International Doctoral School
Medical University of Lodz

Lipid rafts in C6 glioma cell line: role in the regulation of calcium signaling and the secretion of neurotrophic factors

Marta Sobolczyk, Department of Molecular Neurochemistry, Medical University, Lodz, Poland

Introduction

It is presumed that mutual regulation of Ca^{2+} and protein kinase A (PKA) signals is spatially compartmentalized and even slight abnormalities in these mechanisms may have severe neuropathological consequences. Although there is strong evidence linking GABA-activated astrocytes to neuronal synaptic activity, knowledge about GABA-mediated gliotransmission is scarce. High-resolution detection of astrocytic Ca^{2+}/PKA fluxes may help to better understand astrocyte functions in gliotransmission and modulation of the neural microenvironment. Particularly, the proposed study may provide new insight into the functional relationship between astrocyte-specific membrane-located GABA transporter 3 (GAT3) and an essential regulator of intracellular Ca^{2+} concentration, the plasma membrane Ca^{2+} -ATPase 4 (PMCA4) in C6 glioma cell line. The C6 cell line is widely selected as an alternative to primary astrocytes.

AIM:
To investigate the effects of GABA on Ca^{2+} and PKA signaling in discrete plasma membrane microdomains and verify how compartmentalization of these signals may regulate gliotransmission by C6 cells

Methods and Results

1. Research model produces GAT3 and two isoforms of plasma membrane calcium ATPase: PMCA1 and PMCA4

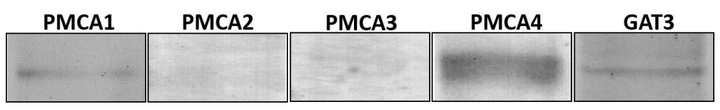
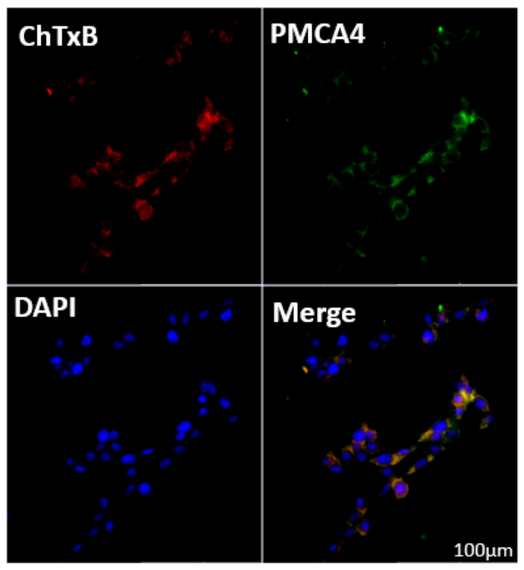
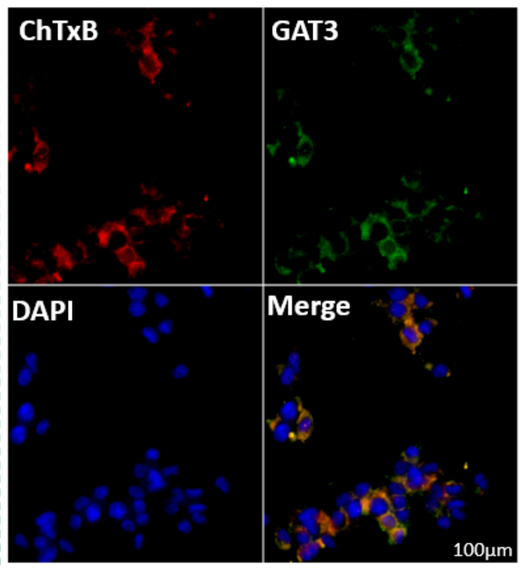


Fig 1. The presence of the GAT3, and PMCA 1-4 isoforms in the C6 glial cell line assessed by Western blot.

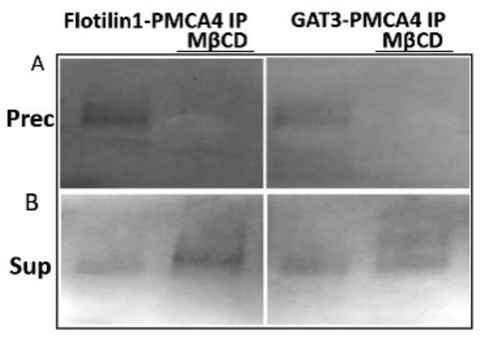
2. Membrane localization of GAT3 and PMCA4 and the effect of raft disruption



Immunofluorescence observation of GM1 labeled with cholera toxin B (ChTxB; 555nm, red), and PMCA4 (488nm, green) distribution in the plasma membrane of C6 cells. Cell nuclei (blue) were stained using DAPI dye.



Immunofluorescence observation of GM1 labeled with cholera toxin B (ChTxB; 555nm, red), and GAT3 (488nm, green) distribution in the plasma membrane of C6 cells. Cell nuclei (blue) were stained using DAPI dye.



Immunoprecipitation of two protein complexes: Flotilin1 (a specific marker of lipid rafts) with PMCA4 and GAT3 with PMCA4 in C6 cells treated or untreated with methyl-β-cyclodextrin (MβCD) by 3h (10mM) (A) C6 lysates were immunoprecipitated using Flotilin-1 or GAT3 antibody, separated by SDS-PAGE, and detected by Western blotting using PMCA4 antibody. (B) The supernatant fraction from each sample was used as a negative control, and next shown by Western blotting using PMCA4 antibody.

3. GABA induces GAT3-dependent intracellular Ca^{2+} signaling

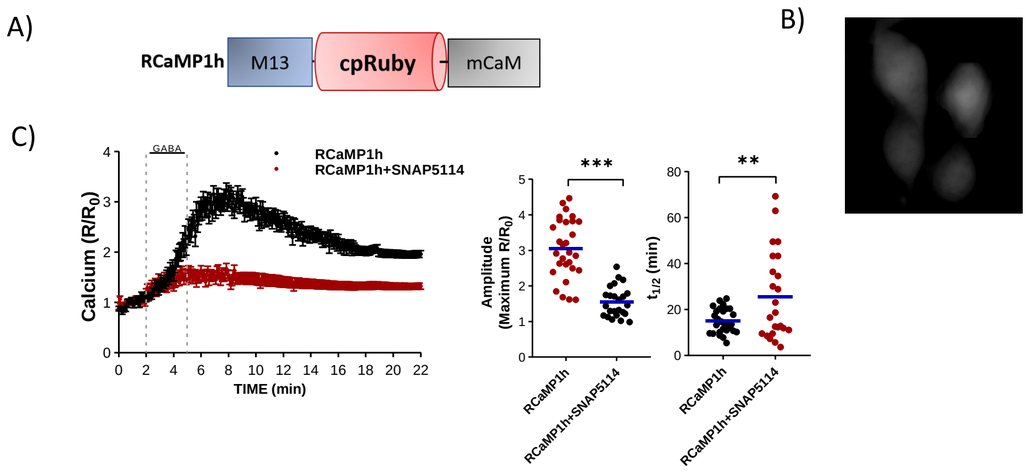


Fig 3. (A) Schematic of RCaMP1h biosensor that was used in the study. (B) Fluorescence image of C6 cells expressing RCaMP1h calcium sensor. Scale bar: 100 µm. **(C) Average tracings (I/I_0±SEM) depict the RCaMP1h response of cells treated with 200 µM GABA for 3 min** (horizontal bars) in control conditions and in the presence of SNAP5114 (inhibitor of GAT3; 25 µM) throughout the experiment. The peak amplitude and half-time of signal decay ($t_{1/2}$) for individual tracings are presented in the scatter plot; blue bars indicate mean. Datasets were compared by unpaired t tests. **p ≤ 0.01, ***p ≤ 0.001.

4. Distinct effects of GABA on temporal dynamics of PKA activity in cytosol and plasma membrane microdomains visualized by high-resolution FRET imaging

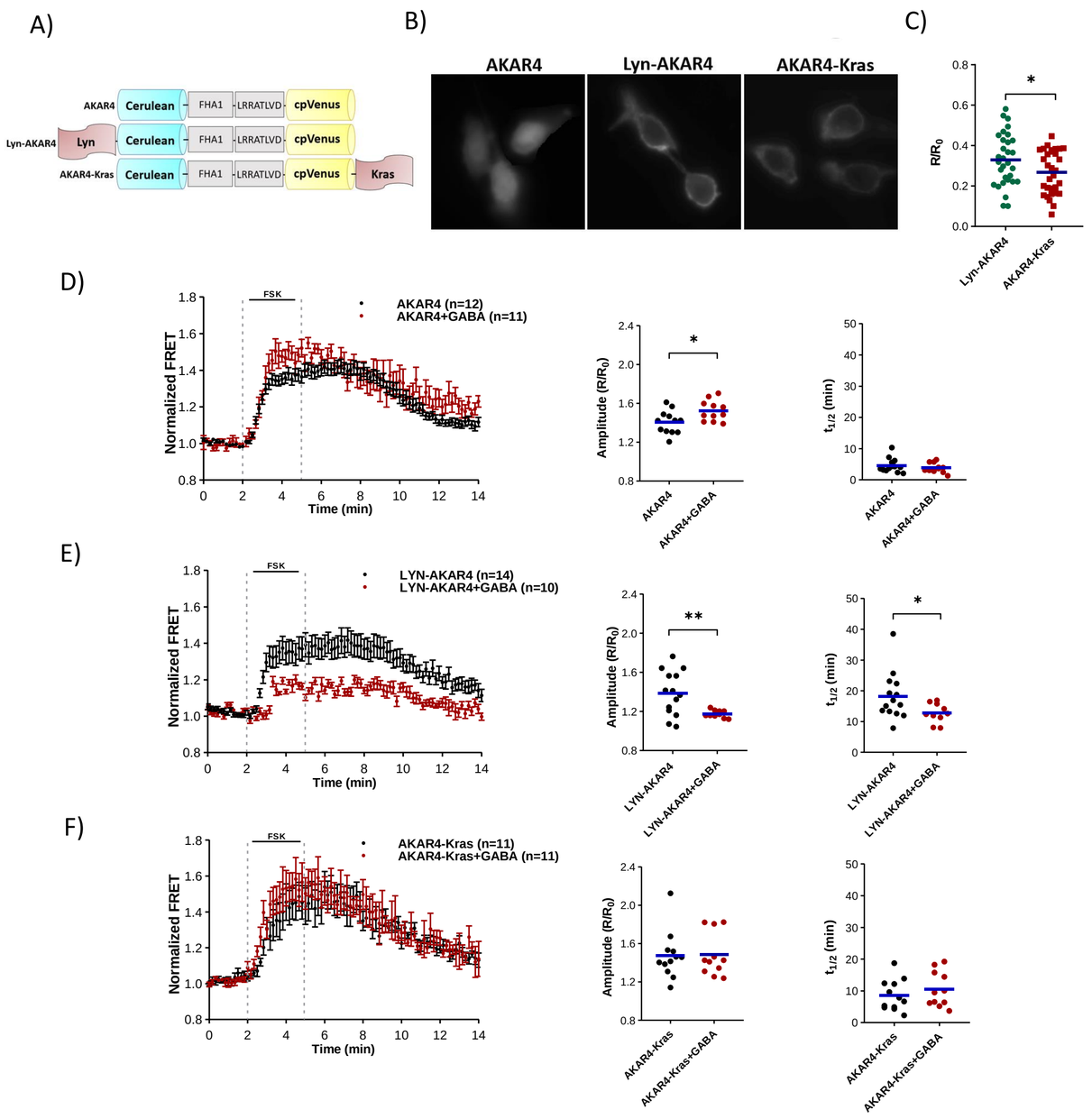


Fig.4. (A) Schematic of AKAR4, Lyn-AKAR4 and AKAR4-Kras biosensors. AKAR4 is a FRET biosensor that exhibits increased signal upon phosphorylation of the LRRATLVD PKA peptide substrate. The Lyn-AKAR4 biosensor provides anchoring to lipid rafts of the plasma membrane via myristoylation and palmitoylation; the AKAR4-Kras biosensor provides targeting to non-lipid-rafts region through C-terminal prenylation sequences derived from K-ras. **(B) Grayscale CFP images of C6 cells expressing AKAR4 or Lyn-AKAR4 or AKAR4-Kras.** Scale bar: 100 µm. **(C) Varied basal PKA activity at the plasma membrane in C6 cells.** All data in scatter graphs are presented as average of Lyn-AKAR4 (n=22) and AKAR4-Kras (n=22) traces that were acquired for 2 min. **Average tracings (R/R_0±SEM) depict the soluble AKAR4 (D) or Lyn-AKAR (E) or AKAR4-Kras (F) response of cells treated with 10 µM of the direct adenylyl cyclase activator forskolin (FSK) alone or 10µM FSK and 200µM GABA for 3 min** (horizontal bars). The peak amplitude and half-time of signal decay ($t_{1/2}$) for individual tracings are presented in the scatter plots respectively; blue bars indicate mean. Datasets were compared by unpaired t tests (for two groups) or one-way ANOVA (for three groups). *p ≤ 0.05, **p ≤ 0.01.

Main conclusions

Visualization of GAT-3 demonstrated its predominant location to lipid rafts and a strong colocalization with PMCA4, the main PMCA isoform found in C6 astrocytic cells. Interestingly GABA-mediated signal transduction is strictly associated with spatial-temporal compartmentalization of PKA activity and an increase of GAT3-dependent intracellular Ca^{2+} signals in astrocyte-like C6 cell. Further studies using advanced microscopy and molecular biology techniques will prove how Ca^{2+}/PKA crosstalk triggered by GABA uptake can modulate gliotransmission. Understanding of how astroglia responds to inhibitory events mediated by GABA in the central nervous system is of paramount importance for developing an efficient therapeutic approach for many brain diseases.