GENERATION AND CHARACTERIZATION OF CELL LINES STABLY EXPRESSING DIFFERENT ISOFORMS OF KCNQ POTASSIUM CHANNELS SUITABLE FOR DRUG SCREENING

Isabella Coletta[1], Barbara Garofalo[1], Rosella Ombrato[1], Claudio Milanese[1], Francesco Paolo Di Giorgio[1], Serena Tongiani[1]

[1]Angelini RR&D (Research, Regulatory & Development), Angelini S.p.A., S. Palomba-Rome - Italy

Introduction: Voltage-gated K+ channels (Kv7.1-Kv7.5), also known as KCNQ1-KCNQ5channels, are involved in physiological processes, including frequency and duration of action potential discharge, muscle contraction. Kv7channels, especially Kv7.2and Kv7.3are key determinants for membrane excitability in the brain. In response to stimuli such as voltage changes, mechanical stress and to neurotransmitters, channels open the pore through which K+ ions diffuse and generate an outward current which has a significant impact on neuronal excitability, limiting the firing rate. Pharmacological activation of Kv7channels in excitable cells reduces excitability. Retigabine a first-in-class Kv7.2-7.5opener, was approved in 2011for the treatment of drug-resistant epilepsy but, following safety issues that have been only recently classified as mostly not related to the drug target, its production was discontinued in 2017. Kv7channels still represent a class of attractive drug targets as for diseases of hyperexcitability. For this reason and with the aim to configure a cell-based assay suitable to identify Kv7.2/Kv7.3openers by a high-throughput screening approach, 3stable cell lines transfected with Kv7.2, Kv7.3and Kv7.2/7.3genes were generated and characterized.

Material and methods: Human genes for Kv7.2and Kv7.3channel subunits were synthetized and cloned in suitable mammalian expression vectors. The Kv7.2and Kv7.3channel constructs were stably transfected into CHO-K1cells (either by cotransfection or single transfection procedures). Empty vectors were used for the generation of control MOCK cell line.
Channel activity was monitored using thallium sensitive dye to evaluate the homo and hetero-tetramers response to channel openers and blockers using a fluorescence signal. To quantify mRNA expression levels of the different isoforms in the selected clones, a qPCR analysis was performed. Heteromeric Kv7.2/7.3and homomeric Kv7.2and Kv7.3activity on selected clones was confirmed by electrophysiology using QPatch16X (with standard whole-cell voltage clamp) and manual patch clamp.

Results: Stable cell lines expressing KCNQ2, KCNQ3and KCNQ2/3were generated, whose channel activity was confirmed by fluorescence signal in the Thallium assay and electrophysiology on automated and manual patch clamp, using reference opener (retigabine, ICA-069673) and blocker compounds (XE-991, linopirdine and TEA-Cl). For each cell line the proper pharmacological response to activator and blocker reference compounds was evaluated. Taking advantage of the high and low sensitivity to TEA of the Kv7.2and Kv7.3subunits, respectively, a comparison between isoforms was assessed. The results suggest the feasibility of a functional discrimination between isoforms and indicates that the two subunits coexist in the heteromeric clone. Moreover, for each cell line, a robust and reproducible thallium-sensitive, fluorescence-based assay for detecting and characterizing K+ channel modulators was configurated.

Discussion and conclusion: In the present work we have developed and characterized cell lines stably expressing KCNQ2, KCNQ3and KCNQ2/3as tools to identify novel KCNQ2/Q3agonists as potential therapeutics for neurological disorders characterized by high excitability.