GHRELIN ATTENUATES THE DEVELOPMENT OF ACUTE PANCREATITIS IN RATS.

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Background: Ghrelin, a circulating growth hormone-releasing peptide isolated from human and rat stomach, stimulates growth hormone secretion, food intake and exhibits gastrophotropic properties. Ghrelin is predominantly produced by a population of endocrine cells in the gastric mucosa, but its presence in bowel, pancreas, pituitary and hypothalamus has been reported. In human fetal pancreas, ghrelin is expressed in a prominent endocrine cell population. In adult pancreatic islets the population of these cells is reduced. The aim of present study was to investigate the influence of ghrelin administration on the development of acute pancreatitis. Methods: Acute pancreatitis was induced in rat by caerulein injection. Ghrelin was administrated twice (30 min prior to the first caerulein or saline injection and 3 h later) at the doses: 2, 10 or 20 nmol/kg. Immediately after cessation of caerulein or saline injections the following parameters were measured: pancreatic blood flow, plasma lipase activity, plasma interleukin-1\(\beta\) (IL-1\(\beta\)) and interleukin 10 (IL-10) concentration, pancreatic DNA synthesis, and morphological signs of pancreatitis. Results: Administration of ghrelin without induction of pancreatitis did not affect significantly any parameter tested. Caerulein led to the development of acute edematous pancreatitis. Treatment with ghrelin at the dose 2 nmol/kg, during induction of pancreatitis, was without effect on pancreatic histology or biochemical and functional parameters. Treatment with ghrelin at the dose 10 and 20 nmol/kg attenuated the development of pancreatitis and the effects of both doses were similar. Administration of ghrelin (10 or 20 nmol/kg) reduced inflammatory infiltration of pancreatic tissue and vacuolization of acinar cells. Also, plasma lipase activity and plasma IL-1\(\beta\) concentration were reduced, and caerulein-induced fall in pancreatic DNA synthesis was reversed. Administration of ghrelin at the dose 10 and 20 nmol/kg was without effect on caerulein-induced pancreatic edema and pancreatitis-related fall in pancreatic blood flow. Conclusions: (1) Administration of ghrelin attenuates pancreatic damage in caerulein-induced pancreatitis; (2) Protective effect of ghrelin administration seems
to be related the inhibition in inflammatory process and the reduction in liberation of pro-inflammatory IL-1β.

Key Words: ghrelin, pancreatitis, interleukin-1β, inflammatory infiltration.

INTRODUCTION

Ghrelin is a 28-amino-acid polypeptide originally isolated from rat stomach (1), where it has been localized in the endocrine X/A-like cells in the gastric mucosa (2). Ghrelin is predominantly produced in the stomach (1, 3), but its presence has been also detected among others in the bowel, pancreas, kidney, pituitary and hypothalamus (1, 4, 5). Although ghrelin is probably able to exert paracrine effects, it is released to the blood from the gastrointestinal source, and most of its actions are believed to be due to endocrine effects (6, 7, 8). Ghrelin strongly and dose dependently stimulates growth hormone (GH) release from the anterior pituitary acting on growth hormone secretagogue receptor (GHS-R) type 1a (1). GHS-R 1a is predominantly expressed in the pituitary. It is also distributed in other central and peripheral tissues, but at much lower levels (5). Apart from the stimulation of GH release, ghrelin stimulates the appetite and fat deposition in rats (9) and humans (10). It has been reported that plasma ghrelin concentration is decreased in obese subjects (11, 12) and after food intake (3, 12), whereas fasting or anorexia nervosa cause an increase in plasma ghrelin concentration (3, 12). In addition, it has been found that fasting plasma level of ghrelin is negatively correlated with body mass index (12).

In the stomach, ghrelin affects gastric acid secretion but its effect is not clear. In anesthetized rats, intravenous (13) or intracerebroventricular (14) administration of ghrelin has been found to stimulate gastric acid secretion, whereas in conscious rats, central administration of ghrelin has been reported to inhibit gastric acid secretion (15). Moreover, ghrelin affects gastric motility (13) and exhibits gastroprotective effect (16).

In fetal pancreas, ghrelin is expressed in a prominent endocrine cell population (17). In adult pancreas, the number of ghrelin-immunoreactive cells is reduced (17) and these cells are localized at the periphery of human and rat pancreatic islets (2). These data suggests that ghrelin is involved in development of pancreas and the regulation of pancreatic secretion. The influence of ghrelin on insulin secretion is unclear. Some studies have reported that ghrelin inhibits insulin secretion in the perfused rat pancreas (18), in anesthetized mice and isolated mouse islets (19) and in humans (20). Other studies have shown that ghrelin stimulates insulin secretion in isolated rat pancreatic islets (2) and anesthetized fed rats (21). Pancreatic exocrine secretion has been found to be inhibited by ghrelin in anesthetized rats and in dispersed rat acinar cells (22).
The gastroprotective effect of ghrelin administration, the presence of ghrelin secreting cell in the pancreas and the influence of ghrelin on exocrine and endocrine pancreatic secretion suggest that ghrelin play a role in the maintenance of pancreatic integrity. The present study was designed to assess the influence of ghrelin administration on the development of acute pancreatitis.

MATERIALS AND METHODS

Animals and treatment

Studies were performed on male Wistar rats weighing 160-180 g and were conducted following the experimental protocol approved by the Committee for Research and Animal Ethics of Jagiellonian University. Animals were housed in cages with wire mesh bottoms, with normal room temperature and a 12-h light-dark cycle.

Acute pancreatitis was induced by five intraperitoneal injection of caerulein at the dose 10 µg/kg/injection with 1 h intervals between injections (Takus, Pharmacia & Upjohn GmbH, Erlangen, Germany). Control animals were intraperitoneally (i.p.) injected with 0.9% NaCl solution. Studies were carried out on the following experimental groups (ten animals in each group): (1) control animals; (2-4) animals injected with 0.9% NaCl solution and treated i.p. twice with ghrelin (30 min before the first injection of 0.9% NaCl and 3 h later) at the doses: 2, 10, or 20 nmol/kg/injection, respectively; (5) animals with caerulein-induced pancreatitis; (6-8) animals with caerulein-induced pancreatitis and treated i.p. twice with ghrelin (30 min before the first injection of 0.9% NaCl and 3 h later) at the doses: 2, 10, or 20 nmol/kg/injection, respectively.

Rat recombinant ghrelin was purchased from Bachem AG, Budendorf, Switzerland.

Determination of pancreatic blood flow

Following the last injection of physiological saline solution or caerulein, animals were anesthetized with ketamine (50 mg/kg i.p., Bioketan, Biowet, Gorzów, Poland) and the abdomen was opened. Pancreases were exposed for the measurement of the blood flow by laser Doppler flowmeter, using PeriFlux 4001 Master monitor (Perimed AB, Järfälla, Sweden), as described previously (23). The pancreatic blood flow was presented as percent change from control value obtained in rats injected with physiological saline solution.

Determination of plasma lipase activity, and plasma interleukin-1β and interleukin-10 concentration

Immediately after measurement of pancreatic blood flow, the abdominal aorta was exposed and blood was taken for determination of plasma lipase activity, and plasma interleukin-1β (IL-1β) and interleukin-10 (IL-10). Plasma lipase activity was determined with a Kodak Ectachem DT II System analyzer (Eastman Kodak Company, Rochester, NY, USA), using Lipa DT Slides (Vitros DT Chemistry System, Johnson & Johnson Clinical Diagnostic, Inc., Rochester, NY, USA). The values of plasma lipase activity were expressed as units/liter (U/L). Plasma IL-1β, and IL-10 were measured in duplicate, using the BioSource Cytoscreen rat IL-1β and IL-10 kit based on a solid phase sandwich Enzyme Linked Immuno Sorbent Assay (ELISA) (BioSource International, Camarillo, California, USA). Concentrations were expressed as pg/ml.
Determination of pancreatic DNA synthesis

After the blood withdrawal, the pancreas was carefully dissected out from its attachment to the stomach, the duodenum, and the spleen. Fat and peripancreatic tissue were trimmed away. Samples of pancreatic tissue were taken for study of DNA synthesis and morphological examination. The rate of DNA synthesis in the portion of minced pancreatic tissue was determined by incubating the tissue at 37°C for 45 min in 2 ml of medium containing 8 µCi /ml of \(^{3}H\)thymidine (\(^{6-3}H\)-thymidine, 20-30 Ci/mmol; Institute for Research, Production and Application of Radioisotopes, Prague, Czech Republic), as described previously (24). The incorporation of \(^{3}H\)thymidine into DNA was determined by counting 0.5 ml DNA-containing supernatant in a liquid scintillation system. DNA synthesis was expressed as \(^{3}H\)thymidine disintegrations per minute per microgram DNA (dpm/µg DNA).

Histological examination

Samples of pancreatic tissue excised from the body portion for morphological examination were fixed in 10% formalin, embedded in paraffin and sections were stained with hematoxilin and eosin. The slides were examined by two experienced pathologists without the knowledge of the treatment given. 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.

Statistical analysis

Comparison of the differences between the mean values of various groups of experiments was made by analysis of variance and the Student’s \(t\) test for unpaired data. A difference with a P value of less than 0.05 was considered statistically significant. Results are expressed as means (± S.E.M.).

RESULTS

Histological examination of pancreatic tissue

Pancreases of saline-injected (control) animals showed macroscopically and at light microscopic level no tissue alteration (Table 1). Also treatment with any dose of ghrelin alone did not affect morphology of pancreatic tissue (Table 1). Administration of caerulein caused 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 more than 50% of acinar cells in all cases.
Treatment with ghrelin, during induction of acute pancreatitis, attenuated the pancreatic tissue damage and this protective effect was dose-dependent (Table 1). Administration of ghrelin at the lowest dose: 2 x 2 nmol/kg slightly reduced caerulein-induced vacuolization of acinar cells but this dose of ghrelin was without effect on caerulein-induced pancreatic edema and inflammatory infiltration (Table 1). Marked protective effect on the pancreas was observed, when ghrelin was used in higher doses. Ghrelin at the dose 2 x 10 nmol/kg and at the dose 2 x 20 nmol/kg attenuated pancreatic damage in similar extent. Leukocytic infiltration and vacuolization of acinar cells were reduced, but pancreatic edema was not affected by any dose of ghrelin.

**Pancreatic blood flow**

Treatment with any doses of ghrelin was without effect on pancreatic blood flow in animals injected with physiological saline solution (Fig. 1). Caerulein-induced pancreatitis caused a reduction of pancreatic blood flow by 55% when compared to saline-injected control group. Treatment with any dose of ghrelin did not significantly affect the pancreatic blood flow in rats injected with caerulein.

**Pancreatic DNA synthesis**

In saline-injected control rats, pancreatic DNA synthesis reached 57.9 ± 4.0 dpm/µg DNA (Fig. 2). Treatment with any dose of ghrelin did not significantly affect the pancreatic DNA synthesis in animals injected with saline. In animals with caerulein-induced pancreatitis, pancreatic DNA synthesis was reduced by 40%. Ghrelin given at the dose 2 x 2 nmol/kg was without significant effect on caerulein-evoked fall in pancreatic DNA synthesis, but administration of ghrelin
at the doses 2 x 10 and 2 x 20 nmol/kg significantly reversed the reduction in DNA synthesis in rats with caerulein-induced pancreatitis by 37 and 42%, respectively.

**Plasma lipase activity and plasma IL-1β and IL-10 concentration**

Plasma lipase activity (*Fig. 3*) in control saline infused rats reached 58.3 ± 9.0 U/L. Administration of any dose of ghrelin did not significantly affect plasma lipase activity in rats injected with physiological saline solution. Injections with caerulein, causing acute pancreatitis, increased plasma lipase activity to 444.7 ± 22.8 U/L. Treatment with ghrelin, at the dose 2 x 2 nmol/kg, did not markedly affect the plasma lipase activity in animals with caerulein-induced pancreatitis. Ghrelin at the dose 2 x 10 and 2 x 20 nmol/kg, significantly reduced caerulein-evoked increase in plasma lipase activity by 35 and 40%, respectively.

In control rats, plasma IL-1β concentration reached value 79.7 ± 6.9 pg/mL (*Fig. 4*). Treatment with any dose of ghrelin was without a significant effect on plasma IL-1β level in saline-injected rats. Caerulein injections caused an increase in plasma IL-1β concentration to value 269.0 ± 9.0 pg/mL. Ghrelin administrated
at the dose 2 x 10 and 2 x 20 nmol/kg led to a significant reduction in caerulein-evoked increase in plasma IL-1β concentration. Ghrelin at the lowest dose 2 x 2 nmol/kg was without significant effect on plasma IL-1β concentration in animals with caerulein-induced pancreatitis.

In control rats, plasma IL-10 reached a value 69.7 ± 7.5 pg/ml (Fig. 5). Injection of caerulein or treatment with ghrelin given alone or in their combination did not significantly affect the plasma IL-10 concentration.

**DISCUSSION**

Previous study has shown that ghrelin exhibits gastroprotective activity (16). For this reason, we suspected that ghrelin might exhibit the similar protective effect in the pancreas. Our present study, for the first time, brings evidences, which support this hypothesis.

Acute pancreatitis is a pathological process dependent on premature activation of inactive zymogens into active digestive enzymes and autodigestion of pancreatic

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**Fig. 2.** Effect of ghrelin administration (given twice at the dose 2, 10 or 20 nmol/kg/injection) on pancreatic DNA synthesis in rats with or without caerulein-induced pancreatitis. Mean ± S.E.M. N=10 in each group of animals. a P<0.05 compared with control, b P<0.05 compared with caerulein given alone.
tissue. Active digestive enzymes act locally within the pancreas as well as they penetrate into blood vessels and through the bloodstream act systemically. Plasma lipase activity estimation has high sensitivity and specificity in the diagnosis of acute pancreatitis (25). In our present study, administration of ghrelin dose-dependently reduced plasma lipase activity. It is the direct evidence of ghrelin protective effect against pancreatic damage in the course of acute pancreatitis.

Activation of leukocytes and release of pro-inflammatory cytokines are responsible for local pancreatic damage and development of systemic inflammatory response syndrome (SIRS) and multiple organ failure (MOF) in the course of acute pancreatitis (26). Pro-inflammatory cytokines such as IL-1β, interleukin-6 (IL-6) and TNF-α (Tumor Necrosis Factor) are produced within pancreas and subsequently within distant organs, which develop dysfunction during severe pancreatitis (27). Pro-inflammatory cytokine production correlates with disease severity (27). IL-1β plays the most important role in the induction of systemic acute phase response and in the release other members of pro-inflammatory cytokine cascade (28). The role of IL-1β in the development of

Fig. 3. Effect of treatment with ghrelin (given twice at the dose 2, 10 or 20 nmol/kg/injection) on plasma lipase activity in rats with or without caerulein-induced pancreatitis. Mean ± S.E.M. N=10 in each group of animals. a P<0.05 compared with control, b P<0.05 compared with caerulein given alone.
acute pancreatitis has been shown by Norman and co-workers (29). They have shown that blockade of IL-1β prevents the rise in serum IL-6 and TNF-α level, and protects against pancreatic damage in the course of experimental acute pancreatitis. Their observation is in agreement with our present data and shows one of mechanisms, which is involved in the attenuation of pancreatic damage by ghrelin administration. Treatment with ghrelin has inhibited the leukocyte infiltration of pancreatic tissue and reduced the production of IL-1β leading to the limitation of pancreatitis severity.

In our present study, induction of acute pancreatitis has reduced the pancreatic DNA synthesis, what is in agreement with previous results from different models of acute pancreatitis (30, 31). Administration of ghrelin has attenuated the pancreatitis-induced fall of pancreatic DNA synthesis. This observation is an additional evidence of protective effect of ghrelin on the pancreas and indicates that ghrelin enhances an ability of pancreatic tissue to regeneration.

Ghrelin strongly and dose dependently stimulates growth hormone (GH) release (1). GH is the first hormone from GH-insulin-like growth factor-1 (IGF-1)-hepatocyte growth factor (HGF) axis (32, 33). It suggests that protective effect

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**Fig. 4.** Effect of ghrelin administration (given twice at the dose 2, 10, or 20 nmol/kg/injection) on plasma interleukin-1β concentration in rats with or without caerulein-induced pancreatitis. Mean ± S.E.M. N=10 in each group of animals. a P<0.05 compared with control, b P<0.05 compared with caerulein given alone.
of ghrelin administration on the pancreas may be dependent on the release of GH, IGF-1 or HGF. Previous studies have shown that treatment with IGF-1 (34) and HGF (35) inhibits development of caerulein-induced pancreatitis. However, in contrast to IGF-1 and HGF, administration of ghrelin has not reversed the pancreatitis-evoked drop of pancreatic blood flow. A disturbance of pancreatic microcirculation plays an important role in the development of acute pancreatitis (36, 37), leading to the formation of thrombi in capillaries, activation of leukocytes, release of proteolytic enzymes, formation of oxygen-derived free radicals and pro-inflammatory cytokines (36). The severity of such experimental pancreatitis has been found to be closely correlated with tissue ischemia (38). Reduction in pancreatic blood flow aggravates pancreatic damage in the course of acute pancreatitis (36, 39-41), whereas a vasodilatation and an improvement of pancreatic blood flow have been found to reduce the development of acute pancreatitis (40-42). The lack of effect of ghrelin administration on pancreatic blood in acute pancreatitis is well correlated with our observation that pancreatic edema has been also not affected by treatment with ghrelin.

Another difference between action of IGF-1 or HGF and ghrelin on the development of acute pancreatitis is their effect on IL-10 release. IL-10 has been
found to be a major anti-inflammatory cytokine. IL-10 reduces activation of macrophages and inhibits the production of inflammatory mediators (43, 44). On the second hand, administration of IL-10 before and during induction of acute pancreatitis decreases the severity of pancreatitis (45). Treatment with IGF-1 (34) or HGF (35) stimulates a release of anti-inflammatory IL-10, whereas treatment with ghrelin does not affect plasma IL-10 concentration in our present study. These differences between effects of IGF-1 or HGF and ghrelin suggest that protective effect of ghrelin administration on the pancreas is, at least in part, independent from GH - IGF-1 - HGF axis.

In summary, our present data demonstrate that pancreatic treatment with ghrelin reduces the severity of caerulein-induced pancreatitis in rats. A decrease in leukocyte activation, limitation of pro-inflammatory IL-1 release, as well as, the preservation of acinar cells integrity are probably the major mechanisms involved in beneficial effect of ghrelin on the pancreas.

REFERENCES


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