

Original Article:**Synergistic effects of serotonin and D-lys³- GHRP-6 on food intakes in food-deprived male rats****Fariba Mahmoudi**

Department of Biology, Faculty of Sciences, University of Mohaghegh Ardabili, Ardabil, Iran

*Corresponding author: email: faribamahmoudi_14@yahoo.com (F. Mahmoudi)**ABSTRACT**

Serotonin exerts an inhibitory effect on food intakes. D-Lys³-GHRP-6 is a ghrelin receptor antagonist which inhibits fasting or ghrelin- induced food intake. In the present study, the effects of serotonin on food intakes were investigated in D-Lys³- GHRP-6- treated food-deprived male rats. Twenty food-deprived male rats in four groups (5 in each) received third cerebral ventricular injection of saline, 10µg serotonin, 20nmol D-Lys³-GHRP-6 or combination of D-Lys³-GHRP-6 and serotonin respectively. Mean of one hour food intakes were measured. Data was analyzed by one-way analysis of variance (ANOVA) with post hoc Tukey's test. In all cases, $P < 0.05$ was considered to be statistically significant. Serotonin, D-Lys³- GHRP-6 or combination of serotonin/D-Lys³- GHRP-6 decreased the mean food intakes significantly when compared to saline. A significant decrease was observed on mean food intakes between combination of D-Lys³-GHRP-6 and serotonin group and serotonin or D-Lys³- GHRP-6 groups. A significant decrease was not observed on food intakes between serotonin and D-Lys³- GHRP-6 groups. Serotonin and D-Lys³-GHRP-6 may exert synergistic inhibitory effects on food intake in rats. Decrease of the ghrelin pathway activity may have a role in mediating the inhibitory effects of serotonin on food intakes.

Key words: Serotonin; D-Lys³- GHRP-6; Food intakes.**INTRODUCTION**

The hypothalamus is a key regulator of feeding behavior. Co-localization of anorexigenic and orexigenic neuronal projections highlights the hypothalamus as a key regulatory site of energy balance and metabolism [1]. Ghrelin is a 28 amino acid peptide which is mainly synthesized by the stomach and hypothalamus nuclei. It acts as endogenous ligand for growth hormone secretagogue receptor (GHSR-1a) and increases food intakes and body weight via GHSR-1a [2]. D-Lys³-GHRP-6 is a synthetic peptide which acts as a GHSR-1a receptor antagonist [3-4]; it has been shown that central or peripheral injection of it inhibits gastric emptying, food intakes and body weight in food-deprived or ghrelin treated mice or rats [5-6].

Serotonin (5-hydroxytryptamine [5-HT]) is a monoamine neurotransmitter which is synthesized

from the essential amino acid tryptophan by the action of hydroxylase and decarboxylase enzymes [7]. Hypothalamus receives the fibers of serotonergic neurons of raphe nuclei [8]. It has been shown that central or peripheral injections of serotonin or serotonergic drugs exert inhibitory effects on food intakes via 5HT_{2C} and 5HT_{1B} receptors [9-13]. The injections of 5HT_{2C} and 5HT_{1B} receptors antagonists increase food intakes [11]. Based on these studies, hypothalamic interneurons may play an important role in mediating the effects of serotonin on food intakes. The present study is aimed at investigating the effects of serotonin on food intakes in D-Lys³- GHRP-6- treated food-deprived male rats.

MATERIALS AND METHODS***Animals and drugs***

Male Wistar rats weighing 230- 250g (provided by the Center of neuroscience Research of Shahid Beheshti University, Iran) were housed individually in the stainless steel cages under controlled temperature (22 ± 2 C°) and 12:12-h light- dark cycle (light on from 0700 to 1900 daily). Animals had free access to standard laboratory food and water all the time except for the beginning of the treatments in which rats were food-deprived for 24 hours. Experiments were executed with the Guide for the Care and Use of Laboratory Animals (National Institute of Health Publication No. 80-23, revised 1996) and were approved by the Research and Ethics Committee of Shahid Beheshti University of Medical Sciences. D-Lys³- GHRP-6 was purchased from Anaspec Company, USA and for the purpose of injections, it was dissolved in distilled water. Serotonin hydrochloride was purchased from Sigma Aldrich, USA; for injection, it was dissolved in distilled water.

Intra cerebral ventricular cannulation

For central injections, a 22- gauge stainless cannulae was implanted 1mm above the third cerebral ventricle according to coordinates of Paxinos and Watson Atlas (AP = - 2.3, ML= 0.0, DV=7.5). The cannula was secured to the skull with three stainless steel screws and dental cement [6]. The animals were kept in individual cages. After one week recovery period, twenty food-deprived rats in four groups (5 in each) received saline, 10µg serotonin, 20nmol D-Lys³ - GHRP-6 or combination of D-Lys³-GHRP-6 and serotonin in a volume of 3µl over one minute respectively. The drugs were injected by a 27-gauge stainless steel injector (protruded 1mm beyond the cannula to reach the third ventricle)

which was connected to Hamilton micro syringe (model 9435, Australia) by PE- 20 tubing via third cerebral ventricle. The doses of drugs used in the present study were selected on the basis of previous studies [5-6].

Feeding tests

Experiments were started at 9:00. Before feeding tests, rats were food-deprived for 24 hours with free access to water. Food intake was measured by subtracting uneaten food from the initially premeasured food at one hour after injections (5-6).

Statistical analysis

All data were expressed as mean $\pm$ SEM. Data was analyzed by one- way analysis of variance ANOVA with post hoc Tukey's test. All statistical analyses were performed with SPSS 16.0 software and the figures were drawn by Microsoft office excel 2007. In all cases, $P < 0.05$ was considered to be statistically significant.

RESULTS

By the injections of serotonin, D- Lys³- GHRP-6 or combination of serotonin and D-Lys³- GHRP-6 decreased the mean significantly with one hour food intake in food-deprived rats compared to food-deprived saline- treated group ($P < 0.05$) (Fig1). Also a significant decrease was observed in food intake in combination of D- Lys³-GHRP-6 and serotonin group compared to serotonin alone or D- Lys³- GHRP-6 alone group ($P < 0.05$) (Fig1). No significant decrease was observed between food-deprived serotonin treated rats compared to food-deprived D-Lys³-GHRP-6 - treated group.

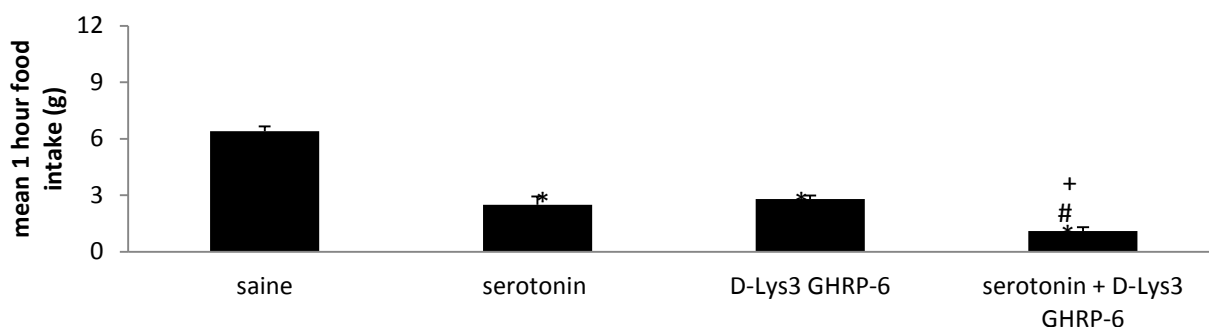


Figure 1 The effects of serotonin (10 μ g), D- Lys³- GHRP-6 (20 nmol) or combination of D-Lys³-GHRP-6 and serotonin on mean of one hour food intake in 24 hour food-deprived rats ($P < 0.05$). *: compared to saline; #: compared to serotonin; +: compared to D-Lys³-GHRP-6.

DISCUSSION

The results showed that ICV injection of serotonin decreased food intakes in rats significantly when compared to food-deprived saline treated ones. This result is consistent with the previous studies which reported that serotonin or serotonergic drugs exerts anorexigenic effect on food intakes in fed or food-deprived rats [9-13]. Previous studies have established that ghrelin is synthesized during fasting and play an important role in the regulation of the food intake [2]. Central injection of ghrelin to fed rats or 24 hour food deprivation resulted in a similar increase in circulating ghrelin concentration and similar increase in daily food intake [2]. It was also demonstrated that GHSR-1a receptor is one of the most important receptors involved in mediating the effects of fasting or ghrelin on food intakes [2-5]; the antagonists of this receptor may play a role in the obesity treatment. The results of the present study showed that injections of D-Lys³- GHRP-6 decreased the food intakes in rats significantly when compared to food-deprived saline treated ones. This result is consistent with the study which reported that D-Lys³- GHRP-6 exerts anorexigenic effect on food intake in 16 hours food-deprived or fed-ghrelin treated male mice [5]. Moreover, these results are consistent with our previous laboratory results which demonstrated that central injection of D-Lys³-GHRP-6 decreases food intakes and NPY gene expression in food-deprived male rats [6]. This study aimed to investigate the interaction of serotonergic and ghrelin pathway on food intake

in 24 hour food-deprived rats for the first time. The results showed that the combined injection of D-Lys³- GHRP-6 and serotonin decreases food intakes significantly when compared to only serotonin or D-Lys³- GHRP-6 groups. It is clearly shown that ghrelin injection or food deprivation exerts their stimulatory effects on food intakes via increasing NPY/AgRP and decreasing α -MSH/CARP neurons activity [2, 10, 14]. Also, our previous study did not report any significant difference on the NPY gene expression level between food-deprived or ghrelin treated rats [2]. This study also showed that NPY/AgRP and α -MSH/CARP play an important role in mediating the inhibitory effects of serotonergic pathway on food intakes and loss of body weight [2, 10, 14]. Recent studies have established that serotonergic drugs including fenfluramine decrease plasma ghrelin concentrations and they inhibit gastric emptying [15-16]. Based on previous information and the results of the present study, ghrelin pathway may have a role in mediating serotonin effects on food intake and serotonin may exert inhibitory effects on appetite partly by decreasing the ghrelin secretion and following decrease of NPY/ AgRP gene expression.

CONCLUSION

The results showed that serotonin and D-Lys³-GHRP-6 exert synergistic inhibitory effects on food intakes in 24 hours food-deprived rats. Decrease of the ghrelin pathway activity may have a role in mediating the inhibitory effects of serotonin on food intakes.

ACKNOWLEDGEMENTS

This work was done through the financial help of University of Mohaghegh Ardabili. The author sincerely appreciates Shahid Beheshti University of Iran for supplying the instruments.

"The authors declare no conflict of interest"

REFERENCES

1. Hillebrand JJG, de Wied D, Adan RAH. Neuropeptides, food intake and body weight regulation: a hypothalamic focus. *Peptides*. 2002;163(12): 2283- 2306.
2. Wren AM, Small CJ, Abbott CR., Dhillo WS, Seal LJ, Cohen MA, et al. Ghrelin causes hyperphagia and obesity in rats. *Diabetes*. 2001;50(11): 2540-7.
3. Holst B, Cygankeiwicz A, Halkjaer JT, Ankersen M, Schwartz, TW. High constitutive signaling of the ghrelin receptor- identification of a potent inverse agonist. *Molecular Endocrinology*. 2003;17(11): 2201-10.
4. Zizzari P, Halem H, Taylor J, Dong JZ, Datta R, Culler MD, et al. Endogenous ghrelin regulates episodic growth hormone (GH) secretion by amplifying GH pulse amplitude: Evidence from antagonism of the GH secretagogue 1a receptor. *Bluet- Pajot* 2006;11(9); 22- 33.
5. Asakawa A, Inui A, Kaga T, Katsuura G, Fujimiya M, Fujino MA, et al. Antagonism of ghrelin receptor reduces food intake and body weight in mice. *Gut*. 2003;52(7): 947-52.
6. Khazali H, Mahmoudi F. The anti-obesity effect of D-Lys3- GHRP-6 peptide, GHSR receptor antagonist in rats. *Iranian Journal of Endocrinology and Metabolism*. 2013; 14(5): 484-91.
7. Sharma SH, Sharma J. Regulation of appetite: role of serotonin and hypothalamus. *Iranian Journal of Pharmacology and Therapeutic*. 2012;11(2); 73- 79.
8. Pollak Dorocic I, Fürth D, Xuan Y, Johansson Y, Pozzi L, Silberberg G, et al. A whole-brain atlas of inputs to serotonergic neurons of the dorsal and median raphe nuclei. *Neuron*. 2014; 83(3): 663-78.
9. Amer A, Breu J, McDermott J, Wurtman RJ, Maher TJ. 5-Hydroxy-L-tryptophan suppresses food intake in food-deprived and stressed rats. *Pharmacology Biochemistry Behavior*. 2004; 77(1): 137-43.
10. Feijó Fde M, Bertoluci MC, Reis C. Serotonin and hypothalamic control of hunger: a review. *Revista Da Associacao Medica Brasileira*. 2011; 57(1): 74-77.
11. Halford JC, Harrold JA. 5-HT(2C) receptor agonists and the control of appetite. *Handbook of Experimental Pharmacology*. 2012; 209: 349-56.
12. Leibowitz SF, Alexander JT. Hypothalamic serotonin in control of eating behavior, meal size, and body weight. *Biological Psychiatry*. 1998;44(9): 851-64.
13. Meguid MM, Fetissov SO, Varma M, Sato T, Zhang L, Laviano A, et al. Hypothalamic dopamine and serotonin in the regulation of food intake. *Nutrition*. 2000;16(10): 843-57.
14. Goto M, Arima H, Watanabe M, Hayashi M, Banno R, Sato I, et al. Ghrelin increases neuropeptide Y and agouti-related peptide gene expression in the arcuate nucleus in rat hypothalamic organotypic cultures. *Endocrinology*. 2006;147(11): 5102-9.
15. Nonogaki K, Ohashi-Nozue K, Oka Y. A negative feedback system between brain serotonin systems and plasma active ghrelin levels in mice. *Biochemical Biophysical Research Communications*. 2006; 341(3): 703-7.
16. Robinson PH, Moran TH, McHugh PR. Inhibition of gastric emptying and feeding by fenfluramine. *American Journal of Physiology*. 1986;250(5); 764-9.