Abstract 1250

Mouse astrocytes exhibit agonist-induced functional S1P1 receptor antagonism

Type: Poster Presentation

Keyword: Disease Modifying Therapies – Mechanism of Action

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Background

Sphingosine-1-phosphate (S1P) receptor subtype 1 ($S1P_1$) plays a key role in regulation of lymphocyte trafficking. In multiple sclerosis patients, $S1P_1$ agonists, such as fingolimod or siponimod, inhibit the egress of pathogenic lymphocytes from lymph nodes and their infiltration into the central nervous system (CNS) via lymphocyte-expressed $S1P_1$ receptor down-modulation, also known as $S1P_1$-functional antagonism. However, there is no evidence of this phenomenon in cells of the CNS.

Objectives

To assess the presence of agonist-induced $S1P_1$ down-modulation in astrocytes using a calcium (Ca^{2+}) signaling assay.

Methods

Murine astrocytes (C8-D1A) were incubated overnight and then loaded with a probe (Fluo-4AM, a dye that becomes fluorescent upon Ca^{2+} binding) for 1 hour, followed by adenosine triphosphate (ATP; 10 µM) over 30 min to activate the Ca^{2+} pumps. The cells were then treated with various $S1P_1$ agonists (S1P [natural ligand for S1P receptors], AUY954 [selective $S1P_1$ agonist], fingolimod [$S1P_{1,3,4,5}$ agonist] or siponimod [$S1P_{1,5}$ agonist]) at different concentrations (0.0001 µM up to 30 µM) to construct dose-response curves using agonist-induced Ca^{2+} signaling, measured as an increase in the intracellular fluorescence (via a FLuorescent Imaging Plate Reader [FLIPR]). To investigate the S1P down-modulation, the astrocytes were pretreated overnight with $S1P_1$ agonists (1 µM) prior to probe loading, ATP priming, and agonist stimulation.

Results

All of the tested $S1P_1$ agonists increased intracellular Ca^{2+} influx in the astrocytes in a dose-dependent manner, with concentration inducing half-maximal effect (EC_{50}) within the range of 2–5 nM. Similar results were obtained after overnight pretreatment with the natural ligand, S1P ($S1P_{1,2,3,4,5}$ agonist), confirming that S1P does not induce down-modulation of its own receptors.However, cells pretreated overnight with AUY954 did not exhibit agonist-induced intracellular Ca^{2+} signaling, suggesting the down-modulation of the $S1P_1$ receptors. Similar outcomes were observed upon pretreatment with either fingolimod or siponimod, indicating their role as functional $S1P_1$ antagonists on the murine astrocytes.

Conclusions

To our knowledge, this is the first report of agonist-induced $S1P_1$ down-modulation in the astrocytes. Additional investigations on other neuronal and glial cells are warranted to establish whether this is a generalized phenomenon in the CNS.

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