THE NEURODIFFERENTIATIVE EFFECT OF ESTROGEN INVOLVES NUCLEAR RECEPTORS AND IS MEDIATED BY TGF-β

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Estrogen is known to affect neuronal maturation and differentiation in the central nervous system. The expression of estrogen receptors (ERs) is developmentally regulated, exhibiting a peak during early stages of neuronal development. In order to obtain an experimental model for neurodifferentiation, stem cells derived from the subventricular zone of adult mouse brain were grown as undifferentiated floating neurospheres in a chemically defined medium supplemented with EGF. Whole neurospheres were then induced to attach to the substrate and start to differentiate by EGF withdrawal and plating on a laminin-coated substrate. The time-course analysis of ERα expression in our model, revealed low basal levels in floating neurospheres and a strong and rapid induction of the protein upon plating, as shown by Western blot. Glial and neuronal populations derived from neurosphere differentiation following treatment with 10 nM 17β-estradiol (17β-E2) were analyzed by western blot, immunocytochemistry and flow cytometry. Exposure to 17β-E2 for 24h induced an increase in the expression of the astrocyte marker GFAP, as detected by western blot analysis. A more intensely GFAP-stained subpopulation expressing high levels of ERα and positive to BrdU uptake was measured by flow cytometry and double immunocytochemistry. 17β-E2 also accelerated the appearance of mature, MAP2-positive neuronal cells after 48h of treatment. Such effects were sensitive to the ER antagonist ICI 182,780, and mimicked by the ERα selective agonist PPT. Pre-treatment with the MAPK inhibitor PD98059 did not modify 17β-E2 effects on GFAP expression, excluding the involvement of rapid membrane signalling by estrogen. In addition, treatment with BSA-conjugated 17β-E2, which is unable to diffuse through the membrane and thus acts at the cell surface, failed to mimic 17β-E2 action. In agreement with these findings, analysis of MAPK1/2 activation after a 10- or 30-minute pulse with 17β-E2 did not show any variation of the phosphorylated form of the protein. The temporal sequence of effects of 17β-E2 on glial and neuronal populations may suggest an indirect neurogenic effect of estrogen through induction and release of astrocytic factors. Accordingly, 17β-E2 increased TGFβ1 expression at 24 h, as by western blot analysis, suggesting a main role for astrocytes in the neurodifferentiative activity of estrogen.