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The effect of luminal ghrelin on pancreatic enzyme secretion in the rat

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Abstract

Ghrelin, a 28-amino-acid peptide produced predominantly by oxyntic mucosa has been reported to affect the pancreatic exocrine function but the mechanism of its secretory action is not clear. The effects of intraduodenal (i.d.) infusion of ghrelin on pancreatic amylase outputs under basal conditions and following the stimulation of pancreatic secretion with diversion of pancreato-biliary juice (DPBJ) as well as the role of vagal nerve, sensory fibers and CCK in this process were determined. Ghrelin given into the duodenum of healthy rats at doses of 1.0 or 10.0 μg/kg increased pancreatic amylase outputs under basal conditions or following the stimulation of pancreatic secretion with DPBJ. Bilateral vagotomy as well as capsaicin deactivation of sensory fibers completely abolished all stimulatory effects of luminal ghrelin on pancreatic exocrine function. Pretreatment with lorglumide, a CCK_1 receptor blocker, reversed the stimulation of amylase release produced by intraduodenal application of ghrelin. Intraduodenal ghrelin at doses of 1.0 or 10.0 μg/kg increased plasma concentrations of CCK and ghrelin. In conclusion, ghrelin given into the duodenum stimulates pancreatic enzyme secretion. Activation of vagal reflexes and CCK release as well as central mechanisms could be implicated in the stimulatory effect of luminal ghrelin on the pancreatic exocrine functions.

Keywords: Ghrelin; Vagotomy; Capsaicin; Sensory nerves; Lorglumide; CCK

1. Introduction

Ghrelin, a 28-amino-acid peptide was isolated from the stomach in 1999 [1]. Ghrelin is mainly produced by X/A-like cells in the oxyntic mucosa, but it was also found in other parts of the gastrointestinal system: duodenum, ileum, colon or pancreas [2]. Apart from the alimentary tract small amounts of that peptide were detected in the hypothalamus, pituitary gland, lung, kidney, placenta and in the immune system [3–8].

Ghrelin is a natural ligand for the growth hormone secretagouge receptor (GHS-R1a) and it is able to stimulate GH secretion [9]. This novel peptide also exhibits the number of other biological activities including increase of food intake and energy expenditure, stimulation of lactotroph and corticotroph secretion, influence on sleep and behavior or modulation of heart rate and blood pressure [10–14].

As ghrelin receptors (GHS-R) have been detected in many central and peripheral tissues, including endocrine cells of the stomach or pancreatic α- and β-cells, these peptides show a number of actions at the gastroenteropancreatic level. Both intravenous (i.v.) and intracerebroventricular (i.c.v.) administration of ghrelin to the anaesthetized rats were reported to stimulate gastric acid secretion and motility [15]. This effect has been reversed by vagotomy or atropine pretreatment, suggesting that ghrelin affects gastric function *via* the activation of the vagus nerve and muscarinic receptors [16]. Recent studies have also demonstrated that ghrelin exerts the gastroprotective actions in the stomach against the stress-induced damage [17]. Ghrelin has been also shown to modulate endocrine as well as exocrine pancreatic secretions, but the physiological role of this peptide in the modulation of exocrine pancreatic function remains unclear. It has been shown that ghrelin given intravenously to the anaesthetized rats is able to inhibit pancreatic exocrine secretion stimulated by cholecystokinin (CCK). This inhibitory effect of ghrelin on pancreatic enzyme secretion has been also observed

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in vitro, in pancreatic lobules [18]. On the other hand central administration of ghrelin stimulated pancreatic exocrine secretion in conscious rats [19]. Both gastric and pancreatic effects of ghrelin have been shown to be mediated *via* the vagus-dependent cholinergic pathway and probably *via* sensory nerves [20,21].

Recent publications have suggested that ghrelin-producing cells occur as two types: closed cells or cells open toward the lumen of the GI tract, with the number of open-type cells increasing in the direction from the stomach to the colon [22,23]. Ghrelin present in the gut lumen could possibly affect pancreatic exocrine secretion, but this hypothesis has not been studied yet.

Pancreatic exocrine function is modulated by neural and hormonal mechanisms. Regulation of pancreatic exocrine secretion occurs at the level of the central nervous system, and involves afferent and efferent vagus fibers, as well as enteric nervous system operating in the gastrointestinal tract [24]. CCK, one of the major gut hormones, released from duodenal mucosa I cells, stimulates pancreatic secretion *via* activation of CCK_1 receptors and enteropancreatic vago-vagal reflex [25,26].

The aims of the present study were:

1) to evaluate the effect of exogenous ghrelin, given intraduodenally (i.d.), on pancreatic enzyme secretion, in the anaesthetized rats with pancreato-biliary fistulas under basal conditions or following stimulation of pancreatic secretion by the diversion of pancreato-biliary juice (DPBJ),
2) to examine the involvement of vagal nerves, sensory fibers as well as CCK in the mechanisms of ghrelin action on pancreatic exocrine function,
3) to determine plasma levels of ghrelin in intact rats and in those pretreated with intraduodenal infusion of ghrelin.

2. Materials and methods

2.1. Materials

The following items were purchased: ghrelin from Bachem (Bubendorf, Switzerland), capsaicin and specific CCK_1 receptor antagonist, lorglumide, were from Sigma Co (St. Louis, MO, USA); CCK radioimmunoassay commercial kit was from DRG International Inc. (Mountainside, WI, USA). Ghrelin radioimmunoassay commercial kit was purchased from Peninsula Laboratories Inc, Division of Bachem (San Carlos, California, USA). The specificity of RIA commercial kit used in our data showed 0% cross-reactivity with motilin, Orexin A and B, secretin, galanin or VIP. Amylase reagent was purchased from Dialab Diagnostic Ges. MBH (Wien, Austria). Vetbutal (Pentobarbitalum) was from BIOWET (Puławy, Poland). PE10 and PE50 polyethylene tubing were purchased from Beckton Dickinson (Sparks, MD, USA).

The experimental protocol was approved by the Jagiellonian University Ethical Committee for Animal Experimentation and run in accordance to the statements of the European Union regarding handling of experimental animals.

2.2. Animal preparation

The study was performed on male Wistar rats weighing 300–350 g. Animals were housed in cages under standard conditions at 24 h light/dark cycle at room temperature with free access to standard laboratory chow and water. Rats were deprived from food for 24 h before the experiment. The surgery was performed under pentobarbiturate anaesthesia (Vetbutal), given intraperitoneally (i.p.) at a dose of 15.0 mg/300 g body weight. The anaesthesia was maintained during the experiment by i.p. pentobarbiturate administration every 2 h as needed.

Following midline laparotomy, the duodenum was identified together with the bile–pancreatic duct entrancing the duodenum. The small incision (about 1 mm) was made in the duodenum at the entrance of the bile–pancreatic duct and the polyethylene tube (PE10) was inserted about 1 cm into the common bile–pancreatic duct for bile and pancreatic juice collection. A second polyethylene cannula (PE10) was placed into the duodenum with its tip fixed proximal to the ampulla for reinfusion of previously harvested bile–pancreatic juice (after dilution with saline 1:2). The abdominal wound was sutured with a double layer suture and the rats were kept under the heating lamps to maintain the right body temperature (37 °C). At the end of the experiment, the abdominal vena cava was exposed and the blood was withdrawn into EDTA containing tubes for determination of CCK by radioimmunoassay.

During the experiment the animals were placed in individual Bollmann cages. The bile–pancreatic juice (BPJ) samples were collected in small preweighted vials in 15 min aliquots to measure the volume of each sample and protein and amylase concentration. Basal secretion of pancreatic juice was measured by collecting BPJ for 60 min to allow stabilization of flow affected by surgical manipulation. Protein and amylase concentration of each sample was measured by enzymatic method as described previously [27]. The results were expressed as total protein (mg/15 min) and amylase (IU/l/15 min) outputs. During the experiments previously collected pancreato-biliary juice was reinfused *via* the duodenal cannula into the duodenum at the rate of 1 ml/h.

2.3. Experimental procedures

The study was divided into five series of experiments.

In series I the effects of ghrelin alone on pancreatic exocrine secretion under basal conditions or following the stimulation of this secretion with diversion of pancreatic juice (DPBJ) were studied. Series II included group of animals with sensory nerves deactivated with capsaicin. For series III of the study rats with bilaterally transected vagal nerves were employed. In series IV, CCK_1 receptor antagonist, lorglumide, was used to determine the involvement of CCK in the secretory effects of ghrelin on exocrine pancreas. Series V was designed to determine plasma ghrelin concentration after intraduodenal administration of that peptide. For each part of the experiment the animals groups of 5–6 rats were used. Ghrelin was dissolved in 0.5 ml of saline and administered intraduodenally (i.d.) into the rats in each test.