IRREVERSIBLE BK CHANNEL BLOCKING MECHANISM REDUCES THE PHOSPHO-AKT.1(PSER473) /PAN- AKT.1 RATIO INDUCING CELL CYCLE ARRESTS IN HUMAN NEUROBLASTOMA SHSY5Y CELLS AND ANTI-PROLIFERATIVE EFFECTS.

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Big calcium activated K+ channels (BK) are involved in a diversity of physiological processes such as metabolism, signaling, phosphorylation, regulation of neurotransmitter release, and modulation of smooth muscle contractions. They are activated by the cooperative effects of two distinct stimuli, membrane depolarization and the elevation in concentration of free cytoplasmic Ca2+ ions concentration. BK channels are composed by the alpha subunit encoded by one gene (slo1/KCNMA1) assembled as tetramer and transmembrane beta subunits and gamma subunits. The BK channel can be located also in the several intracellular compartments including nuclear membrane and the mitochondrial inner membrane. In proliferating tumor cells the BK channels play a role in the regulation of cell proliferation and migration. BK channels have been found in glioma, prostate cancer, breast cancer, and the CD133+ subpopulation of SH-SY5Y cells regulating cell proliferation. BK channel blockers such as resveratrol (RESV) and iberiotoxin (IbTX) are known to reduce cell proliferation in several tumor cells including the SH-SY5Y. Inhibiting BK channels with iberiotoxin (IbTX) arrests D54-MG glioma cells in S phase, and leads to apoptosis suggesting that BK channel affects cell cycle progression. The signaling pathway coupling BK channel function to the cell proliferation is however poorly understood. Emerging evidence suggest that Akt.1 is functionally coupled to BK channel regulating cell proliferation in certain tumor cells. Sequence analyses of the BK C-terminus across different species showed putative binding sites for 14-3-3, RAC-a serine/threonine-protein kinase 1 (Akt.1), glycogen synthase kinase-3b (GSK3b) and phosphoinositide-dependent kinase-1 (PDK1). An elevated phospho-Akt.1(pSer473) /pan- Akt.1 ratio is associated with cell proliferation and Akt.1 interacting protein were observed to change as the cell cycle progressed from G0 to G1-S and then to G2 phase. Perturbation of Akt.1 signaling and cyclin D1 expression is shown to be implicated in tumor genesis due to inappropriate cell cycle entry.

Here the effects of increasing concentrations of RESV (10-7-2x10-4M), IbTX (10-8M-10-6M), paxilline (PAX) (1-50x10-6M) and TEA (1-5 x10-3M) on whole cell K+-currents, cell cycle progression and on phosphorylation of Akt.1(pSer473) were investigated, respectively, using patch-clamp tecnique, multi-parametric assay and ELISA assay in SH-SY5Y cell after 3 h of incubation with the drugs.

We found that IbTX, RESV and PAX caused a concentration-dependent and irreversible block of the whole cell K+-current with an IC50 of 2.06x10-7M, 3.1x10-6M and 1x10-5 M and a maximal current inhibition (Emax) of -53.3%, -67.4%, and -35% respectively. IbTX and RESV reduced cell number with an IC50 of 2.61x10-7M, 5.31x10-5M and 0.8x10-5M and an Emax of -57.32%, -71.98% and -90%, respectively, inducing cell cycle arrest in G2 phase. RESV, IbTX and PAX induced a concentration–dependent reduction of the SER473-Akt1/PAN-Akt1 phosphorylation ratio

without affecting the level of the PAN Akt-1. In contrast, TEA caused a reversible block of whole cell K+ currents without affecting the phospho-Akt.1(pSer473) /pan- Akt.1 ratio, cell cycle progression and cell proliferation.

In conclusion, the observed drug-induced irreversible BK channel blocking mechanism is associated with the deactivation of the Akt1 pathway arresting cell cycle progression with anti-proliferative effects.

Keywords: cell viability, cell cycle, patch-clamp, neuroblastoma cell, calcium activated potassium channels.