Functional characterization of a novel mutation affecting the first Arginine in the S_4 segment of $K_v7.2$ channel causing Early-Onset Epileptic Encephalopathy

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Neonatal seizures are among the most frequent neurological symptoms in the first year of life. In neonatal convulsions, genetic determinants appear to play a relevant role. In particular, mutations in *KCNQ2*, and more rarely *KCNQ3* genes, encoding for the $K_v7.2$ and $K_v7.3$ voltage-gated K^+ channel subunits, have been identified in patients with Benign Familial Neonatal Seizures (BFNS), a rare autosomal-dominant epilepsy of the newborn with mostly benign neurodevelopmental outcome. More recently, *KCNQ2* mutations have been also described in neonates affected with Early-Onset Epileptic Encephalopathy (EOEE), a group of devastating epilepsies characterized by refractory seizures and cognitive arrest or regression that typically carry a poor prognosis (Weckhuysen et al., 2012). $K_v7.2$ and $K_v7.3$ channels are mainly expressed in the Central Nervous System where they form homo- or hetero-tetrameric channels underlying a slow activating/deactivating K^+ current called M-current which regulates neuronal firing (Soldovieri et al., 2011). Functional experiments in $K_v7.2$ and $K_v7.3$ channels carrying disease-causing mutations reveal that most of them cause a loss-of-function effect. However, we have recently studied four mutations, three in $K_v7.2$ and one in $K_v7.3$ channels, that cause EOEE by a gain-of-function (GOF) mechanism (Miceli et al., 2015).

In the present study, mutagenesis, electrophysiological and molecular modeling techniques have been used to investigate the consequences prompted by a novel mutation neutralizing the first Arg in the S_4 segment of $K_v7.2$ channels (R198Q), identified in three unrelated families with epileptic encephalopathy and later-onset seizures reported into a case registry/database (www.rikee.org). To this aim, we introduced the specific mutation in the human *KCNQ2* cDNA and studied their functional properties using the whole-cell configuration of the patch-clamp technique upon their transient expression in CHO cells. Electrophysiological experiments revealed that homomeric $K_v7.2$ R198Q subunits exhibited an approximately 2-fold increase in maximal current density and a robust leftward shift in activation voltage-dependence of about 30 mV, as previously reported (Miceli et al., 2008). When expressed with wild-type $K_v7.2$ and $K_v7.3$, to reproduce the genetic balance of the affected patients, the current density was equal to control, but activation was shifted approximately 10 mV to hyperpolarized potentials. These results suggest that this mutation, similar to those affecting the proximal part of S4, induced a GOF effect on $K_v7.2$ channels. Therefore, in order to attempt to counteract these mutation-induced effects on $K_v7.2$ currents, we evaluated the effects of pH on currents elicited by heteromeric channels incorporating $K_v7.2$ R198Q subunits, since $K_v7.2/3$ currents are inhibited by H^+ ions in a voltage-dependent manner (Prole et al, 2003). A decrease of pH dose-dependently rightwardly shifted (opposite to the mutation effect) the voltage-dependence of current activation, and at pH 6.4 we observed an almost complete restoration of the wild-type voltage-dependence of the channel.

In conclusion, we identified a novel $K_v7.2$ mutation that causes epileptic encephalopathy by a GOF mechanism and we found that a decrease in pH significantly reverts the gain of function of $K_v7.2$ R198Q mutant channel, suggesting that drugs causing a moderate acidosis, such as the carbonic anhydrase inhibitor acetazolamide, may be effective in this specific subgroup of patients.

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