ARTICLE

Ion Channels and Epilepsy

HOLGER LERCHE, KARIN JURKAT-ROTT, AND FRANK LEHMANN-HORN*

Ion channels provide the basis for the regulation of excitability in the central nervous system and in other excitable tissues such as skeletal and heart muscle. Consequently, mutations in ion channel encoding genes are found in a variety of inherited diseases associated with hyper- or hypoexcitability of the affected tissue, the so-called 'channelopathies.' An increasing number of epileptic syndromes belongs to this group of rare disorders: Autosomal dominant nocturnal frontal lobe epilepsy is caused by mutations in a neuronal nicotinic acetylcholine receptor (affected genes: *CHRNA4, CHRNB2*), benign familial neonatal convulsions by mutations in potassium channels constituting the M-current (*KCNQ2, KCNQ3*), generalized epilepsy with febrile seizures plus by mutations in subunits of the voltage-gated sodium channel or the $GABA_A$ receptor (*SCN1B, SCN1A, GABRG2*), and episodic ataxia type 1—which is associated with epilepsy in a few patients—by mutations within another voltage-gated potassium channel (*KCNA1*). These rare disorders provide interesting models to study the etiology and pathophysiology of disturbed excitability in molecular detail. On the basis of genetic and electrophysiologic studies of the channelopathies, novel therapeutic strategies can be developed, as has been shown recently for the antiepileptic drug retigabine activating neuronal KCNQ potassium channels.

KEY WORDS: ion channel; epilepsy; genetics; electrophysiology; patch clamp

INTRODUCTION

Epileptic seizures are induced by abnormal focal or generalized synchronized electrical discharges within the central nervous system (CNS). The equilibrium in communication between neurons is regulated by a network of excitatory and inhibitory circuits. Both enhancement of excitatory and impairment of inhibitory mechanisms will disturb this equilibrium, which may result in epileptic discharges. There are two basic mechanisms underlying the electrophysiological excitability of and the communication between neurons: Axonal conduction is mediated by action potentials and signal transduction from cell to cell by synaptic transmission. Since ion channels provide the basis for these processes, any mutation-induced channel malfunction may directly alter brain excitability and can induce epileptic seizures.

Ion channels are membrane-spanning proteins forming selective pores for Na^+, K^+, Cl^-, or Ca^{2+} ions. During action potentials a precise control of ion channel gating is mediated by membrane voltage, during synaptic transmission by the binding of specific neurotransmitters, such as acetylcholine (ACh). With regard to these basic principles, two distinct and structurally conserved classes of ion channels emerged during evolution, the voltage-gated and the ligand-gated channels [Hille, 1992].

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Since these two are the only channel types that so far have been shown to be affected by mutations causing epilepsy, other classes of ionic channels, e.g., those regulated by intracellular ions such as Ca^{2+}, by nucleotides, or by cell volume, will not be considered in this article.

Over the last 10–15 years, the combination of electrophysiological and genetic studies has revealed an increasing number of inherited diseases associated with mutations in ion channel encoding genes. The first of these so-called ion channel disorders or 'channelopathies'

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were found in skeletal muscle, the myotonias and periodic paralyses, caused by mutations in voltage-gated Na^+, Cl^-, or Ca^{2+} channels. Subsequently, several disorders of the CNS, the episodic ataxias, familial hemiplegic migraine, spinocerebellar ataxia type 6, startle disease, and several epileptic syndromes, were identified as belonging to the growing family of channelopathies [Lehmann-Horn and Jurkat-Rott, 1999, 2000; Ptacek, 1999; Cannon, 2000]. The current review will focus on the pathophysiological mechanisms of the epileptic channelopathies in man. We will start with a short overview of the structure and function of voltage- and ligand-gated ion channels, then summarize the clinical, genetic, and pathophysiological concepts of the known epileptic channel syndromes and finally discuss the implications of the general contribution of mutated ion channels to the genetics and therapy of the more common forms of epilepsy.

STRUCTURE AND FUNCTION OF VOLTAGE-GATED CATION CHANNELS

Voltage-gated K^+, Na^+, and Ca^{2+} channels consist of several subunits, a main α-subunit constituting both the gating and permeation machinery of the channel and one or more smaller subunits with modifying functions, called β, γ, or δ. The α-subunits have a common tetrameric structure of homologous domains (I–IV) each with six transmembrane segments (S1–S6). Whereas K^+ channels are constituted by four identical domains, the about fourfold longer genes of Na^+- and Ca^{2+}-channel α-subunits encode four homologous but distinct domains. In all voltage-gated cation channels, the S4 segments contain four to eight positively charged residues conferring voltage dependence to the channel protein and the S5–S6 loops form the major part of the ionic pore with the selectivity filter (Figs. 3, 5, 7).

There are three main conformational states of voltage-gated channels, a closed, an open, and an inactivated state. At the resting potential the channels are in the closed and activatable state. Upon membrane depolarization, the voltage sensors move outward opening the 'activation gate' of the channel on a time-scale of milliseconds by a yet-unknown mechanism, and with sustained depolarization the channels inactivate spontaneously by closing of a different, 'inactivation' gate. Upon membrane repolarization, inactivated channels remain refractory to further openings for a certain period determined by the time needed for recovery from inactivation (Fig. 1). Typical modifying properties of the smaller β-, γ-, or

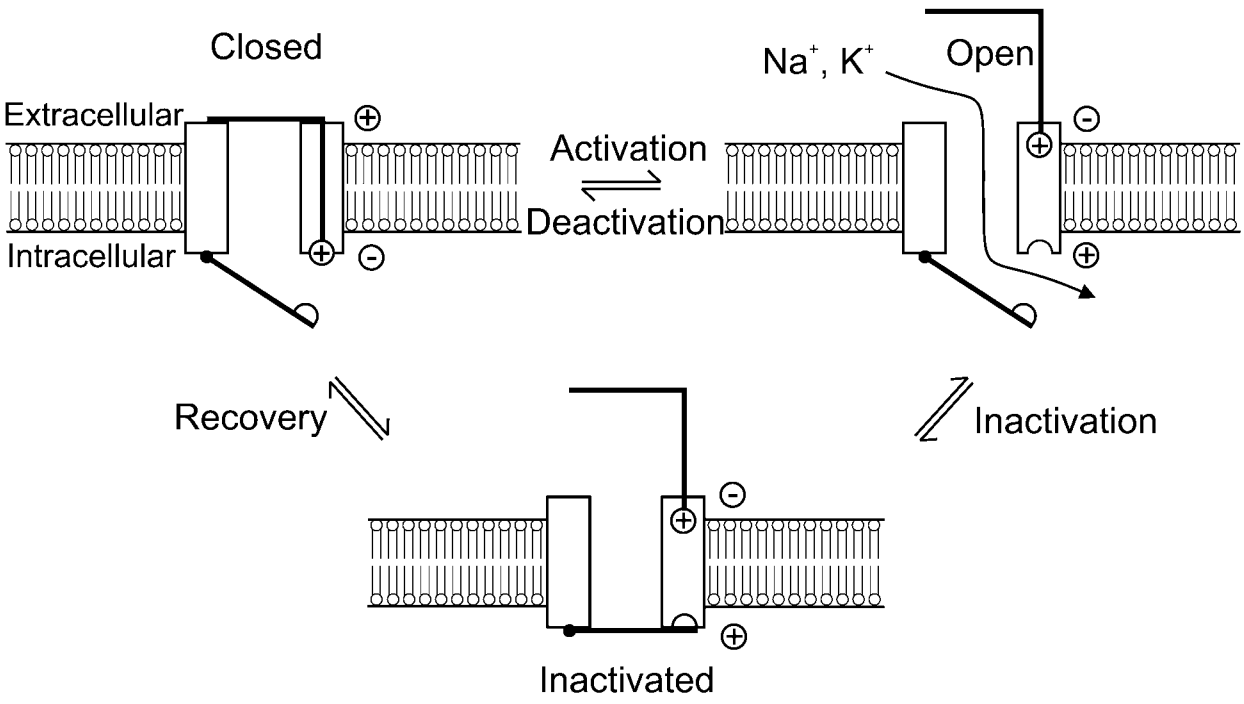

Figure 1. The three main conformational states of voltage-gated ion channels. From a closed state at the hyperpolarized resting membrane potential, channels open upon depolarization during an action potential via outward movement of the voltage sensors that open the activation gate. Some channels, such as the voltage-gated Na^+ channel, inactivate spontaneously via closing of a different, inactivation gate, when depolarization is maintained. From the inactivated state they can only recover upon repolarization of the cell membrane before they are ready for another opening.

δ-subunits are the regulation of the amount of functional protein in the membrane or minor alterations of the kinetics or voltage dependence of channel gating [Lehmann-Horn and Jurkat-Rott, 1999; Catterall, 2000; Siegelbaum and Koester, 2000].

The time course of depolarization and repolarization during an action potential is conveyed by the gating of voltage-dependent Na^+ and K^+ channels: Activation of the Na^+ inward current mediates the steep depolarizing phase, whereas fast inactivation of Na^+ channels and activation of the outward K^+ current are responsible for membrane repolarization. Consequently, disruption of fast Na^+ channel inactivation or a decrease in K^+ conductance leads to slowed or incomplete repolarization of the cell membrane, resulting in hyperexcitability and spontaneous series of action potentials. Both are the most common disease-causing mechanisms in the channelopathies. Main functions of neuronal voltage-gated Ca^{2+} channels are the regulation of transmitter release in presynaptic nerve-terminals.

The different subunits, in particular the channel α-subunits, are expressed tissue specifically. For example, there are several genes encoding different Na^+ channel α-subunits (*SCN1A-SCN11A*) that are expressed in skeletal muscle (*SCN4A*), heart muscle (SCN5A) or neuronal tissue; four of these subunits (*SCN1A, SCN2A, SCN3A,* and *SCN8A*) are considered to be responsible for the sodium current in brain [Goldin et al., 2000]. The tissue specificity explains why there are Na^+ channel disorders with symptoms restricted to skeletal or heart muscle (myotonia or cardiac arrhythmia), or to the CNS (febrile and afebrile seizures). Whereas the relatively few different Na^+ channels are structurally and functionally highly conserved among each other, a large variety of different voltage-gated K^+ channel types with distinct electrophysiological properties is known [Chandy and Gutman, 1995; Lehmann-Horn and Jurkat-Rott, 1999]. For example, there are inactivating (e.g., *KCNA1*) and noninactivating (e.g., *KCNQ1*-5) K^+ channels and large differences in the kinetics of activation and inactivation have been described.

STRUCTURE AND FUNCTION OF LIGAND-GATED ION CHANNELS

Ligand-gated channels are a group of ion channels activated by different neurotransmitters such as acetylcholine (ACh), γ-amino-butyric-acid (GABA), glycine, glutamate, or nucleotides. They are also composed of several subunits, usually four or five. In contrast to the voltage-gated cation channels, all subunits have a similar structure, with two to four transmembrane segments (M1–4, Fig. 2). They form a channel complex with each subunit contributing equally

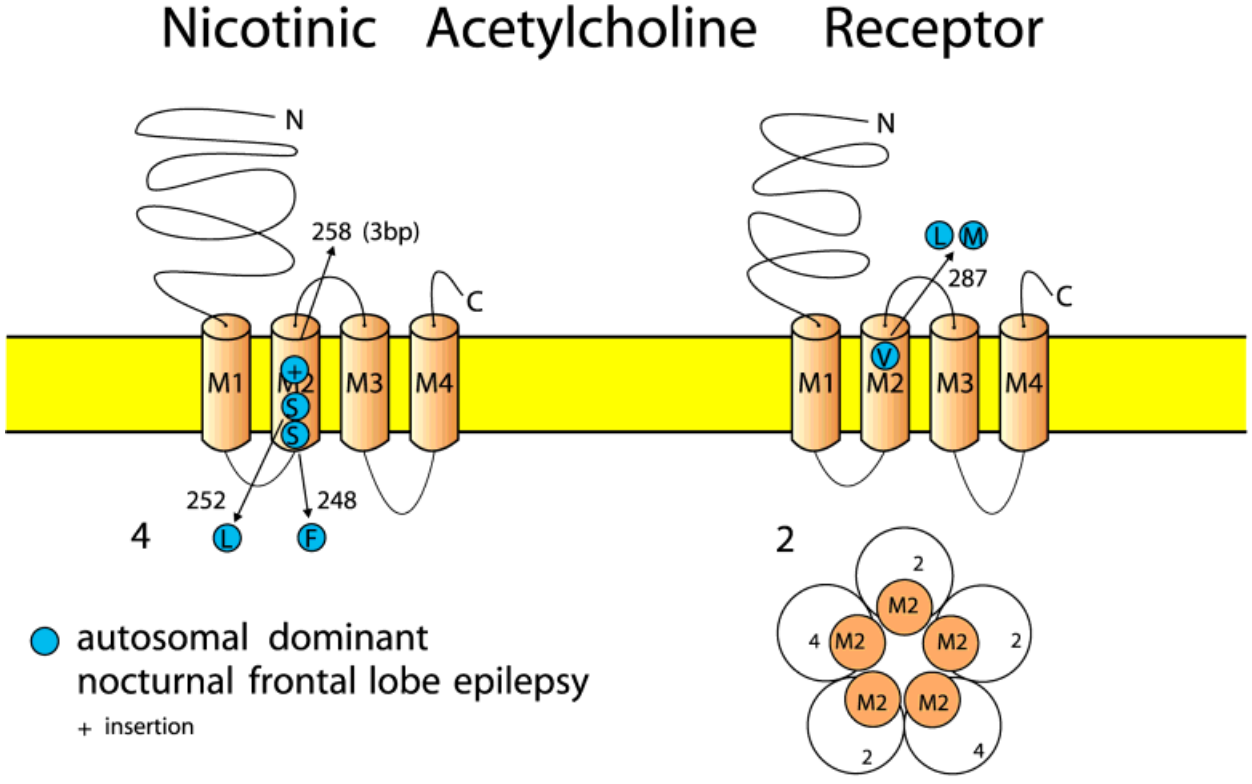

Figure 2. Proposed structure of the nicotinic acetylcholine receptor with mutations found in patients with autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE, see text). Each of the five subunits contains four transmembrane regions (M1–M4), the M2 segments line the channel pore and the long extracellular N-terminal part of the α-subunit contains the binding site for acetylcholine.

to the ion conducting central pore formed by the M2 segments (Fig. 2). The pore is not as selective as in the voltage-gated channels and permeable either to cations, as in excitatory ACh or glutamate receptors, or to anions, such as in inhibitory GABA or glycine receptors.

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The binding sites for transmitters are located in long extracellular loops.

Similar to the voltage-gated channels, there are three main con formational states of the ligand-gated channels: closed, open, and desensitized. Binding of the transmitter opens the channel from the closed state and during constant presence of the transmitter desensitization will occur. Only after removal of the transmitter can the channel recover from desensitization and subsequently be available for another opening [Kandel and Siegelbaum, 2000].

Neuronal nicotinic ACh receptors (nAChR) have a pentameric structure of two α- and three β-subunits (Fig. 2). Eight α-(α_{2-9}) and three β-(β_{2-4}) subunit isoforms are known to be expressed differentially in brain. Most abundantly found in all brain areas are the α_4- and β_2-subunits encoded by the genes *CHRNA4* and *CHRNB2*, which are both affected in autosomal dominant nocturnal frontal lobe epilepsy [Bertrand and Changeux, 1999]. GABA receptors belong to the same family of ligand-gated channels having the same pentameric structure. There are several different subunit classes of $GABA_A$ receptors (α_{1-6}, β_{1-3}, γ_{1-3}, δ, ϵ, π, ρ_{1-3}). The subunit composition most abundantly found in brain is probably $2\alpha_1 2\beta_2 1\gamma_2$ [Mehta and Ticku, 1999; Sieghart et al., 1999].

AUTOSOMAL DOMINANT NOCTURNAL FRONTAL LOBE EPILEPSY (ADNFLE)

This disorder is characterized by frequent brief seizures with hyperkinetic or tonic manifestations occurring typically in clusters at night. Ictal video-electroencephalographic studies revealed partial seizures originating from the frontal lobe. The onset is usually in childhood, inheritance is autosomal dominant, and the penetrance approximately 70–80% [Scheffer et al., 1995; Picard et al., 2000]. ADNFLE has often been misdiagnosed as paroxysmal nocturnal dyskinesia, sleep disorders such as night terrors or nightmares, or hysteria [Scheffer et al., 1994]. In a large Australian family, linkage was found to chromosome 20q13.2 [Phillips et al., 1995] and subsequently a mutation was identified in the gene *CHRNA4* encoding the α_4-subunit of a neuronal nicotinic acetylcholine receptor (nAChR), being the first ion channel mutation in an inherited form of epilepsy [Steinlein et al., 1995]. Two more mutations were found in *CHRNA4* [Steinlein et al., 1997a; Hirose et al., 1999; Phillips et al., 2000] and recently, two groups identified mutations in *CHRNB2* [De Fusco et al., 2000; Phillips et al., 2001], the gene encoding the β_2-subunit of neuronal nAChR, located on chromosome 1. All mutations described so far reside in one of the M2 transmembrane segments lining the ion conducting pore of the ligand-gated channel (Fig. 2).

Functional expression of some of the known mutations in *Xenopus* oocytes or human embryonic kidney (HEK) cells revealed different effects on gating of heteromeric $\alpha_4\beta_2$ channels. The first two studies of the S248F mutation postulated a decrease of the overall channel activity by enhanced desensitization, slowed recovery from desensitization, reduced single channel conductance, and reduced permeability for Ca^{2+} ions [Weiland et al., 1996; Kuryatov et al., 1997]. Further studies of S248F and the 776ins3 mutations also revealed mechanisms that increase the activity of the channel. A use-dependent potentiation to repetitive ACh-expositions, absent in wild-type receptors, was found for both mutations, and the 776ins3 mutation revealed a 10-fold increase in ACh-sensitivity [Steinlein et al., 1997a; Bertrand et al., 1998; Figl et al., 1998]. On the other hand, Ca^{2+} permeability was reduced for both receptors [Bertrand et al., 1998]. First studies of the mutations in the β_2-subunit revealed only functional alterations that enhance channel activity. The V287L mutation showed a profound slowing of desensitization kinetics [De Fusco et al., 2000] and V287M showed a 10-fold increase in ACh-sensitivity [Phillips et al., 2001]. Ca^{2+} permeability for V287L was normal. Thus, pathomechanisms that enhance the activity of the nAChR seem to predominate. A disease-causing hyperactivity of the channel is also supported by a study showing a threefold increase in sensitivity to block by carbamazepine of mutant nAChR, suggesting that the good therapeutic response of ADNFLE patients to this drug is at least in part due to carbamazepine block of the mutant channel [Picard et al., 1999].

ADNFLE has often been misdiagnosed as paroxysmal nocturnal dyskinesia, sleep disorders such as night terrors or nightmares, or hysteria.

How these changes in the electrophysiological properties of the nAChR induce frontal lobe seizures remains to be elucidated. Both the α_4- and β_2-subunits are expressed abundantly in nearly all brain tissues without specificity to the frontal lobe or to projections into this region [Bertrand and Changeux, 1999]. Also, the nocturnal occurrence of the seizures is difficult to explain. Transgenic mice generated with either a knock-out or knock-in of the

α_4-subunit were not reported to develop seizures [Ross et al., 2000; Labarca et al., 2001].

BENIGN FAMILIAL NEONATAL CONVULSIONS (BFNC)

BFNC is a rare dominantly inherited epileptic syndrome characterized by frequent brief seizures within the first days of life that typically disappear spontaneously after weeks to months. Neurological examination, interictal EEG, and development of these children are usually normal. The risk of recurring seizures later in life is about 15%. The penetrance is as high as 85% [Ronen et al., 1993; Plouin, 1994]. The disease was first mapped to the long arm of chromosome 20 [Leppert et al., 1989] and a second locus on chromosome 8 has been described [Lewis et al., 1993].

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Subsequently, mutations in two novel voltage-gated potassium channel genes, *KCNQ2* (20q13.3) [Biervert et al., 1998; Singh et al., 1998; Biervert and Steinlein, 1999; Lerche et al., 1999; Miraglia del Giudice et al., 2000] and *KCNQ3* (8q24) [Charlier et al., 1998; Hirose et al., 2000], have been identified (Fig. 3).

The *KCNQ* gene family encodes delayed rectifier K^+ channels that are mainly expressed in heart muscle (*KCNQ1*), in the CNS (*KCNQ2–5*), the inner ear (*KCNQ4*), and skeletal muscle (*KCNQ5*) [reviewed by Jentsch, 2000]. They are activated upon depolarization of the cell membrane and contribute to the repolarizing phase of the action potential. Mutations in four of the five genes identified cause inherited diseases. *KCNQ1* mutations cause cardiac arrhythmia in the long QT syndrome [Wang et al., 1996], *KCNQ2* and *KCNQ3* mutations cause epileptic seizures in BFNC (see above), and

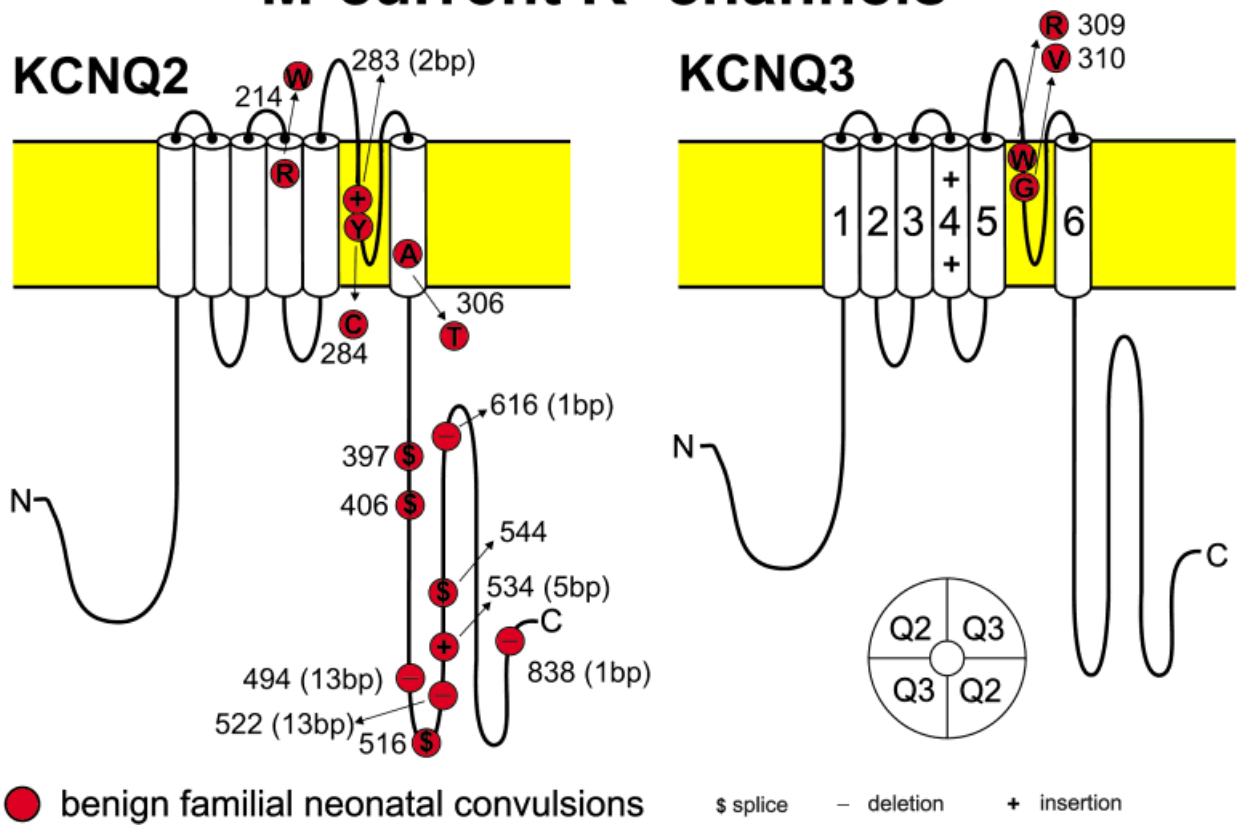

Figure 3. Proposed structure of the voltage-gated K^+ channels *KCNQ2* and *KCNQ3* containing mutations causing benign familial neonatal convulsions (BFNC, see text). The channels are built of six transmembrane segments (S1–S6), the S4 segments containing positively charged residues conferring voltage-dependence to the channel protein and the P-loops between S5 and S6 forming the ion conducting pore. The long cytoplasmic C-terminus is a particular feature of all KCNQ K^+ channels and most probably mediates the formation of heteromeric *KCNQ2/3* channels (see text). Mutations are located within two hot spots: in the pore and in the C-terminus.

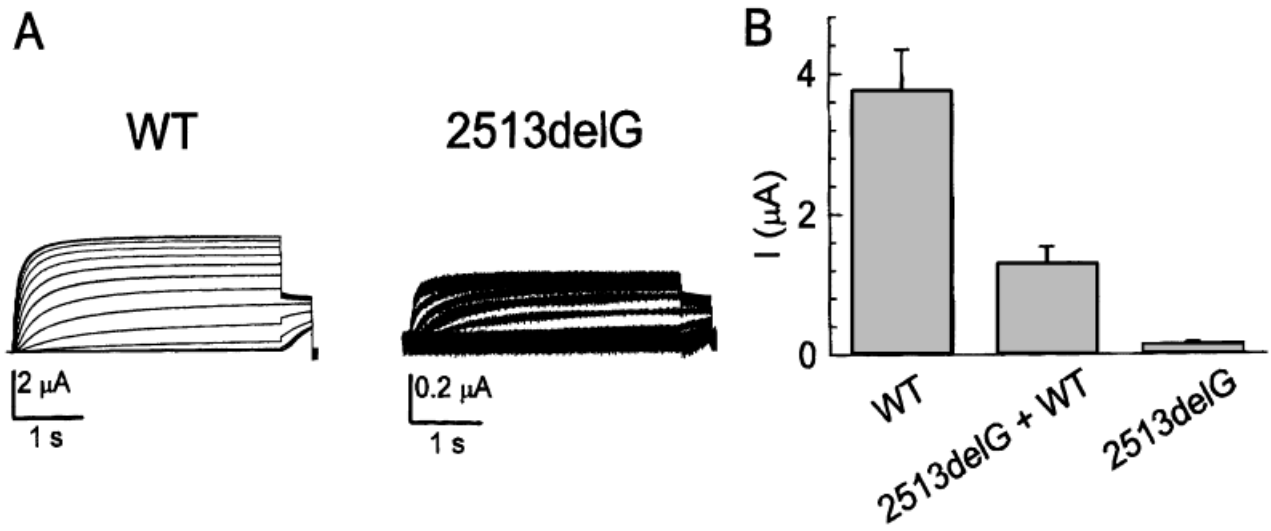

Figure 4. Functional consequences of a BFNC-causing mutation when expressed in *Xenopus* oocytes. The mutation 2513delG is a single basepair deletion located at amino acid (aa) position 838 (Fig. 3), just 7 aa before the regular stop codon. It induces a frame shift, change of the last 7 aa and prolongation by another 56 aa before the new stop codon. This mutation reduces the resulting K^+ current by 20-fold, as seen in the raw current traces in **A** and for the average of many such experiments in **B**. Coexpression of both wild-type (WT) and mutant channels did not result in significantly less than 50% of WT current, suggesting that there was no dominant negative effect of the mutation [modified after Lerche et al., 1999].

KCNQ4 mutations cause congenital deafness [Kubisch et al., 1999]. *KCNQ5* is the only channel in which disease-causing mutations have not been found thus far [Lerche et al., 2000a; Schröder et al., 2000]. Functional expression of the known mutations revealed a consistent reduction of the resulting potassium current in *KCNQ1–4* [Chouabe et al., 1997; Wollnik et al., 1997; Biervert et al., 1998; Schröder et al., 1998; Kubisch et al., 1999; Lerche et al., 1999] (Fig. 4). This leads to an impairment of membrane repolarization, explaining the occurrence of hyperexcitability in the affected tissues.

However, the effects on current reduction were quite different for channels expressed in heart muscle and outer hair cells compared to those expressed exclusively in brain. Whereas *KCNQ1* and *KCNQ4* mutations exhibited strong dominant negative effects on WT channels [Chouabe et al., 1997; Wollnik et al., 1997; Kubisch et al., 1999], *KCNQ2* and *KCNQ3* mutations did not. The latter cause a dominant disease by haploinsufficiency [Biervert et al., 1998; Schröder et al., 1998; Lerche et al., 1999]. Hence, the brain seems to be more sensitive to changes in K^+ conductance inducing hyperexcitability than heart muscle fibers, a fact that apparently applies similarly to the Na^+ channel disorders in muscle and brain (see below).

After the discovery of the neuron-specific *KCNQ2* and *KCNQ3* channels, it was shown that both interact with each other, since the current size of *KCNQ2* is enhanced by about 10-fold upon coexpression with *KCNQ3*, which exhibits only very small currents when expressed alone [Yang et al., 1998]. Both channels most probably constitute the so-called 'M-current,' a neuronal K^+ current known for several decades to play an important role in the regulation of the firing rate of neurons [Wang et al., 1998; Shapiro et al., 2000]. When the in vivo situation for dominant *KCNQ2* and *KCNQ3* mutations was mimicked in vitro by coexpressing, for example, WT and mutant *KCNQ2* with WT *KCNQ3* channels in a 1:1:2 ratio in *Xenopus* oocytes, the reduction in current size was only 20–25% compared to WT *KCNQ2* combined with *KCNQ3* [Schröder et al., 1998]. Thus, as stated above, relatively small changes of the M-current seem to be sufficient to cause epileptic seizures.

Disease-causing mutations in *KCNQ* channels are clustered in two regions of the protein, in the P-loop between segments S5 and S6 constituting the pore region and in the long C-terminus, which is specific for this family of K^+ channels (Fig. 3). The pore mutations should reduce K^+ current by affecting ionic conductance, whereas the C-terminus is most probably responsible for assembly to heteromeric channels. Although the stoichiometry of *KCNQ* channels has not been examined so far, it is well known from other voltage-gated K^+ channels that they assemble to form tetramers. A mutation in the C-terminal part of *KCNQ1* causing Jervell and Lange-Nielson syndrome disrupt assembly of *KCNQ1* channels [Schmitt et al., 2000] and experiments using chimeras between *KCNQ1, KCNQ2*, and *KCNQ3* channels show that the interaction of *KCNQ2* and *KCNQ3* channels is indeed mediated by this region [Lerche et al., 2000b; Maljevic et al., 2001]. Hence, C-terminal mutations probably reduce current size by inhibiting the formation of functional heteromers inserting into the cell membrane. This hypothesis corresponds well to a reduced surface expression of a *KCNQ2* mutant truncating the C-terminus. In contrast, pore mutations in *KCNQ2* and

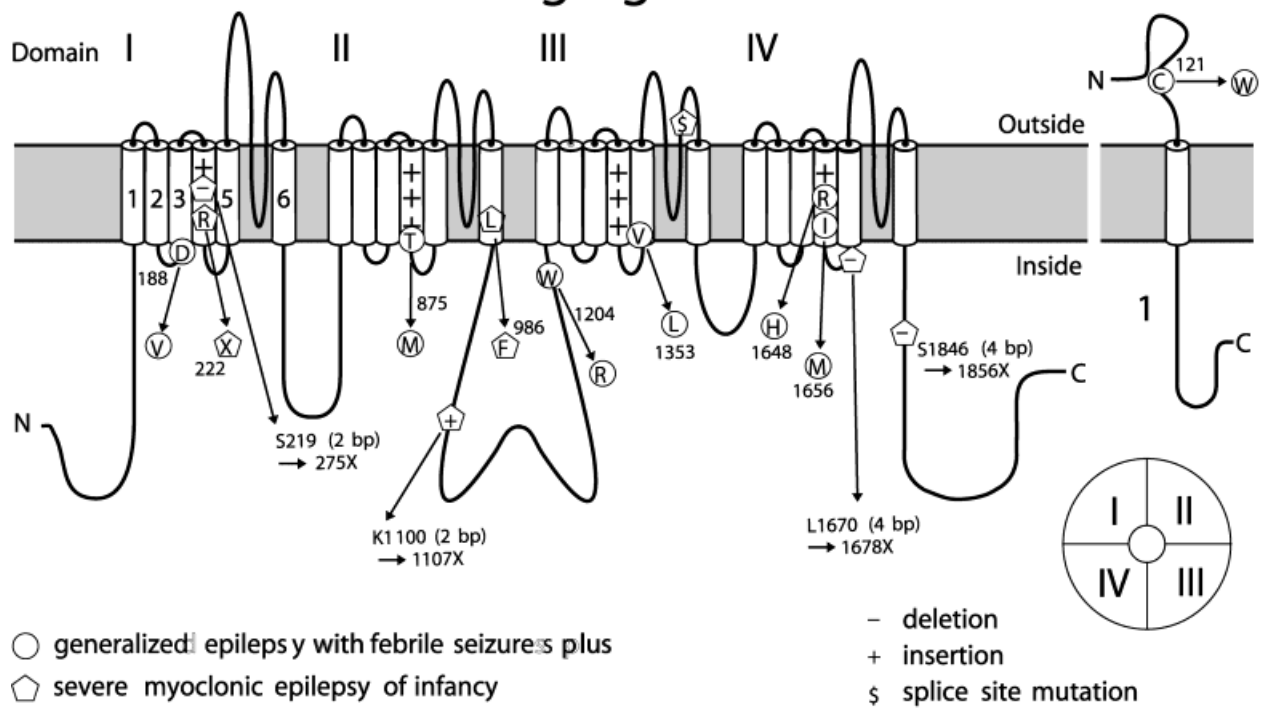

Figure 5. Proposed structure of the voltage-gated Na^+ channel containing mutations causing generalized epilepsy with febrile seizures plus ($GEFS^+$) or severe myoclonic epilepsy of infancy (SMEI) in the α-subunit encoded by the gene *SCN1A* or the β_1-subunit encoded by *SCN1B*. Na^+ channels are built similar to K^+ channels, as shown in Figure 3. They have four highly homologous repeats (I–IV) with six transmembrane segments each (S1–S6) forming a central pore (lower right). The α-subunit mutations that have been functionally characterized so far (Fig. 6) are located in the highly conserved voltage sensors in repeat II and IV, respectively (T875M, R1648H). The β_1-subunit mutation disrupts a disulfide bridge between two cysteine residues in the extracellular loop that is essential for interaction with the α-subunit (see text).

KCNQ3 did not affect surface expression [Schwake et al., 2000].

The question remains why the reduced *KCNQ2/KCNQ3* K^+ current results in seizures preferentially during the neonatal period. One possibility could be that the brain is generally more likely to develop seizures in this premature state than later in life [Swann et al., 1993]. Another explanation might be the differential expression of potassium channels during maturation, which may attribute a dominant role to *KCNQ* channels in central neurons within the first days to weeks of life. Either potassium channels of the *KCNQ* family could be upregulated during this period or other voltage-gated potassium channels could still not have reached their full expression level. Differential expression with reduced expression of *KCNQ3* during the first days of life [Tinel et al., 1998] and expression of a shorter splice variant of *KCNQ2* in fetal brain that attenuates *KCNQ2* and *KCNQ3* channels upon coexpression [Smith et al., 2001] have been reported. However, it remains unclear how these findings contribute to the neonatal seizure phenotype.

GENERALIZED EPILEPSY WITH FEBRILE SEIZURES PLUS ($GEFS^+$) AND SEVERE MYOCLONIC EPILEPSY OF INFANCY (SMEI)

$GEFS^+$ was first described in 1997 and 1999 by the group of Scheffer, Berkovic and colleagues [Scheffer and Berkovic, 1997; Singh et al., 1999] as a childhood-onset syndrome featuring febrile convulsions and a variety of afebrile epileptic seizure types within the same pedigree with autosomal dominant inheritance. Most common was the febrile convulsion syndrome (FS), often with febrile seizures persisting after the sixth year of life or in combination with afebrile generalized tonic-clonic seizures (called 'FS^+'). The phenotypes FS and FS^+ were found in about two-thirds of affected individuals. According to the additional seizure types occurring in the remaining third of the patients, phenotypes such as 'FS^+ with absences,' 'FS^+ with myoclonic seizures,' or 'FS^+ with atonic seizures' were described. The most severe phenotype was myoclonic astatic epilepsy (MAE). Also, partial epilepsies occurred in rare cases ('FS^+ with temporal lobe epilepsy'). The penetrance was about 60%.

Severe myoclonic epilepsy of infancy as first described by Dravet [1978] is characterized by clonic and tonic-clonic seizures in the first year of life that are often prolonged and associated with fever. Later, patients have afebrile generalized seizures such as myoclonic, absence, or tonic-clonic, and also simple and complex partial seizures

occur. Developmental stagnation with dementia occurs in early childhood. In contrast to GEFS$^+$, the syndrome is usually resistant to pharmacotherapy.

The first genetic defect in GEFS$^+$ was found by Wallace et al. [1998]. The authors described linkage to chromosome 19q13 and identified a point mutation within the gene *SCN1B* encoding the β_1-subunit of the voltage-gated Na^+ channel. The mutation predicts substitution of tryptophan for a cysteine residue at position 121 disrupting a disulfide bridge and changing the secondary structure of the β_1-subunit extracellular loop (Fig. 5). This leads to a loss of β-subunit function resulting electrophysiologically in a slight slowing of the inactivation time course of the resulting Na^+ current [Wallace et al., 1998]. Although the β_1-subunit is also expressed in skeletal muscle, interestingly, these patients were not reported to suffer from myotonia like others carrying mutations within the skeletal muscle Na^+ channel α-subunit gene *SCN4A*. Hence, the brain seems to be more sensitive to such changes of excitability than skeletal muscle fibers, or, alternatively, there are different disease-causing mechanisms for both diseases, which is discussed in more detail below.

Subsequently, several groups found linkage to a cluster of genes encoding neuronal Na^+ channel α-subunits on chromosome 2q21-33 and the first two point mutations were detected in *SCN1A* predicting amino acid changes within the voltage sensors (S4 segments) of domains II and IV [Escayg et al., 2000a] (Fig. 5). Recently, several more *SCN1A* mutations have been described [Escayg et al., 2001; Wallace et al., 2001a] (Fig. 5) and there is evidence for further genetic as well as clinical heterogeneity [Lerche et al., 2001].

Heterologous functional expression of the first two *SCN1A* mutations in segments II/S4 and IV/S4 using the highly conserved gene *SCN4A*, human embryonic kidney cells (tsA201), and the whole-cell patch clamp technique revealed only subtle changes in sodium channel fast inactivation and activation and no persistent current [Alekov et al., 2000, 2001] (Fig. 6). The most obvious alteration of the IV/S4 mutant (R1460H) was a threefold acceleration of recovery from inactivation (Fig. 6B), which was also reported in a preliminary study with expression of the mutation in the *SCN1A* gene using *Xenopus* oocytes and two-microelectrode voltage clamping [Escayg et al., 2000b]. In addition, we found little acceleration of the activation time course at potentials more negative than −20 mV for both mutations compared to wild-type channels (Fig. 6C). By shortening the refractory period after an action potential and the time of depolarization needed to elicit an action potential, these alterations would increase membrane excitability.

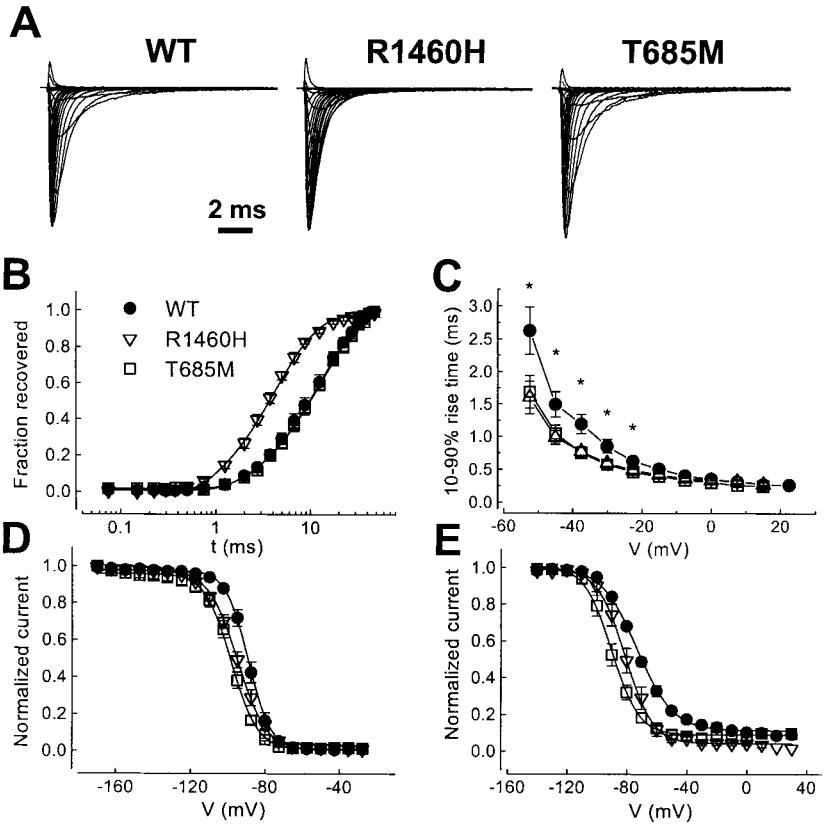

Figure 6. Functional consequences of two *SCN1A* GEFS$^+$-mutations in II/S4 and IV/S4 (T875M, R1648H; Fig. 5). The mutations were introduced in the highly homologous skeletal muscle α-subunit gene *SCN4A* (corresponding mutations T685M, R1460H) and studied in human embryonic kidney cells (tsA201) using the whole-cell patch-clamp technique. **A**: Families of raw current traces show only barely measurable differences in the time course of inactivation and no persistent Na^+ current at the end of the depolarizing test pulses for the mutations compared to wild type (WT) channels. **B**: A strong acceleration of the time course of recovery from inactivation was found for R1460H (shown at −100 mV). **C**: Another significant difference was an acceleration of the time course of activation at potentials more negative than −20 mV, shown as a shortening of the rise time of the whole-cell Na^+ current for both mutations. **D,E**: Steady-state fast (**E**) and slow (**F**) inactivation curves. For both mutations inactivation is enhanced resulting in a loss-of-function and decrease of membrane excitability in contrast to the gain-of-function mechanisms shown in **B,C** [modified after Alekov et al., 2000, 2001].

However, the most obvious difference in gating for the II/S4 mutation (T685M) in comparison to the wild type was an enhancement of both fast and slow inactivation of the channel. The steady-state fast and slow inactivation curves were shifted by −10 or −20 mV, respectively, entry into slow inactivation was accelerated and recovery from slow inactivation significantly slowed. These alterations were also found for the IV/S4 mutation, although less pronounced (Fig. 6D,E) [Alekov et al., 2000, 2001].

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The increase of excitability due to acceleration of recovery from fast inactivation or of the activation time course—which would have to exert its effect on excitatory neurons to explain the occurrence of epileptic seizures—may be to small, in particular for the II/S4 mutation. In contrast, enhancement of both fast and slow inactivation would decrease membrane excitability by reducing the number of available sodium channels. When acting on inhibitory neurons, this effect could be responsible for the occurrence of synchronous activity in neuronal circuits causing epileptic seizures.

These findings are in contrast to the gain-of-function mechanism by a failure of inactivation that has been shown for *SCN4A* mutations causing sodium channel disorders of skeletal muscle, like myotonia and periodic paralysis [Lehmann-Horn and Jurkat-Rott, 1999; Cannon, 2000; Mitrovic and Lerche, 2000]. Nonetheless, such a gain-of-function has also been shown to induce epileptic seizures in a transgenic mouse model in which an *SCN2A* mutation with slowing of the inactivation time course and increased persistent current was introduced. Only 25% of the animals survived beyond 6 months of age; death occurred due to severe status epilepticus [Kearney et al., 2001]. The gating defects of inactivation were much less pronounced than those found for *SCN4A* mutations causing myotonia, suggesting that the CNS reacts much more sensitively to such alterations of excitability than muscle fibers, which seems to apply similarly for K^+ channel defects (see above).

Two recent advances in the genetics of idiopathic epilepsies support the hypothesis that a decrease of excitability of inhibitory neurons is the most important disease-causing mechanism for GEFS$^+$-causing sodium channel mutations. First, only recently mutations in two GEFS$^+$ families were found in the γ_2-subunit of $GABA_A$ receptors. One of these families presented with a typical GEFS$^+$ phenotype (FS and FS$^+$) [Baulac et al., 2001], the other with a frequent combination of FS and absence seizures besides other syndromes described in GEFS$^+$ [Wallace et al., 2001b]. The two mutations are located in different regions of the channel, one in the benzodiazepine binding domain in the N-terminal extracellular loop (R43Q) [Wallace et al., 2001b] and the other in the loop connecting transmembrane segments M2 and M3 (K289M) [Baulac et al., 2001]. Functional expression of the mutant receptor γ_2-subunits together with α_1- and β_2-subunits revealed two distinct gating defects. Whereas mutation K289M reduced GABA-activated currents 10-fold, R43Q revealed normal GABA-activated currents, but abolished the sensitivity to benzodiazepines such that activation by diazepam was no longer present. Thus, both mutations lead to a loss-of-function of $GABA_A$ receptors, although for R43Q it has to be postulated that 'endozepines' do exist and can prevent the development of epileptic seizures in vivo [Wallace et al., 2001b]. For these mutations, undoubtedly a decrease of excitability in inhibitory neurons is the pathophysiological mechanism causing seizures, as it could similarly be explained by enhanced inactivation of sodium channels.

Second, novel mutations were identified in *SCN1A* causing a more severe phenotype than GEFS$^+$, that is SMEI [Claes et al., 2001]. Most of these mutations predict an early stop codon and a truncated protein without function (Fig. 5), with regard to all we know about structure–function relationships of voltage-gated sodium channels [Catterall, 2000]. Therefore, SMEI is a loss-of-function sodium channel disorder caused by haploinsufficiency and, from a genetic point of view, a severe allelic variant of GEFS$^+$.

Finally, the loss of β_1-subunit function by the *SCN1B* mutation could also induce a loss-of-function of the sodium channel, since one of the major effects of the β_1-subunit upon coexpression with the α-subunit is to increase the current amplitude [Catterall, 2000]. Altogether, loss-of-function mechanisms (in inhibitory neurons) seem to predominate and are common to all mutations causing GEFS$^+$ or SMEI.

EPISODIC ATAXIA TYPE 1 WITH MYOKYMIA (AND PARTIAL EPILEPSY)

Another ion channel disorder with disturbed excitability of the CNS is episodic ataxia type 1 with myokymia (EA-1).

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Dysfunction occurs predominantly in the cerebellum. Patients suffer from brief kinesiogenic attacks of gait and limb ataxia or cerebellar dysarthria. Interictally they experience myokymia. In four families, partial epileptic seizures were also reported, occurring in some family members affected by ataxia or myokymia [van Dyke et al., 1975; Brunt and van Weerden, 1990; Zuberi et al., 1999; Eunson et al., 2000]. Zuberi et al. [1999] estimated a 10-fold increased risk to develop epilepsy when affected by EA-1.

Genetic analyses in EA-1 revealed linkage to chromosome 12p13 and mutations within the Shaker homologous gene *KCNA1* encoding the K^+ channel $K_v1.1$ [Browne et al., 1994; Litt et al., 1994] (Fig. 7). Functional expression in *Xenopus* oocytes or mammalian cells resulted in a reduction of the K^+ currents either by diminished expression

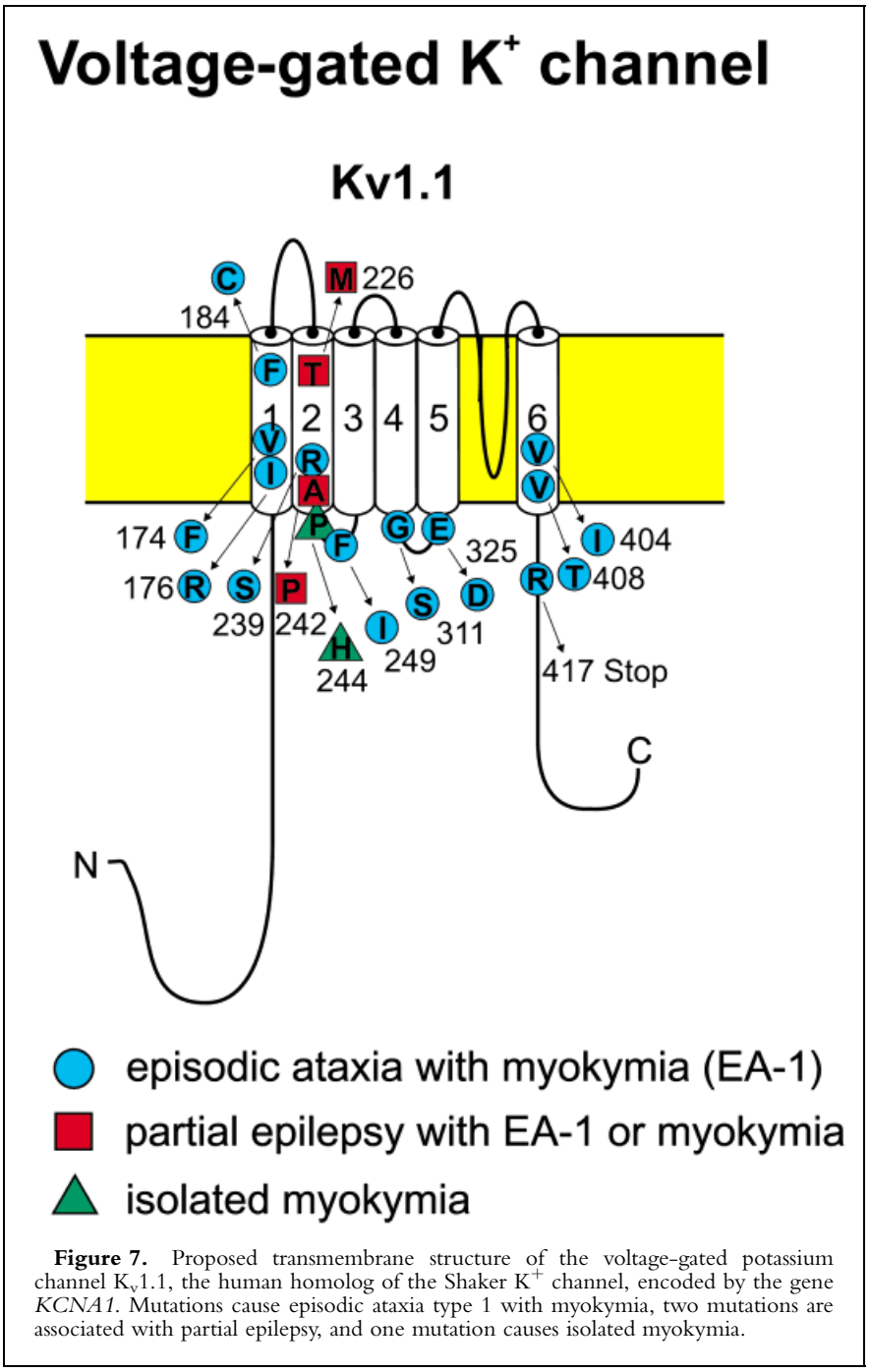

Figure 7. Proposed transmembrane structure of the voltage-gated potassium channel $K_v1.1$, the human homolog of the Shaker K^+ channel, encoded by the gene *KCNA1*. Mutations cause episodic ataxia type 1 with myokymia, two mutations are associated with partial epilepsy, and one mutation causes isolated myokymia.

or shifts in voltage-dependence. According to these studies, both dominant negative effects on WT channels and haploinsufficiency can cause EA-1 [Adelman et al., 1995; Zerr et al., 1998; Bretschneider et al., 1999; Zuberi et al., 1999; Eunson et al., 2000]. A specific defect for those mutations going along with an epilepsy phenotype could not be found [Zuberi et al., 1999; Eunson et al., 2000]. In support of the hypothesis that *KCNA1* mutations can induce epileptic seizures, a knock-out mouse model for $K_v1.1$ presented with an epileptic phenotype [Smart et al., 1998].

ASSOCIATION OF ION CHANNEL DEFECTS WITH COMMON FORMS OF IDIOPATHIC EPILEPSY

Genetic linkage studies in a few large families with a presumably monogenic trait of idiopathic generalized epilepsy (IGE) revealed loci on chromosomes 6p and 15q14 for juvenile myoclonic epilepsy [JME; 6p: Greenberg et al., 1988; Serratosa et al., 1996; Sander et al., 1997; 15q14: Elmslie et al., 1997] and on 8q24 for childhood absence epilepsy [CAE, Fong et al., 1998; Sugimoto et al., 2000]. In two linkage studies using a large number of smaller IGE families, the 8q24 locus was also found, while the 6p locus could not be verified [Zara et al., 1995]; other potential loci were described on 2q36, 3q26, and 14q23; 15q14 was confirmed [Sander et al., 2000]. Significant linkage to the JME locus on chromosome 15 was recently also described for Rolando epilepsy [Neubauer et al., 1998]. Until now, mutations in genes at these locations have not been identified but there are several ion channel or transporter encoding genes that are strong candidates: on chromosome 2q36 the chloride-bicarbonate exchanger gene *SLC4A3,* on 3q26 the voltage-gated K^+ channel β-subunit gene *KCNA1B* [Schultz et al., 1996], and the Cl^- channel gene *CLCN2* [Cid et al., 1995], on 14q24 the Na^+/Ca^{2+}-exchanger gene *SLC8A2* [Li et al., 1994] and on 15q14 the α_7-subunit gene of the neuronal nAChR *CHRNA7* [Chini et al., 1994].

Several ion channel encoding genes were tested in association studies and mutation screenings if the play a role in the genetics of IGE. For *KCNQ2* [Steinlein et al., 1999], *KCNQ3* [Haug et al., 2000a], *KCNJ3* and *KCNJ6* [Girk1 and Girk2: Hallmann et al., 2000], *KCNN3* [hKCa3: Sander et al., 1999], *CACNA1A* [Sander et al., 1998], and *SCN1B* [Haug et al., 2000b], no association could be found. A possible association of a benign polymorphism in *CHRNA4* with IGE [Steinlein et al., 1997b] could not be confirmed in another study [Chioza et al., 2000].

Recently, a mutation was discovered in a patient with juvenile myoclonic epilepsy in the gene *CACNB4,* encoding the β_4-subunit of the high voltage-gated L-type Ca^{2+} channel, and functional studies revealed differences in channel gating for this mutation com-

pared to the WT [Escayg et al., 2000c]. Naturally occurring mutations in different subunits of the same channel complex cause epilepsy with generalized spike and wave discharges in the EEG in several mouse models (Noebels, accompanying article). Mutations in *CACNA1A,* encoding the α-subunit, cause episodic ataxia type II, familial hemiplegic migraine, or spinocerebellar ataxia type 6 in man [Ophoff et al., 1996; Zhuchenko et al., 1997]. Interestingly, the same mutation in *CACNB4* causing JME caused EA-2 in a Canadian family [Escayg et al., 2000c]. It remains to be proven in further studies if *CACNB4* is an 'epilepsy gene' involved in a larger number of IGE families.

IMPLICATIONS FOR THERAPY

The discovery of genetic defects and, in particular, the electrophysiological characterization of mutant ion channels in hereditary forms of epilepsy elucidates pathophysiological concepts of hyperexcitability in the CNS. This knowledge enables new therapeutic strategies by antagonizing the epilepsy-causing mechanisms using the defective proteins as pharmacological targets. In the case of BFNC, a completely novel approach in the treatment of epilepsies emerged from identifying retigabine as an activator of M-currents conducted by *KCNQ2* and *KCNQ3* K^+ channels. Retigabine shifts the voltage dependence of steady-state activation of these channels by about 20 mV in the negative direction so that they are active at the resting membrane potential. This stabilizes the cell membrane via hyperpolarization towards the K^+ equilibrium potential [Rundtfeld and Netzer, 2000; Main et al., 2000a; Wickenden et al., 2000].

It has been shown that for openers of ATP-dependent K^+ channels (K_{ATP} channels) that they reduce hyperexcitability and can reverse paralysis of biopsied skeletal muscle fibers from patients with myotonia or periodic paralysis in vitro by hyperpolarization of the cell membrane [Grafe et al., 1990; Quasthoff et al., 1990; Lerche et al., 1996]. Attempts to treat such patients with K_{ATP} channel openers failed due to intolerable cardiovascular side effects, since K_{ATP} channels are expressed abundantly in heart, smooth muscle cells, and other tissues [Lawson, 2000]. In contrast, *KCNQ2* and *KCNQ3* channels are expressed specifically in neurons and, therefore, side effects of retigabine should be diminished, since it has no effect on *KCNQ1* channels expressed in the heart [Main et al., 2000b]. Clinical trials are currently under way.

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