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The effects of interactions between testosterone and ghrelin on mean plasma thyroid hormones concentration in male rats

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ABSTRACT

Ghrelin is an orexigenic peptide synthesized mainly by stomach and hypothalamus. Ghrelin decreases secretion of thyroid hormones. Testosterone is an esteroidogenic hormone that exerts stimulatory effects on Hypothalamus-Pituitary-Thyroid (HPT) axis activity. The aim of the present study was to investigate the effect of interactions between the central injection of ghrelin and testosterone on mean plasma thyroid hormones concentration. Twenty male Wistar rats (200-250 g) were randomly divided into four groups. The groups received saline, 5 nmol ghrelin, 1 µg testosterone and simultaneous injection of 5 nmol ghrelin and 1 µg testosterone in third cerebral ventricle in volumes of 3 µl. Blood samples were collected one day before injection and until 12 hours after that. Mean plasma thyroid hormones concentrations were determined by radio-immunoassay (RIA). The results indicated that testosterone significantly increased the mean plasma concentration of T3 and T4 hormones after injection compared to before injection, whereas ghrelin significantly decreased the mean plasma concentration of T3 and T4 compared to before injection. The results demonstrated that ghrelin significantly decreased the stimulatory effect of testosterone on mean plasma T3 and T4 concentrations.

Keywords: Stimulatory effect, Radio-immunoassay; Hypothalamus-Pituitary-Thyroid.

Introduction

Androgenic-anabolic steroids (AAS) are steroid molecules derived from testosterone (1, 2). AAS Improve athletic ability, balance the energy levels, increase food intake, muscle mass, sexual function, libido, body hair and body weight and decrease risk of osteoporosis in rats (1, 3, 4). Testosterone is physiologically secreted by testes and adrenal glands and transported by the testosterone binding globulins (TBG) and albumin. TBG is produced in the liver and binds to about two-thirds of serum thyroxine (T4) (5, 6, 7, 8). Peripheral conversion of inactive T4 to biologically active T3 is catalyzed by 5´-deiodinase activity. A recent study showed the specific modulatory effect of testosterone and estradiol on the proliferation of human thyroid carcinoma cell lines, which is independent of Thyrotropin (9). The stimulatory effect of testosterone and estradiol on the expression of TSH mRNA in the pituitary in normal

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Wistar rats is known (10). Ghrelin, a novel 28 amino acid peptide hormone is secreted by the X/A like endocrine cells of stomach, neurons of hypothalamus and some other tissues (11, 12, 13). Ghrelin is involved in a series of biological functions including regulation of energy balance, food intake (14), sleep (15), body weight (16), gastrointestinal motility (17), cardiovascular functions (14) cell proliferation (18), production of pro-inflammatory cytokines (19), and reproduction in many species. Ghrelin is the endogenous ligand for growth hormone secretagogue receptor (GHS-Rs). GHS-R is highly expressed in the ARC with 94% of all NPY-containing neurons co-expressing GHS-R mRNA The co-localization of the growth hormone secretagogue with these hypothalamic neurons further indicates that the hypothalamus is a main target site for ghrelin (20, 21, 22). During fasting, ghrelin is secreted by stomach cells and neurons of the hypothalamus and following food consumption mean plasma level of ghrelin decreases (23). A recent study in male rats has reported a decreased activity of the HPT axis after ICV injection of ghrelin every 24h for 5 days; pituitary TSH cells were smaller and TSH plasma levels were lower and as a result, thyroid follicles were less active and mean plasma concentration of thyroxine (T4) was reduced compared with saline-treated rats (24). It was shown that injection of 50µg acylated ghrelin decreases T3, T4 and TSH from 2 until 7 hour after injection (25). The hypothalamic-pituitary-thyroid (HPT) axis plays an important role in the energy balance via the effect of thyroid hormones to increase oxygen consumption and heat generation. It has been shown that different neural, hormonal and environmental factors interact to modulate thyroid hormones secretions. Hormones of the HPT axis are crucially involved in maintaining body temperature and energy balance in mammals (20, 22).

Earlier studies showed the effect of ghrelin or testosterone on mean plasma thyroid hormones but the simultaneous effect of ghrelin and testosterone on T3 and T4 was unknown. The purpose of this study was to examine the effect of the interaction between ghrelin and testosterone (injected via ICV route) on mean plasma T3 and T4 concentrations in male rats and to assess the potential pathway of this change.

Material and Methods

Animals

Twenty male Wistar rats (200-250 g) used in this study were provided by the Center of Neuroscience Research of Shahid Beheshti University. Animals were housed individually in cages under controlled temperature (25±2˚C) and light (12h light/dark cycle). They had free access to food and water all the time. The rats were divided to four groups and each group included five animals. Group 1 (control) included the animals that received saline (3µl ICV), in group 2 (ghrelin) the animals received 5 nmol ghrelin dissolved in saline, in group 3 (testosterone) the animals received 1 µg testosterone dissolved in sesame oil, and in group 4 (ghrelin+testosterone) the animals received ghrelin and testosterone simultaneously. All injections was done in 3 µl volumes.

ICV cannulation and injection

Animals were anesthetized by intraperitoneal (IP) injection of a mixture of ketamine and xylazine (100 mg/kg BW ketamine+15 mg/kg BW xylazine). For implanted cannulae, animals were placed in a stereotaxic frame and 22-gauge stainless steel intracerebroventricular (ICV) cannulae were implanted in the third cerebral ventricle 4-6 days before the injection experiments. A cannulae were inserted using the following stereotaxic coordinates: AP-2.3, ML-0.0, DV-6.5 according to coordinates of Paxinos and Watson atlas. The cannulae were fixed to the skull with three stainless steel screws and acrylic dental cement. Dummy cannulae were inserted to prevent the entry of foreign material. One week after surgery, the drugs were injected by 27-gauge stainless steel injector which connected to 5 µl Hamilton syringe by PE-20 tubing. Ghrelin dose injected was 5 nmol and testosterone dose injected was 1 µg according to earlier studies (26, 27)

Blood Collection

Blood samples were collected before injection and, 30 minutes, 6 and 12 hours after injection. All injections and blood collections was done between 8-10 am. Correct cannula placement was checked by postmortem dye Nate. Only those animals with properly positioned cannulae were included in data analysis.

Hormones assay

Blood samples centrifuged immediately for 20 min at 4000 rpm and the plasma frozen at -25°C until T3 and T4 concentration were assayed. Plasma T3 and T4 were measured by T3 and T4 kits (Padtangostar, Tehran, Iran) and concentration were determinate by radio-immunoassay (RIA).

Statistical analysis

Data were presented as mean ±SE, and analyzed by repeated measures test followed by post hoc Least Significant Difference and SPSS software. $P<0.05$ was considered to be statistically significant.

Results

Effect of ghrelin on T3 and T4 concentration

The results showed that ghrelin decreased mean plasma T3 concentration 6 and 12h after injection, respectively, 38.35% and 34.24% compared to before injection (Fig. 1) also decreased mean plasma T4 concentration 30 min, 6 and12 h after injection, respectively 24.17%, 31.70% and 30.46% compared to before injection (Fig. 2).

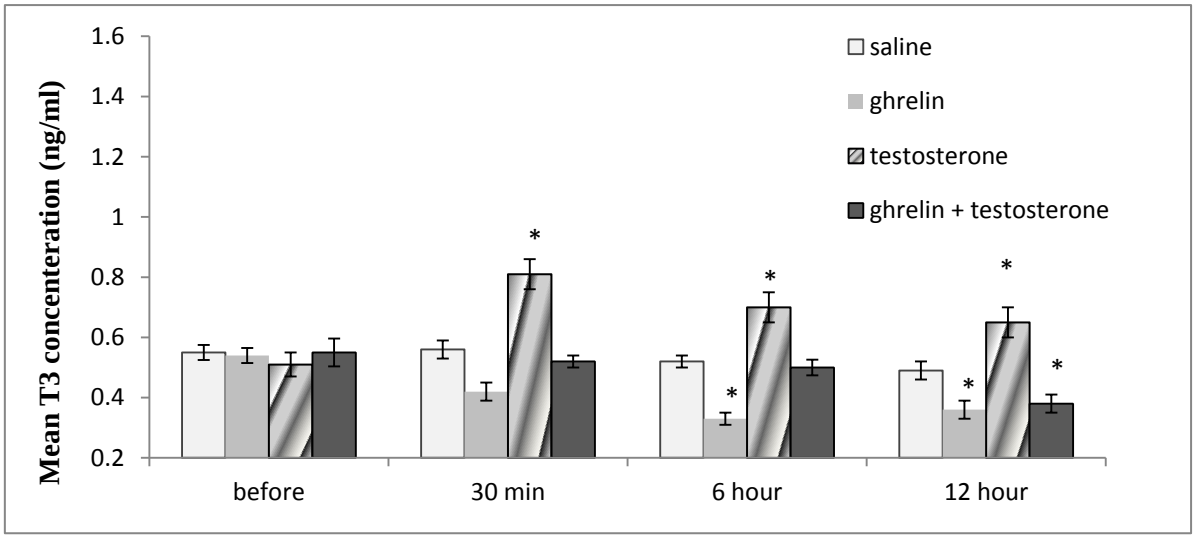

Figure 1. Effect of ghrelin (5 nmol), testosterone (1 µg) and simultaneous injection of ghrelin and testosterone on mean plasma T3 concentration compared to saline in 30 mins, 6 and 12 hours after injection (p<0.05).

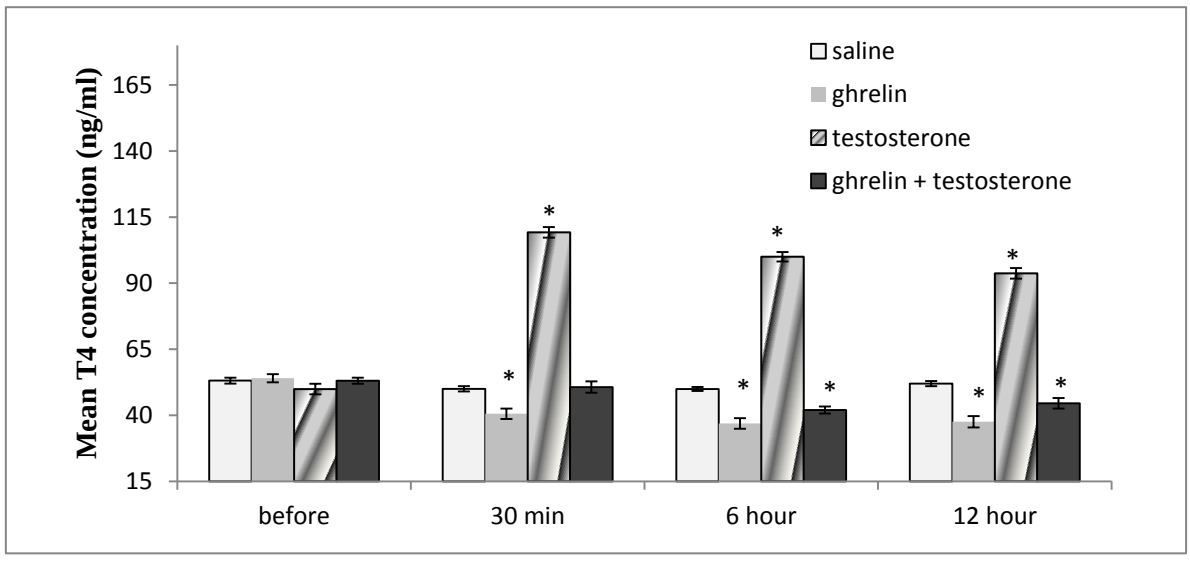

Figure 2. Effect of ghrelin (5 nmol), testosterone (1 µg) and simultaneous injection of ghrelin and testosterone on mean plasma T4 concentration compared to saline in 30 mins, 6 and 12 hours after injection (p<0.05).

Effect of testosterone on T3 and T4 concentration

Testosterone increased mean plasma T3 concentration 30 min, 6 and 12h after injection respectively, 135% , 96.07% and 60.78% compared to before injection (*P<0.05*) (Fig. 1) also increased mean plasma T4 concentration 30 min, 6 and 12h after injection, respectively, 198.91%, 121.61% and 87.70% compared to before injection (*P<0.05*) (Fig. 2).

Effect of ghrelin and testosterone on T3 and T4 concentration

Simultaneous injection of ghrelin and testosterone decreased mean plasma T3 concentration 12h after injection, 30.9% compared to before injection (*P<0.05*) (Fig. 1) also decreased mean plasma T4 concentration, 6 and 12h after injection, respectively, 20.83% and 18.06% compared to before injection (*P<0.05*) (Fig. 2).

Discussion

It seems that significant decrease in T3 and T4 concentration after ghrelin injection was due to effect of ghrelin on different peptides like Agouti-related peptide (AgRP) or neuropeptide Y (NPY) in the ARC of hypothalamus. Previous studies have shown that ghrelin increase the synthesis of AgRP/ NPY in ARC nucleus (26, 27, 28). It was found that chronic central administration of ghrelin increases hypothalamic NPY and AGRP mRNA levels. It has been proven that AgRP/NPY immunoreactive axons densely innervate the thyrotropin-releasing hormone (TRH) neurons in the paraventricular nucleus (PVN) of hypothalamus (26). Other study demonstrated that AgRP and NPY have a similarly potent inhibitory action on the pro-TRH gene expression of hypophysiotropic neurons, indicating that both AGRP and NPY may play a major role in the inhibition of the HPT axis during fasting (29, 30, 31, 32). Earlier observations showed that AgRP and NPY administration *in vivo* decreases TSH levels, while *in vitro* prevents TRH release from hypothalamic explants (29, 30, 33, 34, 35). The effect of NPY in reducing serum TSH is probably due to its impact in increasing dopamine utilization in the median eminence. Dopamine itself might be inhibitory to TRH release from the median eminence (36) and TSH from anterior pituitary thyrotropes (37). So, ghrelin may have an inhibitory effect on HPT axis activity via increasing AgRP and NPY. Some studies have shown that alpha- melanocyte-stimulating hormone (α-MSH) secreting neurons in ARC densely innervate the TRH neurons in PVN. Therefore, there is a significant increase in TSH and thyroid hormones concentration after ICV or paraventricular injection of α-MSH (29, 38, 39). Hence, we could expect inhibitory effect of ghrelin on HPT axis, at least partially, which might be due to an increase in the Agouti level and its antagonist action on α-MSH receptors. It has been recognized that ICV injection of ghrelin blocked the GABA release from AgRP and NPY neurons in hypothalamus. The inhibition of GABA secretion, cause of increasing corticotrophin releasing hormone (CRH) from hypothalamus by activation of CRF neurons and demonstrated CRH and cortisol exert an inhibitory effect of mean plasma T3 and T4 concentration (40, 41, 42).

The stimulatory effect of testosterone on the expression of TSH mRNA in the pituitary in normal Wistar rats is known (43), in other word testosterone would have increased the responsiveness of thyrocytes to TSH in male rats. Nevertheless, one author has reported that testosterone decreased mean plasma TSH concentration (44). Several studies have reported decreased serum TSH concentration after orchidectomy and reversion of this effect by testosterone replacement (45, 46, 47). Oral testosterone administration decreased TBG, but whether testosterone decreases TBG by reduced synthesis or increased clearance is not recognized (48). Testosterone inhibitory effect on HPA axis (49) decreases CRF which is done by increasing in 5-HT (serotonin precursor) concentration (50, 51). Moreover, 5-HT via 5-HT_{1B}Rs, both inhibits AgRP neuronal activity (49). Further evidence illustrates that the direct 5-$HT_{2A/2C}$ receptor agonist DOI injected into the PVN inhibits the effect of NPY on energy intake and metabolism (52) (Fig. 3).

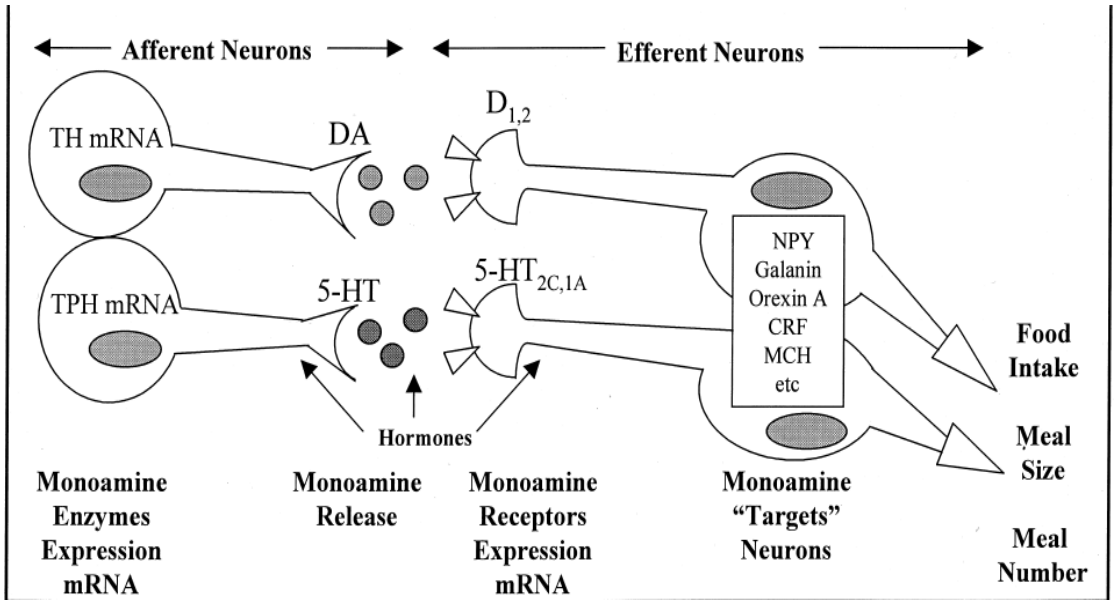

Figure 3. Interaction between NPY, CRF, MCH, 5-HT and dopamine neurons in hypothalamus.

In the present study, the effect of ghrelin+ testosterone on HPT axis was investigated for the first time. We demonstrated that in co-injection of testosterone and ghrelin, ghrelin significantly counteracting effect of testosterone and decreasing mean plasma T3 and T4 concentration.

As mentioned, testosterone decreases CRF by increased 5-HT and eventually 5-HT via 5-HT_{1B}Rs, both inhibit AgRP neuronal activity. Furthermore, we know AGRP plays a major role in the inhibition of the HPT axis. It has also been demonstrated that ghrelin inhibits 5-HT release from rat hypothalamic (53). So, probably ghrelin decreased the stimulatory effect of testosterone on mean plasma T3 and T4 concentrations by inhibiting 5-HT release and it cause more activity of AgRP neuronal on the TRH neurons in the paraventricular nucleus of hypothalamus.

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