THE EFFECT OF 17β-ESTRADIOL ON ASTROCYTE DENSITY IN HIPPOCAMPAL CA1 REGION DURING THE AGING PROCESS

*Zahra Yadegari1, Mohammad Taghi Ghorbanian2, Gholamhassan Vaezi and Vida Hojati1

1Department of Biology, College of Biological Sciences, Damghan Branch, Islamic Azad University, Damghan, Iran
2Department of Biology, College of Basic Sciences, Damghan University, Damghan, Iran

*Author for Correspondence

ABSTRACT

The aging process affects all body tissues including the brain and different hormones are reduced with aging in mammals. This research was performed to study the effects of 17β-estriadiol on astrocyte density in CA1 region of hippocampus during the aging process. Female Wistar rats were divided into the following five groups: immature rats, mature rats in metestrus phase, mature rats treated with 17β-estriadiol, old rats in metestrus phase and old rats treated with 17β-estriadiol. The groups received 50 μg/kg 17β-estriadiol subcutaneously daily injections for a period of 7 days. The groups in metestrus phase were detected on the basis of observed cells in the vaginal smear. The results revealed increased astrocyte density in groups possessing a better condition in estradiol hormone.

Keywords: Astrocyte, 17β-estradiol, CA1, Hippocampus, Metestrus, Aging

INTRODUCTION

Astrocytes are a sub-type of glial cells in the central nervous system. They are star-shaped with many processes envelope synapses made by neurons (Venkatesh et al., 2013). Astrocytes perform many functions, including biochemical support of endothelial cells that form the blood–brain barrier, provision of nutrients to the nervous tissue, maintenance of extracellular ion balance, and a role in the repair and scarring process of the brain. During the embryogenesis, glial cells direct the neuronal migration along with production of molecules that modify the axons and dendrites growth (Allen and Barres, 2005). Recent studies indicate that glial cells in the hippocampus and cerebellum participate in synaptic transmission, regulation of neurotransmitters distance from the synaptic cleft, release of factors including ATP that regulates pre-synaptic function and even release of neurotransmitters. Moreover, unlike neurons, glial cells are capable of mitotic division (Kuffler and Nicholls, 2000). Astrocytes are believed to be the most abundant type of glial cells that outnumber neurons with a proportion of 10 to 1 and having occupied 25 to 50 percent of the brain volume. Astrocytes are formed like continuous bricks throughout CNS with no overlay (Fiaocco et al., 2008). Astrocytes are broadly divided into two groups: protoplasmic astrocytes found in the brain’s grey matter and fibrous astrocytes of the white matter. Protoplasmic astrocytes are intimately associated with neuronal cell bodies and synapses, whereas fibrous astrocytes are associated with neuronal axons (Allen and Barres, 2009). Gonadal hormones participate in regulation of hippocampal morphology and activity using their influence on both neural and glial cells specially astrocytes (Hajszan et al., 2007; Reyna-Neyra et al., 2002; Tanapat et al., 2005). Astrocytes possess perivascular feet covering around 85 percent of the surface of capillaries in the CNS (Sofroniew and Vinters, 2010). These cells possess a bunch of 9-nm-itermediate filaments that are made of Glial fibrillary acidic protein (GFAP) that strengthens their structure. GFAP is the marker of astrocytes identification (Isaacs et al., 1998). GFAP is an intermediate filament (IF) protein that is expressed by numerous cell types of the central nervous system including astrocytes (Jacque et al., 1978), and ependymal cells (Roessmann et al., 1980). The agrin expression, a protein derived from astrocytes involving in formation of synapses is induced by progesterone (Tournell et al., 2006) while estradiol influences astrocytes and enhances neurite outgrowth by repressing GFAP expression and reorganizing laminin (Rozovsky et al., 2002). Estradiol regulates the GFAP expression in hypothalamus and hippocampus of the rat in both in vivo and in vitro (Stone et al., 1998). Astrocytes number is regulated by
Research Article

estradiol and testosterone during the development of hippocampus of rats (Conejo et al., 2005). The development of astrocyte processes increases the GFAP positive reaction in astrocytes of hippocampal sections in an in vitro environment (Del Cerro et al., 1995) and modifies the astrocyte response after the brain injury (Nilsson and Pekny, 2007). Moreover, the astrocyte processes density and GFAP positive reactions in hippocampus of rats fluctuates during the different estrous cycles and increases in proestrus phase when gonadal hormones are at their highest level (Struble et al., 2006). Astrocytes volume fluctuates in the hippocampal CA1 region across the estrous cycle (Klintsova et al., 1995). These findings support the fact that glial cells are the main target for the effects of gonadal hormones in the mammalian brains. CA1 and CA3 regions of the hippocampus mainly have protoplasmic astrocytes with short and highly branched processes. Within the diestrus phase of the estrous cycle, astrocytes having tinier and longer processes with the reduced GFAP content. Instead, there exists an increased number of astrocyte with a marked increase in GFAP immunoreactivity with short process during the proestrus phase which is more distinguishable in CA1 and in hilus region of dentate gyrus. The consequences of such changes in astrocyte processes could be important for the brain operation. It has been proven that astroglial processes show spontaneous motility at active synaptic terminals in situ (Hirrlinger et al., 2004). In addition to regulation of endocrine functions and sexual behaviors, estrogen plays an important role in normal development of the mammal’s CNS with major neurotrophic and protective functions (Belcher, 2008). Estrogen receptors are found in different brain areas like cerebellum, hippocampus, hypothalamus, basal ganglia, forebrain and cortex (Sherwin, 2006; Shughrue et al., 2000). There are three major types of estrogen: estrone, estradiol and estriol where estradiol is the most potent form of estrogen and is found in higher levels in mature females prior to menopause (Rannevik et al., 1995). Estradiol receptors belong to the transcription factors family and nuclear receptor superfamily (Kuiper et al., 1996; Kuiper and Gustafsson, 1997) that are divided into two estradiol receptors named alpha and beta (Bodo and Rissman, 2006). Moreover, steroids conduct their duties through binding to two intracellular receptors named ERα and ERβ. The aromatase enzyme that synthesizes beta-estradiol is activated in active astrocytes of the damaged areas of the brain and increases peripheral estrogen levels after neuronal injury (Brann et al., 2007). It is interesting that ERα and ERβ are located in progenitor cells and immature neurons in dentate gyrus which indicates that estradiol could directly function for cell proliferation or survival (Perez-Martin et al., 2003; Isgor and Watson, 2005). This research was aimed at studying the effects of 17β-estradiol on astrocyte density in hippocampal CA1 region during the aging process.

MATERIALS AND METHODS

In this study, subjects were female Wistar rats kept under a 12-hour-light-dark cycle at an ambient temperature of 23 ± 2 °C and relative humidity of 60-40. The animals had free access to food and water at standard conditions. The animals were divided into five groups (n= 7 per group):

1- Immature rats (Pre-pubertal) rats (3-week-old, body weight 60-80 g).
2- Mature rats (6-8-week-old, body weight 180-220 g) in metestrus phase.
3- Mature rats (6-8-week-old, body weight 180-220 g) + 17β-estradiol subcutaneous injections.
4- Old rats (one-year-old, body weight 230-250 g) in metestrus phase.
5- Old rats (one-year-old, body weight 230-250 g) + 17β-estradiol subcutaneous injection.

Detection of groups in metestrus phase was done based on observed cells in the vaginal smear (Montes and Luque, 1988). The groups 3 and 5 received 50 μg/kg of 17β-estradiol with sesame oil solvent subcutaneously daily injections for 7 days.

In this research, GFAP marker was used for detecting astrocytes. The cells were labeled with GFAP marker: inserting samples in xylenol I, xylenol II, ethanol 100, 95, 70, and 50, respectively; washing with distilled water I and II, each for three minutes; pouring sodium citrate buffer on samples; inserting slides into the Petri dish where the bottom of it covered with wet filter paper; Petri dishes were heat treated at 720, 360, 180 watt microwave, each for five minutes. It should be noted that during this period, the humidity inside the plate and the sodium citrate buffer should never be reduced and therefore, distilled water was incorporated to maintain humidity along with application of sodium citrate to prevent sample

© Copyright 2014 | Centre for Info Bio Technology (CIBTech)
drying. Samples in wet Petri dish with a solution of 10% goat serum were incubated at room temperature for one to two hours. The samples were incubated with the monoclonal anti-GFAP antibody (Sigma, Aldrich-USA, cat: G6171) for 24 h at 4 °C, washing with TBST with three replacements, each time for five minutes; incubation with secondary antibody (Fluorescein Goat anti-Rabbit-HRP Rhodamin) for two hours at 37 °C; washing with TBST (Tris Buffered Saline Tween) with three replacements, each time for 10 minutes; mounting with glycerol phosphate glue.

Terminal deoxynucleotidyl transferased UTP nick end labeling (TUNEL) is a method for detecting DNA fragmentation by labeling the terminal end of nucleic acids. TUNEL is a common method for detecting DNA fragmentation that results from apoptotic signaling cascades. The assay relies on the presence of nicks in the DNA which can be identified by Terminal deoxynucleotidyl Transferase (TdT), an enzyme that will catalyze the addition of dUTPs that are secondarily labeled with a marker. In the present study, TUNEL kit was used to perform the TUNEL assay. The work stages were as follows: Placing the paraffinic samples in xylene for five minutes; hydrating with ethanol 100, 95, 90, 80 and 70 respectively, each of which for a minute; washing samples with PBS for five minutes; using protein kinase K (10 μg/ml) for 15 minutes; washing samples with PBS (Phosphate-Buffered Saline) for five minutes; Incubation the samples for one hour at 37 °C with 50 μl TUNEL reaction mixture (45 μl Lable solution + 5 μl Enzyme solution); washing samples with PBS for five minutes; mounting the sample using a glycerol phosphate glue; photography of sections with a fluorescent microscope (Nikon Eclipse, E 600, Japan) and digital camera (Nikon, DMX 120, USA).

RESULTS AND DISCUSSION

Results

After Immunostaining, it was revealed that GFAP marker showed different responses in treatment groups and in different regions of the hippocampus. In groups with higher levels of hormones (groups 1, 3 and 5) stronger reactions to GFAP marker (immunostaining) were observed compared to those of other groups (2 and 4).

Figure 1: Astrocytes density of CA1 subfield of hippocampus detecting by GFAP marker
Also, the morphology of astrocytes in the DG region was different compared with the hilus of dentate gyrus, CA1 and CA3 regions. Astrocytes have narrow and slender processes in the dentate gyrus (DG) while they have short and branched processes in CA1 and CA3 regions of hippocampus.

Figure 2: Coronal sections of CA1 region of hippocampus
The results of counting astrocytes indicated that cell density in CA1 region of hippocampus in mature rats treated by hormone was significantly more than immature, mature and old in metestrus phase and old rats treated with hormone (Figure 1). In treated groups with 17β-estradiol, the density of GFAP-positive cells is higher in CA1 region of the hippocampus. Yet, the density of GFAP-positive cells was reduced in specimens of metestrus phase without receiving 17β-estradiol (Figure 2).

The results of counting astrocytes indicated that cell density in CA1 region of hippocampus in mature rats treated with hormone (*) was significantly higher than immature, mature and old in metestrus stage (**) and old rats treated with hormone (***) (p<0.05).

The experimental groups were stained by GFAP marker. A and B: immature rats. C and D: old rats in the metestrus phase. E and F: mature rats in the metestrus phase. H and G: old rats treated with hormone. I and J: mature rats treated with hormone

Discussion

As previously mentioned, gonadal hormones regulate the morphology and activity of hippocampus through their effects on neural and glial cells especially astrocytes (Reyna Neyr et al., 2002; Tanapat et al., 2005). Beta receptors were identified in hippocampal astrocytes and this is important due to the ability of astrocytes to divide and produce new neurons and which is an indication of the possible effect of estradiol (Galea et al., 2008). On the other hand, beta receptor agonist, at low, medium and high doses increases cell proliferation and shows a stronger response for neurogenesis than alpha receptor agonist indicating the higher possible involvement of beta receptors in hippocampal neurogenesis (Galea et al., 2008). Estradiol regulates the number of astrocytes during the development of hippocampus in rats (Conejo et al., 2005). Studies have shown the highest rate of astrocytes in proestrus females (with high levels of steroid hormones) compared to females in other phases of estrous cycle or males (Struble et al., 2006). Astrocyte density was lower in old females or in females located in metestrus phase than other groups with higher levels of hormones. Based on previous studies in this field, it is assumed that higher GFAP expression in females in proestrus phase is associated with higher rates of cell proliferation in the hippocampus.

The aging process affects all body tissues including the brain and consequently, different hormones such as growth hormone, IGF-1 and sexual hormones are reduced with age in mammals resulting in neuronal dysfunction and symptoms such as depression and other psychiatric disorders. This suggests that changes in hormone levels due to aging may have negative effects on brain function. Since brain is an important center for endocrine control, brain aging can cause hormonal changes (Garcia-Segura, 2009). The puberty is a period with favorable conditions of sex hormone levels and neurotrophic factors. Clinical studies have suggested that the incidence of certain neurological diseases such as Alzheimer's and memory loss occurs with aging due to possible changes in environmental conditions. Since brain is an important center for endocrine control, brain aging can cause hormonal changes, which subsequently lead to a decrease in neurogenesis (Garcia-Segura et al., 2009). Since after the formation of neurons in different regions of the CNS, astrocytes formation starts, increasing the neurogenesis is associated with increasing the density of astrocytes and decreasing the neurogenesis is associated with decreasing the astrocytic density. Many of new neurons die within two weeks of their production (Cameron et al., 1993). However, exposure to different treatments including steroid hormones can either increase or suppress the survival of these new neurons (Barker and Galea, 2007; Ormerod et al., 2004; Spritzer and Galea, 2007). Some glial cells act primarily as neurons physical support. Neurogenesis includes cell proliferation, differentiation, migration and survival (Prickaerts et al., 2004). Density of astrocytes affects the survival of newly generated neurons. Eventually, the number of new neurons can be increased not only by increasing the cell proliferation, but also by increasing the survival of new neurons where the neuron protective role of astrocytes density is an obvious reason for survival of neurons.

REFERENCES


© Copyright 2014 | Centre for Info Bio Technology (CIBTech)
Research Article

Research Article


Spritzer MD and Galea LA (2007). Testosterone and dihydrotestosterone, but not estradiol, enhance survival of new hippocampal neurons in adult male rats. Developmental Neurobiology 67 1321–133


