ACTIVATION OF GROUP II METABOTROPIC GLUTAMATE RECEPTORS PREVENTS THE DIFFERENTIATION OF CULTURED NEURAL PROGENITOR CELLS ISOLATED FROM THE MOUSE SUBVENTRICULAR ZONE INTO MATURE ASTROCYTES

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Neural progenitor cells isolated from the mouse subventricular zone (SVZ) of postnatal day 4-5-old mice, and cultured as neurospheres under non differentiating conditions (i.e. in the presence of mitogens) expressed functional metabotropic glutamate 2/3 (mGlu2/3) receptors. Plating of neurospheres on poly-ornitine coated-dishes, and culturing in the presence of fetal calf serum (FCS) and in the absence of mitogens, for 7 days, induced differentiation into astrocytes (80%), neurons (5-10%), and oligodendrocytes (5-10%). As mGlu3 receptors are highly expressed by glial cells, we focused on the possible role of these receptors in the differentiation of neurospheres into mature astrocytes, which can be identified in cultures by a flat polygonal morphology and staining for the Glial Fibrillary Acid Protein (GFAP). Pharmacological activation of mGlu2/3 receptors with the selective agonist, LY379268 (100 nM, administered every other day to cultures starting from day 1 of the differentiation protocol) induced a dramatic change in the morphology of $GFAP^+$ cells, which appeared as elongated bipolar/tripolar cells similar to $GFAP^+$ progenitors in zones of active neurogenesis in the adult brain. Co-treatment with either the mGlu2/3 receptor preferential antagonist LY341495 (100nM) or the cell permeable, non degradable, cAMP analogue, 8-Br-cAMP(1mM), greatly reduced the change in morphology elicited by LY379268, suggesting that activation of mGlu2/3 receptors affects glial cell differentiation through a Gi-coupled signalling pathway. Mature astrocytes are mainly quiescent in culture. To assess whether activation of mGlu2/3 receptors induced any difference in the proliferative activity of $GFAP^+$ cells, we challenged our cells with a pulse of 5-bromo-2'deoxyuridine (BrdU), which is incorporated into the DNA of dividing cells. Treatment with LY379268 increased the number of $BrdU^+$ bipolar/tripolar cells. This effect was reverted by the co-administration of LY341495. To assess whether, $GFAP^+$ elongated cells could be considered progenitor cells, we looked for the expression of Sox-1, a neural marker expressed by progenitor cells, but not by mature astrocytes. LY379268 induced the selective expression of Sox-1 in elongated $GFAP^+$ cells, an effect that was reverted by the co-administration of 8-Br-cAMP. Our data suggest that activation of mGlu2/3 receptors alters the differentiation of SVZ-derived neurospheres into mature astrocytes, inducing the appearance of cells similar to $GFAP^+$ progenitors detected in zones of active neurogenesis in the adult brain.