Previous studies have shown that pancreatic polypeptide (PP) inhibits exocrine pancreatic secretion. The aim of present study was to determine the influence of PP administration on gastric growth and blood flow. Methods: Study was performed on regularly fed, fasted or fasted and subsequently refed rats. Rats were treated with saline (intraperitoneally - i.p.), caerulein (0.24 nmol/kg/dose, i.p.), pentagastrin (0.38 µmol/kg/dose, i.p.) or PP (5 nmol/kg/dose, i.p. or 10 pmol/dose intracerebroventricularly - i.c.v.). Saline, caerulein, pentagastrin and PP were administered alone or in combination, 3 times daily during last 48 h of experiment. Results: Treatment with pentagastrin increased gastric mucosa weight, mucosal DNA synthesis and gastric blood flow in all group tested. Intraperitoneal and i.c.v administration of PP alone reduced mucosal DNA synthesis in regularly fed and refed animals, and decreased gastric blood flow in refed animals. Combination of PP i.p. or i.c.v plus pentagastrin significantly reduced the pentagastrin-evoked increase in gastric mucosa weight, gastric DNA synthesis and gastric blood flow in fasted animals, as well as regularly fed animals. In refed animals, influence of PP administration on the pentagastrin-evoked increase in gastric mucosa weight was weak and statistically insignificant, but still i.p or i.c.v administration of PP significantly reduced gastric blood flow and mucosal DNA synthesis in this group of animals. Administration of caerulein caused weak, but significant increase in gastric DNA synthesis, gastric mucosa weight and gastric blood flow in fasted rats. In regularly fed animals, caerulein significantly increased only gastric DNA synthesis and gastric blood flow. In fasted animals with subsequent refeeding, caerulein was without effect on parameters tested in the stomach. Neither i.p. nor i.c.v administration of PP affected the caerulein-evoked effects in the stomach. Conclusions: Peripheral and central administration of PP inhibits food- and pentagastrin-stimulated growth of gastric mucosa. Similar effects of low central doses of PP as the high peripheral doses of PP suggests a crucial role of the central nervous system in the inhibitory effect of PP on gastric mucosa growth.

Key words: pancreatic polypeptide, oxyntic mucosa growth, gastric blood flow, DNA synthesis
INTRODUCTION

Pancreatic polypeptide (PP) is a 36-amino acid polypeptide and belongs to the family of peptides including also peptide YY (PYY) and neuropeptide Y (NPY) (1). PP is produced by PP cells, which are localized in the periphery of pancreatic islets of Langerhans and scattered between the acinar cells of the pancreatic head and uncinate lobe (2). Physiologically, PP is released in response to food ingestion. Postprandial release of PP is mediated by a long vago-vagal reflexes and short local cholinergic pathways (3). Presence of pancreaticobiliary juice in the duodenum, gastrin, cholecystokinin (CCK), gastrin releasing peptide (GRP), neuromedin B and C, and secretin also stimulate PP secretion (4), but vagal cholinergic activity appears to be the most powerful stimulant of PP release (3, 5). The well known physiological function of PP is an inhibition of exocrine pancreatic and biliary secretion (6). Infusion of physiologic concentrations of PP inhibits basal and stimulated pancreatic secretion (6, 7) and this effect appears to be preferentially dependent on inhibition of vagal stimulation (8). In vitro studies with pancreatic lobules suggest that PP inhibits pancreatic enzyme secretion by a presynaptic modulation of acetylcholine release (9).

Recent studies indicate the influence of PP on food intake and energy balance. In humans, intravenous administration of PP reduces food intake in healthy volunteers (10) and a plasma level of PP is elevated in anorexia nervosa (11). In contrast, plasma level and secretion of PP is reduced in case of morbid obesity (12, 13) and in Prader-Willi syndrome, which is characterized by childhood-onset hyperphagia and morbid obesity (14). Also, studies on rodents have shown the influence of PP on food intake. Ueno et al. (15) have indicated that PP-overexpressing mice gain less weight with decreased food intake and fat mass (15). Asakawa et al. (16) have reported that intraperitoneal administration of PP reduces a food intake and increases energy expenditure.

The influence of PP on the gastric mucosa growth has not been tested. Therefore, the aim of present study was to determine the effect of central and peripheral administration of PP on the oxyntic mucosa growth in regularly fed, fasted or refed rats.

MATERIAL AND METHODS

Animals and treatment

Studies were performed on male Wistar rats weighing 260-280 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. Three series of experiments were carried out. Animals from the first series were kept on a regular feeding at the time of experiments, animals from the second series were fasted for 48 h before the end of study, and animals from the third series were fasted for 48 h and refeed for next 16 h before the end of study. All groups of rats had free access to water during the study. In regularly fed and refeed rats, the amount of food was restricted to 20 g/day/rat.
Rats from the each series of study were divided on the following experimental groups (ten animals in each group of each series of study): (1) control animals treated with saline (0.9% NaCl intraperitoneally - i.p.); (2-3) animals treated with PP (bovine pancreatic polypeptide, Lilly & Company, Indianapolis, In, USA; 5 nmol/kg/dose, i.p. or 10 pmol/dose intracerebroventricularly - i.c.v.); (4) animals treated with caerulein (Takus, Pharmacia & Upjohn GmbH, Erlangen, Germany; 0.24 nmol/kg/dose, i.p.); (5) animals treated with pentagastrin (Sigma Chemicals, St. Louis, Mo, USA; 0.38 µmol/kg/dose, i.p.); (6) animals treated with combination of PP (5 nmol/kg/dose, i.p.) plus caerulein (0.24 nmol/kg/dose, i.p.); (7) animals treated with combination of PP (5 nmol/kg/dose, i.p.) plus pentagastrin (0.38 µmol/kg/dose, i.p.); (8) animals treated with combination of PP (10 pmol/dose, i.c.v.) plus caerulein (0.24 nmol/kg/dose, i.p.); (9) animals treated with combination of PP (10 pmol/dose, i.c.v.) plus pentagastrin (0.38 µmol/kg/dose, i.p.) Saline, caerulein, pentagastrin and PP were administered 3 times daily during last 48 h of experiment.

Central administration of PP or saline was performed into right lateral cerebral ventricle, at the volume 5 µl/injection, as described previously (17). Briefly, animals were anesthetized with ketamine (50 mg/kg i.p., Bioketan, Biowet, Gorzów, Poland). An incision was made along the midline of the head, the skull bones were cleaned out from connective tissue with visualization of intersection between the sagittal and coronary sutures. The point at the distance of approximately 2.5 mm from both sutures was defined and in this place a small hole in the skull was made using a needle with a sharp end. The hole was made by a rotary movement of the needle. The effectiveness of cerebral ventricle penetration was verified by injection of 10 µl of 0.1% solution of toludine blue. The visualization of dye on the walls of right lateral cerebral ventricle indicated the exact location of intracerebroventricular injections.

Determination of gastric blood flow

At the end of experiments, animals were anesthetized with ketamine (50 mg/kg i.p., Bioketan, Biowet, Gorzów, Poland), the abdominal cavity was opened and the stomach was exposed. Gastric blood flow was measured by laser Doppler flowmeter using PeriFlux 4001 Master monitor (Perimed AB, Järfalla, Sweden), as described previously (18). Blood flow was measured in five different regions of the stomach and was expressed as percent change from control value.

Determination of gastric oxyntic mucosa weight and DNA synthesis

After measurement of gastric blood flow, the stomach was dissected out from the body, opened along the greater curvature and washed using solution of 0.9% NaCl. The oxyntic gland area was dissected out and weighed. The rate of DNA synthesis in the portion of minced pancreatic tissue oxyntic gland mucosa was determined by incubating the tissue at 37°C for 45 min in 2 ml of medium containing 8 µCi/ml of [3H]thymidine ([6-3H]thymidine, 20-30 Ci/mmol; Institute for Research, Production and Application of Radioisotopes, Prague, Czech Republic), as described previously (19). The incorporation of [3H]thymidine into DNA was determined by counting 0.5 ml DNA-containing supernatant in a liquid scintillation system. DNA synthesis was expressed as [3H]thymidine disintegrations per minute per microgram DNA (dpm/µg DNA).

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

Fasted animals

In fasted control rats, DNA synthesis in the oxyntic gland area of the stomach reached 27.8 ± 2.4 dpm/µg DNA (Fig. 1) and this value was significantly lower than mucosal DNA synthesis observed in regularly fed control rats. Treatment with PP i.p. or i.c.v. alone did not affect mucosal DNA synthesis in fasted animals. Administration of caerulein increased mucosal DNA synthesis by 73%, whereas pentagastrin increased DNA synthesis in gastric mucosa of fasted rats by 179%. PP given i.p. or i.c.v. was without significant effect on the caerulein-induced increase in DNA synthesis in oxyntic gland area in fasted rats, but significantly reduced the pentagastrin-induced increase in mucosal DNA synthesis in fasted rats.

In fasted control rats, the weight of the oxyntic gland area mucosa was significantly lower than the weight of this mucosa in regularly fed control animals.
(447.7 ± 18.2 v. 859.3 ± 23.4 mg) (Fig. 2). Caerulein, as well as pentagastrin increased the gastric mucosa weight in fasted rats by 30 and 110%, respectively. PP alone administered i.p. or i.c.v was without effect on the weight of the oxyntic gland area of the stomach. Also, treatment with PP, in both routes of administration, failed to affect the caerulein-induced increase in the gastric mucosa weight. PP administered i.p. or i.c.v significantly decreased the weight of the oxyntic gland area of the stomach in pentagastrin-treated rats.

In fasted rats, alterations in gastric blood flow generally paralleled the changes in the weight and DNA synthesis in the oxyntic gland area of the stomach (Fig. 3). Fasting significantly decreased gastric blood flow in control rats by 49%, when compared to that observed in control regularly fed rats. Caerulein and pentagastrin increased gastric blood flow in fasted rats by 36 and 75%, respectively. Effect of intraperitoneal or intracerebroventricular PP administered alone or in combination with caerulein was statistically insignificant. PP administered i.p. or i.c.v. significantly reduced the pentagastrin-evoked increase in gastric blood flow.

![Graph showing gastric mucosa weight changes](image.png)

**Fig. 2.** Effect of treatment with pancreatic polypeptide (PP 5 nmol/kg/dose - i.p. or 10 pmol/dose - i.c.v.), caerulein (0.24 nmol/kg/dose - i.p.) or pentagastrin (p-gastrin, 0.38 µmol/kg/dose - i.p.) given alone or in combination on gastric oxyntic mucosa weight in rats fasted for 48 h. Mean ± S.E.M. N=10 in each group of animals. aP<0.05 compared to regularly fed control, bP<0.05 compared to fasted control, cP<0.05 compared to pentagastrin given alone.
Regularly fed animals

In regularly fed control rats, DNA synthesis in the oxyntic gland area of the stomach reached a value 65.5 ± 1.7 dpm/µg DNA (Fig. 4). Administration of caerulein or pentagastrin alone significantly increased DNA synthesis in the oxyntic gland area by 25 and 44%, respectively. In contrast to fasted animals, treatment with PP i.p. or i.c.v. alone significantly decreased DNA synthesis in the oxyntic gland area in regularly fed animals by 13 and 14%, respectively. Also, treatment with PP i.p. or i.c.v. significantly reduced the pentagastrin-evoked increase in DNA synthesis in gastric mucosa in regularly fed rats. Neither PP given i.p. nor PP given i.c.v. affected the caerulein-induced increase in DNA synthesis in the oxyntic gland area of the stomach.

In regularly fed animals, the increase in the weight of the oxyntic gland area in the stomach was only observed in animals treated with pentagastrin (Fig. 5). Treatment with caerulein or PP alone failed to affect the gastric mucosa weight in regularly fed animals, but either PP given i.p. or i.c.v. significantly reduced the

![Graph](image_url)

**Fig. 3.** Effect of treatment with pancreatic polypeptide (PP 5 nmol/kg/dose - i.p. or 10 pmol/dose - i.c.v.), caerulein (0.24 nmol/kg/dose - i.p.) or pentagastrin (p-gastrin, 0.38 µmol/kg/dose - i.p.) given alone or in combination on gastric blood flow in rats fasted for 48 h. Mean ± S.E.M. N=10 in each group of animals. aP<0.05 compared to regularly fed control, bP<0.05 compared to fasted control, cP<0.05 compared to pentagastrin given alone.
pentagastrin-evoked increase in the weight of the oxyntic gland area in the stomach in regularly fed animals.

Treatment with caerulein and pentagastrin significantly increased gastric blood flow in regularly fed animals by 23 and 38%, respectively (Fig. 6). PP given alone i.p. or i.c.v. did not significantly affect gastric blood flow. Also, PP given i.p. or i.c.v. failed to affect the caerulein-induced increase in gastric blood flow. Treatment with PP, in both routes of administration, significantly decreased the pentagastrin-induced increase in gastric blood flow in regularly fed animals.

Refed animals

In control animals with 48-h fasting and subsequent 16-h refeeding, DNA synthesis in the oxyntic gland area of the stomach reached 78.8 ± 2.1 dpm/µg DNA and was higher by 20% than that observed in regularly fed control rats (Fig. 7). In refed animals, PP alone administered i.p. or i.c.v. caused a small but significant decrease in DNA synthesis in the oxyntic gland area by 9 and 10%,

![Graph](image-url)

_Fig. 4. Effect of treatment with pancreatic polypeptide (PP 5 nmol/kg/dose - i.p. or 10 pmol/dose - i.c.v.), caerulein (0.24 nmol/kg/dose - i.p.) or pentagastrin (p-gastrin, 0.38 µmol/kg/dose - i.p.) given alone or in combination on gastric mucosa DNA synthesis in regularly fed rats. Mean ± S.E.M. N=10 in each group of animals. ^P<0.05 compared to control, ^P<0.05 compared to pentagastrin given alone._
respectively. Caerulein alone did not significantly affect DNA synthesis in gastric mucosa in refed rats. Pentagastrin alone increased DNA synthesis in the oxyntic gland area of the stomach in refed animals by 25%. In refed animals treated with combination of PP i.p. or i.c.v. plus caerulein, DNA synthesis in gastric mucosa did not significantly differ than that observed in refed control rats. Administration of PP i.p. or i.c.v. significantly reduced the pentagastrin-induced increase in DNA synthesis in the oxyntic gland area of the stomach in refed animals.

In refed control animals, the weight of the oxyntic gland area in the stomach reached a value of 815.1 ± 35.6 mg (Fig. 8). Neither PP nor caerulein given alone nor their combination significantly affected gastric mucosa weight in refed animals. Pentagastrin significantly increased the weight of the oxyntic gland area in the stomach of refed rats. PP given i.p. or i.c.v. in combination with pentagastrin tented to reduce the pentagastrin-induced increase in oxyntic gland area weight, but this effect was statistically insignificant.

![Fig. 5. Effect of treatment with pancreatic polypeptide (PP 5 nmol/kg/dose - i.p. or 10 pmol/dose - i.c.v.), caerulein (0.24 nmol/kg/dose - i.p.) or pentagastrin (p-gastrin, 0.38 µmol/kg/dose - i.p.) given alone or in combination on gastric oxyntic mucosa weight in regularly fed rats. Mean ± S.E.M. N=10 in each group of animals. aP<0.05 compared to control, bP<0.05 compared to pentagastrin given alone.](image-url)
In refed control rats, gastric blood flow was significantly higher than in control of regularly fed rats (Fig. 9). PP administered alone i.p. or i.c.v. decreased gastric blood flow by 14 and 15%, respectively. Administration of caerulein or pentagastrin increased gastric blood flow by 14 and 27%, respectively. PP given i.p. or i.c.v. in combination with pentagastrin significantly reduced pentagastrin-evoked increase in gastric blood flow. Combination of PP i.p. or i.c.v. plus caerulein tended to reduce the caerulein-induced increase in gastric blood, but this effect was not statistically significant.

**DISCUSSION**

The presence of food in the gut is the most important factor in the regulation of gastrointestinal mucosa growth (20). Constituents of the diet in the gastrointestinal tract may directly (21) and indirectly stimulate growth of the mucosa. Indirect stimulation of gastrointestinal mucosa growth involves a variety of events, such as the release of trophic hormones, stimulation of nerves, and the
activation of exocrine secretion, motility and absorption (22). Contrary, fasting (23) or total intravenous alimentation (24) produces atrophy of gastric and intestinal mucosa.

Our present study confirms and extends previous findings that food intake affects the growth of gastrointestinal mucosa (25). Fasting for 48 h caused the decrease in gastric mucosa DNA synthesis and weight of the oxyntic gland area of the stomach, whereas refeeding of fasted rats markedly increased DNA synthesis in the oxyntic gland area of the stomach and caused restoration of the gastric mucosa weight. These changes were correlated with changes of gastric blood flow. Fasting significantly reduced gastric blood flow, while refeeding enhanced gastric blood flow above a value found in regularly fed animals.

In our present study, either intraperitoneal or intracerebroventricular administration of PP significantly reduced mucosal DNA synthesis in regularly fed and refed control animals, and significantly decreased gastric blood flow in refed control animals. This observation indicates that exogenous PP is able to

Fig. 7. Effect of treatment with pancreatic polypeptide (PP 5 nmol/kg/dose - i.p. or 10 pmol/dose - i.c.v.), caerulein (0.24 nmol/kg/dose - i.p.) or pentagastrin (p-gastrin, 0.38 µmol/kg/dose - i.p.) given alone or in combination on gastric mucosa DNA synthesis in rats fasted for 48 h and subsequently refeed for 16 h. Mean ± S.E.M. N=10 in each group of animals. aP<0.05 compared to regularly fed control, bP<0.05 compared to refeed control, cP<0.05 compared to pentagastrin given alone.
inhibit the food-stimulated growth of gastric mucosa. The mechanism of this effect is not clear. Previous studies have shown that peripheral administration of PP decreases food intake (10, 16), whereas PP administered into the brain increases food intake (26, 27). In our study, food intake was controlled and for this reason all animals from fed and refed groups consumed the same amount of food. It indicates that PP-evoked decrease in gastric DNA synthesis in fed and refed animals is not related to the influence of PP on food intake.

In the present study, we observed the increase in gastric oxyntic mucosa weight and DNA synthesis, and gastric blood flow in all animals treated with pentagastrin. The highest increase was observed in fasted animals. This result is in agreement with previous studies, which have showed a growth-promoting effect of exogenous and endogenous gastrin in the stomach (28-30). Our present study, for the first time, has shown that central and peripheral treatment with PP inhibits pentagastrin-induced increase in gastric oxyntic mucosa growth. Physiologically, PP is released in response to food (3), but also gastrin stimulates

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<tr>
<th>Treatment</th>
<th>Gastric oxyntic mucosa weight (mg)</th>
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<tr>
<td>REGULARLY FED CONTROL</td>
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<td>CONTROL</td>
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<td>PP i.p.</td>
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<td>CAERULEIN</td>
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*Fig. 8. Effect of treatment with pancreatic polypeptide (PP 5 nmol/kg/dose - i.p. or 10 pmol/dose - i.c.v.), caerulein (0.24 nmol/kg/dose - i.p.) or pentagastrin (p-gastrin, 0.38 µmol/kg/dose - i.p.) given alone or in combination on gastric oxyntic mucosa weight in rats fasted for 48 h and subsequently refed for 16 h. Mean ± S.E.M. N=10 in each group of animals. aP<0.05 compared to regularly fed control, bP<0.05 compared to refed control.*
PP release and a postprandial increase in PP level is preceded gastrin (31, 32). These data and our present observation suggest that PP may play a role in the endogenous mechanisms, which prevent the gastric mucosa against gastrin-evoked hyperplasia. The support for this hypothesis is observation that an increase in PP within physiological range reduces the gastrin response to a protein-rich meal (33).

In the stomach, gastrin acts by binding to CCK-B receptors. CCK and its analog-caerulein have a high affinity to CCK-A receptor, but a low affinity to CCK-B receptors, which are present in the stomach (34). In the present study, we have used low doses of caerulein, because high doses of this peptide lead to the development of acute edematous pancreatitis (35). Caerulein was used at the dose 0.24 nmol/kg/dose 3 times daily, and this dose did not significantly affect the growth of oxyntic mucosa in refed animals, when the high levels of endogenous gastrin and CCK are expected (36). In fasted rats, endogenous levels of gastrin and CCK are decreased (36) and for this reason exogenous caerulein caused a

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*Fig. 9. Effect of treatment with pancreatic polypeptide (PP 5 nmol/kg/dose - i.p. or 10 pmol/dose - i.c.v.), caerulein (0.24 nmol/kg/dose - i.p.) or pentagastrin (p-gastrin, 0.38 µmol/kg/dose - i.p.) given alone or in combination on gastric blood flow in rats fasted for 48 h and subsequently refed for 16 h. Mean ± S.E.M. N=10 in each group of animals. aP<0.05 compared to regularly fed control, bP<0.05 compared to refed control, cP<0.05 compared to pentagastrin given alone.*
significant stimulation of gastric oxyntic mucosa growth and gastric blood flow. It is of interest that exogenous PP given peripherally or centrally failed to affect the growth promoting effect of caerulein in the stomach probably because the mucosal growth promoting action of caerulein was rather weak. In contrast, pentagastrin-induced stimulation of mucosal growth was significantly reduced by both central and peripheral application of PP, indicating that the inhibitory effect of PP on gastric mucosal growth depends upon the rate of mucosal proliferation and growth.

Recent studies postulate the role of brain-gut axis in regulation of gut function and appetite control (37). In our present study, the dose of PP administered intraperitoneally was 100-fold higher than the dose of PP applied intracerebrally. However, both doses and routes of PP administration affected gastric mucosa growth and gastric blood flow at the same extent. This observation suggests that peripherally applied PP acts on mucosal growth, at least in part, via central mechanism. This hypothesis is in agreement with the study, which has demonstrated that intraperitoneal administration of PP decreases food intake and increases energy expenditure (16). The mechanism of this effect involves a modification by PP of activity of the vago-vagal and vago-sympathetic reflexes and an alteration of gene expression of hypothalamic feeding regulatory peptides (16). These findings taken together suggest the participation of the central nervous system in the effects of peripherally administered PP on gastric mucosal growth and mucosal blood flow. Further studies are needed to determine whether the effects of PP on gastric mucosal growth and circulation acting via central and peripheral mechanisms involve peripheral neural system, growth factors or both.

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