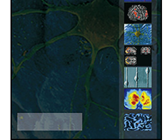
Neuroscience Letters 546 (2013) 1–5

View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet



Stimulatory effect of gonadal hormones on fetal rat hippocampal neural proliferation requires neurotrophin receptor activation in vitro

Meixia Pan [a,b,*], Caixia Zhang [c]

[a] Department of Nutrition, Guangdong General Hospital, Guangdong Academy of Medical Sciences, Guangzhou 510080, China
[b] School of Biological Sciences, The University of Hong Kong, Hong Kong Special Administrative Region
[c] Department of Medical Statistics and Epidemiology, School of Public Health, Sun Yat-sen University, Guangzhou 510089, China

HIGHLIGHTS

- 17β-Estradiol and testosterone significantly increased the neural cell proliferation.
- Estradiol and testosterone increased mature BDNF level and its mRNA expression in neural cell.
- Effect of testosterone on cell proliferation required neurotrophin receptor activation.

ARTICLE INFO

Article history:
Received 11 February 2013
Received in revised form 26 March 2013
Accepted 12 April 2013

Keywords:
Gonadal steroids
Brain-derived neurotrophic factor (BDNF)
Proliferation
Hippocampal neural cell

ABSTRACT

To determine the effects of gonadal hormones on proliferation of the hippocampal neural cells, which are of importance in learning and memory function. 17β-Estradiol or testosterone was added to the culture at various concentrations. Their proliferation and protective effects on the neural cell were determined with BrdU, flow cytometry and MTT assay. Effects of the gonadal hormones on brain-derived neurotrophic factor (BDNF) expression were determined using ELISA and RT-PCR respectively. 17β-Estradiol and testosterone at 20 nM or higher concentrations significantly increased the neural cell proliferation and viability, and induced increasing in the S phase arrest which is essential for cell proliferation. Both estradiol and testosterone significantly increased the neural cell expression of cellular mature BDNF and BDNF mRNA. Effect of testosterone on hippocampal neural proliferation was blocked by Trk neurotrophin receptor inhibitor. 17β-Estradiol and testosterone promoted hippocampal neural proliferation and improved cell viability in vitro. The effect of testosterone on hippocampal neural cell proliferation required neurotrophin receptor activation.

© 2013 The Authors. Published by Elsevier Ireland Ltd. Open access under CC BY-NC-ND license.

1. Introduction

It has been reported that verbal memory declines with the loss of estrogen production in elderly women and the memory loss can be prevented by estrogen replacement therapy (ERT) at the early state of menopause [7]. It has also been reported that early ERT in post-menopause women may reduce the risk of Alzheimer's disease [27]. In both elderly men and women the risk of Alzheimer's disease increases with age [11], and the decline in androgen levels and subsequently diminishing of estrogen in elderly men has been suggested to contribute to the age-associated memory loss [3].

It has been proposed that estrogen may act on central neurons via a direct genomic pathway [24] or indirectly through mitogen-activated protein kinases [20], cAMP-responsive element binding protein [29] or neurotrophic factors [3]. It was reported that estrogen deprivation in rats reduced brain-derived neurotrophic factor (BDNF) expression, while ERT restored the BDNF level in the brain tissue [3]. BDNF is known to play important role in neuronal growth, survival and plasticity formation [1]. The BDNF/tyrosine kinase receptors B (TrkB) system expresses in the hippocampus region and plays a crucial role in memory acquisition and retention [16]. On the other hand, current studies presumed that testosterone may act through androgen (directly or conversion to dihydrotestosteraone), estrogen (via aromatase conversion) [17] pathways, androgen

Abbreviations: BDNF, brain-derived neurotrophic factor; p75NTR, p75 neurotrophin receptor; TrkB, tyrosine kinase receptors B; LTP, long-term potentiation; NGF, neurotrophic growth factor; DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethyl sulfoxide; RT-PCR, reverse transcription polymerase chain reaction.

* Corresponding author at: Department of Nutrition, Guangdong General Hospital, Guangdong Academy of Medical Sciences, Guangzhou 510080, China.
Tel.: +86 20 83827812x10077; fax: +86 20 83827712.
E-mail addresses: meixpan@yahoo.com.cn, meixpan@gmail.com (M. Pan), zhangcx3@mail.sysu.edu.cn (C. Zhang).

0304-3940 © 2013 The Authors. Published by Elsevier Ireland Ltd. Open access under CC BY-NC-ND license.
http://dx.doi.org/10.1016/j.neulet.2013.04.029

receptor activation [4], and increasing neurotrophic growth factor (NGF) levels and induced up-regulation of p75-NGFr in brain in adult male rats [23]. Therefore, testosterone level may account for male's susceptibility to Alzheimer's disease as well as estrogen for female, and the effects of testosterone on hippocampus may be mediated by BDNF expression. In present study, we examined the effects of gonadal hormones on fetal rat hippocampal neural cells proliferation and survival in vitro, BDNF expression, and the subsequent role of BDNF on the neural proliferation.

2. Materials and methods

2.1. Cell culture

Fetal rat hippocumpal neural cells (H19-7/IGF-IR) were purchased from ATCC (No.CRL-2526, USA). The cells were maintained at 34 °C in a humidified atmosphere at 5%CO_2 in phenol-red free Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 200 μg/ml geneticin (Sigma), 0.001 mg/ml puromycin (Sigma) and 2 mM L-glutamine (Sigma). For experiments, neural cells were trypsinized with 0.25% trypsin-EDTA, then suspended in complete DMEM and seeded at a density of 3.0×10^5 cells/ml in 96-well plate. The cells were cultured to approximately 80% confluence, then the complete DMEM was replaced with FBS-free medium with different concentrations of 17β-estradiol (E, Sigma) and testosterone (T, Fluca) respectively.

17β-Estradiol or testosterone was dissolved in pure ethanol with 0.001% ethanol as the final concentration of vehicle in the medium, a concentration without cytoxicity in previous experiments. The cells received treatment of 0.001% ethanol at the final concentration of vehicle in the medium as control group. K252a (Sigma), a selective inhibitor of the tyrosine kinase Trk family of neurotrophin receptors [8], was dissolved in dimethyl sulfoxide (DMSO, Sigma). 200 nM K252a in DMEM containing 0.01%DMSO was added 2 h prior to the addition of gonadal hormones.

2.2. Assessment of cell viability and proliferation

Cell viability was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Sigma) colorimetric assay [15]. Briefly, MTT was added to the culture at a final concentration of 0.5 mg/ml and incubated for 4 h. The intracellular purple formazans in living cells were dissolved in lyses buffer (10%SDS, 0.01 M HCl) overnight. The optical density was measured at 595 nm in micro-plate reader (Bio-Rad).

The percentage of 5-bromo-2′-deoxyuridine (BrdU) incorporated cells were determined using BrdU labeling ELISA kit (Roche, USA). BrdU labeling was determined by immunoassay following the manufacturer's instructions. The reaction product was quantified by the measurement of absorbance at 450 nm/655 nm.

2.3. Cell cycle analysis by flow cytometry

The cell cycle phase distribution was assessed using flow cytometry. Briefly, the cells were harvested with PBS and re-suspended in chilly 75% ethanol at 4 °C overnight, then incubated in 0.5 ml of staining solution containing 50 μg/ml propidium iodide and 50 μg/ml RNAase in PBS at 37 °C for 2 h. Approximately 10,000 cells per sample were collected for analysis with EPICS-XL (Beckman). The percentages of cells in each cell cycle phase were calculated using WinMDI v2.8 software. Each sample was tested three times.

2.4. Measurement of BDNF protein expression

Cells were harvested in 4 °C PBS and centrifuged for 5 min at 1800 rpm at 4 °C. Cell pellets were sonicated for 20 s at 3 W in 50 μl lysis buffer (0.1% (v/v) Triton X-100, 50 mM Tris–HCl (pH 7.0), 200 mM NaCl, 10 mM $CaCl_2$, 0.5% NaN_3, and 1% protease inhibitors cocktail), then centrifuged at 15,000 × g for 30 min at 4 °C. The resulting supernatants were used for measurements of cellular protein [5] and BDNF levels.

Mature BDNF was measured using BDNF-Emax ImmunoAssay System (Promega) following manufacturer's instructions. The cellular concentrations of BDNF in the samples were calculated from the rhBDNF standard curve by linear regression analysis, and expressed as pg/mg protein.

2.5. Semi quantification of BDNF mRNA expression

Reverse transcription polymerase chain reaction (RT-PCR) was used to evaluate the expression levels of BDNF mRNA and using GAPDH mRNA as the internal control. Primers specific to BDNF and GAPDH were designed from public sequences using Primer 3 software and the sequences of the PCR primers were as follows: BDNF Sense: 5′ TGTGACAGTATTAGCGAGTGGGT 3′, anti-sense: 5′ CGATTGGGTAGTTCGGCATT 3′; GAPDH Sense: 5′ AAGTTCAACG-GCACAGTCAAGG 3′, anti-sense 5′ GCACCAGTGGATGCAGGGAT 3′.

Total RNA was extracted using TRIzol reagent (Invitrogen), and reverse transcription (RT) reactions were performed in duplicates in SuperScript First-strand Synthesis System (Invitrogen) according to the manufacture's instructions respectively. Conditions of PCR amplification were as follows: BDNF, denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s, extension at 68 °C for 15 s; GAPDH, denaturation at 94 °C for 30 s, annealing at 57 °C for 30 s, extension at 68 °C for 30 s. 5 μl of PCR products was resolved by 1% agarose gel electrophoresis, stained with ethidium bromide and visualized under UV light. The density of PCR products was analyzed by Quantity One software (Bio-Rad). Results are expressed as the ratios of BDNF mRNA to GAPDH mRNA from the identical sample.

2.6. Statistical analysis

All data are presented as mean ± SEM of two or more repeated independent experiments, and 6–8 replicate per experiment. Statistically significant differences were determined by Student's t-test for two-group comparisons, and by one-way ANOVA followed by Student–Newman–Keuls post hoc analysis for multi-group comparisons. Statistical analyses were performed using SPSS v15.0 (SSPS Inc.). A P value of <0.05 was considered statistically significant.

3. Results

3.1. Gonadal hormones increased cell viability of H19-7/IGF-IR cells

Compared to the control group, treatments with 20, 200 and 2000 nM of 17β-estradiol for 72 h significantly increased the cell viability in H19-7/IGF-IR cells from 7% to 11% ($P < 0.05$, $F = 8.824$, Fig. 1). The cell viability of cultures receiving 200 nM or 2000 nM of testosterone was significantly higher than that of control group respectively ($P < 0.05$, $F = 4.478$), and the magnitude of the testosterone induced reduction of cell viability ranged from 6% to 8%.

3.2. Gonadal hormones promoted H19-7/IGF-IR cells proliferation

To determine whether the increase of cell population is resulted from cell proliferation, the percentage of BrdU incorporated cells in cultures were determined after treatment of 20 nM and 200 nM of 17β-estradiol and testosterone, respectively. We found that 17β-estradiol significantly promoted the cell proliferation by around 33% at 20 nM and 36% at 200 nM compared to the control culture

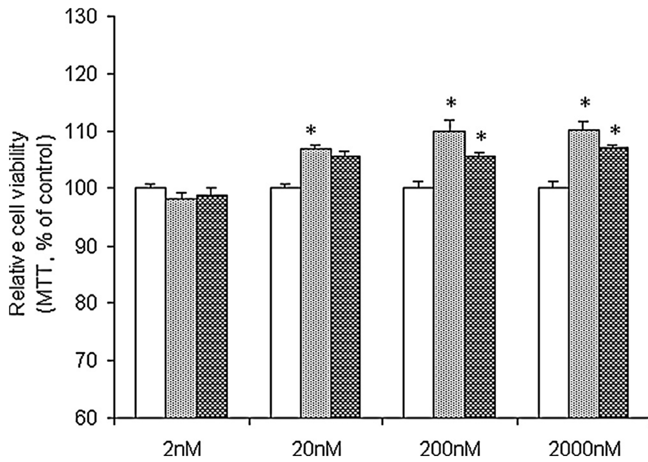


Fig. 1. Cell viability of H19-7/IGF-IR cells determined by MTT assay. Compared to the control cells (white), treatments with of 17β-estradiol (white spotted) for 72 h significantly increased the cell viability from 7% to 11% ($P < 0.05$). The cell viability of cultures receiving testosterone (gray spotted) was significantly higher than that of control cells respectively ($P < 0.05$). *: vs control group, $P < 0.05$.

($P < 0.05$, $F = 9.457$ and $F = 9.913$, Fig. 2A). Testosterone significantly promoted cell proliferation by around 25% at 20 nM and 17% at 200 nM ($P < 0.05$), respectively. The effect of estradiol was more potent than that of testosterone at 200 nM ($P < 0.05$).

Cell cycle analyses showed that 17β-estradiol and testosterone elicited an increase in the percentage of hippocampus neural cells in S phase, an essential phase for cell proliferation, at 200 nM [(17.73 ± 0.51)% and (18.92 ± 0.26)% respectively] as compared to the control group [(14.21 ± 1.75)%] ($P < 0.05$, Fig. 2B).

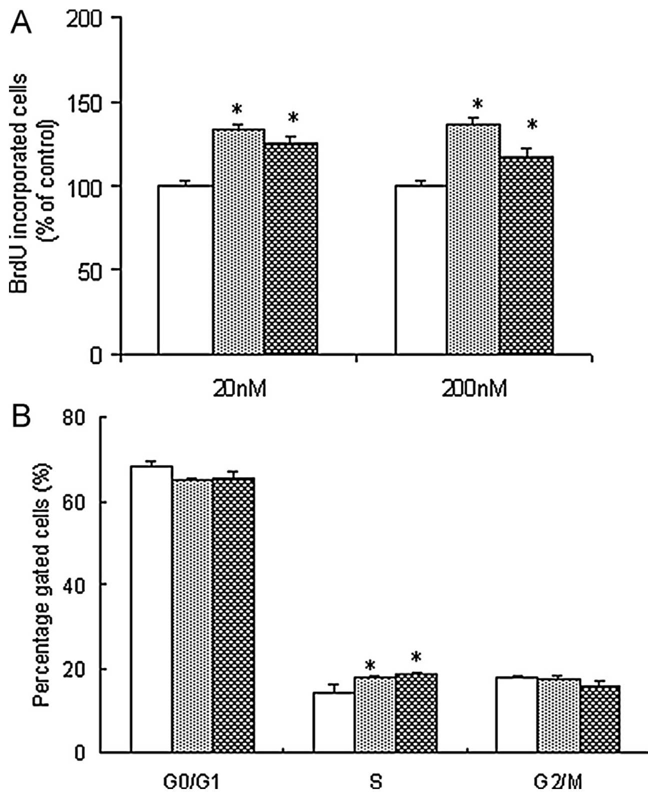


Fig. 2. H19-7/IGF-IR cells proliferation determined by BrdU assay and flow cytometry. 17β-Estradiol (white spotted) promoted the cell proliferation by around 33% at 20 nM and 36% at 200 nM compared to the control cells (white) ($P < 0.05$), while testosterone (gray spotted) promoted cell proliferation by around 25% at 20 nM and 17% at 200 nM ($P < 0.05$, A), respectively. 17β-Estradiol and testosterone elicited an increase in the percentage of cells in S phase as compared to the control group ($P < 0.05$, B). *: vs control group, $P < 0.05$.

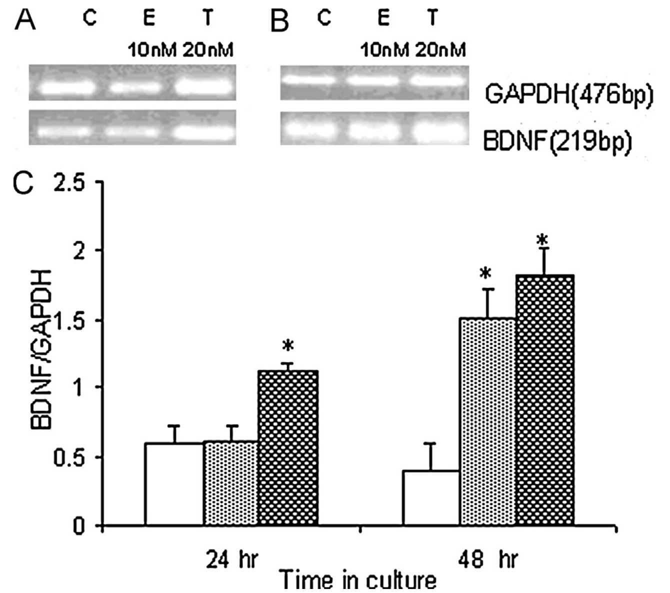


Fig. 3. BDNF mRNA expressions in H19-7/IGF-IR cells. BDNF mRNA level in cells with 17β-estradiol treatment (band E, white spotted) for 48 h was higher than the control cells (band C, white) ($P < 0.05$). BDNF mRNA level in cells with testosterone treatment (band T, gray spotted) for 24 h or 48 h was higher than the control cells respectively ($P < 0.05$). (A) and (B) PCR products with treatment for 24 h and 48 h; (C) Histogram of BNDF mRNA expression. *: vs control group, $P < 0.05$.

3.3. Gonadal hormones increased BDNF expression

Compared to the control group, BDNF mRNA level in H19-7/IGF-IR cells significantly increased with treatment of 17β-estradiol for 48 h ($P < 0.05$, $F = 5.425$, Fig. 3), and testosterone for 24 h and 48 h ($P < 0.05$, $F = 7.539$, Fig. 3), respectively. Compared to the control group, 17β-estradiol increased cellular BDNF level at 24 h after treatment ($P < 0.05$, $F = 4.327$, Fig. 4A). While testosterone increased cellular BDNF level at 24 h and 48 h after treatment ($P < 0.05$, $F = 5.301$, Fig. 4A). No difference in cellular BDNF expression was observed at 72 h after treatment for any groups. And no difference in supernatant BDNF expression was observed after treatment for any groups (Fig. 4B).

3.4. Effect of Trk receptors inhibitor on gonadal hormones-induced H19-7/IGF-IR cells proliferation

Pre-treatment with K252a, a selective Trk receptors phosphorylation inhibitor, significantly decreased cells proliferation ($P < 0.05$, $t = 3.389$, Fig. 5), indicating that Trk pathway contributed to the regulation of cell proliferation in these cells. Pre-treatment with K252a blocked the effect of testosterone on cells proliferation ($P < 0.05$, $F = 3.996$, Fig. 5), suggesting the requirement of Trk receptors activation in testosterone-mediated effects on neural cell proliferation. Pre-treatment with K252a did not abolish the effect of 17β-estradiol on cell proliferation.

4. Discussion

The present study demonstrated that 17β-estradiol and testosterone promoted fetal hippocampal neural cell proliferation and viability. Testosterone and 17β-estradiol increased the cellular BDNF level and BDNF mRNA expression. Effect of testosterone on the cell proliferation was blocked selective inhibitor of

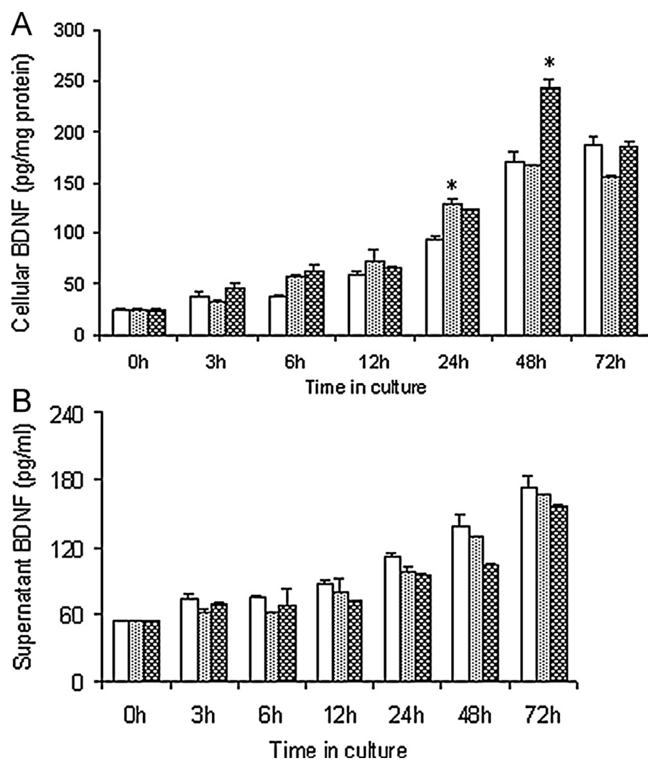


Fig. 4. Mature BDNF level in H19-7/IGF-IR cells. Time course of cellular (A) and supernatant (B) BDNF level with 17β-estradiol (10 nM) and testosterone (20 nM) treatment respectively. Compared to the control (white), 17β-estradiol (white spotted) increased cellular mature BDNF level at 24 h of treatment ($P < 0.05$), while testosterone (gray spotted) increased cellular BDNF level at 24 h and 48 h of treatment ($P < 0.05$, A). *: vs control group, $P < 0.05$.

neurotrophin receptor (Trk), suggesting that the effects of testosterone might require neurotrophin receptor activation.

Hippocampal neurogenesis has been reported to involve in the effects of antidepressants [21], performances on a hippocampal dependent task and recovery from injury [25,26]. New neurons, since they are structurally plastic, are highly susceptible to changes in the environment and different life experiments [12]. Newly generated cells in the adult mouse hippocampus have neuronal morphology and can display passive membrane properties, action potentials and functional synaptic inputs similar to those found in mature dentate granule cells [26]. It was reported that testosterone analog alone increased the number of BrdU labeled neural stem cell (NSC) in embryonic culture [6]. Ovarian steroids increase the proliferation of granule cell precursors in the dentate gyrus of the adult animals [18,22]. It was found here that estrogen and testosterone exert effect on promoted proliferation in H19-7/IGF-IR cells using BrdU assay. These results supported the epidemiological studies demonstrated that women using hormone replacement therapy performed significantly better on a visual memory test in a cross-sectional study [13]. In view of roles of hippocampal neurogenesis, it would be important for the neural development and survival in adults. Our findings raised the possibility that effects of gonadal hormones on promoted proliferation and improved viability could help prevention or improvement of neurodegenerative disease.

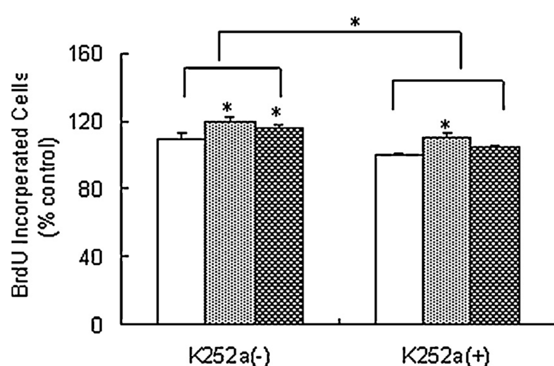


Fig. 5. Effect of K252a on gonadal hormones-induced cell proliferation. Pretreatment with K252a decreased H19-7/IGF-IR cells proliferation (*$P < 0.05$). Pre-treatment with K252a blocked the effect of testosterone (gray spotted) on cell proliferation ($P < 0.05$) but did not abolish the effect of 17β-estradiol (white spotted) on cell proliferation. *: vs control group, $P < 0.05$.

Neurotrophins are key signaling molecules in the development of the nervous system. They elicit diverse cellular responses such as proliferation, differentiation, survival and apoptosis [10]. Initially synthesized as precursors, neurotrophins are cleaved to produce mature proteins, which promote neural survival and enhance synaptic plasticity by activating Trk receptor tyrosine kinases [2]. It was reported that endogenous produced neurotrophins (BDNF and NT-3) and high-affinity Trk-mediated signaling pathways mediated cortical progenitors and microglial cells survival and neurogenesis [28]. Exogenous BDNF has effects on postsynaptic excitability and Ca^{2+} signaling in the dentate granule cell, and the postsynaptic BDNF-TrkB pathway is crucial for regulation of excitatory synaptic transmission and long-term potentiation (LTP) induction, which is an important synaptic connection model of memory formation [14].

Our data showed that BDNF expression displayed a time-dependent increasing in control culture, while estradiol and testosterone had more potent effect on promoting neural cells proliferation compared with the controls. This result indicated that BDNF played an important role in protection and/or reparation on cell model of cytotoxicity and gonadal hormone may have the beneficial effect through enhanced BDNF expression. Testosterone increased BDNF expression [19] and promoted cell growth, differentiation, morphology, survival in center neuronal system [9], suggested that BDNF might media the effects of gonadal hormones on neural cells proliferation. K252a significantly slowed down the neural cell proliferation in the present study, indicating that Trk receptor also contributed to the hippocampal neural cell proliferation. Additionally, K252a blocked the proliferation promoting activity of testosterone, indicating that activation of Trk receptor was required for testosterone to mediate its effect on hippocampal neural cell proliferation. Therefore, testosterone might enhance hippocampal neurogenesis via directly increased cell proliferation through a neurotrophin receptor activation dependent mechanism. While estradiol may promote cell proliferation through BDNF stimulation and other pathways at the same time.

Gonadal hormones replacement therapy has been proved to be an effective for aging related diseases. Although some discrepancy results have been reported, it is probably that the time point of treatment is important to get a beneficial effect. These results showed an important new lead in the prevention and treatment against neurodegenerative diseases with aging of neurodegenerative diseases.

Conflicts of interest

The authors declare that there are no actual or potential conflicts of interest in the manuscript including financial, personal or other relationships with other people or organizations.

Acknowledgements

This research received a fellowship for Ph.D study from Dr. Ruo-Jun Xu and the University of Hong Kong. The authors also express

their deepest thanks to Dr. Ruo-Jun Xu for his important intellectual comment and support. MP participated in the design of the study, cell culture and sample collection, gene and protein expression measurements, statistical analysis, interpretation of data and drafting of the paper. CZ participated in statistical analysis of data, provided language help and proof reading the article.

References

[1] H. Adlercreutz, W. Mazur, Phyto-oestrogens and Western diseases, Ann. Med. 29 (1997) 95–120.
[2] I. Banmeyer, C. Marchand, A. Clippe, B. Knoops, Human mitochondrial peroxiredoxin 5 protects from mitochondrial DNA damages induced by hydrogen peroxide, FEBS Lett. 579 (2005) 2327–2333.
[3] N.C. Berchtold, J.P. Kesslak, C.J. Pike, P.A. Adlard, C.W. Cotman, Estrogen and exercise interact to regulate brain-derived neurotrophic factor mRNA and protein expression in the hippocampus, Eur. J. Neurosci. 14 (2001) 1992–2002.
[4] C. Beyer, J.B. Hutchison, Androgens stimulate the morphological maturation of embryonic hypothalamic aromatase-immunoreactive neurons in the mouse, Brain Res. Dev. Brain Res. 98 (1997) 74–81.
[5] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72 (1976) 248–254.
[6] K. Brannvall, N. Bogdanovic, L. Korhonen, D. Lindholm, 19-Nortestosterone influences neural stem cell proliferation and neurogenesis in the rat brain, Eur. J. Neurosci. 21 (2005) 871–878.
[7] R.D. Brinton, Investigative models for determining hormone therapy-induced outcomes in brain: evidence in support of a healthy cell bias of estrogen action, Ann. N. Y. Acad. Sci. 1052 (2005) 57–74.
[8] J.A. Britton, M.D. Gammon, J.B. Schoenberg, J.L. Stanford, R.J. Coates, C.A. Swanson, N. Potischman, K.E. Malone, D.J. Brogan, J.R. Daling, L.A. Brinton, Risk of breast cancer classified by joint estrogen receptor and progesterone receptor status among women 20-44 years of age, Am. J. Epidemiol. 156 (2002) 507–516.
[9] B.P. Brooks, D.E. Merry, H.L. Paulson, A.P. Lieberman, D.L. Kolson, K.H. Fischbeck, A cell culture model for androgen effects in motor neurons, J. Neurochem. 70 (1998) 1054–1060.
[10] M.E. Evangelopoulos, J. Weis, A. Kruttgen, Neurotrophin effects on neuroblastoma cells: correlation with trk and p75NTR expression and influence of Trk receptor bodies, J. Neurooncol. 66 (2004) 101–110.
[11] M. Gatz, C.A. Reynolds, L. Fratiglioni, B. Johansson, J.A. Mortimer, S. Berg, A. Fiske, N.L. Pedersen, Role of genes and environments for explaining Alzheimer disease, Arch. Gen. Psychiatry 63 (2006) 168–174.
[12] E. Gould, C.G. Gross, Neurogenesis in adult mammals: some progress and problems, J. Neurosci. 22 (2002) 619–623.
[13] C. Kawas, S. Resnick, A. Morrison, R. Brookmeyer, M. Corrada, A. Zonderman, C. Bacal, D.D. Lingle, E. Metter, A prospective study of estrogen replacement therapy and the risk of developing Alzheimer's disease: the Baltimore Longitudinal Study of Aging, Neurology 48 (1997) 1517–1521.
[14] Y. Kovalchuk, E. Hanse, K.W. Kafitz, A. Konnerth, Postsynaptic induction of BDNF-mediated long-term potentiation, Science 295 (2002) 1729–1734.
[15] T. Mosmann, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, J. Immunol. Methods 65 (1983) 55–63.
[16] M.G. Murer, Q. Yan, R. Raisman-Vozari, Brain-derived neurotrophic factor in the control human brain, and in Alzheimer's disease and Parkinson's disease, Prog. Neurobiol. 63 (2001) 71–124.
[17] F. Naftolin, Brain aromatization of androgens, J. Reprod. Med. 39 (1994) 257–261.
[18] B. Ormerod, L. Galea, Reproductive status influences cell proliferation and cell survival in the dentate gyrus of adult female meadow voles: a possible regulatory role for estradiol, Neuroscience 102 (2) (2001) 369–379.
[19] S. Rasika, A. Alvarez-Buylla, F. Nottebohm, BDNF mediates the effects of testosterone on the survival of new neurons in an adult brain, Neuron 22 (1999) 53–62.
[20] E.F. Rissman, S.R. Wersinger, H.N. Fugger, T.C. Foster, Sex with knockout models: behavioral studies of estrogen receptor alpha, Brain Res. 835 (1999) 80–90.
[21] L. Santarelli, M. Saxe, C. Gross, A. Surget, F. Battaglia, S. Dulawa, N. Weisstaub, J. Lee, R. Duman, O. Arancio, C. Belzung, R. Hen, Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants, Science 301 (2003) 805–809.
[22] P. Tanapat, N.B. Hastings, A.J. Reeves, E. Gould, Estrogen stimulates a transient increase in the number of new neurons in the dentate gyrus of the adult female rat, J. Neurosci. 19 (1999) 5792–5801.
[23] P. Tirassa, I. Thiblin, G. Agren, E. Vigneti, L. Aloe, C. Stenfors, High-dose anabolic androgenic steroids modulate concentrations of nerve growth factor and expression of its low affinity receptor (p75-NGFr) in male rat brain, J. Neurosci. Res. 47 (1997) 198–207.
[24] L. Tora, J. White, C. Brou, D. Tasset, N. Webster, E. Scheer, P. Chambon, The human estrogen receptor has two independent nonacidic transcriptional activation functions, Cell 59 (1989) 477–487.
[25] H. van Praag, G. Kempermann, F.H. Gage, Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus, Nat. Neurosci. 2 (1999) 266–270.
[26] H. van Praag, A.F. Schinder, B.R. Christie, N. Toni, T.D. Palmer, F.H. Gage, Functional neurogenesis in the adult hippocampus, Nature 415 (2002) 1030–1034.
[27] K. Yaffe, B. Ettinger, A. Pressman, D. Seeley, M. Whooley, C. Schaefer, S. Cummings, Neuropsychiatric function and dehydroepiandrosterone sulfate in elderly women: a prospective study, Biol. Psychiatry 43 (1998) 694–700.
[28] J. Zhang, C. Geula, C. Lu, H. Koziel, L.M. Hatcher, F.J. Roisen, Neurotrophins regulate proliferation and survival of two microglial cell lines in vitro, Exp. Neurol. 183 (2003) 469–481.
[29] L. Zhao, S. Chen, J. Ming Wang, R.D. Brinton, 17Beta-estradiol induces Ca2+ influx, dendritic and nuclear Ca2+ rise and subsequent cyclic AMP response element-binding protein activation in hippocampal neurons: a potential initiation mechanism for estrogen neurotrophism, Neuroscience 132 (2005) 299–311.