**Calcium imaging of cerebellar granular neurons in culture acutely treated with cerebrospinal fluid of patients with neurodegenerative diseases**

Andjela Laudanović1, Aleksandra Antić1, Aleksa Palibrk2, Pavle Andjus1, Zorica Stević2, David Lutz3, Milena Milošević1

*1 Center for Laser Microscopy, Institute for Physiology and Biochemistry “Jean Giaja”, Faculty of Biology, University of Belgrade, Serbia*

*2 Neurology Clinic, Clinical Center of Serbia, School of Medicine, University of Belgrade, Serbia*

*3 Department of Neuroanatomy and Molecular Brain Research, Ruhr University Bochum, Bochum, Germany*

e-mail:andjela.laudanovic@bio.bg.ac.rs

Neurodegenerative diseases pose a significant burden to patients and their families, and many of them are diagnosed in later stages when most available treatments only alleviate the symptoms. The whole medical field is in the constant need for reliable biomarkers that would enable early diagnostics. Cerebrospinal fluid (CSF) is routinely obtained as a part of a standard diagnostic procedure. As CSF is in contact with neurons that degenerate during the disease, it is thought to contain toxic factors that influence the disease [1]. Here we implemented calcium imaging of cerebellar granular neurons in order to investigate calcium signaling evoked by CSF of patients with different neurodegenerative diseases.

Cerebellar granular neurons were isolated from 5-7 days old *Wistar* rats. Disease diagnostics and CSF sampling was conducted at the Clinic of Neurology, Clinical Center Serbia in Belgrade. Calcium transients evoked by CSF were evaluated utilizing Fluo 4-AM, a calcium-sensitive indicator that changes the intensity of fluorescence depending on the concentration of free calcium ions in the cytosol. To assess the origin of calcium influx to the cytosol, and signaling pathways involved in this process, several blockers of ion channels, receptors or enzymes were used.

CSF of patients with neurodegenerative diseases (amyotrophic lateral sclerosis, progressive supranuclear palsy, and spinal muscular atrophy type III) evoke strong calcium transients in cerebellar granular neurons, and this reaction is dose–dependent. Moreover, neurons respond to repeated CSF treatment with similar calcium transients. External calcium is necessary for the response to CSF. The amplitude and shape of the calcium transients were greatly modified by the use of lanthanum (La3+), which blocks voltage-gated calcium channels [2], but also influences the connexin hemichannels [3]. Depending on the CSF sample and the type of disease, lanthanum lowered the amplitude of CSF evoked calcium transients ~30-95%. It is interesting to note that nifedipine, which specifically blocks L-type calcium channels, negligibly influenced CSF evoked calcium transients. 2-APB, which blocks the IP3 receptors on the endoplasmic reticulum, and ligand-gated TRP channels mostly located on the cell membrane, lowered the amplitude ~20%. Ryanodine, a blocker of calcium channels on the endoplasmic reticulum, lowered the amplitude ~16%. U73122, a blocker of phospholipase C, lowered the amplitude ~12%. On the other hand, blockade of glutamate receptors (AMPA/kainate and NMDA), as well as voltage gated sodium channels, did not influence the amplitude of CSF evoked calcium transients.

Calcium imaging of CSF evoked transients in neurons could be utilized as a part of the complex analysis (ROS imaging, evaluation of inflammation, etc.) that could lead towards the complementary differential diagnostics and/or evaluation of the effectiveness of specific drugs or their combination in a patient-personalized manner.

REFERENCES

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