Beta-estradiol protects cortical neurons from beta-amyloid oligomers toxicity through mediations of the Ras/raf and PI3K pathways.

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1 Introduction

Alzheimer disease (AD) affects mainly people over the age of 65 years, suffering from different clinical symptoms such as progressive decline in memory, thinking, language, and learning capacity. The toxic role of beta amyloid peptide (Aβ) has now shifted from insoluble Aβ fibrils to smaller, soluble oligomeric Aβ (AβO) fractions. Many evidences suggest that the neurodegenerative process would be due to the interaction of AβO with binding targets, activation of stress kinases, hyperphosphorylation of tau (τ) protein, caspase activation, loss of synapse, neuronal death, loss of cholinergic function, generation of reactive intermediates of oxygen (oxidative stress) or glutamate excitotoxicity. Urgent need for efficient new therapies is high, but could only be successful with an extensively comprehension of AβO degeneration process.

By modulating concentration and time of exposure to AβO, it was possible to reproduced their early effect (oxidative stress) and their long term structural alteration development (death of neurons); for review see Callizot et al., 2013.

In the present study we investigated the neuroprotective mechanism of 17-βEstradiol (17-βE) on Aβ induced neurotoxicity.

2 Methods

Culture of cortical neurons: Rat cortical neurons (E15) were cultured as described by Singer et al., 1999. The cells were seeded at a density of 30,000 per well in 96-well plates (for immunostaining) and of 170,000 per well in 24-well plates (for Western-blot (WB)) precoated with poly-L-lysine and cultured at 37°C in an air (95%) – CO2 (5%) incubator. Six wells were used per condition.

Pharmacological treatments: The cortical neurons were treated with Aβ solution (10 µmol/L) after 11 days of culture. The human Aβ-1-42 preparation was done following the procedure described by Callizot et al., 2013. 2h before addition of Aβ, the cells were incubated with differents inhibitors. 17-βE was used at 100 nmol/L and was added as pretreatment (1h before Aβ addition).

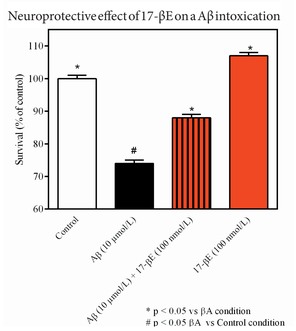
Immunostaining: After 24 h of Aβ treatment, cells were fixed by a solution of ethanol (95%) acetic acid (5%). The cells were incubated with monoclonal anti-microtubule-associated-protein 2 (MAP-2) antibody that was revealed with Alexa Fluor 488 goat anti-mouse IgG. The immuno-labelled cultures were examined with MetaXpress (Molecular Devices) at X 20 magnification.

Westernblotting: After 8 and 24 h of Aβ treatment, cells were lysed with cellytic and immediately frozen at -80°C. All reagents were prepared and used according to manufacturer's recommendations (Simon™ - ProteinSimple - www.proteinsimple.com). Anti-phospho-τ, anti-phospho-AKT primary antibodies were used for WB analysis.

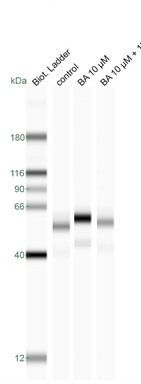
3 Results

17-βE (100 nM) was able to significantly protect neurons from Aβ injuries (10 µM for 24h) and reduced the τ-phosphorylation

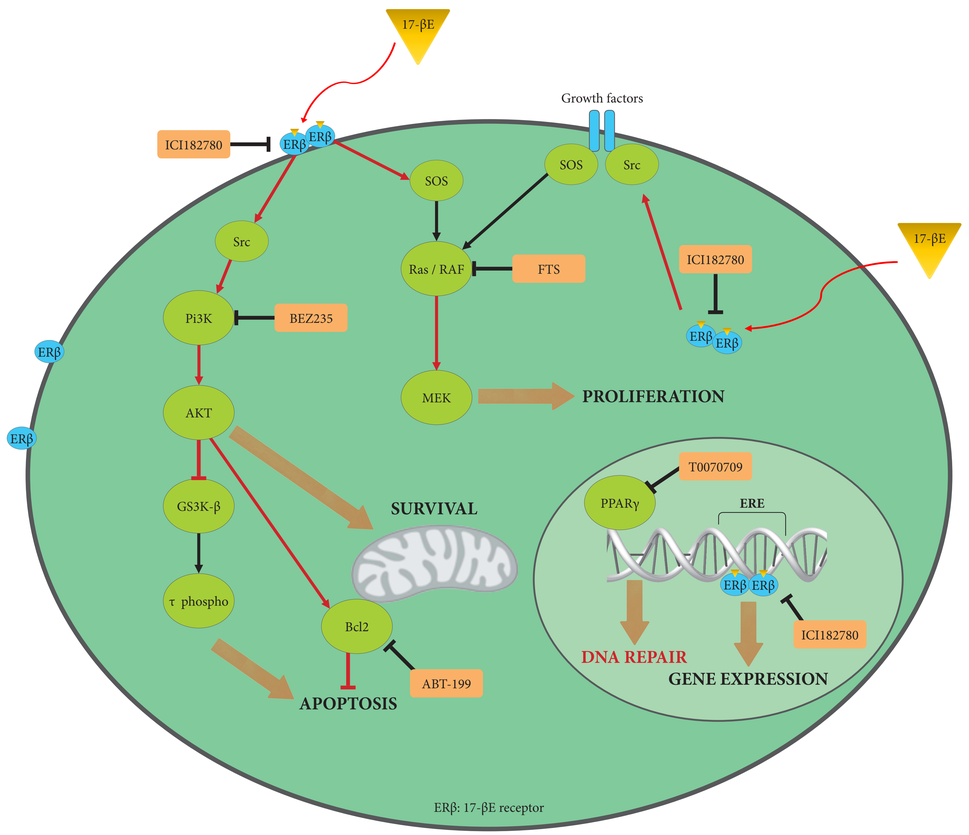
Neuroprotective effect of 17-βE on a Aβ intoxication



Quantification of τ-phospho in cell lysate after a 8h Aβ (10 µM) application: Effect of 17-βE



Investigation of the neuroprotective effect of 17βE in presence of inhibitors

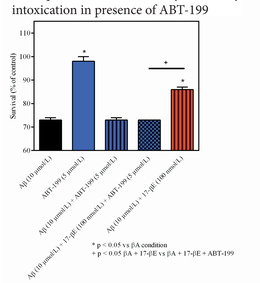


Neuroprotective effect of 17-βE on a Aβ intoxication in presence of BEZ235

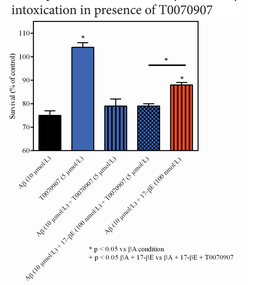
Neuroprotective effect of 17-βE on a Aβ intoxication in presence of FTS

Neuroprotective effect of 17-βE on a Aβ intoxication in presence of ICI 182780

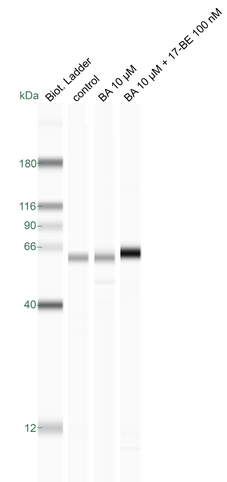
Neuroprotective effect of 17-βE on a Aβ intoxication in presence of ABT-199



Neuroprotective effect of 17-βE on a Aβ intoxication in presence of T0070907



Quantification of AKT-phospho in cell lysate after a 24h Aβ (10 µM) application: Effect of 17-βE



4 Conclusions

We showed in this study :

1. 17-βE was able to protect neurons from AβO injuries;
2. This protective effect involved the receptor Erβ;
3. 17-βE was able via its own receptor to activate the anti-apoptotic and the DNA repair pathway;
4. The putative mode of action of 17-βE was summarized into the drawing below by red arrows.

Ref: Callizot N, Combes M, Steinschneider R and Poindron P. JNR. 2013; 91(5):706-16 / Singer CA, Figueroa-Masot XA, Batchelor RH, Dorsa DM. J Neurosci. 1999; 1;19(7):2455-63.