Regulation of neuronal GIRK channel activity by palmitate turnover

M. Chisari[1], L. Jia[2], C. Sobieski[3], S.J. Mennerick[3-4] and K.J. Blumer[2]

Depts. of [1]Clinical and Molecular Biomedicine, Section of Pharmacology and Biochemistry, University of Catania, Italy, [2]Cell Biology and Physiology, [3]Psychiatry, and [4]Anatomy and Neurobiology, Washington University in St. Louis, MO, USA

G-protein-activated inward-rectifying K^+ channels (GIRKs) are widely expressed in the central nervous system. Activation of these channels leads to neuronal hyperpolarization. It has been shown previously that GIRK channel activity is modulated by the regulator of G protein signaling (RGS) family. Specifically, an allosteric activator, R7-RGS binding protein (R7BP), is responsible for a prolonged activation of GIRK channel in hippocampal neurons (Zhou et al., 2012). Although R7BP is known to undergo rapid palmitoylation turnover, the consequences of this turnover on signaling are unexplored. Blocking the palmitoylation stage of turnover alter protein localization and function. However, tools to manipulate depalmitoylation are sparse. Here we used two new depalmitoylation inhibitors, palmostatin B (palm B) and hexadecylfluorophosphonate (HDFP) to address the role of palmitate turnover in GIRK modulation. We used both drugs in Neuro2a (N2a) cell line and hippocampal neurons. GIRK channel activation was elicited by $GABA_B$ mediated response with 0.05mM baclofen in whole-cell electrophysiology experiments. N2a cells do not endogenously express R7BP and overexpression of this protein by transfection speeds up the offset decay time (4152.61 ± 386.74 ms, n =16, vs. 2258.60 ± 209.97 ms, n = 13, $P < 0.0001$). Treatment with palm B (0.01 mM, 6h) or HDFP (0.005 mM, 2h) in N2a cells transfected with R7BP, reverted the offset decay time to control level (4241.85 ± 550.28 ms, n = 9; 4250.78 ± 402.82 ms, n = 10, respectively). Confocal imaging experiments in N2a cells transfected with GFP-R7BP and treated with both palm B and HDFP showed intracellular accumulation of R7BP after 2h treatment. In hippocampal neurons, palm B treatment slowed GIRK offset kinetics after 2h incubation (control, 560.57 ± 59.79 ms, n = 21; palm B, 1171.64 ± 220.53 ms, n = 13, $P < 0.01$), whereas HDFP effect was observed after overnight treatment (1066.43 ± 213.04 ms, n = 12, $P < 0.01$ compared to control cells). Taken together these data confirm the importance of R7BP in GIRK channel modulation and suggest that such modulation is mediated by S-palmitoylation occurring post-translationally in R7BP. Based on these results, palmitate turnover and enzymes involved in such process might be a novel pathway to be explored in pathological conditions where GIRK modulation is altered, including epilepsy, pain, Down's syndrome and Parkinson's disease.

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