Neuroprotective effects of estrogen against beta-amyloid toxicity are mediated by estrogen receptors in cultured neuronal cells

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Abstract

Although estrogen is known to exert beneficial effects on Alzheimer’s disease, its underlying cellular mechanisms have not been clear. In this study we investigated whether or not neuroprotective effects of estrogen are mediated by estrogen receptors (ERs). Treatment of estrogen (1.8 nM) reduced beta-amyloid (Aβ)-induced death of ER-expressing W4 cells. This effect of estrogen was blocked by a specific ER blocker ICI 182,780. When estrogen was treated to HT22 cells, which lack functional ERs, Aβ-induced cell death was not affected. Transfection of HT22 cells with human ERα, but not ERβ, restored protective action of estrogen against Aβ. Hoechst staining revealed that estrogen protected ERα-expressing cells by blocking Aβ-induced apoptosis. These results indicate that estrogen blocks Aβ-induced cell death via ERα-dependent pathways.

Keywords: Estrogen; Estrogen receptor; Beta amyloid; Alzheimer’s disease; Cell death; Apoptosis

Recently much attention has been given to estrogen for its beneficial effects on Alzheimer’s disease (AD) [17]. Several clinical studies have shown that post-menopausal women who are under estrogen replacement therapy are less likely to develop AD compared to those who are not, and that estrogen use is associated with a delayed onset of AD [8,20]. The underlying mechanisms of the estrogen effects on AD are currently unclear, but estrogen has many actions in the brain that are potentially beneficial. Estrogen is known to boost the production of acetylcholine [3], decrease production of beta-amyloid (Aβ) [19], increase α-secretase derived amyloid precursor protein (sAPPα) production [7], improve blood flow through the brain [6] and maintain the integrity of the hippocampus [18]. In addition, estrogen blocks Aβ-induced neuronal cell death in vitro [1] and increases expression of an anti-apoptotic protein Bcl-xL in cultured hippocampal neurons [14].

In general, estrogen is known to exert its effects through both estrogen receptor (ER)-dependent and -independent pathways [5,10] and there exist two different receptor subtypes, α and β [9]. Currently little is known about the role of ERs in mediating the beneficial effects of estrogen on AD. In this study we investigated whether or not neuroprotective effects of estrogen against Aβ toxicity are dependent upon ERs and, if they are, which receptor subtype plays the major role in this process.

Cultured W4 and HT22 cells were used in this study. W4 cells, which express ERs, are APP-overexpressing B103 cells [15]. In contrast, HT22 cells do not express functional ERs [5]. For selective expression of ERα and ERβ, HT22 cells were transfected with human ERα cDNA with subcloning into pBabe vector containing a puromycin resistant gene for selection (5 μg/ml of puromycin, Sigma, St. Louis, MO) or human ERβ cDNA with 25 μg/ml of G418 selection. All transfections were performed using lipofectamine (Gibco-BRL, Grand Island, NY). Before factor treatment, to make a steroid-free condition, cells were cultured in phenol red-free Dulbecco’s modified Eagle’s medium (DMEM) (Gibco-BRL, Grand Island, NY) containing 10% charcoal stripped fetal bovine serum (FBS) (Hyclone, Irvine, CA) for 48 h. Various combinations of the following factors were treated for 18 h: 25 μM Aβ25-35 (US Peptides, Fullerton, CA); 1.8 nM 17β-estradiol (Sigma, St. Louis, MO) and 100 nM ICI 182,780 (Tocris, United Kingdom).
The degree of cell death was quantified with MTT assay [4], and DNA fragmentation and chromatin condensation were visualized by Hoechst staining [11]. All statistical comparisons in this study were done using one-way ANOVA with post-hoc Tukey test, and data are expressed as mean ± SEM.

Aβ25-35 (25 μM), when added to W4 cell culture for 18 h, reduced survival of W4 cells to 58.7 ± 1.5% of the control culture. A physiological concentration (1.8 nM) of estrogen, when treated with 25 μM of Aβ25-35, significantly enhanced cell survival to 73.5 ± 5.7% of the control (P < 0.05), which amounts to 14.8% blockade of Aβ-induced cell death (Fig. 1C). This result is consistent with our previous finding that a physiological concentration of estrogen blocks Aβ-induced death of cultured B103 cells [12]. Immunocytochemical and RT-PCR analyses revealed that W4 cells contain both ERα and ERβ (Fig. 1A,B), suggesting the possibility that ERs mediated the protective effect of estrogen. When 100 nM ICI 182,780, a specific ER antagonist, was co-treated with estrogen and Aβ to W4 cells, the degree of cell survival was reduced down to 60.2 ± 10.6% of the control culture. Thus the protective effect of estrogen against Aβ-induced cell death was completely blocked by ICI 182,780 (P < 0.001, Fig. 1C). These results indicate that the neuroprotective action of estrogen is dependent upon ERs.

HT22 cells, which lack functional ERs [5], died about 25% (75.1 ± 7% survival of the control) by 25 μM of Aβ25-35 as W4 cells did. Unlike W4 cells, however, co-treatment of estrogen (1.8 nM) did not affect Aβ-induced death of HT22 cells (75.7 ± 6.3% survival of the control, Fig. 2A). ICI 182,780 treatment (100 nM) had no effect on the...
degree of cell survival when treated with estrogen and Aβ (70.1 ± 6.4% survival of the control, Fig. 2A), as expected. ANOVA indicates that there is no statistically significant difference among the three treatment conditions (P > 0.05). These data corroborate the conclusion that the protective effects of estrogen against Aβ toxicity are mediated by ERs.

The brain contains two subtypes of ERs, α and β [9], but currently no subtype-specific inhibitors or activators are available. To examine which subtype mediates the protective effect of estrogen against Aβ toxicity, human ERα and ERβ cDNA were stably transfected to HT22 cells and the clones that expressed high levels of ERα or ERβ proteins were selected. When Aβ25-35 (25 μM) was treated to ERα- or ERβ-expressing HT22 cells, 12.6% (87.4 ± 3.4% survival of the control) and 19.5% (80.5 ± 3.3% survival of the control) of cells died, respectively, which were comparable to Aβ toxicity to untransfected HT22 cells. Estrogen significantly lowered Aβ toxicity in ERα-expressing HT22 cells (93.8 ± 6.0% survival of the control, P < 0.05), but not in ERβ-expressing HT22 cells (78.6 ± 7.1% survival of the control, P > 0.05, Fig. 2B,C). ICI 182,780 treatment attenuated the effect of estrogen for Aβ toxicity in ERα-expressing HT22 cells. The levels of cell survival were 93.8 ± 6.0% of the control in estrogen + Aβ treated culture and 85.7 ± 6.2% in ICI + estrogen + Aβ treated culture (P < 0.01). These results show that the protective effect of estrogen against Aβ toxicity is mediated by ERα, and that ERβ contributes little to this effect.

Hoechst staining revealed that chromatin condensation occurred following Aβ treatment to W4 as well as HT22 cells (Fig. 3), indicating that Aβ induces chromatin condensation regardless of the presence of ERs. When estrogen was treated together with Aβ, the degree of chromatin condensation was reduced only in W4 and ERα-expressing HT22 cells (Fig. 3A,C). In contrast, co-treatment of estrogen did not reduce Aβ-induced chromatin condensation in ER-negative HT22 and ERβ-expressing HT22 cells (Fig. 3B,D). ICI 182,780 blocked the protective effect of estrogen against Aβ-induced chromatin condensation in W4 as well as ERα-expressing HT22 cells, whereas it had no effect on ER-negative HT22 and ERβ-expressing HT22 cells (Fig. 3). These data indicate that Aβ-induced cell death is accompanied by chromatin condensation and estrogen blocks this process in an ERα-dependent manner. It is thus likely that Aβ induces apoptotic cell death under the present experimental condition and estrogen protects ERα-expressing neurons by inhibiting the apoptotic process.

**Fig. 3.** Estrogen blocks Aβ-induced chromatin condensation in an ERα-dependent manner. Results of Hoechst staining are shown for different cell lines after treating various combinations of Aβ (25 μM of Aβ25-35), E2 (1.8 nM of 17β-estradiol) and ICI (100 nM of ICI 182,780). The arrows indicate condensed chromatin. (A) W4 cells which express ERs. (B) ER-negative HT22 cells. (C) ERα-overexpressing HT22 cells. (D) ERβ-overexpressing HT-22 cells. (a) control; (b) Aβ treated alone; (c) E2 + Aβ; (d) ICI + E2 + Aβ.
Although previous studies have demonstrated beneficial effects of estrogen on AD, little is known about its underlying mechanisms. Even such elementary information as whether or not the estrogen effects on AD are dependent upon ERs has not been known. Previous studies by Green et al. [5] and Behl et al. [1] have shown that high concentration of estrogen protects Aβ-induced death of HT22 cells, suggesting that estrogen can protect neurons through ER-independent pathways. On the other hand, results from other studies suggest involvement of ERs in neuroprotection. Estrogen changed Bcl-2 gene expression, which protects neuronal cell death, in a cerebral ischemia model [2]. Estrogen also increased expression of anti-apoptotic protein Bcl-xL, which was colocalized with ERs in cultured hippocampal neurons [14]. Since estrogen induces gene expression through ERs, both reports suggest that estrogen can exert neuroprotective effects through ER-dependent pathways. In the present study we examined the role of ERs in mediating estrogen effects of blocking Aβ-induced cell death. ICI 182,780, a potent ER antagonist, completely blocked neuroprotective effects of estrogen on W4 cells which contain both ERα and ERβ. In addition, estrogen did not influence Aβ-induced death of HT22 cells which lack functional ERs. These results argue strongly that the beneficial effects of estrogen are mediated by ERs.

At the first glance, the present results may seem contradictory to the previous results reported by Green et al. [5] and Behl et al. [1]. However, close examinations of the results reveal that there is actually no contradiction. In the study by Green et al. [5], 2 nM of estrogen did not block Aβ-induced death of HT22 cells as in the present study. It was only after addition of anti-oxidant glutathione that estrogen blocked Aβ toxicity. Another difference is the duration of Aβ treatment. Green et al. [5] examined cell death after 48 h of Aβ treatment whereas it was examined at 18 h in our study. Our recent results indicate that Aβ-induced cell death has multiple mechanisms; Aβ initially induces apoptotic cell death with chromatin condensation which eventually progresses to necrosis [12]. Thus, it is likely that the type of cell death we observed, which is more likely to be apoptotic, is different from that of Green et al. [5]. Regarding the study of Behl et al. [1], they did not find protective effect of estrogen at nM range concentrations after 24 h of Aβ treatment as in our study. Estrogen protected ER-negative neurons from Aβ toxicity only at μM concentrations, which are probably non-physiological.

There exist two receptor subtypes, α and β in the brain [13]. The cerebral cortex and hippocampus of the rat contain both ERα and ERβ mRNA [16]. We addressed which receptor subtype is involved in mediating the protective effect of estrogen against Aβ-induced cell death. Since no subtype specific antagonist is available, we transfected human ERα and ERβ genes into HT22 cells which do not express ERs. Estrogen had no effect on untransfected and ERβ-transfected HT22 cells, but protected ERα-transfected HT22 cells from Aβ toxicity. ICI 182,780 blocked this effect. These results demonstrate clearly that the estrogen effect against Aβ toxicity is mediated by ERα, but not by ERβ, and suggest that selective activation of brain ERα may be targeted as a new treatment strategy for AD.

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