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ORIGINAL ARTICLE

17β-estradiol modulates NGF and BDNF expression through ERβ mediated ERK signaling in cortical astrocytes

Jaldeep M. Langhnoja[1] · Lipi K. Buch[1] · Prakash P. Pillai[1]

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Abstract

17β-estradiol is known to exert neurotrophic and neuroprotective effects through classical estrogen receptors [ERs], ERα and ERβ, on a variety of cell types either by genomic or non-genomic actions. The actions of estradiol on glial cells are important to maintain metabolic functions of the nervous system. Astrocytes are considered to be active participants in brain activity because of their ability to release growth factors, including neurotrophins. Present in vitro studies show that 17β-estradiol modulates NGF and BDNF expression in time-dependent manner and ERK acts as secondary messenger for estradiol's action. 17β-estradiol is involved in survival of cortical astrocytes. In conclusion, this study indicates vital role of ERβ mediated ERK signalling for regulation of NGF and BDNF expression along with cell viability of cortical astrocytes which further confirms the role of ERs, particularly ERβ in glial cells' functions and viability.

Keywords BDNF · ERK · 17β-estradiol · NGF

Abbreviations

E2	17β-estradiol
ERs	Estradiol Receptors
ERα	Estradiol Receptor alpha
ERβ	Estradiol Receptor beta
NGF	Nerve growth factor
BDNF	Brain derived neurotrophic factor
NT3	Neurotrophins 3
NT4/5	Neurotrophins 4/5
ERK	Extracellular regulated kinase
MEK	Mitogen activated protein kinase
GFAP	Glial fibrillary acidic protein

Introduction

17β-estradiol (E2), a steroid hormone plays a diverse role at cellular and molecular levels in brain cells. Secretion of this hormone is mainly by the ovaries, brain and fat tissue through aromatization of testosterones. During development, estradiol regulates cell proliferation, differentiation and survival in the brain (MacLusky et al. 1987; TORAN-ALLERAND 2005). Estradiol regulates neural plasticity and dendritic spine density in the brain regions (Gould et al. 1990; Matsumoto and Arai 1981; Woolley et al. 1990). Two distinct estrogen receptors (ERs), ER alpha (ERα) and ER beta (ERβ) are distributed in the brain regions (McEwen et al. 1997; Mitra et al. 2003; Register et al. 1998; Shughrue et al. 1997) and located in nucleus as well as in cytoplasm, and this localization decides estradiol's mode of action (Lee and McEwen 2001). Nucleus-initiated receptors' signaling activate new gene transcription by association with estrogen response elements (EREs) in the DNA (Nelson and Bulun 2001), also called direct genomic action while membrane associated receptors cooperate with growth factor receptors or G-protein-coupled receptors to activate kinase cascades through indirect genomic action (Hammes and Levin 2007; Levin 2005; Thomas et al. 2005; Vasudevan and Pfaff 2007).

Post receptor activation, estradiol is involved in many of the cascades such as induction of anti-apoptotic gene expression, maintenance of intracellular calcium homeostasis, promoting antioxidant activity and modulating actions of neurotrophins (Lee and McEwen 2001). The major focus in the present study is to elucidate the role of estradiol in neurotrophin expression modulation. Neurotrophins are a family of proteins that are important for neuronal

✉ Prakash P. Pillai
pillaippp@gmail.com; prakash.pillai-zoo@msubaroda.ac.in

[1] Division of Neurobiology, Department of Zoology, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India

development, neuronal survival and neuronal functions. Four members of neurotrophins are present in the mammalian system: (a) Nerve growth factor (NGF), (b) Brain derived neurotrophic factor (BDNF), (c) Neurotrophins 3 (NT3) and (d) Neurotrophins 4/5 (NT4/5) (Allen and Dawbarn 2006). Neurotrophins exert their effects by binding to their receptors, the Trk family of receptor tyrosine kinase (TrkA, TrkB, and TrkC) and p75NTR, a member of the tumour necrosis factor (TNF) receptor super-family. This association triggers a complex series of signal transduction events, which are able to induce neuronal differentiation, neuronal maintenance and neuronal functions (Bucci et al. 2014). NGF and BDNF are most widely distributed neurotrophins and engaged in many of the essential functions of the brain. NGF binds with TrkA and BDNF binds with TrkB receptor along with p75NTR receptor and activate the downstream signalling involved in neural protection (Allen et al. 2013; Cheng and Mattson 1994), neuron outgrowth (Hannan et al. 2015; Labelle and Leclerc 2000), glial cell proliferation ((Douglas-Escobar et al. 2012; Tsiperson et al. 2015) and myelination (Chan et al. 2004; Xiao et al. 2010). There are reports available regarding estradiol mediated modulation of NGF and BDNF levels in neurons (Dittrich et al. 1999; Kwakowsky et al. 2016; Scharfman and MacLusky 2006; Sohrabji et al. 1994; Solum and Handa 2002) along with their receptors (Hasan et al. 2005). Astrocytes are the supportive glial cells in neural tissue and the main source for production of growth factor and neurotrophins (Sofroniew and Vinters 2010). Astrocytes express both ERα and ERβ receptors either on their plasma membranes or intracellularly (Fuente-Martin et al. 2013) and also provide support to estrogen for neuroprotection (Dhandapani and Brann 2007; Johann and Beyer 2013; Karki et al. 2014).

However, the role of E2 in the modulation of NGF and BDNF expression in cortical astrocytes and the underlying mechanism is still unclear. Present study demonstrates the neurotropho-modulatory effect of E2 through its receptors. Involvement of ERK signaling cascade in the neurotrophins expression modulation by estradiol along with its effect on astrocytes cell survival was also explored. The data from the current study may help further in understanding of neuroprotective role of E2 in central nervous system.

Materials and methods

Rat cortical astrocyte culture

Astrocytes were cultured by a previously described method (Chen et al. 2007; Sharma et al. 2015; Tripathi et al. 2017). Briefly; mixed glial cultures were isolated from P0–2 day rat cortices. In the current protocol, both male and female pups were sacrificed and cortices were pooled for establishing astrocyte culture. Dissociated cells were plated on Poly-L-lysine (PLL; Sigma) coated T75 cm2 flasks in Dulbecco's modified Eagle's medium (DMEM; Gibco) containing 10% Fetal bovine serum (FBS; Gibco) and 1%penicillin/streptomycin (1X; Invitrogen) then incubated at 37 °C in the presence 5% CO2. Cell growth medium was changed every third day for 10–12 days to obtain mixed glial cultures containing microglia and Oligodendrocyte precursor cells (OPCs) on an astrocytes monolayer. Flasks were sealed and initially shaken for 1 h at 37 °C on an orbital shaker to remove microglia followed by shaking for 18–20 h to remove OPCs. Enriched cultures containing >95% GFAP-positive astrocytes were obtained. Adherent astrocytes were dissociated with 0.1% Trypsin (Invitrogen) and passaged using the same medium for further experiments.

Experimental strategy

Isolated astrocytes were plated in DMEM containing 10% FBS and 1% penicillin/streptomycin on PLL coated 12 wells culture dish. Each experiments contain six 12 well plates and three 96 well plates. After reaching to confluency (1×10^6 cells) astrocytes were pre-incubated with followings: 10 μM ERα antagonist (MPP Dihydrochloride; Sigma;) or 10 μM ERβ antagonist (4-Hydroxytamoxifen; Sigma) (Bains et al. 2007; Dong et al. 2009) or 25 μM MEK inhibitor (PD098059; Invitrogen) (Blázquez et al. 2000; Choi et al. 2011) for 1 h followed by treatment with 100 nM 17β-Estradiol (1, 3, 5-Estratreine-3,17β-diol; HiMedia) (Kajta et al. 2006; Lee and McEwen 2001; Lorenzo et al. 1992) for three different time points- 6 h, 12 h and 24 h. The time course for the analysis was decided to follow both the genomic and non-genomic action of estradiol (Lee and McEwen 2001).

RNA isolation and reverse transcriptase- polymerase chain reaction (RT-PCR)

Total RNA was isolated using Trizol following the manufacturer's protocol. RNA was quantified using a Qubit RNA assay kit (Invitrogen) in Qubit 2.0 Fluorometer (Invitrogen). Total RNA (1 μg) was used for cDNA preparation in 20 μL reverse transcription (RT) reaction using verso cDNA synthesis kit (Thermo Scientific). Semi-quantitative polymerase chain reaction was carried out using specific primers as in Table 1. GAPDH was used as an internal control. PCR products were separated on 2% Agarose horizontal gel and viewed with Bio-red gel documentation instrument followed by densitometry analysis using Image J software.

Immunoblotting

Immunoblotting was performed following Abcam protocol with minor modifications (Abcam-protocols 2016a). Whole cell

Table 1 Details of primers used for semi quantitative PCR

Gene	Sequences	Annealing Temp.	Product Size	Reference
GAPDH	Forward: GGTCGGTGTGAACGGATTTG Reverse: GTGAGCCCCAGCCTTCTCCAT	60 °C	318 bp	Arita et al. 2008
NGF	Forward: CCCCGAATCCTGTAGAGA Reverse: CACGCAGGCTGTATCTAT	52 °C	504 bp	Aiga et al. 2006
BDNF	Forward: CCATAAGGACGCGGACTTGT Reverse: GAGGCTCCAAAGGCACTTGA	60 °C	103 bp	Fuchikami et al. 2009

lysate was prepared in lysis buffer containing 40 mM Tris pH 7.4, 120mMNaCl, 0.5% Triton X-100, 0.3% SDS and protease inhibitor. 40 μg protein was resolved by SDS-PAGE separation. Protein was transferred on nitrocellulose membrane and blocked using 3% BSA (Bovine Albumin Serum) followed by incubation in following primary antibodies: Rabbit anti–p-ERK1/2 from R&D systems (diluted at 1:750), Mouse anti-β-actin from Pierce (diluted at 1:5000). Bands were visualized using corresponding horseradish peroxidase conjugated secondary antibodies (Sigma). Specific immune-reactivity was visualized using an ECL kit (Invitrogen). Protein bands were imaged on X-ray films, scanned digitally and quantitatively analysed using Image J software. β-actin was used as internal control to normalize the protein expression.

Immunocytochemistry

Immunocytochemistry was performed following Abcam protocol with minor modifications (Abcam-protocols 2016b). Cells were grown on coverslips and fixed in cold 4% paraformaldehyde (*w*/*v*), permeabilized with 0.25% Triton-X-100 in phosphate buffer saline (PBS) for 10 min and then blocked with 1% (w/v) BSA in PBS containing 0.2% (*v*/*v*) Tween 20 for 30 min and incubated overnight with Rabbit anti-GFAP from Pierce (diluted at 1:1000) in 1% BSA in PBS-Tween (PBST) over night at 4 °C. Subsequently, coverslips were washed thrice with PBST solution, incubated with corresponding FITC conjugated secondary antibody in 1% PBST for 1 h, mounted with Anti-fade mounting medium (Invitrogen) and sealed with nail polish. Images were observed under Zeiss confocal laser scanning LSM 510 microscope.

MTT assay for cell viability

Cell survival was assessed by a colorimetric assay using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (Riss et al. 2016) that detects the formation of dark blue formazon product from MTT in active mitochondria. Viability of cortical astrocytes cells in response to estradiol was determined using the MTT assay. In brief, cortical astrocytes were treated with 17-β estradiol with and without ER-α, ER-β or MEK inhibitor for 24 h. 1 mg/mL MTT was added to the culture medium of these cells and incubated for 4 h in dark at 37 °C in a humidified atmosphere with 5% CO2. The medium was removed and 150 μL of DMSO was added to each well. The absorbance of the dissolved dye was measured at 490 nm. Data was expressed in OD units. Cells without any treatment were considered as blank.

Statistical analysis

Results are expressed as mean ± standard error mean (SEM) of three independent experiments and differences between treatment groups were statistically analysed using one way analysis of variance (ANOVA) with Bonferroni post-test. Statistical analysis was performed with Prism 6 software (GraphPad Software Inc.). A value of $P < 0.05$ was considered statistically significant (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$).

Results

Cortical astrocyte primary culture from rat pups:

Mixed glial cultures were harvested from P0-P2 day rat pups. After 10 days of culturing, astrocytes were isolated from mixed glial culture. Enriched astrocytes were immunostained with astrocyte specific marker GFAP (Glial fibrillary acid protein) and nucleus stained with DAPI (Fig. 1). More than 95% GFAP-positive astrocytes were obtained.

17-β estradiol modulates NGF and BDNF transcript levels:

Astrocytes were treated with 100 nM E2 in a time dependent manner (6, 12 and 24 h). Results indicate up-regulation of neurotrophins mRNA expression levels for all time points which indicate that E2 positively regulates neurotrophins transcript levels. To further validate this, E2 receptors were blocked using specific inhibitors- MPP Dihydrochloride (ERα inhibitor, 10 μM) and 4-Hydroxytamoxifen (ERβ inhibitor, 10 μM). Cells were incubated with these inhibitors for 1 h followed by E2 treatment for three different time points-

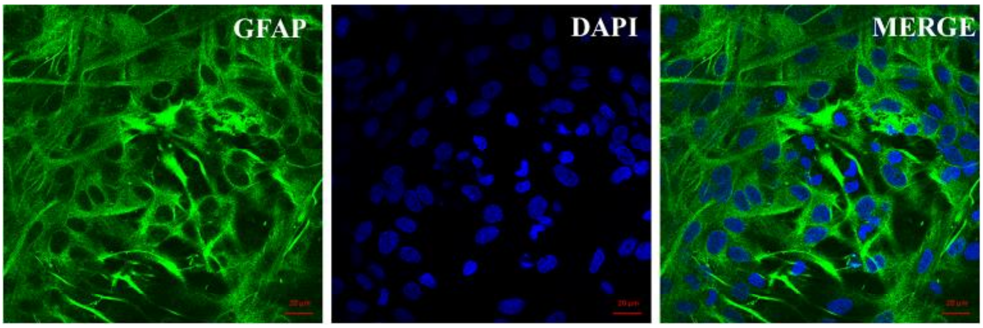

Fig. 1 Dissociation of 0–2 day old neonatal rat cortex yields predominantly GFAP positive astrocytes in primary culture following fibroblast removal.63X immunofluorescent microscopy using Rabbit Anti-GFAP (Green) monoclonal antibody visualized with Anti-Rabbit FITC secondary antibody and DAPI stain for nucleus identification. Scale Bar = 20 μm

6 h, 12 h and 24 h. In 6 h time point, NGF was found to be up-regulated by E2 treatment, with a corresponding decrease as seen in E2 receptor inhibitor treated groups (both ERα and ERβ). On the other hand, BDNF was up-regulated after E2 treatment but surprisingly in the receptor inhibitor groups, the BDNF levels were not significantly declined (Fig. 2a, b).While in 12 h and 24 h time point, both NGF and BDNF levels were found to be elevated in E2 treatment group with a corresponding decrease in E2 receptor inhibitor treated groups (ERα and ERβ) (Fig. 2c, d, e, f) which indicates a crucial role of E2 and its receptors in the regulation of neurotrophins in cortical astrocytes.

Involvement of ERK in E2 mediated modulation of NGF and BDNF transcript levels:

Lee and McEwen (2001), reported that the mode of action of E2 is through indirect genomic action also, which occurs by activating secondary messenger cascade. To study the mechanism of E2 in modulation of NGF and BDNF, we studied the involvement of ERK. Cellswere pre-incubated with specific MEK inhibitor (PD098059) for 1 h before E2 (100 nM) treatment for specific time points- 6 h, 12 h and 24 h. In 6 h (short term exposure), E2 induced neurotrophins levels were not found to be inhibited by MEK inhibitor (25 μM) indicating that the possible mode of E2 action is the direct genomic action (Fig. 3a, b). However, in 12 h and 24 h (long term exposure), pre-treatment with MEK inhibitor caused down-regulation of NGF and BDNF levels, thus suggesting that ERK might be involved in E2 mediated modulation of neurotrophins through the indirect genomic action (Fig. 3c, d, e, f).

Modulatory effects of E2 and its receptors on p-ERK1/2:

ERK is a well studied secondary messenger involved in directing cellular responses to a diverse array of stimuli. We aimed to understand the mode of action of E2 as a neurotrophomodulator in astrocytes. To justify this aim, astrocytes were treated with E2 receptors inhibitors (10 μM) for 1 h followed by treatment of E2 (100 nM) for 6 h, 12 h and 24 h. Results indicate in 6 h treatment p-ERK1/2 levels are greatly affected by ERβinhibition compared to control andonly E2 treatment group (Fig. 4a, d). Surprisingly in 12 h, inhibition of ERα increased p-ERK levels but not significantly while ERβinhibition causes drastic down-regulation of p-ERK levels (Fig. 4b and e). However in 24 h treatment, E2 upregulated the phosphorylation of ERK1/2 compared to control while ERβ inhibition significantly downregulated the levels of p-ERK 1/2 compared to control and only E2 treatment groups (Fig. 4c and f). Thus, overall results demonstrate that p-ERK1/2 levels are down-regulated by ERβinhibition in at all three time points, which indicates ERK acts as a secondary molecule for E2 mediated neurotrophin modulation via ERβ receptor.

ERα and ERβ mediated cell Viablity:

The growth stimulatory effects of 17β-estradiol and its receptors on cortical astrocytes cells was determined by MTT assay. The percentage viablity of astrocytes increased upon incubation with 100 nM 17β-estradiol compared with the untreated control,. Astrocytes were treated with ER inhibitors for 1 h followed by E2 treatment for 6 h, 12 h and 24 h, after which MTT assay was performed. Results demonstrate that the percentage viablity was significantly reduced in inhibitor treated groups compared to E2 and control (Fig. 5).

Role of ERK in E2 treated astrocytes cell viablity:

The growth stimulatory effects of 17β-estradiol along withMEK receptor inhibition on cortical astrocytes cells was determined by MTT assay. The percentage cell viablity of astrocytes increased upon incubation with 100 nM 17β-estradiol compared with the untreated control. Astrocytes were treated with MEK inhibitor for 1 h followed by E2 treatment for 6 h, 12 h and 24 h, after which MTT assay was performed. Results demonstrate that there was no significant difference in the E2 treated groups and MEK inhibitor treated

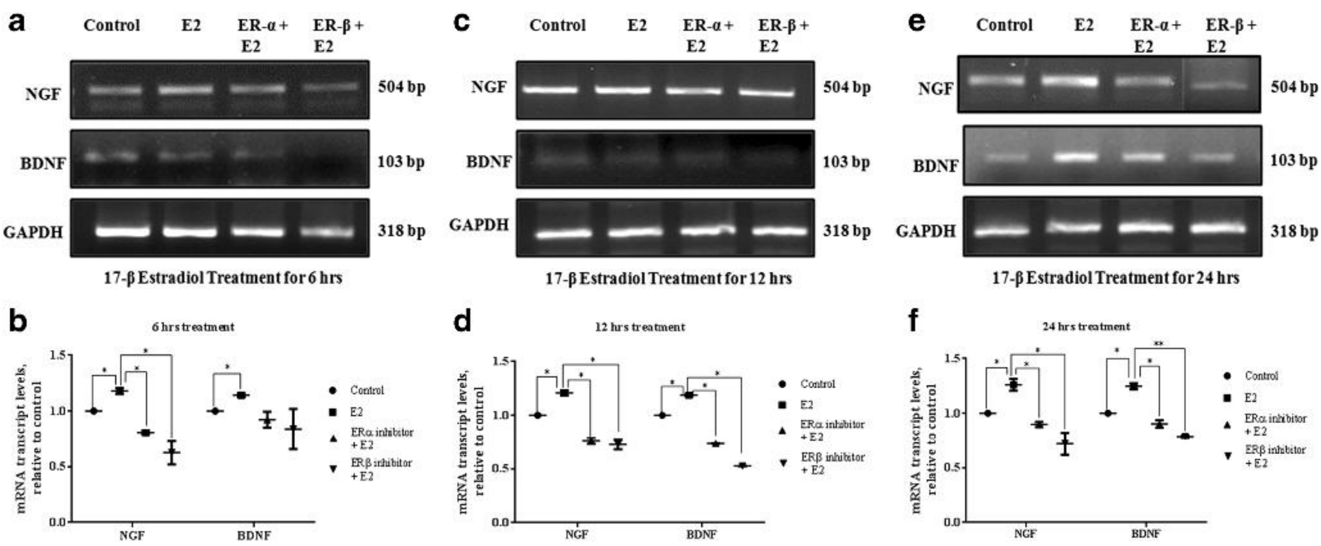

Fig. 2 **Effect of 17β- Estradiol on NGF and BDNF mRNA levels in rat cortical astrocytes cultures:** The cells were treated with 100 nM Estradiol dose for 6 h, 12 h and 24 h, along with receptors inhibitors (10 μM) and the NGF and BDNF mRNA levels were detected by RT-PCR. Typical pattern of RT-PCR products for NGF, BDNF and GAPDH are indicated for 6 h (A), 12 h (C) and 24 h (E). Time course changes in NGF and BDNF mRNA transcript levels after 6 h (B), 12 h (D) and 24 h (F). Expression levels of NGF and BDNF mRNA were normalized to GAPDH mRNA transcript levels. E2 alone group was compare with control and ER-α or β inhibitor groups separately. Results are expressed as Mean ± SEM of three independent experiments and the data were evaluated using One way ANOVA followed by Bonferroni test (**$p < 0.01$, *$p < 0.05$)

groups indicating that ERK does not play any role in cell viablity (Fig. 6).

Discussion

Neurons in the adult rat forebrain of both sexes co-express estrogen and neurotrophin receptors and are also the sites of estrogen and neurotrophin synthesis (Miranda et al. 1994; Toran-Allerand et al. 1992). It has also been shown that estradiol and neurotrophin receptor co-expression leads to convergence of their signaling pathways (Toran-Allerand et al. 1999). Thus, it was interesting to explore the involvement of estradiol in neurotrophin expression in the astrocytes and whether the estradiol mediated modulatory effects on neurotrophins are mediated via direct or indirect genomic

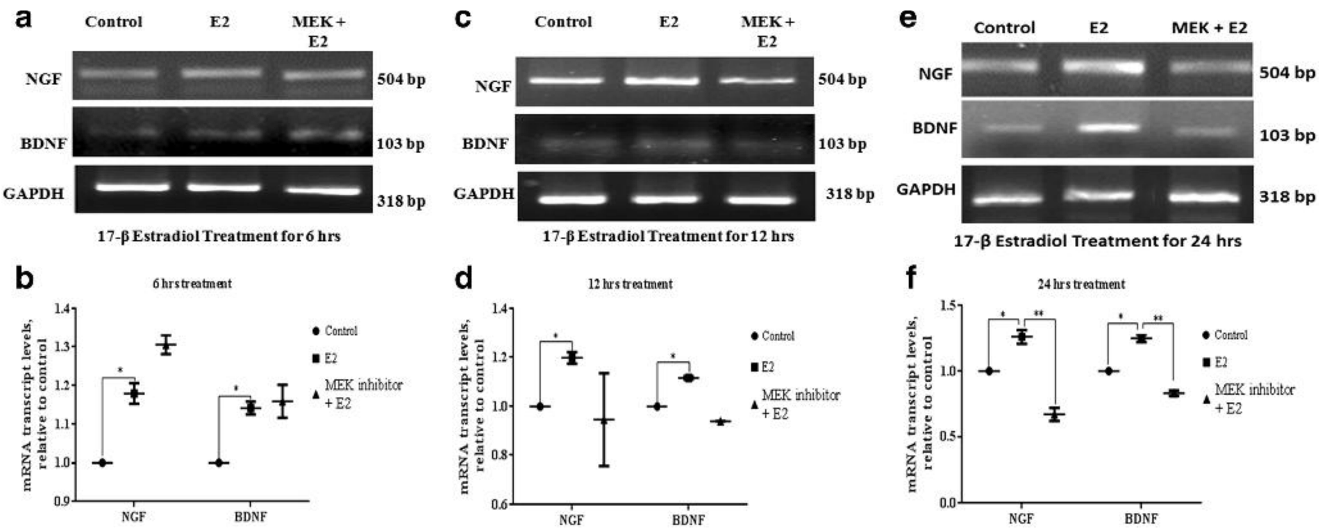

Fig. 3 **ERK involvement in E2 mediated modulation of NGF and BDNF transcript levels:** The cells were treated with 100 nM Estradiol dose for 6 h, 12 h and 24 h, along with MEK inhibitor (25 μM) and the NGF and BDNF mRNA levels were detected by RT-PCR. Typical pattern of RT-PCR products for NGF, BDNF and GAPDH are indicated for 6 h (A), 12 h (C) and 24 h (E). Time course changes in NGF and BDNF mRNA transcript levels after 6 h (B), 12 h (D) and 24 h (F). Expression levels of NGF and BDNF mRNA were normalized to GAPDH mRNA transcript levels. E2 alone group was compare with control and MEK inhibitor groups separately. Results are expressed as Mean ± SEM of three independent experiments and the data were evaluated using One way ANOVA followed by Bonferroni test (**$p < 0.01$, *$p < 0.05$)

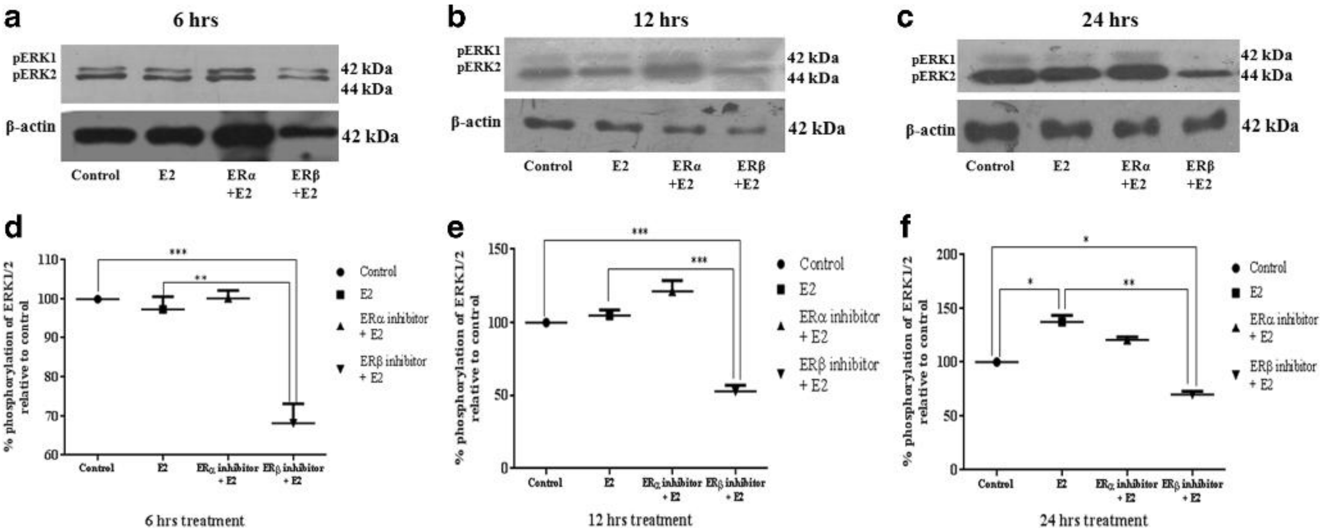

Fig. 4 **Modulatory effect of E2 and its receptors on p-ERK1/2**: Cultured astrocytes were harvested at 6, 12 and 24 h after E2 along with ERs inhibitors' treatment. Western blotting analysis of proteins extracted from astrocytes was performed using antibodies against *p*-ERK1/2 [6 h (A and D), 12 h (B and E) and 24 h (C and F)]. Phosphorylation of ERK1/2 were normalized to β-actin protein levels and mean values in control groups were scaled to 100%. Results are expressed as Mean ± SEM of three independent experiments and the data were evaluated using One way ANOVA followed by Bonferroni test (***$p < 0.001$, **$p < 0.01$, *$p < 0.05$)

actions and to study the signalling pathway, particularly the ERK signalling pathways, activated by estradiol receptors.

17β estradiol, (through both ERα and ERβ), contributes to the modulation of levels of neurotrophins (NGF & BDNF) and our data suggests ERβ is majorly involved and responsible for cortical astrocytes cell growth, proliferation and thus play an important role in neuroprotection. Estradiol induced neurotrophins level in astrocytes was reduced after blocking the ERs which confirms the role of estradiol in the modulation of neurotrophin expression. Estradiol induced neurotrophins expression levels following exposures to E2 (6, 12 and 24 h) and in the case of 24 h exposure to E2, neurotrophins expression levels post MEK inhibitor was significantly reduced which indicates ERK might be involved in estradiol signalling pathway through indirect genomic action. Protein analysis shows ERK1/2 act as a secondary molecule for estradiol mediated neurotrophin modulation, via indirect genomic action and is activated in long term E2 exposure condition for astrocytes survival. Present data strongly indicates ERβ may be majorly responsible in modulating the level of neurotrophins and thus responsible for astrocytes' cell survival compared to ERα.

ER and neurotrophin receptors co-localizations are widespread in neurons of the cerebral cortex, hypothalamus and hippocampus (Sohrabji and Lewis 2006) which explain the fact that estradiol and neurotrophins generally appear to exert reciprocal regulation upon each other's actions at the level of gene transcription. Reduced levels of E2 in post-menopausal women coincide with the high rate of neurodegenerative disorders in this age group, as reported by clinical studies (Rocca et al. 2011). E2 therapy thus may be highly beneficial in protection against certain neurodegenerative diseasesas Alzheimer's (Birge 1997), Parkinson's (Adams and Kumar 2013) and brain ischemia (Ma et al. 2016). Moreover, NGF and BDNF are well studied with respect to their role in treatment of neurological disorders (Balaratnasingam and Janca

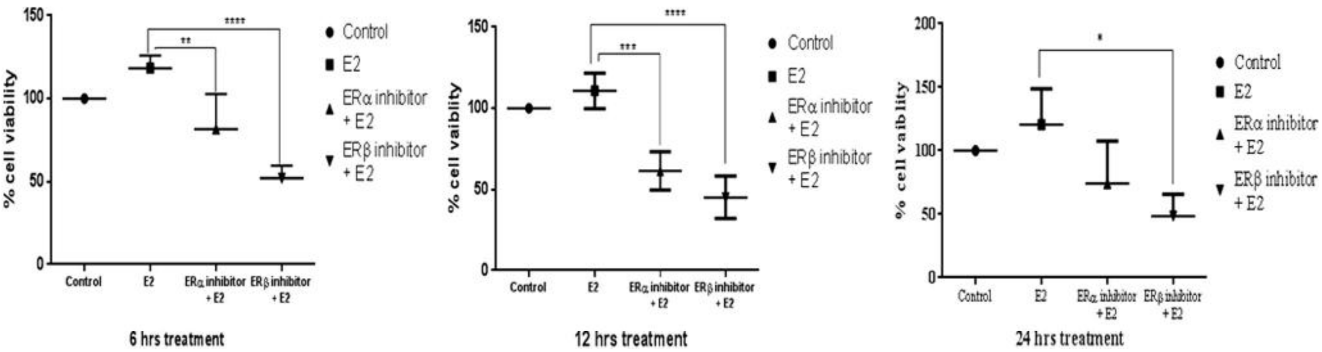

Fig. 5 **ERα and ERβ mediated cell viablity:** Cell viability after 6 h, 12 h and 24 h of E2 treatment along with receptors inhibitor respectively (A, B and C). Cell viability was measured by MTT assay. E2 alone group was compare with control and ER-α or β inhibitor groups separately. Statistical analysis was performed using one-way ANOVA followed by Bonferroni test (***$p < 0.001$, **$p < 0.01$, *$p < 0.05$)

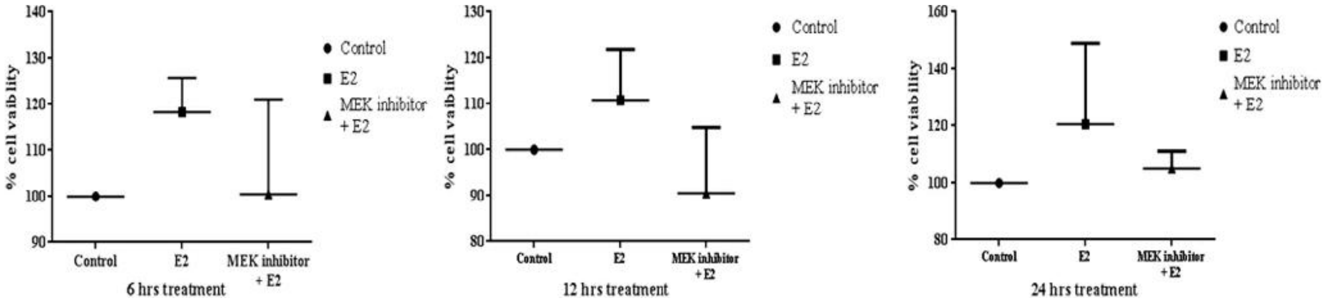

Fig. 6 Role of ERK in E2 treated astrocytes cell viablity: Cell viability after 6 h, 12 h and 24 h E2 treatment along with MEK inhibitor (A, B and C). Cell viability was measured by MTT assay. E2 alone group was compare with control and MEK groups separately. Statistical analysis was performed using one-way ANOVA followed by Bonferroni test

2012; Cattaneo and Calissano 2012). Thus, estrogen and neurotrophin receptors crosstalk gives rise to a possible therapeutic approach of neuroprotection against neurodegenerative diseases. We sought to understand the effect of E2 on NGF and BDNF in astrocytes in a time dependent fashion in 6, 12 and 24 h to elucidate the underlying cellular mechanism. Neurotrophins were significantly up-regulated in all the three time points of E2 exposure in astrocytes compared to control Next, we analyzed if this up-regulation was mediated by E2 receptors, and for which (both ERα and ERβ) were blocked using specific inhibitors (as described in the materials & methods section) wherein neurotrophins levels were significantly down-regulated in 12 and 24 h but not in 6 h. Our results indicate that E2 positively regulates the modulation of neurotrophins in astrocytes through both its receptors- ERα and ERβ.

Estradiol receptors exist in two forms nuclear and membrane-associated and both of which are involved in mediating neurotrophic and neuroprotective estrogen effects. Nuclear or direct genomic actions are independent of secondary messenger whereas non-nuclear or indirect genomic estrogen effect activation of second messenger cascades and transcription factors that are regulated via phosphorylation and dephosphorylation, some of which are also linked to cells survival. Moreover, estrogen and neurotrophins stimulate a common secondary messenger- ERK (Lee and McEwen 2001). In cortical explants cultures, estrogen phosphorylates the MAP kinases ERK1 and ERK2 in a time frame similar to that of the neurotrophins (Singh et al. 1999). In the present study, we focused to understand the mode of E2 action in modulation of neurotrophins levels in astrocytes. To justify this aim, phosphorylation of ERK1/2 was checked to confirm the secondary messenger activation. We found significantly down regulation of p-ERK1/2 levels in ERβ inhibition group in all time points which indicating action of E2 was mediate by ERK pathway only through indirect genomic action. Overall, these results indicate that ERβ mediated ERK is involved in E2 modulated neurotrophins expression through the indirect genomic action.

The first neurotrophic actions of estrogens on cell survival/ growth were demonstrated using organotypic slice cultures of the developing hypothalamus and cerebral cortex (Toran-Allerand et al. 1999). Our results suggest that the growth stimulatory effects of 17β-estradiol in astrocytes via both receptors- ERα and ERβ, predominantly by ERβ. On the other hand, we also observed that ERK is not playing a role in ERα and ERβ mediated astrocytes cell survival.

Conclusion

We conclude that17β-estradiol (E2) acts as a neurotrophomodulator and regulate viability of cortical astrocytes. Estradiol receptor β (ERβ) play a vital role in mediating neurotrophomodulator functions of E2. We also show that E2 mediated modulation of neurotrophins is via ERβ mediated ERK signalling pathway indicating the indirect genomic action of this hormone. Thus, crosstalk between neurotrophins and E2 play a very significant role on glial cells' function and survival suggesting its potential benefits in neuroprotection.

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Compliance with ethical standards

Animal ethical approval All animal experiments were in compliance with the ethical committee approved by the Institutional Animal Ethical Committee, The Maharaja Sayajirao University of Baroda; ZD/02/2013, 2014, 2015.

Conflict of interest The authors declare that they have no conflict of interest.

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RESEARCH ARTICLE

Role of astrocytic MeCP2 in regulation of CNS myelination by affecting oligodendrocyte and neuronal physiology and axo–glial interactions

Buch Lipi[1] · **Langhnoja Jaldeep**[1] · **Pillai Prakash**[1]

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Abstract

Astrocytes perform several critical functions such as promoting neuronal maturation, neuronal survival, maintaining and supporting neurons and oligodendrocytes. Astrocytes participate in the formation of nodes of Ranvier. Recently, studies emphasizing on the role of astrocytes in regulating myelination by secreting pro-myelinating factors like growth factors, neurotrophins and ECM proteins, have been investigated by many researchers. Methyl-CpG-Binding Protein 2 (MeCP2), an epigenetic protein, binds to CpG islands in the genome and induces multiple gene regulatory functions by conforming changes in the chromatin structure and resulting in cell-specific gene expression. MeCP2 deficient astrocytes have been linked with abnormal neuronal function including decreased dendritic arborization and decreased dendritic outgrowth. However, role of astrocytic MeCP2 in central nervous system myelination is largely not known. The data from the current study indicate altered mRNA levels (*Lif, Cntf, Pdgfa, Cxcl10*) of astrocyte-secreted factors involved in myelination. *Bdnf* and *Ngf* mRNA levels were also altered in MeCP2 knockdown astrocytes. Moreover, the secreted BDNF levels were significantly altered whereas there were no significant changes in NGF secretion. We also observed that astrocytic MeCP2 affects the morphology, physiology and survival of oligodendrocytes and neurons—two of the key players in myelination. Further, we report that some of the axo–glial interaction genes, namely *Caspr, Notch1, Nf155* and *Nrg1* are under the regulation of astrocytic MeCP2 along with key myelin genes and proteins.

Keywords Cortical astrocytes · Methyl-CpG-binding protein 2 (MeCP2) · Axo–glial interactions · CNS myelination

Introduction

In all complex nervous systems neuronal cells coexist with glial cells. This suggests that neuron–glia interactions are principal features for proper functioning of both types of cells. The principal neuro-glia interaction is myelination—process by which an axon becomes insulated by the continuous wrapping of the lipid-rich glial membrane known as myelin. The need for myelination occurs to support the effective transduction of action potentials via saltatory impulse propagation and also providing an additional trophic and metabolic support to the axons. The ability of myelinated axons to conduct saltatory conductions depends on the distribution of numerous molecular components into distinct domains—the internode, the paranode and the node of Ranvier which form as the result of specific interactions between axons and myelinating cells (Devaux and Faivre-Sarrailh 2013). The first molecular constituent discovered and associated with nodal junctions is the axonal contactin-associated protein, Caspr or Paranodin (Einheber et al. 1997) and the glial ligand for which is the neurofascin isoform NF155, which is expressed by myelinating glia in the paranodal region (Tait et al. 2000; Bhat et al. 2001). In addition, other molecules—namely Notch1 (Hu et al. 2004), Neuregulin (Brinkmann et al. 2008) and Neural cell adhesion molecule (NCAM) (Charles et al. 2000) have been reported to be expressed at the site of axo–glial interactions.

Buch Lipi and Langhnoja Jaldeep have contributed equally to this work.

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✉ Pillai Prakash
pillaippp@gmail.com

[1] Division of Neurobiology, Department of Zoology, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India