GLIA MEDIATES NEUROPROTECTION BY ESTROGEN AGAINST ß-AMYLOID TOXICITY: EFFECT AMPLIFIED BY ESTROGEN RECEPTOR OVEREXPRESSION

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Estrogen receptors (ERs) are expressed on neurons and estrogen exerts its neuroprotective effects acting directly at neurons. ERs are present also in glial cells and nanomolar concentrations of 17ß-estradiol (17ßE2) protect pure rat cortical neurons against ß-amyloid (Aß) toxicity through astrocytic neurotrophic factors. Thus, conditioned medium from cortical astrocytes previously exposed to 10 nM 17ßE2 reduced neuronal death induced by a 24-48 h-treatment with 25 µM Aß as detected by MTT assay and evaluation of apoptosis by flow cytometry. Glia-mediated neuroprotection by estrogen finds support in the overexpression of ERs detected under conditions of neuronal damage. In order to create a model of enhanced expression of ERs on glia, astrocytes were deprived of serum and grown in the presence of the culture supplement G5. This condition is known to induce morphological differentiation resembling reactive glia similar to that observed following neuronal insults. The expression of ERs was increased in G5-supplemented astrocyte cultures as by western blot analysis and flow cytometry (about 90-100% increase). Conditioned medium from astrocytes cultured in G5 supplement protected cortical neurons against Aß toxicity (as by MTT assay) similarly to conditioned medium from 17ßE2-treated astrocytes. However, when G5-grown astrocytes were treated with 17ßE2, the neuroprotective effect was significantly increased. Searching for a neurotrophic factor mediating the effect of 17ß-E2 in G5-supplemented cultures, attention has been focused on transforming growth factor ß1 (TGFß1) which has been involved in the neuroprotective effect of 17ßE2. These data identify G5-grown astrocytes as a reliable experimental model for the overexpression of ERs occurring during neuronal damage and suggest (i) a potential constitutive activity of ERs and (ii) a role for TGFß1 in 17ßE2. Western blot analysis revealed that TGFß1 concentrations were significantly increased in astrocyte cultures grown in G5 supplement in the presence of 10 nM 17ßE2. The ability of estrogen to exert neuroprotective activity was also evaluated using an alternative experimental paradigm, i.e., excitotoxicity induced by a 10-min pulse with 100 µM NMDA in mixed cultures of cortical neurons. Conditioned medium from 17ßE2-treated astrocytes did not reduce neuronal death induced by brief exposure to NMDA, as measured by neuronal counting after trypan blue staining. However, untreated conditioned medium, per se, was neuroprotective against NMDA-induced excitotoxic death suggesting that factors released by astrocytes may mask the effect of 17ßE2 under these experimental conditions.