

# IS EPILEPSY A DISEASE OF SYNAPTIC TRANSMISSION?

**Masking Epilepsy by Combining Two Epilepsy Genes.** Glasscock E, Qian J, Yoo JW, Noebels JL. *Nat Neurosci* 2007;10(12):1554–1558. Inherited errors in ion channel genes comprise the largest subset of monogenic causes of idiopathic epilepsy, and pathogenic variants contribute to genetic risk in the complex inheritance of this common disorder. We generated a digenic mouse model of human idiopathic epilepsy by combining two epilepsy-associated ion channel mutations with mutually opposing excitability defects and overlapping subcellular localization. We found that increasing membrane excitability by removing Shaker-like  $K^+$  channels, which are encoded by the *Kcna1* gene, masked the absence epilepsy caused by a P/Q-type  $Ca^{2+}$  channelopathy due to a missense mutation in the *Cacna1a* gene. Conversely, decreasing network excitability by impairing *Cacna1a*  $Ca^{2+}$ -channel function attenuated limbic seizures and sudden death in *Kcna1*-null mice. We also identified intermediate excitability phenotypes at the network and axonal levels. Protective interactions between pathogenic ion channel variants may markedly alter the clinical expression of epilepsy, highlighting the need for comprehensive profiling of this candidate gene set to improve the accuracy of genetic risk assessment of this complex disease.

## COMMENTARY

Much work has been done in the past 3 decades to support the notion that seizures and interictal spikes result from the combined action of networks of neurons. Alterations in synaptic transmission appear to play a critical role in generating this network activity. Some of the earliest work investigating the nature of paroxysmal depolarizing shifts, which is the intracellular signature of a focal interictal spike, suggested a role for synaptic activity. These studies demonstrated that in the penicillin focus, paroxysmal depolarizing shifts had properties

similar to those of an EPSP: they were graded and could be reversed in polarity (1). Follow-up studies have established that postdepolarizing shifts are mediated by glutamatergic neurotransmission.

Additional support for the synaptic transmission hypothesis was provided by studies of acquired epilepsies in experimental animals and in human surgical specimens. One of the most intensely investigated mechanisms of altered synaptic transmission in acquired epilepsies is sprouting of axon collaterals. Sprouting of mossy fibers (i.e., axons) of dentate granule cells of the hippocampus was demonstrated in experimental models and later in human specimens. These studies indicated that formation of new glutamatergic synapses on principal neurons was a key feature of temporal lobe epilepsy (2). Other studies have shown the presence of altered GABAergic synaptic transmission

in temporal lobe epilepsy and in neurocortical epilepsies. In addition to alterations, both presynaptic input onto postsynaptic neurons and changes in GABA<sub>A</sub>-receptor structure and function also have been described in various forms of epilepsy.

In contrast to the synaptic transmission theory is the “channelopathy” theory of epileptogenesis, which proposes that defects in ion channels participating in the generation of action potentials lead to epilepsy. Monogenic defects in ion channels, such as mutations of sodium channel or GABA<sub>A</sub> receptor subunits, lead to generalized epilepsy febrile seizures plus syndrome or severe myoclonic epilepsy of infancy. Other examples of ion channel defects that result in human epilepsies have been forthcoming, including mutations in potassium channels, which result in benign neonatal febrile seizures, and in calcium channels, which are associated with absence epilepsy.

Theories of altered synaptic transmission and ion channel defect may appear mutually exclusive because ion channels are believed to modulate neuronal excitability by controlling the generation of action potentials, whereas synaptic transmission is largely modulated by neurotransmitter receptors and neurotransmitters. However, a careful analysis of the relationship between ion channels and neurotransmitter release reveals that defects in ion channel function could result in altered synaptic transmission. Furthermore, as the study by Glasscock et al. shows, if the role of ion channels in modulating synaptic transmission and in generating epilepsy is clearly understood, then certain defects in ion channels can be demonstrated to cancel each other and the development of epilepsy is prevented or attenuated.

Invasion of the presynaptic terminal by an action potential causes Ca<sup>2+</sup> entry, which is typically mediated by the P/Q-type of voltage-gated calcium channels. The amount of calcium entering into the terminal determines the number of synaptic vesicles that fuse and release neurotransmitter from the presynaptic terminal (3). The central pore-forming region of P/Q-type calcium channels is formed by a polypeptide subunit called CACNA1A. Mutations in the gene coding for CACNA1A lead to absence epilepsy in some patients (4). These mutations result in reduced function or loss of function in calcium channels, which is likely to reduce the entry of calcium into presynaptic terminal and depress neurotransmitter release.

The shape of the action potential invading the presynaptic terminal is to a large extent determined by potassium channels. Inhibition of potassium channels at presynaptic terminals leads to prolongation of action potentials (causing activation of more voltage-gated calcium channels) and increases the entry of calcium into the presynaptic terminal (releasing more neurotrans-

mitter) (5). Therefore, inhibition of potassium channels elevates the amount of neurotransmitter released from the presynaptic terminal, whereas inhibition of Ca<sup>2+</sup> channels diminishes it. Interestingly mutations in the Kv1.1  $\alpha$  subunit of potassium channels lead to seizures of hippocampal onset in patients and mice. Thus, there exist two kinds of ion channel mutations in humans—each having an opposite effect on synaptic transmission, yet both leading to epilepsy.

Glasscock et al. reasoned that if both these mutations were present in a single animal they would cancel out each other's effect. In a series of carefully performed experiments, the investigators demonstrate that the presence of both mutations (i.e., double homozygous) in a single transgenic mouse results in reduced seizures, decreased mortality, and increased long-term survival. In addition, there was marked reduction in seizure activity compared with single homozygous mice, including marked attenuation of spike-wave seizures and diminished frequency and intensity of generalized tonic-clonic seizures. The authors found that changes in excitability occur in the hippocampus: in hippocampal slices treated with elevated potassium, the frequency and duration of epileptiform discharges recorded from double homozygous slices was significantly less than that in slices from single homozygous animals. Furthermore, a direct analysis of synaptic transmission suggested that these two mutations exert opposing effects on presynaptic potentials.

This study has implications for understanding the pathogenesis of both inherited and acquired epilepsies. The authors demonstrate that multiple genes contribute to susceptibility to epilepsy. Some current notions about inherited epilepsies have been derived from Mendelian genetics, however the approach is limited by the fact that many genes expressed in the brain can modify actions of a mutation in a single gene, making the hunt for epilepsy genes extremely complex. It is possible that for each epilepsy mutation, multiple modifying genes are expressed in the brain, which may mask or exaggerate effects of single gene defect.

These studies by Glasscock et al. also suggest a mechanism for more common, acquired epilepsies. Indeed, many examples of acquired alterations in structure and functions of ion channels and receptors have accumulated over the past decade, including changes in GABA<sub>A</sub> receptors, chloride transporters, potassium channels, and sodium channels. The sequence of events that links these acquired defects in ion channels to increased susceptibility to seizures and epilepsy remains unclear. The study by Glasscock and colleagues proposes these defects in the structure and function of ion channels and receptors may alter synaptic transmission to contribute to epileptogenesis.

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## References

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