The effects of interaction between ghrelin and substance-P on mean plasma thyroid hormones concentration and body weight

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ABSTRACT

Background and the purpose of the study: Ghrelin increases food intakes and body weight via growth hormone secretagogues receptor (GHSR-Ia). [D-Arg-1, D-phe-5, D-Trp-7,9, Leu-11]Substance-P (an analog of Substance-P) is known as an antagonist of GHSR-Ia receptor which inhibits ghrelin- induced food intakes. Thyroid hormones have also an important role in the regulation of metabolism and body weight. The goal of this study was to determine the effect of different doses of either ghrelin, analog of Substance-P or their interactions on the body weight, mean plasma TSH, Triiodothyronine (T_3) and Thyroxine (T_4) concentration and to investigate whether ghrelin exerts its effects on thyroid axis activity via GHSR-Ia.

Methods: Rats received different doses of ghrelin (1, 5, or 10 nmol), analog of Substance P (5, 10, 20 nmol) or saline via lateral cerebral ventricle. Body weight was measured daily before injection and at 9 h of the day after the final injection. The blood samples were collected at the end of experiment. Plasma was assayed for thyroid hormones concentration.

Results: Ghrelin significantly increased body weight and decreased mean plasma TSH, T_3 and T_4 concentrations in a dose related manner. Analog of Substance-P significantly decreased body weight and increased thyroid hormones concentration in a dose related manner. It blocked the stimulatory effect of ghrelin on body weight and the inhibitory effect of ghrelin on thyroid axis activity.

Conclusion: From the results of this study it appears that analog of substance P may be useful for treatment of obesity.

Keywords: Ghrelin; Substance-P; Triiodothyronine (T_3); Thyroxine (T_4).

INTRODUCTION

Ghrelin, a novel 28 amino acid peptide with an n-octanoyl modification on Ser 3, was first identified in the stomach as an endogenous ligand for growth hormone secretagogues receptor (GHSR-Ia) (1). It is well recognized to have an important role in the maintenance of energy homeostasis. During fasting, ghrelin is secreted by X/A like cells of stomach, neurons of hypothalamus and other tissues (1).Ghrelin increases growth hormone secretions and food intakes via GHSR-Ia (2). It decreases energy expenditure and suppresses TSH secretion (3).It is suggested that different neurotransmitters such as substance-P may interact with ghrelin actions. (4).

Substance-P, a tachykinin peptide with 11 amino acid, was first identified in the intestinal and brain extracts as an endogenous ligand for NKI receptor (5). Previous studies have shown that Substance-P suppresses food intakes (6) and growth hormone (GH) secretions (7). It has been also reported that [D-Arg-1, D-phe-5, D-Trp-7,9, Leu-11]S-P (an analog of Substance-P) acts as a potent inverse agonist or an antagonist of GHSR-Ia receptor (8) which blocks the stimulatory effects of ghrelin on gastric motility, food intakes and body weight (4).

The Hypothalamus- Pituitary- Thyroid axis (H-P-T) plays an important role in the regulation of metabolism and energy homeostasis through thyroid hormones. It has been shown that different neural, hormonal and environmental factors interact to modulate thyroid hormones secretions. This study was designed to determine the effect of either ghrelin or [D-Arg-1, D-phe-5, D-Trp-7,9, Leu-11]S-P on thyroid axis activity and body weight and also, to investigate whether ghrelin exerts its effects on thyroid axis via GHSR-Ia receptor.

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MATERIAL AND METHODS

Animals

Male Wistar Rats (n= 103) weighing 200- 250 g (provided by the Center of neuroscience Research of Shahid Beheshti University) were housed individually in cages under controlled temperature (22± 2 C°) and light (12h light/ dark cycle). Animals had free access to food and water all the time.

ICV cannulation and injections

Animal surgery procedures and handling were carried out as previously described (9, 10). Animals were anesthetized by intraperitoneal (IP) injection of a mixture of Ketamine and Xylezine (Ketamine100 mg/kg BW+ xylezine 15 mg/ kg BW, Alfasan Company, Holand). For intra cerebral ventricle (ICV) injections, animals were placed in a stereotaxic frame (Stoelting, USA) and a 22- gauge stainless cannulae was implanted in to the right lateral cerebral ventricle. The cannulae tip was placed at anterior- posterior = - 0/8, Lateral = - 1/6 and dorsoventral = 3/2 mm according to coordinates of Paxinos and Watson Atlas. The cannula was secured to the skull with three stainless steel screws and dental cement. The animals were kept in individual cages and habituated by handling every day to minimize the stress of surgery. After one week recovery period, 1, 5 or 10 nmol of ghrelin (2,10) or 5, 10 or 20 nmol of analog of S-P (4) [ghrelin and analog of SP provided by Sigma Company, USA] were dissolved in 5µl of 0.9%saline. The peptides were injected by a 27- gauge stainless steel injector which connected to 10µl Hamilton micro syringe (model 9435, Australia) by PE- 20 tubing. Body weight was measured daily before injection and at 9 h of the day after the final injection during 6 days (10). At the end of the experiment the animals were decapitated and blood samples were collected at 20 min after infusions. The dose of ghrelin and the time of blood sampling were chosen based on the previous experiments which found to produce a significant increase in body weight and a decrease on mean plasma T_3 and T_4 (2, 10). Heparin was used in samples to prevent clotting. Blood samples immediately centrifuged for 10 min at 3500 rpm and the plasma stored at –20°C until TSH, T_3 and T_4 concentrations were assayed. The brains were removed and kept in formalin (10%) for two weeks. Correct ICV cannulae placement was confirmed histologically. Only those animals with correctly positioned cannulae were included in the analysis of data.

Initially the effect of different doses of either ghrelin or [D-Arg-1, D-phe-5, D-Trp-7,9, Leu-11]S-P (an antagonist of GHSR-Ia receptor) on body weight and thyroid axis activity was investigated. Sixty three rats in 7 groups (in each group, n=9) received saline, ghrelin (1, 5 or 10 nmol) or analog of S-P (5, 10 or 20 nmol) in a volume of 5µl over one minute once-daily for six days during the early light phase (0800h - 0900 h). Body weight was measured daily before and after the infusion. Blood samples were collected by decapitation. In each group mean plasma TSH, T_3 and T_4 concentration and body weight were measured.

In order to investigate the possibility that whether ghrelin exerts its inhibitory effects on mean plasma TSH, T_3 and T_4 concentration via GHSR-Ia in the brain, the effect of simultaneous administration of ghrelin and [D-Arg-1, D-phe-5, D-Trp-7,9, Leu-11]S-P (an antagonist of GHSR-Ia receptor) was examined. Forty rats in four groups (in each group, n=10) received saline or simultaneous administration of ghrelin (5nmol) and [D-Arg-1, D-phe-5, D-Trp-7,9, Leu-11]S-P (5,10 or 20nmol) in a volume of 5µl for six days during the early light phase (0800h - 0900 h). In each group mean plasma TSH, T_3, T_4 concentration and body weight were measured.

Hormone assays and statistical analysis

Plasma TSH, T_3 and T_4 were measured by using TSH, T_3 and T_4 kits (Tabeshyarnor Company, Iran) and the method of a homologous double antibody radio- immunoassay (RIA).The results are presented as mean ± SEM. The data were analyzed by unpaired t-test, ANOVA test followed by post hoc Least Significant Difference and SPSS software. Also, the multiple repeated measurement analysis was used to determine the effect of time on body weight. In all cases $P<0/05$ was considered to be statistically significant.

RESULTS

The results show that Ghrelin increased body weight in a dose related manner and there was a significant increase in body weight in fifth and sixth days compared to saline (Fig 1). It also, significantly decreased mean plasma TSH, T_3 and T_4 concentration in a dose related manner compared to saline. Ghrelin (1 nmol) did not significantly alter mean plasma thyroid hormones concentration compared to saline but at high doses (5 or 10 nmol) compared to saline decreased TSH, T_3 and T_4 significantly. (Table1). As it is shown in the Table 1, analog of S-P significantly increased

Table 1. The effects of different doses of ghrelin (Gr), analog of Substance-P (SP) or simultaneous injection of ghrelin and different doses of analog of SP on mean plasma TSH, T_3 and T_4 concentration compared to saline.

	Gr 1 nmol	Gr 5 nmol	Gr 10 nmol	SP 5 nmol	SP 10 nmol	SP 20nmol	Gr 5 nmol +SP 5 nmol	Gr 5 nmol +SP 10nmol	Gr 5 nmol +SP 20nmol
TSH	%13	%38	%46	%12	%38	%54	%38	%32	%5
T_3	%23	%50	%59	%22	%41	%63	%41	%34	%10
T_4	%18	%55	%66	%14	%36	%59	%38	%36	%6

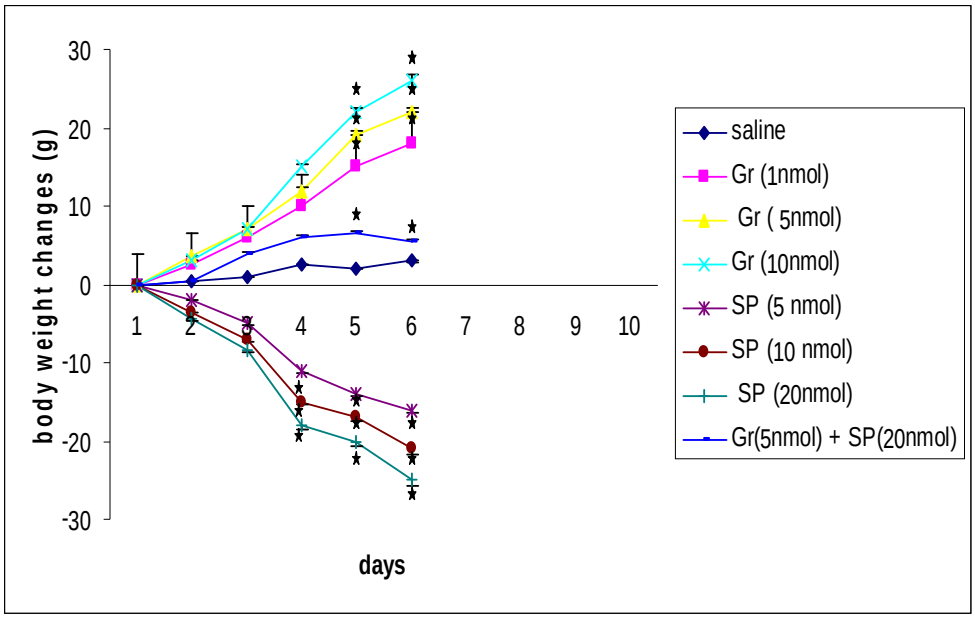

Figure 1. The effects of ghrelin or analog of SP on body weight changes compared to saline or simultaneous administration of ghrelin and analog of SP on body weight compared to ghrelin group (P<0/05).

mean plasma TSH, T_3 and T_4 concentration in a dose related manner compared to saline but at the dose of 5 nmol did not significantly alter the mean plasma thyroid hormones concentration in comparison to salin significantly. However at high doses (10 or 20 nmol) it significantly increased TSH, T_3 and T_4 concentration compared to saline (Table 1). Also, different doses of analog of SP significantly decreased body weight by 4th, 5th, and 6th days compared to saline (Fig 1).

Also, as it is shown in the table 1, [D-Arg-1, D-phe-5, D-Trp-7,9, Leu-11] S-P (5 or 10 nmol) compared to saline didn't abolish the inhibitory effect of ghrelin on mean plasma TSH, T_3 and T_4 significantly). In contrast, [D-Arg-1, D-phe-5, D-Trp-7,9, Leu-11]S-P (20 nmol) significantly blocked the inhibitory effect of ghrelin on mean plasma TSH, T_3 and T_4 comparison to saline and decreased their concentrations significantly (Table1). Analog of SP (20nmol) also, significantly blocked the stimulatory effect of ghrelin on body weight in 5th, and 6th days compared to ghrelin group. (Fig1).

DISCUSSION

The results of this study showed that ICV injection of ghrelin significantly increased body weight and decreased the mean plasma TSH, T_3 and T_4 concentrations in a dose related manner which is consistent with the results of the previous studies which reported a significant decrease in the hypothalamus - pituitary-thyroid axis activity following ICV or IP injection of ghrelin, agouti-related-peptide (AgRP) or neuropeptide-Y (NPY) (3, 10- 13).

In the present study, the effect of interaction between ghrelin and [D-Arg-1, D-phe-5, D-Trp-7,9, Leu-11]S-P on thyroid axis activity was investigated for the first time. The results demonstrated that simultaneous administration of ghrelin and [D-Arg-1, D-phe-5, D-Trp-7,9, Leu-11]S-P significantly blocked the inhibitory effect of ghrelin on mean plasma TSH, T_3 and T_4 concentration.

GHSR-Ia receptor, which is mainly found in the hypothalamus, belongs to a small subset of 7TM G protein- Coupled receptors and exhibits high

constitutive activity. It is active in the presence of ghrelin and inactive in the presence of [D-Arg-1, D-phe-5, D-Trp-7,9, Leu-11]S-P (12). [D-Arg-1, D-phe-5, D-Trp-7,9, Leu-11]S-P exerts its central effects as endogenous antagonist or inverse agonist at GHSR-Ia receptor. (8). Also, It has been found that analog of S-P blocks the stimulatory effects of ghrelin on gastric motility, food intakes and body weight.(4). Although further studies are required to determine the possible effects of GHSR-Ia antagonist on thyroid axis disorders, it is suggested that GHSR-Ia antagonists may be promising targets for treatment of obesity. Thyroid hormones play an important role in regulation of energy homeostasis and body weight. Therefore, GHSR-Ia antagonists may reduces body weight by an increase in T_3 and T_4 levels, and consequently increase the metabolism and energy expenditure.

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