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M-Channels

Neurological Diseases, Neuromodulation, and Drug Development

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Efforts in basic neuroscience and studies of rare hereditary neurological diseases are partly motivated by the hope that such work can lead to better understanding of and treatments for the common neurological disorders. An example is the progress that has resulted from identification of the genes that cause benign familial neonatal convulsions (BFNCs). Benign familial neonatal convulsions is a rare idiopathic, generalized epilepsy syndrome. In 1998, geneticists discovered that BFNC is caused by mutations in a novel potassium channel subunit, *KCNQ2*. Further work quickly revealed the sequences of 3 related brain channel genes *KCNQ3, KCNQ4,* and *KCNQ5*. Mutations in 2 of these genes were shown to cause BFNC *(KCNQ3)* and hereditary deafness *(KCNQ4)*. Physiologists soon discovered that the *KCNQ* genes encoded subunits of the M-channel, a widely expressed potassium channel that mediates effects of modulatory neurotransmitters and controls repetitive neuronal discharges. Finally, pharmacologists discovered that the biological activities of 3 classes of compounds in development as treatments for Alzheimer disease, epilepsy, and stroke were mediated in part by effects on brain KCNQ channels. Cloned human KCNQ channels can now be used for high-throughput screening of additional drug candidates. Ongoing studies in humans and animal models will refine our understanding of KCNQ channel function and may reveal additional targets for therapeutic manipulation. *Arch Neurol. 2003;60:496-500*

Channelopathies are hereditary or acquired disorders resulting from defective activity of particular ion channels. The human genome contains about 200 genes that encode neuronal ion channels, proteins that generate electrical signals by regulating the flow of ions across neuronal membranes. These channels play essential roles in such aspects of neuronal functioning as the release of neurotransmitters, generation of synaptic responses, and propagation of action potentials along dendrites and axons. Inherited channelopathies are disorders caused by mutations in ion channel genes. The first mutations in neuronal ion channel genes were identified in the fruit fly *Drosophila melanogaster*, where they cause phenotypes that include abnormal limb shaking, loss of coordination, and paralytic episodes.[1] We now know that mutations in several classes of human ion channel genes can induce similar symptoms. Although the first such human channelopathies were identified in 1991, more than 20 are known at present, and the list is growing rapidly.[2]

The *KCNQ* genes encode subunits for potassium channels. The term *KCNQ* is a genomic shorthand with K representing potassium; CN, channel; and Q, long QT syndrome. (*KCNQ1*, the first *KCNQ* gene to be identified, is expressed in the heart and is mutated in about half of the hereditary cases of long QT syndrome.[3]) Recent advances concerning the neuronal KCNQ channels have produced new insights into the mechanisms of neurological disease and identified a set of previously unknown therapeutic targets.

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MUTATIONS AND BENIGN FAMILIAL NEONATAL CONVULSIONS

Benign familial neonatal convulsions (BFNC) is an idiopathic generalized epilepsy, inherited in an autosomal dominant

pattern.[4] Full-term infants with BFNC experience seizures that begin during the first week of life, recur frequently for several weeks, and then remit. When birth occurs before term, the onset and remission of seizures is delayed, suggesting that the phenotype is related in a distinctive way to the stages of brain maturation. Although these infants otherwise experience normal growth and development, about 16% experience a recurrence of seizures later in life.

In 1989, Leppert and colleagues[5] mapped the gene causing most cases of BFNC to a region of chromosome 20. Subsequently, Lewis et al[6] and Steinlein and colleagues[7] found additional families in whom the trait mapped not to chromosome 20 but to chromosome 8. A decade later, 2 independent research groups found that the *BFNC* gene on chromosome 20 encoded a previously unknown potassium channel subunit, KCNQ2 (**Figure 1**A).[8,9]

Soon, 3 additional neuronal *KCNQ* genes were identified. Charlier et al[10] identified a close homologue of *KCNQ2* and mapped this gene, *KCNQ3*, to the region of chromosome 8 associated with BFNC. A *KCNQ3* mutation was identified in DNA from a patient with BFNC previously mapped to chromosome 8 (Figure 1A).[10] Jentsch[11] then cloned *KCNQ4* and *KCNQ5* and found that a mutation in *KCNQ4* was associated with a form of dominantly inherited deafness.

Physiologists soon studied the cloned KCNQ2 and KCNQ3 channels with in vitro electrophysiological methods.[11,12] They found that *KCNQ2* and *KCNQ3* coassembled in tetramers (Figure 1B), which resulted in large voltage-dependent potassium currents. Mutations associated with BFNC caused reductions in the size of the potassium currents of 20% to 95%. This finding suggested that epilepsy might result from modest reductions (as little as 20%) in the amount of KCNQ channel activity.[11]

McKinnon and colleagues had been studying a potassium channel called the *M-channel*, which is prominent in sympathetic neurons but also expressed widely in the brain.[12] They discovered that the functional properties and expression pattern in autonomic ganglia of

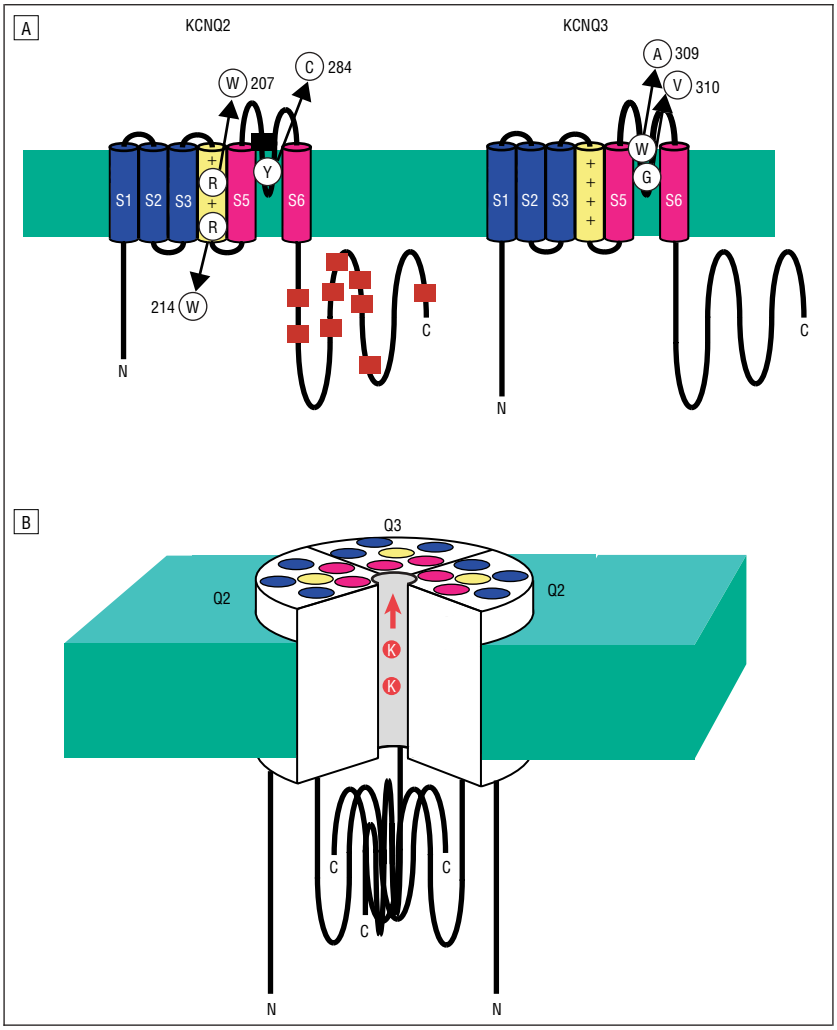

Figure 1. The structure of M-channels. A, Membrane folding pattern of *KCNQ2* and *KCNQ3* subunits and locations of benign familial neonatal convulsions (*BFNC*) gene mutations resulting in amino acid substitutions (circles) or truncation of the polypeptide (red boxes). Numbers indicate the position of the disease-causing mutations in the amino acid sequence of each subunit (A, alanine; W, tryptophan; R, arginine; Y, tyrosine; C, cysteine; G, glycine; and V, valine). B, Cutaway of the tetrameric channel, with 1 subunit removed to reveal the central ion pathway through the membrane. Each subunit consists of intracellular amino and carboxy terminal regions and a membrane component containing 6 transmembrane segments (ie, S1 through S6). Segment S4 is positively charged and moves in response to changes in the membrane potential, leading the channel to open with depolarization. Segments S5 and S6 and the extended loop between them form the walls of the transmembrane ion pathway. Point mutations have been found in the pore or the S4 regions; truncations, the intracellular C terminus.

the newly cloned KCNQ channels appeared identical to those of the M-channel.[12]

M-CHANNELS AND NEUROMODULATION

M-channels are a type of very slowly opening and closing voltage-dependent potassium channel.[13] They can powerfully control the number of action potentials fired by a neuron that is receiving a strong excitatory input. To explain how M-channels control repetitive neuronal firing and how reductions in the activity of M-channels might result in hyperexcitability, we need to first review some cell physiology and then indicate a few details about the M-channels.

Neurons establish ionic gradients across their cell membranes with pumps and cotransporter proteins that extrude calcium and sodium ions and take in potassium ions. As a result, when membrane ion channels that are selectively permeable to sodium or calcium ions open, these positive ions flow into the cell and depolarize the membrane potential. By contrast, when potassium-selective channels open, potassium ions pass from the cell

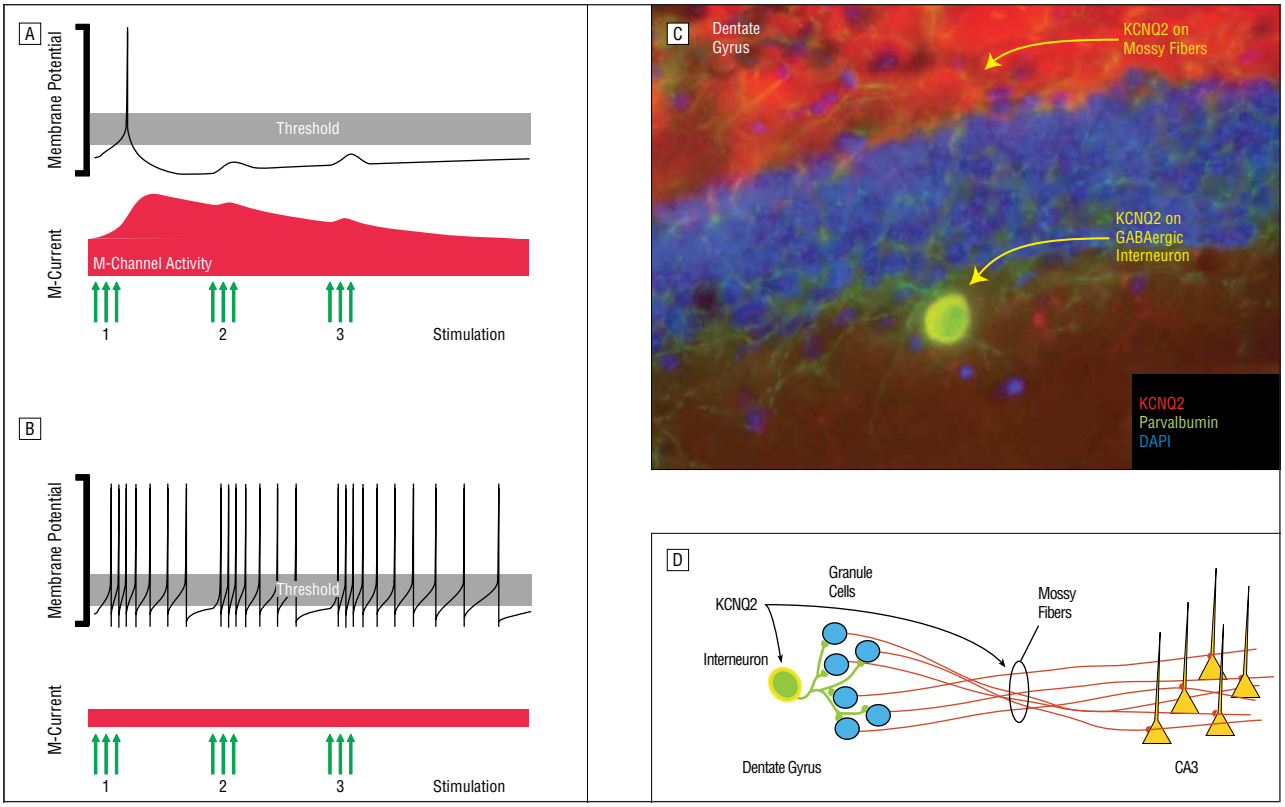

Figure 2. Role of neuronal M-channels in controlling excitability and synchronization. A, Excitatory inputs (green arrows) cause membrane depolarization and a single action potential. Afterward, increased activation of M-channels hyperpolarizes the membrane potential, preventing spiking in response to recurrent excitation. B, When M-channel activity is reduced owing to neurotransmitters (eg, acetylcholine) or to mutations in the gene that causes benign familial neonatal convulsions, excitatory inputs lead to multiple action potentials. C, The KCNQ channels are localized on hippocampal interneurons controlling cellular rhythmic firing and synchronization. The top part shows immunofluorescence staining of mouse dentate gyrus with antibodies directed against KCNQ2 (red). The interneuron and its afferent axonal arbor are labeled with antibodies against parvalbumin (green). Dentate granule neuronal cell bodies are labeled using DAPI (blue). Immunoreactivity of KCNQ2 is concentrated on the cell body of the interneuron, which influences the activity of many neighboring granule cells through its extensive axonal arborization. KCNQ2 staining of granule cell axons (mossy fibers) projecting to CA3 is also strong. D, Synaptic relationships depicted in part C.

into the extracellular space, causing the cell interior to become more negative or hyperpolarized.

Many types of signaling in neurons and neuronal networks are based on sequential opening of ion channels with differing effects on the membrane potential. The propagated action potential, a brief, explosive depolarization based on the rapid opening and closing of sodium channels, is the best known example of this. In some settings, potassium channels open near the peak of the action potential, hastening membrane repolarization and shortening the duration of the action potential.

M-channels, discovered by physiologists David Brown, PhD, and Paul Adams, PhD, about 20 years ago, are a very different type of potassium channel from those that repolarize individual action potentials. M-channels are partially active in the range of the neuronal resting membrane potential and further activated by membrane depolarizations. M-channels open and close at rates about 100 times slower than those of the channels that underlie fast-propagating action potentials. As a result, M-channels tend to allow the firing of single action potentials, but effectively oppose sustained membrane depolarization and repetitive firing of action potentials (**Figure 2**A). M-channels are inhibited by muscarinic acetylcholine receptor agonists (Figure 2B), leading to a profound increase in cellular excitability that reverses when the receptor agonist is withdrawn.

We now know that muscarinic acetylcholine receptors are just one of many types of membrane receptors capable of inhibiting M-channels.[13] Some subtypes of receptors for dopamine, serotonin, glutamate, and several peptide neurotransmitters, including luteinizing hormone-releasing hormone and bradykinin, have also been found to be capable of inhibiting the M-channel. The convergence of many distinct receptors and pathways on M-channels may partly explain why muscarinic agonists are potent convulsants, but muscarinic antagonists are not useful as anticonvulsants.

Thus, M-channels appear to serve 2 different and somewhat opposing roles in the brain. They serve as a restraint on repetitive neuronal discharges that may lead to hyperexcitability (Figure 2A), and more specifically and locally, they mediate transient increases in excitability that result from the release of modulatory neurotransmitters such as acetylcholine (Figure 2B).

SELECTIVE KCNQ CHANNEL OPENERS AND BLOCKERS AND NEUROLOGICAL DISEASES

Linopirdine, a selective M-channel blocker, has been shown to promote acetylcholine release and improved learning abilities in animal

models of cognitive dysfunction. Although phase 3 clinical trials of linopirdine in Alzheimer disease failed to show significant benefit, more potent derivatives of the drug are under development.[14]

Retigabine was discovered in the 1980s, as part of the National Institute of Neurological Disorders and Stroke Anticonvulsant Screening Project. Retigabine was effective in preventing seizures induced by electrical shock or by a broad range of chemical convulsants (eg, pentylenetetrazol, *N*-methyl-D-aspartate, 4-aminopyridine, and picrotoxin). Retigabine also prevented spontaneous and induced seizures in seizure-prone inbred lines and electrically kindled rodents.[15] Recently, several groups showed that retigabine was a potent opener of channels formed by *KCNQ2* and *KCNQ3* subunits.[16] Retigabine caused the channels to open at more hyperpolarized membrane potentials, increased the rate of channel opening, and slowed the rate of channel closing. Retigabine has completed phase 1 and phase 2a clinical trials, and a multicenter Euro-American phase 2b trial was recently conducted.

In rodent models of cerebral ischemia, BMS-204352 is a novel compound that reduces infarct volume.[17] The compound activates the maxi-K (potassium) channel very potently when intracellular calcium levels are abnormally elevated. This mechanism may contribute to its beneficial effects in rodent stroke. More recently, BMS-204352, like retigabine, was shown to strongly activate KCNQ channels.[18]

The safety and efficacy of these KCNQ blockers and openers remain to be proved by human trials. However, 3 classes of compounds identified in drug candidates for neurological disorders have already been shown to act on these channels, which indicates that they are likely to be important targets for further development.

UNANSWERED QUESTIONS

The M-channels of the brain have been relatively little studied by physiologists, who have focused on neurons of sympathetic ganglia, where the M-currents are especially large and prominent. For these reasons, our group has begun to study brain KCNQ channels in human surgical and postmortem samples and in rodents.

In studies of human temporal lobe, Cooper et al[19] found that KCNQ2 and KCNQ3 were coexpressed on the cell bodies and dendrites of many hippocampal and cortical neurons. In addition, staining patterns indicated that KCNQ2 but not KNCQ3 was expressed on neuronal axons, where they might regulate action potential propagation or neurotransmitter release. Indeed, a family with BFNC and a mutation in *KCNQ2* (R207W, Figure 1A) was recently shown to also have myokymia, suggesting a potential role for KCNQ2 on motor axons.[20] KCNQ2 channels are also prominently expressed in the mouse brain by several types of neurons important for synchronizing the patterns of neuronal firing in regions such as the thalamus, septum, and hippocampus[21] (Figure 2C-D).

The identification and initial characterization of neuronal KCNQ channels set the stage for many additional studies. By determining the molecular composition of KCNQ channels expressed in different brain regions, and by characterizing the neurotransmitter and signal transduction systems through which brain KCNQ channels are regulated, these studies will clarify the roles of KCNQ channels in neuroprotection, epilepsy, and normal cognitive function.

Accepted for publication October 8, 2002.

Author contributions: *Study concept and design* (Drs Cooper and Jan); *acquisition of data* (Dr Cooper); *analysis and interpretation of data* (Drs Cooper and Jan); *drafting of the manuscript* (Dr Cooper); *critical revision of the manuscript for important intellectual content* (Dr Jan); *obtained funding* (Drs Cooper and Jan); *administrative, technical, and material support* (Drs Cooper and Jan); *study supervision* (Drs Cooper and Jan).

Dr Cooper and Dr Jan's research is supported by grant NS42100 from the National Institute of Neurological Disorders and Stroke, Bethesda, Md, and by a grant to the Silvio Conte Center for Neuroscience at the University of California–San Francisco from the National Institute of Mental Health, Bethesda, Md. Dr Jan is an investigator of the Howard Hughes Medical Institute, San Francisco.

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