J. JAWOREK, J. BONIOR, S.J.KONTUREK, J.BILSKI, A.SZLACHCIC, W.W. PAWLIK

ROLE OF LEPTIN IN THE CONTROL OF POSTPRANDIAL PANCREATIC ENZYME SECRETION.

Chair of Physiology Jagiellonian University School of Medicine, Kraków, Poland

Leptin released by adipocytes has been implicated in the control of food intake but recent detection of specific leptin receptors in the pancreas suggests that this peptide may also play some role in the modulation of pancreatic function. This study was undertaken to examine the effect of exogenous leptin on pancreatic enzyme secretion *in vitro* using isolated pancreatic acini, or *in vivo* in conscious rats with chronic pancreatic fistulae. Leptin plasma level was measured by radioimmunoassay following leptin administration to the animals. Intraperitoneal (i.p.) administration of leptin (0.1, 1, 5, 10, 20 or 50 µg/kg), failed to affect significantly basal secretion of pancreatic protein, but markedly reduced that stimulated by feeding. The strongest inhibition has been observed at dose of 10 µg/kg of leptin. Under basal conditions plasma leptin level averaged about 0.15 ± 0.04 ng/ml and was increased by feeding up to 1.8 ± 0.4 ng/ml. Administration of leptin dose-dependently augmented this plasma leptin level, reaching about 0.65 ± 0.04 ng/ml at dose of 10 µg/kg of leptin. This dose of leptin completely abolished increase of pancreatic protein output produced by ordinary feeding, sham feeding or by diversion of pancreatic juice to the exterior. Leptin (10^{-10}-10^{-7} M) also dose-dependently attenuated caerulein-induced amylase release from isolated pancreatic acini, whereas basal enzyme secretion was unaffected. We conclude that leptin could take a part in the inhibition of postprandial pancreatic secretion and this effect could be related, at least in part, to the direct action of this peptide on pancreatic acini

Key words: *Leptin, pancreatic enzyme, isolated pancreatic acini, plasma leptin level.*

INTRODUCTION

Leptin, a 167-amino acid product of *ob* gene, is a peripheral adiposity signal for the central regulation of food intake and energy homeostasis and reproduction

(1-3). Leptin is produced by adipocytes, but recent studies have shown that it could also be released from the gastric mucosa in response to feeding and cholecystokinin (1, 4-6). This peptide exerts its biological effects via specific receptors which resemble those of class-I of cytokine family (2) Leptin is involved in a protective mechanism against the aggressive effects of the immune system and it has been shown to protect the gastric mucosa against the acute damage and to prevent the pancreatic tissue damage caused by acute pancreatitis (6-10).

Leptin receptor mRNA has been demonstrated in the pancreatic β cells, suggesting that leptin could take a part in the physiological modulation of pancreatic endocrine function (11). Under normal conditions administration of leptin suppress insulin secretion from pancreatic islets (12). To the contrary, long-term treatment with leptin improves insulin release in leptin-deficient mice (13).

Recently leptin receptors has been detected in pancreatic AR42J cells and leptin receptor gene expression has been shown in the normal pancreatic acini, indicating that this hormone could be implicated in the regulation of pancreatic enzyme secretion (14, 15). The effects of leptin on pancreatic exocrine function have been the subject of recent studies performed on anesthetized rats with acute pancreatic fistulae, but the results of these studies are controversial (16, 17).

For this study we have selected the model of conscious rats with chronic pancreatic fistulae to examine the effects of leptin on exocrine pancreatic
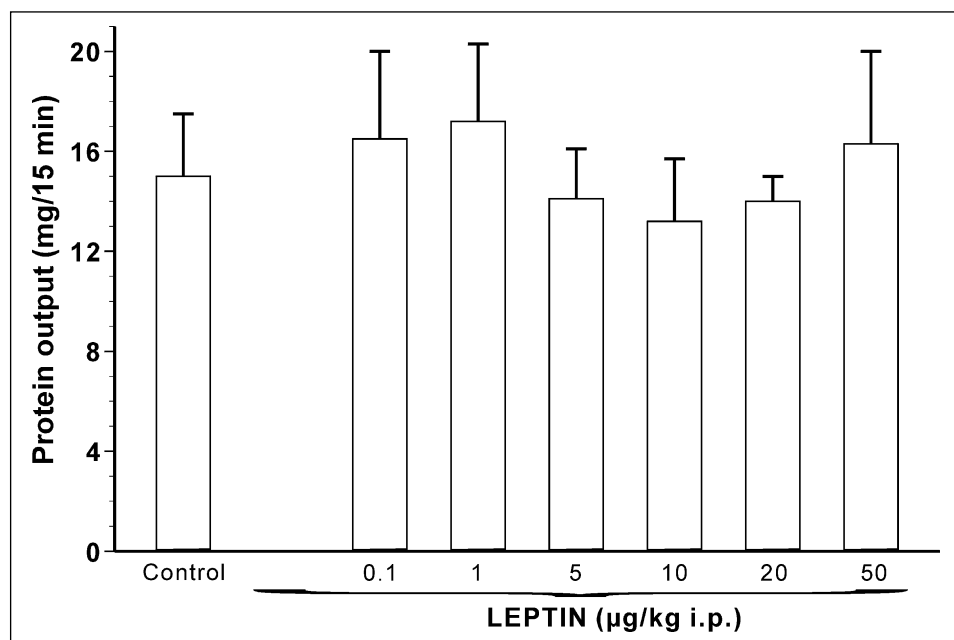

Fig. 1. Effect of increasing doses of leptin on pancreatic protein secretion in conscious rats under basal conditions. The results are the means ± SEM of separate experiments, each performed on 6-8 rats.
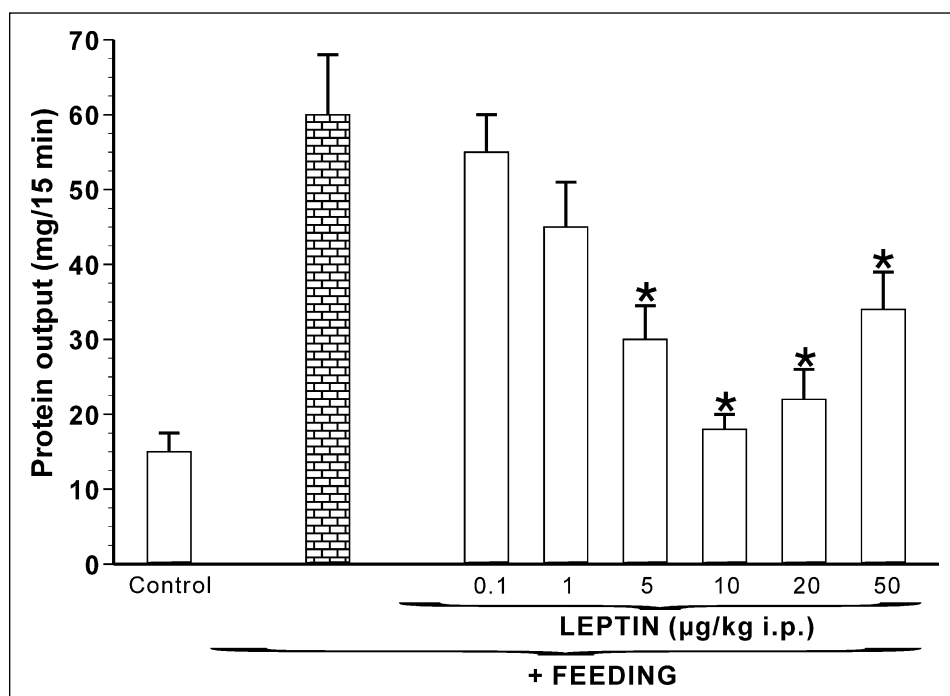
Fig. 2. Effect of increasing doses of leptin on postprandial pancreatic protein secretion in conscious rats. The results are the means ± SEM of separate experiments, each performed on 6-8 rats. Asteriks indicates significant decrease below the control.

function stimulated by sham feeding, ordinary feeding or diversion of pancreatic juice. To assess the direct influence of leptin substance on pancreatic enzyme secretion we have used isolated pancreatic acini for *in vitro* experiments.

MATERIAL AND METHODS

Following items were purchased: caerulein (Takus) from Pharmacia GmbH, Erlangen, Germany, leptin, urecholine and trypsin inhibitor from Sigma Co (St. Louis, MO, USA), essential and nonessential amino acid mixture from Serva Feinbiochemica (GmbH, Heidelberg, Germanty), purified collagenase CLSPA from Worthigton Biochemica Co.(Freehold, N.J., USA). Amylase reagent, was purchased from Dialab Diagnostic Ges. MBH, (Wien, Austria). Morbital (Pentobarbitalum) was from BIOWET (Puławy, Poland). Rat leptin radioimmunoassay kit was from LINCO Research Inc, St Charles, MO, USA.

In vivo study:

Animal preparation: The study was performed on Wistar rats weighing about 300 g. Animals were housed at constant temperature with light-dark cycles of 12 h. To examine pancreatic secretion
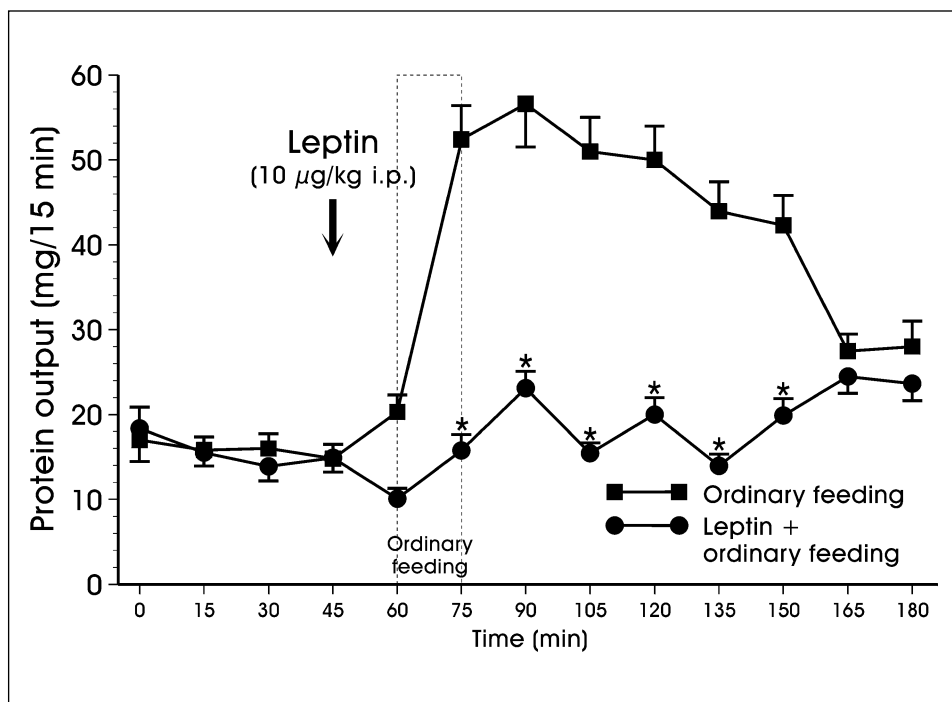
Fig. 3. Time course of the effect of leptin (10 µg/kg i.p.), on pancreatic protein secretion stimulated by ordinary feeding. The results are the means ± SEM of separate experiments, each performed on 5-6 rats. Asteriks indicates significant decrease below the control.

rats were anesthetized with Morbital and equipped with chronic pancreatic fistulae (PF) and gastric fistulae (GF), prepared as described previously (18). Briefly, to prepare gastric fistula the metal cannula was inserted into the stomach and fixed by a suture. End of the cannula was brought to the exterior and closed by a stainless steel thimble between the tests.

PF was prepared by placing polyethylene cannula into common bile pancreatic duct to collect pancreato-biliary juice, whereas another cannula was inserted into the duodenum. Both cannulas were connected to permit the circulation of the pancreatic juice into the duodenum. Cannulas endings were brought to the exterior and protected by a stainless steel thimble to allow for the free movement of the rats in their cages. During the experiment the animals were placed in Bollmann cages, the GF was open, the metal thimble from the PF was removed and the pancreatic and duodenal cannulas were disconnected. The PF was used to collect the pure pancreatic juice. In most of the experiments, except these with stimulation of pancreatic secretion by diversion of pancreatic juice to the exterior (DPJ), the duodenal cannula was employed for the reinfusion of the juice, diluted with saline 1:2, into the duodenum. Following a week of recovery after surgery, the secretory studies started, usually after 18 hours of food, but not water, deprivation. The pancreatic juice was collected in 15 min aliquots to measure the volume and protein concentration of each sample. Protein concentration in each sample was measured by Lowry method. The results were expressed as protein output (mg/15 min). During all tests, except those with diversion of pancreatic juice to the exterior, previously collected bile-pancreatic juice was re-infused *via* duodenal cannula at the rate of 1 ml/h. In the beginning of
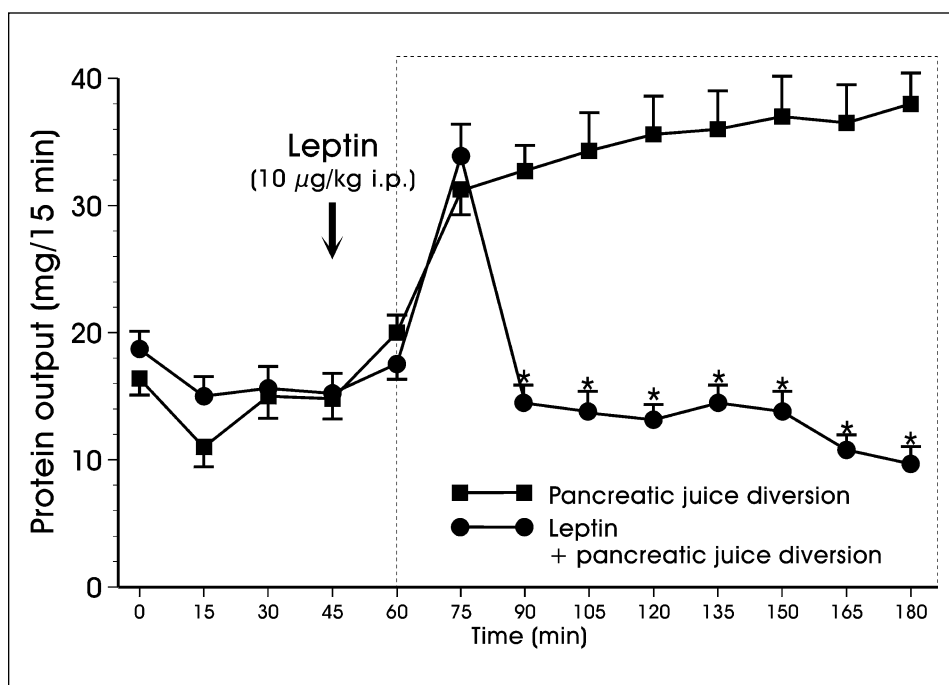
Fig.4. Time course of the effect of leptin (10 μg/kg i.p.), on pancreatic protein secretion stimulated by pancreatic juice diversion. The results are the means ± SEM of separate experiments, each performed on 5-6 rats. Asteriks indicates significant decrease below the control.

each test basal secretion was collected for 60 min to allow for stabilization of basal pancreatic juice secretion.

Experimental protocol:

The experimental protocol has been approved by the Jagiellonian University Ethical Committee for Animal Experimentation.

Leptin, dissolved in 0.5 ml of saline, was administered intraperitoneally (i.p.) into the rats in each test. In control studies vehicle alone was given to the animals, instead of leptin. Several series of tests, each performed on 5-6 rats were carried out, including:

1. Control tests; pancreatic juice returned to the duodenum throughout the experiment.
2. Experiments with pancreatic juice returned to the duodenum, and i.p. administration of leptin (0.1, 1, 5, 10, 20 or 50 μg/kg), to measure the effect of leptin on basal pancreatic secretion. Each dose of leptin was given to the separate group of animals.
3. Tests with feeding of the rats with liquid mixted meal (6 g of white bread mixted with milk in proportion 1:1) for 30 min to examine the postprandial secretion. In these experiments gastric fistula was kept closed and pancreatic juice returned to the duodenum. Leptin at various doses (0.1, 1, 5, 10, 20 or 50 μg/kg) was administered i.p. Each dose was given to the separate group of animals.
4. For the next part of the study the dose of 10 μg /kg of leptin was selected and given i.p. 15 min prior to the start of the stimulation of pancreatic secretion by DPJ or sham feeding. For
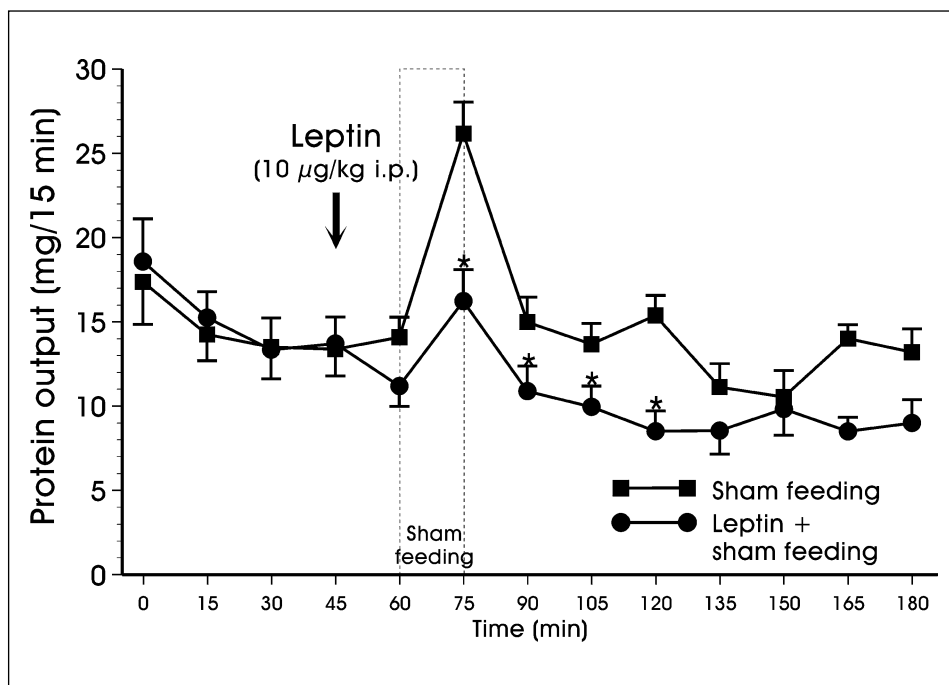
Fig. 5. Time course of the effect of leptin (10 μg/kg i.p.), on pancreatic protein secretion stimulated by sham feeding. The results are the means ± SEM of separate experiments, each performed on 5-6 rats. Asteriks indicates significant decrease below the control.

the experiments with pancreatic secretion stimulated with sham feeding; rats were offered liquid mixted meal for 15 min. In these rats the GF were open, to allow for drainage of consumed food from the rats stomach. Then the volume of gastric content was measured and compared to the ingested food.

Determination of plasma leptin level :

In part of the study leptin blood level was determined after i.p. injection of exogenous leptin (0.1, 1, 5, 10, 20 or 50 μg/kg). Rats were anesthetized with Morbital, the abdominal vena cava was exposed and blood was withdrawn into EDTA containing tubes, at 180 min after leptin administration. Plasma leptin concentration was measured by radioimmunoassay using rat leptin kit LINCO Research Inc., St Charles, MO, USA. as described elsewhere (8, 9).

In vitro study:

Pancreatic acini were isolated from the pancreas of intact rats by collagenase digestion and suspended in KRH medium (pH 7.4), as described previously (8, 19). Acinar suspensions were incubated in shaking bath at 37°C for 30 min in presence of various concentrations of leptin (10^{-10}-10^{-7} M), alone or in the combination with submaximal doses of caerulein (10^{-12} M) or urecholine (10^{-5} M). The concentration of urecholine or caerulein required to cause submaximal stimulation of enzyme secretion *in vitro* was calculated from the previous experiments done in our laboratory.
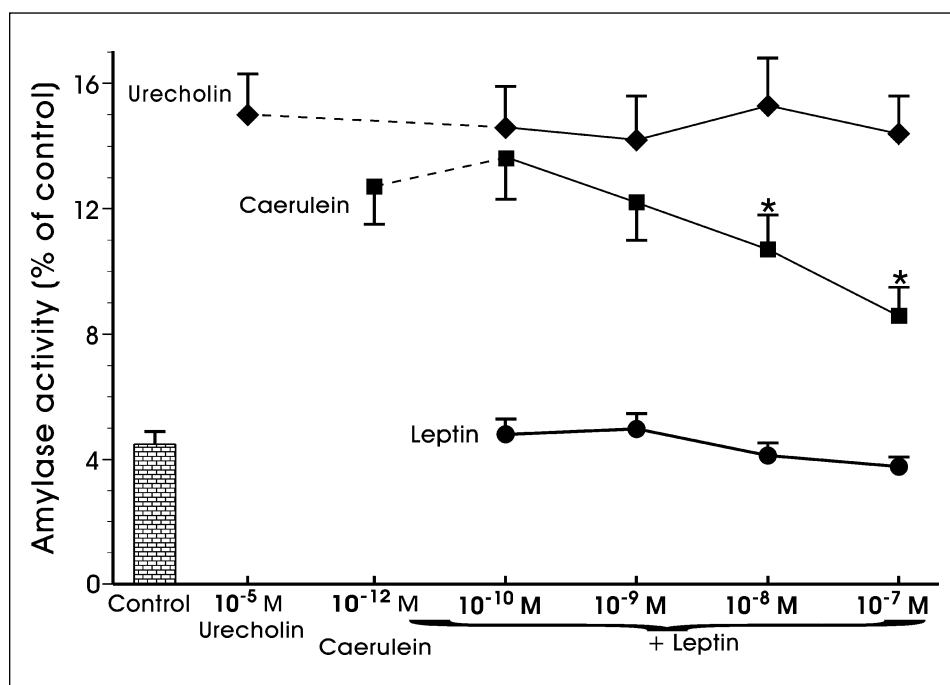
Fig 6. Effect of increasing concentrations of leptin on unstimulated or secretagogue-induced amylase release from isolated pancreatic acini. Results are means ± SEM from 6 separate experiments, each done in duplicate. Asteriks indicates significant decrease below the control.

Amylase release from the acini was determined with the method of Bernfeld (20). The results were expressed as percent of total amylase content released into the supernatant from the acini.

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. A difference with a p. value of < 0.05 was considered statistically significant. Results are expressed as means ± SEM.

<div align="center">RESULTS</div>

Pancreatic secretion in vivo:

In conscious rats with chronic PF and with pancreatic juice returned to the duodenum, the basal pancreatic secretion was relatively well sustained, averaging about 15.0 ± 2.5 mg/15 min (*Fig. 1*). This unstimulated pancreatic secretion was not affected significantly by leptin administration (0.1, 1, 5, 10, 20 or 50 µg/kg) (*Fig.1*). In these rats, feeding caused a strong increase in pancreatic enzyme secretion, ranging about 60 ± 8 mg/15 min (*Fig 2*). Leptin given at doses of 5, 10,
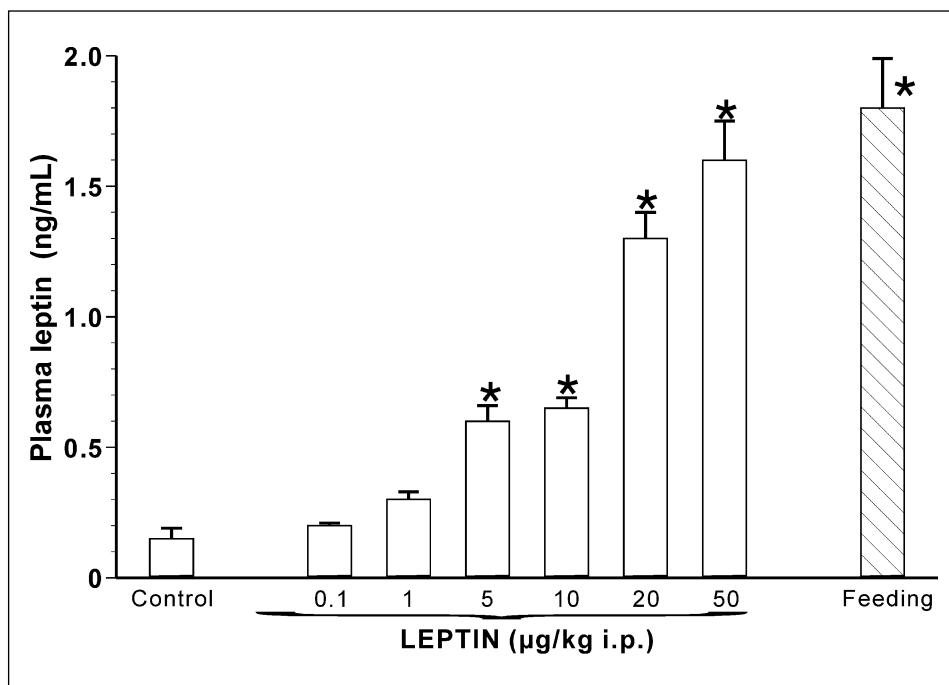
Fig. 7. Plasma leptin level in response to feeding and to increasing doses of leptin, given i.p. (0.1, 1, 5, 10, 20 or 50 μg/kg). Results are means ± SEM from 6 separate experiments. Asteriks indicates significant increase above the control.

20 or 50 μg/kg i.p., resulted in the significant decrease of this postprandial pancreatic protein secretion (*Fig. 2*). The strongest inhibition was observed with the dose of 10 μg/kg of leptin, which almost completely abolished pancreatic protein secretion stimulated by feeding, whereas administration of higher doses of leptin (20 or 50 μg/kg i.p.) reduced this secretion by about 50% and 40 % respectively (*Figs 1 - 3*).

As expected, DPJ caused a strong increase in pancreatic enzyme secretion in conscious PF rats (*Fig. 4*). Leptin given as i.p. bolus injection at dose of 10 mg/kg abolished this stimulatory effect of DPJ (*Fig. 4*).

Sham feeding resulted in the immediate, significant increase of pancreatic protein output over the control level, reaching about $26 \pm 5 .5$ mg/15 min (*Fig. 5*). Pretreatment of these rats with leptin resulted in the dramatic decrease of this protein response to sham feeding (*Fig. 5*).

Pancreatic secretion in vitro:

Incubation of isolated pancreatic acini in presence of various doses of leptin (10^{10} - 10^{-7} M) failed to affect significantly amylase release from these acini (*Fig. 6*). Leptin, at doses of 10^{-8} M and 10^{-7} M produced significant decrease of

caerulein- but not urecholine-induced enzyme secretion from isolated pancreatic acini (*Fig. 6*).

Plasma leptin level

Under basal conditions plasma leptin level averaged about 0.15 ± 0.04 ng/ml (*Fig. 7*). Administration of exogenous leptin (0.1, 1, 5, 10, 20 or 50 µg/kg), resulted in the dose dependent rise of plasma leptin activity, reaching the highest level with the dose of 50 µg/kg of leptin, that was about 1.6 ± 0.15 ng/ml, and was similar to the value obtained from the fed rats. (*Fig. 7*).

DISCUSSION

Our present study clearly shows that leptin inhibits pancreatic enzyme secretion stimulated by ordinary feeding, sham feeding and DPJ, and that leptin could influence pancreatic enzyme secretion acting directly on the pancreatic acinar cells.

The presence of leptin receptor (Ob-R) in the pancreatic islets and leptin receptor gene expression in normal pancreatic acini (8, 11) suggests that this peptide could take a part in the physiological modulation of pancreatic function. Indeed, leptin receptors have been detected on pancreatic AR42J cells and leptin was shown to inhibit CCK-induced amylase release from these cells (14). Herein, we demonstrate that leptin is able to attenuate pancreatic enzyme secretion stimulated by submaximal doses of caerulein, but not urecholine, acting directly on freshly isolated pancreatic acini. The inhibitory effect of leptin on these acini is probably related to the changes of intracellular calcium or other intracellular events, because in AR42J cells leptin does not affect the binding of CCK to its receptors (14).

Our results concerning the effect of leptin on pancreatic secretion *in vitro* are in disagreement with previous data of Matyjek et al., showing that amylase release from normal pancreatic acini is unaffected by leptin (17). However, in our study leptin was applied in increasing doses to the pancreatic acini, and this resulted in the dose-dependent inhibition of enzyme secretion produced by micromolar doses of this peptide. In the previous study of Matyjek et al, low, single dose of leptin failed to affect significantly CCK-induced dose-response curve for amylase release from isolated pancreatic acini (17).

Previous studies yielded contradictory results regarding the effects of leptin on the exocrine pancreas; leptin applied at high doses has been demonstrated to increase pancreatic secretion of enzymes by one group (16), whereas the others have shown that administration of leptin resulted in the significant reduction of pancreatic exocrine function (17). Our study, performed on conscious rats with chronic pancreatic fistulae, confirms the latter observation in anesthetized rats (17), showing that exogenous leptin, administered to the rats at low doses (1 or

10 µg/kg) produced significant inhibition of pancreatic enzyme secretion. We have demonstrated that leptin is able to modulate cephalic phase of pancreatic secretion by reducing enzyme output stimulated by sham feeding. Similar inhibitory effect of leptin was observed in gastric secretion stimulated by sham-feeding in humans (21). Pancreatic secretion stimulated by ordinary feeding, as well as that induced by DPJ, were also attenuated by leptin, indicating that this substance is able to inhibit all phases of postprandial pancreatic secretion.

It should be mentioned that leptin has been shown to produce the decrease of plasma ghrelin, another gastric hormone that has been reported to stimulate the appetite, while inhibiting pancreatic secretion (22 23). Since leptin negatively regulates ghrelin production it is likely, that the observed inhibition of pancreatic secretion by leptin involves also ghrelin.

In this study we have compared plasma leptin activities resulting from the application of various doses of exogenous peptide to the rats with plasma leptin concentration detected after feeding. It is of interest that exogenous leptin markedly inhibits pancreatic secretion at dose which produced lower plasma leptin level than that observed postprandially, whereas high concentration of plasma leptin, similar to that observed after feeding, resulted in the relatively weaker inhibition of pancreatic secretory function. Feeding produced marked increase of leptin blood level. At the end of digestion, plasma activity of leptin decreases, reaching the lowest value during interdigestive period. Plasma leptin level lower than that detected immediately after food ingestion inhibits pancreatic secretion. It is very likely that leptin effect on the exocrine pancreas depends on the amount of circulating leptin. Our results suggest that leptin could take a part in the modulation of pancreatic exocrine function as an inhibitory signal closing the postprandial stimulation of pancreatic enzyme secretion, however further studies are needed to evaluate this hypothesis.

Beside the adipocytes, leptin is produced in the stomach and could be released into the gastrointestinal tract (5). Leptin receptors have been shown in the intestinal sensory endings of the vagal nerve and in the pancreatic neurons (24, 25). These observations strongly recommended the hypothesis that intraluminal leptin of gastric origin could stimulate duodenal receptors and influence pancreatic secretion though activation of neural pathway. In our previous study we have shown that sensory nerves are involved in the protective effect of leptin on the pancreas (15). Herein, we have observed that in the experiments with sham feeding and in these with DPJ the injection of leptin to the rats produced short peak increase in pancreatic secretion which subsequently decreased. It is possible that at the moment of application leptin could activate a stimulatory nervous reflex, resulting in a peak rise of protein output followed by prolonged inhibition of pancreatic secretion.

Recently published paper has demonstrated that pancreatic secretory effects of leptin could be reversed by deactivation of sensory nerves or by vagotomy (17). Taking together, our results and previously published data indicate that leptin

could exert its effect on the pancreatic enzyme secretion acting directly on the pancreatic acini as well as though its indirect action involving vagal enteropancreatic reflex.

We conclude that leptin could take a part in the control of pancreatic enzyme secretion and that low level of plasma leptin level could be an early inhibitory signal, closing the postprandial stimulation of pancreatic secretion.

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Authors address: Jolanta Jaworek MD PhD, Chair of Physiology Jagiellonian University CM, Grzegórzecka Street 16, 31-531 Kraków, Poland, phone: +48+12 424-72-30, fax: +48 +12 421-15-78 E-mail: mpjawore@cyf-kr.edu.pl