

Effect of Pregabalin on Pituitary – Gonad Axis and Testis Histological Changes in Adult Rat

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ABSTRACT

Pregabalin binds potently to the $\alpha 2\text{-}\delta$ subunit of calcium channels, resulting in a reduction in the release of several neurotransmitters, including glutamate, noradrenaline, serotonin, dopamine and substance P. Recently it is approved for adjunctive treatment of partial seizures in adults neuropathic pain from postherpetic neuralgia and diabetic neuropathy. In this research the effect of Pregabalin were studied on pituitary-gonad axis function, spermatogenesis, the concentration of testosterone, FSH and LH level and testis histological changes. The experiments were done on 40 male Wistar rats divided into 5 groups of 8. The control group received nothing. The sham group was given distilled water as a solvent. The experimental groups received 1000, 2000 and 4000 mg/kg of the drug orally for 28 days. Blood samples were taken at 29th day and concentrations of testosterone, FSH and LH were measured by RIA method. In addition, at the 29th day the testes were separated and histological changes were studied among experimental, control and sham group. The results were evaluated using SPSS and ANOVA tests. The results showed that 4000mg/kg of Pregabalin reduced serum testosterone level while it increased FSH and LH levels ($p < 0.05$). Histological investigations of the testes showed a decline on spermatogenesis chain when Pregabalin doses increased to 4000mg/kg. According to our findings, Pregabalin decreases the concentration of testosterone and the number of spermatogenic and Leydig cells, and increases FSH and LH levels at maximum dose.

KEY WORD: Pregabalin, Reproduction, Rat.

INTRODUCTION

Epilepsy is the most common chronic neurological disorders in the world. The lost natural balance between excitement and inhibition in the central nervous system causes convulsions (2). Antiepileptic drugs create natural balance between excitatory and inhibitory postsynaptic potential through different mechanisms (3). Pregabalin has an impressive and superior absorption compared to voltage dependant $\alpha 2\text{-}\delta$ subunit of calcium channels in the central nervous system (4), which inhibits the receptor, prevents the entry of calcium ions into it and reduces stress- induced release of neurotransmitters such as glutamate, noradrenaline, dopamine, serotonin, substance P and calcitonin gene-related peptide (5) and with this action the existing neurons in the central nervous system that have been overstimulated return to their normal position (6). This drug is used as adjuvant treatment of adult partial epilepsy and the treatment of generalized anxiety disorders, neuropathic pains associated with peripheral diabetic neuropathy and postherpetic neuralgia. Pregabalin is a sleep aid. According to studies taking pregabalin 450 mg daily as a single dose treatment is effective in relieving pain in patients with fibromyalgia syndrome. It can also reduce sleep disturbance and fatigue (8,7). In human and animal studies observing many similarities between pathophysiological phenomenon in some models of epilepsy and neuropathic pains is a rational justification for the use of this drug in the treatment of neuropathic pains (9). Using the drug led to a 50 percent reduction in morphine requirements after surgery; however, in addition to saving drug use, it leads to reduced nausea or vomiting and increased levels of sedation (11, 10). Studies conducted on animals have shown that analgesic power of pregabalin is 6 times more than gabapentin. It passes the blood-brain barrier. The side effects include weight gain, breast enlargement in males (12), dizziness and peripheral edema (13). Studies have shown that pregabalin changes sexual desire, erectile dysfunction and delayed ejaculation (14, 15). In the present study the effect of pregabalin on the pituitary – gonad axis and testis was examined to use results to provide appropriate solution regarding regulation, development or limitation of its use.

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MATERIALS AND METHODS

This experimental study was conducted in laboratory and Wistar rats weighing 180-220 g and 2.5-3 months of age were used. All animals were placed in standard lighting conditions of 12 hours of light and 12 hours of darkness at $22 \pm 2^\circ\text{C}$ and had access to food and water indefinitely. Animals were randomly divided into 5 groups of 8 rats in control, sham and experimental groups of 1, 2 and 3. The control group animals received no drug or non-drug treatment. The sham group received 2 ml of distilled water (solvent) orally. The experimental groups 1, 2 and 3 received pregabalin with the values of 1000, 2000 and 4000 milligrams per kilogram of body weight for 28 days orally. After 28 days, after weighting the animals, they were anesthetized with ether and about 5 ml of blood was taken from the heart of each mouse and poured in test tubes. Blood samples were centrifuged for 15 minutes at 3000 rpm to separate the serum from the clot. The samples were kept at -20°C for the measurement of serum concentrations of FSH, LH and testosterone. Measuring hormones was performed using radioimmunoassay (RIA). Hormone kits included standard solutions of radioactive iodine, antibody and wash buffer purchased from Kavosh Yar company as an affiliate of Atomic Energy Organization. After opening the abdomen and scrotum of the rats both testicles were removed from all groups and tissue sections prepared and stained with hematoxylin – eosin. Then using scaled slide for measurement, and Nikon optical microscope, changes in sperm density in seminiferous tubules, interstitial cells changes, Sertoli and spermatogenesis chain were determined between the experimental and control groups in studies of tissue. The mean and standard deviation was expressed as mean \pm standard error. For statistical analysis of test results between the experimental and control groups ANOVA test and SPSS software were used. Statistical inference borderline of $p < 0.05$ was used to analyze significant differences between the experimental and control groups.

RESULTS

Figure 1 presents changes in the plasma concentration of testosterone in the experimental group receiving pregabalin. The comparison of the data shows an average reduction of 38% in average plasma concentration of testosterone in experimental group 3 compared with the control group which was significant at $p < 0.05$. Changes in plasma concentration of LH hormone in the experimental groups are shown in Figure 2. The comparison of the data shows an average increase of 35% in LH hormone in experimental group 3 compared with the control group which was significant at $p < 0.05$. Changes in plasma concentration of FSH hormone in the experimental groups are shown in Figure 3. The comparison of the data shows an average increase of 11% in FSH hormone in experimental group 3 compared with the control group which was significant at $p < 0.05$. The results also showed that the average number of spermatogonial cells in experimental groups 2 and 3 have reduced 28 and 30% compared to their average in control group which was significant at $p < 0.05$. Also the average number of primary spermatocytes cells in different groups taking the drug has reduced compared to the control group as their average in experimental group was 36% less than the control group which was significant at $p < 0.05$. This reduction in compared experimental group to the control group was 29.5 which was significant at $p < 0.05$. The average number of spermatid cells in different groups receiving the drug decreased compared to the control group so that the average of the experimental group 3 was 45% less than the control group which was significant at $p < 0.05$. The reduction in experimental group 2 was 35% less than the control group which was significant at $p < 0.05$ (Table 1).

Table 1. Mean (\pm Standard deviation) number of sperm cells in a Seminifer tube after oral prescription of the pregabalin drug.

Groups	The medication (Mg / kg)	The number of spermatogonia	The number of spermatocytes	The number of spermatide
Control	-	58.83 \pm 3.92	70.83 \pm 2.48	157.17 \pm 3.55
Sham	-	57.83 \pm 4.87	69.17 \pm 2.79	154.5 \pm 3.83
Experimental 1	1000	51.67 \pm 9.03	60.67 \pm 6.09	132.67 \pm 6.52
Experimental 2	2000	42.17 \pm 4.79*	50 \pm 5.33*	102.83 \pm 9.06
Experimental 3	4000	41.17 \pm 2.23*	45.5 \pm 1/05**	86.5 \pm 6.89**

* indicates significant differences at $p < 0.05$

** indicates significant differences at $p < 0.01$

The number of Sertoli cells in the experimental groups did not show a significant difference compared to the control group. The average number of Leydig cells in different groups of receivers The drug also decreased compared to the control group so that the averages of the experimental groups 3 and 2 were 28 and 23% less than the control group which was significant at $P < 0.05$ (Table 2).

Table 2. Mean (\pm Standard deviation) number of Sertoli and Leydig cells in a Seminifer tube after oral prescription of the pregabalin drug.

Groups	The medication(kg/ha)	Number Sertoli cells	Number of Leydig cells
Control	-	14.73 \pm 0.15	17.25 \pm 0.14
Sham	-	14.61 \pm 0.17	17.18 \pm 0.24
Experimental 1	1000	14.57 \pm 0.19	16.23 \pm 0.39
Experimental 2	2000	14.76 \pm 0.18	13.52 \pm 59/0*
Experimental 3	4000	14.72 \pm 0.13	12.47 \pm 0.56*

* indicates significant differences at $p < 0.05$

Photo micro-graph prepared by the cross-section of seminiferous tubules analysis in the experimental groups and its comparison with control group indicates that the number of spermatogonia, primary spermatocytes and spermatids in the experimental groups was reduced compared to the control group. Also the chain process of spermatogenesis did not have the same continuity of the control group. Spermatogenesis in the seminiferous tubules center of the experimental groups was reduced compared to the control group and the changes in the group receiving the highest dose reached a maximum level (figure 1, 2).



Figure 1- photomicrograph of Spermatogonia, Primary Spermatocyte, Spermatid and Sperm cells in the controlled group.

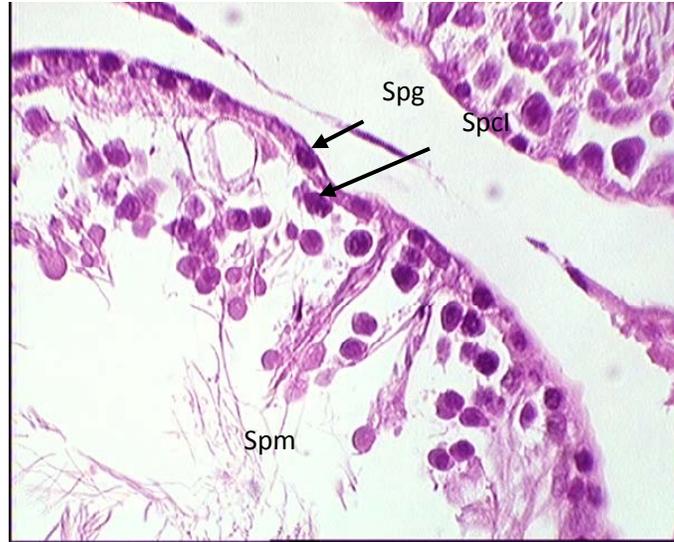


Figure 2- photomicrograph of Spermatogonia, Primary Spermatocyte, and Sperm cells in the group that treated with the maximum amount of drug.

Also Photo micro-graph prepared by the space between seminiferous tubules analysis in control group and its comparison with different groups receiving the drug represents the destruction and loss of interstitial cells in the experimental group compared with the control group, and the changes in the group receiving the highest dose reached a maximum level (figure 3, 4).

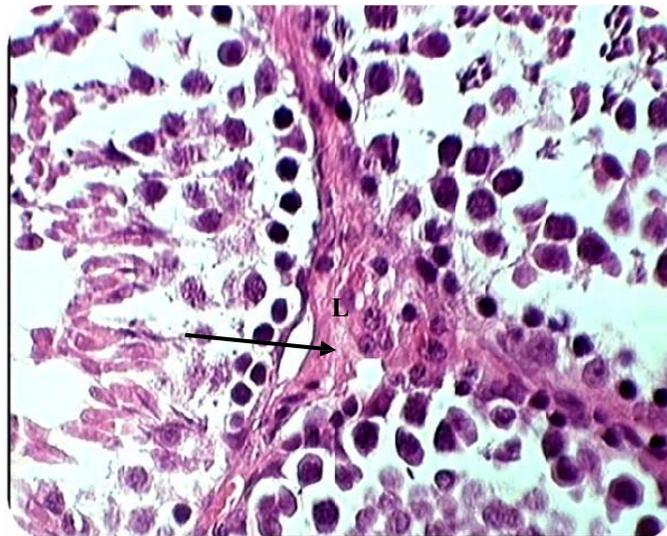


Figure3 - photomicrograph of interstitial cells in the control group.



Figure4- photomicrograph of interstitial cells in the group that treated with the maximum amount of drug.

DISCUSSION

Due to the significant decrease of testosterone in the group receiving the highest dose of pregabalin, this drug increases prolactin by reducing the release of dopamine in the synaptic cleft. Prolactin through increasing nitric oxide inhibits the conversion of cholesterol to pregnenolone which reduces testosterone (16). On the other hand pregabalin drug reduces the release of serotonin in the synaptic cleft. Since serotonin is the necessary precursor of melatonin biosynthesis, this would reduce melatonin. Melatonin as an antioxidant is effective in protecting the testicular tissue and by reducing melatonin, the level of anti - oxidants in Leydig cells reduces and decreases synthesis and secretion of testosterone (17). Reduced melatonin decreases testosterone by reducing the glutathione peroxidase enzyme (18). Pregabalin increases the activity of Glutamate decarboxylase enzyme and GABA synthesis of glutamate. GABA is produced by glutamate decarboxylase enzyme. Pregabalin by reducing serotonin increases glutamate decarboxylase enzyme activity which increases the synthesis of GABA from glutamate. GABA inhibits ACTH hormone secretion by anterior pituitary (19). By reducing ACTH adrenal gland cortex activity to produce steroid is reduced and the most important phase of ACTH excitation to regulate the adrenal gland cortex i.e. the activation of protein kinase an enzyme to convert cholesterol to pregnenolone is weakened. Thus testosterone level is reduced (20). Serotonin increases the secretion of Alpha-melanocyte stimulating hormone. Arcuate nucleus terminal includes alpha-melanocyte stimulating hormone that is closely related with cell bodies of TRH neurons through which regulates TRH biosynthesis in paraventricular nucleus (PVN). Also the increased level of Alpha-melanocyte stimulating hormone increases CREB protein's phosphorylation in many PVN sub-sectors including TRH. Thyroid stimulating hormone that is released from paraventricular nucleus in hypothalamus affects pituitary gland and causes TSH secretion. Thus thyroid hormones are secreted by TSH. It seems that pregabalin decreased thyroid hormone through reducing serotonin (21). Studies show that thyroid hormones are involved in the development and function of Leydig cells (22). Thyroid hormones increase the number of LH receptor Leydig cells and simulate production and secretion of testosterone from these cells (23). Thus, by reducing the thyroid hormones the number of LH receptors is reduced and consequently the amount of testosterone secretion is reduced. FSH and LH hormones are released anterior pituitary FSH cells. ACTH has limiting impact on hypothalamic - pituitary – gonadal axis. Thus pregabalin by increasing GABA decreases ACTH which increases LH (24). Other studies have shown that there is an inverse relationship between the levels of serotonin and LH secretion (25). Thus pregabalin by serotonin increases serum concentrations of LH. On the other hand, by reducing melatonin it increases GnRH volatility of hypothalamus which is followed by an increase in LH and FSH secretion in anterior pituitary (26). Low testosterone secretion based on negative feedback allows the hypothalamus to release large amounts of GnRH resulting in increased LH and FSH secretion from anterior pituitary gland. Reduced level of testosterone may have a negative feedback effect on pituitary gland directly besides its effect on hypothalamus. And it is believed that this pituitary feedback increases LH secretion exclusively (27). Testosterone is an inhibitor factor in monoamine oxidase enzyme activity that is involved in the catabolism of dopamine (28).

According to the results of this study, possibly by reducing testosterone, the inhibitory effect on enzyme activity decreases and dopamine concentration is reduced. Dopamine affects the arcuate nucleus and prevents the production of LHRH. Thus, by reducing the amount of dopamine, the level of LH increases indirectly (29). Studies have shown that testosterone directly affects the Sertoli cells. Sertoli cells help feeding dividing sexual cells by secreting liquid in the tubes. They also secrete several growth factors and transferring proteins each one having a special role in sexual cell division and eventually in sperm production. Testosterone also has another role which is a direct effect on splitting sexual cells (30). With regard to the role of testosterone on spermatogenesis, it is clear that in case of reduction in the secretion of the hormone sperm density is decreased. Mammalian sperm cells contain high amounts of unsaturated fatty acids and sphingomyelin that are important substrates in oxidation. In natural conditions antioxidant mechanisms are involved in reproductive tissues and prevent oxidative damage in gonad cells and mature spermatozoa (31). Studies show that increased free radicals adversely affects sperm proliferation, activity and fertility (32).

Glutathione peroxidase enzyme plays a role as an antioxidant to protect sperms in testis and epididymis. This enzyme by location in plasma membrane and the nucleus of the sperm protects it from damage by free radicals and cause final maturation and evolution of sperm (33). Glutathione peroxidase enzyme prevents the destructive effects of DNA break in sperm and sperm-producing cells. Melatonin as a powerful antioxidant prevents the degeneration of germ cells. Melatonin stimulates the internal antioxidant system and increases glutathione peroxidase, glutathione -S Transferase, superoxide dismutase and other thiols activity in the blood, liver, testis and kidney. These combinations prevent negative impacts and DNA breaking as well as inhibition of mitosis and meiosis division in sperm sperm-producing cells (34, 35, 36). Pregabalin by reducing the release of dopamine in the synaptic space increases prolactin which is followed by a reduction in the activity and division in the epithelial cells of the testes, therefore the reduced number of Spermatogenic cells in the effect of using pregabalin is expected (37). Generally it can be said that one of the side effects of using pregabalin is the reduced steroid production process in testes. Pregabalin in high doses can reduce testosterone levels and may interfere with reproductive activity. Therefore, it is recommended to be used cautiously in patients with impaired production of sexual hormones. Also in order to reduce the side effects of pregabalin in these patients, it is suggested to use them simultaneously with drugs enabling the production of steroids.

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