Studying the effect of surgery stress on HPG axis hormones

Mozafar Ahmad, Kargar Jahromi Hossein, Dehdari Faranak, Rahmanian Koshkaki Elham, Farzam Mohammad

ABSTRACT

Back ground and objection: Stress is a word that we often encounter with this word during our life and we expose to various stress during day. There is a stress that can effect on person seriously. This stress is called surgery stress. Is among the effect on masculine reproduction system is the effect of stress on HPG axis hormones of rats that has been studies in this study. Material and methods: The mature and rats of Wistar race were divided in to three groups of ten, including control group, experimental group 1, experimental group 2. The rats of control group were not affected by any stress. In the experimental group 1, the rats were maintained for seven days after surgery and in the experimental group 2; the rats were maintained for 14 days after surgery in the laboratorial conditions. After the stress period finished, blood sampling was performed. The rate of LH and GnRH hormones concentration and testosterone of blood serum measured by ELISA method. Finally the results were studied by on way variance statistical test and Dunkan at $P \leq 0.05$ level. Results: The result indicated that surgery stress is resulted in significant decrease of testosterone and GnRH hormones concentration, but there was no significant decrease in LH hormone. Discussion: The result of this study indicates that surgery stress is resulted in control effect on HPG axis and is resulted in negative effect on spermatogenesis process.

Key words: Surgery stress, HPG axis, Rats.

Introduction

Studying the inducing reasons and factors of infertility in male and studying the prevention methods of infertility in a society help patients suffered from infertility. Stress is among disorders that all living creatures are encountered with it during their life. However stress is necessary to continue our life, but this disorder is resulted in various unwanted and negative complications on our life. Yearly many people have to suffer surgery. This stress can be induced by fracture a part of body or extremities surgery or damage or the other reasons. Regardless of the patients reason, some complications induced by stress during surgery, can have unwanted complicity for everyone. In some studies performed about stress, there are some evidences on various stresses effect such as sound stress on the secretion of sexual hormones. For example by studies performed in Japan in 2003, it was determined that sound stress decreases testosterone in the males [6]. In the other case, there were some rats stressed by cold water. In this study we observed the decrease of testosterone level in their bodies [3]. In some cases, the rats were suffered from psychic stress. In this study we observed some changes in the level of some hormones such as sexual hormones. The other study was performed to investigate chronic stress effect and exposing cow to OX, by Shan- Talk. In this study it was indicated that this stress was resulted in LH hormone rate decrease [10]. On the other hand we know that the function of reproduction system is affected by secreted hormones of HPG axis. As example, testosterone is secreted by leydigs cells. This testosterone is necessary to develop and divide germinal cells which are the first phase to form spermatozoid [7].

According to the importance of stress effect on human life, and according to this reality that all stresses can effect on the rate of some sexual hormones secretion and on the function of reproduction system, the object of present study is to investigate the effect of surgery stress effect on...
testosterone, GnRH and LH hormones concentration on mature and male rats.

Methods:

The number of 30 mature and male rats of wistar race (200-220g) were used to perform present experimental study. These rats were maintained in the animal’s house located in Islamic Azad university at standard conditions (12 hours light, 12 hours darkness and 22± 2 ºC). These rats were divided into 3 groups randomly as follow:

1. Control group, 2. Experimental group 1, 3. Experimental group 2. In the control group, there was no stress on rats, but the rats of the experimental groups 1,2 were stressed by surgery after 7 days, blood sampling was performed in the experimental group 1 and after 14 days, blood sampling was performed in the experimental group 2.

The method inducing surgery stress:

The rats were anesthetized by ketamine (50 mg/kg) and Xylazine (20mg/kg). Then the hairs of back were cutted. After this phase, between 1-2 vertebra, there was 1 cm longitudinal cutting. Then skin and its musculature tissues were dissected carefully. Again, the skin was stitched. Blood sampling of heart was performed by direct blood sampling method. The test of testosterone, LH and GnRH hormones concentration was performed by ELISA method on serums after separating serum by centrifuging. Statistical analysis SPSS software version 16 were used to analyze the data and one way variance analysis statistical test and Dunkan test were used to compare the groups. Based on Dankan test, There will be no significant difference if there is at least on common letter in every group. The rate of P<0.05 was considered as statistical significant level. The standard deviation and average of data were measured.

Results:

The level of GnRH hormone serum concentration: The findings related to GnRH hormones concentration indicated that we observed significant decrease of hormone level as compared with control group. Also we observed that this decrease was more in experimental group 2 (14 days surgery stress) as compared with experimental group 1 (7 days surgery stress) and this stress was resulted in decrease of GnRH hormone by increasing the stress period (Table 1).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Experimental 1</th>
<th>Experimental 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>GnRH (pg/ml)</td>
<td>102.3±8.66</td>
<td>8.6±102.34</td>
<td>1.15±68.2 *</td>
</tr>
</tbody>
</table>

The data of table have been measured according to $\bar{X} \pm SEM$ in every group. The level of significant variance is P<0.05.

Fig. 1: Comparison of mean serum GnRH levels in the studied groups. Indicates the significant variance to control group.

The level of LH hormone serum concentration: According to obtained findings, we observed no significant decrease of LH hormone level decrease (Table 2).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Experimental 1</th>
<th>Experimental 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH (Iu/ml)</td>
<td>0.013±0.126</td>
<td>0.011±0.116</td>
<td>0.009±0.011</td>
</tr>
</tbody>
</table>

The data of table have been measured according to $\bar{X} \pm SEM$ in every group. The level of significant variance is P<0.05. The lack of * Indicates no significant variance between studying groups.
Fig. 2: Comparison of mean serum LH levels in the studied groups.

The level of testosterone serum hormone concentration:

We observed no significant decrease in the both of experimental groups 1, 2 in testosterone as compared with control group. Also we observed more effect of surgery stress for 14 days on the decrease of testosterone hormone concentration as compared with surgery stress for 7 days.

Table 3: The comparison of testosterone hormone serum concentration average in study groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Groups</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experimental 1</td>
<td>Experimental 2</td>
</tr>
<tr>
<td>Testosterone (pg/ml)</td>
<td>3.9±0.38*</td>
<td>2.81±0.38*</td>
</tr>
<tr>
<td>Control</td>
<td>5.3±0.26</td>
<td></td>
</tr>
</tbody>
</table>

The data of table have been measured BY $\bar{X} \pm SEM$ in every group. The level of significant variance is $P<0.05$.

*Indicates significant variance to control group.

Discussion and conclusion:

According to obtained results in this study, GnRH concentration in experimental groups indicates significant decrease as compared with control group (Table 1, Diagram 1). Based on performed studies, it was determined that the rate of RFRP mRNA, (RF-amide related peptides) RFRP gene in hypothalamus was increased by response to 3 hours Immobilization stress. Also it was determined that RFRP has effective role to control GnRH secretion [4]. In the recent studies in polan country, it was determined that Close up of stress for 5 hours during 4 successive days can be resulted in significant decrease in the rate of GnRH-mRNA in rats [3]. In some studies about psychic stress on human tried by Ferin in 1999, it was determined that these stresses have been resulted in control mechanisms in hypothalamus and GnRH rate decrease [5]. LH serum concentration indicates insignificant decrease in this study (Table 2, Diagram 3).

LH hormone is secreted by gonadotropes in the anterior pituitary. If there is no complete GnRH secretion, no LH will secret [7]. Janson and colleagues in Helsinki University, in 1988 in their studies on students, pre and post stress of exam found that stress was resulted in LH level decrease in subject blood plasma [8]. Some studies were tried by Tilbrook and his colleagues in 1999 in Monash University in Australia country on sheep’s male. In this study, it was determined that Immobilization stress for hours decreases LH rate [11]. In 2009 Kirbi, in Australia tried a study about Immobilization stress on sheep’s (female) that its result was LH level decrease. They expressed in this study that this decrease is due to stress effect by secreting cortisole and its effects by Glucocorticoid receptor type II on GnRH function, that is resulted in LH rate decrease.
by this function [9]. In 2003, Stakpool tried a study on sheep. In this study it was determined that Restraint stress was resulted in LH level rate decrease in the stressed samples. Probably, LH changes were insignificant because of animal species, animal age, stress and inducing time of stress. Testosterone is secreted by Interstitial Leydig cells in testis but its secretion occur when these cells are stimulated by lutein hormones from anterior pituitary gland. Testosterone effects on LH control by effecting on hypothalamus and GnRH secretion regulation [7]. Some studies were tried by Almeida and colleagues in 1988 in Brazil country on immobilization stress for 6 hours a day during a 60 days period in the rats of Wistar race. In this study it was determined that stress was resulted in the decrease of LH rate (29%), testosterone (37%), in rats blood [2].

In the performed studies in 1993, it was determined that cold water stress was resulted in the decrease of blood plasma testosterone rate in the male rats [3].

In some performed studies in Japan, in 2003 ita was determined that sound stress was resulted in the decrease of testosterone in males [6]. Testosterone secreted by Leydig cells is necessary to develop and divide germinal cells which are the first phase of forming spermatozoa [7]. It is clear that some changes have been beginning in spermatogenesis process following stress and because of the decrease of testosterone level. In 2008, John itken expressed that spermatogenesis is a dynamic process and is very sensitive to reactive factors. For example it was determined in this study that oxidative stress was resulted in negative effect on spermatogony and Leydig [1]. Therefore possibly surgery stress can have negative effect on HPG axis hormones secretion and spermatogenesis process.

References