INTRODUCTION

The most important role of the gastrointestinal (GI) tract is the digestion and the absorption of the food. There is a sophisticated regulatory system in the GI tract of the vertebrates regulating the production of digestive enzymes, the motility, the connection with the central nervous system and the proliferation and differentiation of gastrointestinal tissues. The regulatory peptides have an important role in the coordination and controlling of these events and act as soluble extracellular messengers. These peptides are produced by special endocrine cells in the intestinal mucosa and can be found in the central and peripheral nerves where they act as neuromodulators.

I investigated the effects of some gastrointestinal peptides (cholecystokinin /CCK/ and somatostatin) on the GI tract, especially on the regulation of exocrine pancreas and motility of stomach in suckling and adult rats. There are only a few data available on the regulation of the GI tract in the newborns and before weaning. In this period of life mammals adapt to the food intake and digestion, and therefore, the effects of the regulatory peptides are very important at this time. In the ontogenesis there is no CCK-like immunoreactivity in the pancreas of the rat embryo but after birth the serum level of the CCK is highly elevated and decreased after weaning. The elevation of the serum level is parallel with the rapid growth of the exocrine pancreas suggesting that CCK plays a regulatory role in the development of the gland.

CCK is also one of the most important hormones involved in the regulation of gastrointestinal motility. The fact that minute amounts of CCK are sufficient to affect GI motility under different in vivo and in vitro conditions would indicate that this action on the gut is one of the physiological actions of the peptide.

Chronic administration of CCK produced pancreatic hypertrophy and hyperplasia, however, there are only a few contradictory data available on the peptide effect on gastric growth. Specific receptors mediate the biological actions of CCK on exocrine pancreas and GI smooth muscle and they belong to the CCK-1 subtypes. The CCK-1 receptors exist in two (i.e. high and low) affinity states, and CCK occupancy of high and low affinity sites is thought to be related to the initiation
of different intracellular events and consequent biological responses. Being an agonist at high affinity CCK-1 receptors and an antagonist towards the low affinity ones, the synthetic JMV-180 represents a useful tool to functionally distinguish between the two receptor states.

Treatment of rats with high concentration of secretagogues, such as CCK or the CCK analogue caerulein generates interstitial pancreatitis suggesting the pathogenetic role of these peptides in the diseases of the pancreas. Although the exact pathophysiology of this „hyperstimulated” pancreatitis is not well established, it is demonstrated that in this type of pancreatitis digestive and lysosomal enzymes are packaged in large intracellular secretory and autophag vacuoles. Breakdown of the vacuole membranes results in the colocalization of the enzymes, and by the catalization of the lysosomal enzymes (i.e. cathepsin B) the inactive trypsinogen is activated into trypsin. The activation of the trypsin induces the necrosis of the acinar cell. Accordingly, the depletion of the pancreatic trypsin content seems to have a beneficial effect on the onset of the „hyperstimulated” pancreatitis.

Somatostatin inhibits the normal and stimulated enzyme secretion of the exocrine pancreas. On the basis of this inhibitory effect, treatment with somatostatin should be favourable in acute pancreatitis. However, literature data regarding the efficacy of somatostatin treatment in acute pancreatitis are controversial.
AIMS OF THE STUDY

We investigated the effects of some regulatory peptides, cholecystokinin (CCK) and somatostatin on the GI tract in newborn rats and before weaning. We were mainly interested in:

I. effects of these peptides on the secretion and growth of the exocrine pancreas in suckling and adult rats

II. effects of CCK-1 receptor stimulation on the motility and growth of gastric smooth muscle

III. In addition, we investigated the therapeutic preventive effects of these peptides on acute experimental pancreatitis.

METHODS

In all experiments Wistar rats were used. In the experiments performed on newborn rats one dam nurished 8 pups. As a rule, four such litters were used in littermate pairs: pups of each litter were distributed in pairs to all experimental groups to avoid the effect of the dam.

In some experiments we administered the CCK analogue caerulein instead of CCK. Caerulein is extracted from frog skin and its aminoacid sequence only differs from that of CCK in a single residue at the C-terminal octapeptide. The consequence of this difference is that although the biological action of the two peptides are the same, caerulein is more resistant to proteolysis.

Assays

The protein content of the pancreas was determined by the method of Lowry using bovine serum albumin as standard. Pancreatic DNA content was measured by using diphenylamine after extraction the DNA by perchloracid. The method was introduced by Munro, Fleck and Burton.
Trypsin activity was measured by spectrophotometric method of Hummel, trypsinogen was activated by enterokinase. Amylase activity was measured according to the method of Bernfeld using blue starch as standard control.

In vivo experiments
Enzyme secretion and growth of the pancreas: in suckling rats, before weaning the cannulation of the pancreatic duct often leads to fatal complications. In our laboratory there was a new methodology elaborated so called „tubeless method”. The essence of the method is that the enzyme activity in the non-stimulated pancreas is unchanged while in the stimulated pancreas it is reduced due to the secretion into the duodenum. So the difference between the enzyme activity in the stimulated and non-stimulated pancreas corresponds to the secretion. We investigated the effects of some gastrointestinal peptides such as secretin and bombesin on the secretion of the exocrine pancreas by this method in rats before weaning. We removed the pancreas and measured the protein and trypsin content. The decrease of the trypsin/protein ratio was shown to be due to enzyme depletion, the increase of this ratio is an indicator of enzyme accumulation in the gland.

After urethane (1.2 g/kg ip) anesthesia the common bile duct at the hepatic hilum was ligated and a polyethylene cannula was introduced into the duodenal part of the duct for collecting pure pancreatic juice for 30-min periods. The rats were given different doses of CCK-8 and/or dexloxiglumide intravenously and we measured the amylase concentration of the collected juice.

We investigated the trophic action of exogenous and endogenous CCK, somatostatin and ethanol on the pancreas. The animals were treated with the above mentioned peptides and ethanol for 7 or 10 days. At the end of the treatments the rats were decapitated, the pancreata were removed, carefully trimmed free of fat, mesentery and lymph nodes, weighed, and homogenized in Tris buffer. Protein and DNA concentration as well as trypsin and amylase activities were then measured in the pancreatic tissue.

Gastric emptying and growth: Under pentobarbitone anesthesia a Gregory-type gastric cannula was implanted in the forestomach of the rats. Experiments were started after at least 1 week to allow for recovery. Some of the animals were given camostate directly intragastrically, others were treated with different doses of caerulein bolus intravenously. 3 ml of the testmeal (saline with phenol-red (37°C)) was administered slowly directly
to the stomach through the cannula. The cannula was closed for 5 minutes, than we collected the rest of the testmeal from the stomach. Phenol-red content of the collected juice was determined by spectrophotometry (560 nm) after alkalinization. The difference of the content of the phenol red in the administered and the collected juice corresponded with the gastric emptying.

Rats were treated with caerulein, camostate and dexloxiogluimide for 7 days. After overnight fasting, on the eighth day the animals were killed, the stomach was removed, opened along the greater curvature and gently rinsed with water. The oxyntic gland area was carefully separated from the pyloric gland (antrum) and forestomach. The oxyntic gland area and the antrum were homogenized and the protein and DNA concentrations were measured in the gastric tissues.

**In vitro experiments**

**Secretion of the pancreas:** The pancreas was quickly removed then cut into small segments (3-5 mg) and a total weight of about 150 mg was placed in a tissue flow chamber of 1 ml capacity and superfused at a constant rate with a modified Krebs-Henseleit solution. CCK-8 and dexloxiogluimide were added directly to the superfusion solution at known concentrations. The tissue was incubated for 30 minutes, then fractions were collected at 3-min intervals and the amylase concentrations in the effluents were measured.

**Preparation of isolated pancreatic acini:** Isolated pancreatic acini were prepared by a method previously described and validated. CCK-8 and JMV-180 (alone or in combination) were added directly to the incubation vials in known concentrations immediately before the second incubation. At the end of the incubation, acini and media were separated by centrifugation and their amylase contents were measured. The biological activity of CCK-8 and JMV-180 was determined by the release of amylase in 30-min periods.

**Contractions of gastric smooth muscles:** Muscle strips (2-3 mm in width and 8-10 mm in length) were prepared from the corpus, the antrum and the pylorus. Both circular and longitudinal muscles were prepared from the corpus and antrum whereas only circular strips were obtained from the pylorus ring. The strips were suspended in a 10 ml organ bath and connected via surgical silk to a force-transducer for monitoring changes in muscle length. Strips were then exposed to different concentrations
of CCK-8 and JMV-180, alone or in combination, until the tension reached a plateau. Values were expressed as percent of the contractile effect of 10^{-5}M acetylcholine on the same muscle preparation.

**Pancreatitis**

The acute injury of the pancreas was induced by repeated subcutaneous injections of supramaximal doses of caerulein given at hourly intervals, according to Lampel. In the first experiment camostate (400 mg/kg) was given by gavage once at 1 hour before the first caerulein injection. Five hours after the first caerulein injection the rats were exsanguinated and their pancreata removed. In the other experiment octreotide was injected into the rats at a dose of 4 \mu g/kg sc. The first dose was administered 12 hrs before the first caerulein injection with repeated injections every 12 hours, until the rats were sacrificed. The animals were decapitated at different intervals: at 6 and 24 hours following the first caerulein injection inducing the acute injury of the pancreas, and after 72 hours (Day 3) and 120 hours (Day 5) to study pancreatic regeneration. Removed pancreata were weighed and analyzed for protein, DNA, trypsin and amylase content, and the plasma amylase levels were determined as well.

**Evaluation of data**

Mathematical statistical analysis was performed by analysis of variance (ANOVA) and Dunn’s multiple range test.

**NEW RESULTS**

- It was first demonstrated that the synthetic trypsin inhibitor camostate (which releases endogenous CCK) stimulates the pancreatic trypsin secretion even in developing rats, before weaning. The stimulating effect of camostate was partially inhibited by atropine suggesting that the endogenous CCK affects on the exocrine pancreas through vagal nerves also.
- We demonstrated that camostate increases plasma CCK in suckling rats and this elevation induced by camostate is longer in time than that due to exogenous CCK-8 (after 45 min the increase due to camostate was 8-fold higher than in control saline group meanwhile in the CCK treated group at the end of the 45-min test period the plasma CCK concentration turned back to the normal level.)
- In 10-days old rats the secretin acts on the pancreas secretion similar than in adult animals: the peptide significantly reduced the trypsin content of the pancreas by stimulating the enzyme secretion and potentiates the effect of caerulein. Bombesin also stimulated the enzyme output in newborns. These data suggest that beside CCK the secretin and bombesin also plays a role in the regulation of the exocrine secretion in the postnatal life.
- In our experiments we demonstrated that the serin protease inhibitor camostate promotes pancreatic growth in rats before weaning. It induces hyperplasia and hyperthrophy of the pancreas in the postnatal life and treatment for 10 days is enough for the effect, longer treatment has no more advantage.
- In the postnatal life the octreotide given alone decreased the plasma growth hormone level and basal pancreatic trypsin content and concentration, and given in combination with caerulein, it diminished the caerulein-induced increase in DNA and trypsin content.
- We demonstrated that before weaning the ethanol affected neither the stimulated nor the non-stimulated growth of the pancreas. The explanation of this inefficiency may be due to the biological programming which compensates the influences acting against the development.
- We demonstrated that the CCK-1 receptor antagonist loxiglumide is able to antagonize the effects of camostate on the exocrine pancreas.
- In adult rats we characterized the effects of dexloxiglumide, a new CCK-1 receptor antagonist on the exocrine pancreas. Dexloxiglumide proved to be a selective and potent and competitive antagonist on CCK-1 receptors, therefore represents a useful tool to investigate CCK-receptor interactions in peripheral organs.
• Results of our investigations show that like CCK, the camostate also delays dose-dependently the gastric emptying. Chronic administration of CCK did not affect gastric weight and composition in both the oxyntic gland area and antrum, suggesting that the effects of the peptide on gastric and pancreatic growth are different.

• By the use of a special synthetic CCK analogue, JMV-180 we provided clear evidence that on pancreatic acinar cells the CCK-1 receptors are present in two different affinity states (high and low affinity), however, on gastric smooth muscle cells only low affinity receptors are present. In these experiments we indirectly confirmed that only the occupation of the high affinity receptors resulted in proliferation of the tissue.

• We demonstrated the preventive oral application of camostate had beneficial effects on caerulein-induced acute pancreatitis diminishing the trypsin content of the acinar cells.

• Somatostatin treatment – even if given before inducing experimental pancreatitis– can not prevent the onset of the experimentally induced acute injury of the pancreas. The early use of the peptide however has beneficial effect on the injury so the early use may be acceptable as a complimentary treatment at the beginning stage of the injury. The long term use, however, cannot be recommended because it does not speed up the restitution after the injury of the gland.
Az értekezéshez közvetlenül kapcsolódó saját publikációk absztraktok nélkül

Közlemények

   IF: 0.928
   IF: 1.662
   IF: 1.739
   IF: 1.589
   IF: 0.680
   IF: 0.707
   IF: 3.704


Könyvrészlet


Az értekezéshez közvetlenül nem kapcsolódó saját publikációk absztraktok nélkül


Az értekezéshez közvetlenül kapcsolódó idézhető absztraktok közül néhány:


