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The Effect of Brotizolam on Serum Ghrelin Levels in Mice

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Öz

Brotizolamla İndüklenen İştahın Farelerde Ghrelin Seviyeleri Üzerine Etkisi

Amaç: Evcil hayvanlarda iştah, metabolik profil, enfeksiyon ve beslenme şeklinden etkilenmektedir. İştahın düzenlenmesi pek çok karmaşık fizyolojik süreci içerir. Ghrelin en iyi bilinen iştah arttırıcı hormondur. İştah da lateral hipotalamus tarafından yönetilir. Öte yandan, ventromedial çekirdek çoğunlukla tokluk ile ilişkilidir. 1,4-benzodiazepin türevinin bir üyesi olan brotizolam, ventromedial hipotalamusun aktivitesini bloke eder ve evcil hayvanlarda iştahı artırmak için kullanılır. Bu çalışmanın amacı, brotizolamın iştah artırıcı hormon olan serum ghrelin düzeylerine etkisini araştırmaktır.
Yöntem: Toplam 16 fare, kontrol (%0,9 NaCl, IP) ve brotizolam (2 µg/kg, IP) olarak 2 eşit gruba ayrıldı. Her iki grupta da uygulamadan 30 dakika sonra kan örnekleri toplandı. Serum ghrelin seviyeleri, Enzyme-Linked Immunoassay ile belirlendi.
Bulgular: Kontrol ve brotizolam grupları arasında anlamlı bir fark vardı ($P<0.001$). Çalışmanın sonuçları, brotizolam uygulamasının serum ghrelin düzeylerini artırdığını gösterdi.
Sonuç: Ventromedial hipotalamus ve tokluğu baskılayan brotizolam, lateral hipotalamusun aktivasyonu ve ghrelin düzeylerinin artması ile iştahı aktive edebili. Ayrıca ghrelin iştahın endokrin belirteci olarak kullanılabilir.
Anahtar Kelimeler: Brotizolam, Ghrelin, İştah

Abstract

The Effect of Brotizolam Induced Appetite on Serum Ghrelin Levels in Mice

Objective: Appetite is affected by the metabolic profile, infections and nutritional shape in domestic animals. Regulation of appetite involves many complex physiological processes. Ghrelin is well known appetite-enhancing hormone. The appetite is also managed by the lateral hypothalamus. On the other hand, the ventromedial nucleus is most commonly associated with satiety. Brotizolam, member of the 1,4-benzodiazepines derivative, is blocking the activity of the ventromedial hypothalamus and used to increase appetite in domestic animals. The aim of this study was to investigate the impact of brotizolam on serum ghrelin levels, enhancer appetite hormone.
Methods: A total of 16 mice were equally assigned to two groups as control (0.9% NaCl, IP) and brotizolam (2 µg/kg, IP). Blood samples was collected 30 min after the administration in both groups. Serum ghrelin levels were determined by enzyme linked immunoassay.
Results: There was a significance differences between control and brotizolam groups ($P<0.001$). The results of the study showed that the administration of brotizolam increased serum ghrelin levels.
Conclusion: Brotizolam, which suppresses ventromedial hypothalamus and satiety, can activate appetite by activation of the lateral hypothalamus and increase in ghrelin levels.In addition, ghrelin may be used as an endocrine marker of appetite.
Keywords: Appetite, Brotizolam, Ghrelin

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INTRODUCTION

Appetite is a complex mixture of physiological and psychological phenomena which ranges from feelings of hunger, total energy intake, ingestion of particular nutrients, preferences of meals and snacks intake. The course of appetite fluctuates according to these conditions. Satiety is controlled by biological systems such as nutrition, feelings and behavior. Biological systems include hormones signals generated and released by gastrointestinal tract. In addition, the generation of amino acids and glucose after digestion is affected this system (1).

The hypothalamus, involved in Central Nervous System (CNS), plays a role as key region involved in the regulation of appetite (2,3). The ventromedial hypothalamic nucleus controls satiety, while the lateral region of hypothalamus controls feeding (4). Gut hormone receptors are largely located in hypothalamic arcuate nucleus (ARC) regulating appetite and satiety signals (2,5). Ghrelin is known as unique orexigenic peptide (6). Conversely, cholecystokinin (CCK), pancreatic polypeptide (PP), peptide YY (PYY), glucagon-like peptide (GLP)-1, and oxyntomodulin (OXM) are anorexigenic gut hormones. The main function of these circulating hormones is to regulate appetite (7,31).

Brotizolam has antianxiety, anticolvulsant, muscle relaxant and sedative effect in humans. It is a member of 1,4-Benzodiazepines (1,4-BZDs) class of drugs. (8). Brotizolam, which is also used in animal health, successfully induces appetite for food intake in parasitized lambs. This effect is appeared by blocking the activity of the ventromedial hypothalamus (VMH) (1).

Therefore in the present study, we aimed to investigate effects of brotizolam on ghrelin, and its possible appetite induced effect.

MATERIALS AND METHODS

Animals and Chemicals

A total of 16 adult (>10 weeks) male Balb/c mice were purchased from the Hatay Mustafa Kemal University Application and Research Center for Experimental Research (Hatay, Turkey). The animals were housed in polycarbonate cages under standardized conditions (22 ± 2 °C temperature, 55 ± 10 % relative humidity, 12:12 h light/dark cycle). Tap water and mouse chow were provided *ad libitum*. Brotizolam (TORO®, SANOVEL, Turkey) was obtained as ready-to-use solution.

Experimental Design

The animals were randomly divided into groups as Control (n= 8) and Brotizolam (n= 8). All animals were fasted overnight (12 h). Control animals were intraperitoneally (i.p) injected with 0.9% sodium chloride solution (0.2 mL), while brotizolam (2 µg/kg, i.p .) was administered to the animals in BTZ group. Blood was drawn 30 min after the administration from both groups. The animals were anaesthetized with 2% isoflurane in a ventilation chamber immediately before the cardiac puncture to acquire blood. Collected blood was decanted into serum tubes and left to clot for 30 minutes at room temperature. Blood samples were centrifuged to obtain serum at 2,000 x g for 10 minutes in a refrigerated centrifuge. The supernatant was pipetted into microcentrifuge tubes. Serum ghrelin levels were measured by using an ELISA kit (Elabscience Biotechnology , China, E-EL-M0551) following the instructions of the manufacturer. The optical density was measured spectrophotometrically at a wavelength of 450 nm and ghrelin concentrations (ng/mL) were determined by comparing the optical density of the samples to the standard curve.

Statistical Analysis

The two-tailed unpaired Student's t-test was used for treatment and control groups. The normality of data distribution was determined by using Shapiro-Wilk normality test. The statistical significance was considered as $P<0.05$. Data are represented as means ±standard errors of means.

RESULTS AND DISCUSSION

Serum ghrelin concentration was increased by the administration of brotizolam compared to controls as illustrated in Fig. 1. BTZ group displayed a higher level of ghrelin than those control animals ($P<0.001$).

Brotizolam, member of the 1,4-Benzodiazepines (1,4-BZDs), use a sedative-hypnotic and the reduction of restraint stress in veterinary medicine (9). Van Reenen et al. (10) evaluated whether intravenous administration of brotizolam influence on the behavioral and physiological responsiveness of calves. Novel object test increased the time spent interacting with the stimulus. In addition, the study seems that the anxiolytic dose of brotizolam in cattle is higher than the orexigenic (i.e., appetite increasing) dose (0.2 mg/ 100 kg body weight). The studies about orexigenic effects of brotizolam revealed that a single dose injection of brotizolam (Mederantil®, Boehringer Ingelheim Ltd., UK) returned to a normal intake of milk in calf within 30 minutes (11,12). Brotizolam has potential as a treatment for loss of appetite according to the Moloney et al. (13). It provides a temporary stimulation of especially intake concentrate diet by healthy cattle and sheep. In addition, brotizolam stimulate food intake in lambs with intestinal parasitism (14). Brotizolam is successfully stimulates appetite through blocking satiety signals on the ventromedial hypothalamus (15).

Ghrelin is a 28-amino acid peptide hormone and ligand for the growth hormone secretagogue receptor (GSH-R1a). It produced from the oxyntic cells of the stomach. Its function

is the regulation of appetite, feed intake, meal initiator and stimulate gastric motility (16-20). The highly of ghrelin-responsive cells is identified in the hypothalamus (21). Plasma ghrelin levels increase in cases of energy deficiency and activate Arcuate nucleus (ARC) neurons via acting on the growth hormone secretagogue receptor (GHSR). Hypothalamic pathways control food intake depending on energy balance conditions. GHSR is the main regulator of this phenomenon (22).

Subcutaneous injection of ghrelin has been induced appetite and increased food intake in healthy volunteers (23). Circulating ghrelin levels are decreased in obesity and caloric diet intake but increased by fasting in humans and cachectic patients with anorexia nervosa (24-27). Olszewski et al. (28) suggests that the lateral hypothalamus is one of the sites that mediate orexigenic properties of ghrelin and as part of larger central circuitry, integrates orexigenic properties of ghrelin. The ARC is a key hypothalamic nucleus in the regulation of appetite. Destruction of distinct hypothalamic regions, particularly the ventromedial nucleus, stimulates appetite and induces hyperphagia. In contrast, destruction in the lateral hypothalamus reduces food intake and induce anorexia (24,29,30).

The results of the present study indicated a significant increase in ghrelin levels after brotizolam injection (Fig. 1). As a much more mechanistic view, stimulating appetite by increasing ghrelin levels with brotizolam application may be generated from the blocking the activity of the ventromedial hypothalamus (VMH) or activating lateral hypothalamus.

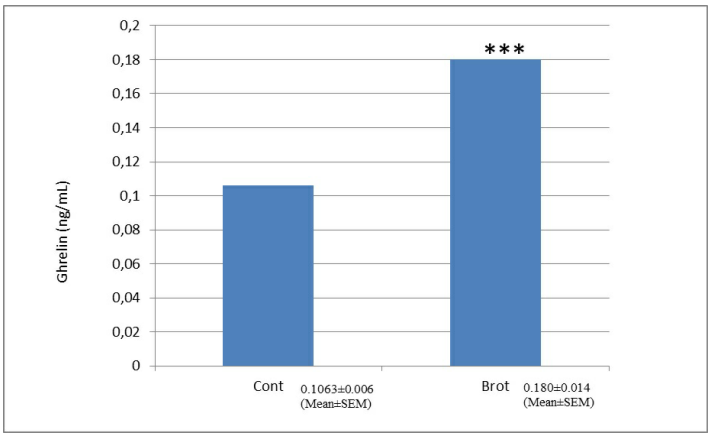

Figure 1. The administration of brotizolam increased ghrelin concentrations. Asterisk (***) indicates the statistical significance (P<0.001) versus control group.

In conclusion, the present study demonstrated that the levels of circulating ghrelin increased by the administration of brotizolam. Therefore, ghrelin could be used as an endocrine marker in the studies of induced of appetite.

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Conflict of Interest

The authors declare that they have no conflict of interests regarding content of this article.

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Ethical Declaration

Ethical approval was obtained from Local Ethics and Animal Care Committee of Mustafa Kemal University with date 18/02/2016 and number 2016/1-6, and Helsinki Declaration rules were followed to conduct this study.

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