The selective stimulation of adenosine A_{2B} receptors decreases outward K^+ currents and increases sphingosine kinase 1 activity in primary purified oligodendrocyte precursor cell cultures

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Current available Multiple Sclerosis (MS) therapies target immune modulation with some efficacy, however, concomitantly with adverse side effects. Oligodendrocytes are the myelin-producing cells in the CNS. Damaged oligodendrocytes no longer generate myelin and remyelination requires generation of new mature oligodendrocytes from the differentiation of oligodendrocytes precursors cells (OPCs). As the disease progresses, this fundamental process fails. 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 treatment target for MS clinical therapy.
A number of pathways have been identified that may contribute to ameliorated/impaired remyelination in MS lesions, among them adenosine (and its receptors A_1, A_{2A}, A_{2B} and A_3) and sphingosine kinase/sphingosine 1-phosphate signaling axis (SphK/S1P). It is known that OPCs express each of the different adenosine receptor subtypes at all maturational stage[1]. To date, a functional role has been attributed only to A_1[1,2,3] and A_{2A} 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 pleiotropic sphingolipid mediator that has been implicated in oligodendrogenetic processes. A relationship between SphK1 activity and A_{2B} adenosine receptor activation has been demonstrated in mouse and human normal and sickle erythrocytes in vitro[5]. In this work the role of adenosine A_{2B} receptors and S1P/SphK signaling on oligodendrogenesis in rat cultured OPCs, at different times of maturation, was investigated.
At this aim we used patch clamp experiments coupled to Real-time PCR and Western Blot analysis. Stimulation of A_{2B} 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 ramp-evoked outward currents are K^+ currents. In particular, BAY60-6583 (0.1-10 μM, n=25), a selective A_{2B} agonist, reduced the amplitude of outward currents. This effect was blocked by VPC96047 (1 μM, n=3), a pan-SphK inhibitor, and inhibited in the presence of the selective A_{2B} antagonists PSB603 (10 μM, n=3) and MRS 1754 (0.5-1 μM, n=6). Similar effects to those observed in the presence of BAY60-6583 were recorded by applying the newly synthetized A_{2B} agonist, P453 (50 nM, n=6). In cultured OPCs, SphK1 phosphorylation was enhanced after 10 minutes treatment with 10 μM BAY60-6583, demonstrating that SphK1 is involved in the action mechanism downstream of A_{2B} activation. Finally, the inhibition of SphK by VPC96047 (1 μM) reduced the expression of mature oligodendrocyte markers after 6 days of cell differentiation, as determined by Real-time PCR analysis, indicating an involvement of this pathway in OPC maturation.
Our findings reveal a novel signaling of adenosine A_{2B} and SphK1, which may be involved in maturation of OPCs.

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