MEDICAL UNIVERSITY OF LODZ

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The role of compartmentalized cAMP/PKA signaling in neuronal survival and regeneration following optic nerve axotomy

Introduction

Ischemic optic neuropathy, a kind of white matter stroke, is the most prevalent type of acute optic neuropathy in people older than 50 years of age. In clinical practice it is a devastating source of irreversible vision loss. Recent evidence suggests that retinal ganglion cells (RGCs), like other neurons of the central nervous system (CNS), die for two main reasons: they are cut off from target-derived trophic signals, and they lose responsiveness to such signals. A major goal of our research is to define the signaling pathways defective in RGC neurons following ischemic injury, so that novel therapeutic regimens may be rationally designed. It is known that tropic responsiveness can be rescued by electrical activity or by elevating intracellular levels of second messenger cyclic AMP (cAMP). Enhanced survival and regeneration elicited by cAMP depends on activation of the cAMP-dependent protein kinase A (PKA) but the source of cAMP and the signal transduction pathways downstream of PKA remain largely unstudied, limiting inroads for therapeutic interventions. Within cells, cAMP, PKA and many other signaling enzymes are organized by scaffold proteins called A-kinase anchoring proteins (AKAPs) into multimolecular signalosomes that confer fidelity and specificity to downstream signaling. Localized to different intracellular compartments, AKAPs permit activation of specific pools of PKA by individual adenylyl cyclases (ACs) in response to upstream stimuli, resulting in the phosphorylation of PKA substrates critical for selected cellular processes. We will test the novel hypothesis that mAKAPα-mediated compartmentation of signaling is critical to the regulation of neuronal survival and axon growth and to the neuronal response to ischemic axon injury.

Aim 1 — **Adenylyl Cyclase Function in Neurons**

Aim 2 — **Characterization of the Signaling Pathways that Crosstalk with cAMP to Promote Neuronal Survival and Neurite Outgrowth**

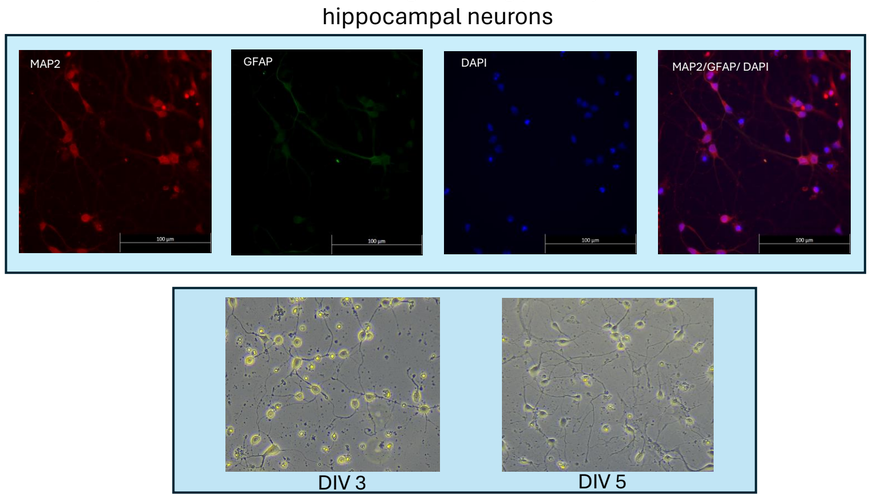
Aim 3 — **Targeting of perinuclear cAMP signaling as a therapeutic approach for RGC protection and optic nerve regeneration in vivo**

Methods and achievements

Western Blot technique was used to confirm the presence of specific adenyl cyclases in the retina. Subsequently, co-immunoprecipitation and western blotting were performed to determine which adenylyl cyclases binds to mAKAPα in the retina. To confirm the interaction between AKAP6 and specific ACs in neurons, cultures of primary hippocampal neurons were transduced with lenti_shRNA-mAKAPα or lenti_scrambled shRNA (serving as negative controls) and co-immunoprecipitation and Western blotting were performed.

It is expected that specific ACs will be found to contribute to cAMP levels in different neuronal compartments. For now, primary neurons were transduced with the recombinant AAV2 virus (pAAV[shRNA]-EGFP-U6>mAdcy10) for silencing of mouse adenylyl cyclase 10 and the level of silencing of adenylyl cyclase bound to AKAP in the perinuclear compartment was confirmed by Co-IP and Western Blotting. Next, cAMP/PKA transients in the perinuclear compartment will be assayed using the FRET-based AKAR4-nesprin1α-fused sensor, and the results will be compared with those obtained with soluble AKAR4. This will allow determination of how external signals elevate global vs. compartmentalized cAMP transients in single neurons by individual ACs.

Optimization of the method for generating primary cultures of mouse hippocampal neurons



Results

Determining which adenylyl cyclases are present in retina and bind mAKAPα

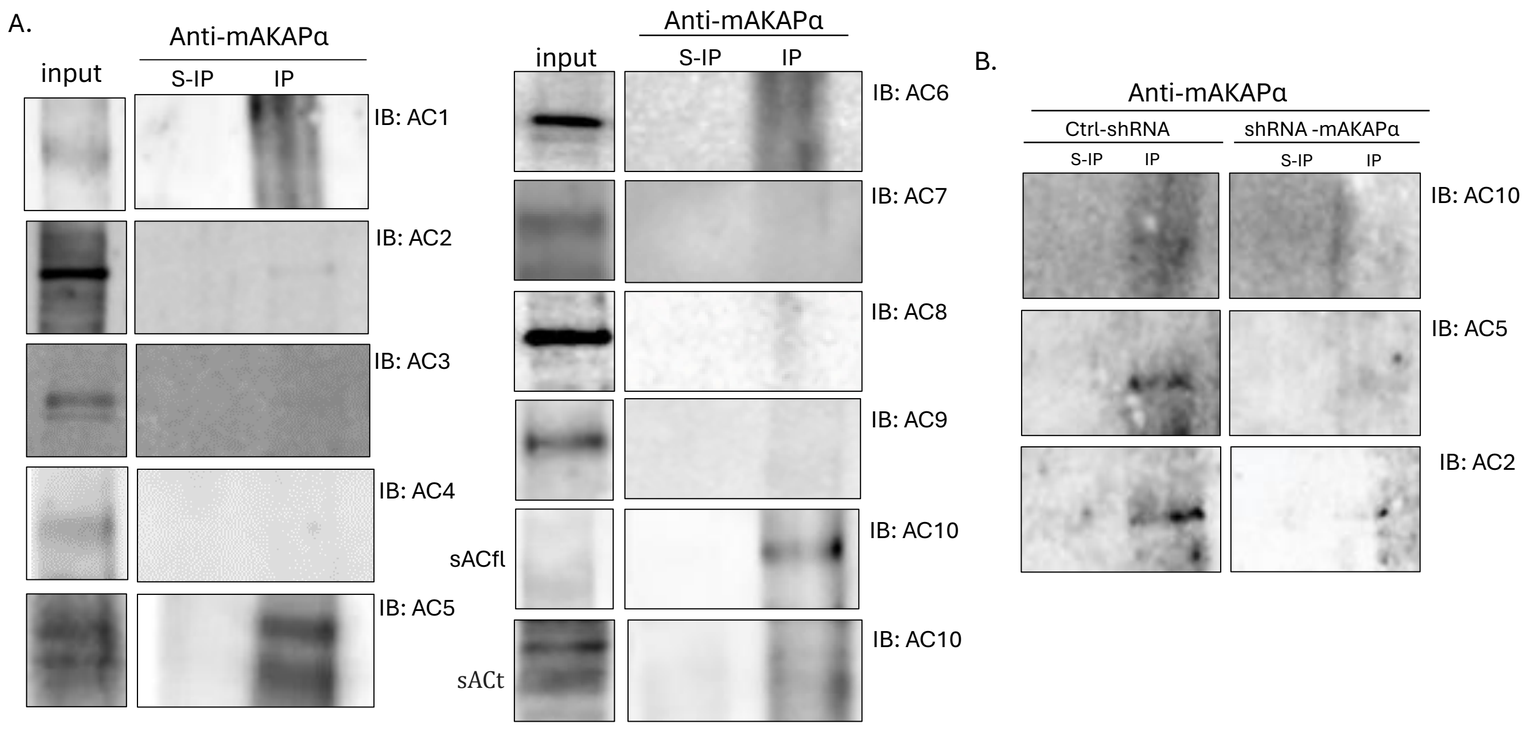


Fig.1 Co-immunoprecipitation (Co-IP) assays showing interactions between mAKAPα and adenylyl cyclase 2 (AC2), adenylyl cyclase (AC5), soluble adenylyl cyclase 10 (truncated sAC and a full-length sAC) in retina and hippocampal neurons . Retinal protein extracts (input) were immunoprecipitated with mAKAP antibody and followed by Western Blot analysis. Protein-protein interactions were immunodetected using AC1-10 antibodies (A). Cultures of primary hippocampal neurons were transduced with lenti_shRNA-mAKAPα or lenti_scrambled shRNA (serving as negative controls) and interaction between mAKAP and AC2, AC5 and sACfl were performed by Co-IP experiments using mAKAPα antibodies and visualized by Western Blot analysis using AC2, AC5, AC10 antibodies (B).

Confirmation of silencing of adenylyl cyclase in mouse primary hippocampal neurons

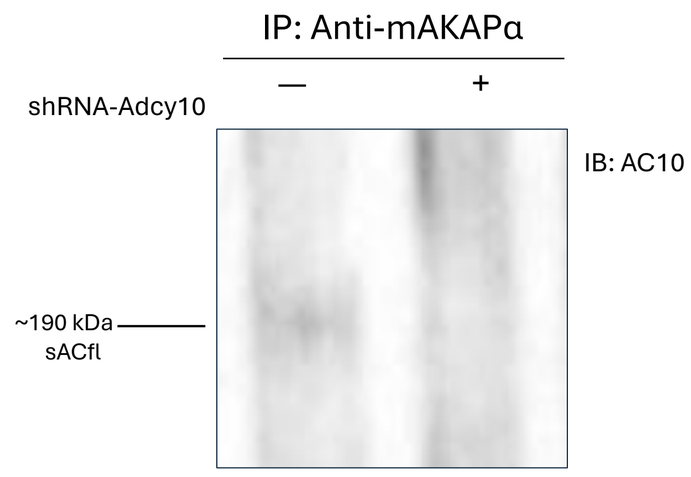


Fig.2 Co-immunoprecipitation (Co-IP) assays showing silencing of adenylyl cyclase in primary hipocampal neurons transducted with recombinant AAV2 virus. Cultures of primary hippocampal neurons were transduced with recombinant AAV2 virus pAAV[shRNA]-EGFP-U6>mAdcy10 or pscAAV[shRNA]-EGFP-U6>Scramble_shRNA (negative control) . Silencing od adenylyl cyclase were performed by Co-IP experiments using mAKAPα antibodies and visualized by Western Blot analysis using AC10 antibodies.

Conclusion

In summary, our investigation has confirmed the presence of transmembrane adenylyl cyclases 1-9 (AC1-9) and soluble adenylyl cyclase 10 (sAC, AC10) in the mouse retina. Moreover, our findings suggest that mAKAPα, situated perinuclearly within the retina, forms complexes with AC2, AC5, soluble adenylyl cyclase (sAC, AC10). Moving forward, our focus will be on elucidating the mechanisms through which these specific adenylyl cyclases induce cAMP-dependent survival signaling within the central nervous system (CNS). The refinement of methodologies planned for this academic year will facilitate the execution of further experiments aimed at achieving this objective. Ultimately, our overarching goal is to advance our understanding of cAMP and AKAP-mediated signaling pathways, particularly regarding their role in regulating neuronal survival and regeneration. By uncovering the intricacies of compartmentalized signaling, we aim to pave the way for the development of novel therapeutic strategies aimed at bolstering neuronal survival and enhancing the regenerative response following ischemic axonal injury, not only within the optic nerves but throughout the entire CNS.