Glucagon-like peptide-1 inhibits gastropancreatic function by inhibiting central parasympathetic outflow

ANDRÉ WETTERGREN, MORTEN WØJDEMANN, AND JENS JUUL HOLST

Wettergren, André, Morten Wøjdemann, and Jens Juel Holst. Glucagon-like peptide-1 inhibits gastropancreatic function by inhibiting central parasympathetic outflow. Am. J. Physiol. 275 (Gastrointest. Liver Physiol. 38): G984–G992, 1998.—Glucagon-like peptide (GLP)-1 inhibits acid secretion and gastric emptying in humans, but the effect on acid secretion is lost after vagotomy. To elucidate the mechanism involved, we studied its effect on vagally stimulated gastropancreatic secretion and motility in urethan-anesthetized pigs with cut splanchnic nerves, in which insulin-induced hypoglycemia elicited a marked stimulation of gastropancreatic secretion and antral motility. In addition, we studied vagally stimulated motility and pancreatic secretion in isolated perfused preparations of the porcine antrum and pancreas. GLP-1 infusion (2 pmol·kg⁻¹·min⁻¹) strongly and significantly inhibited hypoglycemia-induced antral motility, gastric acid secretion, pancreatic bicarbonate and protein secretion, and pancreatic polypeptide (PP) secretion. GLP-1 (at 10⁻¹⁰–10⁻⁸ mol/l) did not inhibit vagally induced antral motility, pancreatic exocrine secretion, or gastrin or PP secretion in isolated perfused antrum and pancreas. We conclude that the inhibitory effect of peripheral GLP-1 on upper gastrointestinal secretion and motility is exerted via interaction with centers in the brain or afferent neural pathways relaying to the vagal motor nuclei.

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MATERIALS AND METHODS

In Vivo Experiments

Animal preparation. Twenty-three pigs, strain LYY, weighing 24–38 kg, were used. They were fasted for 24 h but had free access to water. Anesthesia was induced briefly with 2.5% halothane and maintained with intravenous infusion of urethane (0.8 g·kg⁻¹·h⁻¹) after intubation and start of artificial ventilation. The abdomen was opened through a midline incision. To reduce sympathetically inhibitory effects on pancreatic secretion (18) (in this study elicited by insulin-induced hypoglycemia), the splanchnic nerves were cut bilaterally at the level of the diaphragm. An orogastric tube was placed in the fundic region of the stomach. A baby-feeding tube (no. 8, Argyll; Sherwood Medical Industries, St. Louis, MO) was fixed to the gastric tube allowing intragastric infusion of NaCl (Amersham); 370 kBq were dissolved in 1,000 ml 0.9% saline with 20 ml 5% human albumin (Novo Nordisk A/S Plasma Product Unit PSU) to avoid adhesion to tubes and infused at a rate of 2 ml/min. A large-bore draining tube was passed through the pylorus via a duodenotomy and kept in place by a string tied around the pylorus without damaging the pyloric nerves and blood vessels. The pancreatic duct was isolated and catheterized with a baby-feeding tube (no. 6), and both the duodenal and pancreatic tubes were exteriorized through separate skin incisions in the abdominal wall. Two force transducers (RB Products, Madison, WI) were sutured onto the serosa of the antrum, ~4–5 cm from the pylorus, one in a longitudinal direction, the other perpendicular to the first, to detect circular and longitudinal muscle contractions. The transducers were calibrated using weights.

The femoral veins and a femoral artery were isolated and catheterized (baby-feeding tube no. 8) for blood sampling, infusion of peptide, and intra-arterial measurements of blood pressure, respectively. Electrodes were placed on the thorax, and electrocardiogram and heart rate were monitored continuously. In four pigs prepared as described above, a cervical bilateral vagotomy was carried out.

Peptide GLP-1-(7–36)amide (porcine, human) was purchased from Peninsula, St. Helens, UK. The peptide was dissolved in 0.9% saline containing 1% human serum albumin (pure, dry; Behringwerke, Marburg, Germany) and kept at −20°C until use.

Experimental procedure. After surgery the animals were left undisturbed for 30 min, allowing equilibration of the
The gastric tracer. After a basal period of 20 min, a bolus of insulin (1.0 IU/kg; Actrapid, Novo Nordisk, Denmark) was injected intravenously. When the motor response had reached a constant stimulated activity (~80 min after the injection), intravenous infusions of either porcine GLP-1 (2 pmol·g⁻¹·min⁻¹) (GLP-1 experiment, n = 8) or saline (control experiment, n = 8) were given for a 30-min period. The experiment was stopped 140 min after the insulin injection. Gastric and pancreatic juice were collected on ice for 10-min intervals throughout the study, and blood samples were drawn every 10 min. Antral motility was recorded continuously from the transducers, which were connected to a multichannel pen-writing recorder via an amplifier. The contractile response to insulin-induced hypoglycemia was quantified manually and expressed as the mean frequency and amplitude (force) in 10-min periods.

### Laboratory analysis

#### Acid secretion

The concentration of titratable acid in the gastric samples was determined by titration with 0.1 mol/l NaOH to pH 7.0 using an automatic titrator (Autoburette Radiometer, Copenhagen, Denmark). The radioactive concentration of ⁵⁷Co in each gastric sample was measured in duplicate in a gamma-spectrometer and employed to calculate the volume of gastric juice according to the recovery of marker (11). Gastric output was expressed as milliequivalents of acid per 10 min.

#### Pancreatic secretion

Pancreatic samples were collected in chilled gastight tubes, which were capped immediately after collection and stored frozen until assayed for volume and bicarbonate and protein content as previously described (20).

#### Blood glucose

Blood glucose concentration was measured every 10 min using a glucometer (One Touch II, Lifescan).

#### Radioimmunoassays

Blood samples were collected in chilled tubes containing EDTA (3.9 mmol/l) and aprotonin (500 KIU/ml) and centrifuged at 4°C. The plasma was separated and stored at ~20°C until analyzed. GLP-1 immunoreactivity was measured as previously described (35), using synthetic GLP-1-(7—36) amide as standard (Peninsula), ¹²⁵I-labeled GLP-1-(7—36) amide and antisera 89390, directed against the COOH terminus of GLP-1-(7—36) amide (35). Plasma concentrations of gastrin and pancreatic polypeptide (PP) were measured as previously described (39, 43), using synthetic porcine peptides for standards and antisera code nos. 2609 (gastrin) and 146—5 (PP), and plasma-coated charcoal for separation. For all assays, sensitivity was <5 pmol/l, intra-assay coefficients of variation were <6%, and recovery of added standards was within ±15% of expected values, whether performed in perfusate (in vitro studies) or in porcine plasma.

### In Vitro Experiments

Isolated perfused antrum and pancreas. The direct effect of GLP-1 on vagally induced antral motility and pancreatic secretion was investigated using isolated perfused preparations of the porcine antrum and pancreas with intact vagal innervation (15). In short, seven pigs weighing 16—19 kg were anesthetized with chloralose, and the pancreas and the gastric antrum were isolated together with a segment of the aorta comprising both the celiac and the anterior mesenteric outlets. The venous effluent from the preparation was drained from the portal vein. In the perfusion chamber, the antrum was suspended with rubber bands sutured to the cut edges of the antral wall. The antral part of this preparation shows annular propulsive contractions with regular intervals. To record these, a suture was tied to the serosal surface of the antrum and connected to a force-displacement transducer, which was connected to a Mingograph recorder. The transducer was calibrated by attaching weights to the suture. Motility was recorded continuously. The vagal innervation was preserved by including the lesser omentum and the gastrosplenic ligament in the preparation. The vagal trunks (isolated at the level of the cardia) were threaded through a tunnel electrode and stimulated electrically with square-wave impulses of 4 ms and 10 mA at 8 Hz, previously shown to elicit submaximal effects (18). Pancreatic secretion was collected for intervals of 5 or 10 min by means of a catheter inserted into the pancreatic duct and analyzed for volume and bicarbonate and protein concentrations as described above. The preparation was perfused in a single-pass system (20), with a Krebs-Ringer bicarbonate solution containing in addition 0.1% human serum albumin, 5% dextran T70, 7 mmol/l glucose, and a mixture of amino acids (Vamin, final concentration of amino acids 5 mmol/l; Pharmacia, Uppsala, Sweden). The medium was supplemented with 20% fresh, washed bovine erythrocytes and perfused at a rate of 0.4 ml·g⁻¹·min⁻¹, whereby an oxygen consumption of 9—12 µl·g⁻¹·min⁻¹ was ensured. In four perfusion experiments, 1-min effluent fractions were collected on ice and centrifuged and the supernatants were analyzed for gastrin and PP concentration as described above.

Protocol. After a 30-min equilibration period, electrical vagal stimulation was carried out for 5 min, followed by a 10-min "rest." Then GLP-1 was infused for 15 min at a rate resulting in a final perfusate concentration of 1 nmol/l (7 perfusion experiments). After a 5-min infusion, vagus stimulation was repeated. In three perfusion experiments, a third vagus stimulation was carried out 15 min after the termination of the GLP-1 infusion. For these experiments, the control values in Table 2 are mean values of results from the first and third vagus stimulation. In two of the perfusion experiments, additional vagus stimulations were carried out during infusion of GLP-1 to 10 nmol/l and in three experiments during infusion to 0.1 nmol/l.

### Statistical analysis

Data are expressed as means ± SE. For evaluation of the effect of GLP-1 in vivo, the data for the basal period and the two 10-min periods immediately before, during, and after GLP-1 infusion were pooled. The resulting values are shown in Table 1. The data from each of these four periods were analyzed by two-factor ANOVA for repeated measures followed by contrasting the data at specific time intervals (typically the GLP-1- or saline-infusion period) using the software Statistica (Statsoft, Tulsa, OK). In the in vitro experiments, vagus stimulations were carried out both with and without GLP-1 in all perfusions, and the vagus stimulation results were, therefore, compared using a t-test for paired data. Changes as a function of time were evaluated by repeated-measures ANOVA followed by Bonferroni’s test. Further comparisons were made using t-tests as specified in the text.

### Results

#### In Vivo Experiments

Antral motility. The motor responses recorded by the circular and longitudinally orientated electrodes were similar; the circular responses have been used for statistical analysis. Insulin-induced hypoglycemia resulted in a prolonged stimulation of antral motility lasting 120 ± 21 min and 122 ± 9 min in the control and GLP-1 experiments, respectively (not significant, t-test). The motility responses to hypoglycemia are summarized in Table 1. In the control experiments, the frequency of contractions increased almost twofold and
the amplitude increased more than eightfold corresponding to a contractile force of $63 \pm 10$ g. The response showed little variation with time with respect to amplitude and frequency and no changes were seen during saline infusion. In the GLP-1 experiments, hypoglycemia increased the frequency twofold, and the amplitude increased about sevenfold. Infusion of GLP-1 caused a pronounced inhibition of motility, with frequency being reduced by $43 \pm 6\%$ of the increase ($P = 0.003$) and the amplitude to almost basal values ($P = 0.0002$). After termination of the GLP-1 infusion, the frequency and the amplitude "recovered" to preinfusion levels. A typical GLP-1 experiment is shown in Fig. 1.

Acid secretion. Mean results for both groups are shown in Fig. 2. Recoveries of tracer in the control and GLP-1 experiments were $94 \pm 5\%$ and $103 \pm 7\%$, respectively (NS, t-test). Insulin-induced hypoglycemia resulted in an increase in gastric acid secretion, reaching a plateau $\sim 10$-fold above basal secretion from 80 to 140 min after insulin injection. In the GLP-1 experiments, a similar response was obtained initially [$P = 0.004$ for the effect of hypoglycemia (=time) in the two groups], but the GLP-1 infusion (started at 80 min) inhibited acid secretion by $40 \pm 14\%$ of the 70-min value (and by $56 \pm 12\%$ of the 80-min value) at 120 min. The P value for the inhibition in the GLP-1 infusion

Table 1. Effect of GLP-1 on centrally (insulin hypoglycemia) induced antral motility and gastric acid and pancreatic secretion in anesthetized pigs

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>70–80</th>
<th>100–110</th>
<th>130–140</th>
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<th>P</th>
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<tr>
<td><strong>Amplitude, mm</strong></td>
<td></td>
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<tr>
<td>GLP-1</td>
<td>4 ± 1</td>
<td>28 ± 7</td>
<td>4 ± 1</td>
<td>27 ± 6</td>
<td>26.20</td>
<td>0.0002</td>
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<td>5 ± 1</td>
<td>43 ± 7</td>
<td>4 ± 1</td>
<td>42 ± 6</td>
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<tr>
<td>GLP-1</td>
<td>17 ± 4</td>
<td>38 ± 2</td>
<td>29 ± 3</td>
<td>41 ± 2</td>
<td>5.79</td>
<td>0.0030</td>
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<td>26 ± 4</td>
<td>42 ± 2</td>
<td>41 ± 2</td>
<td>41 ± 3</td>
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<td><strong>Acid secretion, mmol/10 min</strong></td>
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<tr>
<td>GLP-1</td>
<td>0.1 ± 0.04</td>
<td>1.5 ± 0.4</td>
<td>0.9 ± 0.3</td>
<td>0.9 ± 0.3</td>
<td>3.82</td>
<td>0.0711</td>
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<td>0.1 ± 0.01</td>
<td>1.6 ± 0.3</td>
<td>2.0 ± 0.4</td>
<td>1.2 ± 0.3</td>
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<td><strong>Pancreatic juice, ml/10 min</strong></td>
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<tr>
<td>GLP-1</td>
<td>0.2 ± 0.01</td>
<td>1.8 ± 0.2</td>
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<td>1.4 ± 0.3</td>
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<td><strong>Pancreatic bicarbonate, µmol/10 min</strong></td>
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<td>GLP-1</td>
<td>4 ± 1</td>
<td>162 ± 28</td>
<td>26 ± 5</td>
<td>110 ± 27</td>
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<td>Saline</td>
<td>8 ± 2</td>
<td>97 ± 29</td>
<td>108 ± 31</td>
<td>105 ± 41</td>
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<td><strong>Pancreatic protein, mg/10 min</strong></td>
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<td>GLP-1</td>
<td>4 ± 1</td>
<td>206 ± 51</td>
<td>16 ± 6</td>
<td>82 ± 33</td>
<td>17.01</td>
<td>0.0010</td>
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<td>103 ± 41</td>
<td>73 ± 24</td>
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<tr>
<td>GLP-1</td>
<td>34 ± 7</td>
<td>135 ± 19</td>
<td>96 ± 19</td>
<td>94 ± 19</td>
<td>2.17</td>
<td>0.1630</td>
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<td>Saline</td>
<td>32 ± 4</td>
<td>83 ± 16</td>
<td>100 ± 19</td>
<td>85 ± 21</td>
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<tr>
<td><strong>Gastrin, pmol/ml</strong></td>
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</tr>
<tr>
<td>GLP-1</td>
<td>10 ± 1</td>
<td>34 ± 10</td>
<td>23 ± 5</td>
<td>36 ± 9</td>
<td>3.09</td>
<td>0.1007</td>
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<tr>
<td>Saline</td>
<td>13 ± 1</td>
<td>23 ± 2</td>
<td>21 ± 2</td>
<td>20 ± 2</td>
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</tbody>
</table>

Values are means ± SE. Values in 70–80 column represent mean values of last 2 10-min periods just before start of glucagon-like peptide (GLP)-1 or saline infusion. Values in 100–110 column represent mean values of last 2 10-min periods with either GLP-1 or saline infusion. Values in 130–140 column represent mean values of last 2 10-min periods of experiments after cessation of GLP-1 and saline infusion. F and P values are given for statistical analysis (2-factor repeated-measures ANOVA) across 100- to 110-min time interval (=GLP-1). Degree of freedom was 14. PP, pancreatic polypeptide.

Fig. 1. Effect of glucagon-like peptide (GLP)-1 on hypoglycemia-induced antral motility. Recording is from a circularly orientated transducer attached to serosal surface of the antrum in a single, typical experiment. Amplitude is in g (contractile force). Figure should be read from right to left. Bottom trace is direct continuation of top trace. Insulin and GLP-1 were given intravenously as indicated.
period was 0.07 (Table 1). The effect of GLP-1, however, was, as expected, somewhat delayed. The P value for a comparison of the secretion in the period of 90–120 min was thus 0.019 [degrees of freedom (df) = 14, F = 7.008]. The acid secretion hereafter recovered to a level that was not significantly different from that observed in the control experiment.

Pancreatic secretion. Insulin-induced hypoglycemia resulted in an eightfold increase in the output of juice (Fig. 3). A similar response was seen at the beginning of the GLP-1 experiments (P = 0.001 for the combined groups), but infusion of GLP-1 almost abolished the increase (P = 0.0016). After termination of the infusion, the output of juice recovered to the same level as in the control experiment (Fig. 3). Protein and bicarbonate outputs also increased significantly after hypoglycemia, and both decreased significantly in response to the subsequent GLP-1 administration (Table 1), whereas in the control experiments a plateau (protein) or a steady increase (bicarbonate) was observed. Neither the concentration of protein nor the concentration of bicarbonate in the juice differed significantly between saline and GLP-1 experiments (not shown).

Plasma peptide and blood glucose concentrations. The plasma concentrations of GLP-1, gastrin, and PP are shown in Fig. 4. Infusion of GLP-1 (2 pmol·kg⁻¹·min⁻¹) resulted in a plateau plasma concentration of 125 ± 27 pmol/l. The basal levels of GLP-1 did not differ between the experiments, and GLP-1 concentrations were not affected by the hypoglycemia. The plasma gastrin concentration increased significantly by ~100% during hypoglycemia in the GLP-1 and control experiments. During GLP-1 infusion, the gastrin concentrations fell compared with preinfusion values but were not significantly different from the concentrations in the corresponding period in the control experiments. The plasma levels of PP also increased significantly during hypoglycemia (P < 0.001 for the combined groups). In the controls, a steady increase in the plasma concentration was seen throughout the experiment, whereas during GLP-1 infusion a prolonged decrease in the PP plasma level was seen, but the concentrations did not differ significantly (Table 1). However, when the individual concentrations in the 80–110-min period were analyzed by linear regression, the slopes of the regression lines (−12.2 ± 4.4 and 3.15 ± 4.3) differed significantly between GLP-1 and saline experiments (P = 0.019, df = 14, F = 7). The basal blood glucose levels in the two experiments were identical (4.9 ± 0.2 mmol/l in each series). Insulin decreased the glucose level similarly to 1.7 ± 0.2 mmol/l, a level that remained almost constant throughout the experiment (Fig. 5).

Vagotomy. In four experiments, bilateral cervical vagotomy was carried out before induction of hypoglycemia. In these experiments, hypoglycemia had absolutely no effect on antral motility or gastropancreatic secretion (not shown). In three experiments involving
hypoglycemia-induced stimulation of gastropancreatic motility and secretion, euglycemia was restored by glucose infusion. This immediately brought motility and secretion back to basal levels (not shown).

**In Vitro Experiments**

Electrical stimulation of the vagus nerves in all cases strongly stimulated antral motor activity and pancreatic secretion (by 24%) and bicarbonate and protein output. The secretion of both gastrin and PP increased in response to vagus stimulation. GLP-1 had no effect on these responses (Table 2). Higher (10 nmol/l, n = 2) and lower (0.1 nmol/l, n = 3) concentrations of GLP-1 were equally ineffective with respect to inhibiting vagally induced motility and secretion (not shown).

**DISCUSSION**

The distal part of the small intestine participates in the regulation of upper GI functions (25, 41). Experiments have shown that intraluminal perfusion of the ileum with fat and carbohydrate-containing solutions inhibits gastropancreatic secretion and motility (9, 25, 26, 41, 42). How this endocrine inhibition, the so-called ileal brake effect, is mediated is not yet clear.

GLP-1 inhibits gastric acid and pancreatic secretion and gastric emptying when infused in amounts that result in plasma concentrations similar to those observed after meals (30, 37, 46). Moreover, ileal perfusion with carbohydrates and lipids in physiological amounts induces increases in GLP-1 plasma levels that reach or exceed those required to inhibit gastric secretory function (24). These results suggest that GLP-1 may be at least partly responsible for the ileal brake effect.

Regarding the mechanism of action of GLP-1, we have previously shown that GLP-1 infused intravenously almost abolished sham feeding-induced gastric acid secretion in humans (45). In addition, we have recently shown that the effect of GLP-1 on pentagastrin-induced acid secretion is lost in vagotomized humans (47), suggesting that the gastric effects of GLP-1 in humans are mediated through neural pathways. GLP-1 may act differently in different species. In the perfused rat stomach, GLP-1 was found to stimulate somatostatin and inhibit gastrin release, indicating that GLP-1 in this species could act directly on the gastrin cells or via local release of paracrine transmitters (3). In contrast, in the pig stomach, GLP-1 affects neither somatostatin nor gastrin secretion (33). In humans, GLP-1 has no effects on hypoglycemia-induced stimulation of gastropancreatic motility and secretion, euglycemia was restored by glucose infusion. This immediately brought motility and secretion back to basal levels (not shown).

GLP-1 at 1 nmol/l had no effect on antral motility but significantly enhanced the vagally stimulated rate of pancreatic secretion (by 24%) and bicarbonate and protein output. The secretion of both gastrin and PP increased in response to vagus stimulation. GLP-1 had no effect on these responses (Table 2). Higher (10 nmol/l, n = 2) and lower (0.1 nmol/l, n = 3) concentrations of GLP-1 were equally ineffective with respect to inhibiting vagally induced motility and secretion (not shown).

**Fig. 4.** Effect of GLP-1 on hypoglycemia-induced secretion of pancreatic polypeptide (PP; A) and gastrin (B). GLP-1 concentrations are shown in C. Means and SE of 8 control experiments (●) and 8 GLP-1 experiments (○) are shown. See text and Table 1 for statistical evaluation.

**Fig. 5.** Plasma glucose levels in response to insulin injection in 8 control experiments (●) and 8 experiments with GLP-1 infusion (○).
on circulating concentrations of gastrin and somatostatin (45, 46), while still effectively inhibiting gastric acid secretion stimulated by intragastric meal instillation (46). Part of the inhibitory effect of GLP-1 on meal-induced pancreatic secretion observed previously was probably due to its inhibitory effect on gastric emptying, because the linear relationship between gastric emptying and pancreatic exocrine secretion was unchanged by GLP-1 infusion (46). However, GLP-1 infused intravenously also effectively inhibits pancreatic secretion stimulated by intraduodenal perfusion with amino acids (6). In earlier experiments employing isolated perfused preparations of the porcine pancreas, GLP-1 had no inhibitory effect on vagally induced secretion and release of the pancreatic neurotransmitter vasoactive intestinal polypeptide (VIP) (16). The lack of effect of GLP-1 on vagally induced pancreatic secretion was confirmed in the present studies using isolated perfused combined preparations of the antrum and the pancreas. In fact, the responses to vagus stimulation were significantly increased by GLP-1. The enhancing effect was observed only at clearly supra-physiological concentrations (1 nmol/l and above) and is unlikely to be of physiological relevance, but presumably reflects the close homology of GLP-1 to the pancreaticotropic hormones, secretin, VIP, and pituitary adenylate cyclase-activating polypeptide. In addition, GLP-1 had absolutely no effect on antral motility, which was strongly stimulated by electrical vagus stimulation and/or the secretion of gastrin and PP, both of which are tightly regulated by efferent vagal activity (17, 38, 39). It may be argued that electrical vagal stimulation is unphysiological and cannot be compared with central activation by meals or hypoglycemia. An inhibitory effect of GLP-1 might therefore be concealed by unphysiological activation of stimulatory mechanisms (e.g., antidromic stimulation of afferent fibers). However, we have previously shown that in this preparation, administration of the nicotinic cholinergic blocker hexamethonium abolishes all effects of vagus stimulation, proving that ganglionic transmission is involved in all effects (15, 18). In addition, somatostatin, which inhibits cho-

Table 2. Effect of GLP-1 on vagally induced antral motility and pancreas secretion using isolated perfused preparations of porcine antrum and pancreas with intact vagal innervation

<table>
<thead>
<tr>
<th></th>
<th>Control Experiment (n = 7)</th>
<th>+1 nM GLP-1 Experiment (n = 7)</th>
<th>Vagus Response With GLP-1 (% of controls)</th>
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<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>Stimulated</td>
<td>Basal</td>
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<tr>
<td>Antral motor activity</td>
<td></td>
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<tr>
<td>Frequency, (contractions/10 min)</td>
<td>14.6 ± 2.9</td>
<td>40.3 ± 1.3*</td>
<td>16.4 ± 2.8</td>
</tr>
<tr>
<td>Amplitude, mm</td>
<td>9.9 ± 2.7</td>
<td>32.4 ± 4.8*</td>
<td>9.6 ± 2.8</td>
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<tr>
<td>Pancreatic exocrine secretion</td>
<td></td>
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<tr>
<td>Flow of juice, ml/10 min</td>
<td>0.16 ± 0.01</td>
<td>5.3 ± 1.2*</td>
<td>0.16 ± 0.02</td>
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<tr>
<td>HCO₃⁻ output, µmol/10 min</td>
<td>ND</td>
<td>700 ± 164</td>
<td>ND</td>
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<td>Protein output, mg/10 min</td>
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<td>Pancreatic endocrine secretion</td>
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<tr>
<td>PP, pmol/min</td>
<td>3.0 ± 0.2</td>
<td>49.0 ± 18</td>
<td>3.8 ± 0.6</td>
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<td>Gastrin, nmol/min</td>
<td>33 ± 7</td>
<td>74 ± 12</td>
<td>36 ± 8</td>
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</table>

Values are means ± SE (gastrin and PP results were only obtained in 4 perfusion experiments). ND, not determined because of small volumes. For amplitude measurements, calibration of transducer with weights generated a straight line with slope of 2.67 g/mm. Bicarbonate and protein outputs were calculated as flow × concentration. *Significantly different from basal (ANOVA + Bonferroni). †Significantly different from control (paired t-test).
linergic and peptidergic neurons, powerfully inhibits the effects of electrical vagus stimulation in this model (16). Thus, if GLP-1 had similar effects, they should not escape notice. In addition, GLP-1 has no direct effect on the exocrine secretion of the isolated perfused pig pancreas (16) and does not inhibit secretin- and cholecystokinin-induced secretion (unpublished studies). Furthermore, in preparatory experiments in anesthetized pigs, infusions of GLP-1 (same doses as described here) had no effect on secretin-stimulated (0.5 clinical units/kg) pancreatic secretion or secretion brought about by electrical stimulation (18) of the peripheral cut ends of the vagus nerves (same parameters as in the present in vitro studies). We conclude, therefore, that the GLP-1 inhibitory mechanism, which may be similar in humans and pigs, involves the vagus nerves but neither peripheral transmission of vagal impulses to the ganglia of the stomach and the pancreas nor the function of their intrinsic excitatory neurons.

Further studies of the mechanism of the inhibitory effects of GLP-1 necessitated a new experimental approach. For the experimental animal we chose the pig because of its similarity to humans with respect to GI physiology (27) and because of the similar apparent lack of direct effects of GLP-1 on the stomach and the exocrine pancreas. The combination of urethan anesthesia, cutting of the splanchnic nerves, and insulin-induced hypoglycemia turned out to provide a reproducible and robust model for centrally induced stimulation of antral motility as well as gastric and pancreatic secretion. The stimulated pancreatic secretion amounts to about one-half of the secretion elicited by maximal electrical stimulation of the vagus nerves (9), and the gastric secretory response is similar to that obtained with maximal pentagastrin stimulation in conscious pigs of similar size (49). The frequency and strength of the insulin-induced antral contractions are very similar to those observed in response to maximal electrical stimulation of the vagus nerves in vitro (14). Arterial gastrin and PP concentrations increased to levels similar to those observed after maximum vagus stimulation in anesthetized pigs (31, 38). All effects were completely abolished by truncal vagotomy (and intravenous glucose), proving their central origin. In a recent study in conscious pigs with permanent pancreatic duct cannulas, Karlsson et al. (22) failed to demonstrate cephalic stimulation of the exocrine pancreatic secretion when neuroglycopenia was induced with 2-deoxy-D-glucose, a finding that contrasts with the present results and with the profuse pancreatic secretion that may be elicited by electrical stimulation of the vagus nerves (10, 18). The sympathopathy included in our preparation probably provides the explanation. We have shown previously that concomitant stimulation of the splanchnic nerves almost abolished the pancreatic response to electrical vagus stimulation, and, in addition, cutting of the splanchnic nerves increased the vagus response (18). In pigs, therefore, activation of the sympathetic nervous system induced by central hypoglycemia may counteract or obscure its stimulatory effect on pancreatic secretion. In the intact pig, the exocrine pancreatic response to electrical vagus stimulation is greatly potentiated by simultaneous acidification of the duodenal bulb (18). In the present study, pyloric ligation prevented acidification of the duodenum, and the pancreatic response to central hypoglycemia therefore represents a "pure" pancreatic response. However, if the stomach was allowed to drain to the duodenum, as occurs under natural circumstances, the pancreatic response to central stimulation would probably be markedly enhanced. Thus our results support a prominent role for central regulation of pancreatic secretion (12, 23).

Our results clearly show that GLP-1 not only inhibits cephalically induced acid secretion but also strongly inhibits cephalically induced antral motility and pancreatic exocrine secretion as well. These effects were observed at plasma concentrations around 125 pmol/l, values that are only slightly higher than those observed in response to ingestion of a meal in normal subjects (35) and similar to those observed in patients with accelerated gastric emptying (13). Taken together, our results suggest that the inhibitory effect of GLP-1 on upper gastric functions involves receptors located either in the central nervous system or associated with afferent pathways to the brain stem. Recently, cephalic stimulation of antral motility induced by sham feeding in humans was shown to approximate 70% of the motor response to a standard meal (23). The finding that the cephalic phase may account for more than two-thirds of the motor response to meal ingestion combined with the observation in the present study that GLP-1 nearly abolished the cephalically induced antral motility could explain the pronounced effect of GLP-1 on gastric emptying in humans, where infusion of doses similar to those employed here may cause a complete arrest of gastric emptying for several hours (48). The inhibition of cephalically induced pancreatic secretion, independent of gastric emptying, suggests that GLP-1 may also inhibit the cephalic phase of the pancreatic secretory response to meal ingestion in addition to the inhibition caused by delayed gastric emptying. The inhibition by GLP-1 of pancreatic secretion stimulated by duodenal amino acid perfusion in human volunteers (6) is probably due to a lowering of vagal, cholinergic tone, known to be essential for intraduodenally stimulated secretion (1).

The precise site of the inhibitory action of GLP-1 remains obscure. However, high-affinity receptors for GLP-1 in the rat brain were demonstrated previously (7, 21). In addition, we have recently reported that specific binding sites in various regions around the third ventricle in rats, in particular the subfornical organ and the area postrema, were accessible to GLP-1 from the systemic circulation (34). GLP-1 might interact with these receptors. However, interaction with afferent pathways to the brain stem, similar to those mediating the satiating effect of cholecystokinin (40), is another possibility, supported by recent findings in rats in which intraperitoneal administration of GLP-1 was demonstrated to evoke a vagal hepatopancreatic reflex (29). Similarly, vagal deafferentation abolished the
inhibitory effect of peripheral GLP-1 on gastric emptying [but, surprisingly, also the effects of intracerebroventricularly administered GLP-1 (19)]. It is of interest that activation of the cerebral GLP-1 receptors leads to inhibition of food intake in experimental animals (44). In addition, intravenous infusion of GLP-1 was recently demonstrated to promote satiety and inhibit food intake in healthy volunteers (5). Possibly, the central actions of GLP-1 are part of a spectrum of activities whereby the distal gut helps to regulate food intake.

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REFERENCES


