Early-onset epileptic encephalopathy caused by gain-of-function mutations in the voltage sensor of Kv7.2 and Kv7.3 potassium channel subunits

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The heteromeric assembly of two voltage-gated K^+ channel subunits, Kv7.2 and Kv7.3, underlies the neuronal M current which stabilizes the membrane potential and controls neuronal excitability. Structurally, these channels show six transmembrane segments (S_1-S_6) and intracellular N- and C-termini. Mutations in the respective genes, *kcnq2* and *kcnq3*, have been associated to early-onset epileptic disorders, such as Benign Familial Neonatal Seizures *(BFNS;* ***1,2****).* More recently, mutations in *kcnq2* gene have been also associated to Neonatal Epileptic Encephalopathies *(NEE;* ***3,4****); s*evere phenotypes have been also associated with mutations in *kcnq3* (***5***). In the present work, electrophysiological, biochemical, multistate and computational modeling techniques have been used to investigate the consequences prompted by mutations affecting the second arginine (R201, R2) along the S_4 segment in Kv7.2 (R2C, and R2H) or Kv7.3 (R230C), recently found in patients affected by epileptic encephalopathies and/or intellectual disability. Electrophysiological studies performed on Chinese Hamster Ovary (CHO) cells expressing each mutant subunit homomerically or in heteromeric configuration with wild-type Kv7.2/3 channels revealed that the neutralization of the second arginine in either Kv7.2 or Kv7.3 subunits destabilized the resting state of both Kv7 channels, thereby producing gain-of-function effects. Notably, similar results were obtained when the same residue was substituted by charged (D or E) or polar (Q) residues, suggesting that a positive charge at R2 is critical for the stabilization of the voltage sensor of Kv7.2 subunits. In fact, multistate structural modeling revealed that the R2 residue in Kv7.2 forms a network of electrostatic interactions with different negatively-charged residues (E130 and E140 in S_2, D172 in S_3); the occurrence of these interactions was studied by coupled charge reversal and disulfide trapping. In these experiments, we observed that, although single mutant channels (D172R-, R2D-, D172C-, and R2C-Kv7.2 mutant subunits) were functional, channels carrying R2D/D172R or R2C/D172C double mutations failed to elicit measurable currents; the absence of currents was not due to a reduction in plasma membrane expression, as revealed by western-blotting experiments performed on biotinylated fractions of CHO cells expressing R2D/D172R or R2C/D172C mutant channels. Notably, Kv7.2-R2C/D172C mutant channels were rescued to active channels by the reducing agent DTT, an effect prevented by the oxidant H_2O_2; this channel reactivation was presumably due to the disruption of the disulfide bridge formed between cysteine residues introduced in Kv7.2-R2C/D172C double mutant channels, as DTT was ineffective in channels carrying the R2C or D172C single mutations. These results suggest that the electrostatic interaction between D172 and R2 is necessary to stabilize the resting VSD state in native Kv7.2 subunits. Finally, to find a plausible explaination for the neuronal hyperexcitability caused by the described gain-of-function Kv7.2 or Kv7.3 mutations, an inhibitory microcircuit between interneurons and CA1 hippocampal cells, incorporating the experimentally-defined values for Kv7.2/3 or Kv7.2/Kv7.2-R2C/Kv7.3 currents was modeled. Using this approach, the Kv7.2-R2C mutation was found to prompt only small effects on the membrane properties of the CA1 pyramidal cells whereas it significantly silenced interneurons, resulting in an effective disinhibition of the CA1 cell.
In conclusion, the results obtained suggest that, in addition to more commonly found loss-of-function mutations, also mutations in Kv7.2 that increase Kv7.2 functionality can be found in patients with Epileptic Encephalopathy (**6**).

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