STARGAZIN REGULATES THE BIOCHEMICAL AND FUNCTIONAL PROPERTIES OF Kv7.2 VOLTAGE-GATED POTASSIUM CHANNELS

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The stargazin gene (also referred to as Cacng2, Calcium channel, voltage-dependent, gamma subunit 2) encodes for stargazin (also known as TARP□□), a synaptic adaptor protein that binds AMPA receptors and regulates their synaptic trafficking and functional properties1.

Stargazin (STG) is mutated in stargazer mice, an animal model that shows head-elevating movements (hence the name stargazer) and episodes of spike-wave discharges associated to an epileptic phenotype2.

Kv7.2 (kcnq2) and Kv7.3 (kcnq3) subunits underlie the M-current (IKM), a slowly activating and deactivating K+ current that regulates neuronal excitability. In particular, mutations in kcnq2 gene have been associated with a wide spectrum of early onset epileptic disorders ranging from benign familial neonatal seizures3 to severe epileptic encephalopathies4.

Both Kv7.2 and STG are located presynaptically on axons and nerve terminals5 and influence neuronal excitability; therefore, in the present study we have used biochemical and functional approaches to investigate their possible interaction.

Mass spectrometry experiments performed on STG-immunoprecipitated samples obtained from cortical neurons identified the presence of Kv7.2 subunits. Therefore, to study a possible functional regulation prompted by STG on Kv7.2 channels, patch-clamp experiments were performed in the whole-cell configuration on CHO (Chinese Hamster Ovary) cells transiently expressing Kv7.2 subunits and stargazin at different transfection cDNA ratios. These experiments revealed that stargazin is able to induce a significant Kv7.2 current potentiation (in fact, current densities were 33.2□3.7 pA/pF, 42.2□5.6 pA/pF, 71.2□6.2 pA/pF, or 75.1□7 pA/pF for Kv7.2 or Kv7.2+STG at 1:1, 1:5, or 1:10 transfection ratios, respectively; $p<0.05$ versus Kv7.2). No change in the voltage-dependence of Kv7.2 channels was measured in each experimental condition.

To investigate whether this STG-induced Kv7.2 current potentiation was due to an increase in the expression and/or trafficking of Kv7.2 channels to the plasma membrane, Western-blotting experiments were performed on both total lysates and plasma membrane fractions of CHO cells expressing Kv7.2, STG or Kv7.2+STG. The results obtained indicate that the co-expression with STG fail to increase total or plasma membrane levels of Kv7.2 subunits, suggesting that the observed STG-induced current potentiation was likely due to changes in the single-channel properties or in the availability to gate of a fraction of Kv7.2 channels normally expressed at the plasma membrane5.

To further expand the pathophysiological meaning of STG/Kv7.2 interaction beyond epilepsy, we performed additional functional experiments using STG isoforms incorporating mutations recently

identified in patients affected by intellectual disability (STG/ID)6 or schizophrenia (STG/SCZ). When Kv7.2 subunits were co-expressed with Kv7.2+STG/ID or Kv7.2+STG/SCZ (at 1:5 or 1:10 transfection ratios), the STG-induced current potentiation was completely lost (current densities were 39.7□2.5 pA/pF, 71.3□6.2 pA/pF, 50,8□3.7 pA/pF and 49.5□3.4 pA/pF for Kv7.2, Kv7.2+STG, Kv7.2+STG/ID and Kv7.2+STG/SCZ at 1:5 tranfection ratio, respectively, and 39.8□3.5 pA/pF, 66.1□4.6 pA/pF, 41.4□5.3 pA/pF and 44.7□4.4 pA/pF for Kv7.2, Kv7.2+STG, Kv7.2+STG/ID and Kv7.2+STG/SCZ at 1:10 transfection ratio, respectively), again with no effect on the voltage-dependent properties of Kv7.2 channels.

Altogether, these results suggest that STG is able to modulate Kv7.2 currents and that such modulation is severely affected by rare STG mutations found in neuropsychiatric disorders.

Reference

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