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Oxyntomodulin from Distal Gut

Role in Regulation of Gastric and Pancreatic Functions

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We studied the effects of intravenous infusion of synthetic oxyntomodulin (proglucagon 33–69), a potential hormone from the ileal mucosa, on fasting and postprandial gastric acid secretion, gastric emptying, gastroduodenal motility, and pancreatic secretion of trypsin and lipase measured simultaneously in six normal volunteers using multilumen tubes for infusion of markers, manometry, and aspiration of gastric and duodenal contents. The infusion resulted in plasma concentrations of 203 ± 21 pmol/liter (mean ± SEM) of oxyntomodulin, regarded as high but not unphysiological concentrations of the peptide. Oxyntomodulin almost abolished basal acid secretion and inhibited postprandial acid secretion by 35 ± 10%. Gastric emptying decreased significantly; the time for 50% to leave the stomach increased from 17.3 ± 2.2 min to 34.7 ± 8.0 min. The postprandial gastroduodenal motility was massively inhibited by oxyntomodulin. Postprandial trypsin and lipase output was significantly inhibited by 56 ± 12% and 42 ± 11%, respectively, during oxyntomodulin infusion. However, pancreatic enzyme output was linearly related to gastric emptying and oxyntomodulin did not influence this relationship, suggesting that oxyntomodulins effect was due to its effect on gastric emptying. Oxyntomodulin seems to play an important role in the small intestinal inhibitory control of gastropancreatic functions.

KEY WORDS: enteroglucagon; enterogastron; pancreatone; gastric emptying; intestinal motility; pancreatic secretion.

Oxyntomodulin (glicentin 33–69) consists of the entire glucagon sequence (1–29) plus a basic octapeptide extending from its C-terminus (1, 2). Oxyntomodulin belongs to the enteroglucagons. It is secreted by the L cells in the ileum and the colon (3–7) and can be released into the circulation (8, 9) by intraluminal stimulation with glucose (8–10) and other nutrients (9–11). Oxyntomodulin may be measured in plasma by radioimmunoassay (8) or, more specifically, using receptor assays and high-pressure liquid chromatography (6, 10).

Pancreatic glucagon (glucagon 1–29) and oxyntomodulin are derived from the same precursor molecule, proglucagon (12, 13). Tissue-specific post-translational processing of the N-terminal part of proglucagon gives rise to mainly glucagon 1–29 and glicentin-related pancreatic peptide (GRPP) in the pancreas and to mainly glicentin, oxyntomodulin and GRPP in the gut (10, 12–14). Oxyntomodulin exerts glucagon-like biological effects on the liver and on the pancreatic beta cells, stimulating the hepatic glucose production (15) and the insulin secretion of the islets (11, 16). However, it has been suggested that the primary targets for this peptide are the gastric oxyntic glands (17). In rats, both

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pentagastrin-stimulated and meal-stimulated gastric acid secretion are almost abolished by oxyntomodulin (18–20). In humans, synthetic oxyntomodulin is a potent and efficient inhibitor of pentagastrin-stimulated acid secretion (21). Furthermore, recently oxyntomodulin was shown to inhibit basal and cerulein-stimulated pancreatic exocrine secretion in conscious rats (22).

To further elucidate the role of oxyntomodulin as a regulator of gastrointestinal functions we studied simultaneously the effects of oxyntomodulin on gastric acid secretion, duodenogastric reflux, gastric emptying, gastroduodenal motility, and pancreatic exocrine secretion in healthy volunteers in the fasting state and in response to a meal.

MATERIALS AND METHODS

Six healthy volunteers (two women, four men; median age 29 years, range 22–45) participated in the study. All gave informed consent. The study was approved by the regional ethical committee.

Two nonabsorbable markers commonly used for gastrointestinal perfusion studies were used. To the meal was added 0.05 mCi ^{99m}Tc chelated to DTPA (diethylene triaminopentaacetic acid). As a marker for duodenal perfusion we used 0.05 mCi ^{111}In (Amersham, Buckinghamshire, UK) chelated to DTPA, dissolved in 500 ml saline with 5 ml 20% human albumin (Statens Serum-Institute, Copenhagen, Denmark) to avoid adhesion to tubes. Perfusion rate was 2 ml/min.

The liquid meal was composed of 10 g albumin, 10 g lipid emulsion (Kabi-Vitrum, Stockholm, Sweden), which has been shown to prevent flocculation (23), and 20 g sucrose dissolved in water to 300 ml at room temperature. The caloric content was 200 kcal (837 kJ). The meal marker [^{99m}Tc]DTPA was mixed with the meal. Previous studies have shown less than 5% dissociation of this marker from the undigested meal (23).

Tubes. These were: (1) A gastric sump tube (Andersen tube AN 10, Andersen, New York) with large aspiration holes and an airvent to facilitate aspiration of gastric contents for determination of meal marker, duodenal marker, and H^+ concentrations; and (2) a duodenal multilumen sump tube (William Cook Europe, A/S, Denmark) consisting of a four-lumen tube cemented to an airvent sump tube with tetrahydrofuran; total diameter was 14 F. At the tip was tied a fingercot filled with 0.75 ml of mercury to facilitate aboral transport. Three of the lumina (10, 20, and 35 cm proximal to the tip of the tube) were perfused with water at a rate of 3 ml/hr and connected to a strain gauge transducer (model M 5 F, Simonsen & Weel, Copenhagen, Denmark) for motility recordings (1) in the gastric antrum, (2) 15 cm distally in the duodenum, and (3) 25 cm distally in the duodenum, close to the ligament of Treitz. One lumen was used for [^{111}In]DTPA perfusion at the papilla of Vater (25 cm proximal from the tip of the tube) to quantify passage of the marker ingested with the meal. The tip of the duodenal sump tube with airvent was positioned at the angle of Treitz for continuous aspiration of duodenal contents for determination of meal marker, duodenal marker, trypsin, and lipase concentrations.

Infusion of Oxyntomodulin. Crude oxyntomodulin, mol wt 4423, was obtained by custom synthesis from Peninsula Laboratories, Europe Ltd. (St. Helens, Merseyside, UK, lot No. 008373; peptide purity by amino acid analysis 78%, factory's chromatogram No. (Cl-8167) and purified in our laboratory to homogeneity by isocratic reverse-phase HPLC (high-pressure liquid chromatography) on a 8 × 250-mm Nucleosil C-18 column (particle size 10 μm) using 37.7% ethanol in water containing 0.5% trifluoroacetic acid (11). The assumed structure of the synthetic peptide was confirmed by gas-phase sequence analysis (11). Purified oxyntomodulin, 0.5 mg, was dissolved in 10 ml 0.5 mol/liter acetic acid, containing, in addition, 100 mg human serum albumin. The solution was passed through sterile 0.22-μm Millipore filter cartridges (Millipore, Bedford, Massachusetts) and stored under sterile conditions. Approval for infusion of this synthetic oxyntomodulin into humans was given by the Pharmaceutical Division of The Danish National Board of Health. The concentration of oxyntomodulin in this solution was determined by radioimmunoassay (see below). The calculated amount of oxyntomodulin to be infused in each volunteer was dissolved in 250 ml saline containing in addition 2500 mg human serum albumin. The amount of oxyntomodulin required to produce plasma concentrations of 130 pmol/liter corresponding to the enteroglucagon concentrations observed in plasma in response to meals or nutrients (24, 25) was calculated to correspond to a priming dose of 57.6 ng/kg followed by a continuous infusion of 400 ng/kg/hr. The calculation was based on single-compartment kinetics, assuming that the apparent volume of distribution (VD_{app}) was 10% of body weight (0.1 × BW) and that the half-life in plasma was approximately 6 min, as found for the related peptide glucagon 1–29 (26). Cp_{ss} = concentration in plasma at steady state = infusion rate/$VD_{app} \times k_e$, where k_e is the rate constant for elimination. Priming dose = $VD_{app} \times Cp_{ss}$. Metabolic clearance rate (MCR) = infusion rate/$CP_{ss} = VD_{app} \times k_e$.

Experimental Protocol. The volunteers were randomly assigned to receive intravenous infusion of either saline or oxyntomodulin on separate days. After an overnight fast (12 hr) the volunteer first swallowed the duodenal and then the gastric tube. Under fluoroscopic control the tubes were positioned as described above.

The person was positioned in a 45° recumbent position, and the gastric contents were aspirated. Infusion of the duodenal marker was started and continued throughout the study. Manometric recordings from the antrum and the duodenum were performed throughout the study. Continuous aspiration with intermittent suction (0–40 mm Hg) on the gastric and duodenal sump tubes were initiated; the aspirate was collected for 20-min periods. Duodenal aspirates were collected on ice. A basal period of 45 min followed, allowing duodenal mixing to reach an equilibrium (27). From then on, appearance of a migrating motor complex (MMC) on the manometric recorder determined initiation of the study (time −50 min). From this time and onwards gastric and duodenal aspirates were collected for 10-min periods. Twenty minutes later (time

−30 min) the intravenous infusion of either saline or oxyntomodulin began. This time was chosen because gastric and pancreatic secretions were then believed to be at a minimum (28). At time 0 min the gastric aspiration was stopped and the liquid meal installed through the gastric tube over 2 min. From time 0 duodenal pooled samples were collected at time 5, 15, 25 min, etc. At time 10, 20, 30 min, etc, gastric contents were mixed using a large bore syringe, and 6 ml was withdrawn for analysis. Thus, there was a 5-min lag period between gastric and duodenal sampling to allow the gastric marker leaving the stomach to reach the aspiration site in the duodenum. When the stomach was empty, 250 ml saline were instilled for lavage and aspirated. The duodenal sampling continued for another 5 min.

Validation of Trypsin and Lipase Stability in Duodenal Aspirates. After an overnight fast (12 hr) a volunteer with a duodenal tube in place swallowed the test meal. The following 30-min duodenal contents were aspirated on ice. Two aliquots were analyzed for trypsin and lipase activity; other aliquots were stored at −20° C until thawed and analyzed for trypsin and lipase on days 2, 4, 9 and 10.

Analysis of Gastric and Duodenal Samples. Concentrations of titratable acid in gastric samples were determined by titration with 0.1 mol/liter NaOH to pH 7.40 at 37° C using an autoburet (titrator TT2, ABU 80 autoburet, Radiometer, Copenhagen, Denmark). Duodenal samples were collected on ice and stored at −20° C to prevent degradation. Aliquots of 1.5 ml were analyzed for lipase and trypsin. Concentrations of lipase were determined titrimetrically (pH stat) and trypsin by modification of the method by Schwert and Takenaka as previously described (29). The radioactive concentrations in each gastric and duodenal samples of ^{99m}Tc and ^{111}In were measured in duplicate in a gammaspectrometer. The results were corrected for background, decay, and cross-talk (determined as the relative amount of ^{111}In detected in the ^{99m}Tc window; this averaged 7%).

Analysis of Blood Samples. Blood samples were drawn at time −45, −30, −15, 0, 15 min, etc, until the termination of the study. For pharmacokinetic studies blood samples were drawn 2.5, 5, 10, 20, and 30 min after the termination of the oxyntomodulin infusion. Blood samples were drawn into chilled tubes containing heparin and aprotinin (Trasylol, Bayer, Leverkusen, FRG, 1000 KIU/ml) and centrifuged at 4° C within 30 min. Plasma was kept at −20° C until analyzed for enteroglucagon, pancreatic glucagon, somatostatin, and glucose contents.

Radioimmunoassays. Assays for the glucagon sequences 6–15 and 19–29 were performed on ethanol-extracted plasma using antisera 4304 and 4305, respectively, glucagon standards and monoiodinated glucagon as in Holst et al (30). Antiserum 4304 measures all forms of glucagon in plasma including the enteroglucagons glicentin and oxyntomodulin. Antiserum 4305 only measures forms with an exposed C-terminus, mainly pancreatic forms. Oxyntomodulin concentrations were calculated by subtraction of values obtained with antiserum 4305 from values obtained with antiserum 4304. The recovery of oxyntomodulin, added to plasma and measured in this way (including ethanol extractions) was 55 ± 5.6% (mean ± SD) at four concentration levels (20, 100, 200, and 1000 pmol/liter). Somatostatin was assayed as in Hilsted and Holst (31). Glucose concentrations were determined by the hexokinase method (32).

Calculations. Recovery of gastric marker in duodenal samples, total acid output, and secretory outputs (volume secretion) from the stomach and duodenum were calculated as described in detail by Malagelada et al (33). Fasting reflux was calculated from the volume aspirated from the stomach per 10 min and its ^{111}In concentration. Postprandial reflux was calculated using equations for calculation of acid output in 10-min periods (33), the ^{111}In concentration of each gastric sample being substituted for acid concentration. The amount of ^{111}In refluxed in 10-min periods was expressed as a percentage of the amount of ^{111}In infused into the duodenum in 10-min periods. Gastric emptying was characterized as follows: The total amount of gastric marker recovered in duodenal samples was taken to represent the volume of the given meal. The amounts of ^{99m}Tc radioactivity calculated (as in reference 33) to remain in the stomach in the 10-min periods were expressed in percent of the total amount of marker recovered; thus, gastric emptying was characterized quantitatively by these percentages.

Antroduodenal motor activity was estimated on the basis of the recorded frequency and amplitude of the pressure waves. The number of pressure waves with an amplitude above 50 cm H_2O were counted from time 4 to 10 min after installing the meal.

Statistical Analysis. Wilcoxon's nonparametric test for paired data was used to test the significance of differences between the two experimental days. Half-hour preprandial (time −30 min to 0) and half-hour postprandial (time 0 to 30 min) as well as results obtained at each of the individual time points were compared. Results are given as mean ± SEM. Differences were considered significant if the P value was less than 0.05.

RESULTS

The recovery of the gastric marker in duodenal samples was 83 ± 4%, which is comparable to that found by others (27).

Gastric Acid and Volume Secretion. Gastric acid secretion in the fasting state and postprandially is shown in Figure 1, upper panel. Infusion of oxyntomodulin strongly inhibited basal acid secretion (time −30 min to 0): from 1.8 ± 0.8 mmol H^+/10 min (saline) to 0.4 ± 0.1 mmol H^+/10 min (oxyntomodulin), an inhibition of 69 ± 9%, $P < 0.05$. Also postprandial acid secretion (0–30 min) was significantly inhibited by oxyntomodulin. Acid secretion decreased from 3.8 ± 0.8 mmol H^+/10 min (saline) to 2.2 ± 0.4 mmol H^+/10 min (oxyntomodulin), an inhibition of 35 ± 10%, $P < 0.05$. Simultaneous measurement of gastric volume secretion (lower panel) showed a similar decrease resulting from infusion of oxyntomodulin. Basal volume secretion

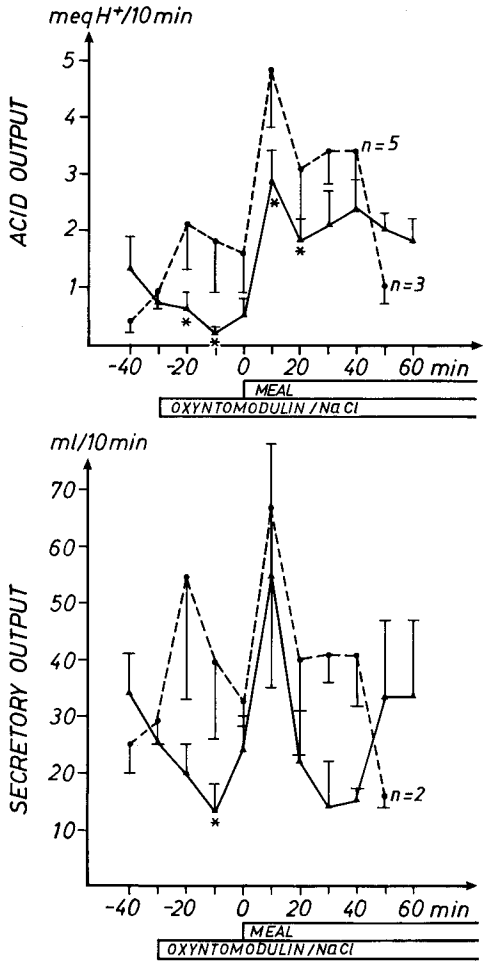

Fig 1. Gastric acid and secretory output (volume secretion) in the fasting state and after instillation of a liquid meal. The dashed lines indicate results from the saline infusion control experiment. Solid lines show the results from the oxyntomodulin infusion experiment. Asterisks indicate statistical significant differences between the two experimental days at individual time points.

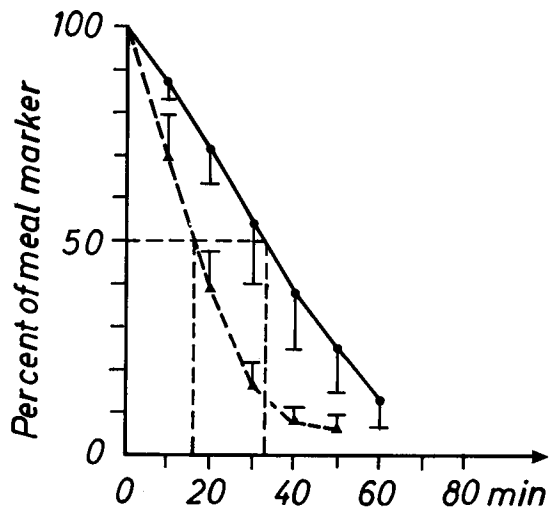

Fig 2. Gastric emptying expressed as percent of the meal (as indicated by measurement of the meal marker ^{99m}Tc) still remaining in the stomach. Dashed lines are from the saline experiment, solid lines from the oxyntomodulin infusion. Oxyntomodulin significantly delayed emptying of the meal marker. The average time for 50% of the meal marker to leave the stomach was 17 min (saline) and 34 min (oxyntomodulin).

decreased from 43 ± 8 ml/10 min to 18 ± 3 ml/10 min, a significant inhibition of 48 ± 9%. Thirty-minute postprandial secretory volumes decreased from 49 ± 20 ml/10 min to 30 ± 16 ml/10 min, an inhibition of 23 ± 17%, which did not reach statistical significance.

Duodenogastric Reflux. Duodenogastric reflux did not differ significantly between saline and oxyntomodulin studies either during fasting or during postprandial periods. In the fasting period (time −30 min to 0) duodenogastric reflux averaged 24 ± 9%/10 min (saline) and 9 ± 5%/10 min (oxyntomodulin). Postprandial values (time 0 to 30 min) averaged 9 ± 3%/10 min and 13 ± 6%/10 min, respectively.

Gastric Emptying. Gastric emptying of the liquid meal during saline and oxyntomodulin infusions is depicted in Figure 2. The rate of gastric emptying was significantly delayed by oxyntomodulin. The time for 50% of the meal marker to leave the stomach was delayed from 17.3 ± 2.2 min (saline) to 34.7 ± 8.0 min (oxyntomodulin), $P < 0.05$. The pattern of gastric emptying did not change.

Gastroduodenal Motility. The interdigestive motor activity in antrum and duodenum during saline and oxyntomodulin infusions exhibited no difference. The fed pattern obtained after instillation of

TABLE 1. POSTPRANDIAL ANTRODUODENAL MOTOR ACTIVITY

Subject	*Infusion*	*Number of pressure waves exceeding 50 cm H_2O 4–10 min postprandial*	
1	NaCl	11	
	oxyntomodulin	0	
2	NaCl	9	
	oxyntomodulin	2	
3	NaCl	2	
	oxyntomodulin	0	
4	NaCl	7	
	oxyntomodulin	0	
5	NaCl	4	
	oxyntomodulin	0	
6	NaCl	2	
	oxyntomodulin	0	
Mean ± SEM	NaCl	5.8±1.5	
	oxyntomodulin	0.3±0.3	$P < 0.05$

TABLE 2. STABILITY OF PANCREATIC ENZYMES AFTER STORAGE*

Day	*Lipase activity (units)*	*Trypsin activity (units)*
0	496	20.0
2	514	17.9
4	499	
9		21.3
10	499	

*Neither enzyme activity decreased within 9–10 days of storage.

the meal was markedly changed by oxyntomodulin (Table 1). The number of pressure waves of more than 50 cm H_2O observed in the postprandial 4- to 10-min period decreased from 5.83 ± 1.54 (saline) to 0.33 ± 0.33 (oxyntomodulin), $P < 0.05$.

Stability of Pancreatic Enzymes. Stability of trypsin and lipase activity in postprandial duodenal aspirates stored at −20° C is shown in Table 2. Neither enzyme activity decreased within 9–10 days of storage.

Pancreatic Enzyme Secretion. Trypsin and lipase concentrations in duodenal aspirates from volunteers receiving saline or oxyntomodulin are shown in Figure 3. Fasting values of both trypsin (upper panel) and lipase (lower panel) were slightly lower during infusion of oxyntomodulin compared to saline. The difference was insignificant. During the initial 30-min postprandial period, responses of both enzymes were strongly inhibited by oxyntomodulin infusion. Trypsin values decreased from an average of 137 ± 21 units/min (saline) to 50 ± 18 units/min (oxyntomodulin), an inhibition of 56 ± 12%, $P < 0.05$. Lipase values from the same period decreased from 2799 ± 220 units/min to 1605 ± 292 units/min, an inhibition of 42 ± 11%, $P < 0.05$.

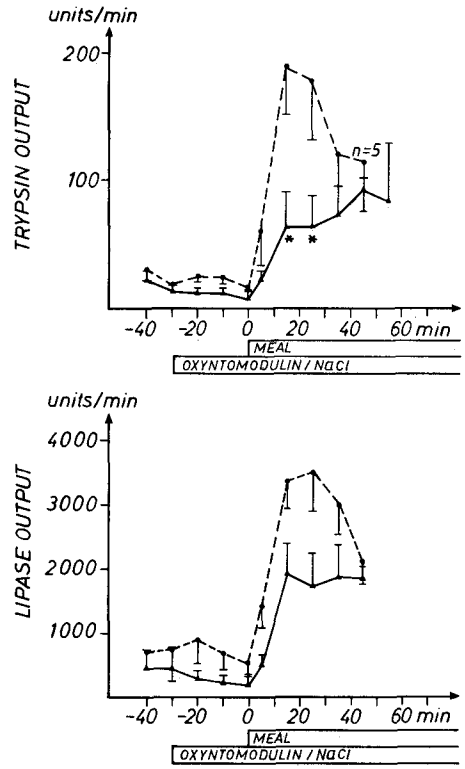

Fig 3. Pancreatic enzyme secretion in the fasting state and after installation of a liquid meal. Dashed lines indicate results obtained with infusion of saline, solid lines results from infusion of oxyntomodulin. Asterisks indicate statistical significant differences between two experimental days at individual time points.

To analyze the relationship between gastric emptying and pancreatic secretion, we plotted accumulated gastric emptying against accumulated lipase output with and without oxyntomodulin (Figure 4). We found a linear relationship between gastric emptying and pancreatic secretion that was unchanged by infusion of oxyntomodulin.

Pharmacokinetics. Plasma concentrations of immunoreactive enteroglucagon and of pancreatic glucagon during saline or oxyntomodulin infusion are shown in Figure 5. Infusion of oxyntomodulin at a rate of 400 ng/kg/hr resulted in increases in the plasma concentrations of immunoreactive enteroglucagon from 13.3 ± 2.5 pmol/liter to a plateau of 203 ± 21 pmol/liter. The half-life in plasma was measured to be 8.4 ± 2.0 min ($N = 4$). The metabolic clearance rate (MCR) was calculated to

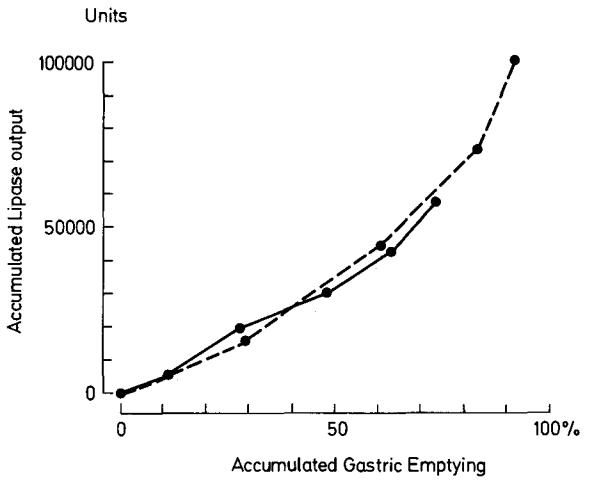

Fig 4. Relationship between gastric emptying and pancreatic secretion measured as accumulated lipase output. Dashed line represents mean values from saline infusion experiments, solid line represents mean values from oxyntomodulin infusion experiments. A linear relationship between gastric emptying and lipase secretion was observed with no significant difference between the two experimental days.

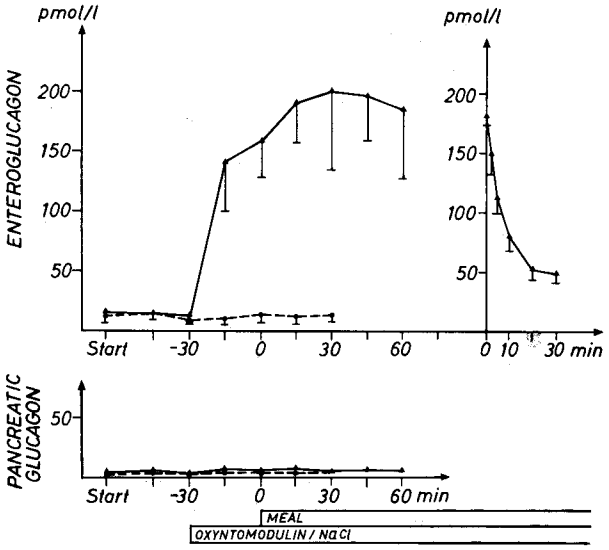

Fig 5. Plasma concentration of immunoreactive enteroglucagon and pancreatic glucagon in volunteers receiving a liquid test meal and intravenous infusion of either saline (dashed lines) or oxyntomodulin (solid lines).

be 8.8 ± 1.0 ml/kg/min, and the apparent volume of distribution was 0.12 ± 0.03 × BW.

The plasma concentration of pancreatic glucagon remained below 10 pmol/liter throughout the study, with no significant differences due to infusion of saline or oxyntomodulin. Somatostatin also remained below 10 pmol/liter (data not shown). Plasma glucose rose slightly after ingestion of the liquid meal (Figure 6); significant differences between results obtained during oxyntomodulin and saline infusion were not observed.

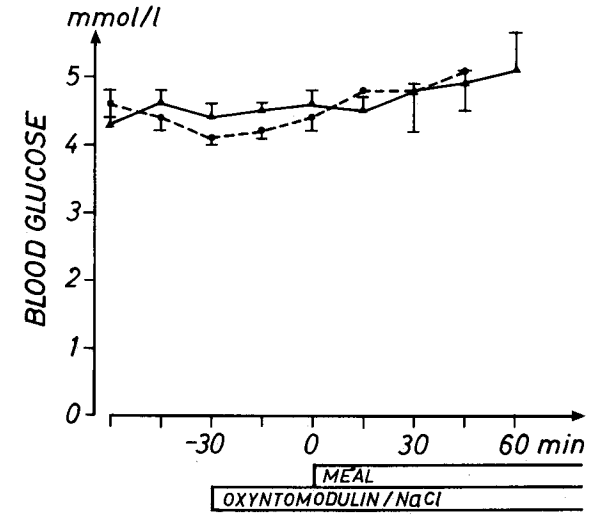

Fig 6. Plasma concentrations of glucose in volunteers receiving a liquid test meal and intravenous infusion of either saline (dashed lines) or oxyntomodulin (solid lines).

DISCUSSION

The oxyntomodulin molecule comprises the entire glucagon sequence plus a basic eight amino acid extension at the C-terminus (1, 2). Because of its homology with pancreatic glucagon while being a hormone from the distal gut, we decided to study the effects of oxyntomodulin on meal-induced gastroduodenal and pancreatic functions. Both peptides have been reported to inhibit gastric acid secretion in man (21, 34). Pancreatic glucagon inhibits gastric emptying, gastroduodenal motility, and pancreatic enzyme secretion (34–37) in addition to its potent stimulatory effects on hepatic glucose production and insulin secretion (38). However, in this study we purposely used a mixed meal with a low caloric content, which is not expected to affect pancreatic glucagon secretion (25). Similarly, the use of continuous aspiration of chyme from duodenum and the fact that the study did not last more than 50 min after ingestion of the meal makes it unlikely that significant amounts of nutrients would reach the distal ileum and stimulate endogenous enteroglucagon secretion. Indeed, in the saline studies no increase in enteroglucagon concentrations was observed.

Saline or oxyntomodulin infusions were initiated 20 min after the occurrence of an MMC on the manometric recorder, that is in phase 1 of the interdigestive motor activity. At this time both pancreatic and gastric secretions are believed to be at a minimum (28). Infusion of oxyntomodulin resulted in an elevation of the plasma enteroglucagon concentration from 10 pmol/liter to a plateau of about 200 pmol/liter. Immunoreactive enteroglucagon comprises both glicentin and oxyntomodulin (10, 14), but since it is unlikely that the endogenous enteroglucagon secretion was stimulated by the oxyntomodulin infusion or by the meal, we assume that the increase of immunoreactive enteroglucagon represents oxyntomodulin. The values obtained are similar to those observed in response to large mixed meals (25) or nutrient loads (24). In those cases, however, glicentin is likely to contribute significantly to the response. In plasma samples obtained in the fasting state in man, oxyntomodulin was found to constitute about one third of the total amount of immunoreactive enteroglucagon (8); a similar ratio was found in pancreatectomized patients after a carbohydrate meal (9). In a recent study of rat plasma, analyzed with a combined radioimmunological and chromatographic tech-

nique, Kervran et al (10) found that oxyntomodulin was the predominant enteroglucagon. Although the issue cannot be settled definitively until specific assays that distinguish between glicentin and oxyntomodulin are available, the evidence suggests that the present concentrations are high but not necessarily unphysiological. Furthermore, in various pathological states with abnormal exposure of unabsorbed nutrients to the distal small intestine, very high concentrations has been found (14).

The calculated metabolic clearance rate of 8.8 ml/kg/min and the half-life in plasma of 8.4 min are close to those of glucagon, 9–11 ml/kg/min and 5–6 min, respectively (26), suggesting that oxyntomodulin is metabolized at a similar rate. The apparent volume of distribution was $0.1 \pm 0.03 \times$ BW, in good agreement with the value of $0.1 \times$ BW, we used in our calculation of the infusion rate.

Oxyntomodulin significantly inhibited gastric acid secretion in the fasting state and postprandially. The reduction in acid output was paralleled by a similar decrease in the gastric volume secretion (although significantly so during the fasting period only). The lower acid secretion observed during oxyntomodulin infusion could theoretically be explained by reflux of less acidic duodenal contents, lowering the gastric acidity; but the average reflux was lower during oxyntomodulin than during saline infusion. The calculated amount of reflux during fasting and postprandial periods is in agreement with results from others (39). Our results are also in agreement with other very recent studies showing that both meal-stimulated and pentagastrin-stimulated gastric acid secretion can be inhibited by both natural oxyntomodulin extracted from the porcine ileum and by the synthetic octapeptide of oxyntomodulin in rats (18, 20) and with studies showing that synthetic oxyntomodulin inhibits pentagastrin-induced acid secretion in man (21).

Gastric emptying of the liquid meal was also significantly inhibited by infusion of oxyntomodulin, which increased the time for 50% of the meal marker to leave the stomach from 17 to 35 min. In this respect, oxyntomodulin seems to be more potent than pancreatic glucagon. Studies in our laboratories have shown that only pharmacological doses of glucagon are capable of inhibiting to the same extent the gastric emptying of a similar meal (40). Studies in humans have shown that instillation of lipids or carbohydrates in jejunum/ileum results in decreased gastric secretion and emptying (41). At the same time, lipids or carbohydrates in the jejunum/ileum cause a release of enteroglucagon (glicentin plus oxyntomodulin) into the circulation (8–11, 42). The enteroglucagons may thus contribute to the observed inhibitory effects on gastric functions.

Very low motor activity in the antrum and the duodenum during interdigestive phase I precluded registration of possible inhibitory changes in motility due to oxyntomodulin. Pancreatic glucagon may inhibit interdigestive motility (36), but whether this may occur also after infusion of physiologically relevant doses of glucagon has not been established yet.

The postprandial motor activity was inhibited significantly by oxyntomodulin. Possibly the inhibition may contribute to the observed delay in gastric emptying.

Our results show that postprandial duodenal aspirates collected on ice did not loose tryptic or lipolytic activity upon storage at −20° C for up to 10 days. The fact that we determined the enzyme activity of the duodenal aspirates in frozen samples after the experiments (for practical reasons) is therefore unlikely to influence our results in an untoward manner. Postprandial values of trypsin and lipase were significantly decreased by oxyntomodulin.

Validation studies were not made for trypsin and lipase activity in the fasting samples due to lack of appropriate amounts of aspirate. Degradation of enzyme activity in the frozen preprandial samples therefore cannot be excluded, and these results must be interpreted with caution.

The mechanism by which oxyntomodulin brings about the described inhibitory effects on gastroduodenal and pancreatic functions is unclear. Gastric acid secretion, emptying, and other motor activity and pancreatic enzyme secretion are controlled by both neuronal and endocrine mechanisms. Oxyntomodulin may exert its inhibitory effects indirectly via a modulatory effect on the activity of gastropancreatic intrinsic nerves, or by preganglionic inhibition of excitatory vagal fibers, via the CNS, or both; or it may act directly on cell membrane receptors on the parietal cells, the acinar cells, and maybe even smooth muscle cells. Pancreatic exocrine secretion is dependent upon the presence of nutrients in duodenum (43) and thereby dependent upon the rate of gastric emptying. Our finding that the relationship between gastric emptying and pancreatic secretion is unchanged during intravenous infusion of saline or oxyntomodulin suggests that oxyntomodulin may not influence postprandial exocrine

pancreatic secretion in humans. In conscious rats, however, oxyntomodulin inhibited both basal and cerulein-stimulated pancreatic secretion (22).

In conclusion, the present study reports potent inhibitory effects of the synthetic enteroglucagon, oxyntomodulin, on fasting and postprandial gastric acid secretion, gastric emptying, postprandial gastroduodenal motility, and pancreatic secretion of trypsin and lipase—simultaneously determined using an intraduodenal marker dilution technique. Oxyntomodulin is therefore a potential enterogastrone and pancreotone from the distal gut.

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