INTRODUCTION

Obestatin is a 23-amino acid peptide derived from the same prohormone as ghrelin and it has been reported to bind and activate the orphan G protein-coupled receptor GPR39 (1). This peptide has been named obestatin due to its inhibitory effect on food intake (1). However, many of subsequent studies (2-6) could not reproduce the anorexic effects of obestatin. On the other hand, Lagaud et al (7) have confirmed the observation that obestatin reduces food intake and suppresses body weight gain in rodents. Obestatin, like a ghrelin has been originally extracted from rat stomach (1), and the stomach seems to be a major source of circulating obestatin. Previous studies have shown that administration of ghrelin exhibits protective effect in the pancreas, inhibiting the development of acute pancreatitis. Recent study has shown that obestatin promotes survival of β-cells and pancreatic islets. Aim of the present study was to investigate the influence of obestatin administration on the development of cerulein-induced pancreatitis. Studies were performed on male Wistar rats. Acute pancreatitis was induced by cerulein given intraperitoneally 5 times at a dose of 50 µg/kg/dose with 1-h intervals. Obestatin was injected twice intraperitoneally at the dose of 4, 8 or 16 nmol/kg/dose. In control saline-treated rats, obestatin was without effect on pancreatic morphology, serum activity of pancreatic enzymes, serum level of pro-inflammatory interleukin-1β or pancreatic cells proliferation. In animals with induction of acute pancreatitis, morphological examination showed that administration of obestatin decreased pancreatic leukocyte infiltration and vacuolization of acinar cells. These effects were accompanied by reduction in the pancreatitis-evoked increase in serum level of pancreatic digestive enzymes, lipase amylase and poly-C ribonuclease. Obestatin administered at the highest dose of 16 nmol/kg/dose reduced serum activity of these enzymes by 33, 42 and 44%, respectively. Also serum concentration of pro-inflammatory interleukin-1β was decreased by obestatin in rats with acute pancreatitis; whereas the pancreatitis-evoked decrease in pancreatic blood flow and pancreatic DNA synthesis was partially reversed. Administration of obestatin reduces the severity of cerulein-induced acute pancreatitis. This effect is related, at least in part, to the improvement of pancreatic blood flow and reduction in pro-inflammatory interleukin-1β release.

Key words: acute pancreatitis, pancreatic damage, interleukin-1β, lipase, amylase, poly-C ribonuclease, pancreatic blood flow

MATERIALS AND METHODS

Animals and treatment

Studies were carried out series on male Wistar rats weighing 160-180 g. Animals were housed in cages with wire mesh bottoms, at normal room temperature (22 ± 1°C) and a 12-h light-dark cycle. The experimental protocol was approved by the Committee for Research and Animal Ethics of the Jagiellonian University.
Experiments were carried out in the following eight experimental groups (ten animals in each group): (1) saline-treated control; (2-4) animals treated intraperitoneally with obestatin given twice at the dose of 4, 8 or 16 nmol/kg/dose, respectively, with 3-h interval between doses; (5) animals with cerulein-induced pancreatitis; (6-8) animals treated with obestatin before and during induction of acute pancreatitis; obestatin was administered twice at the dose of 4, 8 or 16 nmol/kg/dose, respectively (the first dose was given 0.5 h before the first injection of cerulein, the second dose 3 h later).

Acute pancreatitis was induced by cerulein (Sigma-Aldrich, GmbH, Steinheim, Germany) administered intraperitoneally 5 times with 1 h intervals at a dose of 50 µg/kg per injection.

Rat obestatin was synthesized in Yanaihara Institute by a solid phase methodology with Fmoc-strategy using automated peptide synthesizer (Applied Biosysytem 9030 Pioneer, Foster, CA, USA). Analytical HPLC and MALDI-TOF MS confirmed the homology of the product.

**Determination of pancreatic blood flow**

Two and half hour after last injection of obestatin (rat without induction of acute pancreatitis) or immediately after the last injection of cerulein (rats with induction of acute pancreatitis) animals were anesthetized with ketamine (50 mg/kg i.p., Bioketan, Vetoquinol Biowet, Gorzow Wielkopolski, Poland). The abdominal cavity was opened and pancreatic blood flow was measured by a laser Doppler flowmeter using PeriFlux 4001 Master monitor (Perimed AB, Jarfalla, Sweden), as described previously (15). Data were presented as percent of change from control value obtained in saline-treated rats.

**Biochemical analysis**

After measurement of pancreatic blood flow, blood was taken from the aorta and serum was collected and frozen at -60°C. Serum lipase and amylase activity was determined with a Kodak Ectachem DT II System analyzer (Eastman Kodak Company, Rochester, NY, USA) using a commercially available LIPA and AMYL DT Slides (Vitros DT Chemistry System, Johnson & Johnson Clinical Diagnostic, Inc., Rochester, NY, USA).

Serum poly-C ribonuclease activity was determined using Warshaw and Lee's procedure (16), employing polycytidylic acid (poly-C) as a ribonuclease substrate, as described previously in detail (17).

Serum concentration of interleukin-1β was measured using the commercial BioSource Cytoscreen rat IL-1β kit (BioSource International, Camarillo, California, USA) based on ELISA.

After blood withdrawal, the pancreas was carefully dissected out from the body. Pancreatic DNA synthesis was determined in samples of pancreatic tissue by measurement of [3H]thymidine incorporation ([6-3H]-thymidine, 20-30 Ci/mmol, Institute for Research, Production and Application of Radioisotopes, Prague, Czech Republic) into DNA as previously described (18). DNA synthesis was expressed as [3H]thymidine disintegrations per minute per microgram DNA (dpm/µg DNA).

**Histological examination of pancreatic damage**

Morphological examination of pancreatic tissue was performed in hematoxilin and eosin stained slides as previously described in detail (19). Slides were examined by two experienced pathologists without knowledge of the treatment given (four slides per animal). The histological grading of edema was made using a scale ranging from 0 to 3 (0 = no edema, 1 = interlobular edema, 2 = interlobular and moderate intralobular edema, and 3 = interlobular edema and severe intralobular edema). Leukocytic infiltration was also graded from 0 to 3 (0 = absent, 1 = scarce perivascular infiltration, 2 = moderate perivascular and scarce diffuse infiltration, 3 = abundant diffuse infiltration). Grading of vacuolization was based on the appropriate percentage of acinar cells involved: 0 = absent, 1 = less than 25%, 2 = 25-50% and 3 = more than 50% of acinar cells. Hemorrhagia was graded: 0 = no hemorrhagia, 1 = 1-2 hemorrhagic foci per slide, 2 = 3-5 hemorrhagic foci per slide, 3 = more than 5 hemorrhagic foci per slide. Necrosis was graded: 0 = no necrosis, 1 = less than 15% of pancreatic cells involved, 2 = 15-35% of cells involved, 3 = more than 35% of cells involved.

**Statistical analysis**

Biochemical results and pancreatic blood flow are expressed as mean ± SEM. Statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Tukey’s multiple comparison test using GraphPadPrism (GraphPad Software, San Diego, CA, USA). Differences were considered to be statistically significant when P was less than 0.05.

**RESULTS**

**Histological findings**

Pancreases obtained from saline-treated control rats showed a lack of any alteration in macroscopic and microscopic examination (Table 1). Also treatment with any dose of obestatin given alone did not affect morphology of pancreatic tissue (Table 1). Administration of cerulien induced acute edematous pancreatitis in all rat tested (Table 1). Pancreases were grossly swollen and enlarged with a visible collection of edematous fluid. At light microscopic level, prominent interlobular and moderate intralobular edema was accompanied with moderate perivascular and scarce diffuse inflammatory leukocyte infiltration. Vacuolization was observed in 25 to more than 50% of acinar cells. No hemorrhagia or necrosis was observed.

Treatment with obestatin, before and during administration of cerulien, reduced the pancreatitis-induced pancreatic damage (Table 1). Maximal protective effect was observed after obestatin given at the dose of 8 and 16 nmol/kg/dose. In some animals from these groups, pancreatic edema was limited to interlobular space; in other animals interlobular and moderate intralobular edema was observed. Moreover, treatment with obestatin at the dose of 8 and 16 nmol/kg/dose reduced inflammatory infiltration of the pancreas and vacuolization of acinar cells. Vacuolization was seen in less than 50% of acinar cells.

**Biochemical examinations**

In saline-treated control rats, serum lipase activity reached 58.7 ± 10.2 U/L (Fig. 1). Obestatin given alone did not significantly affect serum activity of lipase. Administration of cerulien, causing acute pancreatitis, increased plasma lipase activity to 887.5 ± 536.7 U/L. Treatment with obestatin reduced the pancreatitis-induced increase in serum activity of lipase and this effect was statistically significant after obestatin given at the dose of 8 and 16 nmol/kg/dose (Fig. 2).

Serum activity of amylase reached a value of 2568 ± 187 U/L in control rats treated with saline (Fig. 2). In these rats, administration of any dose of obestatin failed to affect serum activity of amylase. Induction of acute pancreatitis by cerulien administration caused four-fold increase in serum activity of amylase. In rats with induction of acute pancreatitis, administration of obestatin reduced the pancreatitis-evoked...
increase in serum activity of amylase and this effect was statistically significant after administration of obestatin at the dose of 8 and 16 nmol/kg/dose (Fig. 2).

In control rats infused with saline, serum activity of poly-C ribonuclease was 161 ±16 U/L (Fig. 3). In saline-treated rats, obestatin was without any effect on serum activity of poly-C ribonuclease. Induction of acute pancreatitis caused nine-fold increase in serum activity of this enzyme. Obestatin administered at the dose of 8 or 16 nmol/kg/dose partly, but significantly reversed the pancreatitis evoked increase in serum activity of poly-C ribonuclease. Effect of obestatin given at the dose of 4 nmol/kg/dose was statistically insignificant (Fig. 3).

In control rats, serum concentration of pro-inflammatory interleukin-1β was 66.3 ± 4.2 pg/mL (Fig. 4). Treatment with
any dose of obestatin was without a significant effect on serum interleukin II-1β level in saline-treated rats. Administration of cerulein increased serum interleukin-1β concentration to a value of $254.5 \pm 17.2 \text{ pg/mL}$ and this increase was significantly reduced by treatment with obestatin at the dose of 8 and 16 nmol/kg/dose. (Fig. 4).

In saline-treated control rats, pancreatic DNA synthesis reached a value of $58.9 \pm 2.6 \text{ dpm/µg DNA}$ (Fig. 5). Treatment with any dose of obestatin did not significantly affect pancreatic DNA synthesis in animals injected with saline. In animals with cerulein-induced pancreatitis, pancreatic DNA synthesis was reduced by 48%. In this group of animals, treatment with...
obestatin partly reversed the cerulein-induced fall in pancreatic DNA synthesis and this effect was statistically significant after obestatin given at the dose of 8 and 16 nmol/kg/dose (Fig. 5).

**Pancreatic blood flow**

Treatment with any dose of obestatin was without any effect on pancreatic blood flow in saline-injected rats (Fig. 6). Induction of acute pancreatitis by cerulein administration reduced pancreatic blood flow by 46% when compared to salinetreated control rats. Treatment with obestatin caused a partial reversion of the cerulein-induced reduction in pancreatic blood flow. This effect was statistically significant after obestatin given at the dose of 8 and 16 nmol/kg/dose (Fig. 5).

**DISCUSSION**

According to our knowledge, our present study is the first report that administration of obestatin inhibits the development of acute pancreatitis. Protective effect of obestatin on the pancreas was found as an improvement of pancreatic histology and a decrease in biochemical indexes of the severity of acute pancreatitis. We observed the reduction in pancreatic edema, inflammatory infiltration and vacuolization of acinar cells. A decrease in leukocyte infiltration of pancreatic tissue was in harmony with the reduction in the pancreatitis-induced increase in serum concentration of pro-inflammatory interleukin-1β. Interleukin-1β is a well-known mediator of acute inflammation and plays a crucial role in the release of other members of pro-inflammatory cytokine cascade (20). Interleukin-1β stimulates the synthesis and release of inflammatory mediators such as TNF-α, PAF, prostaglandins and pro-inflammatory interleukins (20, 21). The essential role of interleukin-1β in inflammatory process is evidenced by the observation that administration of interleukin-1β receptor antagonist prevents the release of pro-inflammatory mediators and reduces the severity of inflammation and tissue damage (21). The same mechanism of inflammation is involved in acute pancreatic. In this disease, pro-inflammatory cytokines are produced within the pancreas and subsequently within distant organs, leading to the development of systemic inflammatory response syndrome (SIRS) and multiple organ failure (MOF) in the course of severe acute pancreatitis (22). For this reason serum level of pro-inflammatory cytokine is well-correlated with severity of acute pancreatitis (23).

The increase in serum activity of lipase and amylase is a well established index of acute pancreatitis severity with high sensitivity and specificity (24). Serum activity of poly-C ribonuclease has been reported to be closely correlated with degree of pancreatic necrosis (16), but serum level of this enzyme is also elevated in mild edematous pancreatitis (25). In our present study, administration of obestatin reduced the pancreatitis-evoked increase in serum activity of pancreatic digestive enzymes: lipase, amylase and poly-C ribonuclease. This observation is an additional evidence that obestatin exhibits protective effect on the pancreas and inhibits the development of acute pancreatitis.

Pancreatic DNA synthesis is an index of pancreatic cell proliferation in the pancreas. Induction of acute pancreatitis, leading to damage of pancreatic cells reduces pancreatic DNA synthesis and grade of this reduction is well-correlated with the severity of acute pancreatitis. Development of mild edematous acute pancreatitis reduces pancreatic DNA synthesis (26), but this effect is much smaller than in acute hemorrhagic pancreatitis (19). In our present study, administration of obestatin alone was without effect on pancreatic DNA synthesis, but higher doses of obestatin (8 abd 16 nmol/kg/dose) administered in combination with cerulein partly but significantly reversed the pancreatitis-evoked fall in pancreatic DNA. These data indicate that obestatin does not stimulate pancreatic cells proliferation but reduces pancreatic damage leading to the increase in vitality of pancreatic cells. This effect of obestatin on vitality of pancreatic cells is in agreement with study performed by Granata et al. (14). They have found that obestatin exerts proliferative, survival and anti-apoptotic effects in β-cells and human pancreatic islets under serum deprived condition and treatment with IFN-γ, TNF-α and interleukin-1β.

Clinical and experimental studies have shown that pancreatic ischemia plays an important role in the initiation of pancreatitis, or the progression to necrotizing pancreatitis (27-29). Microvascular perfusion failure is known to be essential in the development of acute pancreatitis in various clinical settings including cardiac (28) or aortic (30) surgery, hypovolemic shock (31) and transplantation of the pancreas (32). Experimental studies have show that the severity of acute pancreatitis is closely related to tissue ischemia. The moderate and severe pancreatitis is accompanied by a progressive decrease in pancreatic blood perfusion (33). Also in mild edematous acute pancreatitis induced by cerulein, the initial hyperemia (33) is followed by a severe reduction in pancreatic circulation (34, 35). In harmony with these findings is study performed by Furukawa et al. (34). They have
shown that the exposure of rats with mild pancreatitis to stress causes additional reduction in pancreatic blood flow and increases the severity of acute pancreatitis. On the other hand, vasodilatation and the improvement of pancreatic blood flow leads to reduction in the development of acute pancreatitis (36) and protects against systemic circulatory failure in this disease (37).

In our present study, induction of acute pancreatitis by cerulein reduced the pancreatic blood flow. Administration of obestatin failed to affect pancreatic blood flow in saline-treated control rats, but obestatin given at the highest doses of 8 and 16 nmol/kg/dose significantly reversed the decrease in pancreatic blood flow in rats with acute pancreatitis. The mechanism of this effect is unclear. Probably the improvement of pancreatic blood flow is a result of reduction in the pancreatitis-induced pancreatic edema and inflammatory infiltration.

In our present study, we have not examined the influence of obestatin on pancreatic exocrine secretion. However, previous study performed by Kapica et al. (38) has shown that obestatin stimulates the secretion of pancreatic enzymes. This observation suggests that protective effect of obestatin on the pancreas is not related to its influence on pancreatic exocrine secretion.

Finally, our present study has demonstrated that pretreatment with obestatin reduces the pancreatic damage and decreases the severity of cerulein-induced acute pancreatitis. This effect is related, at least in part, to improvement of pancreatic blood flow, reduction in the liberation of pro-inflammatory interleukin-1β and improvement of pancreatic cells vitality.

Conflict of interests: None declared.

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Received: June 6, 2008
Accepted: July 15, 2009

Author’s address: Professor Artur Dembinski, MD, PhD, Department of Physiology, Jagiellonian University Medical College, 16 Grzegorzecka Street, 31-531 Cracow, Poland; Phone: +48-12-4211006; Fax: +48-12-4225478; e-mail: mpdembis@cyf-kr.edu.pl