ABSTRACT

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Title of diploma thesis: Microglia control adenosine A_{2A}-receptor mediated astrogliosis

In the central nervous system, astrocytes and microglia are the main cells coordinating the inflammatory response. During inflammation, dying or temporarily damaged cells release ATP, as a danger-associated signal molecule, that contributes to the induction of astrogliosis and promotes clearance of the debris by immune cells such as microglia. Adenosine that results from ATP metabolism also stimulates astrogliosis. However, the effects of adenosine on astrogliosis may be more complex, since it also modulates microglia phenotype and microglia have been shown to prevent excessive astroglial proliferation mediated by nucleotides. In this context, ATP and adenosine are assumed as relevant signalling molecules in the control of astrogliosis and its modulation by microglia. However, it is still unknown whether and how microglia modulate adenosine-mediated astrogliosis. The present study aims to clarify the impact of microglia in the control of adenosine-induced astrogliosis.

Two types of primary glial cultures were prepared from cortical hemispheres of newborn rats (age: 0-2 days): co-cultures of astrocytes containing approximately 15% of microglia and "pure" cultures of astrocytes, where microglia were almost absent (< 1%). These cultures were used to evaluate the effect of P1 agonists on methyl-[^{3}H]-thymidine incorporation and to evaluate A_{2A} receptors expression by Western blot.

In "pure" cultures of astrocytes adenosine (0.001-0.3 mM) increased astroglial proliferation up to 172 ± 5% ($n=7$; $P<0.05$), but the effect was attenuated to 131 ± 5% ($n=5$; $P<0.05$) by 30 nM of the selective A_{2A} antagonist SCH 58261 or to 125 ± 6%

(n=5; P<0.05) by 10 nM of the selective A_{2B} antagonist MRS 1706. The selective agonists of A_{2A} receptor CGS 21680 (1-100 nM) induced astroglial proliferation up to 155 ± 3% (n=4; P<0.05), while the A_1 agonist CPA (1-100 nM) and the A_3 agonist 2-Cl-IB-MECA (1-100 nM) had no effect. Furthermore, the proliferative effect of adenosine (100 microM; 179 ± 4%; n=5, P<0.05) was attenuated to 107 ± 7% (n=3; P<0.05) by inhibition of protein kinase A (PKA) with 1 μM of H-89 and to 120 ± 6% (n=4, P<0.05) by inhibition of mitogen-activated protein kinase kinase 1/2 (MEK1/2) with 10 μM of U0126.

In co-cultures, the proliferative effects induced by adenosine and CGS 21680 (concentrations as above) were lower than those obtained in “pure” cultures. Adenosine increased the proliferation to 142 ± 8% (n=4; P<0.05) and CGS 21680 to 126 ± 5% (n=4; P<0.05).

Western blot indicated that A_{2A} receptors are expressed either in pure cultures of astrocytes and in co-cultures being present in both types of cells.

The results show that astroglial proliferation induced by adenosine is mediated by A_{2A} and A_{2B} receptors coupled to the intracellular PKA-ERK pathway and this effect can be attenuated by microglia.