THE EVALUATION OF SUBICULUM NEURONAL DENSITY IN OVARECTOMIZED RATS COMPARED TO NORMAL RATS

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Summary

Steroidal hormones were express in different part of brain such as hippocampus. Then any changes in sex hormones induce neuronal changes in hippocampus and their part. The aim of this study is the evaluation of subiculum neuronal density in ovarectomized rats in compare with normal rats. Eighteen female Wistar Rats weight (300-350 g) were completely divided into two experimental groups and one control group. Animals were anesthetized under interaperitoneal injection of a mixture (1:2) of 10% ketamin and 2% xylazine then the gonads were removed. After one month and two, Animals were decapitated and their brain dissected, fixed in 10% formalin, sectioned in 7µm thickness and stained by H.E.By applying stereological techniques and systematic random sampling scheme the neuronal density of cubiculum were estimated. Statistical analyses showed significant increase ($p<0/01$) in the subiculum neuronal density in experimental groups. Then reduce the steroidal hormones induced neuronal degeneration in subiculum in way that reduced the neuronal density of subiculum.

Key words: subiculum, stereo logy, ovarectomy

Running title: subiculum neuronal density in ovarectomized rats

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Introduction

The hippocampus is an important structure for memory processing. It is a particularly vulnerable and sensitive region of the brain that is also very important for declarative and spatial learning and memory(1). Hippocampal neurons are vulnerable to seizures, strokes, and head trauma, as well as responding to stressful experiences. At the same time they show remarkable plasticity, involving long-term synaptic potentiation and depression, dendrite remodeling (synaptic turnover, and neurogenesis in the case of the dentate gyrus (2).The hippocampus has been implicated in certain short-term memory. Indeed hippocampal lesions often produce short-term memory deficits (3).The hippocampus is preferentially susceptible to a wide variety of toxic insults and disease processes, including hypoxia-ischemia and hypoglycemia (4).Metabolic diseases such as diabetes and obesity have been associated with increased vulnerability to stress (5) and cognitive dysfunction (6).

Gonadal hormones affect the nervous system in ways that extend beyond their essential actions of regulating gonadotropin and PRL secretion and modulating sexual behavior(7). Confronting the efficacy of a regenerative therapeutic is the degenerative environment that is characterized by neuronal loss, physical plague and glial scar barriers and inflammation(8,9), But perhaps more fundaments from a regenerative prospective, are changes in the biochemical milieu of steroid and peptide growth factors, cytokines and neurotransmitter systems(10,11). Data from multiple researches indicate that gonadal steroid hormones and their metabolites can promote neural health whereas their decline or absence is associated with reduction in neural health and increased risk of neurodegenerative diseases including Alzheimer's (12,13). In ovariectomized mice with or without estrogen replacement, regeneration of the sciatic nerve after crush injury was studied. Functional recovery, quantified with sciatic functional index was significantly accelerated in estrogen – treated mice throughout the regeneration (14). Sexosteroid hormones are potential neuroprotective candidates following CNS injury (15) and can play an important role in promoting and enhancing repair after traumatic brain injury and stroke. Although many of it's specific actions on neuroplasticity remain to be discovered. There is growing evidence that these hormones may be a safe and effective treatment for traumatic brain injury and other neural disorders in humans (16). In this research, the neuroprotective actions of testosterone on populations of injured rat spinal motoneurons will be discussed. The aim of present experimental design was to evaluation of subiculum neuronal density in ovarectomized rats in compare with normal rats.

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Materials and methods

All experiment was conducted in faculty of science, Islamic Azad University of Mashhad, Iran (2011-2011).

Animal subjects:

Eighteen female, Wistar rats weighting between 300-350 g served as subjects for these experiments. All animals were housed individually and maintained on a 12/12 light/dark cycle, with lights on at 6.00h. Ambient temperature in the animal facility was kept at 22±2C$^{\circ}$ .Food and water was given ad libitum.

Groups:

1. Controls (N=8)

2.,3. Ovariectomized groups (N=8)

(In group2, the time for brain sampling was 1 month after ovariectomy but in group 3 was 2 month.)

Surgery:

Animals were anesthetized under interaperitoneal injection of an initial dose of 60mg kg^{-1}ketamine and 6 mg kg^{-1} xylazine (ip). Then gonads were removed. In group 2 after one month and in group 3 after 2 month, Animals were anesthetized with sodium pentobarbital(64mg/kg) and decapitated. The whole brain was removed and fixed in 10% paraformaldehyde. NaCl was added to the fixative to make the tissue float in order to overcome deformities during the fixation period. Paraffin embedded tissue blocks were sectioned at 7mμ thickness coronally and stained. A uniform random sampling scheme was employed so that about 10 sections from each block were sampled. With each section thus selected its immediately preceding neighbor was also collected. Sections were stained with toluidine blue staining method with special buffer of acetic acid, sodium acetate and distilled water (PH=4.65). Neuronal density in subiculum was determined, using stereological counting technique; physical dissector (17).

The dissector principle was used to determine the numbers of neurons in each section. Form each section and it's adjacent neighbor two photos were taken, one from each section with a final magnification of 100. A two-dimensional unbiased counting frame was overlaid in a uniform, random manner on to regions of any two photos taken of both sections. Those cell nuclei selected by the frame on the reference plane but not appearing on the adjacent look-up frame section were deemed to have their tops in the volume described by the product of the area of the counting frame and the distance between sections. These nuclei were counted (Q) to provide the numerical density of cells (NV) in the ventral horns of 100-spinal cord according to the equation:

$$NV = \frac{\sum a}{\sum frame \times V_{di\,\mathrm{sec}\,tor}}$$

Where $\sum a$ is the sum of counted neurons, h is the depth of the dissector equal to the section thickness (7 micron) and a (frame) is the scaled area of the dissector frame (18).

Statistical analyze

The ratio of numerical density of neurons in samples of brain was then used as an index of neuronal death. All quantitative data were analyzed using ANOVA and t-test. Student's t test was used for comparison when only 2 groups were analyzed. Statistical significance was chosen as $p<0.05$.All results are reported as mean ± SEM.

Results

The effects of gonad hormone on the numbers of intact neurons in the subiculum region of hippocampus after ovarectomy in rats are shown in (Fig.1,2,3).

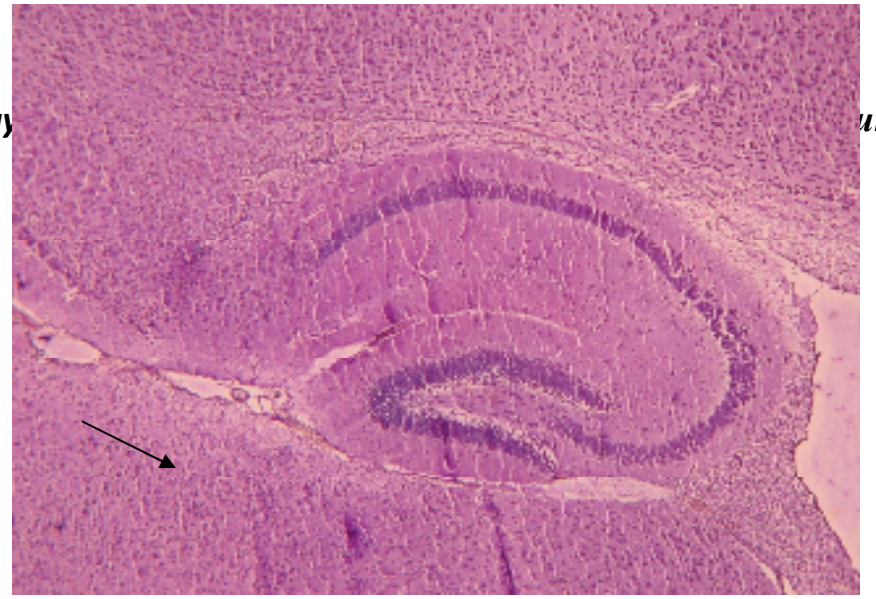

Fig.1.Photomicrograph illustrates neurons of the subiculum region of hippocampus at magnification of (20×). spiks show the subiculum.

1-The control group revealed healthy neuronal cells amounted by (21414×10^{-9} ± 585) intact neurons. The neuronal cells in ovarectomized (1) was (17995×10^{-9} ± 485) and ovarectomized (2) group (16690×10^{-9} ± 479). The numerical density in control group has significant increased ($P<0.05$) in comparison to ovarectomized groups (Fig.2).

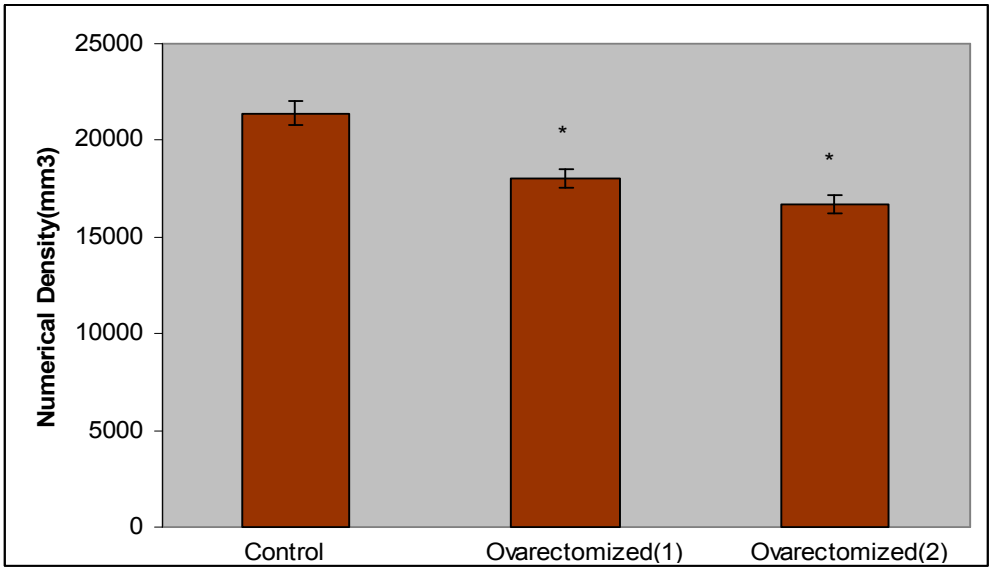

Fig.2: Effects of gonad hormone on the intact neurons numerical density of hippocampus subiculum region in rats. Results are expressed as Mean± SD of 8 rats and data were analyzed by one-way ANOVA followed by Tukey-kramer multiple comparisons test.

2- As has shown in fig.2, ovarectomy resulted in massive neuronal damage manifested as a significant ($P<0.05$)25% decrease in the number of normal appearing neurons after one month.

3- After two month this reduction was more than 30%. There was a remarkable decrease in this group in compare with control ($P<0.01$).

4- When compare ovarectomzed (1) with (2), there was not any significant changes in numericl density (Fig.3).

4- In ovarectomized groups the shape of the neurons and the number of that is obviously changed .

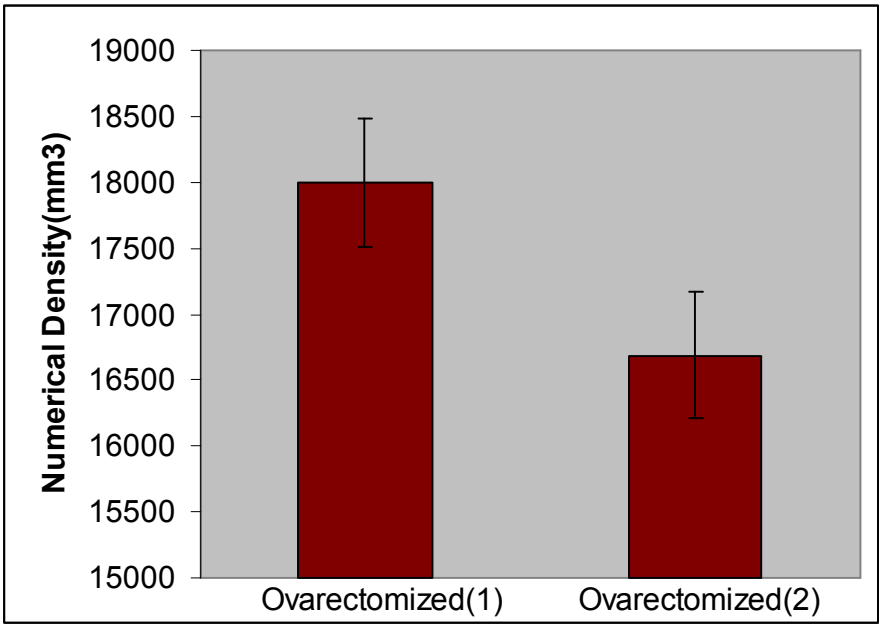

Fig.3.Photomicrographs illustrate neurons of hippocampus subiculum region in rats at magnification of (20×), 30 and 60 days after injury.

Discussion

Our findings demonstrate that endogenous estrogen plays an important role in the maintenance and survival the cell. The data show that there is a remarkable change in the number of neurons in different groups. Endogenous estrogen in control animals resulted in a significant ($P<0.05$) increase in the number of intact neurons, respectively as compared to ovarectomized groups (Fig. 2). Result show that the rate of degeneration was increased in ovarectomized groups during the experiment. It means that in control group endogenous estrogen acts as a survival factor and suppresses the degeneration

phenomena. It is also recommended that whenever in the animal gonad hormone decreased program cell death was happened. In ovarectomized groups even omitting the hormone was longer, the numerical density was reduced more (Fig.3).

Previous researches showed that gonadal androgens (testosterone) can act as a regulator of the expression of receptors for trophic factors, proteins critical for the maintenance of normal structure and function. The cellular effects of gonadal hormone can be grouped into genomic and non genomic categories. Genomic effects are related to transcription and translation of genes, but non genomic effects occur very rapidly and involve in ion movements and/or initiation of signal transduction cascades (19). Testosterone might act directly through androgen pathways or indirectly via conversion to estrogen (6). There is a consensus that these hormones increase secretion of neuromediators, stimulate formation of new synapses and activation of certain genes, responsible for production of anti-apoptotic proteins and growth factors (20). Further more, accumulated evidence suggests that TP manifests its effect on neuronal regeneration in the emendate post operative or pre regenerative phase by altering the cellular stress response (21).

Also, antioxidant action is an important role of the complex neuroprotetive effect of Gonadal hormones. A report argues for a previously unrecognized antioxidant cycle for estrogen derived compounds (22). Gonadal hormones can dilate cerebral vessels, here acting through increased synthesis of nitric oxide and by stimulating such compounds as prostacycline that is a potent vasodilator. There are many evidences which suggest that during brain ischemia the physiological steroidal hormones stimulation can affect metabolism and cerebral blood flow, via release of vasodilating substances (20). Other researchers reported that exogenous administration of testosterone immediately after nerve injury impacted positively on functional recovery through actions mediated by the androgen receptors (AR) (23). They have suggest that mechanism, by which steroidal enhancement of the regenerative properties of the injured motoneurons occurs, may involve pre-existing AR, modulation of the cellular stress response and heat shock proteins. It is interesting that steroid hormones regulate ribosomal gene expression and nuclear ultra structure in target tissues (6). Transcriptional activation of the rRNA gene occurs almost immediately and is maintained regardless of the presence or absence of the steroid. The rRNA transcription is rapidly activated by axotomy. After TP administration, the time interval between rRNA transcription and processing is significantly shortened (21).

So, at least there are several steroid actions involving membranes either coupling via G proteins or generation of a second messenger (2). Such actions may raise the possibility that a membrane steroid

receptor can regulate gene expression indirectly via a second messenger-regulated DNA-binding protein such as a member of the cAMP response element binding protein (CREB) family (25).

Estrogens and other steroids affect the activity of second messenger systems and may do so via genomic as well as nongenomic mechanisms (26). The categories of second messengers will be considered from the standpoint of evidence for receptor mechanisms involved, both genomic and nongenomic. Generaly, the results of present study indicated that endogenous estrogen may change the media for regenerating neurons or survival the cell. It is concluded that estrogen with the protective role is clinically beneficial in the cases of neuronal death. If estrogen provides neuroprotection as seen in rats, estrogen treatment would act to save a number of patients from CNS damage.

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