Abstract  A predominance of the pancreatic cholecystokinin (CCK) receptor of the B/gastrin subtype (CCK-B/G) was reported in calves older than 1 month. Specific CCK-A and CCK-B/G receptor antagonists (SR 27897 and PD 135158, respectively) were used to identify the CCK receptor subtype involved in exogenous CCK- and gastrin-induced exocrine pancreatic responses. Conscious calves (2 months old) with catheterized pancreas, jugular vein and duodenum were used; the pancreatic juice was continuously reinfused. CCK (30 pmol kg\(^{-1}\) min\(^{-1}\), 40 min) evoked an increase in pancreatic juice flow and enzyme secretion, while the same dose of gastrin increased enzyme secretion alone. CCK-induced pancreatic secretion was abolished by SR 27897 (15 nmol kg\(^{-1}\) min\(^{-1}\), 55 min) and reduced by PD 135158 (0.15 nmol kg\(^{-1}\) min\(^{-1}\), 55 min). Gastrin-induced enzyme secretion was reduced by PD 135158 (50% to 90%) and to a lesser extent by SR 27897 (50% to 60%). These results demonstrate that CCK and gastrin in the physiological range stimulate pancreatic exocrine secretion in calves and that these effects are partly mediated by CCK-B/G receptors. Although CCK-A receptors are not predominantly expressed, they seem to play a major role in the response of pancreatic exocrine secretion to CCK.

Key words  CCK-A and CCK-B/G receptors · Exocrine pancreatic secretion · Gut regulatory peptides

Introduction

Cholecystokinin (CCK) and gastrin are related peptides distributed in the gastrointestinal tract and in the central and peripheral nervous systems. These peptides are characterized by an identical carboxyl-terminal amidated pentapeptide which is essential for biological activity [25]. CCK has a sulfated tyrosyl residue at the seventh position from the amidated carboxyl terminus, while gastrin has this tyrosyl residue at the sixth position which is sulfated in 50% of the circulating molecules. CCK and gastrin have common and also specific biological functions [25]. Their receptors have been classified into CCK-A and CCK-B/gastrin (CCK-B/G) subtypes on the basis of their pharmacological selectivity towards a series of agonists and antagonists. The CCK-A receptor, characterized by its high affinity for CCK over gastrin, is present in the pancreas, gall-bladder, vagus nerve and in localized areas of the central nervous system. The CCK-B/G receptor subtype, found in the brain, gastric parietal cells and smooth muscle, binds gastrin and sulfated CCK with a nearly equal high affinity [33].

CCK and gastrin are gut regulatory peptides, the plasma concentrations of which increase postprandially. It has long been accepted that CCK stimulates pancreatic exocrine secretion and gall-bladder contraction, and that gastrin stimulates gastric acid secretion. Previous studies in dogs [13], sheep [11] and humans [32] suggest that gastrin could be involved in the regulation of pancreatic exocrine secretion, but the physiological significance of this effect remains controversial [33]. CCK-B/G receptors, which are capable of mediating gastrin’s actions, are present in the dog pancreas but are in a minority compared to CCK-A receptors [6]. More recent data obtained in studies of other mammals suggest that gastrin might have a physiological role in the exocrine functions of the pancreas [5, 16, 24, 30]. This hypothesis is based
on data showing that the CCK-B/G receptor is predominantly expressed in the pancreas of calves and pigs. Indeed, in a previous study of calves, we demonstrated that CCK-A receptors are the only CCK receptors present in the pancreas at birth, whereas CCK-B/G receptors predominate in animals older than 28 days [5, 16]. In 2.5-month-old pigs, pancreatic CCK receptors are composed of 30% CCK-A and 70% CCK-B/G receptors [24]. Interestingly, it was recently reported [30] that CCK-B/G receptors predominate in the human pancreas. In this study, a diffuse autoradiographic signal for the CCK-B/G receptor was detected in the acinar cell, which is the main cell type in the pancreas. The physiological function of the acinar cell is to synthesize and secrete digestive enzymes destined for discharge into the duodenal lumen in response to luminal nutrients. In spite of these recent findings, no physiological function has yet been assigned to pancreatic CCK-B/G receptors, although Wank [33] argues that they are unrelated to the physiological regulation of enzyme release.

In the present study, pancreatic exocrine secretion evoked by exogenous CCK and gastrin agonists was analyzed in vivo in 2-month-old milk-fed calves. Specific CCK-A and CCK-B/G receptor antagonists were used to identify pharmacologically the CCK receptor subtype involved in CCK- and gastrin-induced pancreatic responses.

**Materials and methods**

**Chemicals**

Nle-human gastrin-13S (G-13S) and Thr,Nle-cholecystokinin [25–33] (CCK-9) were purchased from the Max-Planck-Institut, Munich, Germany. The CCK-A receptor antagonist (SR 27897) was obtained from Sanofi Recherche, Toulouse, France. The CCK-B/G receptor antagonist (PD 135158) was kindly supplied by Parke-Davis, Cambridge, UK. Heparin was Choay heparin, and aprotinin was Iniprol, both purchased from Sanofi Winthrop, France. Specific substrates N-α-benzoyl-L-arginine-p-nitroanilide (L-BAPNA, B 3133) and succinyl-L-alanyl-L-alanyl-L-prolyl-L-phenylalanine-p-nitroanilide (Suc-Ala2-Pro-Phe-pNa, S 7388) for trypsin (EC 3.4.21.4) and chymotrypsin (EC 3.4.21.1), respectively, were obtained from Sigma Chemical, St Louis, Mo., USA.

**Animals**

Treatments and experiments were conducted according to the European Union regulation concerning the protection of experimental animals. Experiments were carried out on seven 60- to 90-day-old Holstein-Friesian male calves. They were fed colostrum (25 g/kg live weight per meal) for the first 2 days of life and then a milk substitute based on spray-dried skim-milk powder and talc (222 g crude protein, 206 g fat, 460 g lactose, 39 g starch and 73 g ash/kg on a dry matter basis). Calves were fed twice daily at 8:30 and 16:30 hours. The dry matter intake was 66 g/kg0.75 live weight per day; the live weight during the experiment was 103±6 kg.

Pancreatic juice collection and analysis

Two weeks before the experiment, calves were fitted with two permanent cannulae (pancreatic duct and duodenum) under halothane anesthesia, as previously described [15]. Throughout each experiment, all the pancreatic juice was collected and its volume measured. Most (92%) of the secretion was reinfused into the duodenum but 8% was pooled as 5-min aliquots and stored at −20°C for analysis. The pancreatic juice was analyzed for protein concentration [19] and trypsin and chymotrypsin activities [14]. Trypsin and chymotrypsin activities were expressed as nmol p-nitroaniline released per minute at 20°C (U).

**Blood collection and analysis**

Two permanent catheters introduced into an external jugular vein were available to infuse products and to collect blood samples into heparin (500 IU/ml) and aprotinin (10,000 IU/ml). Plasma samples were stored at −20°C. Four plasma gut regulatory peptides (CCK, gastrin, secretin, and somatostatin) were analyzed by radioimmunoassay after ethanol extraction (2/1, v/v). The antigen–antibody complex was separated from free antigen by adsorption on activated charcoal. CCK was assayed with an antisera (final dilution 1/16,000) that recognizes equally the 33- and 39-residue peptides and carboxyl-terminal fragments of nine or more residues [20]. Reactivity was 30% for sulfated octapeptide and 1% for desulfated octapeptide and gastrin I, 1–17 (on a molar basis). CCK-33 was used as standard. The dose of peptide required for 50% inhibition of binding (ID50) was 3.8 fmol/tube and variations were 9% (inter-assay) and 13.5% (intra-assay). The antiagastatin serum (final dilution 1/1400,000) was obtained from a rabbit immunized against synthetic human gastrin I, 1–17, conjugated to bovine serum albumin through carbodiimide condensation [3]. Synthetic human gastrin I, 1–17 was used as standard. Reactivity was 42%, 31% and 1.9% for natural gastrin I, 1–34, natural gastrin II, 1–17 and highly purified porcine CCK, respectively. The estimated ID50 and variations were 1.2 fmol/tube, 13.4% (inter-assay) and 9.3% (intra-assay), respectively. The antisomatostatin serum (final dilution 1/400,000) was obtained from a rabbit after an 11th injection with porcine secretin conjugated to bovine serum albumin through carbodiimide condensation using ethyl carbodiimide and emulsified in complete Freund’s adjuvant after a 24-h dialysis [23]. Highly purified porcine secretin was used as standard. The ID50 was 0.21–0.42 fmol/tube, and variations were 6.2% (inter-assay) and 5.8% (intra-assay). The antisomatostatin serum (final dilution 1/300,000) was obtained from a rabbit twice injected with synthetic cyclic somatostatin conjugated to bovine serum albumin I [3]. Ovine somatostatin was used as a standard. The sensitivity of the assay was 0.9–1.83 pmol/tube and variations were 12.5% (inter-assay) and 7.1% (intra-assay). The results are expressed as pmol equivalent of standard per liter of plasma (pmol/l).

**Experimental procedure**

In order to obtain plasma CCK and gastrin concentrations fairly similar to physiological levels, we first collected blood samples at different times, from 75 min before to 90 min after the morning meal on the day preceding each experiment. Experiments were carried out between the two daily meals, from 4.5 to 7.0 h after the morning meal. Since this period has been previously shown to be constant in terms of pancreatic exocrine secretion [15], we chose to infuse the CCK receptor agonists and variations were 12.5% (inter-assay) and 7.1% (intra-assay). The results are expressed as pmol equivalent of standard per liter of plasma (pmol/l).