Apelin is considered as important gut regulatory peptide ligand of APJ receptor with a potential physiological role in gastrointestinal cytoprotection, regulation of food intake and drinking behavior. Circulating apelin inhibits secretion of pancreatic juice through vagal-cholecystokinin-dependent mechanism and reduces local blood flow. Our study was aimed to determine the effect of fundectomy and intraperitoneal or intragastric administration of apelin-13 on pancreatic and gastric enzymes activities in adult rats. Fundectomy is a surgical removal of stomach fundus - main site apelin synthesis. Three independent experiments were carried out on Wistar rats. In the first and second experiment apelin-13 was given by intragastric or intraperitoneal way twice a day for 10 days (100 nmol/kg b.w.). Control groups received the physiological saline respectively. In the third experiment the group of rats after fundectomy were used. Fundectomized rats did not receive apelin and the rats from control group were ‘sham operated’. At the end of experiment rats were sacrificed and blood from rats was withdrawn for apelin and CCK (cholecystokinin) radioimmunoassay analysis and pancreas and stomach tissues were collected for enzyme activity analyses. Intragastric and intraperitoneal administrations of apelin-13 increased basal plasma CCK level and stimulated gastric and pancreatic enzymes activity in rats. In animals after fundectomy decreased activity of studied enzymes was observed, as well as basal plasma apelin and CCK levels. In conclusion, apelin can effects on CCK release and stimulates some gastric and pancreatic enzymes activity in adult rats while fudectomy suppresses those processes. Changes in the level of pancreatic lipase activity point out that apelin may occurs as a regulator of lipase secretion.

Key words: apelin, cholecystokinin, fundectomy, pancreatic enzymes activity, gastric enzymes activity

INTRODUCTION

Apelin is an endogenous ligand of the orphan G protein-coupled receptor APJ (1). Native peptide of apelin is produced through processing from the C-terminal portion in the pre-proprotein consisting of 77 amino acid residues. During posttranslational processing of pre-proprotein several molecular active forms of apelin are synthesized, containing of 12, 13, 17, or 36 amino acids and the pyroglutamated apelin-13 (Pyr(1)-apelin-13) (2). Apelin-13 and apelin-36 differ in receptor binding affinity and in their ability to affect the intracellular trafficking of the apelin receptor (2-4). The APJ is a 380 amino acid 7-transmembrane domain G-coupled receptor showing a close sequence homology to the angiotensin II receptor type 1 (30 – 40% identity in amino acid sequence), but angiotensin II does not interact with the APJ receptor when expressed in Chinese hamster ovary cells or in fibroblasts (4, 5).

Apelin and APJ receptor have a widespread distribution in the body (central nervous system, heart, lung, placenta, mammary gland, adipocytes) (6, 7). In the gastrointestinal tract the expression of apelin peptide was found chiefly in the stomach mucosa, and to a lesser degree in the duodenum and in the villi in jejunum (8). APJ receptor was reported in gastric fundic glands, duodenal tunica mucosa and in the upper half of jejunal villi (8-10). In the pancreas APJ receptor is expressed in pancreatic acinar, duct and islets cells (8).

Apelin is involved in various physiological functions, such as regulation of homeostasis and food intake (11), cardiovascular system development and cardiac muscle contraction, reduction of blood pressure (12, 13) and modulation of the pituitary hormone secretion (11). In the gastrointestinal tract, apelin may stimulate gastric acid (14), and duodenal electrolyte and cholecystokinin (CCK) secretion (8, 15, 16). In the pancreas, it may influence the response to insulin (17). Apelin was shown to control the epithelial cell turnover in vitro, since it stimulated proliferation of gastric epithelial cell line (SIIA) (16), and suppressed apoptosis, mitosis and 8-oxoguanine (OGG 1,2) of rat intestinal crypt cell line IEC-6 (18). Recently, these findings were confirmed in vivo in rats (19). However, the role of apelin in controlling the digestive enzyme secretion remains still unclear. The present study is aimed to better understand the role of apelin in controlling the activity of major gastric and...
pancreatic digestive enzymes. In our in vivo rat model, apelin-13 was either administered into the gastric lumen or intraperitoneally or a part of the stomach was surgically removed (fundectomy) to eliminate a part of endogenous apelin production.

MATERIALS AND METHODS

Animals

The experiments on animals were approved by the Local Ethics Committee. Three independent experiments were carry out on Wistar male rats (200 ± 10 g of body weight (b.w.)). In each experiment rats were divided into control and experimental groups for 6 rats in each group. Rats were housed in a light- and temperature-controlled room with free access to standard feed and water. In the first and second experiment apelin-13 (Hokuriku University, Japan) was given by intragastric or intraperitoneal way twice a day for 10 days (100 nmol/kg b.w.). Control groups received physiological saline in the corresponding way. In the third experiment rats after fundectomy were used. The surgery comprising of partial removal of the gastric fundus (fundectomy) was performed under general anaesthesia (atropine sulfate, s.c., 0.15 mg/kg body weight, b.w. Polfa, Poland; 2% xylasine, s.c., 3 mg/kg b.w., Rometar, PPHU INEX, Poland; and ketamine, s.c., 25 mg/kg b.w., 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. The remaining stomach was sutured with the two layers of non-absorbable sutures and laparotomy was closed. The rats from control group were ‘sham operated’, it means that animals underwent sham laparotomy involving exposure of the gastrointestinal tissues and gentle palpation for 20 min. Antibiotics (Amoxycillin s.c., 150 mg/kg b.w., 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. The fundectomized rats did not receive any apelin during experiment. At the end of each experiments about 12 hours after apelin administration, the rats were sacrificed with barbiturate overdose. Next blood was withdrawn from the heart of unconscious animals for hormones analysis, and gastric and pancreatic tissues were immediately collected and frozen for total protein content and enzymes activity analysis. For comparability of results in fundectomized and ‘sham operated’ animals the level of protein content in stomach was determined only in the cardiac and pyloric regions. Gastric enzymes activity was not measured in fundectomized rats because in this group the fundus (main portion of the stomach) was removed.

Apelin and cholecystokinin (CCK) concentration in plasma

Blood samples 1 – 2 ml were withdrawn from the heart of unconscious animals at the end of study immediately before collecting gastric and pancreatic tissues. Blood samples were preserved using 10% EDTA (Sigma, USA), centrifuged for 10 min at 4500 rpm at 4°C, and plasma aliquots were stored frozen (–20°C) until further analysis. Plasma concentrations of apelin and CCK were measured by radioimmunoassay using a commercially available RIA-kits. Plasma apelin was measured using Apelin - 36 Rat, Mouse a RIA-kit (Phoenix Pharmaceutical Inc, Germany). This kit allow us measure overall level of apelin in blood because has cross reactivity with all molecular isoforms of apelin. Plasma CCK was measured using a RIA-kit for CCK (Phoenix Pharmaceutical Inc, Germany).

Gastric and pancreatic enzymes activity

Samples of gastric wall and pancreas tissue were frozen in liquid nitrogen and stored at –80°C until further assays. Before enzymatic analysis pancreas was homogenized. In the obtained homogenates total protein content and the activity of trypsin, amylase and lipase were analyzed. Total protein content was measured by the Lowry method modified to be performed in 96-microwell plates (20). Trypsin activity was measured with use of Trypsin Activity Assay Kit (Bio Vision, USA); amylase activity was measured using QuantiChrom™ G-Amylase Assay Kit DAMY-100 (Bioassays Systems, USA) and lipase activity was measured using a Lipase-PS kit QuantiChrom™ Lipase Assay Kit (Bioassays Systems, USA).

For gastric enzymatic analysis stomach tissues were homogenized and in the homogenates total protein content and pepsin and rennet activity were analyzed. Total protein content was measured like described above. Pepsin activity was determined according to the Walker method (21, 22). Rennet was measured according to Erlanger et al. using BAPNA (N-benzoyl-DL-arginine-p-nitroanilide, Sigma, USA) as the substrate (23).

Statistical analysis

Data are expressed as their means ± standard errors of mean (S.E.M.). The unpaired Student’s t-test was used to indicate the statistical differences between the groups in each experiment (Statistica v.10.0, StatSoft Poland). In all statistical analysis P < 0.05 was taken as the level of significance.

RESULTS

Plasma apelin and cholecystokinin concentrations were lower in the fundectomized rats as compared to their ‘sham operated’ controls (Table 1). Intragastric and intraperitoneal administrations of apelin-13 had no effect on plasma apelin concentration, but increased (P < 0.001) cholecystokinin level in blood (Table 2).

In fundectomized animals an increased (P < 0.001) total protein content (Fig. 1A) and decreased (P < 0.001) amylase (Fig. 1C) and lipase (Fig. 1D) activity was observed in rat pancreatic homogenates as compared with control group. No effect of fundectomy on trypsin (Fig. 1B) activity in pancreatic homogenates was determined.

Intragastric administration of apelin-13 increased (P < 0.001) total protein content (Fig. 2A) and trypsin (Fig. 2B), amylase (Fig. 2C) and lipase (Fig. 2D) activity in rat pancreatic homogenates as compared with control group.

In rats which received apelin-13 intraperitoneally increased (P < 0.001) amylase (Fig. 3C) and lipase (Fig. 3D) activity was observed in pancreas tissue, but no effect on total protein content (Fig. 3A) and trypsin (Fig. 3B) activity was stated as compared with control group.

The fundectomy significantly decreased (P < 0.001) level of total protein content in stomach in comparision with control ‘sham operated’ group (Fig. 4). For comparability of the obtained results in fundectomized and ‘sham operated’ animals level of total protein content was determined only in the cardiac and pyloric regions of stomach.

Both intragastric (Fig. 5C) and intraperitoneal (Fig. 5D) administrations of apelin-13 increased (P < 0.001) the rennet activity.
activity in the stomach tissue, but had no effect on total protein content and pepsin activity as compared with control group.

**DISCUSSION**

The results presented in this study show that intragastric or intraperitoneal administration of apelin-13 increases basal plasma cholecystokinin but no effect on plasma apelin level. Moreover apelin-13 stimulates the activity of gastric rennet and pancreatic enzymes in adult rats. The inhibitory effect is observed in animals after fundectomy. The luck of result in increased plasma apelin level in groups after apelin administration could be connected with circulating half-life of apelin in blood. In our experiments blood samples were withdrawn from the rats heart about 12 hours after apelin administration, after the animals were sacrificed. We did not take blood intravital directly during experiment, because of the high

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**Table 1.** Plasma apelin and cholecystokinin (CCK) levels in control and fundectomized rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Apelin pmol/l</th>
<th>CCK pmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5472 ± 238</td>
<td>40.6 ± 6.9</td>
</tr>
<tr>
<td>Fundectomized</td>
<td>4062 ± 251</td>
<td>21.3 ± 2.4</td>
</tr>
</tbody>
</table>

Value are given as means ± S.E.M. (n = 6). *** - indicates value significantly different from control value according to Student's t-test (P < 0.001).

**Table 2.** Plasma apelin and cholecystokinin (CCK) levels in rats after intragastric (i.g.) and intraperitoneal (i.p.) infusion of apelin-13 (100 nmol/kg b.w. twice per day for 10 days).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Apelin pmol/l</th>
<th>CCK pmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5585 ± 266</td>
<td>33.1 ± 5.5</td>
</tr>
<tr>
<td>Apelin, i.g.</td>
<td>5760 ± 251</td>
<td>50.9 ± 4.0***</td>
</tr>
<tr>
<td>Control</td>
<td>5244 ± 242</td>
<td>42.4 ± 3.9</td>
</tr>
<tr>
<td>Apelin, i.p.</td>
<td>5437 ± 227</td>
<td>55.1 ± 3.1***</td>
</tr>
</tbody>
</table>

Value are given as means ± S.E.M., (n=6). *** - indicates value significantly different from control value according to Student's t-test (P < 0.001).

Fig. 1. Protein content (mg/g of tissue) (A) and pancreatic enzymes: trypsin (B), amylase (C), lipase (D) activity (U/g of tissue) in fundectomized rats. Values are given as means ± S.E.M., (n = 6). Student's t-test (***P < 0.001).
mortality amount the rats after intravital blood collection is occurred. The cause of a significant decrease of plasma apelin level in rats after fundectomy may be due to removal of the main site of endogenous apelin and many hormones synthesis such as leptin, obestatin, grelin and gastrin. All these hormones have a significant impact on physiological processes occurring in organism. But there are reports that shows negative effects hormones produced in stomach on gastrointestinal function. Such, intraperitoneal gastrin significantly increase intestinal polyposis in APC(Min+/−) mice and gastrin receptor may mediate...
proliferative signaling in macrophages cells and fostering tumor microenvironment (24). Surgical fundectomy also causes reduction of hydrochloric acid secretion in the stomach which stimulates the bile and pancreas flows through circulating secretin. These facts explain the cause of pancreatic enzymes secretion reduction in fundectomized rats. Other reason of decreased activity of pancreatic enzymes could be due to removal of neural gastro-pancreatic reflexes that originate in the stomach fundus.

Results obtained from experiments where apelin-13 was administrated intragastric showed increasing plasma CCK level and increasing all studied pancreatic enzymes activity. Similar data was obtained from experiments where apelin-13 was administrated intraperitoneal way, excluding trypsin activity and protein content level. Intraperitoneal administration of apelin-13 was not effect on the trypsin activity and protein content level. The reason for such results may be connected with different signaling pathways involved in the pancreatic enzymes secretion. For example inhibition of phosphodiesterase 3B (PDE3B) by amrinone in pancreatic islets in rats significantly augments insulinotropic action of physiological glucose in β-cells (25). It is known that pancreatic secretion is regulated by a complex neurohormonal system involving the central nervous system, the afferent and efferent vagus, the sympathetic nerves and enteric nervous system operating in the stomach, duodenum and in the pancreas (12, 26-29). To the exactly explain why intraperitoneal administration of apelin-13 no effect on enzyme which responsible for protein digestibility the further studies are required to clarify the mechanism via apelin affects in this proces.

Recent in vitro studies demonstrated that apelin-13 stimulated CCK release from a murine small-intestinal cells line (STC-1) expressing and secreting CCK (16). Also in in vitro experiment on dispersed acinar cells obtained from rat pancreas the highest dose of apelin-13 stimulated basal and potentiated CCK-8-stimulated amylase release whereas lower doses of apelin-13 were ineffective (8). These results suggest that apelin can act directly on CCK cells and exerts stimulatory effects on the digestibility enzymes activity. Kapica et al. (8) studied effect of intravenous and intraduodenal administration of apelin on pancreatobiliary juice secretion in rats. They found out that intravenous injection of apelin reduced pancreatobiliary volume and pancreatic output. While intraduodenal boluses of apelin led to dose-dependent stimulation of pancreatic juice volume and protein (trypsin) output (8). It is suggested that apelin effect dependents on intact mucosal CCK1-vagal mechanism that operates in the duodenum (30-32). Similar effect was noticed in the present study, both intragastric and

**Fig. 4.** Protein content (mg/g of tissue) in rats stomach after fundectomy. Values are given as means ± S.E.M., (n = 6). Student’s t-test (**P < 0.001). Gastric enzymes activity was not measured in fundectomized rats because in this group the fundus (main part of the stomach) was removed.

**Fig. 5.** Protein content (mg/g of tissue) (A) and gastric enzymes (U/g of tissue): pepsin (B), rennet (C), activity (U/g of tissue) in rats after intragastric (i.g.) administration of apelin-13. Values are given as means ± S.E.M., (n = 6). Student’s t-test (**P < 0.001).
intraperitoneal administration of apelin acts stimulatory on some digestive enzymes secretion in stomach and pancreases. In our previous experiment carried out on young rats intraperitoneal injection of apelin -13 stimulated total protein output and pancreatic enzymes activity, but had no effect on stomach protein output and the activity of gastric enzymes. Whereas intragastric administration of apelin-13 to young rats stimulated only the activity of pancreatic lipase and also had no effect on the protein output and the gastric enzymes activity (data in press). In these experiments we also discovered that intragastric and intraperitoneal administrations apelin-13 increased, but fundectomy decreased, level of pancreatic lipase activity. These data point out that gastric apelin may modulates of lipase secretion and takes part in lipid metabolism. The literature data shown that apelin also takes part in regulation of body adiposity (33, 34). Than et al., in experiment on transfected by apelin-siRNA or APLNR-siRNA vectors preadipocytes cells line (3T3-L1) discovered that apelin is able to inhibit lipolysis by stabilizing the lipid droplets. They also observed that long-term exposure of exogenous apelin stimulated the expression of lipid-droplet-coating protein and reduced the release of free fatty acids (34).

In conclusion, regardless the way of administration apelin-13 stimulates basal plasma cholecystokinin release and some pancreatic and gastric enzymes activity in adult rats. The reduction of gastric apelin may act inhibitory on cholecystokinin release and pancreatic enzymes activity. Changes in the level of pancreatic lipase activity point out that apelin may occurs as a regulator of lipase secretion.

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