Identification of different splicing variants of Kv7.4 potassium channels in the F11 neuronal cell line

A. Medoro[1], M.V. Soldovieri[1], I. Vinciguerra[1], and M. Taglialatela[1,2]

[1]Dept. of Medicine and Health Science, University of Molise, Campobasso (Italy)
[2]Dept. of Neuroscience, University of Naples 'Federico II', Naples (Italy)

Among voltage-gated K^+ channels, the Kv7 (KCNQ) gene subfamily includes five members (Kv7.1-5), each encoding for subunits showing distinct tissue expression and functional properties[1]. These subunits are structurally similar, showing six transmembrane segments (S_1-S_6); while the S_1-S_4 segments form the voltage-sensing domain (VSD), the S_5-S_6 and the intervening linker constitute the K^+-selective pore, and both N- and C-termini are intracellular. The long C-terminus contains the binding site for many regulatory molecules, such as calmodulin (CaM)[2], syntaxin[3], PIP_2[4], and others. Kv7 channels are slowly activating/deactivating delayed-rectifier K^+ channels expressed in a wide range of excitable and non excitable cells, where they control their membrane potentials and appear as promising pharmacological targets for hyperexcitability diseases. In the inner ear, Kv7.1 and Kv7.4 channels are critical for auditory function: indeed, mutations in these genes cause nonsyndromic sensorineural deafnesstype 2 (DFNA2, OMIM_603537[5]), an autosomal dominant form of progressive hearing loss. Although four different mouse Kv7.4 variants, each showing a specific functional modulation by CaM, have been reported in the mouse cochlea[6], little is known on the expression and function of different Kv7.4 variants in neurons.

In the present study, we have attempted to identify the specific KV7.4 splice variants expressed in F11 cells, a hybrid somatic cell line obtained by the fusion of rat embryonic dorsal root ganglion (DRG) and mouse neuroblastoma cells[7]. In particular, the mouse KCNQ4 cDNA variant A (vA) sequence and its correspondence with topological protein domains were obtained from ENSEMBL and UNIPROT databases, while sequences of the other mouse variants (vB, vC and vD) were manually reconstructed from protein sequences; notably, genomic sequences corresponding to two mutually exclusive exons, called 10 and 11, have been identified in the vB or vC, respectively. In the shortest vD, both these sequences are spliced out. By contrast, among the five rat variant sequences, vEn was extracted from the ENSEMBL database, while vX1, vX2, vX3 and vX4 predicted sequences were extracted from the NCBI database.

Rat or mouse sequences were aligned using ClustalΩ. The results obtained suggest that, while sequences corresponding to the N-terminus and to S1-S6 segments appear to be identical in all mouse Kv7.4 variants, those in the C-terminal regions can be heterogeneous because of the presence of mutually exclusive exons (9, 10 and 11), leading to sequence differences in the CaM binding site6. By contrast, rat Kv7.4 variants appear to differ, not only at this C-terminal region, but also at the exon sequences corresponding to the N-terminus and S1 segment.

To identify Kv7.4 variants expressed in F11 cells, primers specific for each of the three mouse mutually exclusive exons and for the divergent regions at both N- and C-termini of rat Kv7.4 variants were engineered. RT-PCR experiments were then performed on the cDNA retro-transcribed from F11 cells total RNA: the expression of a specific variant was assessed by the correspondence between the length or the presence/absence of PCR products versus those predicted. The results obtained suggest that F11 cells express vA, vB and vD mouse variants and all predicted rat variants, although the expression of the vX4 shortest isoform remains uncertain. Based on the high homology between rat and mouse isoforms (~90%), further experiments on non-hybrid cells (mouse or rat cells), allowing to define the specie-specificity of the primers used, will lead to a more accurate identification of the Kv7.4 isoforms expressed in neuronal cells.

REFERENCE

1.Soldovieri et al., Physiology 2011. 2.Yus-Näjera et al., J Biol Chem 2002. 3.Regev et al., PLoS One 2009. 4.Li et al., J Neurosci 2005. 5.Kubisch et al., Cell 1998. 6. Xu et al., J Biol Chem 2007. 7. Platika et al., PNAS 1985.