RELEASE-REGULATING SPHINGOSINE-1-PHOSPHATE RECEPTORS IN NERVE ENDINGS AND GLIAL PARTICLES OF MICE CENTRAL NERVOUS SYSTEM

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Introduction: Multiple sclerosis (MS) is an autoimmune inflammatory disease with unknown etiology. Besides overt demyelination and inflammation, diffuse loss of synaptic contacts and astrocytosis are observed in the central nervous system (CNS) of patients suffering from MS and in mice suffering from experimental autoimmune encephalomyelitis (EAE mice), compatible with the role of astrocytes and neurons in the onset and the progression of the disease. Data exists in literature showing that sphingosine-1-phosphate receptors (S1PRs), which represent the targets of disease modifying therapies (DMTs) for MS, including fingolimod, siponimod, ponesimod and ozanimod, exist in neurons and astrocytes, but their contribution to control chemical transmission is far to be elucidated. By using isolated nerve endings (synaptosomes) and glial particles (gliosomes) we investigated the existence and the role of S1P receptors as modulator of glutamate exocytosis.

Material and methods: Synaptosomes and gliosomes were purified from the cortex of adult male mice (C57BL/6J strain), preloaded with [3H] D-aspartate ([^{3}H]D-Asp), an unmetabolizable analogue of glutamate that allows a reliable measure of glutamate exocytosis, and superfused to monitor the tritium release. After 39min of superfusion to equilibrate the system, synaptosomes or gliosomes were exposed to a mild depolarizing stimulus (i.e. 15mM KCl containing medium) in the absence or in the presence of S1P1, S1P3and S1P5receptor ligands, namely CS2100, CYM5541, A971432.

Results: The results unveiled that CS2100, the S1P1selective agonist slightly although significantly inhibited glutamate exocytosis when added in the nanomolar range. The agonist failed to affect significantly the release of [^{3}H]D-Asp from cortical gliosomes. Western blot analysis of cortical synaptosomal lysates unveiled the presence of a clear immunopositivity for the S1P1, the S1P3and S1P5receptor proteins with an appropriate mass, compatible with the hypothesis that also S1P3and S1P5receptors could be present in these particles.

Conclusions: Our findings could give the rationale of the use of fingolimod and derivatives in central diseases typified by altered glutamate transmission.