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Central injection of CDP-choline suppresses serum ghrelin levels while increasing serum leptin levels in rats

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ABSTRACT

In this study we aimed to test central administration of CDP-choline on serum ghrelin, leptin, glucose and corticosterone levels in rats.

Intracerebroventricular (i.c.v.) 0.5, 1.0 and 2.0 μmol CDP-choline and saline were administered to male Wistar-Albino rats. For the measurement of serum leptin and ghrelin levels, blood samples were obtained baseline and at 5, 15, 30, 60 and 120 min following i.c.v. CDP-choline injection. Equimolar doses of i.c.v. choline (1.0 μmol) and cytidine (1.0 μmol) were administered and measurements were repeated throughout the second round of the experiment. Atropine (10 μg) and mecamylamine (50 μg) were injected intracerebroventricularly prior to CDP-choline and measurements repeated in the third round of the experiment. After 1 μmol CDP-choline injection, serum ghrelin levels were suppressed significantly at 60 min ($P=0.025$), whereas serum leptin levels were increased at 60 and 120 min ($P=0.012$ and $P=0.017$ respectively). CDP-choline injections also induced a dose- and time-dependent increase in serum glucose and corticosterone levels. The effect of choline on serum leptin and ghrelin levels was similar with CDP-choline while no effect was seen with cytidine. Suppression of serum ghrelin levels was eliminated through mecamylamine pretreatment while a rise in leptin was prevented by both atropine and mecamylamine pretreatments.

In conclusion; centrally injected CDP-choline suppressed serum ghrelin levels while increasing serum leptin levels. The observed effects following receptor antagonist treatment suggest that nicotinic receptors play a role in suppression of serum ghrelin levels,whereas nicotinic and muscarinic receptors both play a part in the increase of serum leptin levels.

1. Introduction

Appetite is regulated by a complex system of central and peripheral signals. Satiety signals from the gastrointestinal tract act through the arcuate nucleus of the hypothalamus and solitary tract nucleus of the brain system. Ghrelin, a potent gut–brain orexigenic peptide, has a role in the stimulation of food intake and long term regulation of body weight. Ghrelin regulates, in an antagonistic manner to leptin, the synthesis and secretion of several neuropeptides in the hypothalamus that regulate feeding and energy balance (Näslund and Hellström, 2007).

CDP-choline is an endogenously-synthesized nucleotide that exerts numerous cellular actions in different experimental models. Exogenous administration of CDP-choline has been shown to affect brain metabolism and exhibit cardiovascular, respiratory, neuroendocrine and neuroprotective actions and has beneficial effects in the treatment of some neurodegenerative and neurovascular disease (Weiss, 1995, Dávalos and Secades, 2011, Topuz et al., 2014, Hurtado et al., 2011). Administered orally, intravenously or intracerebroventricularly (i.c.v.), CDP-choline is rapidly metabolized to choline and cytidine/uridine which results in the elevation of plasma and tissue levels of these metabolites (Lopez et al., 1987, Wurtman et al., 2000). Treatments that raise plasma and tissue choline levels increase the synthesis of acetylcholine and enhance cholinergic transmission (Cohen and Wurtman, 1976, Ulus et al., 1989). Results of studies which investigate the role of the cholinergic system and vagus on serum ghrelin levels were controversial. There is also little data about the role of cholinergic system on serum leptin levels. In a previous study, it has been shown that appetite ratings decline significantly after CDP-choline treatment (Killgore et al., 2010). But there is no data on whether

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there are any alterations in serum ghrelin and leptin levels, or if these alterations are involved in the effect of CDP-choline on appetite. The dense cholinergic innervation of the hypothalamopituitary system and the presence of cholinergic receptors in this area are very well known.When the dense cholinergic innervation of hypothalamus, which has an important role in feeding control (Mason, 1985, Michels et al., 1986), and the effect of vagal stimulus on feeding are taken into consideration, CDP-choline treatment could have an effect on serum ghrelin and leptin levels.

Therefore, the present study was designed to determine the effect of centrally injected CDP-choline on serum ghrelin and leptin levels and the involvement of central cholinergic receptors that mediate the effect of CDP-choline. We also aimed to measure serum glucose and corticosterone levels that can interact with serum concentrations of these peptides after central CDP-choline injection.

2. Materials and methods

Male Wistar-Albino rats (300–350 g; Experimental Animal Breeding and Research Center, Uludag University Medical Faculty, Bursa, Turkey) were housed under a 12 h light/dark cycle with free access to food and water. The surgical and experimental protocols were approved by the Animal Care and Use Committee of Uludag University and are in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

2.1. Surgical procedures

Animals were allowed to acclimate to the animal care facility for 7 days before the experiment. Following the acclimatization period, rats were anesthetized with ether. The left carotid artery was cannulated with PE-50 tubing filled with heparinized saline (250 U/ml) and the cannulas were exteriorized at the nape of the neck and sealed until use. For i.c.v. drug administration, a 21-gauge stainless steel guide cannula was implanted in the right lateral ventricle through a burr hole drilled into the skull. The tip of the guide cannula was positioned 1.5 mm lateral to the midline, 1.0 mm posterior to bregma and 4.5 mm below the skull surface. The cannula was fixed to the skull with acrylic cement. At the end of the surgical procedure, rats were housed in individual cages and allowed to recover from anesthesia for 4–5 h. During this period, rats did not exhibit any sign of pain.

2.2. Experimental procedures

In the first set of experiments, dose and time courses of serum ghrelin, leptin, corticosterone, and glucose responses to i.c.v. CDP-choline were studied. Rats were randomized to four groups and various doses of CDP-choline 0.5 ($n=14$), 1.0 ($n=16$), 2.0 ($n=16$) μmol and 5 μl ($n=16$) saline (0.9% NaCL) were administered. Blood samples (0.5 ml) were taken just before the i.c.v. injection (0 min) of CDP-choline or saline and at 5, 15, 30, 60 and 120 min following administration. Each set of animals was used for the three time intervals and each blood sample replaced with equal volume of saline. Blood glucose was detected immediately from samples by a commercially available glucometer using test strips. Blood samples were centrifuged at 3000 g for 15 min at 4 °C and serum samples were separated and stored at −20 °C until analysis. Rats were killed with high dose ether anesthesia after the first set of experiments.

In the second set of experiments, in order to determine the effects of hydrolysis product of CDP-choline, choline, and cytidine, on serum ghrelin, leptin and glucose responses, equimolar dose of choline (1.0 μmol), cytidine (1.0 μmol) or saline (5 μl) was injected i.c.v.

In the third set of experiments, the effects of the blockade of central muscarinic or nicotinic acetylcholine receptors on serum ghrelin, leptin and glucose responses were investigated. Rats were pretreated i.c.v. with atropine sulfate (10 μg), mecamylamine HCL (50 μg) or saline (5 μl) 15 min before i.c.v. injection of CDP-choline (1.0 μmol).

In the second and third sets of experiments, surgical procedures were performed as described in the first set of experiments. Blood samples (0.5 ml) were taken just before the i.c.v. injections and at 60 and 120 min following the treatments. Time intervals were assessed according to the maximum effect of CDP-choline on serum ghrelin and leptin levels were observed in the first set of experiments. Each blood sample was replaced with equal volume of saline. Blood glucose was detected immediately from samples by a commercially available glucometer using test strips. Blood samples were centrifuged at 3000 g for 15 min at 4 °C and serum samples were separated and stored at −20 °C until analysis. Rats were killed with high dose ether anesthesia after the completion of experiments.

2.3. Drugs

The following drugs were used: CDP-choline, choline, cytidine, atropine sulfate, mecamylamine HCL (Sigma, St Louis, MO, USA). Drugs were dissolved in saline (0.9% NaCl).

2.4. Measurements

Serum ghrelin, leptin and corticosterone levels were measured by using commercially available RIA kits (Millipore's Ghrelin [Total] Radioimmunoassay Kit, MO, ABD, sensitivity 93 pg/ml), leptin (Linco's Rat Leptin Radioimmunoassay Kit, MO, ABD, sensitivity 0.5 ng/ml) and corticosterone (ImmuChem Double Antibody Corticosterone RIA Kit, Orangeburg, NY, sensitivity 7.7 ng/ml).

2.5. Data and statistical analysis

Comparisons between groups were performed using Student's *t*-test for normally distributed variables, and Mann–Whitney *U*-test for non-normal variables. Paired *t* test and Wilcoxon signed-rank test were used for within-group comparisons. The data were analyzed using SPSS for Windows, Version 11.5 and shown as mean ± standard deviation. A *P* value of less than 0.05 was considered statistically significant.

3. Results

3.1. Effects of intracerebroventricularly injected CDP-choline on serum ghrelin, leptin, glucose and corticosterone levels

There was no difference between basal serum ghrelin and leptin levels in the groups. Serum ghrelin levels were suppressed significantly at 60 min following 1.0 μmol CDP-choline i.c.v. injection ($P=0.025$) (Fig. 1). When area under the curve (AUC) was calculated, serum ghrelin levels were found significantly suppressed after 0.5 μmol (AUC = 160 ± 11.4 pg dk/ml) and 1.0 μmol (AUC = 147.5 ± 11.1 pg dk/ml) of i.c.v. CDP-choline injection compared with the saline group (AUC = 190 ± 18 pg dk/ml) ($P=0.035$ and P = 0.013, respectively).

Administration of 0.5, 1.0 and 2.0 μmol dose of i.c.v. CDP-choline significantly increased serum leptin levels at 60 min ($P=0.036$, $P=0.012$ and $P=0.043$; respectively) compared with basal values and a significant increase was also found at 120 min following 1.0 and 2.0 μmol dose of i.c.v. CDP-choline injection