

Physiological role of cholecystokinin on postprandial insulin secretion and gastric meal emptying in man. Studies with the cholecystokinin receptor antagonist loxiglumide

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Summary. Cholecystokinin was previously proposed to play an important role in the regulation of postprandial insulin secretion either indirectly, by inhibiting gastric meal emptying, or directly, by acting as an incretin promoting the release of insulin. The aim of this investigation was therefore to clarify the role of endogenous cholecystokinin in the regulation of insulin release and gastric emptying applying the highly potent and specific cholecystokinin receptor antagonist loxiglumide. Five healthy volunteers were examined after an overnight fast. Gastric meal emptying was measured by the double indicator technique using a multiple lumen tube in the duodenum and ^{99m}Tc-diethylenetriamine pentaacetate as a meal marker and polyethylene glycol 4000 as a duodenal perfusion marker. Postprandial insulin, C-peptide, cholecystokinin and glucose levels were measured after ingestion of two isocaloric meals of a) Ensure (containing fat, protein and glucose), and b) a pure glucose meal (1.11 mol/l). The meals were given either with an intravenous infusion of loxiglumide (22 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) or placebo. The infusion of loxiglumide markedly accelerated the gastric emptying of the mixed meal (area under curve, 5576 \pm 352 min vs 3498 \pm 109 min; $p < 0.001$) and the pure glucose meal (area under curve

5662 \pm 537 min vs 3551 \pm 534 min; $p < 0.05$). Simultaneously, loxiglumide induced a more rapid rise in postprandial insulin levels after both meals resulting in significantly higher ($p < 0.05$) insulin levels during the first postprandial hour, but similar insulin levels in the second postprandial hour. Accordingly, we found a close correlation between meal emptying and insulin release ($r = 0.748$, $p < 0.01$). Integrated insulin and C-peptide levels for the whole 2-h experimental period tended to be higher during loxiglumide infusion, but the difference did not reach statistical significance. Similar plasma glucose levels at all time periods were observed with and without loxiglumide infusion. Higher cholecystokinin levels were measured during loxiglumide infusion after the mixed ($p < 0.01$) and the pure dextrose ($p < 0.05$) meals. We conclude that postprandially released cholecystokinin exerts an important role in the regulation of gastric meal emptying and consecutively the postprandial pattern of insulin release. In contrast, no evidence was found for an insulinotropic role for cholecystokinin in man.

Key words: Cholecystokinin blocker, plasma glucose, insulin, C-peptide, plasma cholecystokinin.

Postprandial plasma insulin and glucose levels are controlled by two major components: a) the gastric emptying rate of ingested nutrients and thus the rate of glucose delivery from the stomach to the duodenum, and b) the potency of glucose to release insulin from the pancreatic Beta cell. Previous studies in man demonstrate that infusion of physiological doses of cholecystokinin (CCK) results in a distinct inhibition of gastric emptying [1, 2]. CCK may therefore exert an important physiological action by regulating insulin release and blood glucose levels indirectly through the retardation of gastric meal emptying in man [3].

Among several other gut factors which have been proposed to act as incretins, thus facilitating the release of insulin [4], CCK has previously been shown to stimulate the

release of insulin during intravenous infusion of amino acids [5]. CCK might therefore act as an incretin, an additional action possibly contributing to postprandial glucose homeostasis.

The importance of postprandial released, endogenous CCK on postprandial insulin release and plasma glucose levels in relation to gastric meal emptying in man has not been studied and remains as yet poorly defined. The aim of this study was therefore to clarify the role of endogenous CCK in the regulation of postprandial insulin release and glucose homeostasis in man. For this purpose we employed loxiglumide, a highly potent and specific CCK receptor antagonist [6–11], which is available for use in humans. We evaluated postprandial plasma glucose levels, the release of insulin and C-peptide in relation to gastric

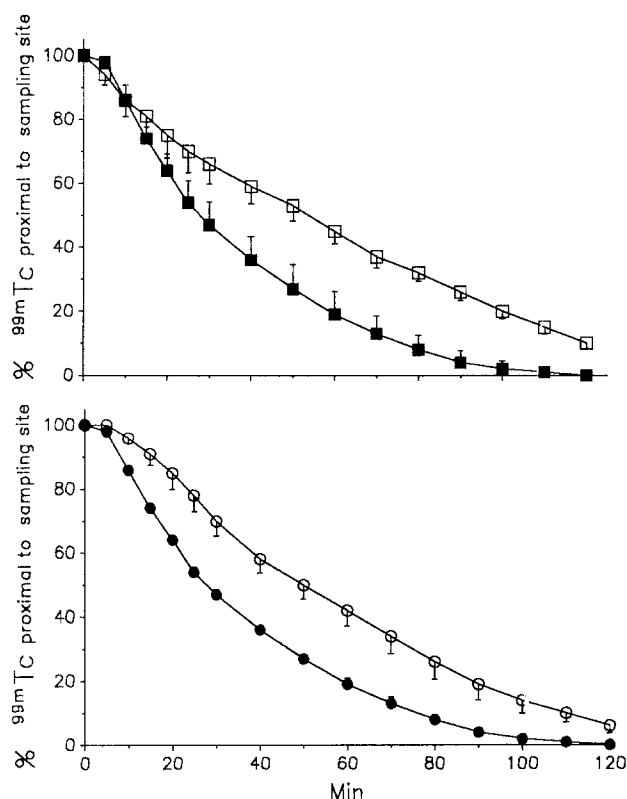


Fig. 1. Gastric emptying (% ^{99m}Tc proximal to sampling site) of 500 ml meals of Ensure (circles) or glucose 1.11 mol/l (squares) during infusion of placebo (empty symbols) or loxiglumide (filled symbols; 22 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) in five healthy subjects (mean \pm SEM)

meal emptying during infusion of loxiglumide or placebo in healthy volunteers.

Subjects and methods

Experimental design

Subjects. Five healthy volunteers, 23–51 years old, participated in the experiments. None was taking any medication nor had a history of gastrointestinal symptoms or prior surgery. The studies were approved by the local ethical committee and all subjects gave written, informed consent. All studies were performed after an overnight fast.

Experiments. Each experiment consisted of an 80-min pre-meal period and a 120-min post-meal period. Loxiglumide was given through a separate indwelling intravenous catheter at 22 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ the infusion being started 60 min before meal administration. This dose and time schedule has been previously shown to result in a marked suppression of pancreatic enzyme secretion during intraduodenal perfusion of mixed meals [12]. In control experiments 154 mmol/l NaCl was infused instead of loxiglumide.

Two isocaloric 500 ml meals were administered intragastrically: a) a mixed meal, containing fat, protein and glucose (Ensure, Abbott, Chicago, Ill., USA) diluted with water, containing 16.2 g protein, 12.9 g fat and 51.4 g carbohydrate per 500 ml and a total caloric value of 387 kcal, 300 mOsm, pH 7, and b) a pure glucose meal (100 g/500 ml, 400 kcal, 1190 mOsm, pH 7) were given either with loxiglumide or a placebo infusion (154 mmol/l NaCl). The four tests were performed in all five subjects in strict random order. Only one test was done on each day with a minimum of 48 h between two experiments in each individual.

Blood sampling. Blood samples were taken through an indwelling venous catheter at 30-min intervals just before each meal and at 10, 20, 30, 45, 60, 90 and 120 min after, in ice-chilled ethylenediaminetetraacetic acid tubes that contained 500 Kallikrein Inactivator units aprotinin per ml of blood. Samples were immediately centrifuged and the plasma stored at -20°C until assay.

Experimental procedure

Measurement of gastric emptying. Gastric emptying rates were measured by the double indicator technique [13, 14]. The subjects were intubated with a multiple lumen tube, the tip being placed at the ligament of Treitz. A marker perfusion site was located 20 cm proximal to the tip at the papilla Vateri. One canal of the tube ended in the antral portion of the stomach. The final position of the tube was verified by fluoroscopy. With the subjects comfortably seated, an infusion of polyethylene glycol 4000 (PEG) (1.5 g PEG/100 ml 154 mmol/l NaCl) was commenced through the proximal port of the duodenal tube at a rate of 1.5 ml/min. After allowing 60 min for equilibration, two pre-meal samples were collected from the tip of the tube at 10-min intervals. The subjects were then given one of the 500 ml liquid meals through the gastric channel, each containing 20–50 μCi ^{99m}Tc-diethylenetriaminepentaacetate (^{99m}Tc-DTPA) as a second non-absorbable marker. Duodenal contents were continuously siphoned as 7–8 ml samples from the distal sampling site, at 5-min intervals in the first 30 min, and at 10-min intervals thereafter. At 15-min intervals throughout the experiments, 5 ml samples of gastric contents were collected from the gastric tube, the first sample being taken 5 min after meal ingestion. Duodenal samples were analysed for concentration of PEG [15] and ^{99m}Tc. Gastric samples were analysed for ^{99m}Tc and PEG. PEG determinations of gastric aspirates proved PEG reflux of the duodenal contents into the stomach to be either lacking or negligible.

Flow rates passing the jejunal sampling site were calculated based on known infusion rates and PEG concentrations at the infusion and sampling ports. The rate of ^{99m}Tc passage at the distal sampling site was calculated from the product of flow rates and ^{99m}Tc concentrations and was used as an index of gastric emptying. The amount of ^{99m}Tc proximal to the distal sampling site was calculated by subtracting the cumulative amount of marker recovered at each timepoint from the total amount of marker recovered during the whole experiment at the duodenal sampling site. This amount of ^{99m}Tc marker was expressed as a percentage of the total ^{99m}Tc recovered during the experiment. Individual emptying curves were plotted using these percentage values. The mean \pm SEM ^{99m}Tc recovery (marker given with the test meals corrected for the amount of ^{99m}Tc aspirated from the stomach) was $86 \pm 5\%$.

The areas under the emptying curves (AUC; min) were calculated for each individual curve. The emptying curves were furthermore fitted to a power exponential model as described earlier [16].

Radioimmunoassay of insulin, C-peptide and CCK. Plasma insulin concentrations ($\mu\text{U}/\text{ml}$) were measured using a commercial RIA kit (CIS Bioindustries, Gif-Sur-Yvette, France) with a sensitivity of 3.6 $\mu\text{U}/\text{ml}$. Plasma C-peptide levels (pmol/l) were measured using antibody 1221 from Novo (Bagsvaerd, Denmark) [17]. Plasma glucose (mmol/l) was determined by a glucose oxidase technique.

Plasma CCK concentrations were estimated by a sensitive and specific RIA [18]. Antibody T204 binds to all carboxy-terminal specific CCK peptides containing the sulphated tyrosine. The antibody shows < 2% cross-reactivity to sulphated gastrins and does not bind to unsulphated forms of gastrin nor does it cross-react with structurally unrelated gastrointestinal peptides. Synthetic CCK 8 coupled to ¹²⁵I-hydroxyphenylpropionic acid succinimide ester (Bolton Hunter reagent) was used as standard. All plasma samples were treated by an ethanol extraction procedure. A two-step incubation procedure was used. Separation between free and antibody-bound hormone was performed by a charcoal method. The detection limit of the assay was 0.5 pmol/l. The 50% inhibition dose was 2.0 pmol/l. The intraassay and interassay variation was 8 and 11%, respectively.

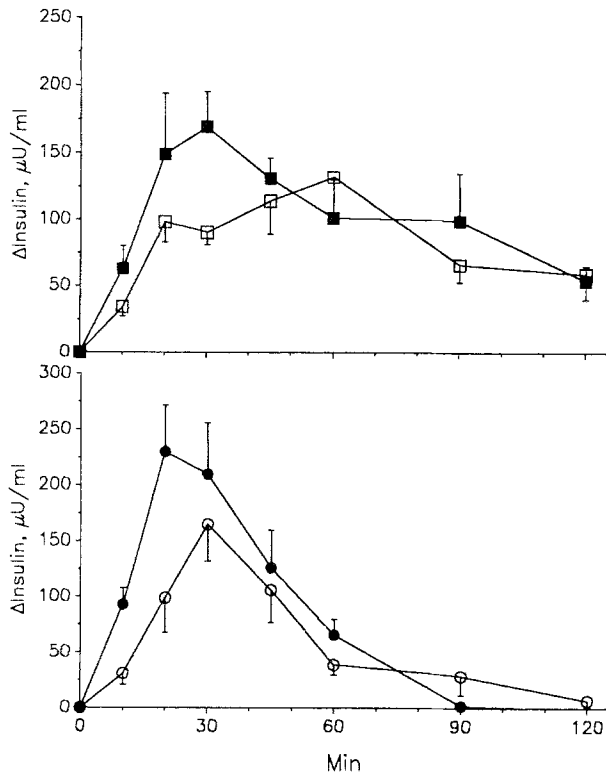


Fig. 2. Plasma insulin levels ($\mu\text{U}/\text{ml}$) after gastric instillation of a mixed meal (Ensure; circles) or glucose 1.11 mol/l (squares) during infusion of loxiglumide (filled symbols) or placebo (empty symbols) in five healthy subjects (mean \pm SEM)

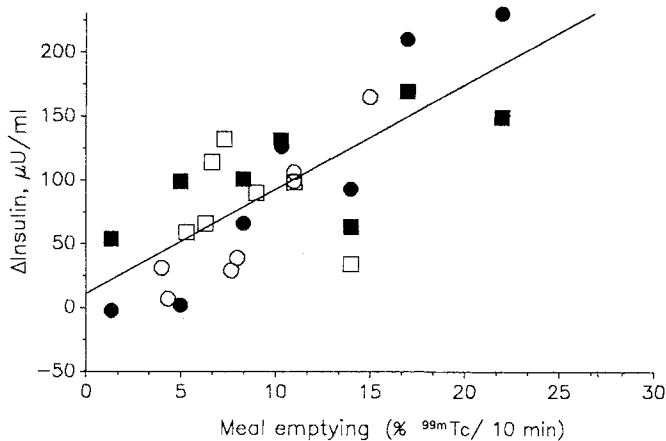


Fig. 3. Linear correlation ($y = 10.7 + 8.2x$; $r = 0.748$; $p < 0.01$) between meal emptying and insulin levels ($\mu\text{U}/\text{ml}$) in five healthy volunteers using single mean timepoints of $^{99\text{m}}\text{Tc}$ -DTPA recovery (% recovery/10 min) after a mixed meal (Ensure; circles) and a glucose 1.11 mol/l (squares) meal during infusion of loxiglumide (filled symbols) or placebo (empty symbols)

Statistical analysis

Results are expressed as mean \pm SEM. Increments of insulin, C-peptide, CCK and plasma glucose were computed by subtracting basal values from postprandial levels. The integrated increments of insulin, C-peptide, CCK and plasma glucose were calculated. Responses in experiments with and without infusion of the CCK blocker were compared using a Student's *t*-test for paired values after logarithmic transformation of the data, if appropriate. Linear regression analysis was performed using single timepoints for emptying data and hormone levels. Differences were considered significant if $p < 0.05$.

Results

Gastric emptying

The mixed meal and the glucose meal were emptied at comparable rates (Fig. 1), both being completely ($> 90\%$) evacuated within 120 min. The infusion of loxiglumide resulted in a marked acceleration of the gastric emptying rates of both meals. Thus, distinctly lower areas under the emptying curves were found during loxiglumide infusion after the mixed meal (AUC 5576 ± 352 min vs 3498 ± 109 min; $p < 0.001$) and the pure glucose meal (AUC 5662 ± 537 min vs 3551 ± 534 min; $p < 0.05$).

Release of insulin, C-peptide, and CCK

The infusion of loxiglumide did not alter basal plasma insulin (4.9 ± 2.8 $\mu\text{U}/\text{ml}$ placebo; 3.4 ± 0.9 $\mu\text{U}/\text{ml}$ loxiglumide, respectively; NS) and plasma glucose (4.9 ± 0.4 mmol/l placebo; 4.6 ± 0.4 mmol/l loxiglumide, respectively; NS) levels. During loxiglumide infusion insulin concentrations rose more rapidly after both meals resulting in significantly higher integrated insulin levels in the first postprandial hour ($p < 0.05$), but similar insulin levels in the second postprandial hour (Fig. 2). Accordingly, we found a close correlation ($r = 0.748$; $p < 0.01$) between meal emptying and insulin release in experiments with and without loxiglumide as shown in Figure 3. The mixed meal resulted in maximal insulin levels of 169 ± 33 $\mu\text{U}/\text{ml}$ after 30 min during placebo infusion and 237 ± 43 $\mu\text{U}/\text{ml}$ after 20 min during loxiglumide infusion; after instillation of the glucose meal, insulin levels rose to reach maximal levels of 138 ± 32 $\mu\text{U}/\text{ml}$ after 60 min during placebo and 173 ± 26 $\mu\text{U}/\text{ml}$ after 30 min during loxiglumide infusion followed by a continuous decline (Fig. 2). Integrated insulin and C-peptide levels for the whole 2-h experimental period tended to be higher during loxiglumide infusion (Figs. 4 and 5), but the difference did not reach statistical significance.

Similar increases of plasma glucose were observed at all timepoints whether the meals were given with or without loxiglumide infusion (Fig. 6). Plasma glucose con-

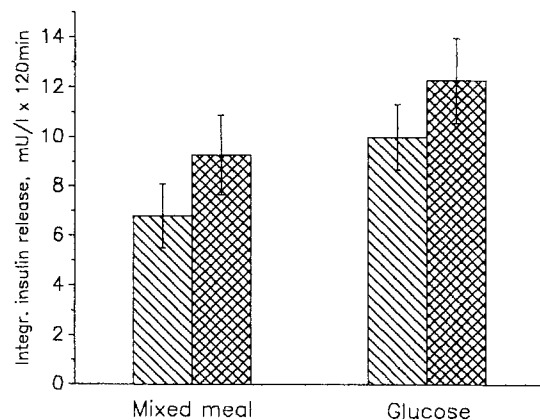


Fig. 4. Integrated plasma insulin levels ($\text{mU}/\text{l} \times 120\text{min}$) after gastric instillation of a mixed meal (Ensure) or glucose 1.11 mol/l during the infusion of loxiglumide (cross-hatched bars) or placebo (diagonal bars; mean \pm SEM; $n = 5$)

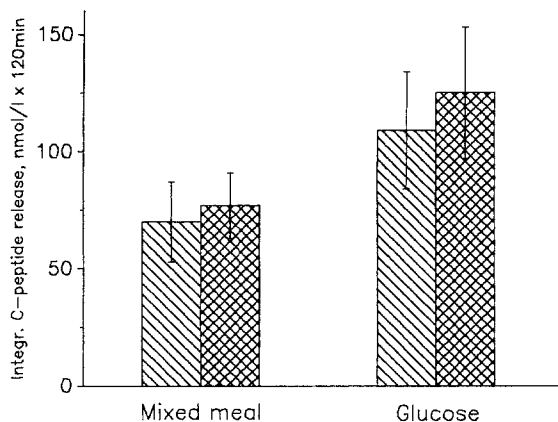


Fig. 5. Integrated plasma C-peptide levels (nmol/l × 120 min) after gastric instillation of a mixed meal (Ensure) or pure glucose 1.11 mol/l during the infusion of loxiglumide (cross-hatched bars) or placebo (hatched bars; mean ± SEM; $n = 5$)

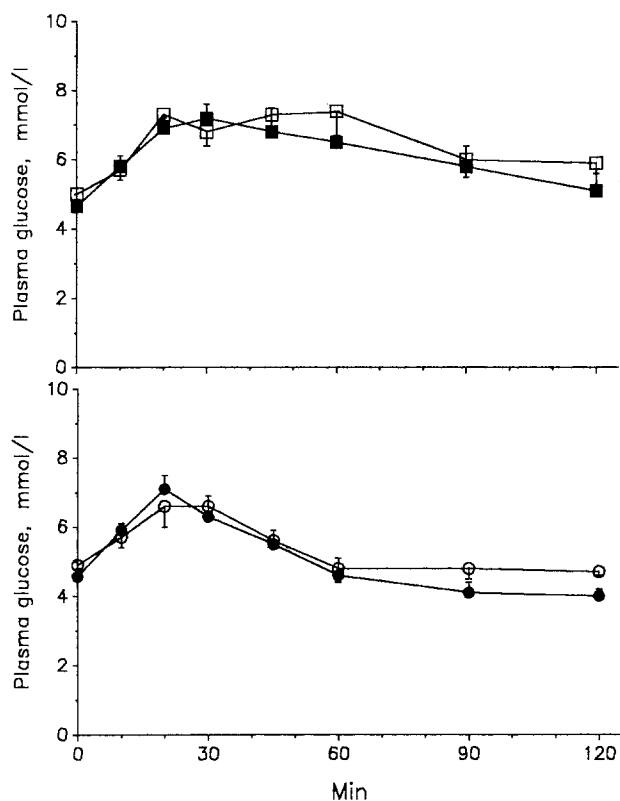


Fig. 6. Plasma glucose concentrations (mmol/l) after intragastric meals of a mixed meal (Ensure; circles) or glucose 1.11 mol/l (squares) during the infusion of placebo (empty symbols) or loxiglumide (filled symbols; mean ± SEM; $n = 5$)

centrations reached a maximum of between 6.9 and 7.3 mmol/l 20 min after ingestion of the mixed and the pure glucose meal declining thereafter towards basal levels.

Plasma CCK levels (not shown) rose to reach maximal increments within 20 min of 6.9 ± 2.6 pmol/l after the mixed meal and 2.8 ± 0.6 pmol/l after the glucose meal, respectively. Higher postprandial CCK concentrations were found during loxiglumide infusion with integrated CCK levels (pmol/l · 120 min) of 523 ± 62 after the mixed meal and 1334 ± 165 during loxiglumide infusion ($p < 0.001$);

after administration of glucose, integrated CCK release was 230 ± 69 , and increased to 493 ± 90 during loxiglumide infusion ($p < 0.05$).

Discussion

CCK is an established gut hormone released after food ingestion exerting a major role in the regulation of pancreatic secretion, gallbladder contraction, and gastrointestinal motility [19–22]. Recently we have shown that CCK is the common regulator of gastric emptying and pancreatic secretion [23]. This study suggested a new integrative role of CCK in the upper gastrointestinal tract contributing to the synchronization and coordination of two closely linked functions. As CCK was previously found to influence both gastric emptying [19, 23] and insulin release [5] we postulated that CCK might also play an integrative role in the regulation of postprandial glucose homeostasis by controlling both the gastric emptying rate of glucose and the release of insulin.

We found a distinct acceleration of gastric meal emptying during the infusion of the highly potent and specific CCK blocker loxiglumide. Our results confirm previous animal [8, 24–30] and human [19, 31] studies using loxiglumide and its related compounds proglumide and lorglumide [8, 19, 24–27, 31] as well as other types of CCK antagonists such as MK-329 [28–30] demonstrating a marked acceleration of the gastric emptying rate by CCK blockade. The only two negative studies [32, 33] used a solid-liquid meal while we employed a liquid meal. As the emptying of the solid and the liquid phase is controlled by different mechanisms [34], it is conceivable that the effect of a CCK antagonist depends upon the type of the meal, probably accounting for the contrasting results.

The faster gastric emptying of both meals during loxiglumide infusion entailed a higher duodenal load of carbohydrates, which was fully compensated for by the augmented release of insulin in the first postprandial hour, explaining similar plasma glucose levels in experiments with and without loxiglumide infusion. Accordingly, we found a close linear correlation between insulin release and meal emptying. The results of this investigation show that CCK regulates insulin release indirectly by controlling the gastric emptying rate and thus the delivery of nutrients to the duodenum. Our results confirm an earlier study demonstrating a dose-dependent retardation of insulin release by intravenous infusion of CCK in physiological doses [3]. We could demonstrate, to our knowledge for the first time, that endogenously released CCK markedly affects the postprandial release pattern of insulin by controlling gastric meal emptying in man. In addition, we found that the effects of endogenously released CCK on the delivery of glucose to the duodenum are fully compensated for by an increased release of insulin early after meal ingestion resulting in relatively uniform plasma glucose levels. These findings explain previous investigations reporting that postprandial blood glucose levels remain regulated within a narrow range, despite wide variations in the content of glucose, fat and protein in the meals [35, 36]. For example, in one study similar plasma glucose

levels were observed after ingestion of whole and skimmed milk despite a marked difference in the fat content of the meals [36].

The fact that orally administered glucose evokes a greater insulin response than intravenously administered glucose is well established. Endocrine factors, incretins released from the small gut, are supposed to be responsible for this phenomenon [4]. However, the gut hormone mainly involved has not been as yet clearly determined [37]. Gastric inhibitory polypeptide (GIP) was hitherto considered to be the best candidate incretin [4]. Recent studies, however, have challenged its physiological role as an insulinotropic hormone [38–41] and other peptides such as truncated glucagon-like peptide 1 (GLP 1) have been introduced as new, more potent candidate incretins [42]. In this investigation no reduction of insulin release by CCK blockade after either a mixed meal or a pure glucose meal was found. Thus, our results do not support a role for CCK as an insulinotropic hormone as reported recently [5, 43]. One group [5] observed an augmented insulin secretion during a combined infusion of CCK 8 and amino acids, whereas CCK alone had no effect on insulin release. However, it is unclear if a physiological dose of amino acids was applied as no data were presented on postprandial plasma levels of amino acids. For example, no significant rise of plasma amino acid levels after a casein meal was found in a study in rats [43]. In the latter study an incretin effect of CCK was reported by use of the specific CCK antagonist L364798 in response to intraduodenal infusion of casein. Species differences account probably for the contrasting results. Thus, it is known that casein but not albumin is a potent stimulant of CCK release and pancreatic secretion in the rat [44], whereas albumin markedly releases CCK in man. Our results, excluding a physiological role of CCK as a incretin in man, are confirmed by several preliminary publications applying the CCK antagonist loxiglumide [45–47] and MK-329 [48, 49], where no augmentation of insulin release by CCK blockade was found.

The infusion of loxiglumide caused a marked increase of plasma CCK levels after both meal types corresponding to a previous study [6]. This phenomenon may be explained by the activation of an enteropancreatic feedback mechanism [50] during CCK blockade resulting in lower duodenal enzyme concentrations. The acceleration of gastric emptying during loxiglumide infusion may have contributed to this phenomenon by accelerating the spread of nutrients over the small intestine and causing an augmented release of CCK.

In summary, by applying the specific and potent CCK receptor antagonist loxiglumide, we could demonstrate, to our knowledge for the first time, that postprandially released CCK exerts a major role in the regulation of gastric meal emptying, and consecutively the postprandial pattern of insulin release. Thus, CCK inhibits gastric meal emptying, simultaneously retarding the release of insulin. In contrast, we found no evidence for a direct insulinotropic role of CCK in man.

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