THE EFFECT OF ENDOGENOUS TESTESTERONE ON NEURONAL SURVIVAL IN RATS

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Summary

Testosterone (tes) is a gonadal sex steroid hormone that plays an important role in the central nervous system (CNS) development. One of the less known testosterone actions is neuroprotection. In this research the effects of endogenous testosterone on neuronal survival in rat was investigated. Twenty-four male wistar rats divided to 3 group (control, compression, compression +castrated n=8). After 4 weeks the lumber segments of spinal cord were sample, processed, sectioned serially and stained with toluidine blue (PH=4.65). By using sterological quantitative technique (physical dissector), the number of alpha motoneurons in the right ventral horns of spinal cord were counted and compared with each other. Statistical analyses showed that the number of motor neurons in compression group reduced significantly. In castration animal compression induced remarkable reduction in compare with control group. This in fact show that testosterone has a survival rule as neuroprotective factor

Key words: Neuronal degeneration, testosterone, survival factor

Running title: Endugenus testosterone on neuronal survival

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Introduction

Neurons have androgenous receptors; androgens as well as testosterone are important for nerve cell. Gonadal hormones affect the nervous system in ways that extend beyond their essential actions of regulating gonadotropin and PRL secretion and modulating sexual behavior(1). For example androgens have been reported to influence verbal fluency, performance spatial tasks, verbal memory tests, and fine motor skills (2). Testosterone has various effects on numerous body tissues, including the brain (3). Testosterone acts via androgen receptors (AR). Regulation of AR protein and/or AR mRNA by androgens has been observed in mammals in multiple androgen-responsive tissues, such as brain (4). AR_s are found in neurons of the brain (5). One of the less known testosterone actions is neuroprotection. By definition, the neuroprotection is an effect that may result in salvage the structure and function of neuronal cells. Testosterone as an endogenous agent, may in the free form cross the blood-brain barrier and influence neuronal cells (6).

Following crush injury to the sciatic nerve changes occur in the cell bodies of the most types of neurons if the neuron successfully regenerates its axon and restores connections with other cells, the cell body usually returns to its former appearance. But failure to contact a new target cell leads to atrophy and death. Studies on neural development have identified several neurotrophic factors that are released by the targets of neurons and retrogradly transport to neuronal cell body. These factors are necessary and important for neuronal survival and growth (4).

In vitro investigations has been demonstrated that the ability of androgen to enhance neurite outgrowth in motoneurons is dependent on neuritin-a protein that is involved in the re-establishment of neuronal connectivity following traumatic damage to the central nervous system and that is under the control of several neurotrophic and neurodegenerative factors(7). It has been hypothesized that, neuritin is a mediator of the ability of androgen to increase peripheral nerve regeneration rates in vivo. (1). There is accumulating body of evidence in the literature suggesting that testosterone may be neuroporotective and therefore have therapeutic value in the treatment of neurodegenerative diseases(8). 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 (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). Data from multiple researches indicate that gonadal steroid hormones(11) 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). In this research, the effects of endogenous testosterone on neuronal survival in rat will be discussed. We have extrapolated concepts from the neuroendocrine field regarding the trophic effects of testosterone on target neural tissue to the nerve regeneration field.

Materials and Methods

All experiment was conducted in faculty of science, Islamic Azad University of Mashhad, Iran (2011-2010).

Animal subjects:

Twenty-four male, 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)

For baseline measurement in this group on the right side an operation was performed which exposed the sciatic nerve but did not disturb it. (Just for induced stress effect of operation.)

2. Compression or Sham-operated controls groups (N=8)

In this group after operation the right sciatic nerve was crushed.

3. Castrated male rat (N=8)

In this animal Coordinated with sciatic nerve crush were castrated.

Surgery:

Animals were anesthetized under interaperitoneal injection of an initial dose of 60mg kg^{-1}ketamine and 6 mg kg^{-1} xylazine (ip). Right sciatic nerve was exposed through a gluteal muscle splitting incision. At this location the nerve trunk was crushed for 30 seconds period between prongs of #5 clamp forceps. The muscle and skin were then closed with 14mm stainless steel sutures (13).

At the selected post-operative time (4weeks), rats were anesthetized and intracardially perfused with formaldehyde. Immediately following perfusion a block of the spinal cord segments L4 toL6 (approximately 8mm length) was removed while sciatic nerve roots of both sides were still attached it. The spinal blocks were placed in the same fixative for post sampling fixation overnight and then processed and embedded in paraffin. The blocks were sectioned serially at 7 micrometer. 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). After permanent mounting the number of motoneurons in right sides of ventrolateral regions of the spinal cord ventral horns (L4 to L6) were determined, using stereological counting technique; physical dissector (13).

The dissector principle was used to determine the numbers of motoneurons 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 from right sides 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(14).

Statistical analyze

The ratio of numerical density of neurons in samples of spinal cord was then used as an index of neuronal death. All quantitative data were analyzed using ANOVA and t-test.

Results

The effects of testosterone on the numbers of intact neurons in the right ventral horn of spinal cord region at 28 days after sciatic nerve compression in rats are shown in (Fig.1).

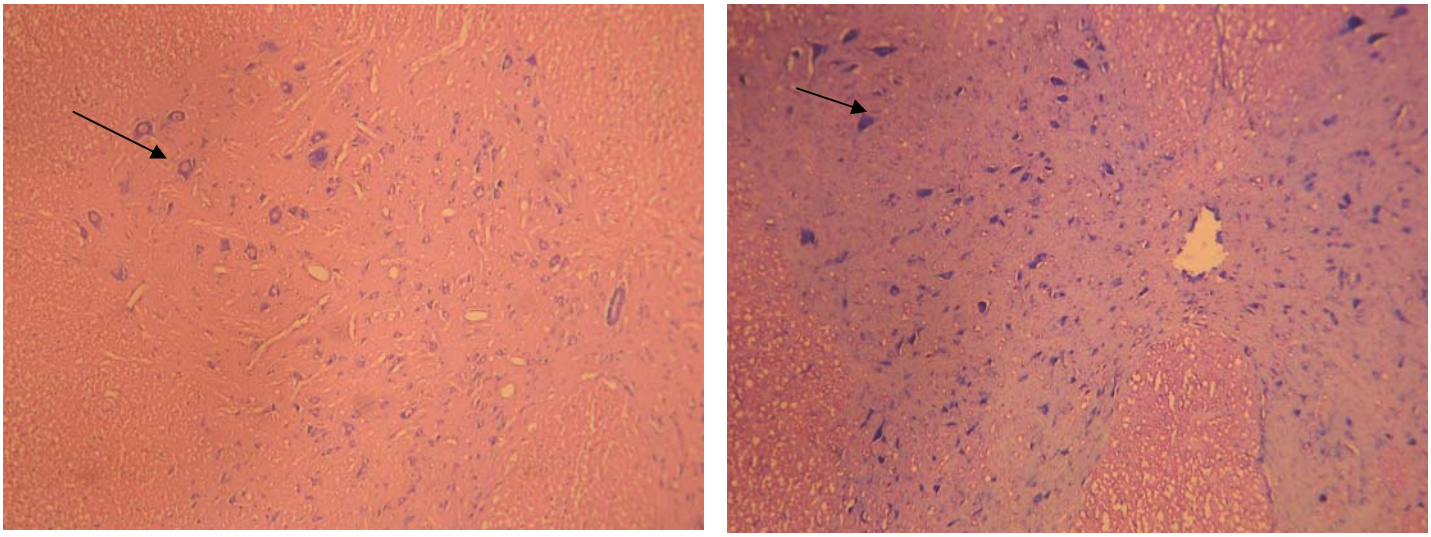

Fig.1.Photomicrographs illustrate neurons of the anterior horn of spinal cord stained with tuloidin blue and eosin at magnification of (20×) 28 days after injury. Left panel: control Right panel: compression. spiks show the alpha neurons.

1-The control group revealed healthy neuronal cells amounted by ($11000\times10^{-9} \pm 400$) intact neurons. The neuronal cells in compression ($8200\times10^{-9} \pm 250$) and compression+ castrated group ($6100\times10^{-9} \pm 200$) were reduced. The numerical density in control group has significant differences ($P<0.05$) in comparison to compression and compression+ castrated groups (Fig.2).

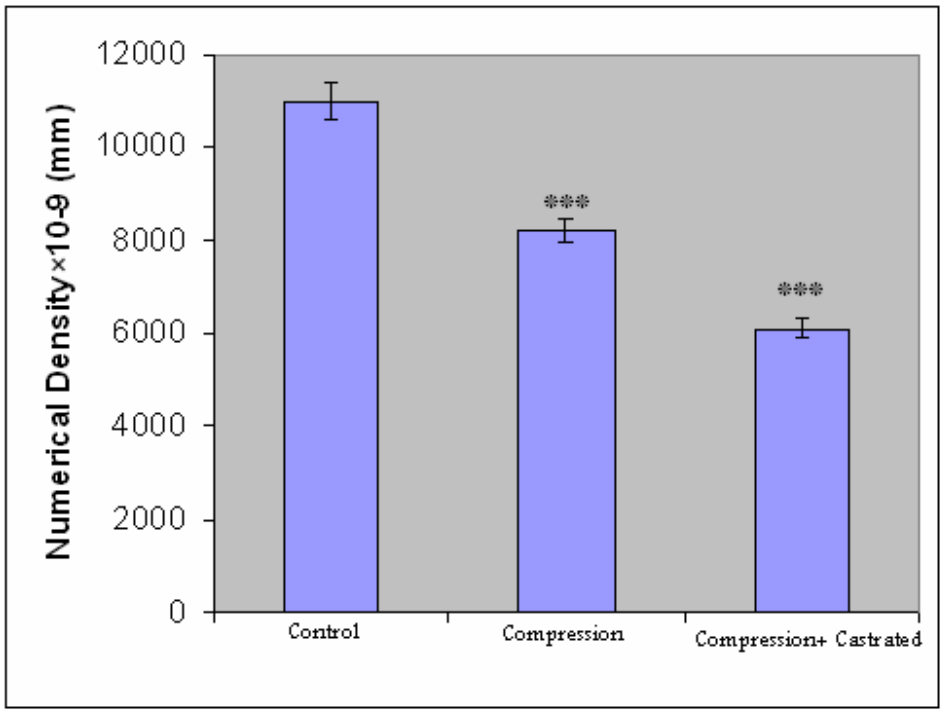

Fig.2: Effects of compression and testosterone on the number of intact neurons of right ventral horn of spinal cord in rat. Results are expressed as Mean± SD of 8 rats and data were analyzed by one-way ANOVA followed by Tukey-kramer multiple comparisons test. Significantly different from control, compression, compression +castrated groups.

3- As has shown in fig.2, sciatic nerve crush resulted in massive neuronal damage manifested as a significant ($P<0.05$)25% decrease in the number of normal appearing neurons. In castrated group this reeducation increases (50%).

4- In castrated groups the shape of the neurons and the number of that is obviously changed (Fig.3).

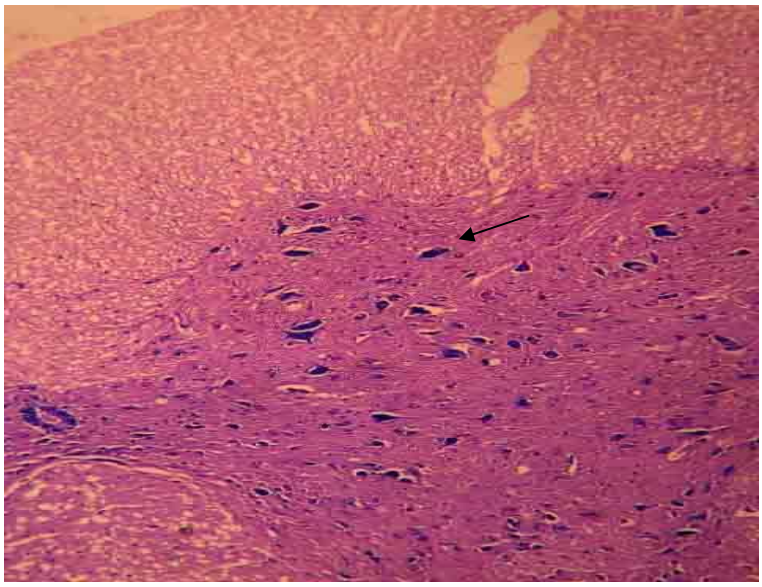

Fig.3.Photomicrographs illustrate neurons of the anterior horn of spinal cord stained with tuloidin blue and eosin at magnification of (20×) 28 days after injury in compression+ castrated groups. Spike shows the alpha neurons

Discussion

Our findings demonstrate that endogenous testosterone plays an important role in the maintenance and survival the cell. Also endogenous testosterone acts as a neuroprotective factor for repairing the nervous system after injury or disease. The data show that there is a remarkable change in the number of Alpha motoneurons in different groups. Endogenous testosterone in control animals resulted in a significant ($P<0.05$) increase in the number of intact neurons, respectively as compared to compression and compression+ castrated groups (Fig1, 2). Result show that the rate of degeneration was increased in castrated groups in compare with compression groups. It means that in compression group endogenous testosterone act as a survival factor and suppresses the degeneration phenomena.

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 testosterone 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 (15).

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 (16). 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 (17).

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 (18) and we can suggest that testosterone also may have such effects. 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 (16). 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) (19). 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 (17).

So, at least there are several steroid actions involving membranes either coupling via G proteins or generation of a second messenger (20). 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 (21).

Estrogens and other steroids affect the activity of second messenger systems and may do so via genomic as well as nongenomic mechanisms 22). 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 testosterone may change

the media for regenerating motoneurons after sciatic nerve compression or survival the cell. So, such new chemical condition may have protection against degeneration of alpha motoneurons. The most important mechanism that we can suggest for such effect is antioxidative function of testosterone. It is concluded that testosterone with the protective role is clinically beneficial in the cases of neuronal death that result from sciatic nerve injury. If testosterone provides neuroprotection against sciatic nerve injury in humans, as seen in rats, testosterone treatment would act to save a number of patients from CNS damage.

Acknowledgments

Authors would like to thanks the Islamic Azad university of Mashhad for financial Supports.

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