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7th CONGRESS OF SERBIAN NEUROSCIENCE SOCIETY
with international participation

BOOK OF ABSTRACTS

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Appearance of second ecto-5'-nucleotidase isoform during experimental autoimmune encephalomyelitis is caused by changes in glycosylation pattern

A24

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Introduction. Ecto-5'–nucleotidase (eN) catalyzes terminal step of extracellular ATP hydrolysis, producing anti-inflammatory adenosine. We reported significantly increased eN activity in lumbar spinal cord during experimental autoimmune encephalomyelitis (EAE), together with increased protein expression connected mainly with reactive astrocytes and appearance of new isoform at ~75kDa at the peak of the disease, besides usual ~71kDa isoform. Since eN is glycoprotein with five potential *N*-glycosylation sites and predicted molecular weight of 57-59 kDa, we hypothesized that occurrence of second isoform during EAE is due to changes in glycosylation pattern, possibly affecting kinetic properties of the enzyme. **Methods.** Lumbar parts of the spinal cords were obtained from Dark Agouti rats at the onset (Eo), peak (Ep) and the end of symptoms (Er) during EAE and from naïve control animals (C). **Results**. We here report significant changes of kinetic properties regarding AMP-hydrolysis during EAE, with almost 50% increase of maximal velocity at Ep (92.35±1.86nmolPi/min/mg) and Er (90.68±2.17nmolPi/min/mg), compared to C, whilst Km increased double at Ep (0.041±0.003mmol/l). Enzymatic deglycosylation caused triple decrease of Vmax (33.6±1.8nmolPi/mg/min) at Ep, and double decrease of Km (0.022±0.008mmol/l), whilst immunoblot probed with anti-eN antibody revealed triple protein band at ~60kDa at all investigated time-points. **Conclusion.** Our results show that changes of kinetic properties during EAE, at least partially, are governed by modification of glycosylation pattern. Also, appearance of new isoform at the peak of EAE is direct consequence of glycosylation changes. In summary, besides gene and protein expression changes of eN, glycosylation might be additional route of inflammation control conducted by astrocytes.

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