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THE EFFECT OF ANTAGONIST OF RECEPTORS FOR GASTRIN, CHOLECYSTOKININ AND BOMBESIN ON GROWTH OF GASTRODUODENAL MUCOSA AND PANCREAS

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The effects of gastrin, cholecystokinin (CCK) and bombesin on the DNA synthesis, as a biochemical indicator of trophic action in the gastroduodenal mucosa and the pancreas, have been examined in rats fasted for 48 h and in rats refed for 16 h with or without administration of specific receptor antagonists for bombesin, gastrin and CCK. Bombesin and gastrin administered three times daily for 48 h in fasted rats significantly increased the rate of DNA synthesis as measured by the incorporation of [3H] thymidine into DNA in each tissue tested. CCK significantly increased DNA synthesis in the duodenal mucosa and pancreatic tissue, but not in the gastric mucosa. The stimulation of DNA synthesis induced by bombesin in the gastroduodenal mucosa and pancreas was abolished by bombesin/GRP receptor antagonist, RC-3095. RC-3095 did not affect DNA synthesis stimulated by gastrin and CCK in these tissues. L-365,260, a receptor antagonist for gastrin suppressed the DNA synthesis induced by gastrin but not by CCK or bombesin in the gastrointestinal mucosa and pancreas. L-364,718, a specific antagonist for CCK receptors was effective only against CCK stimulated duodenal mucosa and pancreatic growth. Refeeding of 48 h fasting rats strongly enhanced the DNA synthesis in all tissues tested, and this effect was significantly reduced in the gastroduodenal mucosa by blocking only gastrin receptors (with L-365, 260) and that in the duodenal mucosa and the pancreas by antagonizing of CCK receptors (with L-364, 718). Antagonism of bombesin receptors (with RC-3095) did not significantly affect the stimulation of DNA synthesis induced by refeeding in all tissues tested. This study indicates that the stimulation of DNA synthesis can be achieved by exogenous gastrin, CCK and bombesin acting through separate receptors, but that only gastrin and CCK play the major role in the postprandial stimulation of the growth of gastroduodenal mucosa and pancreatic tissue.

Key-words: growth, gastroduodenal mucosa, pancreas, DNA synthesis, bombesin, gastrin, CCK.
INTRODUCTION

Ingestion and digestion of a meal influence gastrointestinal mucosal growth (1) and play an important role in maintaining the pancreatic mass and structure in animals and in humans (2). Conversely, fasting (3) produces mucosal hypoplasia and mucosal atrophy. Fasting of rats for more than 48 h caused a marked decrease in the pancreatic mass, total content of DNA and protein in the pancreatic tissue (4, 5). Fasting for 3 weeks resulted in over 50% reduction in the pancreatic mass and in the RNA and DNA contents (6, 7). Parenteral hyperalimentation developed gastric and duodenal atrophy (8). In the pancreas, a liquid diet also resulted in a significant decrease in pancreatic weight and this was accompanied by a significant reduction in plasma gastrin concentration (9). The presence of food in the gut is the primary stimulus for gastrointestinal hormone release (10) and the decrease in the growth of gastroduodenal mucosa caused by fasting or liquid diet was reversed by pentagastrin (9, 11). In the pancreas the decrease in the growth caused by liquid diet or fasting was partly prevented by infusion of pentagastrin (9, 11) or a combination of secretin and CCK (12). These data suggest that feeding-related alterations in the growth of gastroduodenal mucosa and pancreatic tissue might be attributed, at least in part, to the release and action of gut hormones such as gastrin and CCK.

Another hormonal peptide, bombesin, isolated from the amphibian skin (13), which has a mammalian counterpart, a gastrin-releasing peptide (GRP) present in the brain and gut (14), was shown to release gastrin and CCK (15, 16) and to stimulate directly the growth of the gastroduodenal mucosa and pancreas (17, 18). Despite numerous studies on the effect of exogenous peptides on the growth of the gastroduodenal mucosa and pancreas, the relative contribution of these peptides to the physiological control of the trophic function of the gut has not been established.

This study was undertaken to compare the trophic responses to hormonal peptides and feeding, and to determine the growth effects of antagonism of receptors for these peptides using highly specific antagonists.

MATERIALS AND METHODS

Studies were performed on male Sprague-Dawley rats weighing 150—175 g. The animals were housed in cages with wire-mesh bottoms in a room with a 12 h light-dark cycle. The trophic effects of the substances tested were examined in two separate series of experiments (A and B).

In the A series rats were randomly divided into sixteen groups. The animals were fasted for 46 hours, drinking water was available ad libitum. All rats were injected intraperitoneally (i. p.) three times daily at 8 h intervals during the experiment with the
following agents: 1) L-364, 718 (1 mg/kg), L-365, 260 (1 mg/kg) or RC-3095 (10 μg/kg); 2) CCK-8 alone (320 ng/kg); 3) CCK (320 ng/kg) combined with L-364, 718, L-365, 260 or RC-3095; 4) bombesin alone (10 μg/kg); 5) bombesin combined with L-364, 718, L-365, 260 or RC-3095; 6) pentagastrin (250 μg/kg) alone; 7) pentagastrin combined with L-364, 718, L-365, 260 or RC-3095 and 8) 154 mM NaCl in a volume (1 ml) equal to that of the other compounds.

In the B series, the rats were divided into five groups. In groups 1—4 the animals were fasted for 48 h and refed for the last 16 h. During this period of time they received injections of L-364, 718, L-365, 260, RC-3095 or 154 mM NaCl in the same doses and intervals as in series A. The fifth group received food ad libitum without any fasting period (normally fed group) and 154 mM NaCl solution i. p. was given. All animals received a final injection of the tested substances one hour before sacrifice. Each experimental group included five rats and each study was repeated twice so that N = 10 for each series of experiments.

After treatment, the animals were killed and the stomach, duodenum and pancreas were removed and placed in ice cold saline. The stomach was opened along the greater curvature and washed. The oxyntic gland area was dissected out. A segment 2 cm long of the duodenum was removed, opened and rinsed. The 2 cm duodenum segment was taken about 1 cm past the pylorus. The mucosa was scraped from tissue samples, using a glass microscope slide over a glass plate. The pancreas was dissected free of mesentery and fat, and minced. The rate of DNA synthesis in the mucosa and minced pancreatic tissue was determined by incubating the tissue at 37°C for 30 min in an incubation medium (Gibco Lab., Grand Island, NY, USA) containing 2 μCi/ml [3H]thymidine (19.3 Ci/nmol, New England Nuclear, Boston, MA, USA) as described by Johnson et al (19). During incubations, the flasks were gassed continuously with 95% O2 and 5% CO2. The reaction was stopped with 0.4 M-perchloric acid containing carrier thymidine et a concentration of 5 mM. Samples were washed in 0.2 M-perchloric acid and RNA was removed by hydrolysis in 0.3 M-KOH for 60 min at 37°C. DNA supernatant was measured using orcinol reaction (20). After standing on ice for 10 min, the DNA-containing tubes were centrifuged and the supernatant discarded. DNA in the residual pellet was solubilized in 10% perchloric acid (3 ml/100 mg tissue), heated to 70°C for 20 min and then centrifuged (3000 r. p. m. for 5 min). Using calf thymus DNA as a standard, the DNA content of the samples was determined by the procedure described by Giles and Myers (21). The incorporation of [3H]thymidine into DNA was determined by counting 0.5 ml DNA-containing supernatant in a Beckman liquid scintillation system (Beckman Instruments, Palo Alto, CA USA). DNA and RNA contents were expressed as milligrams per total pancreas weight. DNA synthesis was expressed as disintegrations per min [3H]thymidine per microgram DNA.

In rats with 48 h fasting and subsequent refeeding and in the bombesin-treated group, blood samples were collected from the aorta immediately after killing and mixed with 10 U heparin and 400 KIU aprotonin (Trasylol) per ml. The mixture was centrifuged to separate plasma that was used for immediate bioassay of CCK as described (16, 22) frozen (at —20°C) for subsequent radioimmunoassay of gastrin as described (9). The assay systems were sufficiently sensitive to detect 0.5 pM of plasma CCK and 1 pM of plasma gastrin.

CCK-8, and pentagastrin were purchased from Bachem Feinchemikalien AG (Bubendorf, Switzerland). Bombesin and its antagonist RC-3095, [D-Tyr6, Leu13] ψ(CH2NH)Leu15] bombesin (6—14), was obtained by the solid phase method in the New Orleans Laboratories by one of us (R.Z. C.). After purification of the crude product by HPLC, this antagonist showed a purity greater than 95%. It inhibited bombesin binding to 3T3 cells at
an IC50 = 5.88 nM and was denoted with the laboratory code number RC-3095. It showed similar potency in suppressing the bombesin-induced amylase secretion from the dispersed pancreatic acini to that of [D-Phe1, Leu10 ψ(CH2NH) Leu16] bombesin (6–14) originally synthesized by Coy et al. L-364, 718 (17, 23, 24) and L-365, 260 (25) were kind gifts from Dr P.S. Anderson, Merck Sharp and Dohme Res. Labs, West Point, PA.

The results are expressed as means (±SEM). Differences between means are evaluated by Student's test for unpaired data and were considered significant if P < 0.05.

RESULTS

Rats fasted for 48 h had a low level of DNA synthesis in the oxyntic gland area of the stomach, which averaged 31.7 ± 3.8 DPM/μg DNA and this rate of DNA synthesis was not significantly affected by the administration of RC-3095, L-365, 260 or L-364, 718 (Fig. 1). Administration of pentagastrin at 250 μg/kg, CCK at 320 ng/kg or bombesin at a dose of 10 μg/kg, every 8 h for 48 h to fasted rats had no effect on the gastroduodenal mucosa and pancreatic weight and RNA, DNA contents, so these results have not been included. In the oxyntic gland area the most effective stimulant was pentagastrin, which increased DNA synthesis by about

![Diagram](image)

Fig. 1 DNA synthesis in the oxyntic gland area following 48 h fasting and injections of RC-3095 or L-365, 260 or L-264, 718 alone or combined with pentagastrin (p-gastrin). Bars represent means, vertical lines SEM of 10 animals in each group.
DNA synthesis in the oxyntic gland area following 48 h fasting and injections of CCK or bombesin alone or combined with blockers: RC-3095, L-365, 260 or L-364, 718. Bars represent means, vertical lines SEM of 10 animals in each group.

250%, while bombesin resulted in an increase of DNA synthesis reaching about 75% of that obtained with pentagastrin (Figs 1 and 2). CCK remained without any effect in these experiments. Addition of L-365, 260 (1 mg/kg) almost completely abolished the increase in DNA synthesis induced by pentagastrin but failed to affect significantly that induced by bombesin. The combination of RC-3095 (10 µg/kg) and L-364, 718 (1 mg/kg) with pentagastrin did not affect the DNA synthesis in the stomach mucosa when compared with pentagastrin alone. DNA synthesis stimulated by bombesin in the oxyntic gland area was abolished completely by addition of RC-3095 but not by L-365, 260 or L-364, 718. (Fig. 2).

Refeeding of 48 h fasted rats carried out for 16 h almost doubled the DNA synthesis in the oxyntic mucosa. The increment in the rate of DNA synthesis in fed rats reached about 230% of that observed in normally fed rats (Fig. 3). Only injection of L-365, 260 significantly inhibited DNA synthesis after refeeding in the oxyntic gland area, whereas RC-3095 and L-365, 718 were ineffective in this respect.

DNA synthesis in the duodenal mucosa in rats fasted for 48 h averaged 43.4 ± 3.5 DPM/µg DNA and was not significantly affected by the administration of RC-3095, L-365, 260 or L-364, 718 (Fig. 4). Pentagastrin significantly stimulated DNA synthesis. Increase in DNA synthesis reached 147% of that obtained with fasted animals. Administration of L-365,260 significantly suppressed this stimulation. Addition of RC-3095 or L-364,718 remained without any influence on pentagastrin-stimulated growth of the duodenal mucosa.
Fig. 3 DNA synthesis in the oxyntic gland area in 48 h fasted rats, normal fed rats or in 48 h fasted rats subsequently refeed for 16 h. Refeeding was combined with RC-3,995, L-365, 260 or L-364, 718. Bars represent means, vertical lines ±SEM of 10 animals in each group.

Fig. 4 DNA synthesis in the duodenal mucosa after treatment as described in Fig. 1. Mean ± SEM of 10 animals.
Addition of L-364, 718 almost completely abolished the increase in DNA synthesis in the duodenum induced by CCK (Fig. 5); RC-3095 and L-365,260 did not affect CCK stimulated duodenal mucosa growth. Bombesin significantly stimulated DNA synthesis in the duodenal mucosa and only RC-3095 significantly reversed this stimulatory effect. L-364,718 was without any effect.

![Graph]

*Fig. 5 DNA synthesis in the duodenal mucosa after treatment as described in Fig. 2. Mean±SEM of 10 animals.*

A significant stimulation of the DNA synthesis in the duodenal mucosa was observed in animals fasted for 48 h and refed for 16 h (Fig. 6). This stimulatory effect was significantly inhibited by L-365,260 and L-364,718, but still the remaining rate of DNA synthesis in the duodenum was significantly higher than the rate reached in rats fasted for 48 h without refeeding. Both antagonists only partly reversed the effect of refeeding. Bombesin receptor blocker RC-3095 partly and insignificantly inhibited DNA synthesis stimulated by 16 h refeeding.

In the pancreatic tissue, DNA synthesis in 48 h fasted animals averaged $56.5 \pm 4.7$ DPM/μg DNA and this rate of DNA synthesis was not significantly affected by any of the three blockers used (Fig. 7).

Administration of pentagastrin stimulated DNA synthesis in pancreatic tissue. Addition of L-365, 260 to pentagastrin significantly reduced the DNA synthesis by about 36%. The most effective stimulant in the pancreas was CCK (Fig. 8). The combination of L-364,718 with CCK decreased the DNA synthesis by about 50%. No significant changes in DNA synthesis were observed when RC-3095 or L-365,260 were combined with CCK administration. Bombesin was a weak but significant stimulant of DNA
**Fig. 6** DNA synthesis in the duodenal mucosa after treatment as described in Fig. 3. Mean ± SEM of 10 animals.

**Fig. 7** DNA synthesis in the pancreas after treatment as described in Fig. 1. Mean ± SEM of 10 animals.
synthesis in the pancreatic tissue. Addition of RC-3095 almost completely abolished the increase in the rate of DNA synthesis induced by bombesin. The combination of L-365,260 or L-364,718 failed to affect the DNA synthesis induced by bombesin in the pancreas (Fig. 8).

16 h refeeding of 48 h fasted animals resulted in a significant stimulation of DNA synthesis. Injections of RC-3095 or L-365, 260 to 48 h fasted and then refed rats did not affect DNA synthesis. In contrast the administration of L-364,718 produced a significant decrease (by about 70%) in the DNA synthesis induced by refeeding (Fig. 9).

In 48 h fasted animals serum gastrin and CCK levels were low and administration of bombesin significantly increased both hormone levels in the plasma, even above the level reached by animals refeed for 16 h. Administration of specific antagonists for bombesin, gastrin or CCK receptors failed to affect these increments in refeed rats (Table I).

DISCUSSION

This study confirms that refeeding of 48 h fasted rats resulted in a marked enhancement of the DNA synthesis in the gastroduodenal mucosa and pancreas. The major factor responsible for this trophic action of
Fig. 9 DNA synthesis in the pancreas after treatment as described in Fig. 3. Mean ± SEM of 10 animals.

Table 1. Plasma gastrin and CCK concentration after 48 h fasting period and after 16 h refeeding period without and with administration of specific receptor antagonists for gastrin, CCK and bombesin. Means ± SEM of 8—10 determinations on 8—10 rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Gastrin (pM)</th>
<th>CCK (pM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting</td>
<td>47.4 ± 5.2</td>
<td>1.2 ± 0.4</td>
</tr>
<tr>
<td>Fasting + Bombesin</td>
<td>124.2 ± 16.8*</td>
<td>14.3 ± 2.8*</td>
</tr>
<tr>
<td>Refeeding Alone</td>
<td>94.8 ± 11.5*</td>
<td>7.8 ± 1.8*</td>
</tr>
<tr>
<td>Refeeding + RC-3095</td>
<td>83.8 ± 8.2*</td>
<td>8.4 ± 2.2*</td>
</tr>
<tr>
<td>Refeeding + L-365,260</td>
<td>104.6 ± 12.6*</td>
<td>9.0 ± 2.2*</td>
</tr>
<tr>
<td>Refeeding + L-364,718</td>
<td>96.6 ± 12.8*</td>
<td>11.4 ± 2.6*</td>
</tr>
</tbody>
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* Significant (< 0.05) increase above the value obtained in fasted rats.

Refeeding is gastrin in the gastroduodenal mucosa, and CCK in the pancreas. Bombesin showed the trophic effect in all tissues tested, but its physiological role is questionable. This conclusion has been reached by using specific hormonal receptor antagonists out of which gastrin receptor antagonist, L-365,260 and CCK receptor antagonist L-364,718 were capable of sup-
pressing DNA synthesis induced by refeeding, respectively gastrin in the gastric and duodenal mucosa and CCK in the duodenal mucosa and pancreas.

Previous study showed that exogenous gastrin prevented the reduction in gastroduodenal mucosa and pancreatic mass caused by fasting of rats (9) or by the removal of the endogenous source of gastrin (antrectomy) (9, 26). The study of Blom and others using light and electron microscopy provided additional evidence that the effect of gastrin is direct in stem cells (27). Pentagastrin induced a 2.3-fold increase in the parietal cell volume density in the mucosa, and this hormone also altered the pancreatic nucleic acid metabolism and resulted in pancreatic acinar cell hypertrophy (28). Parenteral feeding or liquid diet was accompanied by a strong reduction in plasma gastrin as well as in gastroduodenal and pancreatic tissue mass (6, 7) and these changes were partly prevented by the treatment of animals with pentagastrin (9, 11). These studies suggested the important role of endogenous gastrin in the maintenance of gastroduodenal mucosa and pancreatic growth in the rat. Other studies with endogenous hypergastrinemia induced by the transplantation of the antrum to the transverse colon (29), by antrum exclusions or by vagotomy (30) failed to support the concept of the predominant role of gastrin in pancreatic growth. Reversibly, the gastroduodenal mucosa is probably the main physiological target for gastrin. Evidence for this is that decreasing endogenous serum gastrin levels by fasting, feeding liquid diets or antrectomy caused significant decreases in gastrin receptor numbers and in the growth of gastroduodenal mucosa (31). Since hypergastrinemia led to an excessive gastric acid secretion and this in turn stimulated the release of CCK and secretin from the acidified duodenum (32), the latter hormones might contribute to the pancreatic growth observed in these rats (33). Our present study demonstrates that fasting results in a reduction in plasma gastrin and that refeeding greatly increases the plasma hormone concentrations, accompanied by a marked increase in the DNA synthesis in all tissues tested. Exogenous pentagastrin also strongly stimulated pancreatic growth in fasted rats. Blockade of gastrin receptors with a highly specific antagonist, L-365,260 failed to affect the refeeding-induced stimulation of DNA synthesis in the pancreas. These data provide evidence that gastrin may not play any physiological role in the pancreas, but they support the evidence for the physiological involvement of gastrin in the stomach and the duodenum because L-365,260, antagonizing gastrin receptor, abolishes the trophic action of pentagastrin in these tissues.

The specificity of this gastrin receptor antagonist was confirmed in this study by demonstrating that it was capable of abolishing the trophic effects of pentagastrin in the same dose that was effective in fasting-refe-
eding experiments. Thus, our results confirm previous findings that endogenous gastrin is capable of stimulating the gastroduodenal mucosa (9, 11) and support the notion that endogenous gastrin plays an important role in the growth of gastroduodenal mucosa, but fail to confirm that endogenous gastrin is important in the pancreatic tissue growth in physiological conditions.

CCK is generally recognized as a major hormonal stimulant of the pancreas including the stimulation of pancreatic tissue growth (33, 34). In our study CCK failed to affect gastric mucosa growth in the doses used, which is in agreement with earlier results (35). Some studies have reported that CCK administered to animals on total parenteral nutrition decreased intestinal atrophy and loss of absorptive function normally seen in the absence of food in the gut (36, 37). In our study CCK significantly stimulated DNA synthesis in the duodenal mucosa after 48 h fasting. Using specific CCK receptor antagonist L-364,718, which eliminates the action of circulating CCK, we showed that such CCK antagonism abolished the enhancement of DNA synthesis in the duodenal mucosa caused not only by exogeneous CCK but also by refeeding. This suggests the direct action of CCK on the growth of duodenal mucosa, but it may also be indirect (37), mediated by CCK-stimulated pancreatobiliary secretion (37)

In our study CCK strongly stimulated DNA synthesis, as observed before (34), and L-364,718 eliminated the trophic action of CCK in the pancreas. The main aim of this part of the study was to determine the role of CCK in feeding-induced pancreatic growth. As reported by others (16), we observed that refeeding with typical diet significantly elevated plasma CCK levels and also increased DNA synthesis in the pancreas. Pretreatment with L-364,718 greatly reduced the enhancement of tissue DNA synthesis caused by refeeding. The antagonism of gastrin and bombesin receptors with L-365,260 and RC-3095, respectively, failed to affect the refeeding stimulated DNA synthesis in the pancreatic tissue. Thus, this study provides direct evidence for the major role of CCK in feeding-induced pancreatic growth. As CCK receptor antagonist significantly reduced (by about 70%) but did not eliminate feeding-induced increase in DNA synthesis, it is likely that besides CCK other neuro-hormonal factors were involved in the trophic effect of feeding on duodenal mucosa and pancreatic tissue growth.

This study confirms that bombesin is an effective stimulant of gastroduodenal mucosa and pancreatic tissue growth (17) and that it also raises the plasma levels of both gastrin and CCK (16). These hormones do not, however, seem to mediate the trophic action of bombesin on the gastroduodenal mucosa and pancreas because the blockade of specific receptors for gastrin and CCK failed to affect the increase in DNA synthesis by
bombesin. This is in agreement with previous findings that bombesin predominantly exerts direct action on the gastroduodenal mucosa and pancreas without mediation of gastrin or CCK (17, 38).

Bombesin-like immunoreactivity has been found to be present in the brain and in the nerves of the gastrointestinal tract and the pancreas (13, 14, 39). This widespread distribution of bombesin suggests that the peptide may play an important role in the modulation of gastrointestinal functions. Bombesin has been found to increase the number of gastrin producing cells. Since gastrin itself does not increase gastrin (G) cell proliferation (40) it is probable that this represents a direct effect of bombesin, although other trophic factors released by bombesin cannot be ruled out. Despite the fact that exogenous bombesin administered for 48 h was found to stimulate the synthesis of DNA, the blockade of bombesin receptors by specific antagonist failed to affect refeeding-induced DNA synthesis in all tissues tested. This militates against any major role of bombesin in the physiological mechanism controlling the growth of the gastroduodenal mucosa and pancreatic tissue.

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