Pharmacological rescue of KCNQ2 channels carrying Early-Onset Epiletic Encephalopathy mutations

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Kv7.2/3 channels underlie the M-current (I_{KM}), a potassium-selective neuronal current characterized by low activation threshold, slow activation and deactivation kinetics, and absence of inactivation. Mutations in Kv7.2/3 are responsible for genetically-determined epileptogenic diseases in neonates, showing a wide phenotypic heterogeneity, ranging from Benign Familial Neonatal Seizures (BFNS)[1] to severe Early-Onset Epileptic Encephalopathy (EOEE)[2]. The molecular basis for such phenotypic heterogeneity is unknown, but the mutation-induced functional changes in the ionic currents seem to play a major role[3].

In the present study, we investigated the biochemical and functional consequences prompted by Kv7.2 mutations (A265T, R325G, or S195P) found in EOEE-affected patients[4]; in parallel, we evaluated the ability of Kv7 modulators to counteract mutation-induced alterations. To this aim, mutations were engineered in a plasmid for mammalian expression containing the cDNA for the human isoform of Kv7.2 subunits and mutant plasmids were used to transiently transfect Chinese Hamster Ovary (CHO) cells.

Patch-clamp recordings performed 24 h after transfection revealed that homomeric KCNQ2 A265T or R325G mutant channels were non-functional. When mutant channels were expressed in heteromeric configuration with WT KCNQ2 and KCNQ3 subunits to reproduce the genetic balance of affected indiduals, a significant reduction in maximal current density was measured, suggesting a loss-of-function as a pathogenetic mechanism. Biotinylation assays revealed that mutations-induced KCNQ2 currents reduction was not due to changes in plasma membrane levels of mutant subunits. Exposure to the Kv7 opener retigabine[5] (10 μM) significantly increased KCNQ2/KCNQ2A265T/KCNQ3 or KCNQ2/KCNQ2R325G/KCNQ3 current density, rescuing defective channels to wild-type levels. By contrast, KCNQ2 S195P mutant subunits, both when expressed in homomeric or heteromeric configuration with KCNQ2/3 subunits, showed a significant increase in the maximal current density and a robust leftward shift in the voltage-dependence of activation, revealing that a gain-of-function mechanism is instead associated to this mutation, as it occurs with other mutations affecting the proximal S_4 region[6]. Exposure to the potent and selective KCNQ2 blocker ML252[7] (100 nM) similarly reduced by about 50% the currents carried by WT or KCNQ2 S195P homomeric channels.

Altogether, these results suggest that EOEE-associated mutations can lead to divergent biophysical consequences on KCNQ2 channels, ranging from gain- to loss-of function effects. These mutation-specific effects should be taken into account when planning patient-tailored pharmacological treatments.

REFERENCES

1. Bellini *et al.*, 2010. GeneReviews® [updated 2013 Apr 11].
2. Weckhuysen *et al.*, 2012. *Ann Neurol* **71**(1):15-25.
3. Miceli et al., 2013. *Proc.Natl.Acad.Sci.USA* **110**(11):4386-4291.
4. Weckhuysen et al., 2013. *Neurology* **81**(19):1697-1703.
5. Barrese et al., 2010. *Clin Pharmacol* **2**:225-236.
6. Miceli et al., 2015. *J. Neuroscience* **35**(9):3782–3793.
7. Cheung *et al.*, 2012. *J Med Chem* **55**(15):6975-6979.