elevated risk of epilepsy later in life, but development and behaviour between seizures are unaffected.

BFNC has a high penetrance, and was linked to two loci on chromosomes 8 and 20 (Leppert et al., 1989). The potassium channel \( KCNQ2 \) was discovered by positional cloning on chromosome 20 (Biervert et al., 1998; Singh et al., 1998), and homology screening then revealed that a closely related channel \( KCNQ3 \) occurs at the locus on chromosome 8 (Charlier et al., 1998). Mutations of both of these channel subunits are associated with BFNC.

\( KCNQ2 \) and \( KCNQ3 \) are homologous with \( KCNQ1 \), mutations of which are associated with two inherited cardiac arrhythmias: autosomal dominant long QT syndrome and
Jervell and Lange-Nielsen syndrome (autosomal recessive long QT with bilateral deafness) (reviewed in Jentsch, 2000). A further member of this new family of potassium channels, KCNQ4, is also associated with inherited deafness. These channels share many structural and functional features with 

Interestingly, they only mediate a very small current depolarization, and do so with relatively small depolarizations. Interestingly, they only mediate a very small current depolarization, and do so with relatively small depolarizations. 

Jervell and Lange-Nielsen syndrome (autosomal recessive when expressed as homotetramers, but when coexpressed, the receptors in the brain (Wang et al., 1998; Yang et al., 1998). They colocalize in the somatodendritic membrane of principal neurones of the cerebral cortex, and can be coimmunoprecipitated, so the two channel subunits are intimately related in vivo (Cooper et al., 2000).

A major surprise was the discovery that KCNQ2/KCNQ3 channels are a major effector mechanism of muscarinic receptors in the brain (Wang et al., 1998). Cholinergic agonists acting through these G protein-coupled receptors inhibit the so-called ‘M current’, a low-threshold, non-inactivating, voltage-dependent potassium current (Brown and Adams, 1980). The current mediated by KCNQ2/KCNQ3 channels has an activation threshold and kinetics similar to the M current, and is suppressed when expressed together with muscarinic receptors (Wang et al., 1998; Selyanko et al., 1999; Shapiro et al., 2000).

Missense, amino acid deletion, splice site and frame-shift mutations (mainly causing truncations of the cytoplasmic C-terminus) and gene deletion have been reported in association with BFNC (Biervert et al., 1998; Charlier et al., 1998; Singh et al., 1998; Lerche et al., 1999; Hirose et al., 2000; Lee et al., 2000; Miraglia del Giudice et al., 2000). Fewer of these mutations have been identified in KCNQ3 than in KCNQ2.

All the mutations that have been studied result in a reduction in current amplitude (Biervert et al., 1998; Schroeder et al., 1998; Lerche et al., 1999). Although some of these mutations result in complete loss of function, the heterozygous situation can only be approximated in vitro by coexpressing the mutant channel (KCNQ2 or KCNQ3) together with its wild-type allele, as well as its wild-type partner. Such experiments have yielded the surprising conclusion that a 25% reduction in current amplitude is sufficient to produce the disease (Schroeder et al., 1998). Dominant negative effects have not been observed. Thus, only a modest reduction in current amplitude uncovers the epileptic phenotype.

Several major questions remain, most notably why seizures resolve spontaneously. Among the possible explanations are that wild-type alleles are upregulated with development, and that other potassium conductances become relatively more effective in preventing abnormal neuronal firing. An insight into the disease mechanism comes from deletion of KCNQ2 in mice. Although homozygous deletion is lethal soon after birth, heterozygous mice develop normally without spontaneous seizures (Watanabe et al., 2000). They do, however, have a lowered threshold to chemoconvulsants.

Interestingly, the novel antiepileptic drug retigabine lowers the activation threshold of potassium currents mediated by KCNQ2/KCNQ3 (Tatulian et al., 2001). By acting on the residual M current, this drug may offer a relatively selective method to correct the disorder in neuronal excitability in the minority of patients who continue to have seizures.

Calcium channelopathies

Cav2.1

The CACNA1A gene on chromosome 19 codes for the 

subunit of the P/Q-type calcium channel, also known as Cav2.1 P/Q-type calcium channels activate rapidly upon strong depolarization and inactivate relatively slowly. The confusing nomenclature reflects the subtly different pharmacological profiles and kinetics of currents originally recorded in cerebellar Purkinje cells (P-type currents) and granule cells (P- and Q-type currents), both of which express 

and 

. The differences result from variable splicing of the same channel subunit (Bourinet et al., 1999). 

is widely expressed not only in cerebellar neurones, but also in the cell bodies and dendrites of hippocampal and other neurones (Westenbroek et al., 1995). P/Q-type channels are also expressed in presynaptic terminals, both in the brain and in the periphery, where they are responsible for a large part of the calcium influx that triggers neurotransmitter release. The subunit forms the voltage sensor and pore of the channel, and is associated with 

and possibly 

accessory subunits, which target and/or anchor it to the membrane, modulate its voltage-dependent kinetics, and play a role in G protein-mediated modulation (Catterall, 2000b).

Three autosomal dominant allelic diseases are associated with mutations of this channel (Ophoff et al., 1996; Jodice et al., 1997; Zhuchenko et al., 1997; Denier et al., 1999; Jen et al., 1999). Familial hemiplegic migraine (FHM) is a severe variant of migraine with aura. It presents in the first or second decade as episodes of severe, generally unilateral migraine, associated with paraesthesia, hemiplegia, hemianopia and/or dysphasia. Some attacks of hemiplegia can last >24 h, and reversible coma has also been reported.

Episodic ataxia type 2 (EA2) tends to present in the second decade, and is associated with disabling attacks of midline cerebellar disturbance, with prominent vertigo, diplopia and nystagmus. Paroxysms are triggered by similar stimuli (emotional or physical stress) as in EA1, but they last longer (several hours) (Gancher and Nutt, 1986). Myokymia, although reported, is not a common feature of EA2. Patients tend to have subtle and slowly progressive interictal cerebellar signs, in particular nystagmus, although it is uncommon for the progressive ataxia to prevent walking without assistance. Cerebellar atrophy, especially of the anterior vermis, can be detected on MRI (Hawkes, 1992; Vighetto et al., 1988). The beneficial effect of acetazolamide is more marked than in EA1 (Griggs et al., 1978). Abnormalities on cerebellar MRI spectroscopy have been
slowly progressive cerebellar degeneration, without the prominent pyramidal signs and other disturbances frequently seen in the other dominantly inherited cerebellar ataxias (Zhuchenko et al., 1997; see review by Klockgether et al., 2000). SCA6 is relatively more common in Japan (Yabe et al., 1998).

Although these three diseases are conventionally described as distinct, they do overlap (Yue et al., 1997; Friend et al., 1999; Denier et al., 2001; Guida et al., 2001). Thus, the combination of FHM and progressive ataxia is recognized (Balogh et al., 1997; Battistini et al., 1999). Conversely, attacks in EA2 have migrainous features in ~50% of cases. Moreover, kindreds have been reported in which different affected members appear to suffer from different syndromes within this spectrum. Additional features such as mental retardation or seizures have also been reported in some EA2 families (Denier et al., 1999; Jouveneau et al., 2001), implying that the boundaries of this disorder may actually stretch beyond hemiplegic migraine and progressive ataxia. A recent surprising finding is the association of CACNA1A mutations with fatal cerebral oedema triggered by minor head trauma (Kors et al., 2001).

Although the distinction between the disorders is blurred, FHM, EA2 and SCA6 are generally caused by distinct defects of CACNA1A (Ophoff et al., 1996; Zhuchenko et al., 1997). FHM is associated with several missense mutations affecting conserved residues (Ophoff et al., 1996; Battistini et al., 1999; Ducros et al., 2001). In contrast, EA2 is generally associated with premature stop codons and splice site mutations, predicted to result in a truncated peptide (Ophoff et al., 1996; Denier et al., 1999). SCA6 is unique among the channelopathies in that it is generally caused by an expansion of a polyglutamine repeat in the intracellular C-terminus of the channel (Zhuchenko et al., 1997). This expansion is relatively small compared with other CAG-repeat disorders, and unlike many of them it appears to be relatively stably transmitted from one generation to the next. The expansion is also in a region that is only present in some splice variants of the gene product. Some kindreds with episodic and/or severe progressive ataxia have been found to carry missense mutations (Yue et al., 1997; Jen et al., 1999; Denier et al., 2001; Guida et al., 2001) and a 3-amino acid insertion has also been identified (M. G. Hanna, personal communication).

Possibly reflecting the phenotypic variability, the functional consequences of the mutations are variable. Surprisingly, different FHM mutations cause either an increase or a decrease in current density when expressed in vitro (Hans et al., 1999). They also cause a variety of alterations in kinetics, although all the mutations that have been examined appear to shift the voltage threshold of activation to more negative potentials (Kraus et al., 1998, 2000; Hans et al., 1999). Although this result implies that the channels open more readily upon depolarization, this is unlikely to result in a greater Ca$^{2+}$ flux in those cases associated with a reduction in current density. It is far from clear how these alterations can explain the episodes of migraine and hemiparesis.

In the case of EA2, the premature stop codons and splice site mutations are unlikely to allow any channel function at all, because the truncations almost always encroach on one or more of the domains of the α$_{1A}$ peptide. Consistent with the hypothesis that EA2 arises from loss of function, a missense mutation associated with this phenotype was also found to produce a non-functional channel (Guida et al., 2001). These data suggest that haploinsufficiency may underlie the EA2 phenotype. As for SCA6, several conflicting alterations in the voltage sensitivity of activation or inactivation have been reported (Matsuyama et al., 1999; Restituito et al., 2000), as well as changes in surface expression of channels (Piedras-Renteria et al., 2001). Part of the difficulty in interpreting these effects is that the polyglutamine expansion is in a region of the C-terminus that is only present in some splice variants. The alteration in channel function may not actually be at the root of the phenotype, because the polyglutamine expansion itself may have a cytotoxic effect akin to that seen in Huntington’s disease. However, intracellular inclusions are not as prominent in SCA6 as in several other neurodegenerative diseases caused by polyglutamine expansions (Ishikawa et al., 2001).

Assuming that EA2 arises from loss of function, an animal model is available. This is the heterozygous α$_{1A}$ knockout mouse, of which two strains exist (Jun et al., 1999; Fletcher et al., 2001). However, no discernible phenotypic abnormalities have yet been reported in these mice, even though homozygous null mutants develop ataxia and dystonia and die prematurely. In one of the strains, the P/Q-type calcium currents in cerebellar neurones are reduced by half in the heterozygotes (Fletcher et al., 2001). This strain could be a close model of the haploinsufficiency thought to underlie EA2. Among the possible explanations why the mice are phenotypically normal is that attacks are too infrequent or subtle to be detected. However, compensatory adjustment in the expression of other calcium channels has been reported in homozygous knockouts, so the consequences of loss of function may be far more complex (Jun et al., 1999). Indeed, upregulation of other calcium currents and consequent neuronal death could conceivably explain the cerebellar degeneration seen in EA2.

To complicate matters further, several recessive mutations of α$_{1A}$ and accessory subunits contributing to Ca$_{v}$.2.1 channels in mice cause not only cerebellar abnormalities and ataxia but also absence seizures (reviewed in Fletcher and Frankel, 1999). Although the ataxia may reflect perturbation of dendritic Ca$_{v}$.2.1 in the Purkinje and granule cells of the cerebellar cortex, absence epilepsy is more likely to reflect the role of this channel in transmitter release. Absence seizures are generally thought to arise as a result of abnormal reverberation in thalamocortical loops, as opposed to an increase in the ratio of excitatory (glutamatergic) versus inhibitory (GABAergic) signalling. Interestingly, a relatively
selective decrease in glutamate release from thalamic synapses has been reported in one of the mutant mouse strains (Caddick et al., 1999).

The observations in mouse strains have made $\alpha_{1A}$ a candidate gene for absence epilepsy. Although absence seizures are not a consistent feature of the known human CACNA1A mutations, EEG abnormalities have been reported in patients with acetazolamide-responsive ataxia (Van Bogaert and Szliwowski, 1996), and polymorphisms in the CACNA1A gene are in linkage dysequilibrium with primary generalized epilepsy (Chioza et al., 2001). A combination of seizures and episodic ataxia has also been tentatively linked to the accessory $\beta_2$ calcium channel subunit (Escayg et al., 2000). Because ataxia in patients with epilepsy is usually ascribed to drug toxicity, its incidence may be underestimated. Recently, we identified an $\alpha_{1A}$ mutation in association with a combination of seizures, mild mental retardation and episodic ataxia. The mutation is a premature stop codon that is predicted to cause a relatively conservative truncation of $\alpha_{1A}$, in that only the C-terminus is lost. However, the mutant subunit was found to be non-functional upon heterologous expression (Jouvenceau et al., 2001). Surprisingly, coexpression with the wild-type allele revealed a dominant negative effect on the calcium current, implying that ion channel mutations may have complex actions on the cellular processing of other membrane proteins. A missense mutation has also been identified in a further family affected by the combination of episodic ataxia and absence epilepsy (M. G. Hanna, personal communication). The functional consequence of this mutation, and the broader role of CaV2.1 in human absence epilepsy, remain to be determined.

As in the case of EA1, the episodic nature of FHM and EA2 and the beneficial effects of carbonic anhydrase inhibitors are difficult to explain.

Sodium channelopathies

beta1

The first sodium channel to be linked to human epilepsy was the $\beta_1$ accessory subunit, coded for by SCN1B on chromosome 19. A missense mutation was identified in a large family affected by a combination of childhood febrile seizures, and febrile and afebrile generalized seizures continuing into adult life (Wallace et al., 1998). A major advance that contributed to this breakthrough was the recognition by Berkovic and colleagues of this novel pleomorphic familial epilepsy syndrome, which was named generalized epilepsy with febrile seizures plus (GEFS+). Although the mutation is transmitted in a dominant manner, the elucidation of this linkage was further complicated by incomplete penetrance. Conversely, febrile seizures occurred among several family members not carrying the mutation, probably reflecting the high background frequency of this condition.

A highly striking feature of this, and subsequently identified GEFS+ families (see below), is the variability of the seizure types affecting different members. These include not only febrile and afebrile generalized tonic-clonic seizures, but also myoclonic, absence and atonic seizures. Some members were also affected by myoclonic-astatic epilepsy, a relatively severe disorder associated with intellectual impairment and drug resistance. It remains unclear whether the variability in phenotype reflects the action of environmental factors or modifying genes.

The mutation identified in the original GEFS+ family disrupts the disulphide bond that holds the extracellular portion of the $\beta_1$ subunit in a loop (Wallace et al., 1998). The wild-type $\beta_1$ subunit associates with sodium channels both in the brain and in skeletal muscle, and a major effect is to accelerate the kinetics of the sodium current, notably the inactivation rate. An additional $\beta_2$ subunit is also associated, although this appears to perform a distinct function. Coexpression of the mutant $\beta_1$ subunit with a brain $\alpha$ subunit revealed it to be non-functional: the sodium current inactivated as fast in the presence of the mutant $\beta_1$ subunit as in the absence of a $\beta$ subunit altogether (Wallace et al., 1998). Thus, loss of function of the $\beta_1$ subunit paradoxically gives rise to a gain of function of the channel, i.e. a prolongation of sodium flux. This result forms a striking parallel with the channelopathies caused by mutations of the skeletal muscle sodium channel. Many of these mutations also cause impaired inactivation, resulting in an abnormally persistent sodium current. Depending on the severity of the impairment of inactivation, this can either cause the muscle fibre to depolarize and become inexcitable (leading to periodic paralysis), or cause it to fire repetitively (resulting in paramyotonia). Whether one or other of these phenomena also occurs in neurones of patients with GEFS+ can only be speculated upon.

Although the $\beta_1$ subunit is expressed in skeletal muscle, neither periodic paralysis nor myotonia occurs in association with GEFS+. The reasons for this are not clear.

NaV1.1 and NaV1.2

Following the discovery of the mutation in the $\beta_1$ subunit, and in the light of the similarities with muscle sodium channelopathies, the $\alpha$ subunits of brain sodium channels became strong candidates for other cases of GEFS+ not linked to the $\beta_1$ subunit. This prediction was vindicated by the discovery of heterozygous missense mutations in SCN1A on chromosome 2, which codes for the NaV1.1 subunit, among several GEFS+ families (Escayg et al., 2000b, 2001; Wallace et al., 2001b). The phenotype is indistinguishable from that linked to the $\beta_1$ subunit, providing a striking example of genetic heterogeneity underlying a single phenotype.

At least three NaV1.1 mutations associated with GEFS+ occur in the voltage sensing transmembrane segments (S4) of the second and fourth domains (Wallace et al., 2001b; Escayg et al., 2001). The functional consequences of these mutations have not yet been reported. However, they are highly likely to
result in impaired inactivation, because closely similar mutations in related sodium channels have this effect.

Another closely related sodium channel α subunit, Na\textsubscript{v}1.2, coded for by SCN2A also on chromosome 2, has also been tentatively linked to primary generalized epilepsy. One heterozygous missense mutation, affecting a highly conserved amino acid in an intracellular loop, was found in a patient with febrile and afebrile seizures, and also caused a slowing of inactivation upon expression in vitro (Sugawara et al., 2001). Interestingly, although Na\textsubscript{v}1.1 and Na\textsubscript{v}1.2 have very similar biophysical properties, their distributions show strikingly different expression patterns, at least in the rodent brain (Gong et al., 1999). These differences are apparent not only among different areas of the brain, but also between cell bodies and axons. Age-dependent changes in expression also differ between the two α subunits. It is therefore perhaps surprising that mutations of the two channels should converge on a similar phenotype. However, epilepsy only affected one of two carriers (Sugawara et al., 2001), so the status of this subunit in primary generalized epilepsy, let alone GEFS+, remains uncertain.

Animal models of GEFS+ caused by missense mutations of sodium channels are not available. However, a gain of function Na\textsubscript{v}1.2 mutation causing a slowing of inactivation has been introduced into transgenic mice, resulting in a severe epileptic phenotype (Kearney et al., 2001). This observation further supports the hypothesis that the seizures in GEFS+ patients arise from an abnormally prolonged sodium current. In the light of this evidence, a recent study of several patients with severe myoclonic epilepsy of infancy (SMEI) presents surprising results (Claes et al., 2001). SMEI manifests in the first year of life with tonic, clonic and tonic-clonic seizures, frequently associated with febrile illnesses. Other seizure types occur later, including myoclonic, absence and partial seizures, akin to a severe form of GEFS+. However, developmental speech and motor arrest occur, as does ataxia, which are not features of GEFS+. The seizures are resistant to medication and life expectancy is severely curtailed. Claes and colleagues reported heterozygous mutations of SCN1A in each of seven patients analysed, most of which were predicted to cause major truncations of the Na\textsubscript{v}1.1 peptide (Claes et al., 2001). They are likely to give rise to a non-functional channel, akin to the suspected disease mechanism in EA2. Does SMEI therefore arise from loss of function of Na\textsubscript{v}1.1? Heterozygous deletion of various sodium channels in mice has not been reported to cause epilepsy (although Na\textsubscript{v}1.1 has not been reported upon). Among alternative possibilities are that the residual peptide perturbs the expression of the wild-type gene product, or that it leads to developmental alterations.

Apart from raising fundamental questions about the mechanisms by which sodium channel mutations lead to epilepsy, an important finding in the study of SMEI was that the mutations were de novo (Claes et al., 2001). Although this is an uncommon phenomenon among the channelopathies, it has been reported for CACNA1A (Jouvenceau et al., 2001). The fact that these severe mutations have not been reported in kindreds probably reflects the severity of the phenotype, which is not compatible with normal lifespan and reproductive success. It may also indicate that SCN1A has a high spontaneous mutation rate.

**Ligand-gated channelopathies**

**GABA\textsubscript{A} receptors**

$\gamma\textsubscript{2}$

The mechanisms underlying GEFS+ have recently taken a further unexpected twist with the discovery of heterozygous missense mutations of the $\gamma\textsubscript{2}$-subunit of GABA\textsubscript{A} receptors in two families affected by a mixture of febrile and afebrile generalized seizures (Baulac et al., 2001; Wallace et al., 2001a). The phenotype in both families again varied widely among different carriers of the mutations within each family, with evidence for incomplete penetrance.

GABA\textsubscript{A} receptors mediate the vast majority of fast inhibitory signalling in the brain, and most receptors at inhibitory synapses are thought to contain the $\gamma\textsubscript{2}$ subunit, coded for by GABRG2 on chromosome 5. This subunit not only contributes to the GABA sensitivity of the receptor, but also helps to target it to the synapse (Essrich et al., 1998). It also affects the sensitivity of the receptor to allosteric modulators such as zinc and benzodiazepines. Although both mutations affected highly conserved residues predicted to lie in extracellular parts of the subunit, they were found to have quite distinct actions on receptor function. One mutation was found to reduce the maximal current evoked by GABA application to *Xenopus* oocytes coexpressing α and β subunits (Baulac et al., 2001). The other mutation, which occurs in a part of the subunit previously implicated in binding benzodiazepines, had no effect on the GABA response, but abolished the sensitivity of the receptor to diazepam (Wallace et al., 2001a). The latter observation is surprising, because it implies that endogenous benzodiazepine receptor agonists (‘endozepines’) may normally play a role in enhancing GABA\textsubscript{A} receptor function: an impairment of this phenomenon because of the presence of a mutant $\gamma\textsubscript{2}$ allele might lower seizure threshold. However, before taking this observation as definitive evidence for the existence and physiological role of endozepines, it will be important to verify that the mutation does not have other effects that could not be detected in the oocyte expression system, such as an impairment of anchoring of the receptor at synapses.

An additional puzzle arising from the association of $\gamma\textsubscript{2}$ mutations with epilepsy is that some affected members had absence seizures (Wallace et al., 2001a). This is difficult to reconcile with the predominant view that 3 Hz spike and wave seizures arise not from a failure of neocortical inhibition, but from abnormally reverberating intrathalamic and/or thalamocortical loops. Potentiation of GABAergic transmission by barbiturates or vigabatrin can actually worsen absence epilepsy, so it is paradoxical that a loss of
function mutation of a GABA<sub>3</sub> receptor should be associated with this type of seizure.

Genetic deletion of γ<sub>2</sub> in mice sheds little light on the mechanism of GEFS+ in association with γ<sub>2</sub> mutations; homozygotes have growth retardation and die by P18, but do not have obvious seizures, while heterozygotes, whose benzodiazepine binding sites are reduced by ~20%, develop normally (Gunther et al., 1995).

**Nicotinic receptors**

α<sub>4</sub> and β<sub>2</sub>

Another receptor implicated in an epileptic channelopathy is the brain nicotinic acetylcholine receptor. This is homologous to the nicotinic receptor at the neuromuscular junction, and has similar biophysical properties. However, the subunit composition of nicotinic receptors in the brain is different—several types of receptor exist: homopentamers composed of α<sub>7</sub>, α<sub>6</sub> or α<sub>9</sub>, and heteropentamers composed of various combinations of α<sub>2-6</sub> and β<sub>2-4</sub> (reviewed in Cordero-Erausquin et al., 2000). α<sub>4</sub> and β<sub>2</sub> coassemble to form the most common heteromeric receptor subtype in the thalamus and cortex (Flores et al., 1992). These receptors have a high affinity for acetylcholine and nicotine, desensitize slowly, and appear to be suited to detect acetylcholine released relatively remotely (‘spillover’ neurotransmission). Exogenous nicotinic agonists enhance electrically evoked neurotransmitter release (McGehee et al., 1995), and such a presynaptic facilitatory effect may be the main function of α<sub>4</sub>β<sub>2</sub> receptors.

A role for the α<sub>4</sub> subunit in epilepsy was demonstrated by the finding that a family with autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) harbours a heterozygous missense mutation in the gene CHRNA4 on chromosome 20 (Steinlein et al., 1995). This is a rare disorder characterized by clusters of seizures occurring during sleep, especially upon falling asleep or waking. Prominent and often violent movements of the limbs occur, often with preserved consciousness. These features have caused the disorder to be misdiagnosed as a paroxysmal dyskinesia, parasomnia or functional disorder (Scheffer et al., 1994). Secondary generalization can occur, and ictal EEG abnormalities point to the underlying process, although these can be overlooked because they are often restricted to the frontal leads and/or concealed by movement artefact. The interictal EEG and brain imaging are usually normal. The disease generally presents in childhood, although there is considerable variability within families, both with respect to the age of onset and to the severity of the seizures. Penetration has been estimated at 75%. The disorder tends to respond to carbamazepine.

Since the original mutation was identified, an insertion mutation and two further missense mutations have been reported in other families (Steinlein et al., 1997; Hirose et al., 1999; Saenz et al., 1999). Mutations of β<sub>2</sub> have subsequently been identified (De Fusco et al., 2000; Phillips et al., 2001).

All of the mutations reported in either α<sub>4</sub> or β<sub>2</sub> occur in the M2 transmembrane domain, which forms the pore of the nicotinic channel. The functional consequences of several of these mutations have been examined. Several distinct effects have been reported for different mutations, including a decrease in maximal current amplitude (Bertrand et al., 1998), an increase (Kuryatov et al., 1997; Bertrand et al., 1998) or decrease (De Fusco et al., 2000) in desensitization rate, and both increases (Steinlein et al., 1997; Phillips et al., 2001) and decreases in apparent acetylcholine affinity (Bertrand et al., 1998). A reduction in calcium permeability has also been reported for two mutations (Kuryatov et al., 1997; Steinlein et al., 1997). Both β<sub>2</sub> mutations appeared to exert a dominant effect when the mutant allele was coexpressed with the wild-type allele in addition to α<sub>4</sub> (De Fusco et al., 2000; Phillips et al., 2001). Finally, one mutation has been shown to cause currents that ‘wind up’ with repeated applications of acetylcholine (Kuryatov et al., 1997). No single alteration in function was seen across all the mutations, and the available data do not point to whether ADNFLE can be thought of as arising from a loss or gain of function.

An indirect argument against a simple gain of function mechanism for ADNFLE is that nicotine, which is a potent agonist of α<sub>4</sub>β<sub>2</sub> receptors, is not a powerful chemoconvulsant. Conversely, targeted deletion of either the α<sub>4</sub> or the β<sub>2</sub> subunit in mice does not lend support to the hypothesis that the disease arises from simple loss of function. These animals have reduced nicotine-induced antinociception, in keeping with the postulated role of these receptors in mediating some of the pharmacological actions of nicotine, but they do not obviously have seizures (Cordero-Erausquin et al., 2000). Overall, these observations suggest that the consequences of human mutations may be more complex. A mechanistic understanding of this disease is still far away. Assuming that the main role of α<sub>4</sub>β<sub>2</sub> receptors is to regulate neurotransmitter release, it will be necessary to introduce the mutations into a system where presynaptic modulation can be studied. And assuming that this approach is successful, it will still be necessary to determine which synapses are preferentially affected in vivo.

**Glycine receptors**

GlyRα<sub>1</sub>

We will finish with the first CNS channelopathy in which a mutation was identified: familial hyperekplexia (FH) (Shiang et al., 1993). This disorder is characterized by an excessive startle reaction, and is usually inherited in an autosomal dominant manner, although several recessive mutations have been identified. The conventional description of the syndrome comes from studying a mixture of familial and sporadic cases, not all of whom have been shown to have mutations of the glycine receptor α<sub>1</sub> subunit. When presenting in infancy it is generally characterized by stiffness, which disappears during sleep, and excessive, non-habituating...
startle responses evoked by sensory or auditory stimuli (Shiang et al., 1993). Reduced intrauterine movements have also been described (Leventer et al., 1995). The startle response consists of a stereotyped sequence of grimacing, neck flexion and arm abduction and flexion, thought to originate in the brainstem reticular formation. In severe cases, myoclonus, and prolonged spasms leading to apnoea and even death have been described (Giacoia and Ryan, 1994). Cases presenting later in life tend to be milder, and patients are generally only affected by excessive startle, although falls and even death have been described (Giacoia and Ryan, 1994). The stiffness and startle responses tend to resolve with age, and the symptoms are often attenuated by clonazepam, other benzodiazepines and sodium valproate. Hyperekplexia is sometimes misdiagnosed as epilepsy or functional disorder.

Several missense mutations of the GLRA1 gene on chromosome 5 have been identified in families with dominantly inherited FH (Shiang et al., 1993, 1995; Milani et al., 1996; Saul et al., 1999) (Fig. 2A). One mutation was associated with FH together with spastic paraparesis, suggestive of some phenotypic heterogeneity associated with the GLRA1 gene (Elmslie et al., 1996). Mutations have also been identified in sporadic cases (Shiang et al., 1995), as well as families showing a recessive pattern of inheritance (Rees et al., 1994). Interestingly, a frame-shift mutation, predicted to delete almost the entire channel subunit, is inherited in a recessive manner (Brune et al., 1996). This implies that the gene product of one wild-type allele is sufficient for normal development and function, and that dominant mutations must therefore cause a gain of abnormal function. The finding of this mutation is also surprising in the light of the mouse mutant oscillator, which also has a frame-shift mutation causing loss of most of GLRA1 (Buckwalter et al., 1994): homozygous mice develop spasms and die by 3 weeks, while the homozygous patient only suffers from a moderately severe form of hyperekplexia (Brune et al., 1996). Recently, a compound heterozygous family was reported (Vergouwe et al., 1999): the unaffected parents were heterozygous for two different, presumably recessive mutations, and two children affected by hyperekplexia inherited the two mutant alleles.

GlyRα1 assembles with β subunits to form the predominant strychnine-sensitive glycine receptor in the spinal cord and brainstem (Fig. 2B). Embryonal glycine receptors are predominantly α2 homomers, implying that dysfunction of α1 may only emerge as the switch-over from α2 to α1β takes place. However, the time course of this phenomenon is not known. Moreover, it is not clear whether the foetal form can persist into adult life under conditions where the α1 receptor is deficient.

Several missense mutations have been studied in vitro, by expressing them alone or together with the wild-type allele (and in some cases with the β subunit). The functional consequences of mutations in the M1–M2 cytoplasmic loop, the M2 segment and the M2–M3 loop are surprisingly similar: they tend to reduce the apparent affinity of the receptors for glycine and the maximum chloride current (Langosch et al., 1994; Lynch et al., 1997; Lewis et al., 1998; Saul et al., 1999). Although some of these mutations are relatively far from the agonist binding site, they probably interfere with the transduction of ligand binding to channel opening. These mutations are all inherited in a dominant manner, implying that the subunits are able to incorporate into functional but defective channels.

We have recently found that two mutations occurring in a family with compound heterozygosity derange subunit function to such a degree that no current is observed when the mutant subunit is expressed alone (R. Rea, M. Tijssen and D. M. Kullmann, unpublished). Upon coexpression with the wild-type allele, the glycine-evoked currents were indistinguishable from those obtained by expressing the wild-type allele alone, implying that the mutants fail to interact with normal subunits. The mutations probably cause such severe perturbations of subunit structure that they fail to assemble into pentamers with the wild-type subunits. Both these mutations occur at the interface between a cytoplasmic loop and a transmembrane segment, so it can be speculated that they prevent normal insertion of the subunit in the membrane. Thus, paradoxically, the more severe mutations are inherited in a recessive manner because they fail to contribute to channels mediating glycineergic inhibition.

Together with GABAA receptors, glycine receptors mediate fast inhibition, especially of motor neurones. Failure of such a feedback regulation may well explain the ‘overflow’ of brief motor discharges that underlie startle reactions.

Other channels and diseases

There are many other channels and channel subunits, of which some are strong candidates for channelopathies yet to be identified. Other than conventional linkage analysis in families showing Mendelian inheritance, what general principles can be used to narrow the search for mutated channels? One potentially fruitful approach is to look for neurological phenotypes in spontaneous mutant strains of inbred mice. If these are linked to ion channel genes, a search for mutations of the same channel in patients with a similar phenotype may be warranted. A potential pitfall is that there may be species differences in the roles of individual channels in brain circuit function. Nevertheless, the discovery of several calcium channel mutations in association with the combination of absence seizures and ataxia in mice (Fletcher and Frankel, 1999) successfully anticipated the association with a similar human phenotype (Escayg et al., 2000a; Jouveneau et al., 2001). Another promising approach is to examine subunits that are homologous to, or associated with, channels mutated in known channelopathies. For example, closely similar phenotypes are associated with mutations of KCNQ2 and KCNQ3, which are known to coassemble, or with the nicotinic receptor α4 and β2 subunits. However, a potential weakness of this approach is that some channel subunits can substitute for one another. Loss of function of
one subunit might therefore be silent if another closely related subunit can take its place. A full explanation of this phenomenon requires the developmental and regional expression of different subunits to be understood.

The above approaches have pointed to some obvious candidates. For instance, the spontaneous murine strain \textit{spasmodic} carries a mutation of glycine receptor \( \beta \) subunit, and has a phenotype reminiscent of FH (Ryan et al., 1994). It remains to be determined whether mutations of this subunit will emerge in patients with this or a similar movement disorder.

Many other murine strains have been created by deliberate manipulation of ion channel genes. Notably, seizures have been elicited by the targeted modification of ionotropic glutamate receptors (Brusa et al., 1995), which in consequence must be candidates for genetic causes of epilepsy.

Although the channelopathies have been discovered by looking for Mendelian inheritance patterns, several mutations of sodium, calcium and glycine receptor channels have been identified in sporadic cases (Shiang et al., 1995; Brune et al., 1996; Claes et al., 2001; Jouvenceau et al., 2001). This raises the possibility that further sporadic mutations will be found, possibly among the vast majority of ion channel genes not hitherto known to be associated with neurological disease.

Many of the channelopathies are associated with epilepsy, either because they have been found to cause rare familial epilepsy phenotypes (BFNC, ADNFLE, GEFS+), or because seizures occur as in association with a complex syndrome (EA1, EA2). Will this breakthrough lead to a better understanding of common sporadic primary generalized epilepsy? Although the vast majority of primary generalized epilepsy does not show Mendelian patterns of inheritance, concordance is elevated among first degree relatives, suggesting a major genetic contribution (reviewed in Gardiner, 1999). Ion channel genes are strong candidates for susceptibility factors that may underlie the complex inheritance of epilepsy. However, susceptibility genes that have been identified for other diseases with similar complex inheritance have generally been distinct from those responsible for rare families showing Mendelian inheritance. For instance, APP (amyloid precursor protein) and presenilin mutations account for very rare dominantly inherited Alzheimer’s disease, but the genes coding for these proteins are not loci for susceptibility factors for the disease in the general population. Nevertheless, the discovery of these mutations has provided powerful support for the amyloid hypothesis, thus yielding an unprecedented insight into the pathogenesis of sporadic Alzheimer’s disease. By analogy, the rare Mendelian epilepsies underline the importance of subtle derangements in ion fluxes in the initiation and propagation of seizures.

It is too early to tell whether the new understanding of disease mechanisms that comes from studying the channelopathies will lead to new therapies. One potential development mentioned above is that retigabine may be an especially effective anti-epileptic treatment in BFNC, because it may act selectively on the residual KCNQ2/3-mediated potassium current (Tatulian et al., 2001). Another important avenue of research is to determine whether carriers of CACNA1A mutations associated with cerebral oedema and coma should be offered special prophylaxis in the event of head injury (Kors et al., 2001).

\textbf{Conclusions}

The field of CNS channelopathies is moving rapidly, and many other mutations and new target channels will no doubt emerge in the next few years. However, there are still major obstacles to be overcome before the mechanisms of these diseases are fully understood. At present, most effort has been devoted to documenting the consequences of identified mutations for the amplitude and kinetics of membrane currents upon heterologous expression. The consequences of the mutations for neuronal function can generally be inferred only by extrapolation; for instance, a decrease in potassium flux is predicted to impair membrane repolarization, and thus render neurones more excitable. This approach can be of relatively little use if the effects of mutations have more subtle effects on ion currents, or if the channels interact with proteins with functions that are difficult to study \textit{in vitro}. For instance, the consequences of missense mutations of Ca\(v\)2.1 for neurotransmitter release are unknown. Indeed, the kinetic alterations that have been documented hitherto may be of little direct relevance to some disease manifestations. It is even more difficult to extrapolate from these results to predict the consequences for the function of neuronal circuits.

A potentially promising approach is to express human disease-associated mutations in mice. Numerous strains of mice with targeted mutations of ion channels have already been generated. However, these have almost exclusively been either knockouts, or mutations designed to test hypotheses about the roles of individual amino acids or regions of the channels, which do not necessarily coincide with human mutations. At the time of writing, no mice have been described where a human disease-associated missense mutation has been introduced, although several laboratories have made progress in this field. Some of the knockouts are arguably disease models for human channelopathies that result from loss of function mutations. Rather disappointingly, the heterozygous mice have tended to have little or no neurological abnormality. As for the homozygous animals, it is difficult to relate them directly to the known channelopathies, because these tend to be dominantly inherited and patients are therefore generally heterozygous. The exception is that one GLRA1 mutation in recessively inherited FH is effectively a knockout, because the frameshift is predicted to disrupt almost the entire subunit (Brune et al., 1996). As mentioned above, the corresponding mouse mutant \textit{oscillator} is rather more severely affected than the homozygous patients.
(Buckwalter et al., 1994), prompting the speculation that individual channel subunits play different roles in different species.

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