THE EFFECT OF PHYTOHAEMAGGLUTININ AND DERAMCICLANE ON GASTRIC AND PANCREATIC SECRETION

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INTRODUCTION

Cholecystokinin (CCK) plays an important role in the regulation of gastrointestinal tract, mainly in physiological processes of stomach and pancreas. CCK is regulated by feedback mechanism and this mechanism can be modulated by different compounds such as kidney bean lectin phytohaemagglutinin (PHA) and 5-HT2A/2C receptor antagonist deramciclane. We observed the effect of PHA and deramciclane in gastric and pancreatic secretion in vivo.

Lectins, including phytohaemagglutinin (PHA) are glycoproteins of non-immunoglobulin nature, capable of specific recognition of, and reversible binding to carbohydrate moieties of complex glycoconjugates without altering the covalent structure of the glycosyl ligands. Lectins in our diet are resistant to breakdown during gut passage and are bound and endocytosed by epithelial cells. Although lectins, such as PHA or soybean agglutinin (SBA), are well known as powerful exogenous growth factors for the small intestine, their actions on gastrointestinal secretions are relatively unexplored. Recent studies revealed that PHA was able to attach directly to the gastric surface, especially to mucosal and parietal cells of the gastric pits. PHA binding was reversible to mucous cells but the kidney bean lectin remained attached to parietal cells even after several days of lectin-free diet. Therefore, the purpose of the present study was to investigate how intragastric PHA affects gastric acid and pepsin secretion in conscious rats.

Recent studies suggested that serotonin receptors may be involved in modulating the actions of cholecystokinin CCK in the gastrointestinal tract. The present work was designed to compare the effects of deramciclane, a recently developed serotonin-2 (5-HT2A/2C) receptor antagonist, and lorglumide, a CCK-A receptor antagonist, on exogenous and endogenous CCK-induced pancreatic enzyme secretion and pancreatic growth, as well as on the emptying of the stomach.
MATERIALS AND METHODS

Gastric acid secretion in conscious rats:
Male Wistar rats were prepared with chronic gastric cannulas and jugular vein catheters using aseptic surgical methods under pentobarbital anaesthesia. Acid concentration was measured by titration of 0.2 ml gastric juice with 0.02 N NaOH to pH 7.0. Output of acid was calculated as mmol per unit time. Pepsin was determined by the activity of the enzyme using a haemoglobin assay described by Berstad (1970).

Pancreatic enzyme secretion in conscious rats
Rats weighing 280–360 g were prepared surgically with gastric cannula under pentobarbital anaesthesia. An indwelling catheter (PE-50) was inserted into the jugular vein. Both cannulas were tunneled to the neck under the skin. Two more catheters (PE-50) were used to cannulate the bile-pancreatic duct at its entry to the duodenum, and to insert a cannula to the duodenum 2 cm distal to the pylorus. These catheters were exteriorized through the abdominal wall. Volume of bile-pancreatic juice was measured gravimetrically. Amylase activity was measured according to the method of Bernfeld (1955) using maltose as a standard.

Pancreatic enzyme secretion in anaesthetized rats
Rats were prepared surgically with duodenal and pancreatic cannulas under urethane or halothane anesthesia. For the replacement the duodenum was infused with T-NaTC solution. Amylase activity was measured (Hummel, 1959), while trypsin activity was evaluated after activation of trypsinogen (Solomon, 1978).

Trophic studies with the pancreas
After animals were killed the pancreas was carefully isolated and trimmed. The weight of pancreas was measured gravimetrically. Trypsin and amylase activities were measured from homogenate of the organ.

Gastric emptying
Under pentobarbital anaesthesia gastric cannula was implanted in the forestomach of rats. An indwelling catheter was inserted into the jugular vein, and another cannula was inserted into the duodenum. Both cannulas were tunnelled to the neck under the skin. A 0.9 M NaCl
solution containing phenol red 0.6 g/l was used as noncaloric liquid test meal. Five minutes later the cannula was opened by removing the plug and the remaining gastric content was collected by gravity in graduated tubes. The phenol red concentration in the mixture was then measured spectrophotometrically at 560 nm by adding 0.1 N NaOH and the total amount of the marker recovered from the stomach was calculated. The data for emptying are expressed as percentages of liquid emptied over 5 min.

Statistical analysis
Values represent mean ± SEM. Experiments were performed using at least six parallel samples. Comparison among the groups was performed by analysis of variance (ANOVA). Calculations were performed using the InStat program package (GraphPad Software, Inc., San Diego, USA).
RESULTS

Effect of PHA on basal and stimulated gastric acid secretion:
In conscious rats PHA significantly inhibited basal acid secretion when compared to vehicle-treated controls. This effect was reversible, maximal inhibition was observed in the first 30-min period after administration of 100 mg/kg PHA, then it gradually disappeared returning to control level of acid output in about 2 h. The inhibitory effect of PHA on gastric acid secretion was dose-dependent.
Pentagastrin firmly elevated the acid output in stomach, but this level of acid output was decreased after the administration of 100 mg/kg PHA. This inhibition disappeared in about three periods. PHA also significantly inhibited basal acid secretion in conscious rats and this effect was reversible. Maximal inhibition (72%) was observed in the first 30-min period after administration of 100 mg/kg PHA.
Afterwards the inhibitory effect gradually disappeared returning to control level in about 2 h. When comparing the inhibitory responses, two way analysis of variance revealed that the inhibition of pentagastrin-stimulated secretion was more than the inhibition of basal acid output. These data suggest that PHA has the ability to diminish not only the basal component of acid secretion, but also that of activated by the peptide.
Pepsin output in gastric juice was also measured in the same experiments. Pentagastrin infusion did not affect pepsin output. Furthermore, no significant change in pepsin secretion was found during either basal or pentagastrin-stimulated conditions in response to PHA. To determine the dose-dependence of the effect of PHA on pentagastrin-stimulated acid secretion 33, 100 and 300 mg/kg doses were administered intragastrically. The effect of PHA depended on the dose, and it was reversible at all doses applied. Once more, PHA did not significantly affect pepsin output in gastric juice in doses which effectively inhibited acid secretion. PHA inhibited histamine-stimulated acid output as well. This inhibition was also dose-dependent. Increasing doses of PHA caused increasing inhibition of acid output as indicated. The action of PHA on histamine-stimulated acid secretion was also reversible: the inhibitory effect disappeared in four periods after administration even when the highest PHA dose was applied.
Effect of PHA on pancreatic secretion:
Given in a dose (100 mg/kg) that effectively inhibits acid secretion, PHA stimulated pancreatic amylase discharge in conscious rats prepared with chronic pancreatic cannula. Maximal increase in amylase output was found in the first 30-min period following PHA treatment. This effect was significantly smaller in magnitude when compared to stimulation induced by diversion of bile-pancreatic juice from the duodenum releasing endogenous CCK. The stimulatory action of PHA on amylase secretion was completely blocked by devazepide, a selective CCK-A receptor antagonist, indicating a CCK-dependent mechanism.

Effect of PHA and anaesthesia on pancreatic secretion:
In the final experiment we tested the modulatory role of anaesthesia on PHA-induced amylase secretion. In rats receiving halothane anaesthesia, intraduodenal PHA infusion (10 mg·kg\(^{-1}\)·h\(^{-1}\)) stimulated pancreatic amylase secretion. When compared to control, the increase of amylase output became significant during the third collection period after starting the infusion of the lectin. During urethane anaesthesia, however, no stimulatory effect of PHA was observed. Under the same experimental conditions, pancreatic trypsin output changed parallel with amylase secretion. It was stimulated by PHA in halothane-anaesthetized rats. Secretory responses to submaximal CCK analogue caerulein stimulation were similar in the halothane anaesthetized rats, but significantly lower during urethane anaesthesia.

Effect of deramciclane on pancreatic secretion:
Diversion of bile-pancreatic juice from the duodenum resulted in a significant increase in pancreatic amylase secretion via release of endogenous CCK. The two lower doses of deramciclane (3 and 10 mg/kg) did not modify this effect of diversion. On the contrary, 30 mg/kg deramciclane and 10 mg/kg lorglumide, given intravenously, almost completely counterbalanced the secretory response to diversion. As expected, exogenous CCK 200 pmol·kg\(^{-1}\)·h\(^{-1}\) also stimulated pancreatic enzyme secretion. In this case, administration of 10 mg/kg lorglumide completely abolished the stimulatory action of the peptide, while 30 mg/kg deramciclane did not significantly modify the effect of exogenous CCK.
Effect of deramiciclane on pancreatic growth:
Chronic administration of proteinase inhibitor camostate increased the weight of the pancreas. Lower doses of deramiciclane 3 and 10 mg/kg did not modify these effects of camostate. At a dose of 30 mg/kg, deramiciclane and lorglumide inhibited the camostate-induced increase in pancreatic weight. Camostate treatment also stimulated the pancreatic trypsin content, an effect that was abolished by both 30 mg/kg deramiciclane and 10 mg/kg lorglumide. No significant changes were observed, however, in pancreatic amylase content among the different experimental groups.

Effect of deramiciclane on gastric emptying:
Five min after instillation of physiological saline into the rat stomach, 32±4% of the fluid was recovered. When intralipid 5, 10 and 20% was given intraduodenally together with the test meal, it delayed gastric emptying in a concentration-dependent manner. For further studies, we used a 20% intralipid infusion because this dose induced the most pronounced effect. 20% Intralipid infusion into the duodenum resulted in a delay in gastric emptying. The two lower doses of deramiciclane (3 and 10 mg/kg) did not affect this action of intralipid. On the contrary, 30 mg/kg deramiciclane and lorglumide, given intragastrically, significantly inhibited the delay induced by the lipid. It is worth mentioning, however, that none of these compounds was able to completely counterbalance the intralipid-induced delay in gastric emptying. Exogenous CCK 10 nmol·kg⁻¹·h⁻¹ also induced a delay in the emptying of the noncaloric test meal. In this case, administration of 10 mg/kg lorglumide completely abolished the action of the peptide while 30 mg/kg deramiciclane did not modify it.
CONCLUSION

Phytohaemagglutinin inhibits basal and stimulated gastric acid secretion in conscious rat, but in the same dose PHA do not affect on pepsin secretion of the stomach. However, PHA increased the enzyme secretion of pancreas. These effects are mediated by CCK. The 5-HT2A/2C receptor antagonist deramciclane inhibited pancreatic enzyme secretion, gastric growth and emptying via inhibition of endogenous CCK.

Effect of phytohaemagglutinin and deramciclane on gastrointestinal tract.
The phytohaemagglutinin inhibits gastric acid secretion but increases pancreatic secretion via CCK. The deramciclane inhibits 5-HT2A receptors on sensory neurons, resulted inhibition of CCK-RP secretion.
PUBLICATIONS

Original articles published in international journals:


Abstracts published in international journals:


Conference proceedings:


