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# Direct effects of estrogens on cholinergic primary neurons from the human fetal nucleus basalis of Meynert

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Epidemiological studies have indicated that Alzheimer's disease (AD) is more common in females and that post-menopausal women are at increased risk than their male counterpart, thus suggesting that estrogens could play a protective role to counteract neurodegenerative processes (1). However, the mechanisms underlying this association remain to be clarified. Since the nucleus basalis of Meynert (nbM) is the major source of cholinergic innervation selectively vulnerable to degeneration in AD, our study is aimed at investigating the effects of estrogens on human cholinergic primary neurons (hfCNs) isolated from the nbM of 12-week old fetuses. The primary culture obtained was immunophenotyped with flow cytometry and resulted almost totally positive (97±2 %) for the neuronal marker MAP2 and for the choline acetyltransferase (ChAT). We demonstrated that hfCNs express receptors for hormones of the reproductive axis (ERs, LHR, GnRHR). In particular, besides to classical estrogen receptors (ERa and ERb), hfCNs express the transmembrane receptor GPR30, which is known to mediate rapid non-genomic estrogen actions. Increasing concentrations of 17-β estradiol (E2, 0.1-100 nM) determined a dose-dependent significant increase in cell number after 24h exposure, which was antagonized by tamoxifen treatment. In addition, E2 exposure determined a significant increase in ChAT expression, thus indicating a direct positive effect of E2 on cholinergic phenotype. Given that substantial evidence now indicates that estrogens exert an anti-inflammatory activity even in the central nervous system (2), we exposed hfCN cells to the proinflammatory cytokine TNFα. E2 treatment (1nM) was able to significantly counteract the TNFα-induced nuclear NF-kB p65 translocation. Interestingly, this effect was mimicked by G1, a GPR30 agonist, and abolished by pretreating cells with the GPR30 antagonist G15, but not by tamoxifen, which usually antagonizes classical ERs. Overall, our results indicate that estrogens exert direct neuroprotective mechanisms on hfCNs through the activation of either classical (trophic) and non-classical (anti-inflammatory) receptors.

### References

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