Leptin, 16- kDa protein produced and secreted from white adipocytes is known to regulate food intake and energy expenditure. Leptin receptors have been detected in the pancreas and it has been shown that systemic application of this protein diminished postprandial pancreatic secretion. Leptin is also produced in the stomach and released into the gastrointestinal lumen but the implication of luminal leptin in the regulation of pancreatic enzyme secretion has not been elucidated. The aim of our study was to evaluate the effects of intraduodenal (i.d.) leptin administration on pancreatic enzyme secretion and to assess the involvment of afferent nerves and CCK in above effects. The secretory studies were carried out on anaesthetized Wistar rats with acute pancreatic fistulae. Leptin was administered to the animals at doses of 0.1 1.0 or 10.0 µg/kg i.d. Tarazepide (2.5 mg/kg i.d.), a CCK, receptor antagonist, was given to the rats prior to the application of leptin. Rats with capsaicin deactivated sensory nerves were used in part of the study. Samples of pancreatic juice were taken at 15 min intervals to measure the volume flow and protein and amylase concentrations. CCK plasma level was measured by radioimmunoassay (RIA) following administration of leptin to the rats. Intraduodenal administration of leptin (1.0 or 10.0 µg/kg) to the fasted rats significanly and dose-dependently increased pancreatic protein and amylase outputs. Pancreatic secretory responses to leptin were totally abolished by prior capsaicin deactivation of sensory nerves or by pretreatment of the rats with tarazepide. Under basal conditions plasma CCK level averaged about 15.46 ± 1.4 pg/ml. Exogenous leptin, given i.d. at doses of 0.1 1.0 or 10.0 µg/kg i.d. to the rats with intact or capsaicin-deactivated sensory nerves resulted in dose-dependent rise of plasma CCK level, reaching the highest value at the dose of 10.0 µg/kg i.d. We conclude that leptin given i.d. stimulates pancreatic enzyme secretion and this effect could be related to the stimulation of CCK release and activation of duodeno-pancreatic reflexes.

Key words: leptin, pancreatic enzyme secretion, CCK plasma level, sensory nerves
INTRODUCTION

Leptin, 16 kDa product of the ob gene, discovered in 1994 by Zhang et al (1) is known to regulate food intake, energy expenditure and body weight homeostasis (2, 3). Leptin is produced and secreted mainly by the white adipocytes, but recent studies have shown that other tissues and organs like muscles, stomach and reproductive tract could be also the source of leptin (4-6). The biological effects of leptin are exerted via specific leptin receptors which have been detected in gastric mucosa, small intestine, liver, as well as in the pancreatic β-cells and pancreatic acini and afferent nerves (6-11). Despite of the presence of leptin in the gastrointestinal lumen the role of luminal leptin is largely unknown.

Leptin has been shown to protect the gastric mucosa and the pancreas against acute lesions, and above beneficial effects of leptin could be related to the activation of afferent nerves, generation of nitric oxide (NO) and modulation of cytokine production (12-24).

Leptin receptors have been detected on pancreatic AR42J cells and their gene expression has been documented in pancreatic acini, suggesting that this protein could take a part in the regulation of pancreatic exocrine secretion (9). On the other hand the presence of leptin receptors on the pancreatic β-cells suggests that leptin could be involved in the regulation of pancreatic endocrine function as well (8, 10, 11).

The effects of leptin on pancreatic enzyme secretion have been the subject of recent studies, but contradictory results have been obtained. Leptin has been shown to either stimulate pancreatic enzyme secretion, or to diminish the pancreatic exocrine functions (13, 19, 20).

Although leptin has been shown to be produced in the stomach by fundic epithelium and released in response to food or to CCK (6,12), the implication of luminal leptin to the regulation of pancreatic enzyme secretion remains unclear.

The aim of present study were: 1) to investigate the effects of intraduodenal (i.d.), application of low doses of exogenous leptin on pancreatic enzyme secretion, in the anaesthetized rats with pancreato-biliary fistulas and 2) to assess the involvement of CCK and sensory nerves in the effect of luminal leptin on pancreatic exocrine secretory function.

MATERIAL AND METHODS

Following items were purchased: leptin and capsaicin was from Sigma Co (St. Louis, MO, USA). Specific CCK, receptor antagonist; tarazepide was the kind gift from Solvay Co (Solvay, Hannover, Germany), CCK radioimmunoassay commercial kit was from DRG International Inc. (Mountainside, WI, USA). Amylase reagent was purchased from Dialab Diagnostic Ges. MBH, (Wien, Austria). Morbital (Pentobarbitalum) was from BIOWET (Pulawy, Poland). PE10 and PE50 polyethylene tubing was purchased from Beckton Dickinson (Sparks, MD, USA).
In vivo study:

The experimental studies have been approved by the Jagiellonian University Ethical Committee for Animal Experimentation.

The study was performed on male Wistar rats weighing 300-350 g. Animals were housed at 24 hours light/dark cycle at constant temperature with free access to laboratory chow and water. Rats were deprived from food for 24 h before the experiment. The surgery was performed under pentobarbiturate anaesthesia (Morbital), given intraperitoneally (i.p.) at a dose of 15 mg/kg. The anaesthesia was maintained during the experiment by i.p. pentobarbiturate administration every 2 h as needed.

Following midline laparotomy, the duodenum was identified and the bile-pancreatic duct was isolated at its entrance to the duodenum. A polyethylene tube (PE10) was inserted into the common bile-pancreatic duct for bile and pancreatic juice collection. A second polyethylene cannula (PE10) was placed into the duodenum and its tip fixed proximal to the ampulla for reinfusion of previously harvested bile-pancreatic juice to maintain feedback control of pancreatic secretion (after dilution with saline 1:2). The abdominal wound was sutured with a double layer suture and the rats were kept under the heating lamps to maintain the right body temperature (37°C). At the end of experiment, the abdominal vena cava was exposed and the blood was withdrawn into EDTA containing tubes for determination of plasma CCK by radioimmunoassay.

During the experiments the animals were placed in individual Bollmann cages. The bile-pancreatic juice (BPJ) samples were collected in small preweighted vials in 15 min aliquots to measure the volume of each sample and protein and amylase concentrations. Basal secretion of pancreatic juice was measured by collecting of BPJ for 60 min to allow for stabilization of flow affected by surgical manipulation. Protein and amylase concentrations of each sample were measured by enzymatic method, as described previously (21). The results were expressed as total protein (mg/15 min) and amylase (IU per 15 min) outputs. During the experiments previously collected bile-pancreatic juice was re-infused via duodenal cannula into duodenum at the rate of 1 ml/h.

Experimental protocol:

The study consisted of three parts. In part I the effect of leptin alone on basal pancreatic secretion was studied. Part II of the study involved rats with sensory nerves deactivated with neurotoxic dose of capsaicin applied 2 weeks before the study. In the part III of the study, the CCK₁ receptor antagonist tarazepide was used. For each part of the study, the groups of 5-6 rats were used. Leptin was dissolved in 0.5 ml of saline and administered i.d. into the rats in each test.

Part I

The following experiments, with BPJ returned to the duodenum throughout the experiment were performed: 1. Control (i.d. bolus injection of vehicle saline); 2. Leptin dissolved in saline given i.d. at doses of 0.1 1.0 or 10.0 µg/kg. Each dose of leptin was administered to the separate group of the animals.

Part II

The involvement of sensory nerves in the effect of leptin on the basal pancreatic enzyme secretion was studied in the rats with sensory nerves deactivated with capsaicin. Capsaicin was given to the rats at total dose of 100.0 mg/kg 10 days before the secretory tests, as described previously (22). During the experiment BPJ was returned to the duodenum and selected dose of
Leptin (1.0 or 10.0 µg/kg) was given i.d. to the rats. In the control tests bile-pancreatic juice was returned to the duodenum but vehicle instead of leptin was applied i.d. to the animals.

Part III

The effect of leptin on BPJ secretion was investigated in rats pretreated with CCK receptor antagonist; tarazepide in following tests: 1. Control tarazepide (2.5 mg/kg) given i.d. followed 15 min later by i.d. injection of vehicle; 2. Tarazepide (2.5 mg/kg i.d.) followed 15 min later by selected doses of leptin (1.0 or 10 µg/kg i.d.)

Determination of plasma CCK radioimmunoreactivity:

Plasma CCK level was measured by radioimmunoassay (RIA) using CCK radioimmunoassay commercial kit, according to the manufacturer's protocol. CCK radioimmunoassay commercial kit detected CCK in plasma or tissue extracts. CCK was extracted from plasma by an ethanol extraction method. The lowest detectable concentration was 0.3 pmol/l. The intra-assay and inter-assay shift were 4.4-20.6 and 4.2-20.6 pmol/L respectively.

Statistical analysis:

Comparison of the differences between the mean values of various groups of experiments were made by analysis of variance and the Student's t test for unpaired data. Differences with a p value of < 0.05 were considered statistically significant. Results are expressed as means ± SEM.
RESULTS

Pancreatic secretion in vivo:

In anaesthetized rats with acute pancreatic fistula and with BPJ returned to duodenum, the basal protein and amylase output were well sustained and averaged about 4.55 ± 0.52 mg/15 min or 430.0 ± 46.0 IU/15 min, respectively (Fig. 1). Leptin at dose of 0.1 µg/kg i.d. failed to affect significantly basal pancreatic secretion. In contrast, leptin at doses of 1.0 or 10.0 µg/kg i.d. markedly increased protein and amylase outputs. Highest stimulation of pancreatic protein and amylase secretion was observed following application of leptin at dose 1.0 µg/kg. This dose of leptin produced the rise of protein and amylase up to 8.42 ± 0.8 mg/15 min and 1480.0 ± 135.0 IU/15 min, respectively (Figs. 1, 2). For comparison, pancreatic protein or amylase secretions in response to exogenous CCK (1 µg/kg i.p.) were 25 ± 1.8 mg / 15 min, or 3.050 ± 250 IU/15 respectively.

The increases of protein or amylase secretion produced by leptin given at doses of 1.0 or 10.0 µg/kg i.d. were completely abolished by previous deactivation of sensory nerves with capsaicin (Figs. 3, 4). Pretreatment with

![Fig. 2. Time course of pancreatic amylase secretion in response to increasing doses of leptin (0.1 1.0 or 10.0 µg/kg i.d.) administered to the animals at 60 min. Control - amylase secretion in rats injected with vehicle instead of leptin. The results are means ± SEM of separate experiments, each performed on 5-6 animals. Asterik (*) indicates significant (p< 0.05) increase above the control value.](image-url)
CCK₁ receptor antagonist tarazepide also completely reversed the stimulatory effect of leptin (1.0 or 10.0 µg/kg i.d.) on pancreatic protein or amylase secretions (Figs. 3, 4).

Fig. 3. Effect of selected doses of leptin (1.0 or 10.0 µg/kg i.d.) on protein output in the rats with intact or capsaicin-deactivated (CD) sensory nerves and in the rats pretreated with CCK₁ receptor antagonist; tarazepide (TA). Results are means ± SEM from 5 separate tests. Asterik (*) indicates significant (p< 0.05) increase above the control value.

Fig. 4. Effect of selected doses of leptin (1.0 or 10.0 µg/kg i.d.) on amylase secretion in the rats with intact or capsaicin-deactivated sensory nerves (CD) and in the rats pretreated with CCK₁ receptor antagonist, tarazepide (TA). Results are means ± SEM from 5 separate tests. Asterik (*) indicates significant (p< 0.05) increase above the control value.
Plasma CCK level:

Under basal conditions plasma CCK level averaged about 15.46 ± 1.4 pg/ml (Fig. 5). Administration of exogenous leptin, given i.d. at doses of 0.1, 1.0 or 10.0 µg/kg resulted in the dose-dependent rise of CCK plasma concentration, reaching the highest level at the dose of 10.0 µg/kg i.d. of leptin (Fig. 5). Deactivation of sensory nerves as well as administration of tarazepide failed to affect significantly the CCK release stimulated by luminal leptin (Fig. 6).

DISCUSSION

The present study demonstrates that: 1) exogenous leptin given into the duodenal lumen stimulates basal pancreatic enzyme secretion in the anaesthetized rats, 2) topical administration of exogenous leptin to intestinal mucosa results in dose-dependent rise of plasma CCK immunoreactivity and 3) deactivation of sensory nerve fibres with neurotoxic dose of capsaicin or pretreatment of the rats with CCK receptor antagonist tarazepide completely abolished the stimulatory effect of luminal leptin on pancreatic exocrine secretion.

To determine the mechanism of the stimulatory effect of leptin on exocrine pancreas we have investigated the possible role of CCK, which is the major and well-known gastrointestinal pancreatic secretagogue. According to Li and
Owyang (27) low, physiological doses of CCK stimulate the pancreatic enzyme secretion mainly via neural mechanism depending on the activation of vagal afferent fibres. Our study demonstrates that intraduodenal administration of leptin results in a dose dependent rise of plasma CCK level. The involvement of CCK in the stimulatory effect of luminal leptin on exocrine pancreas was confirmed by the observation that the blockade of CCK receptors by tarazepide completely abolished the stimulatory effect of luminal leptin on exocrine pancreas. To determine the role of neural reflex pathways in the stimulation of pancreatic enzyme secretion by luminal leptin we assessed the involvement of sensory nerves in the above stimulation. Deactivation of sensory fibres with capsaicin completely abolished the increase of pancreatic protein and amylase outputs produced by luminal leptin. This observation suggests that the increase of pancreatic enzyme secretion induced by leptin depends on the activation of sensory nerves.

Leptin has been previously detected in the stomach and released into the gastric lumen (6). The major question remains whether the amount of leptin in the gut originating from the stomach is sufficient to activate CCK release and/or sensory nerves. The answer to this question requires further studies.

Previous reports have shown that parenteral application of leptin resulted in the significant inhibition of pancreatic enzyme secretion (19, 20). These observations are in disagreement with the results of our present study. Matyjek et al administered leptin intravenously to anaesthetized animals and such application of leptin at doses ranging from 0.1 to 10 µg/kg resulted in dose-

![CCK plasma level](image)

Fig 6. Plasma CCK level in response to selected doses of leptin, given i.d. (0.1, 1.0 or 10.0 µg/kg) in the rats pretreated with CCK-1 receptor antagonist, tarazepide (TA). Results are means ± SEM from 8 separate tests. Asterik (*) indicates significant (p<0.05) increase above the control value.
dependent inhibition of pancreatic protein and trypsin outputs (20). However, in that study the rate of pancreatic secretion was very low and neither plasma leptin nor CCK levels have been measured (20). Also in our previously published report leptin was given to the rats as a bolus intraperitoneal injection resulting in the significant reduction of pancreatic exocrine function, but the rate of pancreatic secretion was rather low (19). This reduction was most pronounced with 10 µg/kg dose of leptin, whereas higher doses of leptin i.p. were less effective in the inhibition of pancreatic secretory function (19). It is likely that leptin exerts biphasic effect on pancreatic protein secretion; inhibitory at lower doses and stimulatory at higher doses. Furthermore, secretory efficacy of leptin may depend on the route of administration. As was shown in this report leptin given into the duodenal lumen acts locally to stimulate CCK release and to activate enteropancreatic reflex stimulation of exocrine pancreas. Our present data confirm the results reported by Guilmeau et al (13, 26). They have demonstrated that high doses of exogenous leptin given i.v. are able to increase basal as well as CCK- stimulated exocrine pancreatic secretion in rats with acute pancreatic fistulae. Furthermore they demonstrated that duodenal infusion of leptin to the rats increased plasma CCK levels and the values of plasma CCK were comparable to those induced by feeding (26). It is not clear, whether the doses of leptin used in this study raised plasma leptin to physiological levels such as observed postprandially or whether they represented purely pharmacological doses of this protein.

In summary: our study demonstrates that in anaesthetized rats low doses of exogenous leptin given intraduodenally are able to stimulate the secretion of pancreatic juice protein and amylase via the mechanism depending on CCK release and activation of afferent nerves fibres, suggesting the involvement of neural reflex pathway in this process.

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REFERENCES


