RESEARCH NEWS

Investigating an epileptogenic mutation
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*JGP* study examines how a mutation in KCNQ3 affects channel behavior.

There are more than 100 naturally occurring mutations that cause neuronal hyperexcitability, which results in a variety of disorders. For example, mutations in KCNQ voltage-gated potassium channels are commonly associated with epilepsy. One such mutation, the substitution of a cysteine in place of an arginine at residue 230 in the KCNQ3 subunit, is linked with a particularly severe form epilepsy called epileptic encephalopathy. A new *JGP* paper by Rene Barro-Soria explores how this mutation affects the channel’s function (1).

In the nervous system, KCNQ3 assembles into an ion channel with another KCNQ subunit, KCNQ2. Shifts in membrane voltage prompt a conformational change in the channel’s voltage-sensing domain that controls the opening of the transmembrane pore. The channel is normally closed at the resting neuronal membrane potential (about −70 mV), but prior studies (2, 3) have established that the R230C mutation, found in the voltage-sensing region of KCNQ3, causes the channel to remain open and act as if it were constitutively active. Although this might be expected to inhibit neuronal excitability by driving membrane hyperpolarization, the R230C mutation instead generates hyperactivity in the brain, possibly by impairing the inhibitory interneurons responsible for tamping down the excitability of other neuronal types (3). But this is just one of the mysteries regarding this mutation.

“There is a really big need to understand how these channels work, but surprisingly little is known about how they open and close,” explains Barro-Soria, an assistant professor at the University of Miami in Florida. “The mutated channel remains open at physiological voltages. One hypothesis to explain this is that the voltage sensor may be locked in its activated position so that the gate is always open. Alternatively, the mutation may let the voltage sensor move up and down but uncouple it from the gate’s opening, or it could shift the membrane voltage at which the channel closes.”

To explore these hypotheses, Barro-Soria expressed KCNQ3 as a homomer in frog oocytes and used voltage clamp fluorometry (4) to simultaneously monitor the channel’s electrophysiological properties and movement of its voltage sensor. These experiments demonstrated that R230C KCNQ3 is not locked open. It is capable of closing, but only does so at membrane voltages lower than −100 mV. At these nonphysiological voltages, however, movement of the channel’s voltage sensor was still tightly coupled to opening of its gate.

“This implies that compounds that shift the voltage dependency to more positive voltages would actually promote gate closure, and hence may have therapeutic potential,” notes Barro-Soria.

For insight into the physical mechanism that drives this behavior, Barro-Soria examined the structural features of the mutation. It is expected that having a positively charged amino acid, like arginine, at position 230 may be important because charged residues can both sense electric fields and form electrostatic interactions with other moieties within the molecule. To confirm this, Barro-Soria substituted R230 with lysine, another positively charged amino acid. Surprisingly, lysine only partially restored the normal gating behavior of the channel. Barro-Soria hypothesized this could be because lysine, though charged, is physically smaller than arginine. To test this idea, he substituted in an artificial amino acid called citrulline that is structurally similar to arginine but lacks a positive charge (5). Citrulline also shifted channel opening back toward less-negative voltages (albeit much less than lysine did), suggesting that both the charge and physical size of the arginine at position 230 is important for channel gating.

Determining whether the R230C mutation affects channel gating by destabilizing its closed state or by stabilizing its open state will require additional work. Having just started his own laboratory, Barro-Soria is also interested in identifying compounds that could help treat epilepsy. “I’m looking for students!” he says.

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2. Miceli, F., et al. 2012. *Biophys. J.* 102:1372–1382.
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5. Infield, D.T., et al. 2018. *J. Gen. Physiol.* 150:1017–1024.