Zagazig J. Pharm. Sci., June 2000
Vol. 9 , No. 1, pp. 38 - 42

ISSN 1110 - 5089

Serum hormonal parameters as biomarkers For the human choionic gonadotropin (hCG) and Cerastes cerastes cerastes venom on immature female rats.

Nabil A. Soliman and Mahmoud R. Mahmoud.

Department of Zoology , Faculty of Science. Zagazig University, Zagazig- Egypt.

ABSTRACT

Studies were conducted to examine the effects of human gonadotrophin (hCG) and snake venom (Cerastes cerastes cerastes) on the serum levels of 17β- estradiol, progesterone, follicular stimulating hormone (FSH) and leuteinizing hormone (LH). Immature female rats were injected two doses weekly for one month with different doses of hCG (10 and 50 IU) , crude venom (1/10 and 1/20 LD_{50}) and venum fractions (F_1 , F_2 and F_3 with sublethal doses). The results revealed a significant increase in 17β- estradiol and progesterone in female immature rats treated with hCG, crude venom and F_1 as compared with that of the control animals.

Furthermore, the treatment with the hCG increased the serum levels of FSH and LH, whereas the venom and its fraction (F_1) caused a significant decrease in circulating levels of FSH and LH compared with that of the control. On the other hand, venom fractions F_2 and F_3 did not affect the levels of these hormones. This suggests that hCG and Cerastes cerastes cerastes venom stimulated the gonadal secretion of progesterone and 17β- estradiol. On the other hand, venom did not modify the production of FSH and LH hormones

INTRODUCTION

In 1978, Audi[1] found that the human chorionic gonadotropin administration stimulated the gonadal secretions of progesterone and estrogens. Prepubertal rats of both sexes showed very high concentration of estrogens [2-4]. They showed that this plasma peak of estrogens which occure in prepubertal rats was related to adrenal steroidogenesis.

An *in vitro* bioassay on progesterone production by enzymatically dispersed immature rat ovary cells was used by Brand[5] to investigate the effect of sialidase treatment on the biological activity by human chorionic gonadotrophin (hCG). They found that when equal amounts of ^{125}I-labelled hCG and asialo-hCG were specifically bound to the ovarian cells, the latter stimulated progesterone production less effectively.

Reddy[6] found that the administration to female rats of the LHRH agonist, ethylylamide resulted in a decrease in the binding of ^{125}I-labeled hCG to ovarian plasma membranes. They demonstrated that the inhibition of ovarian steroidogenesis observed in response to the injection of the LHRH agonist is primarily due to down-regulation of ovarian hCG receptors with a resultant decrease in cAMP accumulation.

On the other hand, Smith and Perks[7-8] reported that the bradykinin may be involved in the ovulatory process. This bradykinin level and the kinin-forming enzyme were found to change with ovulation. The ovarian kinin-generation activity increases significantly during ovulation. This openion is supported by Espey[9]. Yoshimura[10] suggested that the nonpeptide hormone bradykinin can induce ovulation in perfused rabbit ovaries. The authors reported that kinins may be important mediators of the ovulatory process and that BK can increase 6-keto-$PGF_{1\alpha}$ in perfused rabbit ovary.

In 1965, Ferreria[11] found that the venom of the snake; *Bothrops jararaca:* contains an alcohol soluble fraction which potentiates the effect of bradykinin *in vivo* and *in vitro* . This fraction was called bradykinin potentiating factor (BPF).

Sato[12] prepared a bradykinin releasing factor from the venom of *Agkistradon halys blomhoffi* . suzuki[13] reported that the venom of the Japanese snake contains two or more bradykinin potentiating peptides. However, Oshima[14] found that kinin is released by a kininogenase isolated from various kinds of snake venoms. Mohamed and khalid[15] interpreted the hypotensive effect of Cerastes cerastes venom to result from a kinin peptide present in a free form in the venom or resulting from a kinin releasing enzyme that activates the kinin from its inactive precursors in the plasma. On the other hand, five bradykinin potentiating peptides potentiators A,B, C,D and E) were isolated from the venom of the Japanese snake. These five bradykinin potentiating peptides were characterized by having a high proline content. They have some resemblance in their terial structures[16].

The biological activities of the five peptides were studied using guinea pig ileum and rat uterus. Kato and Suzuki[16] found that only potentiator E shows a high bradykinin potentiating activity on rat uterus. Furthermore, the investigation was conducted to test the probable effect of this venom on gametogenesis and on circulation of sex hormones in premature female animals under experiment. These experiments were aimed to detect a probable beneficial utilization of such a venom fraction on follicular growth response and uterine enhancement for physiological activity in females.

Russelli venom injection in albino rats is indicative of cell or tissue damage. High incidence of intravascular hemnolysis in addition to hemostasis, haemoptysis and haematuria are observed as the most prominent features of russelli viper venom[17].

Assi and Nasser[18] concluded that *Sistrarus malarius barbouri* crude venom produced significantly impaired motor coordination, learning and retention, spontaneous activity and produced

behavioural chainges, muscle weakness and loss of righting refelx in mice. The some dose also produced a signficant decrease in body temperature and inhibited acetylcholine – induced contraction of the isolated smooth (rabbit intestine) and skeletal (frog rectus abdominis) muscle and impaired transmission at the nerve muscle synapse of the rat phrenic nerve diaphragm preparation. The effects of the acute sublethal and chronic doses on carbohydrate metabolism revealed a hyperglycemic effect associated with a dimination of liver and muscle glycogen, while its effects on blood electrolytes (sodium and potassium) showed a significant elevation in the blood sodium level and a signcficant reduction in that of potassium. Serum enzymes were also affected. Levels of alkaline phodphatase (ALP), aspartake aminotransferase (ALT) activities were moderately increased. The crude venom had an aggregatory effect on platelets and had also a phospholipase A_2 activity while, on the other hand, it showed no L-amino acid oxidase activity. Testing of the effect of the venom on the plasma recalcification time showed that the venom had an anticoagulant effect in case of high dose (200 µg) while a coagulant effect at a low dose of the venom (2.5 µg). SMB venom at a dose level of 1.94 µg/g. b.w. (LD_{10}) was found to exhibit significant inhibitory effect on tumor growth when injected into mice[18].

MATERIAL AND METHODS

White albino rats were used. They arrived in the laboratory at 25-30 days of age. They were housed in group cages (5 rats per cage) under controlled conditions of light and temperature. Free access to food and water was provided.

Human chorionic gonadotrophin (Pregny1) was produced by the Nile Company For Pharmaceuticals And chemical Industries Cairo A.R.E. under Licence of organon, Oss Holand.

The crud venom used was milked from Cerastes cerastes cerastes snake species. The *in vivo* LD_{50} value was determined by the method of Abu-Sinna[19].

Collection of Venoms:

Cerastes cerastes cerastes venom was collected from adult snakes kept in the serpentarium in the laboratory of physiology, Department of Zoology, Faculty of science, Ain shams University. The snakes were allowed to inject their venom through a nylon diaphragm into-ice cold beakers. The venom was dried in adessicator containing anhydrous calcium chloride in the dark till complete dryness. The dried venom was stored at room temperature in a vacuum dessicator as it has been reported that storage under these conditions for long periods does not effect the toxicity of the venom [20].

Gel fltration of C. C. cerastes crude venom:

Gell fltration technique was carried out according to the methed of soliman [21] using sodium chloride solution (0.3 M) and sephadex G-75 (pharmacia, sweeden).

The better separation were achieved using a column with dimensions of 1.8 X 60 cm and flow rate of 20 ml/h. effluents of 5 ml were collected by an automatic fraction collector (LDB. Company – Sweden) at 4^0C. After pooling each group of tubes together, the fractions were dialyzed against distilled water and lyophilized to complete dryness.

Determination of the approximate acute median lethal dose (LD50) of some C. C. cerastes venom fractions:

Toxicity studies were carried out on the fraction (F_1, F_2 and F_3). Determination of the appreximate acute median lethal dose (LD_{50}) was carried according to Meier and Theakston[22].

LD_{50} of F_1 = 2 ug/g. b.w

F_2 = 1.2 ug/g. b.w

F_3 = 1.80 ug/g. b.w.

It was found that the LD_{50} of (F_1) , (F_2) and (F_3) were 2, 1.2 and 1.8 ug/g body weight respectively.

HCG (10 and 50 IU) and venom (1/10 and 1/20 LD_{50}) doses were injected i.p. for month two doses weekly. The animals were killed 24 h after the last injection.

Serum 17β- estradiol, progesterone, FSH and LH were determined by radio - immunoassay techniques using coat-A-count kits. The plasma 17β- estradiol progesterone FSH and LH were measured according to Rosemberg[23],and BatZer[24]. The results were statistically analyzed using student't test Snedecor[25].

RESULTS

The plasma levels of 17β - estradiol (pg/ml) and progesterone (ng/ml) are presented in the following tables. The previously mentioned hormones revealed a significant increase in all groups injected with hCG in the doses of 10 and 50 IU. Also the plasma level of 17β- estradiol and progesterone in the group injected with venom revealed a significant increase at doses of 1/10 and 1/20 LD_{50} and fraction F_1 . The groups injected with fractions F_2 and F_3 venom was nonsignificant.

The groups injected with hCG revealed a significant increase in both doses at levels 13% , 25% , 16% , and 29% in plasma 17β -estradiol and progesterone respectively. Also, the groups injected with venom and F1 revealed significant increase in 17β -estradiol and progesterone at levels 7% and, 15% and 28% , 18% , 21% and 37% respectively (table 1 and 2).

Tables (3 and 4) show that a significant increase in the levels of both FSH and LH hormores under the effect of hCG at doses 10 and 50 IU at 72% , 153%, 25% and 51% respectively. On the other hand , the levels of FSH and LH were decreased significatly at 46% , 39% , 68% , 14% , 25% and 24% under the effect of crude venom (1/10 and 1/20 LD_{50}) and fraition (F_1) respectively.

In the immature rats the effect of hCG (10 and 50 IU) and venom (1/10 and 1/20 LD50) and venom fractions (F_1 , F_2 and F_3) with sublethal doses on sex hormones were studied, where each animal was given

two doses weekly and investigated 30 days after treatment.

DISCUSSION

An interesting finding was the appearance of grafian follicles in about 50% of scorpion venom treated mice while in control mice there were no grafian follicles which is the usual case with the fecale mice that are under 40 days old[26].

Ovarian growth was accompanied by an increase in the plasma concentration of estradiol. Characteristic signs of uterine growth were also detected in mice treated with isolated venom fraction. Consequently it suggested that endogenous bradykinin that was probably activated the action of the venom enhanced the release of gonadotropin regulatory hormones, resulting in the elevation of leutinizing hormone (LH) and follicular stimulating hormone (FSH)[27].

Since the secretion of progesterone begins with the formation of corpus luteum, the lack of effect of the venom on pregesterone is suggests that the activation of endogenous bradykinin had no effect the formation and regulation of the corpus luteum. Also, it is expected that bradykinin can induce prostaglandin release in a variety of animal tissue[28-29].

However, one can suggest that the administered venom might enhance the endogenous bradykinin leading to prostaglandin and promote the observed effect on ovarian and uterin growth. Nassar[30] indicated that some prostaglandin administration exhibit an estrogen effect on uterus.

Administration of hCG to female rats is followed by an increase of plasma concentrations of progesterone and estradiol. Steroidogenesis seems to be necessary for the synthesis of these compounds since the administration of hCG and venom for month inhibited this synthesis. However, short acting hCG and venom did not significantly modify plasma concentration of progesterone and estradiol[1]. Indeed, the decrease of plasma estradiol and progesterone levels after hCG and venom administration to intact immature female rats was due exclusively to a decreased production of these hormones by gonads. Furthermore, Audi[1] showed that ^{125}I-hCG was not able to bind to adrenal plasma membranes while it bound to ovary plasma membranes. Morris[31] reavealed that the rabbit received 75 IU of hCG each day for 7 days increased tolal body weight, hemetocrit and ovaria weight significatly. Moreover, Gregoraszczuk and Zieba[32] concluded that progesterone production was enhanced by hCG in a dose dependent manner.

Wherever, Mau[33] studied the effect of wasp venom peptide on the secretion of prolactin from the anterior pituitary cells. They concluded that the secretion of prolaction was increased significantly.

This investigation was conducted to test the probable effect of the resemblance between the hCG and Cerastes cerastes cerastes venom in their activities on circulating female sex hormones in immature female rats.

Table (1): Comparison between hCG (10 and 50 IU), Cerastes cerastes cerastes venom (1/10and 1/20 LD_{50}) and venom fractions F_1 , F_2 and F_3 (sublethal dose) on serum 17 *B* – estradiol level (ng/ml) on immature female rats .

Parameters	Control	hCG 10 IU	hCG 50 IU	Control	Venom 1/10LD50	Venom 1/20 LD50	F_1 1ug/gb.w	F_2 0.6 ug/g b.w	F_3 0.9ug/gb.w
Mean ± S.E	760 ± 25	856 ± 19	947 ± 34	785 ± 9	838 ± 19	903 ± 28	1004 ± 44	771 ± 20	767 ± 19
% change	-	+13%	+25%	-	+7%	+15%	+28%	02%	02%
p.value	-	P< 0.01	P<0.001	-	P<0.05	P<0.01	P<0.001	n.s.	n.s

P<0.05, P<0.01 and P<0.001 denoted to significance, high significance and very highly significance. n.s denoted to non significance.

Table (2): Comparison between hCG (10 and 50 IU), Cerastes cerastes cerastes venom (1/10and 1/20 LD_{50}) and venom fractions F_1 , F_2 and F_3 (sublethal dose) on serum progesterone level (ng/ml) on immature female rats .

Parameters	Control	hCG 10 IU	hCG 50 IU	Control	Venom 1/10LD50	Venom 1/20LD50	F_1 1ug/gb.w	F_2 0.6 ug/g b.w	F_3 0.9ug/gb.w
Mean ± S.E	8.6 ± 0.21	10.0 ± 0.18	11.1 ± 0.14	9.0 ± 0.27	10.0 ± 0.21	10.9 ± 0.10	12.3 ± 0.60	9.1 ± 0.30	9.4 ± 0.19
% change	-	+16%	+29%	-	+18%	+21%	+37%	+1%	+4%
p.value	-	P<0.001	P<0.001	-	P<0.001	P<0.001	P<0.001	n.s.	n.s

P<0.001 denoted to very highly significance. n.s denoted to non significance.

Table (3): Comparison between hCG (10 and 50 IU), Cerastes cerastes cerastes venom (1/10and 1/20 LD_{50}) and venom fractions F_1 , F_2 and F_3 (sublethal dose) on serum FSH level (ng/ml) on immature female rats .

Parameters	Control	hCG 10 IU	hCG 50 IU	Control	Venom 1/10LD50	Venom 1/20LD50	F_1 1ug/gb.w	F_2 0.6 ug/g b.w	F_3 0.9ug/gb.w
Mean ± S.E	3.2 ± 0.24	5.5 ± 0.32	8.1 ± 0.29	5.6 ± 0.35	3.0 ± 0.21	3.4 ± 0.24	1.8 ± 0.13	5.1 ± 0.17	4.7 ± 0.28
% change	-	+72%	+153%	-	-46%	-39%	-68%	-9%	-16%
p.value	-	P<0.001	P<0.001	-	P<0.001	P<0.001	P<0.001	n.s.	n.s

P<0.001 denoted to very highly significance. n.s denoted to non significance.

Table (4) : Comparison between hCG (10 and 50 IU), Cerastes cerastes cerastes venom (1/10and 1/20 LD_{50}) and venom fractions F_1 , F_2 and F_3 (sublethal dose) on serum LH level (mIU/ml) on immature female rats .

Parameters	Control	hCG 10 IU	hCG 50 IU	Control	Venom 1/10LD50	Venom 1/20LD50	F1 1ug/gb.w	F2 0.6 ug/g b.w	F3 0.9ug/gb.w
Mean ± S.E	8.8 ± 0.25	11.0 ± 0.14	13.3 ± 0.36	9.5 ± 0.21	8.2 ± 0.30	7.1 ± 0.68	7.2 ± 0.50	8.6 ± 0.60	8.9 ± 0.70
% change	-	+25%	+51%	-	-14%	-25%	-24%	-10%	-6%
p.value	-	P<0.001	P<0.001	-	P<0.01	P<0.01	P<0.001	n.s.	n.s

P<0.01 and P<0.001 denoted to high significance and very highly significance. n.s denoted to non significance.

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Received : Feb., 10, 2000
Accepted : Apr., 15 , 2000

Zagazig J. Pharm. Sci., June 2000
Vol. 9 , No. 1, pp. 38 - 42

قياسات هرمونية في المصل كدلالات لتأثير هرمون المشيمة وسم السيراستس سيراستس سيراستس على إناث الفئران الغير ناضجة

نبيل عباس سليمان ومحمود رابح محمود

قسم علم الحيوان – كلية العلوم – جامعة الزقازيق – الزقازيق – مصر

تضمنت الدراسة تأثير هرمون المشيمة بتركيز ١٠ , ٥٠ وحدة دولية وسم السيراستس سيراستس سيراستس بجرعات ١٠/١ ، ٢٠/١ من نصف الجرعة المميتة وكذلك تأثير ناتج مصل السم الخام F_1 , F_2 , F_3 وبتركيزات ٢ ، ١,٢ ، ١,٨ ميكروجرام لكل جرام من وزن الجسم وكانت المعالجة كل على حده مرتين أسبوعيا ولمدة شهر. وتم قياس هرمونات الاستراديول والبروجيستيرون وهرمون الحاثة الجرابية وهرمون الجسم الأصفر.

وسجلت النتائج أن هناك زيادة واضحة في هرمون الاستراديول والبروجستيرون في إناث الفئران المعالجة بهرمون المشيمة والسم الخام والمفصول الأول F_1. وكانت نسب الزيادة ١٣% ، ٢٥% ، ١٦% ، ٢٩% ، ٧% ، ١٥% ، ٢٨% ، ١٨% ، ٢١% و ٣٧% على التوالي وذلك بالمقارنة بإناث الفئران الغير معالجة.علاوة على ذلك كانت هناك زيادة واضحة في مستويات هرموني الحاثة الجرابية والجسم الأصفر تحت تأثير هرمون المشيمة وكانت نسبة الزيادة ٧٢% ، ١٥٣% ، ٢٥% و ٥١% على التوالي.

بينما كان هناك نقص ملحوظ في مستوى هرموني الحاثة الجرابية والجسم الأصفر في الفئران المعالجة بالسم والمفصول الأول F1 وسجل النقص ٤٦% ، ٣٩% ، ٦٨% ، ١٤% و ٢٥% و ٢٤% على التوالي. بينما في حالة الفئران المعالجة بأجزاء السم المفصولة F3 ، F2 لم يكن لها أي تأثير على تلك الهرمونات.