

INDIVIDUAL SUBTYPES OF METABOTROPIC GLUTAMATE RECEPTORS REGULATES NEURAL DIFFERENTIATION OF CULTURED MOUSE EMBRYONIC STEM CELLS

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Embryonic stem (ES) cells are pluripotent cells isolated from the pre-implantation embryo, that can be cultured in vitro as a self-renewing population, or can be induced to differentiate in cells of the three germ layers. Several differentiation protocols are available to generate neural progenitors from ES cells. These may be roughly divided into 2 main types: (i) differentiation mediated by the formation of floating aggregates, termed embryoid bodies (EBs); and (ii) differentiation in monolayer cultures, that skips the step of EB formation. Starting from our previous data showing the expression of metabotropic glutamate receptors (mGluRs) in both ES cells and EBs, we have studied the role of individual mGluRs in both models of differentiation. Floating EBs were used to study the role of mGluR4 (the only mGluR subtype expressed by these cells) during neural differentiation. Neural differentiation was induced by culturing EBs in the presence of 1 μ M retinoic acid for 4 days and subsequent plating on gelatine-coated dishes for 6 additional days. Addition of the selective group III mGluR agonist L-AP4 (30 μ M) during treatment with retinoic acid, increased the expression of β III-tubulin, a marker of differentiated neurons, but did not effect the expression of Glial Fibrillary Acidic Protein (GFAP) or chondroitin sulfate proteoglycan, NG2, that identify astrocytes and oligodendrocytes, respectively. To further characterize the neuronal phenotype enriched in cultures treated with L-AP4, we performed western blot and immunocytochemical studies using antibodies against: glutamic acid decarboxylase 65/67 (GAD 65/67), a marker of GABAergic neurons, Tyrosine Hydroxylase (TH), a marker of dopaminergic neurons, and Choline acetyltransferase (ChAT), a marker of cholinergic neurons. L-AP4 increased the number of GAD65/67⁺ cells, without affecting the expression of other differentiation markers. Monolayer cultures of ES cells were used to study the role of mGluR5 (the only mGluR subtype expressed by these cells) during neuronal differentiation. ES cells were induced to differentiate by plating on gelatine-coated dishes, in the presence of a chemically defined medium, for 6 days followed by re-plating onto fibronectin-coated dishes for additional 6 or 12 days. Pharmacological blockade of mGluR5, which is tonically activated by endogenous glutamate, with the selective non-competitive antagonist MPEP (1 μ M), increased the number of GAD 65/67⁺ cells, without affecting the number of TH⁺ cells. Our data show that sub-type selective ligands of mGlu5 and mGlu4 receptors may be used to optimise in vitro protocols aimed at implementing the number of cells differentiating towards a specific lineage.