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Effect of some neurotransmitters on the testes and reproductive hormones in albino rats

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The present study has been carried out to investigate the effect of three neurotransmitters (Glutamate, L-Arginine and GABA) on some aspects of the reproductive performance of mature male Albino rats. For this purpose, a total of 100 mature male Albino rats were used. Rats were divided into 4 comparable groups; the first consists of 10 rats, was left as control. The second was administered glutamate 10 mg/ kg, the third group was injected by L- Arginine 20 mg/ kg while the fourth was injected by GABA 1 mg / rat. The results showed that administration of glutamate was concomitant with increase in synthesis and release of pituitary LH causing increase in its serum level as well as decrease serum level of testosterone. On the other hand, prolonged L-Arginine administration led to remarkable elevation in both pituitary and serum LH and significant decrease of serum testosterone. While, GABA administration led to remarkable decrease in pituitary and serum LH with significant decrease in serum testosterone level.

Reproduction in farm animals is considered the backbone of the economy of any country so the efforts of the scientists are directed toward this object in order to improve the animal reproductive performance.

The reproductive patterns are regulated mainly by hormonal system begins from the hypothalamus which secrets gonadotropin-releasing hormone (GnRH) that stimulates anterior pituitary to release gonadotropins (GnH) including luteinizing hormone (LH) and follicle stimulating hormone (FSH). gonadotropins act on gonads regulating the secretion of gonadal steroids which in turn regulate the hypothalamic-hypophyseal function by feedback action through indirect mechanism. However, several studies have demonstrated that GnRH neurons in hypothalamus do not have steroid receptors (Donoso *et al.*, 1994; Tillbrook and Clark, 2001), hence steroid control of GnRH secretion appear to be mediated by other inhibitory or excitatory neurotransmitter neurons, which, in effect, relay steroid signals to the GnRH neurons (Bhat *et al.*, 1998).

Neurotransmitters (NTS) appear to play an important role in regulating the reproductive function. L-Glutamic acid (GLU), the major representative of the excitatory amino acids (EAA) system, stimulates LHRH release from arcuate nucleus-median eminence (AN-ME) fragments in vitro, in the same time gamma-aminobutyric acid (GABA) is a major inhibitory amino acids (IAA) neurotransmitter, as another regulator of LHRH secretion (Donoso *et al.*, 1994). Also, Nitric Oxide gas (NO) is an important neurotransmitter which has established itself as a polyvalent molecule that plays a decisive role in regulating multiple functions within the female as well as the male reproductive system (Paul *et al.*, 1998).

The present study was designed to clarify the effect of three neurotransmitters (Glutamate, L – Arginine "the precursor of nitric oxide" and GABA) on some aspects of reproduction of male rats.

Materials and methods

Experimental Design. This study included 100 sexually mature male Albino rats weighing from 150-170 g obtained from lab animal house, Faculty of medicine, Assuit University. Animals were kept for two weeks for acclimatization. Throughout the experimental period, rats were kept under the same environmental and hygienic conditions as well as offered food and water ad. libitum. Food consisted of cereal standard diet supplemented with minerals and vitamins mixture. Rats under experiment were divided into 4 groups and treated as follow.

Control group. Consists of 10 rats and injected with normal saline. The rest of animals; 90 rats were equally divided into 3 comparable groups.

Second group. Animals were injected with Glutamate (LOBA CHEMIE, Mumbai, India) 10

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mg/ kg (Estienne *et al.*, 2000).Third group. Animals were injected with L- Arginine (the British Drug House, B.D.H. Laboratory Chemicals Division, England) 20 mg/ kg (Anderson *et al.*, 2005).Fourth group. Animals were injected with GABA (Merck – Schuchardt, Germany) 1 mg / rat (Jones *et al.*, 1976). For all groups, the injected dose of corresponding drug was freshly prepared in 0.5 ml saline and injected by I.M. route. This process was repeated every 3 days for 2 successive months.

Sample collection. Individual blood samples (for obtaining serum) and pituitaries were collected, from retro-orbital plexus, every 10 days from 5 animals of each treated group (2nd, 3rd and 4th group). Those of control group collected at the end of experimental period; 60 days. Sera and pituitaries, immediately after collection, were kept frozen at -20° C until the immunological assay of the studied hormones (FSH, LH and Testosterone "T").

The serum and pituitary levels of FSH, LH and LH were determined using indirect enzyme linked immunosorbent assay (ELISA) as outlined by Voller *et al.*, (1979).

Serum levels of testosterone in the control and treated rats were assayed using radioimmunoassay technique as described by Jaff and Behrman, (1974) in the Middle Eastern Regional Radioisotope Center for the Arab countries, Dokki, using the specific RIA kits (Diagnosis Products Corporation Immunotech, France).Throughout the current study, the statistical analysis of the obtained data was done using t- test as outlined by Snedecor and Cochran, (1987).

Results

The results showed that the control levels of pituitary FSH and LH were (8.63 ± 0.83 and 2.05 ± 0.17 i.u. / ml, respectively) while that of serum were (8.37 ± 0.520 and 2.02 ± 0.13 i.u. / ml, respectively). The results showed that glutamate administration had no significant effect on pituitary and serum FSH. On the other hand, regarding pituitary LH levels, results in table 1 showed that pituitary levels significantly decreased in comparison with control. However, serum levels in glutamate – injected groups were significantly higher than control.

Results in Table 2 showed that L-Arginine administration had no significant effect on serum and pituitary FSH levels. Furthermore, it was shown that, L-Arginine administration resulted in significant increase of both pituitary and serum LH levels in all groups and significant decrease in pituitary LH after 60 days of injection.

Table (3) showed that GABA induced no significant effect on pituitary FSH but caused significant decrease in its serum level (6.14 ± 0.360 i.u./ml) after 60 days of administration at $P<0.05$. Moreover, the data showed that, there was significant decrease in both pituitary and serum levels of LH at $P<0.01$ following GABA administration.

The maximum serum testosterone reduction was detected after injection of Glutamate, GABA and L-Arginine in the last 10 days of the administration period (2.10 ± 0.21, 0.61 ± 0.07 and 0.26 ± 0.04 ng/ml, respectively).

Discussion

The results showed that glutamate administration had no significant effect on pituitary and serum FSH. On the other hand, regarding LH pituitary levels, results in Table 1 showed that pituitary levels were significantly decreased in comparison with control. However, serum levels in glutamate – injected groups were significantly higher than control. These results agreed with previous ones obtained by Ondo *et al.*, (1976). The authors demonstrated that glutamate treatment markedly increased LH release in adult male rats without affecting FSH release. They suggested that the effect of the glutamate on LH was due to hypothalamic site of action since direct pituitary injection of glutamate was found to have no effect on LH or FSH plasma levels. It was also demonstrated that DL-α-hydroxy-5-methyl-4-isoxypropionic acid "AMPA" which is glutamate agonist; increased LH secretion in the male farm animals without affecting FSH level (Estienne *et al.*, 2000).

To elucidate the action of glutamate on the control of LH, some studies mentioned that glutamate effect on LH secretion is produced through stimulation of hypothalamic GnRH secretion (Donoso *et al.*, 1990). Moreover, Bhat *et al.*, (1995) found that central administration of glutamate agonist through either direct injection into hypothalamus or injection into the third cerebroventricule was shown to induce a significant elevation of serum LH levels in male and female animals. However, direct injection of glutamate into the anterior pituitary was found to have no effect on LH secretion (Ondo *et al.*, 1976; Tal *et al.*, 1983). The present study showed that prolonged administration of glutamate exhibited no effect on serum testosterone level during the first month then the level decreased during the second month

Table (1): Effect of Glutamate on FSH and LH level of Pituitary (i.u. / mg dry weight) and Serum (i.u. / ml) (Mean ± SE).

Sample	FSH		ICHS	
	Pituitary (i.u. /mg dry weight)	Serum (i.u./ml)	Pituitary (i.u. /mg dry weight)	Serum (i.u./ml)
Control	8.63 ± 0.83	8.37 ± 0.520	2.05 ± 0.17	2.02 ± 0.13
10 days from last injection	8.20 ± 0.80	8.11 ± 0.82	3.52 ± 0.82	3.110 ± 0.01**
20 days after last injection	7.92 ± 0.71	7.85 ± 0.17	3.52 ± 0.82	3.47 ± 0.38*
30 days after last injection	7.85 ± 0.85	7.88 ± 0.72	1.12 ± 0.13*	3.85 ± 0.25**
40 days after last injection	7.92 ± 0.71	8.63 ± 0.89	0.69 ± 0.08**	4.14 ± 0.19**
50 days after last injection	8.73 ± 0.96	8.63 ± 0.89	0. 25 ± 0.02**	4.46 ± 0.00**
60 days after last injection	8.69 ± 0.09	7.37 ± 0.95	0. 25 ± 0.02**	4.46 ± 0.00**

**: Significant at (P < .01) within the same column *: Significant at (P < .05) within the same column

Table (2): Effect of L- Arginine on FSH and LH levels of Pituitary (i.u. / mg dry weight) and serum (i.u. / ml) (Mean ± SE),

Sample	FSH		LH	
	Pituitary (i.u. /mg dry weight)	Serum (i.u./ml)	Pituitary (i.u. /mg dry weight)	Serum (i.u./ml)
Control	8.63 ± 0.83	8.37 ± 0.520	2.05 ± 0.17	2.02 ± 0.13
10 days after last injection	8.63 ± 0.83	7.98 ± 0.27	4.33 ± 0.44**	3.26 ± 0.16**
20 days after last injection	8.69 ± 0.65	8.11 ± 0.183	5.92 ± 0.61**	4.01 ± 0.22**
30 days after last injection	8.76 ± 0.70	8.37 ± 0.654	6.89 ± 0.58**	4.23 ± 0.19**
40 days after last injection	8.63 ± 0.83	8.50 ± 0.431	8.11 ± 0.22**	4.46 ± 0.090**
50 days after last injection	7.88 ± 0.17	8.37 ± 0.654	5.92 ± 0.67**	3.11 ± 0.120**
60 days after last injection	7.49 ± 0.65	7.91 ± 0.712	1.12 ± 0.14*	4.69 ± 0.110**

**: Significant at (P < .01) within the same column. *: Significant at (P < .05) within the same column.

Table (3): Effect of GABA on GH and LH levels of Pituitary (i.u. / mg dry weight) and Serum (i.u. / ml) (Mean ± SE).

Sample	FSH		LH	
	Pituitary (i.u./mg dry weight)	Serum (i.u./ml)	Pituitary (i.u./ mg dry weight)	Serum (i.u./ml)
Control	8.63 ± 0.83	8.37 ± 0.520	2.05 ± 0.17	2.02 ± 0.13
10 days after last injection	8.37 ± 0.59	7.37 ± 0.95	0.81 ± 0.04**	0.12 ± 0.08**
20 days after last injection	8.37 ± 0.59	7.15 ± 0.92	1.06 ± 0.11**	0.08 ± 0.01**
30 days after last injection	7.85 ± 0.39	7.10 ± 0.81	0.81 ±0.04**	0.07 ± 0.01**
40 days after last injection	8.63 ± 0.83	8.19 ± 0.63	0.81 ± 0.04**	0.06 ± 0.01**
50 days after last injection	7.25 ± 0.25	7.16 ± 0.420	0.81 ± 0.04**	0.17 ± 0.03**
60 days after last injection	7.12 ± 0.22	6.14 ± 0.360*	0.97 ± 0.11**	0.22 ± 0.02**

**: Significant at (P < .01) *: Significant at (P < .05) N.B: This significance within the same column.

Table (4): Effect of Glutamate, L-Arginine and GABA on the level of serum testosterone (ng/ ml) of male rats (Mean ± SE).

Sample	GLU	L-Arg.	GABA
Control	4.56 ± 0.23	4.56 ± 0.23	4.56 ± 0.23
After 10 days	4.71 ± 0.36	3.04 ± 0.33*	2.65 ± 0.22**
After 20 days	4.67 ± 0.41	2.65 ± 0.22**	2.33 ± 0.17**
After 30 days	4.67 ± 0.41	1.48 ± 0.13***	1.48 ± 0.13***
After 40 days	3.97 ± 0.31	1.19 ± 0.09***	1.19 ± 0.09***
After 50 days	2.21 ±0.23**	0.69 ± 0.11***	0.85 ±0.11***
After 60 days	2.10 ± 0.21**	0.26 ± 0.04***	0.61 ± 0.07***

***: Significant at (P< .001) within the same column. **: Significant at (P < .01) within the same column.
*: Significant at (P < .05) within the same column.

compared with control. This finding is similar to that reported by Nagata *et al.*, (1999) who mentioned that L-glutamate has no effect on testosterone production in rat cultured Leydig cells. In the current study, the decreased testosterone production after the first month can be attributed to the fact that high concentration of glutamate can be toxic to Leydig cells of rat or it may inhibit the process of steroidogenesis leading to decreased testosterone production and subsequently, decreased serum testosterone level. Regarding the effect of L-Arginine administration, the present study disclosed that its prolonged administration caused no significant variations in pituitary and serum FSH levels. However, it caused an increase in pituitary and serum LH levels with exception of the pituitary level of LH after 60 days which was lower than the control (Table 2). These results come in accordance with previous studies of Rosselli *et al.*, (1998); Kasperska-Zajac and Rogala (2003) that attributed the increase of LH in pituitary and serum levels after arginine administration to the stimulating effect of liberated NO on hypothalamus LHRH. It was reported that FSHRH not affected by inhibitors to NOS or NO donors. Moreover, the direct evidence supporting the notion that NO regulates LHRH synthesis comes from the in vitro studies of Rettori *et al.*, (1994) which demonstrated that the treatment of AN-ME "Arcuate nucleus-Median Eminence" explants with sodium nitroprosside, a NO donor, increased LHRH release. The present study showed that long term administration of L-Arginine (for 2 months) caused significant decrease in serum testosterone level of male rats (Table 4). Similarly, Adams *et al.*, (1993) reported that, in rats, alcohol- induced suppression of the testosterone synthesis was reversed in the presence of L-NAME (NO synthesis inhibitors) suggesting that ethanol induced its effects via NO generation. Moreover, it was found that administration of L-NAME to male rats increased testosterone suggesting that NO down regulates testosterone synthesis (Adams *et al.*, 1996). The above mentioned authors reported that the effect of NO on testosterone synthesis is produced by local action directly in the testes and not dependent on LH secretion. In study of Lue *et al.*, (2003), the reproductive hormonal profile in the adult iNOS-deficiency mice "mice with the gene producing iNOS is deficient" (iNOS is one of the enzymes which produce NO) and normal mice was studied. The authors found that there were no significant difference in plasma LH, FSH and testosterone levels between the mice groups.

Table (3) showed that GABA induced no significant effect on pituitary FSH but caused significant decrease in serum level of FSH (6.14 ± 0.360) after 60 days of administration at $P<0.05$. Moreover, the data showed that, there was significant decrease in both pituitary and serum levels of LH at $P<0.01$ following GABA administration. These results agreed with previous studies of Lamberts *et al.*, (1983) and Masotto *et al.*, (1989) who stated that GABA and GABA agonist (muscimol) reduced LH secretion Moreover, it was reported that that endogenous GABA release suppressed GnRH (Seong-Kyu *et al.*, 2004). The inhibitory effect of GABA on LHRH is produced through the inhibition of NO synthesis (NO stimulates LHRH release) (McCann *et al.*, 1998 and Rosselli *et al.*, 1998). In addition, it is well known that GABA is the principle inhibitory neurotransmitters in CNS and acts postsynaptically on $GABA_A$ and $GABA_B$ receptors to induce neural inhibition (Hu *et al.*, 2004). The obtained results showed that GABA administration for 2 months induced significant decrease in serum testosterone level along the experimental period (Table 4). In this respect, Geigerseder *et al.*, (2004) studied the effect of testicular GABA on testosterone production, where they found that GABA stimulated the proliferation and testosterone production by Leydig cell culture. Moreover, GABA is known to play important role as a major inhibitory neurotransmitter in mammalian CNS; it may also play important roles in the peripheral non-neuronal tissues as testes (Kanbara *et al.*, 2005).

It could be concluded that exposure to 3 neurotransmitters (Glutamate, L-Arginine and GABA) led to disturbance in serum gonadotropins (especially LH) as well as impaired gametogenic and steroidogenic performances of the testes. The central effect of these neurotransmitters differs from their peripheral or local action. However, further studies are required to clarify if this effect is reversible or not.

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تأثير بعض الناقلات العصبية على الخصى والهورمونات التناسلية فى الفئران

هذه الدراسة أجريت لدراسة تأثير٣ من مختلف الناقلات العصبية (الجلتاميت ، الأرجينين وحمض الجاما امينو بيوترات) على بعض جوانب الأداء التناسلى فى ذكور الفئران. لهذا الغرض ، تم استخدام عدد ١٠٠ من ذكور الفئران والتى قسمت إلى ٤ مجموعات مماثلة ، الأولى ، تتكون من ١٠ فئران والتى اعتبرت كمجموعة ضابطة. والثانية تناولت الجلتاميت ١٠ مجم / كجم ، والمجموعة الثالثة تم حقنها بالأرجينين ٢٠ مجم / كجم بينما كانت الرابعة تحقن بحمض الجاما امينو بيوترات ١ مجم / فأر. وأظهرت النتائج أن الجلتاميت أدى إلى زيادة مستوى الهرمون الحاث للخلايا البينية فى أنسجة الغدة النخامية والأمصال مع انخفاض مستوى هرمون التستوستيرون فى المصل. من ناحية أخرى ، الأرجينين أدى إلى ارتفاع ملحوظ فى مستوى الهرمون الحاث للخلايا البينية فى كل من الغدة النخامية والمصل وانخفاض ملحوظ لهرمون التستوستيرون فى المصل. فى حين ، استخدام حمض الجاما امينو بيوترات أدى إلى انخفاض ملحوظ فى مستوى الهرمون الحاث للخلايا البينية فى الغدة النخامية والمصل مع انخفاض ملحوظ لهرمون التستوستيرون فى الأمصال.