The aim of present study was to evaluate the influence of surgical fundectomy and exogenous leptin on the secretion of pancreatic juice in anesthetized rats. In male Wistar rats major part of the gastric fundus was surgically removed, and 60 days afterwards the external jugular vein and the pancreatic-biliary duct were catheterized under general anesthesia. The pancreatic-biliary juice (PBJ) was collected in 15 min intervals without introducing it into the duodenum. Intravenous leptin infusions (0.1, 1.0 and 10 µg/kg body weight) were made every 30 min. The same protocol was used in control non operated rats. The PBJ volume was significantly lower in fundectomized rats as compared to control rats and showed no significant effect to exogenous leptin. The PBJ protein output but not trypsin activity was lower in fundectomized rats as compared to control. Leptin reduced the PBJ protein and trypsin outputs in a dose-related manner in the control and experimental group. The inhibition was, however, more evident in the fundectomized rats. Plasma gastrin was higher in fundectomized rats, while plasma leptin and ghrelin were lower. In conclusion, fundectomy seems to reduce the non stimulated pancreatic secretion and modifies the response to leptin in anesthetized rats.

Key words: pancreatic protein secretion, leptin, ghrelin

INTRODUCTION

Gastric mucosa is known to be a rich source of gastrointestinal regulatory peptides and hormones including gastrin, substance P, somatostatin and gastrin releasing peptide (GRP) (1). Recently, it was shown that leptin (2) and ghrelin (3) are produced in the stomach mucosa, mostly in the fundus region. Leptin was
first discovered as a protein product of ob gene secreted into the blood circulation by the fat tissue adipocytes (4). More recently, however, leptin was demonstrated in the chief cells and endocrine P cells located in the lower half of fundic glands, and following stimulation leptin was released into the gastric lumen and blood circulation (2, 5). Accordingly, leptin mRNA levels in the stomach decreased in fasting conditions and increased shortly following stimulation by food. Leptin seems to be resistant against the degradation in the gastric lumen (6) what enables it's action in the small intestine (7), however, it could also enter into the stomach circulation to act locally on gastric mucosa and generally on distant organs (8).

Ghrelin is synthesized by oxyntic mucosa cells that secrete hydrochloric acid, and its concentration decreases toward the small intestine in rat (3, 9). Besides many other biological effects, leptin (10) as well as ghrelin (11) were found to inhibit pancreatic secretion in anesthetized rats. Exogenous leptin was shown to inhibit the unstimulated and CCK-8 stimulated output of pancreatic proteins and enzymes through a local neurohormonal mechanism mediated by CCK and vagal nerves (10), but the contribution of gastric leptin relative to adipose tissue leptin and the other leptin pools in the body to control the exocrine pancreas remains unclear.

The aim of study was to evaluate the secretion of rat pancreatic juice following the surgical removal of the major part of gastric fundus (12), a major source of gastric leptin and ghrelin. This paper describes the effect of fundectomy on the unstimulated basal pancreatic secretion and the secretion inhibited by exogenous leptin in anesthetized rat model.

**MATERIAL AND METHODS**

Animal studies have been approved by the Local Ethical Committee. Total, 32 Wistar rats weighing 250 ± 10 g were used. Animals were housed in a light and temperature-controlled room with free access to laboratory food and tap water. The night before fundectomy, the rats received a half of their daily food intake. The surgery comprising of partial removal of the gastric fundus was performed in 16 rats under the general anaesthesia (atropine sulfate, sc, 0.15 mg/kg body weight, b. wt, Polfa, Poland; 2% xylasine, sc, 3 mg/kg b. wt, Rometar, PPHU INEX, Poland; and ketamine, sc, 25 mg/kg b. wt, Bioketan, Biowet, Poland) supported by local anesthesia with lidocaine (0.003 mg per stomach, Polfa, Poland) injected into the gastric fundic tissue. A major portion of the gastric fundus was cut off thereby preserving a small portion on the smaller curvature (Fig. 1). The remaining stomach was sutured with the two layers of nonabsorbable suture and laparotomy was closed. Antibiotics (Amoxycillin sc, 150 mg/kg b. wt, Betamox, Biowet, Poland) were administered intramuscularly after the surgery and repeated after 48 hours. Rats received only little water with glucose on the first postoperative day. After the surgery, rats gradually returned to normal ratios of feed and water within a week and started to gain their body weight.

In 8 fundectomized rats (320 ± 15 g b. wt., approximately 60 days after fundectomy) as well as in 8 control, untreated rats (320 ± 20 g b. wt.) the external jugular vein and the biliary-pancreatic duct were catheterized under mixed azaperone (12 mg kg⁻¹, Stresnil, Janssen, Belgium) and ketamine (35 mg kg⁻¹, Bioketan) general anaesthesia. The right external jugular vein was prepared,
and a silicone tubing was inserted and fixed with ligatures. Continuous intravenous infusion of saline (0.9% NaCl, peristaltic pump speed 2 ml/h) started immediately after the cannulation to replace the fluid loss, and it was continued until the end of experiment. Following the midline laparotomy, a polyethylene tubing was inserted into the common pancreatico-biliary duct for collection of bile and pancreatic juice (PBJ). Collection of PBJ started immediately after completing the cannulation and the experimental protocol started 30 min thereafter. PBJ was collected in 15 min intervals into 1.5 ml polyethylene tubing kept in an ice-cold bath, no PBJ was infused into the duodenum. Three leptin boluses (0.1, 1.0, 10 µg/kg b. wt., Leptin mouse recombinant, Sigma-Aldrich, St. Louis, USA) were given intravenously every 30 min in 0.5 ml of 0.9% NaCl as vehicle. After experiments the animals were killed by intravenous thiopental (Biochemie GmbH, Kundl, Austria) overdose. The PBJ samples were checked for their weight and 0.1 ml stored at -20 C for further analyses. Samples were analyzed for total protein using the Lowry method, performed on 96-well microwell plates with bovine serum albumin (Sigma-Aldrich, USA) as standard. Intra- and inter-assay for the protein determination were 3.1 and 3.6%, respectively. Trypsin (EC 3.4.21.4) activities were estimated according to a modified Erlanger method (13) after activation with enterokinase (Sigma) and using N-alpha-benzoyl-DL-arginine-p-nitroanilide (Sigma) as a substrate. Intra- and inter-assay for the trypsin determination were 2.8 and 3.2%, respectively.

Blood samples were withdrawn during the interdigestive period from the control (n=8) and fundectomized (n=8) rats. Blood plasma was measured radioimmunologically for the concentration of gastrin (EURIA-Gastrin, Eurodiagnostica, Denmark), leptin (RIA-kit Leptin Rat, Linco, USA) and ghrelin (Ghrelin Total RIA-kit, Linco). The respective assays were performed in triplicates in one run.

Results were calculated as means and standard errors of mean (SEM). A one-way variance analysis for repeated measures followed by a Tukey post-test, linear trend analysis, and t-test were performed (GraphPad Prism v.4.0, Graph Pad Software, San Diego, CA, USA) to investigate the effect of leptin dose and the differences between the control and fundectomized rats, respectively. A value of P < 0.05 was considered statistically significant.

RESULTS

In anaesthetized rats, the volume of secreted PBJ was significantly lower in fundectomized rats as compared to control non-operated rats. The infusion of saline iv as vehicle did not affect the PBJ volume flow, protein and trypsin outputs in both groups of rats. Infusions of leptin had no effect on PBJ volume in fundectomized rats though in control rats it showed a tendency toward the
reduction (Fig. 2). Leptin does not affect bile flow (14) thus changes in PBJ flow concern the secretion of pancreatic juice. Leptin reduced the PBJ protein and trypsin outputs (Fig. 2) in a dose-related manner in the control and fundectomized rats. The effect was more evident in the fundectomized rats, however, the inhibition was seen after 15 min whereas in the control rats it was evident during the first 15 min post-infusion period (Fig. 3).

In rats before the fundectomy, the interdigestive concentration of plasma leptin was 2.29 ± 0.25 ng/ml, whereas 2 months after the fundectomy it was 2.66 ± 0.11 ng/ml. In contrast, in the control non operated rats of similar weight it was 3.57 ± 0.15 ng/ml (fundectomized vs. non-operated P<0.01). Two months after the fundectomy, the concentration of plasma ghrelin was 0.25 ± 0.03 ng/ml versus 7.71 ± 0.3 ng/ml in the control non operated rats (P<0.001). The most dramatic reduction in plasma ghrelin was observed within the first 6 weeks after the fundectomy. The interdigestive concentration of plasma gastrin in rats gradually rose following fundectomy, thereby being 523 ± 29 pmol/l two months after the fundectomy versus 146 ± 9 pmol/l in the control non operated rats.

DISCUSSION

In the present study, surgical removal of a major part of the gastric fundus was shown to markedly reduce the PBJ volume and pancreatic protein output
in anaesthetized rats as well as to augment the inhibitory effect of exogenous leptin.

Earlier studies on dogs showed that total removal of neural supply to the pancreas by its autotransplantation, reduced the secretion of non-stimulated and stimulated pancreatic juice (15). In the present rats, the smaller curvature of the stomach was left untouched thus the vagal supply to the pancreas was presumably not disrupted whereas any gastro-pancreatic reflexes that originate from the fundus (16) were apparently eliminated. Surgical fundectomy was employed in order to remove a major part of circulating gastric leptin pool. It resulted in a reduction of plasma leptin in the interdigestive conditions by about 25 % what should be considered a substantial contribution into the total pool of circulating leptin. A steady increase in plasma gastrin was the result of substantial reduction of gastric acid secretion in turn to fundectomy (12, 17), and a dramatic decrease in plasma ghrelin further confirmed the effectiveness of surgical fundectomy. Thus lowered PBJ volume following fundectomy could be the result of long lasting reduction of hydrochloric acid secretion in the stomach that stimulate the bile and pancreas flows through circulating secretin. The secretion of pancreatic protein was also reduced in fundectomized rats, and could be ascribed due to removal of neural gastro-pancreatic reflexes that originate in the stomach fundus. It appears that exclusion of a part of circulating leptin and most of circulating ghrelin, known for their inhibitory effect on pancreatic juice secretion (10, 11) did not reverse this effect. The effect of gastrin on the rat exocrine pancreas secretion

**Figure 3.** The dynamics of pancreatic protein and trypsin output responses to increasing doses of leptin boluses (0.1, 1.0 and 10 µg/kg body weight) as indicated by arrowheads. Values are shown as means, each point represents the secretion during 15 minutes.
is still a matter of debate; it seems doubtful in the conscious dog (18) but not in the calf (19). Pancreas hypertrophy have been found in response to chronic pentagastrin administration in rats (20) and in transgenic CCK$_2$gastrin receptor mice with CCK$_2$ receptor overexpressed in pancreatic acinar cells (21). In our study we did not see, however, the differences in the pancreas wet weight between fundectomized and control rats. It is possible also that in fundectomized rats the CCK$_2$gastrin receptor expression in adipose tissue may have changed, however, we don't provide this data. Attoub et al. (22) suggested that circulating gastrin may be involved in long term stimulation of adipocyte leptin expression and secretion mediated through a CCK$_2$ receptor in rat. Therefore, in the present fundectomy model, the elevated gastrin in the blood plasma might affect leptin production by fat tissue thereby masking a part of reduction in circulating leptin due to fundectomy. Finally, the inhibition by exogenous leptin in fundectomized rats was delayed but stronger as compared to control rats, the later may be explained by the up regulation of leptin receptor since plasma leptin was low in fundectomized rats. Concerning the delay in response, it seems possible that partial removal of the gastric fundus along with its neural supply could contribute, since it was shown previously that leptin may control pancreatic secretion through a vagal and capsaicin-sensitive pathways (11, 23). In conclusion, fundectomy seems to reduce the non stimulated pancreatic secretion and modifies the pancreatic response to exogenous leptin in anesthetized rats.

**Acknowledgments:** This work was supported by grants from the State Committee for Scientific Research (KBN, Poland, grants nr 3P06K 003 24 and 3P06D 019 24).

**REFERENCES**


Received: 27 March 2004
Accepted: 28 May 2004

Author's address: Rafał Matyjek, The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, 05-110 Jabłonna, Poland, tel.: +48 22 782 44 22, Fax.: +48 22 774 20 38. E-mail: r.matyjek@ifzz.pan.pl