**Adenosine $A_{2B}$ receptors and sphingosine kinase/sphingosine 1-phosphate signaling axis control maturation of oligodendrocyte precursor cells in vitro.**

A.M. Pugliese, Dept. of Neuroscience, Psychology, Drug Research and Child Health NEUROFARBA- Section of Pharmacolog University of Florence, Florence A.M.
I. Fusco, Dept. of Neuroscience, Psychology, Drug Research and Child Health NEUROFARBA- Section of Pharmacolog University of Florence, Florence
F. Cencetti, Dept. of Experimental and Clinical Biomedical Sciences University of Florence, Florence
I. Dettori, Dept. of Neuroscience, Psychology, Drug Research and Child Health NEUROFARBA- Section of Pharmacolog University of Florence, Florence
L. Gaviano, Dept. of Neuroscience, Psychology, Drug Research and Child Health NEUROFARBA- Section of Pharmacolog University of Florence, Florence
C. Donati, Dept. of Experimental and Clinical Biomedical Sciences University of Florence, Florence
P. Bruni, Dept. of Experimental and Clinical Biomedical Sciences University of Florence, Florence
D. Catarzi, Dept. of Neuroscience, Psychology, Drug Research and Child Health NEUROFARBA- Section of Pharmaceut University of Florence, Florence
E.Coppi, Dept. of Neuroscience, Psychology, Drug Research and Child Health NEUROFARBA- Section of Pharmacolog University of Florence, Florence
F. Pedata, Dept. of Neuroscience, Psychology, Drug Research and Child Health NEUROFARBA- Section of Pharmacolog University of Florence, Florence

Oligodendrocyte progenitor cells (OPCs) are present throughout the adult brain and spinal cord and can replace oligodendrocytes lost due to injury, aging or disease such as multiple sclerosis (MS), the most frequent demyelinating disease in the Central Nervous System (CNS). Remyelination does occur, but is limited especially in chronic disease stages. Multiple causes seem to contribute to such transient decline, including the failure of OPCs to differentiate and enwrap the vulnerable neuronal axons. Thus, OPCs are a viable target for MS clinical therapy. A number of pathways have been identified that may contribute to ameliorate/impair remyelination in MS lesions, among them adenosine and sphingosine kinase/sphingosine 1-phosphate signaling axis (SphK/S1P). OPCs express each of the different adenosine receptor subtypes (A1, A2A, A2B and A3) at all maturational stage1. To date, a functional role has been attributed only to A11,2,3 and A2A receptors,1,2,4 whose stimulation modulates OPCs proliferation, differentiation, migration and ionic channels activity. S1P, produced by the action of SphK (two isoforms: SphK1 and SphK2), is a bioactive lipid that regulates remyelination and cell injury. An unexpected finding is that fingolimod (FTY720), approved as orally active drug for relapsing MS, modulates S1P receptors. A relationship between SphK1 activity and A2B adenosine receptor activation has been demonstrated in mouse and human normal and sickle erythrocytes in vitro5. In this work the role of adenosine A2B receptors and SphK/S1P signaling on oligodendrogenesis in rat cultured OPCs, at different times of maturation, was investigated.

To this aim patch clamp experiments coupled to Real-time PCR and Western Blot analysis were carried out. Stimulation of A2B receptors reduced the amplitude of outward currents elicited by a voltage ramp protocol. These currents were abolished when K+ was replaced by equimolar Cs+, indicating that, in OPCs, ramp-evoked outward currents are K+ currents. In particular, BAY60-6583 (0.1-30 μM, n=43), a selective A2B agonist, reduced the amplitude of outward currents in a concentration dependent manner. This effect was prevented by the A2B antagonists MRS1706 (10

µM, n=5) and was reduced by VPC96047 (500 nM, n= 4), a pan-SphK inhibitor. Similarly, FTY720 phosphate (1 µM, n=4), the active metabolite of FTY720, mimicked and partially occluded the effect of 10 µM BAY60-6583 on ramp-evoked current. In cultured OPCs, SphK1 phosphorylation was enhanced after acute (10 minutes) treatment with 10 µM BAY60-6583, demonstrating an interaction between SphK/S1P pathway and A2B activation. Finally, chronic A2B stimulation (incubation for 6 days with 10 µM BAY60-6583) reduced the expression of mature oligodendrocyte markers, as determined by Real-time PCR analysis, indicating an involvement of this pathway in OPC maturation.

Our findings reveal that novel pathways activated by adenosine A2B and SphK/S1P are involved in the maturation of OPCs.

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