Pakistan J. Zool., vol. 43(4), pp. 799-803, 2011.

Possible Modulation of Testosterone Secretion by Obestatin in Pubertal Male Rats

Sarwat Jahan*, Shakeel Ahmed, Nazifa Taqvim and Hizbullah
Reproductive Physiology Lab, Department of Animal Sciences, Quaid-i-AzamUniversity, Islamabad.

Abstract.- The objective of the present study was to examine the effect of different doses of obestatin on testosterone secretion in pubertal male Sprague Dawley rats. Animals were divided into three groups. One group served as control and the other two groups were treated with 8 and 16 nmol/kg of obestatin, respectively. Prior to sample collection, teflon cannula was implanted in lateral tail vein of rat and the sequential blood samples were collected 10 min before, at the time of obestatin treatment (0 min) and then at 10 min intervals upto 40 min. in heparinized syringes. The whole sampling was carried out under diethyl ether anesthesia. Plasma testosterone levels were determined by using Enzyme Immunoassay (EIA). Administration of obestatin (8 nmol/kg) in animals resulted in a significant ($p<0.05$) increase in mean plasma testosterone concentrations at 10 and 20 min after obestatin administration. Highly significant ($p<0.001$) increase in testosterone concentrations were observed at 30 min which then declined at 40 min. At dose of 16 nmol/kg in pubertal animals, a significant increase ($p<0.05$) was noticed in testosterone secretion at 20 and at 30 min after obestatin administration. Plasma testosterone concentrations then declined after 40 min. The present study suggests that this peptide may be involved in the regulation of testosterone secretion in pubertal male rats.

Key words: Obestatin, testosterone, reproduction, pubertal male rats

INTRODUCTION

Obestatin is a 23-amino acid peptide encoded by the ghrelin gene, was isolated from rat stomach. It is produced by C terminal cleavage of preproghrelin by convertase (Kojima *et al*., 1999; Bednarek *et al*., 2000; Cassoni *et al*., 2001). The name 'obestatin' was given because of its appetite-suppressing potential. The C-terminal Gly–Lys motif is almost available for amidation, which is considered as a prerequisite for its biological activity (Zhang *et al*., 2005). Obestatin seems to be a functional part of a complex gut–brain network where hormones and substances from the stomach and intestines signal the brain about satiety or hunger. Zhang *et al*. (2005) first examined effect of human obestatin on food intake and body weight in adult male fed mice. Intraperitoneal and intracerebroventricular treatment of obestatin suppressed food intake in a time-dependent and dose-dependent manner, but treatment with ghrelin increased body weight, whereas the same dose of obestatin suppressed body weight gain. Obestatin was suggested to bind to an orphan G-protein coupled receptor (GPCR), named GPR39 (Pan *et al*., 2006; Chanoine *et al*., 2006). High levels of GPR39 mRNA have been found in the gastrointestinal tissues, amygdala, hippocampus, auditory cortex and hypothalamus (Moechars *et al*., 2006; Zhang *et al*., 2005). However, GPR39 is also present in pituitary (Kojima *et al*., 2001). It was found that GPR39 has sequence homology to GH secretagogue receptor, the receptor for ghrelin (McKee *et al*., 1997).

Using radioimmunoassay obestatin-specific antibodies, obestatin was found in large and small intestines, stomach, spleen, cerebral cortex of rats and in the perinatal rat pancreas (Chanoine *et al*., 2006; Dun *et al*., 2006). With the use of an antiserum directed against the mouse/rat obestatin, immunoreactivity of obestatin was detected in cells of the gastric mucosa, myenteric plexus, and in the Leydig cells of the testis in rodents (Dun *et al*., 2006). Reproductive function is regulated by the interplay of the hypothalamus, pituitary and gonads, which form the so-called gonadotropic or reproductive axis (Tena-Sempere *et al*., 2004; Huhtaniemi *et al*., 1989). Proper function of the gonadotropic axis, and hence reproductive capacity, is regulated by metabolic and nutritional factors (Kennedy and Mitra, 1963; Frisch and Revelle,

* Corresponding author: drsarwatjahan@gmail.com
0030-9923/2011/0004-0799 $ 8.00/0

1970; Frisch and McArthur, 1974).

Data regarding obestatin effects on reproductive function are very limited. It was observed earlier that obestatin stimulates testosterone secretion but has no effect on prolactin secretion in adult male rats (Jahan *et al*., 2010). The present study was designed to examine the dose dependant response of obestatin on plasma testosterone secretion in pubertal male Sprague Dawley rats.

MATERIALS AND METHODS

Animals

Pubertal male Sprague Dawley rats 45-50 days (n=14) were used in this study. Animals were maintained in groups of 5,5 (treated) and 4 (control) per cage at room temperature under 12 h light/12 h dark cycle. The animals had free access to pelleted food and water was available *ad libitum*. All animal protocols were approved by the committee on Animal Experimentation, Quaid-i-Azam University, Islamabad, Pakistan.

Experimental protocol

Working solution of obestatin (AnaSpec, USA) was made in normal saline (0.9% sodium chloride). One mg of obestatin was dissolved in one ml saline and a stock solution was prepared and kept at -70^{o}C. All the doses were administered intravenously.

Prior to blood sampling, animals were anesthetized using diethyl ether (Liu *et al*., 1992) which was purchased from Sigma Aldrich, USA. The animals tail was tied with a band and warmed with a lamp to visualized the vein. A Teflon cannula (Vasocan Branule, B. Braun Melsungen AG, Belgium; 0.7 mm/24 G O.D) was inserted in the lateral tail vein (Staszyk *et al*., 2003; Omaye *et al*., 1987). The cannula was tightly tied with tail by adhesive tape. Blood sampling and infusion of drugs were carried out using heparinized syringes. Heparin was purchased from Sinochem Ningbo, Ningbo, P.R. China. Animals were divided into three groups *i.e.* control, obestatin 8 nmol/kg and obestatin 16 nmol/kg. Sequential blood samples (250-300µl) were obtained at 10 min intervals in heparinized syringes at -10, 0, 10, 20, 30 and 40 min relative to obestatin challenge at 0 min. All sampling were carried out between 10 am to 12 pm to minimize diurnal variation. Following each sample, an equal volume of heparinized saline (250-300µl) was injected. Blood samples were immediately centrifuged at 3000 rpm at 4°C for 10 min. Plasma was separated and stored at -20°C until analyzed. Testosterone was quantitatively determined by using Enzymatic Immunoassay (EIA) kits (Amgenix, USA) according to manufacturer's instructions.

Statistical analysis

Data are expressed as Mean ± SEM. Student's 't' test was applied to compare pre treatment testosterone concentrations at 0 min with post treatment obestatin induced testosterone concentrations at 10, 20, 30 and 40 min. ANOVA followed by Tukey's test was employed to compare pre treatment testosterone concentrations and post treatment testosterone concentrations of all treated and control groups. Statistical significance was set at $p \leq 0.05$.

Table I.- Plasma testosterone concentrations (ng/ml) before and after *i.v.* injection of saline and obestatin (8 and 16 nmol/kg) in pubertal male rats.

Time (min)	Plasma testosterone concentration (ng/ml) (Mean±SEM)		
	Saline	8 nmol/kg	16 nmol/kg
-10	3.07±0.81	3.28±0.61	2.74±0.12
0	2.49±0.78	3.65±0.71	2.21±0.12
10	2.39±0.91	5.00±1.11*	2.56±0.33
20	2.46±0.82	5.45±1.05*	3.53±0.17*
30	2.32±0.53	5.68±0.67***	3.84±0.33*
40	1.85±0.54	4.84±0.46*	2.64±0.32

*p<0.05, ***p<0.001 vs 0 minute sample of the respective group (Student's 't' test).

RESULTS

Following saline administration in control rats, the plasma testosterone concentrations (ng/ml) remained almost at the same level (Table I, Fig. 1).

After obestatin administration, the plasma testosterone concentrations (ng/ml) were significantly ($p<0.05$) increased from 0 min to 20

min. Highly significant ($p<0.001$) effect was observed at 30 min. Plasma testosterone concentrations then declined at 40 min after obestatin administration (Table I, Fig. 1). Mean pre-treatment plasma testosterone concentrations were significantly high ($p<0.005$) as compared to the mean post-treatment samples (Fig. 2).

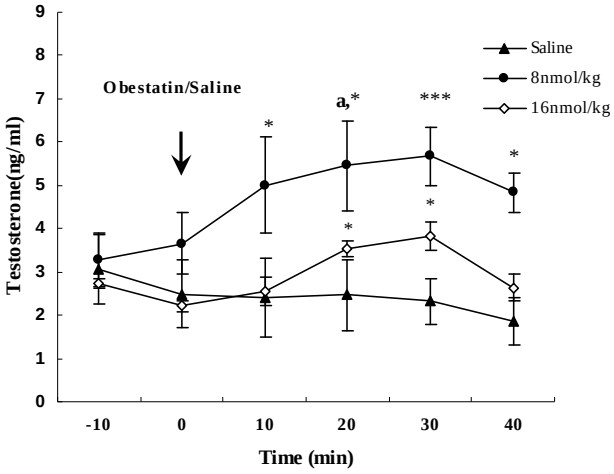

Fig. 1. Mean±SEM plasma testosterone concentrations before and after iv injection of obestatin/saline in three pubertal groups, control, 8 and 16 nmol/kg, respectively. (n=14) *$p<0.05$, ***$p<0.001$ vs values of 0 min sample (student t test). [a]$p<0.001$ vs saline treated group (ANOVA followed by Tukey's test).

After obestatin administration (16 nmol/kg), in the plasma testosterone concentrations there was a slight but not significant ($p> 0.05$) increase from 0 to 10 min. Significant increase ($p<0.05$) was noticed at 20 and 30 min, respectively. Plasma testosterone concentrations then declined at 40 min (Table, I, Fig. 1).

In all three groups, different responses were observed in mean plasma testosterone concentrations after obestatin and saline administration. Post treatment plasma testosterone concentrations in 8 nmol/kg obestatin group were significantly higher ($p<0.001$) as compared to the saline treated group. While no significant difference was observed after giving 16 nmol/kg in pubertal animals as compared to the saline treated group. In all the three groups, pre-treatment plasma testosterone concentrations were not significantly different (Fig. 2).

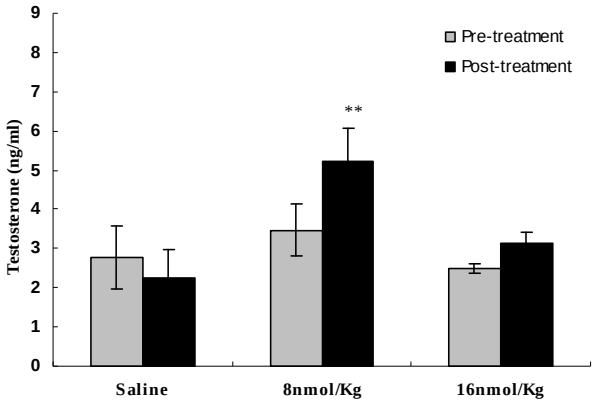

Fig. 2. Mean±SEM plasma testosterone concentrations in pre and post treated (Saline, 8 and 16 nmol/kg obestatin) in pubertal male rats. (n=14)
**$p<0.005$ vs corresponding pre treatment samples (Student's 't' test).

DISCUSSION

In the present investigation, intravenous administration of the peptide obestatin elicited a significant increase ($p<0.05$) in plasma testosterone secretion in pubertal rats at a dose of 8 nmol/kg which is in line with the previous observation in rats (Nogueiras *et al*., 2007) that this dose has an effect on food intake when administered centrally. In the present study, single obestatin (8 nmol/kg) injection caused a significant increase in plasma testosterone concentrations. Obestatin effect was observed within 10 minutes after its administration and reached its maximum value at 30 min and there after the level decreased gradually. The plasma testosterone concentrations showed its pronounced effect at 30 min of obestatin administration. Previous findings also demonstrated that the same dose of obestatin stimulates testosterone secretion in adult male rats but this dose has no effect on prolactin secretion (Jahan *et al*., 2010). In current investigation, significant changes were observed in plasma testosterone concentrations after administration of 16 nmol/kg of obestatin in pubertal animals. After obestatin administration, the plasma testosterone concentrations were increased but not significantly at 10 min as compared to 0 min. But it was increased significantly ($p<0.05$) at 20 and 30 min, respectively. After this the concentrations of plasma testosterone were declined

at 40 min. In this study, two doses were used in order to determine the dose dependent effect of obestatin on testosterone concentrations like ghrelin that significantly inhibited plasma testosterone concentrations dose dependently (Tena-Sempere and Barreiro, 2002). The inhibitory effect of ghrelin upon plasma testosterone concentrations was associated with human choriogonadotropin (hCG). The fact that ghrelin equally decreased hCG- and cAMP-induced plasma testosterone concentrations indicates that this inhibitory action must take place in a step beyond cAMP formation (Tena-Sempere *et al*., 2004). Alternatively, such an inhibitory effect upon plasma testosterone concentrations might be conducted by the ghrelin. Thereby, elevated ghrelin levels (as those observed in energy insufficiency) might contribute to the suppression of male reproductive axis in situations of negative energy balance, such as starvation (Dornonville *et al*., 2005). As because obestatin counteracts the effects of ghrelin on food intake and gastrointestinal motility, in this study, it was also reported that obestatin increases the plasma testosterone level at 8 nmol/Kg in pubertal male rats, opposite to the effect of ghrelin.

The neuroendocrine circuitry responsible for such a positive effect of obestatin on plasma testosterone concentrations remains to be elucidated. However, Dong *et al*. (2009) found that the receptors of obestatin are present in hypothalamus and central nervous system at moderate levels. Gonadotropin releasing hormone (GnRH) is secreated from hypothalamus which control leutinizing hormone and follicle stimulating hormone from pituitary which in turn control plasma testosterone concentrations from Leydig cells (Topari *et al*., 2007). These findings show that obestatin may be involved in modulating GnRH secretion.

CONCLUSIONS

In conclusion, this study demonstrates the effects of obestatin on plasma testosterone concentrations in rodents, which is dose as well as age dependent. The data suggests that metabolic hormones including obestatin and intracellular mediators of their action could provide new explanations for the interrelationships between metabolism, nutrition and reproduction, as well as new approaches for controlling these processes in the treatment of reproductive and metabolic disorders. Further studies are still required concerning the role of obestatin to be involved in physiological regulation of hypothalamic pituitary gonadal (HPG) axis.

ACKNOWLEDGMENT

We are grateful to Department of Animal Sciences, Quaid-i-Azam University, Islamabad. Pakistan for funding and providing all the facilities for this research work.

REFERENCES

BEDNAREK, M.A., FEIGHNER, S.D., PONG, S.S., MCKEE, K.K., HRENIUK, D.L., SILVA, M.V., WARREN, V.A., HOWARD, A.D., VANDERPLOEG, L.H. AND HECK, J.V., 2000. Structure–function studies on the new growth hormone-releasing peptide, ghrelin: minimal sequence of ghrelin necessary for activation of growth hormone secretagogue receptor 1a. *J. med. Chem.*, **43**:4370–4376.

CASSONI, P., PAPOTTI, M., GHE, C., CATAPANO, F., SAPINO, A., GRAZIANI, A., DEGHENGHI, R., REISSMANN, T., GHIGO, E. AND MUCCIOLI, G., 2001. Identification, characterization, and biological activity of specific receptors for natural (ghrelin) and synthetic growth hormone secretagogues and analogs in human breast carcinomas and cell lines. *J. clin. Endocrinol. Metab*., **86**:1738–1745.

CHANOINE, J.P., WONG, A.C. AND BARRIOS, V., 2006. Obestatin acylated and total ghrelin concentrations in the perinatal rat pancreas. *Horm. Res*., **66**:81–88.

DONG, X.Y., HE, J.M., TANG, S.Q., LI, H.Y., JIANG, Q.Y. AND ZOU, X.T., 2009. Is GPR39 the natural receptor of obestatin ? *Peptide.* **30**: 431-438.

DORNONVILLE, D.L.C., LINDQVIST, A., EGECIOGLU, E., TUNG, Y.C., SURVE, V., OHLSSON, C., JANSSON, J.O., ERLANSON-ALBERTSSON, C., DICKSON, S.L. AND HAKANSON, R., 2005. Ghrelin treatment reverses the reduction in weight gain and body fat in gastrecto mised mice. *Gut*, **54**: 907–913.

DUN, S.L., BRAILOIU, G.C.B.E., YANG, J., CHANG, J.K. AND DUN, N.J., 2006. Distribution and biological action of obestatin in the rat. *J. Endocrinol*., **191**:481–9.

FRISCH, R.E. AND REVELLE, R., 1970. Height and weight at menarche: a hypothesis of critical body weights and adolescent events. *Science*, **169**:397–399.

FRISCH, R.E. AND MCARTHUR, J.W., 1974. Menstrual

cycles: fatness as a determinant of minimum weight for height necessary for their maintenance or onset. *Science*, **185**:949–951.

HUHTANIEMI, I., PAKARIENEN, P., SOKKO, T. AND KOLHO, K.L., 1989. Pituitary gonadal function in the fetus and neonate. In: *Control of the onset of puberty* (third edition) (eds. D van de Waal, G.P. Rees and J. Schoemaker), Amsterdam. pp. 101–109.

JAHAN, S., AHMED, S., TAQVIM, N. AND HIZBULLAH, 2010. Obestatin induces testosterone secretion but not prolactin secretion in male Sprague Dawley rats. *Pakistan J. Zool*., **42**:836-839

KENNEDY, G.C. AND MITRA, J., 1963. Body weight and food intake as initiating factors for puberty in the rat. *J. Physiol*., **166**:408–418.

KOJIMA, M., HOSODA, H., DATE, Y., NAKAZATO, M., MATSUO, H. AND KANGAWA, K., 1999. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature*, **402**:656–660.

KOJIMA, M., HOSODA, H., MATSUO, H. AND KANGAWA, K., 2001. Ghrelin: discovery of the natural endogenous ligand for the growth hormone secretagogue receptor. *Trends Endocrinol. Metabol*., **12**:118–122.

LIU, P.T., KENTISH, P.A., SYMONS, A.M. AND PARKE, D.V., 1993. The effects of ether anaesthesia on oxidative stress in rats – dose response. *Toxicology*, **80**:37–49

MCKEE, K.K., PALYHA, O.C., FEIGHNER, S.D., HRENIUK, D.L., TAN, C.P., PHILLIPS, M.S., SMITH, R.G., VAN DER PLOEG, L.H. AND HOWARD, A.D., 1997. Molecular analysis of rat pituitary and hypothalamic growth hormone secretagogue receptors. *Mol. Endocrinol.*, **11**:415–423.

MOECHARS, D., DEPOORTERE, I., MOREAUX, B., DESMET, B., GORIS, I., HOSKENS, L., DANEELS, G., KASS, S., VER, D.L., PEETERS, T.L. AND COULIE, B., 2006. Altered gastrointestinal and metabolic function in the GPR39-obestatin receptor-knockout mouse. *Gastroenterol.*, **131**: 1131–1144.

NOGUEIRAS, R., PFLUGER, P., TOVAR, S., MYRTHA, A., MITCHELL, S., MORRIS, A., PEREZ-TILVE, D., VAZQUEZ, M.J., WIEDMER, P., CASTANEDAZ, T.R., MARCHI, D.R., TSCHOP, M., SCHURMANN, A., JOOST, H.G., WILLIAMS, L.M., LANGHANS, W. AND DIEGUEZ, C., 2007. Effects of obestatin on energy balance and growth hormone secretion in rodents. *J. Endocrinol*., **148**: 21–26.

OMAYE, S.T., SKALA, J.H., GRETZ, M.D., SCHAUS, E.E. AND WADE, C.E., 1987. Simple method for bleeding the unanaesthetized rat by tail venipuncture. *Lab. Anim*., **21**: 261-264.

PAN, W., TU, H. AND KASTIN, A.J., 2006. Differential BBB interactions of three ingestive peptides: Obestatin, ghrelin, and adiponectin. *Peptide*, **27**: 911-916.

STASZYK, C., BOHNET, W., GASSE, H. AND HACKBARTH, H., 2003. Blood vessels of the rat tail: a histological re-examination with respect to blood vessel puncture methods. *Lab. Anim*., **37**:121-125.

TENA-SEMPERE, M. AND BARREIRO, M.L. 2002. Leptin in male reproduction: the testis paradigm. *Mol. cell. Endocrinol*., **188**:9–13.

TENA-SEMPERE, M., AGUILAR, E., FERNANDEZ-FERNANDEZ, R. AND PINILLA, L., 2004. Ghrelin inhibits prolactin secretion in prepubertal rats. *Neuroendocrinology*, **79**:133–141.

TOPARI, J., KALEVA, M., VIRTANEN, H.E., MAIN, K.M. AND SKAKKEBAEK, N.E. 2007. Luteinizing hormone in testicular descent. *Mol. cell. Endocrinol*., **269**: 34-37.

ZHANG, J.V., REN, P.G., KRETCHMER, O.A., LUO, C.W., RAUCH, R., KLEIN, C. AND HSUEH, A.J., 2005. Obestatin, a peptide encoded by the ghrelin gene, opposes ghrelin's effects on food intake. *Science*, **310**: 996–999.

(Received 30 September 2010, revised 15 November 2010)