

Effect of obestatin on body weight, serum glucose and insulin levels in albino rats

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SUMMARY

Obestatin, a peptide encoded by the ghrelin precursor gene, is said to exert actions opposite to that of ghrelin. While ghrelin is said to increase appetite and decrease energy expenditure, thus causing weight gain, obestatin acts like an anorexic hormone, decreasing appetite and reducing body weight gain, besides other effects such as reducing serum insulin and glucose levels. However, these actions have been submitted to serious contests with many laboratories opposing each others' arguments. In our studies on albino rats, obestatin was administered for two different periods of time. One group received intraperitoneal obestatin for one week, while the other got it for two weeks. The control animals received the vehicle alone. It was found that obestatin brought about a reduction in the final body weight, while the control rats continued to gain weight during the period of the experiments. The more the duration of administration of the hormone, more pronounced are the results. There was a fall in the serum glucose and insulin levels in the obestatin-treated rats in comparison with the control rats. Therefore, it was concluded that the anti-obesity hormone obestatin decreases the food intake and the body weight by lessening the appetite in the experimental rats. The study may have im-

plications for its use in obesity.

Key words: Obestatin – Appetite – Weight – Glucose – Insulin – Rats

INTRODUCTION

Obestatin is a putative peptide hormone (Zhang et al., 2005) that is potentially produced in the cells lining the stomach and small intestine and testis of several mammals including humans. Obestatin is encoded by the same gene that also encodes ghrelin, a peptide hormone that increases appetite. Initial studies showed that treatment with ghrelin increased body weight, whereas the same dose of obestatin suppressed food intake and reduced weight gain. At the same time, serum leptin levels were not affected by treatment with either hormone, suggesting that body-fat content was not changed.

Acute administration of obestatin inhibited feeding in mice as well as in lean and Zucker fatty rats. Interestingly, the dose-response relationship was U-shaped to the extent that both low and high doses were without effect in either species (Dun et al., 2006). Treatment of mice with obestatin over a 7-day period decreased body weight gain and food consumption. Overall, obestatin suppressed food intake and body-weight gain in rodents. However, there is evidence that obestatin injected intraperitoneally influenced neither acute food intake nor gastric emptying in rats (Bassil et al., 2007).

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Obestatin inhibited insulin secretion from rat islets in a dose-dependent fashion. Therefore, under glucose-stimulated conditions, exogenous obestatin acts as a potent inhibitor of insulin secretion in anaesthetized rats *in vivo* (Ren et al., 2008).

Because of the controversy (Gourcerol and Tache, 2007; Kobelt et al., 2008; Nogueiras et al., 2007; Seoane et al., 2006) regarding the actual effect of obestatin, we thought that there is a need to re-examine and clarify whether exogenously administered obestatin will bring about changes in the body weight, the serum glucose, and insulin levels in normal adult albino rats, and whether the duration of administration of the hormone will influence the changes in the said parameters.

MATERIALS and METHODS

Inbred strains of adult male Wistar rats were used. The animals were individually housed in a temperature-controlled room in a 12h/12h dark/light cycle, and a standard dry diet (pellets) and water was made available *ad libitum*. The rats were divided into 2 groups, one control and the other experimental with each containing six animals. The experimental rats were deprived of food for 16 hours before administration of the drug, obestatin. The experimental rats were divided into 2 subgroups in order to find out whether the duration of exposure to obestatin would make any difference on its effects. One subgroup received the hormone obestatin for 7 days while the other received the same for 14 days. The experimental rats received obestatin, dissolved in normal saline, at a dose of 64 µg/kg body weight, contained in a volume of 0.1 ml. Control rats received isovolumetrical amounts of physiological sterile saline for similar periods of time. Obestatin was dissolved in normal saline (0.9% NaCl, sterile). The required quantity of the hormone was prepared everyday fresh, just before administration, so reconstituted to contain the required dosage in a volume of 0.1 ml. Injections were given at the beginning of the light phase (Gourcerol et al., 2007).

Food intake and body weight were measured daily. All rats, housed singly, were given free access to a pre-weighed amount of food. Before administering obestatin, the remaining food and spillage were collected on papers placed at the bottom of the animal cages and weighed. Food intake was calculated as the difference between the food weight before and after the feeding period every day.

All experiments were performed according to the guidelines of the Institutional Animal Ethics Committee, which approved the study. All efforts were made to minimize pain and suffering and to reduce the total number of animals used which was required under the Committee for Prevention, Control and Supervision of Experiments on Animals (CPCSEA) guidelines which are followed in India.

Statistical analysis

Data were analysed using unpaired Student's *t* tests to compare the control and experimental groups within each group (7 days or 14 days). The mean daily weight of all rats for seven days and fourteen days were compared using ANOVA followed by Tukey Kramer Multiple Comparisons test. A *p* value of <0.05 was taken as significant. The absolute difference in body weight was taken by subtracting the weight on Day 7 or 14 from the weight on Day 1 in all four groups.

RESULTS

Daily food intake

While the daily food intake steadily increased in 7-day-treated control rats, the experimental rats in the corresponding group showed a gradual decline (Table 1). We also observed a corresponding significant reduction in absolute body weight in both groups, as seen in Table 1. There was a mean difference of body weight -25.5 (95% CI -28.2 to -22.7) in the 7-day group and -66.8 (95% CI -69.4 to -64.2) in the 14-day group. This reduction was also seen when the mean body weight on each day for each of the groups were

Table 1. Composite table showing all the values of the corresponding parameters studied.

Parameter	7 day control	7 day exptl.	<i>p</i> value	14 day control	14 day exptl.	<i>p</i> value
Change in Body weight (g)	12.50±1.87	-13.00±2.36	<0.0001	31.83±1.16	-35.0±2.60	<0.0001
Body Weight(g)	193.85±4.54	181.92±4.84	0.0005	203.38±10.16	171.88±11.70	<0.0001
Food intake (g)	26.38±0.70	24.21±1.20	<0.01	43.45±1.69	39.32±1.65	<0.0001
Blood glucose (mg/dl)	123.66±3.50	100.66±7.25	<0.0001	135.5±1.87	111.16±3.12	<0.0001
Serum insulin (µU/ml)	52.56± 2.37	35.09±1.25	<0.0001	67.44±2.81	56.52±2.87	<0.0001

Abbreviations: g, grams; exptl, experimental.

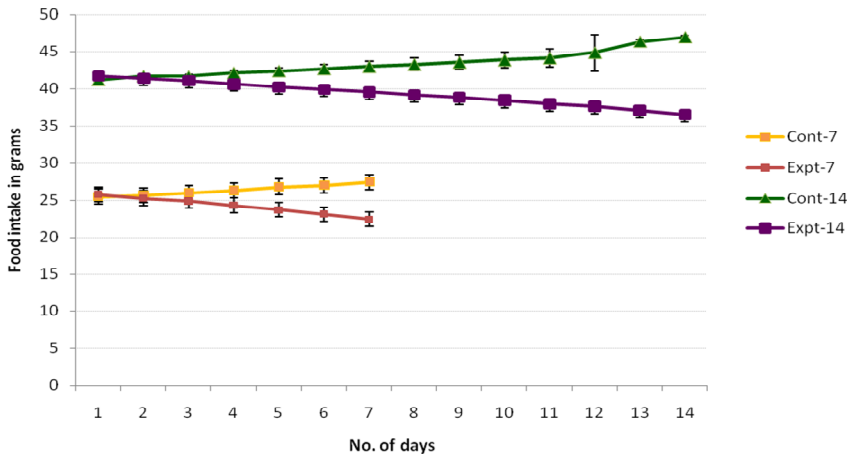


Fig. 1. Average daily food intake in control and obestatin-treated rats in the 7- and 14 day groups. All values are mean \pm S.D. Food intake is significantly different in Control and Experimental Groups from Day 3 onwards. $p < 0.001$. ANOVA with Tukey's post hoc test.

taken and averaged (Table 1). All rats in the control groups steadily gained weight as expected throughout the 7 and 14 days. The food intake decreased gradually in the obestatin-treated rats (Fig.1). There was a significant difference between the experimental and control groups from day 3 onwards (ANOVA followed by Tukey Kramer Multiple Comparison's test, $p < 0.0001$; $F = 119.94$).

Regarding the serum glucose level changes, it was observed that, in the 7-day-treated rats, there was a general fall in the values. The changes in the serum glucose values in the 14-day-treated rats showed a slightly up-and-down pattern in the control group, while in the experimental rats the values were uniformly and gradually falling. The serum insulin levels, revealed lower values in the experimental group in comparison with the control groups (Table 1).

DISCUSSION

The researched actions of obestatin are not without scientific challenges from other laboratories. These varied from denial of its effects on food intake and gastric emptying parameters (Seoane et al., 2006; Kobelt et al., 2008) to challenging inhibition of the pro-kinetic effects of ghrelin (Bassil et al., 2007). The controversy regarding the role of obestatin as a new regulator of appetite and gastrointestinal mobility is so severe that Gourcerol and Tache (2007), in an editorial, go to the extent of suggesting renaming it merely as a ghrelin-associated peptide!

Our study proves that obestatin decreases food intake and reduces body weight, leading us to believe that obestatin appears to act as an ano-

rex hormone by decreasing food intake, gastric emptying, jejunal motility, and body-weight gain, although Bassil et al. (2007) demonstrated that obestatin (125 nmol kg^{-1}) did not inhibit fasting-induced hyperphagia, suggesting that peripheral obestatin is not a satiety signal that plays a role in the regulation of gastric emptying.

However, in our experiments involving obestatin, it has been clearly shown that administration of obestatin led to a loss of body-weight gain as the rats grew up. The serum glucose levels were also significantly low, probably due to the reduced appetite. The serum insulin levels were also low in the same experimental rats, due to the direct inhibitory influence of exogenous obestatin. Thus our values are in agreement with the general role of obestatin as an anorexic agent. Although the effects on food intake seem to be impressive, leading to a significant change in body weight, it is not known yet if it involves an actual reduction in fat mass. It may be that the actual decrease in body weight could well be due to a decrease in muscle mass, which would then make it not a very good potential drug against obesity.

Therefore, just as what brings about the balance between obestatin and ghrelin is not known, it can only be surmised that perhaps there are other players that are involved in this process. Nevertheless it is interesting to note that there are serious attempts to produce a vaccine that may prevent obesity (Zorrilla et al., 2006).

Hormones are classically defined as chemical mediators that are released in the circulation to incite action away from their site of origin. Furthermore, the synthesis of hormones is regulated by feedback mechanisms, and they act on cells through their specific receptors. In recent years, this definition has become blurred. Hormones are now known to be secreted by tissues other than classical endocrine organs, such as the central nervous system, the gastrointestinal tract, and the adipose tissue. Hormones have been shown to cause paracrine and local effects as well. Even considering this broader definition of a hormone, controversies about obestatin and its action indicate that the weight of evidence does not support its designation as a hormone until more conclusive data emerge (Garg, 2007).

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REFERENCES

- BASSIL A K, HÄGLUND Y, BROWN J, RUDHOLM T, HELLSTRÖM PM, NÄSLUND E, LEE K, SANGER GJ (2007) Little or no ability of obestatin to interact with ghrelin or modify motility in the rat gastrointestinal tract. *Br J Pharmacol*, 150: 58-64.
- DUN SL, BRAILOIU GC, BRAILOIU E, YANG J, CHANG JK, DUN NJ (2006) Distribution and biological activity of obestatin in the rat. *J Endocrinol*, 191: 481-489.
- GARG A (2007) The ongoing saga of obestatin: is it a hormone? *J Clin Endocrinol Metab*, 92: 3396-3398.
- GOURCEROL G, TACHÉ Y (2007) Obestatin – a ghrelin-associated peptide that does not hold its promise to suppress food intake and motility. *Neurogastroent Motil*, 19: 161-165.
- GOURCEROL G, COSKUN T, CRAFT LS, MAYER JP, HEIMAN ML, WANG L, MILLION M, ST-PIERRE DH, TACHÉ Y (2007) Preproghrelin-derived peptide, obestatin, fails to influence food intake in lean or obese rodents. *Obesity*, 15: 2643-2652.
- KOBELT P, WISSER AS, STENGEL A, GOEBEL M, BANNERT N, GOURCEROL G, INHOFF T, NOETZEL S, WIEDENMANN B, KLAPP BF, TACHÉ Y, MÖNNIKES H (2008) Peripheral obestatin has no effect on feeding behavior and brain FOS expression in rodents. *Peptides*, 29: 1018-1027.
- NOGUEIRAS R, PFLUGER P, TOVAR S, ARNOLD M, MITCHELL S, MORRIS A, PEREZ-TILVE D, VÁZQUEZ MJ, WIEDMER P, CASTAÑEDA TR, DIMARCHI R, TSCHÖP M, SCHURMANN A, JOOST HG, WILLIAMS LM, LANGHANS W, DIÉGUEZ C (2007) Effects of obestatin on energy balance and growth hormone secretion in rodents. *Endocrinology*, 148: 21-26.
- REN AJ, GUO ZF, WANG YK, WANG LG, WANG WZ, LIN L, ZHENG X, YUAN WJ (2008) Inhibitory effect of obestatin on glucose-induced insulin secretion in rats. *Biochem Biophys Res Commun*, 369: 969-972.
- SEOANE LM, AL-MASSADI O, PAZOS Y, PAGOTTO U, CASANUEVA FF (2006) Central obestatin administration does not modify either spontaneous or ghrelin-induced food intake in rats. *J Endocrinol Invest*, 29: 13-15.
- ZHANG J, REN P, AVSIAN-KRETCHMER O, LUO C, RAUCH R, KLEIN C, HSUEH A (2005) Obestatin, a peptide encoded by the ghrelin gene, opposes ghrelin's effects on food intake. *Science*, 310: 996-999.
- ZORRILLA EP, IWASAKI S, MOSS JA, CHANG J, OTSUJI J, INOUE K, MEIJLER MM, JANDA KD (2006) Vaccination against weight gain. *Proc Natl Acad Sci USA*, 103: 13226-13231.