NEURONAL KCNQ POTASSIUM CHANNELS: PHYSIOLOGY AND ROLE IN DISEASE

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Humans have over 70 potassium channel genes, but only some of these have been linked to disease. In this respect, the KCNQ family of potassium channels is exceptional: mutations in four out of five KCNQ genes underlie diseases including cardiac arrhythmias, deafness and epilepsy. These disorders illustrate the different physiological functions of KCNQ channels, and provide a model for the study of the 'safety margin' that separates normal from pathological levels of channel expression. In addition, several KCNQ isoforms can associate to form heteromeric channels that underlie the M-current, an important regulator of neuronal excitability.

SPLICE VARIANTS
Further forms of a protein derived from alternative processing of its mRNA.

Ion channels form pores that allow the passive diffusion of ions across biological membranes. They have several key functions in nervous system physiology, including the generation and propagation of action potentials. The net flow of electric charge carried by the ions as they move along their electrochemical gradient through the pores leads to changes in the membrane voltage. The rapid propagation of these voltage changes is the basis for the electrical signalling in the brain and other tissues. Ion channels can also change the concentrations of second messengers such as calcium, and are important in the ionic homeostasis of the cytoplasm, of intracellular organelles and of extracellular compartments. The latter function is most obvious in the transport of ions and water across epithelia, but is also important in the homeostasis of the extracellular space between neurons and glia.

Potassium channels are probably the most diverse class of ion channels. For instance, the *Caenorhabditis elegans* genome encodes over 60 potassium channels[1], and there are probably more in mammals. This diversity is enhanced by SPLICE VARIANTS, and by the formation of heteromeric channels, which complicates the study of their individual contributions to the function of the organism.

In humans, the physiological functions of ion channels are sometimes illuminated by genetic diseases in which their role is abolished or modified ('channelopathies')[2]. About ten potassium channel genes are known to be mutated in human disease and, interestingly, four of these genes encode potassium channels of the small KCNQ gene family.

Mark Keating and colleagues[3] identified KvLQT1 (now called KCNQ1) as one of the ion channels underlying the long-QT syndrome (LQTS), a potentially fatal inherited cardiac arrhythmia. Four further related channel proteins (KCNQ2, -3, -4 and -5) have yielded surprising insights in the past few years. Mutations in KCNQ genes underlie diverse diseases, and cause electrical hyperexcitability in cardiac arrhythmia[3] and epilepsy[4–6], defects in transepithelial transport in congenital deafness[7] and probably cell degeneration in progressive hearing loss[8].

KCNQ1 has different functions in different tissues: when associated with the β-subunit KCNE1, it repolarizes cardiac action potentials[9,10], and provides a pathway for transepithelial potassium secretion in the inner ear[7,11]. Depending on the magnitude of the resulting loss of function, mutations in either subunit can lead to cardiac arrhythmia in the dominant LQTS, also called Romano–Ward syndrome (RWS), or, with a more severe loss of function, to the recessive Jervell and Lange-Nielsen syndrome (JLNS), in which cardiac arrhythmia is associated with congenital deafness. With

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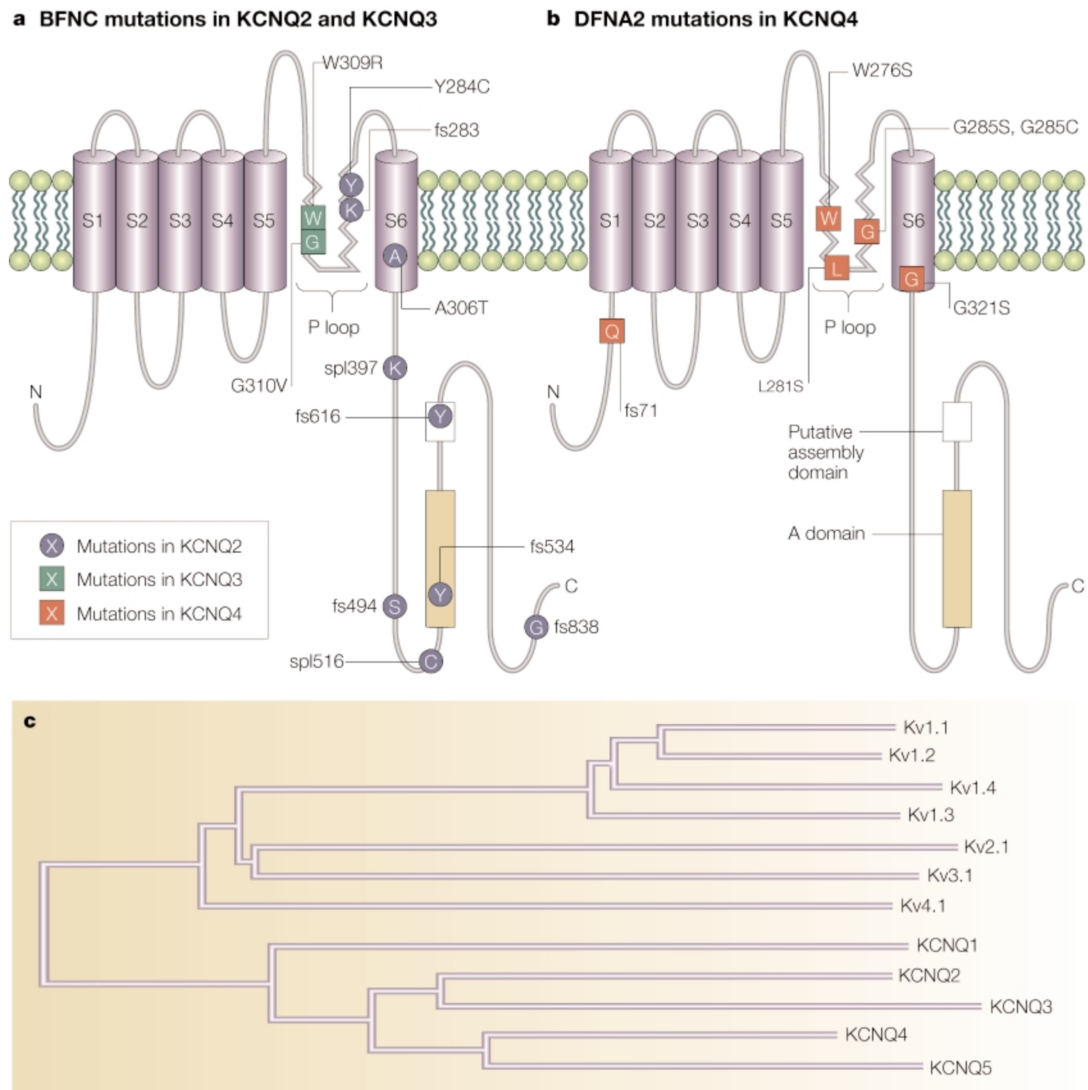

Figure 1 | **The structure of KCNQ channels. a** | Basic structure of KCNQ2/3 proteins and mutations leading to BFNC. KCNQ proteins have six transmembrane domains (TMDs) and a pore-forming P-loop. Mutations found in KCNQ2 (blue circles): Y284C, A306T, fs283, fs494 and spl516 are described in REF. 5, spl397 in REF. 39, fs534 in REF. 4, fs616 in REF.38 and fs838 in REF. 37. Numbering of KCNQ2 residues is according to REF. 4. KCNQ3 mutations (green squares): W309R is described in REF. 40 and G310V in REF. 6. Several mutations truncate the channel before or in the A domain or the putative assembly domain, shown as beige and white boxes respectively. **b** | KCNQ4 mutations (red squares) identified in people with progressive dominant hearing loss DFNA2. The G285S mutation is described in REF. 8, and Fs71, W276S, G285C and G321S were reported in REF. 72. With the exception of Fs71, these mutants exert dominant-negative effects. The L281S mutation[73] has not been tested functionally. The equivalent tryptophan residue is mutated in KCNQ3 (W309R) and KCNQ4 (W276S), as well as in KCNQ1 in JLNS (W305S)[74], indicating that it has no strong dominant-negative effect. G285 is the first glycine of the GYG pore signature sequence, which is also mutated in KCNQ1 in the dominant long-QT syndrome (G314S)[75] and suppresses wild-type KCNQ1 currents[74,76]. Fs, frameshift mutation; spl, splice site mutation. Both types of mutations are expected to truncate the protein. **c** | Dendrogram of KCNQ and selected Kv channels.

a different subunit (KCNE3), KCNQ1 recycles potassium at the basolateral membrane of intestinal crypt cells, a process that is required for intestinal chloride secretion[12].

The observation[13] that KCNQ2/KCNQ3 HETEROMERS are a molecular correlate of the M-current has caused further interest in these channels. This current, which was first described 20 years ago[14], is involved in determining the subthreshold excitability of neurons and their responsiveness to synaptic input. It is subject to a complex and poorly understood regulation by several neurotransmitters. The importance of M-channels is illustrated by the fact that a 25% decrease in M-current leads to neonatal epilepsy in humans[15], and that a disruption of *kcnq2* in mice is lethal[16]. The recent identification of KCNQ5 (REFS 17,18), which may form functional heteromers with KCNQ3 in the central nervous system (CNS) and in peripheral ganglia, indicates that M-type channels may be more diverse than previously expected. This provides opportunities to develop specific drugs that may be useful for the treatment of epilepsy and other neurological disorders.

HETEROMERS
Channels formed by the assembly of two or more different subunits.

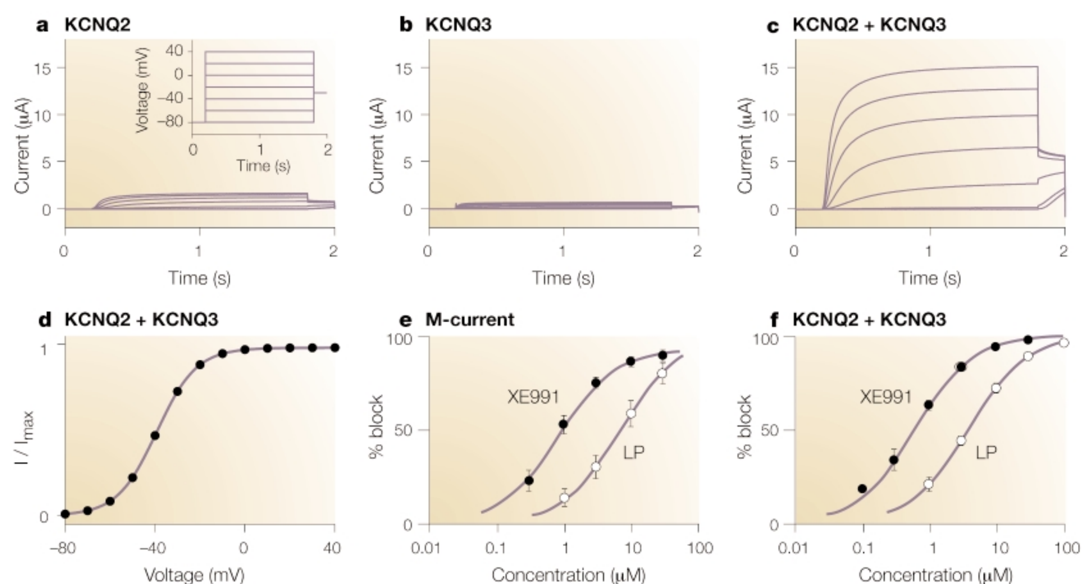

Figure 2 | **KCNQ2/KCNQ3 heteromers yield currents with the properties of the M-current. a–c** | Two-electrode voltage-clamp traces of homomeric KCNQ2 (**a**), KCNQ3 (**b**) and KCNQ2/KCNQ3 (**c**) co-injected at a 1:1 ratio. The voltage-clamp protocol is shown in the inset of **a**. Note the large increase in current observed upon co-expression. **d** | Voltage-dependence of steady-state KCNQ2/KCNQ3 currents in *Xenopus* oocytes. **e**, **f** | Sensitivity of native M-current (**e**) and of co-expressed KCNQ2/KCNQ3 (**f**) to the inhibitors XE991 and linopirdine (LP). Data in **a–c** are from Schroeder and T.J.J., unpublished observations. Similar effects were reported in REFS 13,15,27. Panels **e** and **f** are from REF .13.

KCNQ4 can also associate with KCNQ3 and yield M-type currents, but its expression pattern is much more restricted. It is prominently expressed in sensory hair cells in the inner ear[8], and in certain tracts and nuclei of the central auditory pathway[19]. Mutations in *KCNQ4* lead to a slowly progressive, dominant hearing loss[8].

KCNQ1 and its role in the LQTS have been discussed in recent articles[2,20–23], so this review will focus on the neuronal isoforms.

Structural features of KCNQ channels

KCNQ proteins have six transmembrane domains and are structurally related to Kv potassium channels (FIG. 1a). The degree of homology between different KCNQ proteins is less than that observed within Kv family branches (for example, within Kv1 channels; FIG. 1c). Like other Kv channels, KCNQ subunits have a single P-loop that forms the selectivity filter of the pore (in four copies provided by four subunits), a positively charged fourth transmembrane domain (S4) that probably acts as a voltage sensor, and intracellular amino and carboxy termini. The C terminus is quite long, and contains a conserved domain (the 'A domain'[24]) closely followed by a short stretch thought to be involved in subunit assembly, at least in KCNQ1 (REF. 25). The A domain is highly conserved between different KCNQ proteins and may be a distinctive feature of the family.

Although it has not been shown directly, four KCNQ subunits probably combine to form functional potassium channels. All five known KCNQ proteins can form HOMOMERIC CHANNELS *in vitro*, and the formation of heteromers seems to be restricted to certain combinations[8,15,17,18]. KCNQ1 may not be able to form heteromers with KCNQ2–5, but KCNQ3 can associate with KCNQ2, -4 and -5. Additionally, KCNQ1 can interact with KCNE1 (REFS 9,10) (originally called IsK or minK) or with KCNE3 (REF. 12), small proteins with a single transmembrane domain. Both interactions lead to distinct changes in channel gating: the interaction with KCNE1 enhances current amplitudes, and slows activation[9,10]; in contrast, KCNE3 nearly abolishes KCNQ1 gating, leading to constitutively open channels[12]. Similar interactions have not been found with the neuronal isoforms yet. The only exception is the marked inhibition of KCNQ4 currents by KCNE3 (REF. 12). The physiological relevance of this effect is unclear.

KCNQ2, KCNQ3 and KCNQ5

KCNQ2 and KCNQ3 in neonatal epilepsy. KCNQ2 and KCNQ3 were identified by homology to KCNQ1 (REFS 4,15), and also by POSITIONAL CLONING in families with benign familial neonatal convulsions (BFNC), a neonatal form of epilepsy[4–6]. Both subunits are expressed mainly in neuronal tissue[4,15,26,27], including sympathetic ganglia[13], and their expression patterns in the brain overlap extensively[4,15,26–28]. However, *in situ* hybridization indicates that they are not always expressed in the same ratio[15], and immunocytochemistry has shown that some neurons stain only for one or the other subunit[28]. Both KCNQ2 and KCNQ3 can form homomeric potassium channels when expressed alone[13,15,27], but currents are much smaller with KCNQ3, at least in oocytes[13,15] (FIG. 2a,b). Like KCNQ1, both KCNQ2 and KCNQ3 yield potassium currents that activate slowly on depolarization. There are several splice variants in the C terminus of KCNQ2 (REFS 4,26,29), but their relevance is unclear.

HOMOMERIC CHANNELS
Channels formed by several copies of a single subunit.

POSITIONAL CLONING
A strategy for identifying a gene associated with a genetic disease in which the phenotype is correlated with a chromosomal site and the DNA cloned and compared with that in normal individuals.

Mutations in either *KCNQ2* or *KCNQ3* can cause the same phenotype (BFNC), and the expression of both subunits overlaps extensively, so they may combine to form a single channel. Expression of both subunits leads to larger currents[13,15,27] (FIG. 2a–c) with slightly changed gating kinetics and sensitivity to inhibitors[13,30]. Furthermore, a KCNQ3 mutant modelled on a dominant-negative KCNQ1 mutation found in RWS inhibited KCNQ2 currents in a DOMINANT-NEGATIVE fashion[15], and KCNQ2 and KCNQ3 could be precipitated together from human brain extracts[28]. However, *in vitro* expression did not reveal a functional interaction with KCNQ1 (REF. 15), indicating that KCNQ channels do not mix indiscriminately. Although Yang *et al.*[27] found that KCNE1 slowed KCNQ2/3 currents and decreased their magnitude, Schroeder *et al.*[15] did not detect significant effects when KCNE1 was co-expressed at levels that suffice to alter KCNQ1. An interaction of KCNQ2/3 with either KCNQ1 or KCNE1 would also seem physiologically irrelevant as neither has been detected in the CNS.

The increase in macroscopic currents observed on co-expression of KCNQ2 and KCNQ3 is predominantly caused by increased surface expression[24]. Co-expression causes an increase in overall protein levels in transfected HEK293 cells[28], but not in *Xenopus* oocytes[24]. Single-channel and NOISE ANALYSIS indicated that homomeric KCNQ2 and KCNQ3 channels have conductances of roughly 18 and 7 pS, respectively. Co-expression did not increase the single-channel conductance, which varied between 8 and 22 ps. This variation suggested the formation of heteromers with different stoichiometries. Like KCNQ1/KCNE1 currents[31], KCNQ2/KCNQ3 currents can be increased by the cyclic AMP-dependent protein kinase A (PKA). This activation requires a PKA consensus site at the N-terminus of KCNQ2 (REF. 15). Indeed, KCNQ2 and KCNQ3 co-purify with the RIIB subunit of PKA when they are immunoprecipitated from human brain extracts[28].

BFNC results from a loss of KCNQ2/KCNQ3 currents. Although epilepsy has a large genetic component, only rare specific syndromes are single-gene disorders[32,33]. In view of the crucial role of ion channels in neuronal excitability, it is not surprising that many of these monogenic forms result from mutations in ion channel genes[32]. One of these rare forms is BFNC, an autosomal dominant idiopathic epilepsy characterized by unprovoked partial or generalized clonic convulsions, sometimes with ocular symptoms and apnoea[34]. BFNC typically begins around three days after birth, and remission occurs after 3–10 weeks. About 15% of patients have seizures later in life.

BFNC was linked to two different loci (20q13.3 (REF. 35) and 8q24 (REF. 36)), which are now known to harbour the *KCNQ2* and *KCNQ3* potassium channel genes, respectively[4–6]. There are about ten known mutations in KCNQ2 (REFS 4,5,37–39), but only two in KCNQ3 (REFS 6,40) (FIG. 1a). They include MISSENSE, FRAME-SHIFT, and SPLICE-SITE mutations and, in one family, the KCNQ2 gene is deleted on one chromosome[5]. A truncating[4] and several missense[15] mutations have been tested in *Xenopus* oocytes for dominant-negative effects on co-expressed wild-type subunits. None of these yielded currents by themselves or exerted dominant-negative effects, indicating that the dominant inheritance of BFNC is due to HAPLOINSUFFICIENCY. Currents from heteromeric KCNQ2/KCNQ3 channels were estimated to be reduced by only 25% in people suffering from BFNC[15]. A reduction of the same magnitude is also predicted[15] for people with deletions of one *KCNQ2* allele[5]. If KCNQ2 and KCNQ3 need a C-terminal domain for their assembly, as suggested for KCNQ1 (REF. 25), the lack of dominant-negative effects with truncating mutations[4] could be explained by a failure to assemble with other subunits.

A reduction of roughly 25% in KCNQ2/KCNQ3 currents is sufficient to increase neuronal excitability to epileptogenic levels in early infancy[15]. This small safety margin may be related to the physiological importance of increases in neuronal excitability resulting from neurotransmitter-mediated inhibition of the 'M-channel' KCNQ2/KCNQ3. The high degree of sensitivity required for this tuning of excitability has the drawback that slightly greater inhibition leads to epilepsy. So far, no dominant-negative mutations have been identified in BFNC, leading to the speculation[15] that such mutations would result in a more severe phenotype. The homozygous deletion of *Kcnq2* in mice leads to death a few hours after birth because of inability to breathe properly[16]. Heterozygous animals develop normally and lack spontaneous epileptic activity, but are more susceptible to pentylenetetrazole-induced seizures[16]. So *Kcnq2*$^{+/-}$ mice may be a valuable model in which to study the effects of a slight reduction in currents mediated by KCNQ2.

Explaining why seizure activity in BFNC generally stops several weeks after birth[34] will require detailed knowledge of the neuronal populations that are responsible for the initiation and propagation of seizure activity. The expression of ion channels and transporters, neurotransmitter receptors, and synaptic connections are still subject to developmental changes during this period, as may be the regulation and expression of M-channels. In the mouse brain, KCNQ3 expression needs more time than KCNQ2 to reach steady-state levels[26], but this observation alone cannot explain the time course of human BFNC.

It was hoped that the genes mutated in BFNC might also be important in POLYGENIC forms of generalized epilepsy, especially as some BFNC patients are prone to seizures later in life. However, an initial study[41] indicates that this may not be the case.

KCNQ2/KCNQ3 heteromers and neuronal M-currents. The M-current was first described by Brown and Adams[14] in frog sympathetic neurons as a non-inactivating potassium current that could be slowly activated by depolarization and was inhibited by muscarinic stimulation (hence its name M-current). It was subsequently found in many central and peripheral neurons and shown to be modulated by an array of receptor types[42]. Many types of second messengers have been implicated in this regulation, including G proteins, intracellular

DOMINANT NEGATIVE
A mutant protein that interacts with the normal form and blocks its function.

NOISE ANALYSIS
A technique in which the random fluctuations in membrane potential are used to give information on the properties of single channels.

MISSENSE MUTATION
A mutation in which an incorrect amino acid is incorporated into the protein.

FRAME-SHIFT MUTATION
The addition or deletion of a nucleotide such that the protein sequence from that point onwards is altered.

SPLICE-SITE MUTATION
A mutation at the site where the mRNA is processed to generate protein variants.

HAPLOINSUFFICIENCY
Loss of one copy (one allele) of a gene is sufficient to give rise to disease. Haploinsufficiency implies that no dominant-negative effect of the mutated gene product has to be invoked.

POLYGENIC
A characteristic controlled by different genes, each of which have only a small role in the phenotype.

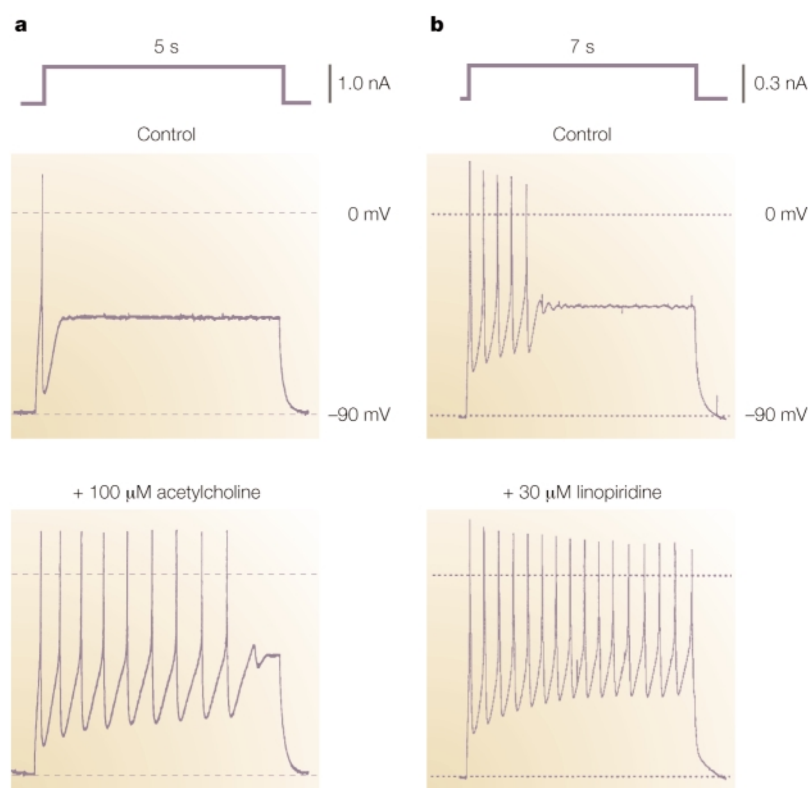

Figure 3 | **Inhibition of neuronal M-currents leads to hyperexcitability. a** | NG108-15 cells that were transfected with an M1-receptor are depolarized by injecting a constant current (shown at top). Under control conditions, this leads to a single action potential. However, if M-currents are inhibited by applying acetylcholine (below), a train of action potentials is observed. NG108-15 cells have endogenous M-currents, and express KCNQ2 and KCNQ3 (REF. 49), as well as KCNQ5 (REF. 17). Unpublished data from J. Robbins and D. A. Brown (for methods, see REF. 77). **b** | The inhibition of endogenous KCNQ M-currents of NG108-15 cells by linopirdine also leads to neuronal hyperexcitability. (Adapted from REF. 49.)

calcium, arachidonic acid, tyrosine kinases and cyclic ADP-ribose. Despite extensive efforts, the regulatory mechanisms are still poorly understood.

The modulation of M-currents has profound effects on neuronal excitability, because M-currents are the only sustained current in the range of action potential initiation. Their slow activation and deactivation is important for their function as a brake for repetitive action potential firing. The inhibition of M-currents therefore leads to enhanced neuronal excitability (FIG. 3).

The molecular identity of M-channels was obscure until David McKinnon and colleagues[13] showed that currents induced by KCNQ2/KCNQ3 heteromers resembled the gating kinetics and pharmacology of M-currents. Linopirdine and XE991, specific inhibitors of M-currents[43], blocked KCNQ2/KCNQ3 channels with similar affinity (FIG. 2e,f). Furthermore, both subunits were shown[13] to be expressed in sympathetic ganglia, which are known to express M-currents. KCNQ2, KCNQ3 and heteromeric KCNQ2/3 channels could be modulated by muscarinic stimulation when expressed with M1-receptors in mammalian cells[44,45]. The cloning of an M-channel now provides the opportunity to dissect the mechanisms involved in M-channel activation. Unfortunately, however, the related channels KCNQ1, KCNQ4 and KCNQ5 are also inhibited by M1-receptor stimulation[17,45], precluding simple chimaeric strategies to identify regulatory domains. Whereas the enhancement of KCNQ2/3 currents by PKA is well understood, activation of M-currents by cAMP has been described in smooth-muscle cells[46] but only sketchily in central neurons.

Mediation of M-currents by KCNQ2/KCNQ3 fits perfectly with the role of KCNQ2 and KCNQ3 in epilepsy. M-current modulators may therefore be important in treating this disease. But inhibitors of M-currents such as linopirdine and XE991, which may be useful as cognition enhancers[43,47], could cause epilepsy at higher dosages. Interestingly, the anticonvulsant retigabine activates KCNQ2/KCNQ3 channels by shifting their voltage-dependence to more negative voltages[48]. This convergence of human genetics and pharmacology shows that KCNQ2 and KCNQ3 are suitable targets for the development of new drugs for the treatment of epilepsy.

However, it seems that KCNQ2/KCNQ3 is not the only channel that yields M-type currents. *Eag*-related channels also give currents that resemble those from M-channels, but opinions differ as to whether they really qualify as such[49,50]. More importantly, other KCNQ subunits such as KCNQ4 and KCNQ5 yield currents that kinetically resemble M-currents and can also be inhibited by M1-receptor agonists[17,45].

KCNQ5 increases M-type potassium channel diversity. As with KCNQ2 and KCNQ3, *in situ* hybridization has revealed broad expression of KCNQ5 in the brain, including the cortex and the hippocampus[17]. It is also expressed in rat superior cervical ganglia and in NG108-15 cells[17], in which many studies on M-currents have been done.

Like other KCNQ subunits, KCNQ5 yields currents that activate slowly upon depolarization. It can form heteromeric channels with KCNQ3, but probably not with KCNQ1, KCNQ2 or KCNQ4 (REFS 17,18), a conclusion supported by using dominant-negative mutants, and mutants with different sensitivity to inhibitors[17]. Co-expression of KCNQ5 with KCNQ3 slightly changed its kinetic properties[18], and increased current amplitudes when KCNQ5 was injected in molar excess[17]. This stimulation was smaller than that observed when KCNQ2 and KCNQ3 were co-expressed[13,15,27], and a larger amount of KCNQ3 actually decreased overall currents[17]. So in cells expressing all three subunits, the overall magnitude of currents is difficult to predict and will also depend on possible assembly preferences. As KCNQ5 could be inhibited by M1-receptors[17] and showed pharmacological properties[17,18] that fit into the general picture of M-type currents, KCNQ5 contributes to the molecular diversity of M-channels. Depending on their relative expression levels, particular neurons may express KCNQ2/KCNQ3, and KCNQ3/KCNQ5 channels, probably together with homomeric channels. This situation may be even more complex if the channels interact with β-subunits. However, KCNQ5 tested negative for interactions with KCNE1, and KCNE2 and KCNE3 had only slight effects on KCNQ5 (REF. 17).

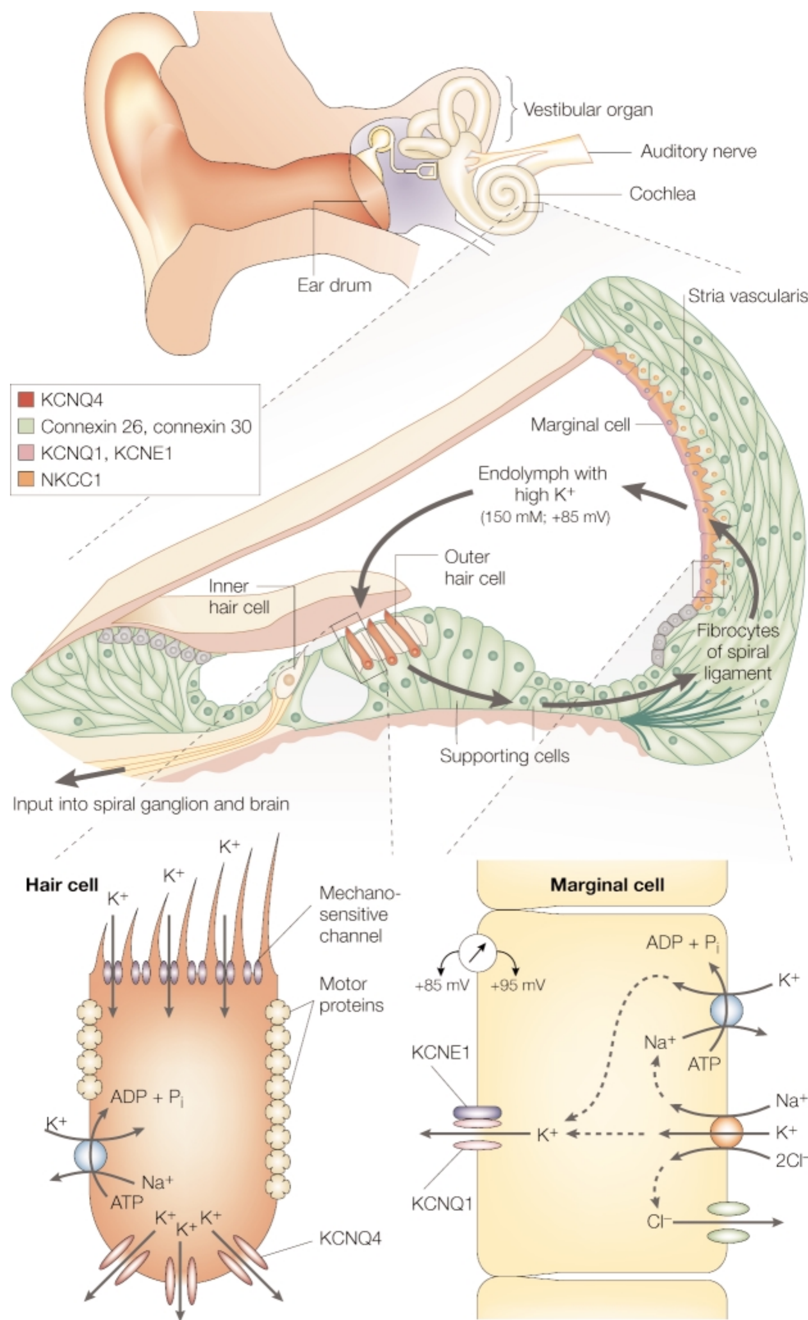

Figure 4 | **Potassium recycling in the scala media of the inner ear.** The endolymph of the scala media has a high K^+ concentration and a positive potential. Potassium secreted into the endolymph by the stria vascularis enters the hair cells through apical mechanosensitive channels, and probably leaves outer hair cells (OHCs) through KCNQ4. It is recycled back to the stria vascularis through supporting cells and fibrocytes of the spiral ligament for another round of secretion. Bottom right: transport model for K^+ secretion in the stria vascularis. A basolateral Na^+/K^+-ATPase pumps K^+ and creates a Na^+ gradient to drive K^+ and Cl^- into the cell in a co-transport process[56]. Chloride is recycled by basolateral Cl^- channels, and K^+ is secreted apically by KCNQ1/KCNE1 K^+ channels, which are open at the unusual apical membrane voltage of marginal cells (inside positive by about +10 mV)[78]. This allows for a net secretion into the endolymph in spite of its high K^+ concentration. The positive potential inside the scala media is probably generated by an epithelium of 'basal' and 'intermediate' cells that lie below the 'marginal' cells[79]. The importance of KCNQ1/KCNE1 channels in K^+ secretion is underscored by the deafness after mutations in either subunit in JLNS[7,55,80] and in *KCNE1* knockout mice[11]. Bottom left: diagram of an outer hair cell. Potassium enters through apical mechanosensitive channels whose opening is coupled to the deflection of stereocilia[83]. In the proposed model, it leaves the cell through KCNQ4 K^+ channels exclusively present in the basal membrane[19]. The lateral motor protein prestin[84] leads to the electromechanical amplification of acoustic vibrations by OHCs.

KCNQ5 localizes to chromosome 6q14 (REFS 17,18). Loci for several retinal diseases map to this region, but no *KCNQ5* mutations have been reported in any of these yet. Given that its expression pattern is similar to KCNQ2 and KCNQ3, and that it can form heteromers with KCNQ3, it can be speculated that KCNQ5 mutations also cause epilepsy. However, making predictions is difficult, as association with KCNQ3 leads to current increases only in certain stoichiometries, while preventing KCNQ3 from forming the more 'efficient' KCNQ2/KCNQ3 heteromers. An initial mutation screen was negative[51], but in view of the low frequency of *KCNQ3* mutations in epilepsy this finding should not discourage further studies.

Unlike other known KCNQ genes, KCNQ5 is also prominently expressed in skeletal muscle[17,18], although its function is unclear. The predominant muscle isoform is a splice variant that contains a further short exon in the C terminus[17], which changes gating, yielding currents that kinetically no longer resemble M-currents. Nonetheless, this isoform of KCNQ5 is also inhibited by co-expressed M1-receptors.

KCNQ4 is important for hearing

KCNQ4 was cloned[8] by homology to KCNQ3. Its expression pattern (localized to the inner ear and certain nuclei and tracts of the brainstem)[8,19] is much more restricted than those of the other KCNQ channels. It forms homomeric potassium channels that activate slowly on depolarization[8] and it can form heteromers with KCNQ3 that activate slightly faster and are more sensitive to inhibition by linopirdine[8]. However, it is not clear whether they occur *in vivo*. Like other KCNQ channels, it may qualify as an 'M-type' channel and can be inhibited by muscarinic stimulation[45].

KCNQ4 was mapped to human chromosome 1p34 (REF. 8). DFNA2, one of over 30 loci for dominant deafness, had been mapped to this region[52]. *KCNQ4* seemed to be a good candidate gene for this slowly progressive hearing loss, as mutations in *KCNQ1* can cause deafness in JLNS (REF. 7). People with DFNA2 did have mutations in this gene[8] (FIG. 1b), but the mechanism leading to deafness turned out to be different from the transepithelial transport defect found in JLNS. Most KCNQ4 mutations, including those published later[53], exert a dominant-negative effect on wild-type subunits (REF. 8, and T. Friedrich and T.J.J., unpublished observations). However, one mutation that truncates the protein before the first transmembrane span[53] must act by haploinsufficiency. The hearing deficit in this family differed only slightly from that in other people with DFNA2.

KCNQ4 in cochlear sensory hair cells. In mouse cochlea, KCNQ4 is mainly expressed in sensory outer hair cells[8,19] (FIG. 4). In the guinea pig, inner hair cells of the apical turn of the cochlea also express KCNQ4 (REF. 19). So the localization of KCNQ4 is different from that of KCNQ1, which is expressed together with the β-subunit KCNE1 in the marginal cells of the STRIA VASCULARIS[7,54] (FIG. 4).

What might be the function of KCNQ4 in sensory hair cells? Hearing loss in DFNA2 patients progresses

over decades, implying that KCNQ4 currents are not essential for the hearing process *per se*, but that reduced currents might lead to a slow, degenerative process. The discovery of several other genes underlying deafness in humans and mice supports an attractive model for inner-ear potassium recycling (FIG. 4).

The inner and outer hair cells in the ORGAN OF CORTI (FIG. 4) form one and three rows of sensory cells, respectively, that extend from the (high-frequency sensitive) base of the cochlea to its (low-frequency sensitive) apex. The inner hair cells provide the brain with the electrical input that mediates hearing. In contrast, outer hair cells (OHCs) do not send many afferents to the brain, but increase the sensitivity of hearing by amplifying acoustical vibrations in an electromechanical feedback loop. The apical stereocilia of both types of hair cells are deflected in response to the acoustical signal and open mechanosensitive transduction channels (FIG. 4). The influx of potassium through these channels depolarizes the hair cells because of an unusual electrochemical potassium gradient across their apical membranes. In contrast to the composition of the normal extracellular space, the ENDOLYMPH (the fluid that fills the SCALA MEDIA of the cochlea, which is contiguous with the tubular system of the vestibular organ) has an unusually high potassium concentration (150 mM) and a positive potential (+85 mV). This provides a large driving force for the entry of potassium into hair cells. This electrochemical gradient is established by ion transport across the stria vascularis, and three of the participating transport proteins are mutated in deafness (KCNQ1 (REF. 7), KCNE1 (REF. 55) and the secretory NaK2Cl co-transporter NKCC1 (REFS 56,81,82)) (FIG. 4).

Whereas the apical membranes of hair cells are bathed in the endolymph, the basolateral side is in contact with the perilymph, which contains the usual high concentration of sodium and low concentration of potassium. So electrochemical gradients dictate that apical potassium channels can lead only to an influx of potassium, whereas basolateral channels may mediate its efflux. KCNQ4 may be responsible for potassium efflux across the basal membrane[8,19], consistent with its localization in the basal portion of the OHC plasma membrane[19]. Loss of KCNQ4 might result in a chronic potassium overload of OHCs, causing their slow degeneration. Noise could accelerate this process by leading to a larger apical potassium influx. Higher expression of KCNQ4 in basal turns of the cochlea[19], which are responsible for sensing high frequencies, is compatible with the DFNA2 hearing loss that starts in this frequency range. However, as a selective loss of OHCs results in a hearing loss of only about 30–50 dB (REF. 57), other processes must contribute to the final, severe deafness.

The proposed role of KCNQ4 in potassium recycling is indirectly supported by the roles of connexins (components of GAP JUNCTIONS) in other forms of genetic deafness. Mutations in connexin 26 (REF. 58), connexin 30 (REF. 59), connexin 31 (REF. 60) and connexin 32 (REF. 61) underlie different forms of deafness. Gap junctions that comprise at least connexin 26 (REF. 62) and connexin 30 (REF. 63) connect the supporting, non-sensory cells in the organ of Corti. It has been suggested that the potassium leaving the OHCs by KCNQ4 is siphoned into supporting cells and transported through the cytoplasm and the gap junctions of this cellular system back to the stria vascularis. There, it is secreted again into the scala media, completing the recycling pathway (FIG. 4).

KCNQ4 in vestibular hair cells. Although people with DFNA2 have no vestibular symptoms, KCNQ4 is also expressed in sensory hair cells of the vestibular organ. Its expression is restricted to the basolateral membrane of type I cells and the nerve endings contacting these cells[19]. KCNQ4 is present both in presynaptic and postsynaptic membranes of these large, calyx-like synapses. Because model calculations predict a significant increase in potassium concentration in this large synaptic cleft, it has been proposed that potassium could cause excitation directly by depolarizing the postsynaptic membrane during repetitive synaptic activity[19].

Comparison of the electrophysiological properties of hair-cell currents, and their upregulation during postnatal development, with data obtained for KCNQ4 indicated that this channel mediates the so-called $I_{K,n}$ or $I_{K,L}$ outwardly-rectifying currents in OHCs and vestibular type I cells, respectively[19]. This fits with the localization of the $I_{K,n}$ current to the base of OHCs[64,65], and the observation that linopirdine selectively blocks the $I_{K,n}$ current in OHCs[66], but seems to conflict with the voltage dependence of activation. KCNQ4 is half-maximally activated at −10 mV and −20 mV in oocytes[8] and CHO[45] cells, respectively, whereas $I_{K,n}$ is half-maximally activated at about −80 mV[66]. But the voltage-dependence of the $I_{K,L}$ current of vestibular type I cells varies widely and can shift by more than 20 mV to positive potentials during measurements[67]. The reason for this variability is not clear. In any case, the proposed role of KCNQ4 in potassium efflux from OHCs requires that, like $I_{K,n}$, it is open at the resting potential of OHCs.

Brainstem KCNQ4 expression and deafness. KCNQ4 is also expressed in many nuclei and tracts of the central auditory pathway in the brainstem, but not in more rostral structures of the brain[19]. Although KCNQ4 is expressed in some other nuclei that have no role in auditory function, its expression pattern is enriched on a single sensory pathway. Neurons in the auditory pathway have fast chemical synapses, not only because they encode high-frequency auditory information, but also because precise timing is crucial for localization of sounds by comparison of the time difference between sounds reaching the right and the left ears[68]. To achieve short electrical time constants, many neurons in the auditory pathway have a high potassium conductance at or close to the resting potential. KCNQ4 may contribute to these currents (which could be tested by applying M-current inhibitors such as linopirdine). So is M-type channel regulation involved in processing auditory information? A knockout mouse model could answer this question, and would be useful for investigating whether there is a central component in DFNA2-type hearing loss. It may also reveal whether a

STRIA VASCULARIS
The lateral part of the scala media, which contains the epithelia that secrete the endolymph.

ORGAN OF CORTI
The primary sensory organ of hearing. It is located on the floor of the scala media, and consists of sensory hair cells (one row of inner hair cells and three rows of outer hair cells) and supporting cells. The stereocilia of the hair cells touch the gelatinous tectorial membrane, which covers the organ.

ENDOLYMPH
The fluid filling the scala media of the cochlea and the cavities of the vestibular organ. In contrast to the perilymph, which fills the scala tympani and scala vestibuli, it has an unusual ion composition with a high potassium concentration.

SCALA MEDIA
The central cavity of the cochlea that is sandwiched between the scala vestibuli (which receives acoustic input through the ossicles of the middle ear) and the scala tympani. It is filled with endolymph and contains the organ of Corti.

GAP JUNCTIONS
Channels that connect adjacent cells and allow for the free passage of small molecules. They are formed by proteins called connexins.

Table 1 | **KCNQ channels: expression data and role in disease**

Gene	Locus	Expression	Partner	Disesase	Inheritance	Remaining function
KCNQ1	11p15.5	Heart, cochlea, intestine, kidney	KCNE1*, KCNE3	LQTS, (dominant RWS)	Dominant	50% > *x* > 0% Dominant negative on one allele Often 0% function in homomers
				LQTS, (recessive RWS)	Recessive	50% > *x* > 0% Residual function of mutated homomers, or mutant1/mutant2 heteromeric channels
				LQTS + congenital deafness (JLNS)	Recessive	~0% Loss of function of both alleles
KCNQ2 *KNCQ3*	20q13.3 8q24	Brain Brain	KCNQ3* KCNQ2*, KCNQ5	BFNC	Dominant	~75% (in heteromer) Loss of function of one allele, haploinsufficiency
KCNQ4	1p34	Inner ear, brainstem nuclei	KCNQ3	DFNA2 (dominant progressive hearing loss)	Dominant	~10–50% Dominant negative on one allele or haploinsufficiency
KCNQ5	6q14	Brain, muscle	KCNQ3	?	–	–

*Indicates that this protein is known to associate with the subunit in the tissue that is affected in the disease, and that its mutation causes the same disease (for example, KCNE1 mutations also cause the RWS and the JLNS). BFNC, benign familial neonatal convulsions; JLNS, Jervell and Lange-Nielsen syndrome; LQTS, long-QT syndrome; RWS, Romano–Ward syndrome.

loss of KCNQ4 leads to a slow degeneration of outer (and maybe inner) hair cells, and may be a useful model for the slow, progressive hearing loss of the elderly.

Channelopathies as a model for dosage effects

KCNQ channelopathies not only highlight the importance of ion channels for diverse cellular functions, but also provide an example of the importance of GENE DOSAGE in inherited disease (TABLE 1). KCNQ1 is expressed in many tissues, including the heart muscle and several epithelia, but *KCNQ1* mutations cause severe effects in just one or two tissues, depending mainly on the degree of current reduction. So dominant-negative mutations in KCNQ1 cause cardiac arrhythmia but do not affect hearing. These mutations reduce currents below 50%, but leave at least 6% of wild-type current intact, assuming that the insertion of one mutant subunit into the tetramer is sufficient to abolish currents totally. Deafness occurs when the function of the channel is further compromised, requiring loss-of-function mutations on both alleles (as in JLNS). Mutations in the β-subunit KCNE1, with which KCNQ1 associates in both the heart and the inner ear, can also lead to dominant or recessive LQTS. However, recessive KCNQ1 mutations leading to cardiac arrhythmia are not always associated with deafness[69–71]. In these cases, homomeric mutant channels still yielded measurable, albeit reduced, currents[69,71].

BFNC (caused by mutations in either KCNQ2 or KCNQ3) shows dominant inheritance, but the mutant proteins lack dominant-negative effect, so haploinsufficiency accounts for the phenotype. *In vitro* studies indicated that a roughly 25% reduction in current is enough to cause epilepsy, an observation compatible with the role of the M-type channel KCNQ2/3 in regulating neuronal excitability. However, the existence of KCNQ5 as a further partner for KCNQ3, and the lack of a fixed stoichiometry that applies to all neurons, make exact predictions impossible. This problem is compounded by the fact that we do not know which neuronal populations are responsible for the initiation and propagation of the epileptic activity. Whereas the slight loss of channel function in BFNC leads to a benign and mostly transient phenotype, a total loss of function may be lethal in humans as it is in mice[16].

Finally, most KCNQ4 mutations in DFNA2 exert a dominant-negative effect. In contrast to the congenital deafness with KCNQ1 (or KCNE1) mutations in JLNS, patients hear normally at early age, and deafness progresses slowly over many years or decades. This indicates that these mutations may cause a slow, degenerative process, as observed with many other genetic diseases affecting the nervous system.

The clinical phenotype of diseases caused by changes in neuronal or muscular excitability is often paroxysmal in nature, but permanent hyperexcitability in the CNS or in cardiac muscle may be lethal. For KCNQ channels, this is shown by *Kcnq2* knockout mice, which die shortly after birth[16].

Future directions

The identification of KCNQ channels and channelopathies has opened many doors for future investigations. First, in the field of channel biophysics and structure–function relationships, the effects of KCNE subunits on KCNQ and other potassium channels is poorly understood. The availability of several KCNQ and KCNE proteins that interact differently should help to solve this problem. Second, the search continues for further proteins that may interact functionally with KCNQ channels or determine their localization. Third, the discovery that KCNQ2, KCNQ3 and KCNQ5 yield M-type currents should aid in the dissection of the mechanisms by which this important regulator of neuronal activity is itself regulated. Fourth, the discovery that KCNQ2 and KCNQ3 are involved in a rare form of neonatal epilepsy will yield new insights into the pathophysiology of epilepsy. This is supported by the observation that an antiepileptic drug can act as a specific activator of KCNQ2/KCNQ3 channels. The more recent observation that KCNQ5, which is broadly expressed in the

GENE DOSAGE
The number of times a gene appears in the genome.

brain, can also form M-type channels alone or together with KCNQ3 makes all of these subunits and their various heteromers interesting targets for drug development. Last, transgenic mouse models are expected to give us insights into the function of this family of channels in the nervous system and other organs. The young field of KCNQ channels will certainly continue to expand at a rapid pace.

Links

DATABASE LINKS KCNQ1 | LQTS | JLNS | KCNE1 | KCNE3 | KCNQ2 | KCNQ3 | BFNC | KCNQ5 | KCNQ4 | DFNA2 | connexin 26 | connexin 30 | connexin 31 | connexin 32

FURTHER INFORMATION Potassium channel nomenclature | Thomas Jentsch's laboratory

ENCYCLOPEDIA OF LIFE SCIENCES Hair cells

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