NOVEL MUTATIONS RESPONSIBLE FOR BENIGN FAMILIAL NEONATAL CONVULSIONS AFFECT UNCHARGED AMINOACIDS IN THE S_4 REGION OF KCNQ2 POTASSIUM CHANNEL SUBUNITS AND REDUCE I_{KM} FUNCTION BY MODIFYING ITS GATING PROPERTIES

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The M-current (I_{KM}) is a K^+-selective neuronal current that activates and deactivates slowly and does not inactivate; this current is mostly formed by heterotetrameric assembly of KCNQ2 and KCNQ3 subunits. Mutations in KCNQ2 and KCNQ3 genes have been found in patients affected by Benign Familial Neonatal Convulsions (BFNC), a rare autosomal-dominant epilepsy of the newborn. In the present study, we identified a novel BFNC-associated mutation in the KCNQ2 gene, leading to an alanine to valine substitution (A196V; A/V) at the N-terminal end of the S_4 segment, and investigated the functional consequences prompted by the KCNQ2 A/V mutation, in parallel with those of another previously-described, but not functionally characterized, BFNC allele in which the KCNQ2 A/V mutation was associated to a leucine to proline substitution on the following residue (L197P; AL/VP) (1). To this aim, wild-type or mutant KCNQ2 subunits were expressed in CHO cells by transient transfection, and the resulting currents were recorded with both the whole-cell (macroscopic currents) and the on-cell (single channel currents) configurations of the patch-clamp technique. When compared to KCNQ2 channels, homomeric KCNQ2 channels carrying the A/V or AL/VP mutations showed a 20 mV rightward shift in their activation voltage-dependence. At the single channel level, a 20 mV rightward shift in the voltage-dependent open probability, without significant changes in the single-channel conductance, was introduced by the two mutations. Intriguingly, KCNQ2 A/V channels, but not KCNQ2 or KCNQ2 AL/VP double substituted channels, displayed an unusual dependence of their activation kinetics on the conditioning pre-pulse voltage, being markedly slower when conditioning pre-pulses to more depolarized potentials (from -80 mV to +40 mV) were applied. Furthermore, these mutations did not significantly modify channel sensitivity to blockade by TEA (0.3-3 mM) or to activation by retigabine (10 μM); surprisingly, in KCNQ2 A/V, but not in KCNQ2 wild-type or KCNQ2 AL/VP channels, retigabine significantly slowed current activation kinetics, irrespectively of the conditioning pre-pulse voltage. Finally, heteromeric channels formed by KCNQ2 A/V and KCNQ3 subunits displayed gating changes qualitatively and quantitatively similar to those of KCNQ2 A/V homomeric channels, consistent with the dominant inheritance pattern of the disease in the affected family. Taken together, these alterations produce a significant I_{KM} functional impairment which could be responsible for a decreased neuronal repolarization reserve in the individuals carrying these mutations, possibly predisposing them to convulsive manifestations.

Reference

1. Moulard B., Picard F., le Hellard S., Agulhon C., Weiland S. and Favre I. (2001) Brain Res. Rev. 36: 275-284.