

# ASTROCYTE RESPONSE UNDER MODIFIED RESTRAINT WATER IMMERSION STRESS AND ALPHA-KETOGLUTARATE SUPPLEMENTATION

Olena Dovban, Galyna Ushakova

Department of Biochemistry and Physiology, Oles Honchar Dnipro National University, Ukraine  
[dovbanelena@gmail.com](mailto:dovbanelena@gmail.com)

Stress research is one of the relevant issues of modern biology and medicine. Stress is a condition of the organism that responds to various types of exogenous and endogenous effects that lead to physical and psychological dysfunctions [1]. The most widely used experimental stress model is the restraint water-immersion stress (RWIS), a compound stress model that includes both psychological and physical stimulations [2, 3]. Several stressors, such as exposure to temperature, biorhythmic changes, and restriction, often work in combination. The reaction of glial cells that support neuron function under stress is actual today. Astrocytes behavior can be evaluated by the level of specific proteins - Ca<sup>2+</sup>-binding protein S-100b and glial fibrillary acidic protein (GFAP).

Our study aimed to investigate the changes in the levels of glial fibrillary acidic protein (GFAP) and Ca<sup>2+</sup>-binding protein S-100b in different rat's brain areas under modified RWIS condition, during physiological recovery and alpha-ketoglutarate supplementation.

The brains of 24 Wistar rats were used for investigation. Animals were kept under standard vivarium conditions, and all manipulations were performed according to the animal protection convention used in the experiments. The experimental model was created within 3 days using prolonged and modified restraint water-immersion stress [4]. Animals were divided into four groups (n = 6). 1 - rats of the control group; 2 - rats subjected to modified RWIS for 3 days using a stress protocol with immersion in water with changing of the temperature and under permanent light, 3 - rats that were kept under normal conditions for 14 days without stress factors after 3days RWIS, 4 - rats receiving 2% alpha-ketoglutarate with drinking water during 14 days after stress exposure. The animals were decapitated under mild anesthesia; four brain areas were isolated: cerebellum, visual cortex, thalamus, and hippocampus. Quantification of S-100b and GFAP was performed according to the competitive ELISA. The results were statistically processed using one-way ANOVA followed by post-hoc Tuckey test to compare different groups. Data at P<0.05 were considered as significant.

It was shown that under modified RWIS exposure the level of S-100b protein increased in cerebellum by 61% compared to control. In other brain areas were no significant changes after RWIS. In the thalamus, visual cortex and hippocampus increase of the level of S-100b protein was shown in the group with 14 days of the physiological recovery by 97%, 81% and 78% compare to control animals. Obtained data of the S-100b level indicate a delayed effect of modified RWIS.

The GFAP level was shown with 31% deficit in the hippocampus, an 18% increase in the thalamus, and a 23% increase in the visual cortex after 3-days stress exposure.

Supplementation for 14-days with 2% alpha-ketoglutarate dissolved in drinking water had a positive impact on astroglial cells after exposure to 3-days stress.

Previous data from our lab had shown the positive impact of alpha-ketoglutarate on the astrocyte condition with age-dependent changes in GFAP concentration [5], and under ischemic conditions [6].

The findings suggest that alpha ketoglutarate as a natural compound may serve as a protector against stress impact.

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[1] McEwen, B. S. Mechanisms of Stress in the Brain. *Nature neuroscience*, 18(10), 1353–1363. (2015)

[2] Sun, H., Li, R., Xu, S., Liu, Z., & Ma, X. Hypothalamic Astrocytes Respond to Gastric Mucosal Damage Induced by Restraint Water-Immersion Stress in Rat. *Frontiers in behavioral neuroscience*, 10, 210. (2016).

[3] Fan F., Yang M., Geng X., Ma X., Sun H. Effects of Restraint Water-Immersion Stress-Induced Gastric Mucosal Damage on Astrocytes and Neurons in the Nucleus Raphe Magnus of Rats via the ERK1/2 Signaling Pathway. *Neurochem Res.* 44:1841–1850. (2019).

[4] Ushakova GO, Dovban OO. Method of modeling of acute combined water-immobilization stress with change of light biorhythm. UA Patent 133796, 25 Apr 2019

[5] Kovalchuk YP, Ushakova HO. The changes of glial fibrillary acid protein level in the different brain areas of gerbils under development, aging and alpha-ketoglutarate effect. *Med Clin Chem.* 1, 29-35 (2016).

[6] Kovalenko T.N., Ushakova G.A., Osadchenko I., Skibo G.G., Pierzynowski S.G. The neuroprotective effect of 2-oxoglutarate in the experimental ischemia of hippocampus. *J Physiol Pharmacol.*; 62, 239–46. (2011).