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The Voltage-Sensor S4 Rises to the Occasion in KCNQ2 Channel Activation

Distinctive Mechanisms of Epilepsy-Causing Mutants Discovered by Measuring S4 Movement in KCNQ2 Channels

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Neuronal KCNQ channels mediate the M-current, a key regulator of membrane excitability in the central and peripheral nervous systems. Mutations in KCNQ2 channels cause severe neurodevelopmental disorders, including epileptic encephalopathies. However, the impact that different mutations have on channel function remains poorly defined, largely because of our limited understanding of the voltage-sensing mechanisms that trigger channel gating. Here, we define the parameters of voltage sensor movements in wt-KCNQ2 and channels bearing epilepsy-associated mutations using cysteine accessibility and voltage clamp fluorometry (VCF). Cysteine modification reveals that a stretch of eight to nine amino acids in the S4 becomes exposed upon voltage sensing domain activation of KCNQ2 channels. VCF shows that the voltage dependence and the time course of S4 movement and channel opening/closing closely correlate. VCF reveals different mechanisms by which different epilepsy-associated mutations affect KCNQ2 channel voltage-dependent gating. This study provides insight into KCNQ2 channel function, which will aid in uncovering the mechanisms underlying channelopathies.

Commentary

Mutations in the gene *KCNQ2* are being increasingly recognized in neonates and young children with epilepsy. A fascinating feature of *KCNQ2* mutations is that they can result in either a mild transient form of epilepsy as in benign familial neonatal seizures (BFNS)[1] or in a severe developmental epileptic encephalopathy (DEE).[2] Therefore, disorders of KCNQ2 are likely to be more than just seizure-generating channelopathies, with widespread neuronal network involvement implicated by the presence of encephalopathy, cognitive impairments, and autism.[3] Unraveling the mechanisms by which *KCNQ2* mutations lead to various epilepsy syndromes is of paramount importance in the era of personalized medicine, when specific gene mutations might be amenable to different therapies.

KCNQ2 codes for the voltage-dependent potassium channel $K_V7.2$ which mediates an outward potassium current called the M-current (because it is inhibited by muscarinic acetylcholine receptors).[4] This current is active around resting potential, activates and deactivates slowly, and does not inactivate. These properties allow the M-current to regulate resting potential and neuronal excitability in the near-threshold voltage range. Moreover, the channel is highly expressed at the axon initial segment, an ideal location for its role in the modulation of neuronal firing. Mutations in *KCNQ2* increase the likelihood of action potential generation and enhance repetitive firing, thereby increasing seizure susceptibility. Indeed, animals with *KCNQ2* knocked out are prone to spontaneous seizures and cognitive and behavioral comorbidities.[5,6]

The channel mediating the M-current consists of a heteromeric combination of 4 subunits (KCNQ2 and related KCNQ3), each having 6 transmembrane segments (designated S1-S6). S4 comprises the voltage sensor. When the membrane is depolarized, S4 undergoes conformational change, allowing the channel to pass outward potassium current, thereby countering the depolarization and constraining neuronal firing. Numerous mutations in the human gene have been identified, in S4 and elsewhere along the molecule, resulting in a panoply of different mechanisms and clinical consequences. For example, mutation at the site M547V decreases axonal surface expression of KCNQ2 and results in an EE phenotype.[5]

A knowledge gap in understanding KCNQ2 function is exactly how S4 conformational changes relate to pore opening and how this dynamic relationship is altered in *KCNQ2* mutations. During S4 activation, amino acid residues that are ordinarily intramembranous become exposed (extramembranous), suggesting that part of S4 has risen above the membrane plane into the extracellular space. In the current article, Edmond et al investigate biophysical properties of KCNQ2 to elucidate how different mutations alter channel function.[7] They used 2 methods to investigate the components that are

involved in voltage sensing. In each case, mutated KCNQ2 channel proteins are expressed in oocytes for electrophysiological analysis. First, a technique called cysteine accessibility allows modifying substituted cysteines along the S4 domain in a state-dependent manner; addition of cysteine-reactive membrane-impermeable thiol reagents allows mapping their extra- versus intramembranous location. By this method, the investigators can assess the degree of S4 outward motion as a function of voltage and determine how much of S4 is newly exposed during channel activation.

The authors show that depolarization and KCNQ2 channel opening causes S4 to extrude slightly above the membrane surface, exposing only certain amino acid sites. These experiments verify that S4 moves outward during membrane depolarization and channel activation. The second method by which they study S4 movement utilizes a technique called voltage-clamp fluorometry. Here, the authors attach fluorometric dyes at various cysteine substituted sites along S4 or the S3-S4 interlink. They found a position where the fluorescence changes correlated with current flow. There was excellent correspondence at the cysteine-modified F192 site, correlating with S4 outward motion.

Having established that the voltage sensor S4 moves outward from the membrane during depolarization and channel activation, the investigators then studied the effects of 2 known human *KCNQ2* mutations on channel gating and kinetics, as an attempt to correlate the mutation with the clinical phenotype. In this sense, they invoke the possibility of personalized medicine, in that mutation-specific channel alterations may or may not respond to a particular intervention. One human epilepsy-associated *KCNQ2* mutation occurs at the R198 site of S4 (R198Q). It was already known that this gain-of-function mutation neutralizes the first gating charge of S4 in KCNQ2 channels, shifting the conductance-voltage curve to more hyperpolarized potentials and slowing the kinetics of deactivation (clinical phenotype: infantile spasms).[8] Edmond et al confirmed these findings and further showed that fluorescence changes also shift in the negative direction and overlap with ionic current in terms of both voltage-dependence and kinetics, suggesting that this mutation alters KCNQ2 channel function by directly affecting S4 activation. The second mutation, R214W, was previously reported to shift the conductance-voltage curve to more depolarized voltages, thus slowing the kinetics of current activation and accelerating the kinetics of current deactivation (clinical phenotype: BFNS).[9] Using their novel techniques, Edmond et al again confirmed the previously reported results, and showed as well that the conductance-voltage curve is separable from the fluorescence-voltage curve in this mutation—the fluorescence-voltage curve does not shift in the depolarized direction with channel activation like the conductance-voltage curve does. This observation suggests that voltage sensor (S4) movement is dissociated from channel opening. That is, the R214W mutation affects gating activation without directly affecting S4 movement.

The authors conclude that distinct mutations in the same molecular subunit, S4, result in different pathophysiological mechanisms. Whether these have clinical therapeutic relevance remains uncertain. The only available potassium channel opener, retigabine, has poor specificity among KCNQ channel subunits and causes undue off-target side effects.[10] Retigabine causes a hyperpolarizing shift in the voltage-dependence of activation, which the authors propose may be beneficial in mutations like R214W but not in other mutations. Other potassium channel openers that target the voltage-sensing domain are under development and may be more applicable to mutations like R198Q. Clearly, the wide diversity of mechanisms even within KCNQ channelopathies implies a vast spectrum of possible mutation-related treatments. This work is only in its infancy but has the potential to distinguish between and therefore treat channel dysfunction not just in *KCNQ* disorders but more broadly among the wide array of ionic channels that regulate neuronal excitability and epileptic firing. Furthermore, the role of these and numerous other ion channel- and synapse-related mutations opens a huge realm of potential treatments for epileptic encephalopathies, in which neural networks are altered beyond simply channel dysfunction. Creative experimental methods such as those employed by Edmond et al will be necessary to unravel the multiplicity of molecular and physiological mechanisms.

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Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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