Intravenous infusion of glucagon-like peptide-1 potently inhibits food intake, sham feeding, and gastric emptying in rats

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Chelikani, Prasanth K., Alvin C. Haver, and Roger D. Reidelberger. Intravenous infusion of glucagon-like peptide-1 potently inhibits food intake, sham feeding, and gastric emptying in rats. Am J Physiol Regul Integr Comp Physiol 288: R1695–R1706, 2005. First published February 17, 2005; doi:10.1152/ajpregu.00870.2004.—Glucagon-like peptide-1(7–36)-amide (GLP-1) is postulated to act as a hormonal signal from gut to brain to inhibit food intake and gastric emptying. A mixed-nutrient meal produces a 2 to 3-h increase in plasma GLP-1. We determined the effects of intravenous infusions of GLP-1 on food intake, sham feeding, and gastric emptying in rats to assess whether GLP-1 inhibits food intake, in part, by slowing gastric emptying. A 3-h intravenous infusion of GLP-1 (0.5–170 pmol·kg⁻¹·min⁻¹) at dark onset dose-dependently inhibited food intake in rats that were normally fed with a potency (mean effective dose) and efficacy (maximal % inhibition) of 23 pmol·kg⁻¹·min⁻¹ and 82%, respectively. Similar total doses of GLP-1 administered over a 15-min period were less potent and effective. In gastric emptying experiments, GLP-1 (1.7–50 pmol·kg⁻¹·min⁻¹) dose-dependently inhibited gastric emptying of saline and ingested chow with potencies of 18 and 6 pmol·kg⁻¹·min⁻¹ and maximal inhibitions of 74 and 83%, respectively. In sham-feeding experiments, GLP-1 (5–50 pmol·kg⁻¹·min⁻¹) dose-dependently reduced 15% aqueous sucrose intake in a similar manner when gastric cannula was closed (real feeding) and open (sham feeding). These results demonstrate that intravenous infusions of GLP-1 dose-dependently inhibit food intake, sham feeding, and gastric emptying with a similar potency and efficacy. Thus GLP-1 may inhibit food intake in part by reducing gastric emptying, yet can also inhibit food intake independently of its action to reduce gastric emptying. It remains to be determined whether intravenous doses of GLP-1 that reproduce postprandial increases in plasma GLP-1 are sufficient to inhibit food intake and gastric emptying.

GLUCAGON-LIKE PEPTIDE-1(7–36)-AMIDE (GLP-1) is a 30-amino acid peptide that is produced by endocrine cells along the gut from stomach to rectum, pancreatic α-cells, and discrete populations of neurons in the nucleus of the solitary tract (NTS), the adjacent dorsomedial medullary reticular formation, and the olfactory bulb (13, 15, 17, 22, 28). Several studies have now clearly demonstrated that exogenous GLP-1 potently reduces food intake, gastric emptying, and body weight when administered systemically (27, 54, 59) or into the brain (9, 23–25, 44, 51). GLP-1 actions appear to be mediated by a single GLP-1 receptor (48). In rodents, GLP-1 receptor mRNA and high-affinity GLP-1 binding sites have been identified in numerous tissues including pancreatic islets, lungs, gastrointestinal tract, and throughout the brain (6, 12, 14, 28, 48, 52).

Endogenous GLP-1 appears to play an essential role in reducing gastric emptying, food intake, and energy reserves. Systemic administration of the GLP-1 receptor antagonist exendin(9–39) to rats has been shown to block GLP-1-induced inhibition of gastric emptying and small intestinal motility, and to increase gastric emptying and intestinal motility after a meal (16, 49, 50). Acute and chronic intracerebroventricular administration of exendin(9–39) has been reported to increase food intake, body weight, and adiposity in rats (25, 44, 51). It remains to be determined whether systemic administration of exendin(9–39) blocks the anorectic response to peripheral administration of GLP-1 or increases food intake when given alone.

The mechanism by which food intake stimulates release of GLP-1, and the source (gut, pancreas, brain), mode (endocrine, paracrine, neurocrine), and site of action (brain, visceral sensory nerves, gastrointestinal tract) of GLP-1 to decrease food intake, gastric emptying, and energy reserves remains to be determined. One likely mechanism is an endocrine one involving meal-induced secretion of GLP-1 from the gut into the circulation. Food intake increases plasma levels of GLP-1 immunoreactivity in humans from about 10 to 20 pM within 40 min, a level which is sustained for at least 2 h (55, 57). In rats, a liquid, mixed-nutrient meal administered intraduodenally increases plasma GLP-1 immunoreactivity from about 4 pM to 27 pM within 30 min, which then gradually declines to basal levels during the next 3 h (2). If GLP-1 acts as a blood-borne signal to control food intake and gastric emptying, then it would be important to determine whether intravenous doses of GLP-1 that reproduce postprandial increases in plasma GLP-1 are sufficient to inhibit food intake and gastric emptying. Numerous studies, all employing continuous intravenous administration of GLP-1 to humans, suggest that postprandial increases in plasma GLP-1 are sufficient to decrease food intake and gastric emptying (10, 26, 32, 33, 45, 54, 60). However, Vilsboll and Holst (56) recently stated that “most published (plasma GLP-1) data show results from assays that do not discriminate between the intact and degraded forms and, therefore, do not reflect the concentrations of bioactive hormones.” Most of the assays used in these studies also used antiseras specific for the C-terminal end of GLP-1(7–36) amide, which does not recognize the bioactive glycine-extended form of GLP-1. Thus it remains to be determined whether meal-induced changes in plasma levels of specific molecular forms of GLP-1 are sufficient either alone or when combined to inhibit food intake and gastric emptying. Furthermore, bolus subcutaneous (43), intraperitoneal (16, 47, 51), and intravenous

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GLP-1, FOOD INTAKE, AND GASTRIC EMPTYING

(21) injections of GLP-1 have been shown to have little (21, 43) or no effect (16, 47, 51) on food intake in rats. No previous study has examined the effects of intravenous infusion of GLP-1 on food intake in rats.

Factors that promote gastric distention by inhibiting gastric emptying can reduce food intake (29, 38, 42). If GLP-1 reduces food intake in part by inhibiting gastric emptying, then it would be important to determine whether intravenous infusion of GLP-1 reduces food intake and gastric emptying with a similar potency and efficacy, and whether GLP-1 is less effective in reducing food intake in sham feeding animals in which ingested liquid food rapidly drains from a gastric cannula. Numerous studies, mostly using single intravenous doses of GLP-1 to humans, suggest that GLP-1 reduces gastric emptying and food intake with a similar potency (10, 26, 32, 33, 45, 54, 60). No study has directly compared the dose-dependent effects of intravenous infusions of GLP-1 on food intake, gastric emptying, and sham feeding.

The present study assessed whether GLP-1 inhibits food intake in part by decreasing gastric emptying. We conducted the following experiments. First, a wide range of GLP-1 doses was administered intravenously during the first 3 h of the dark period to rats that were normally fed to determine the potency and efficacy for GLP-1-induced inhibition of food intake. Second, similar total doses of GLP-1 were administered intravenously for 15 min just before dark onset to determine whether the duration of GLP-1 administration affects its potency and efficacy for reducing food intake. Third, a wide range of GLP-1 doses was administered intravenously to rats to determine the potency and efficacy for GLP-1-induced inhibition of gastric emptying of saline and ingested chow, and to assess whether GLP-1 inhibits gastric emptying and food intake with a similar potency and efficacy. Fourth, several doses of GLP-1 were administered intravenously to rats consuming liquid diet with gastric cannulas closed (real feeding) and open (sham feeding) to determine whether GLP-1 can inhibit food intake independently of its action to inhibit gastric emptying. Fifth, GLP-1 and GLP-1 receptor antagonist exendin(9–39) were administered intravenously to rats to determine whether GLP-1-induced inhibition of food intake and gastric emptying is mediated by the GLP-1 receptor subtype that is blocked by exendin(9–39).

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (Charles River, Kingston, NY) weighing 300–485 g were housed individually in hanging wire-mesh cages in a room with controlled temperature (19–21°C) and a 12:12-h light-dark cycle (lights off at 1600 h). Animals were provided pelleted rat chow (Labdiet, 5001 Rodent diet, PMI Nutrition International, Brentwood, MO) and water ad libitum. The Animal Studies Subcommittee of the Omaha Veterans Affairs Medical Center approved the experimental protocol.

Surgical procedures. The procedure for implantation of a jugular vein catheter for peptide infusions has been described previously (61). Catheters were kept patent by flushing with 0.2 ml of 50% dextrose on alternate days. The procedure for implantation of a stainless steel gastric cannula for instilling saline and retrieving gastric contents was described previously (41). Animals were allowed at least 1 wk to recover from surgery before being subjected to experimental procedures.

Peptides

Rat GLP-1 was purchased from Bachem (Torrance, CA). Exendin(9–39) was synthesized and purified as follows. Synthesis and purification of exendin(9–39). A 5-(4-Fmoc-aminoethyl-3,5-dimethoxyphenoxy) valeric acid linker attached to a polyethylene-graft polystyrene support (PAL-PEG-PS) for solid-phase peptide synthesis was purchased from Applied Biosystems (Foster City, CA). O-(benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU), 1-hydroxybenzotriazole (HOBr), piperezine, trifluoroacetic acid (TFA), and protected amino acid derivatives were purchased from Chem-Impex International (Wood Dale, IL). Dimethylformamide (DMF), acetonitrile and diethyl ether were purchased from Fisher Scientific (Pittsburgh, PA). Thioanisole, anisole, and ethanolthiol were purchased from Aldrich (St. Louis, MO).

Exendin(9–39) was synthesized by continuous flow solid-phase methodology on the PAL-PEG-PS support at a 0.1 mmol scale using a Pioneer Peptide Synthesizer (Applied Biosystems). α-Amino groups were protected with the fluorenlymethylcarbonyl (Fmoc) group, and side chains were protected with the trityl group for Glu and Asn. tert-Butyl group for Ser, Asp, and Glu. tert-butylxycarbonyl group for Lys and Trp, and pentamethyldihydrobenzofuransulfonyl group for Arg. After removal of the Fmoc group from the resin with 20% piperidene/DMF (vol/vol), HBTU and HOBt activated Fmoc-Ser(tBu)-OH in DMF was coupled to the resin. This process was repeated with each amino acid derivative. After assembly, the peptide-resin was washed with diethyl ether and dried under vacuum. Cleavage of peptide from the resin and removal of side chain protecting groups was accomplished in a 10 ml mixture of TFA, thioanisole, methanol, and anisole (9:0.5:3:0.2, vol/vol). After stirring 2 h at room temperature, the peptide was precipitated by adding the solution to cold diethyl ether. The crude peptide was filtered, dissolved, and lyophilized.

Purification of exendin(9–39) was accomplished by reverse-phase high-performance liquid chromatography on a Waters (Milford, MA) model 600 HPLC system. The crude peptide was dissolved in 71% solvent A (0.1% TFA/water), 29% solvent B (0.09% TFA/acetonitrile), and subjected to a gradient of 29% B to 41% B over 50 min on a semipreparative Vydac (Hesperia, CA) C18 column (10 × 250 mm). Flow rate was 4 ml/min and the peptide was detected by UV absorbance at 230 nm. Fractions containing the pure peptide were collected and lyophilized. Proof of structure was provided by coelution with a known sample and by electrospray mass spectrometry.

Experiments

Effects of 3-h intravenous infusions of GLP-1 on solid-food intake in normally fed rats. For each animal used in these experiments, the jugular vein catheter was connected to a 40-cm length of tubing, which passed through a protective spring coil connected between a syringe infusion pump (PHD2000, Harvard Apparatus, South Natick, MA) and water ad libitum. Rats had ad libitum access to ground chow that was provided fresh each day at 1300 h. Animals were adapted to experimental conditions for at least 1 wk before the start of experiments. Rats that were normally fed (n = 15) received a 3-h jugular vein infusion of a single dose of GLP-1 (0, 0.5, 1.7, 5, or 17 pmol·kg⁻¹·min⁻¹) in 0.15 M NaCl, 0.1% bovine serum albumin; 50 µl/min) beginning 15 min before dark onset (1600 h). Food intake cumulated hourly for 17 h after dark onset, and meal parameters [mean meal size, number of meals, and mean satiety ratio (postmeal interval per meal size)] cumulated hourly during the 3-h infusion period were determined, as described previously, from continuous computer recordings of changes in food bowl weight (61). GLP-1 was administered via a syringe infusion pump (PHD2000, Harvard Apparatus, South Natick, MA). Syringes were installed in pumps and infusion lines were connected to the rats at 1300 h, the same time fresh food was provided, 3 h before dark onset. Pumps were turned on and off by a
computer program. Each rat received each dose of GLP-1 in random order at intervals of at least 48 h. At the end of the experiment, the patency of jugular vein catheters was determined by intravenous injection of 0.2 ml of the short-acting anesthetic propofol (Abbott Laboratories, North Chicago, IL). A catheter was considered patent if the rat lost consciousness immediately on injection of the anesthetic; only data from such propofol-positive rats were included in statistical analyses. In separate experiments of identical design, rats \( (n = 15) \) received either vehicle or a higher dose of GLP-1 (50 or 170 pmol·kg\(^{-1}\)·min\(^{-1} \) in 0.15 M NaCl, 0.1% BSA).

**Effects of 15-min intravenous infusions of GLP-1 on solid food intake in normally fed rats.** Experiments were identical to those described above for the 3-h infusions of GLP-1 except for the doses and duration of GLP-1 infusion. Rats \( (n = 16) \) received a 15-min jugular vein infusion of GLP-1 (0, 60, 200, or 600 pmol·kg\(^{-1}\)·min\(^{-1} \) in 0.15 M NaCl, 0.1% BSA; 50 µl/min) beginning 15 min before dark onset (1600 h). Food intake was determined as described above. The total amounts of GLP-1 delivered at the 60, 200, and 600 pmol·kg\(^{-1}\)·min\(^{-1} \) infusion rates (0.9, 3, and 9 nmol/kg, respectively) were equivalent to those delivered in the preceding experiment when GLP-1 was infused for 3 h at 5, 17, and 50 pmol·kg\(^{-1}\)·min\(^{-1} \), respectively.

**Effects of intravenous infusions of GLP-1 on gastric emptying of saline.** Rats with jugular vein and gastric cannulas were adapted to the following procedures, which took about 1 wk. Every other day after an overnight food-deprivation (\( \sim 18 \) h), rats were restrained in Bollman cages for \( \sim 60 \) min, gastric cannulas were opened, residual gastric contents were flushed with warm water, 5 ml of 0.9% saline containing 60 mg/l phenol red were instilled into the stomach, and cannulas were closed. Ten minutes later, gastric contents were collected, the stomach was flushed with 5 ml of 0.9% saline, cannulas were closed, and rats were returned to their cages. Rats were then provided free access to rat chow and liquid diet (Ensure Plus, Abbott Laboratories, Columbus, OH; 1.5 kcal/ml). Following adaptation to these procedures, rats \( (n = 12) \) received a 20-min intravenous infusion of a single dose of GLP-1 (0, 1.7, 5, 10, 17, or 50 pmol·kg\(^{-1}\)·min\(^{-1} \) in 0.15 M NaCl, 0.1% BSA; 50 µl/min). Ten minutes after infusion onset, 5 ml of 0.9% saline containing phenol red were instilled into the stomach. Ten minutes later, gastric contents were collected, the stomach was flushed with 5 ml of 0.9% saline, and the total volume of fluid recovered from the stomach was determined. Concentrations of phenol red in instilled saline and recovered fluid were determined spectrophotometrically. The amount of phenol red in the instilled saline was determined by multiplying phenol red concentration in the saline by volume of saline instilled into the stomach. The amount of phenol red recovered from the stomach was determined by multiplying phenol red concentration in recovered fluid by volume of fluid recovered. Gastric emptying of saline during the 10-min period was calculated as percent emptied \( = \frac{1}{n} \times 100 \times \left( \frac{\text{volume of saline instilled} - \text{volume of saline recovered}}{\text{volume of saline instilled}} \right) \times 100 \times \left( \frac{\text{volume of gastric content recovered}}{\text{volume of saline instilled}} \right) \times 100 \). Each rat received each dose of GLP-1 in random order at intervals of at least 48 h. At the end of each experiment, the patency of jugular vein catheters was determined by intravenous injection of propofol.

**Effects of intravenous infusions of GLP-1 on gastric emptying of ingested chow.** An experimental model was developed and validated to repeatedly measure gastric emptying of ingested solid food in the same rat. Three groups of rats were used in three experiments to determine the temporal profile of gastric emptying of ingested chow. Rats with gastric cannulas to recover gastric contents through the cannulas, rats with gastric cannulas that were decapitated to recover gastric contents, and rats without gastric cannulas that were decapitated to recover gastric contents. All rats were fasted overnight (\( \sim 18 \) h) and trained to consume at least 10 g of moistened ground rat chow (1.61 wt/wt water to chow) within 10 min. In the first experiment, rats with gastric cannulas \( (n = 16) \) were first adapted to the following procedures, which took about 2 wk. Every other day after an overnight food-deprivation, rats were brieﬂy restrained in Bollman cages, gastric cannulas were opened, residual gastric contents were flushed with warm water, cannulas were closed, and rats were returned to their cages where they were provided access to moistened ground chow (\( \sim 13 \) g wet wt) for 10 min. The amount of food consumed was determined from the difference in the weight of food offered and left-over. The animals were then immediately transferred to Bollman cages. At 0, 1, 2, or 3 h after the 10-min eating period, gastric cannulas were opened and gastric contents were recovered by flushing the stomach 6 to 20 times with 5 ml of warm water until effluent was clear. Recovered contents and samples of moistened chow were lyophilized. Gastric emptying of ingested chow was calculated as percent emptied \( = \left[ 1 - \frac{\text{dry wt of stomach contents}}{\text{dry wt of food ingested}} \right] \times 100 \). Gastric emptying at each time-point was determined randomly in each rat at intervals of at least 48 h.

Gastric emptying determined in this manner was compared with the traditional method of killing rats at specific times after meal ingestion to recover gastric contents. Rats with gastric cannulas \( (n = 5 \text{ to } 7 \text{ per group}) \) were randomly decapitated at 0, 1, 2, or 3 h after the 10-min eating period, abdomens were opened, and stomachs were clamped at the pylorus and cardia and rapidly removed. Stomachs were then cut along the greater curvature and gastric contents were recovered by flushing with water. Gastric emptying of the ingested food was determined as described above. A third experiment of identical design was conducted to determine the temporal profile of gastric emptying of ingested chow in rats without gastric cannulas \( (n = 8 \text{ per group}) \).

The temporal profiles of gastric emptying of ingested chow were similar in the three experiments using the two methods of recovery of gastric contents, with nearly 50% of ingested food emptied by 2 h (see **RESULTS** below and Fig. 7). Therefore, in the subsequent study examining the effects of intravenous infusion of GLP-1 on gastric emptying of ingested chow, rats with gastric cannulas \( (n = 16) \) received a 2-h intravenous infusion of a single dose of GLP-1 (0, 1.7, 5, 17, or 50 pmol·kg\(^{-1}\)·min\(^{-1} \) in 0.15 M NaCl, 0.1% BSA; 50 µl/min) immediately after the 10-min eating period, and gastric contents were recovered 2 h after infusion onset by flushing the stomach as described above. The amount of ingested chow emptied was determined as described above. Each rat randomly received each dose at intervals of at least 48 h.

**Effects of intravenous infusions of GLP-1 on ingestion of 15% aqueous sucrose in food-deprived rats with either gastric cannula closed (real feeding) or open (sham feeding).** Rats with jugular vein and gastric cannulas were adapted to the following procedures, which took about 1 wk. Every other day after an overnight food deprivation (\( \sim 18 \) h), rats were restrained in Bollman cages for \( \sim 60 – 90 \) min, gastric cannulas were opened, stomachs were flushed with warm water, and 15% aqueous sucrose solution was provided for 30 min. Rats were then returned to their cages and provided free access to rat chow and Ensure Plus. After adaptation to these procedures, in separate experiments, rats \( (n = 15) \) with either an open or closed gastric cannula received intravenous infusion of a single dose of GLP-1 (0, 5, 17, or 50 pmol·kg\(^{-1}\)·min\(^{-1} \) in 0.15 M NaCl, 0.1% BSA; 50 µl/min) for 40 min beginning 10 min before sham access to 15% sucrose. The volume of sucrose consumed during the 30-min period was measured. Each rat received each treatment in random order at intervals of at least 48 h. Data from a sham-feeding rat was considered valid only if the volume of fluid passing through the gastric cannula during the 30-min sham-feeding period was equal to the volume of liquid consumed during that period (±10%). At the end of each experiment, the patency of jugular vein catheters was determined by intravenous injection of propofol.

**Effects of intravenous infusion of GLP-1 receptor antagonist exendin(9–39) on GLP-1-induced inhibition of gastric emptying and food intake.** Rats with jugular vein and gastric cannulas were adapted to procedures as described above. In gastric emptying experiments, rats \( (n = 12) \) received a 20-min intravenous infusion of vehicle (0.15 M NaCl, 0.1% BSA; 50 µl/min), GLP-1 (17 pmol·kg\(^{-1}\)·min\(^{-1} \)), or GLP-1 (17 pmol·kg\(^{-1}\)·min\(^{-1} \) plus exendin(9–39) (1,700 pmol·kg\(^{-1}\)·min\(^{-1} \)).
pmol·kg⁻¹·min⁻¹). Ten minutes after infusion onset, 5 ml of 0.9% saline containing phenol red was instilled into the stomach. Ten minutes later, gastric contents were collected, and gastric emptying of saline was determined as before. Rats received each treatment in random order at intervals of at least 48 h. A separate experiment of identical design determined the effects of intravenous infusion of exendin(9–39) (1,700 pmol·kg⁻¹·min⁻¹) on gastric emptying of saline. In feeding experiments, rats (n = 15) confined to Bollman cages and with closed gastric cannulas, received a 40-min intravenous infusion of vehicle (0.15 M NaCl, 0.1% BSA; 50 µl/min), GLP-1 (17 pmol·kg⁻¹·min⁻¹), or GLP-1 (17 pmol·kg⁻¹·min⁻¹) plus exendin(9–39) (1,700 pmol·kg⁻¹·min⁻¹), beginning 10 min before 30-min access to 15% sucrose. The volume of sucrose consumed during the 30-min period was measured. Rats received each treatment in random order at intervals of at least 48 h.

Statistical analyses. Values are presented as group means ± SE. Data from each of the experiments determining the dose-response effects of peptide infusions on food intake, meal parameters (meal size, number of meals, and satiety ratio), and gastric emptying were analyzed separately by one-way repeated-measures ANOVA. Planned comparisons of treatment means were evaluated by paired t-tests; differences were considered significant if P < 0.05. A general nonlinear, least-squares curve fitting method was used to fit the dose-response data for the effects of GLP-1 on food intake and gastric emptying to the following sigmoidal dose-response equation: 

\[
Y = a + (d - a) \frac{1}{1 + 10^{(X - b)}}
\]

where Y is the response, X is the logarithm of the administered dose, a is the response for 0 dose, d is the response for infinite dose, b is the logarithm of the mean effective dose producing a response halfway between a and d (ED₅₀), and b denotes the steepness of the dose-response curve.

RESULTS

Effects of 3-h intravenous infusions of GLP-1 on solid-food intake in normally fed rats. GLP-1 infusion during the first 3 h of the dark period dose-dependently inhibited cumulative food intake (Figs. 1A–C, 2, and 3A). The minimal effective dose (0.5 pmol·kg⁻¹·min⁻¹, 0.09 nmol/kg) produced a maximal inhibition of 26% at 2 h, which decreased to 6% inhibition by 10 h. The maximal effective dose (170 pmol·kg⁻¹·min⁻¹, 30.6 nmol/kg) produced a sustained inhibition of cumulative intake for 17 h, with a peak inhibition of 92% at 3 h, decreasing to 19% at 17 h. Nonlinear regression fitting of the dose-response data to the sigmoidal equation gave the following relationship between GLP-1 and change in cumulative food intake during the 3-h infusion period: change in cumulative intake (g) = -5.84 + 5.27/[1 + 10^{(X - 1.36 - \log(dose))} × (-1.36)] \ [r^2 = 0.50; F(4,75) = 51.8, P < 0.0001]. The estimated potency (ED₅₀) and efficacy (maximal % inhibition) were 23 pmol·kg⁻¹·min⁻¹ (4.1 nmol/kg) and 82%, respectively (Fig. 2).

GLP-1 reduced food intake during the 3-h infusion period by reducing meal size and frequency, and increasing the satiety ratio (postmeal interval per meal size) (Fig. 3, A–D). GLP-1 at 0.5 pmol·kg⁻¹·min⁻¹ reduced cumulative food intake at 2 h by 26%, yet it had no significant effect on meal parameters. GLP-1 at 1.7 pmol·kg⁻¹·min⁻¹ reduced cumulative food intake at 3 h by 8% and increased mean satiety ratio at 1 h by 65%. GLP-1 at 5 pmol·kg⁻¹·min⁻¹ reduced cumulative intake at 3 h by 8%, yet it had no significant effect on meal parameters. GLP-1 at 17 pmol·kg⁻¹·min⁻¹ reduced cumulative food intake at 1, 2, and 3 h by 28, 40, and 33%, reduced number of meals ingested at 2 and 3 h by 31 and 28%, reduced mean meal size at 2 h by 28%, and increased mean satiety ratio at 1, 2, and 3 h by 94, 106, and 72%, respectively. GLP-1 at 50 pmol·kg⁻¹·min⁻¹. Ten minutes after infusion onset, 5 ml of 0.9% saline containing phenol red was instilled into the stomach. Ten minutes later, gastric contents were collected, and gastric emptying of saline was determined as before. Rats received each treatment in random order at intervals of at least 48 h. A separate experiment of identical design determined the effects of intravenous infusion of exendin(9–39) (1,700 pmol·kg⁻¹·min⁻¹) on gastric emptying of saline. In feeding experiments, rats (n = 15) confined to Bollman cages and with closed gastric cannulas, received a 40-min intravenous infusion of vehicle (0.15 M NaCl, 0.1% BSA; 50 µl/min), GLP-1 (17 pmol·kg⁻¹·min⁻¹), or GLP-1 (17 pmol·kg⁻¹·min⁻¹) plus exendin(9–39) (1,700 pmol·kg⁻¹·min⁻¹), beginning 10 min before 30-min access to 15% sucrose. The volume of sucrose consumed during the 30-min period was measured. Rats received each treatment in random order at intervals of at least 48 h. Statistical analyses. Values are presented as group means ± SE. Data from each of the experiments determining the dose-response effects of peptide infusions on food intake, meal parameters (meal size, number of meals, and satiety ratio), and gastric emptying were analyzed separately by one-way repeated-measures ANOVA. Planned comparisons of treatment means were evaluated by paired t-tests; differences were considered significant if P < 0.05. A general nonlinear, least-squares curve fitting method was used to fit the dose-response data for the effects of GLP-1 on food intake and gastric emptying to the following sigmoidal dose-response equation: 

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Y = a + (d - a) \frac{1}{1 + 10^{(X - b)}}
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where Y is the response, X is the logarithm of the administered dose, a is the response for 0 dose, d is the response for infinite dose, b is the logarithm of the mean effective dose producing a response halfway between a and d (ED₅₀), and b denotes the steepness of the dose-response curve.
pmol·kg\(^{-1}\)·min\(^{-1}\) reduced cumulative food intake at 1, 2, and 3 h by 50, 48, and 54%, reduced mean meal size at 1, 2, and 3 h by 57, 53, and 54%, and increased mean satiety ratio at 1, 2, and 3 h by 270, 320, and 310%, respectively. GLP-1 at 170 pmol·kg\(^{-1}\)·min\(^{-1}\) reduced cumulative food intake at 1, 2, and 3 h by 89, 90, and 92%, reduced number of meals ingested at 3 h by 46%, reduced mean meal size at 1, 2, and 3 h by 85, 85, and 84%, and increased mean satiety ratio at 2 and 3 h by 920 and 1,140%, respectively.

Effects of 15-min intravenous infusions of GLP-1 on solid-food intake in normally fed rats. GLP-1 infusions during the 15-min period just before dark onset dose-dependently inhibited cumulative food intake, albeit with a reduced potency, and with an efficacy that was less than half of that for the 3-h infusions of the same total doses of GLP-1 (Figs. 4 and 5). The minimal effective dose (60 pmol·kg\(^{-1}\)·min\(^{-1}\), 0.9 nmol/kg) for the 15-min infusions produced a transient inhibition of cumulative intake at 2 h of 18%. The maximal effective dose (600 pmol·kg\(^{-1}\)·min\(^{-1}\), 9 nmol/kg) produced a sustained inhibition of cumulative intake for 17 h, with a peak inhibition of 52% at 1 h, decreasing to 8% at 17 h. This same total dose of GLP-1 (9 nmol/kg) when delivered over a 3-h period, produced a twofold greater reduction in food intake during the first 3 h of the dark period (Fig. 5).

Effects of intravenous infusions of GLP-1 on gastric emptying of saline. GLP-1 infusion dose-dependently reduced the volume of saline emptied from the stomach (Fig. 6). When vehicle was infused, 80% of the 5 ml of saline instilled into the stomach emptied from the stomach within 10 min. The minimal effective dose of GLP-1 (5 pmol·kg\(^{-1}\)·min\(^{-1}\), 0.1 nmol/kg) reduced gastric emptying to 73% of the instilled load; the maximal effective dose (50 pmol·kg\(^{-1}\)·min\(^{-1}\), 1 nmol/kg) reduced gastric emptying to 28% of the instilled load. Nonlinear regression fitting of the dose-response data to the sigmoidal equation gave the following relationship between GLP-1 and gastric emptying of saline: percent emptied = 19.9 + 57.2/[1 + 10 \((\log_{10}(dose)) \times (-1.980)] \), \[r^2 = 0.71; F(4,69) = 420, P < 0.0001\]. The estimated potency (ED\(_{50}\)) and efficacy (maximal % inhibition) were 18.3 pmol·kg\(^{-1}\)·min\(^{-1}\) (0.4 nmol/kg) and 74%, respectively.

Effects of intravenous infusions of GLP-1 on gastric emptying of ingested chow. The two methods of measuring gastric emptying of ingested chow, decapitation to recover gastric contents vs. recovery of gastric contents through the gastric cannula, gave similar temporal profiles in gastric emptying of the chow meals (Fig. 7). The presence of the closed gastric cannula also did not significantly affect gastric emptying, as emptying profiles were similar in cannulated and noncannulated rats that were decapitated to recover gastric contents. Chow meals were similar in size in each treatment group, averaging 4.5 ± 0.2 g dry wt in the noncannulated rats that were decapitated, 4.7 ± 0.2 g dry wt in the cannulated rats that were decapitated, and 5.0 ± 0.1 g dry wt in the cannulated rats that were flushed with warm water. Only a small amount of ingested chow (0.4 to 5%) had emptied by the end of the 10-min eating period; by 1 h, 28 to 39% of the ingested chow had emptied; and by 3 h, 54 to 64% had emptied.

Intravenous infusion of GLP-1 for 2 h beginning immediately after the 10-min meal, dose-dependently reduced gastric emptying of ingested chow during the infusion period (Fig. 6). Chow meals were similar in size for each of the dose groups; 5.1 ± 0.2, 5.1 ± 0.2, 5.0 ± 0.1, 5.0 ± 0.2, and 5.3 ± 0.2 g dry wt for 0, 1, 7, 5, 17, and 50 pmol·kg\(^{-1}\)·min\(^{-1}\), respectively. When vehicle was infused, 51% of the ingested chow emptied from the stomach by the end of the 2-h infusion period. GLP-1 at the minimal effective dose of 5 pmol·kg\(^{-1}\)·min\(^{-1}\) (0.6 nmol/kg) reduced gastric emptying to 30% of the ingested chow. The maximal effective dose of 17 pmol·kg\(^{-1}\)·min\(^{-1}\) (2 nmol/kg) completely blocked emptying of the chow from the stomach. Nonlinear regression fitting of the dose-response data to the sigmoidal equation gave the following relationship between GLP-1 and gastric emptying of chow: % emptied = 8.1 + 40.1/[ 1 + 10 \((0.74 - \log(dose)) \times (-6.5)] \), \[r^2 = 0.45; F(3,59) = 17.8, P < 0.0001\]. The estimated potency (ED\(_{50}\)) and efficacy (maximal % inhibition) were 5.5 pmol·kg\(^{-1}\)·min\(^{-1}\) (0.7 nmol/kg) and 83%, respectively.

Effects of intravenous infusions of GLP-1 on ingestion of 15% aqueous sucrose in food-deprived rats with either gastric cannula closed (real feeding) or open (sham feeding). GLP-1 infusion inhibited 30-min sucrose ingestion dose-dependently and to a similar degree in real-feeding and sham-feeding rats (Fig. 8). GLP-1 at 5 pmol·kg\(^{-1}\)·min\(^{-1}\) (0.2 nmol/kg) inhibited real feeding by 24%, yet had no significant effect on sham feeding. GLP-1 at 17 pmol·kg\(^{-1}\)·min\(^{-1}\) (0.7 nmol/kg) inhibited real feeding by 31% and sham feeding by 47%. GLP-1 at 50 pmol·kg\(^{-1}\)·min\(^{-1}\) (2 nmol/kg) inhibited real feeding by 50% and sham feeding by 57%.

Effects of intravenous infusion of GLP-1 receptor antagonist exendin(9–39) on GLP-1-induced inhibition of gastric emptying and food intake. Intravenous infusion of GLP-1 at 17 pmol·kg\(^{-1}\)·min\(^{-1}\) inhibited gastric emptying of saline by 49%; coadministration of exendin(9–39) at 1,700 pmol·kg\(^{-1}\)·min\(^{-1}\) completely blocked this response (Fig. 9A). Exendin(9–39) infusion alone had no effect on the volume of saline emptied [3.80 ± 0.1 ml in response to exendin(9–39) vs. 3.84 ± 0.08 ml in response to vehicle]. Intravenous infusion of GLP-1 at 17 pmol·kg\(^{-1}\)·min\(^{-1}\) inhibited 30-min sucrose ingestion by 31%; coadministration of exendin(9–39) at 1,700 pmol·kg\(^{-1}\)·min\(^{-1}\) completely blocked this response (Fig. 9B).
DISCUSSION

Our results demonstrate several important properties of the effects of intravenous infusion of the gut-brain peptide GLP-1 on food intake and gastric emptying in rats. First, GLP-1 potently inhibits food intake in a dose-dependent manner when administered by continuous intravenous infusion during the first 3 h of the dark period to normally fed rats. The estimated potency (ED50) and efficacy (maximal % inhibition) are 23 pmol·kg⁻¹·min⁻¹ (4.1 nmol/kg) and 82%, respectively. Second, similar total doses of GLP-1 infused during the 15-min period just before dark onset also dose-dependently inhibit food intake, albeit with a significantly reduced potency and efficacy. Third, GLP-1 reduces food intake by reducing meal size and by decreasing meal frequency and/or increasing the satiety ratio. Fourth, GLP-1 dose-dependently inhibits gastric emptying of saline and ingested chow with estimated potencies of 18 and 6 pmol·kg⁻¹·min⁻¹ and maximal inhibitions of 74 and 83%, respectively. And fifth, GLP-1 inhibits real feeding and sham feeding with a similar potency and efficacy. Thus GLP-1 may inhibit food intake in part by reducing gastric emptying, yet can also inhibit food intake independently of its action to reduce gastric emptying.

Using the same experimental models, we previously determined the effects of intravenous infusions of CCK-8, amylin, calcitonin (CT), salmon calcitonin (sCT), calcitonin gene-related peptide (CGRP), adrenomedullin (ADM), PYY(3–36), and PYY(1–36) on food intake and gastric emptying in rats (7, 8, 39, 40). The rank order of potencies [ED50 in pmol·kg⁻¹·min⁻¹] of these peptides, including GLP-1 in reducing food intake are sCT [1], amylin [6], PYY (3–36) [15], CCK-8 [18], GLP-1 [23], CGRP [26], ADM [35], and PYY(1–36) [≥100]. For inhibition of gastric emptying of saline, the rank order of potencies of these peptides are sCT [1], amylin [3], GLP-1 [18], CCK-8 [35], PYY (3–36) [37], CGRP [130], ADM [160], PYY (1–36) [470], and CT [730]. If in vivo clearance rates of these peptides differ significantly, then relative potencies of infused doses will not reflect potencies of plasma or tissue levels of the peptides. Nevertheless, these studies do show that intravenous doses of GLP-1 inhibit food intake and gastric emptying with potencies that are similar to those for the putative satiety peptides CCK, amylin, and PYY(3–36).

Bolus subcutaneous (43), intraperitoneal (16, 47, 51), and intravenous (21) injections of GLP-1 have previously been reported to have little (21, 43) or no effect (16, 47, 51) on food intake in rats. This is thought to be due to rapid degradation of GLP-1 in blood by the enzyme dipeptidyl peptidase-IV (DPP-IV); 50% of intravenously infused GLP-1 is converted to GLP-1(9–36) within 2 min (20). GLP-1 receptor agonists and GLP-1 analogs that are DPP-IV resistant—exendin-4, a non-mammalian GLP-1 receptor agonist from lizard venom, and NN2211, an acylated GLP-1 derivative with a half-life of 4 h

Fig. 3. Effects of 3-h intravenous infusions of GLP-1 on change in cumulative food intake (A), change in number of meals consumed (B), change in mean meal size (C), and change in mean satiety ratio in 12–14 rats (D). Values are means ± SE. *P < 0.05, †P < 0.01, ‡P < 0.001, compared with the vehicle-treated data. Vehicle-treated values (averaged across the three experiments) at 1, 2, and 3 h, respectively, were 1.9, 4.4, and 7.1 g for food intake; 1.0, 2.3, and 3.5 for number of meals; 1.9, 2.2, and 2.3 g for mean meal size; and 31 min/g each for mean satiety ratio.
The present study demonstrates that in rats, 2-h infusions of GLP-1 inhibit gastric emptying of ingested chow during the infusion period with a minimal effective dose of 5 pmol·kg⁻¹·min⁻¹, ED₅₀ of 6 pmol·kg⁻¹·min⁻¹, and efficacy (maximal inhibition) of 83%, which are comparable to those observed in the present study for the effects of 3-h infusions of GLP-1 on ingestion of chow in rats (minimal effective dose of 5 pmol·kg⁻¹·min⁻¹, ED₅₀ of 15 pmol·kg⁻¹·min⁻¹, and efficacy of 82%). Our results also demonstrate that 20-min GLP-1 infusions inhibit gastric emptying of a liquid (saline) during the last 10 min of the infusion period with a similar minimal effective dose, ED₅₀, and efficacy: 5 pmol·kg⁻¹·min⁻¹, 18 pmol·kg⁻¹·min⁻¹, and 74%, respectively. Only one other study has examined the effects of intravenous infusion of GLP-1 on gastric emptying in rats. GLP-1 at 20 pmol·kg⁻¹·min⁻¹ reduced gastric emptying of a liquid in rats by 30% (49), which is identical to that produced by GLP-1 at 17 pmol·kg⁻¹·min⁻¹ on saline emptying in the present study. Together, these results suggest that GLP-1 may inhibit food intake in part by inhibiting gastric emptying.

To further assess the role of gastric emptying in mediating the anorexic response to GLP-1, we determined the effects of 40-min intravenous infusions of GLP-1 on real feeding and in rats—have been shown to inhibit food intake in rats when administered peripherally by bolus injections (3, 4, 21, 37, 43, 46). No previous study has examined the effects of intravenous infusion of GLP-1 on food intake in rats. We show here that 3-h intravenous infusions of GLP-1 at dark onset, which are more likely than bolus injections to mimic postprandial increases in plasma GLP-1, dose-dependently inhibit food intake in normally fed rats. Similar total doses of GLP-1 infused during the 15-min just before dark onset also dose-dependently inhibited food intake, albeit with a significantly reduced potency and efficacy. These studies indicate that intravenous infusion of GLP-1 is a more reliable method than bolus injection for reducing food intake.

Factors that promote gastric distention by inhibiting gastric emptying can reduce food intake (29, 38, 42). If circulating GLP-1 reduces food intake in part by inhibiting gastric emptying, then it would be important to determine whether intravenous administration of GLP-1 reduces gastric emptying and food intake with a similar potency and efficacy. Numerous studies, mostly using single intravenous doses of GLP-1 in human subjects, suggest that GLP-1 reduces gastric emptying and food intake with a similar potency and efficacy (10, 26, 32, 33, 45, 54, 60). A meta-analysis of the results of these studies indicated that GLP-1 decreases food intake comparably in normal-weight, obese, and type 2 diabetic subjects, the anorexic response to GLP-1 is dose-dependent, and a mean GLP-1 infusion rate of 0.89 pmol·kg⁻¹·min⁻¹ reduces food intake by about 12% (54). Three of these studies, which administered similar doses of GLP-1 to obese humans, demonstrated that GLP-1 decreases gastric emptying. A meta-analysis of the results indicated that GLP-1 reduced gastric emptying dose-dependently, yet it showed no correlation between reductions in food intake and gastric emptying (51). In a separate study, Nauck et al. (33) showed that intravenous infusion of GLP-1 at 0.4, 0.8, and 1.2 pmol·kg⁻¹·min⁻¹ potently reduces gastric emptying of a mixed-liquid meal in normal-weight humans. The present study demonstrates that in rats, 2-h infusions of GLP-1 inhibit gastric emptying of ingested chow during the infusion period with a minimal effective dose of 5 pmol·kg⁻¹·min⁻¹, ED₅₀ of 6 pmol·kg⁻¹·min⁻¹, and efficacy (maximal inhibition) of 83%, which are comparable to those observed in the present study for the effects of 3-h infusions of GLP-1 on ingestion of chow in rats (minimal effective dose of 5 pmol·kg⁻¹·min⁻¹, ED₅₀ of 15 pmol·kg⁻¹·min⁻¹, and efficacy of 82%). Our results also demonstrate that 20-min GLP-1 infusions inhibit gastric emptying of a liquid (saline) during the last 10 min of the infusion period with a similar minimal effective dose, ED₅₀, and efficacy: 5 pmol·kg⁻¹·min⁻¹, 18 pmol·kg⁻¹·min⁻¹, and 74%, respectively. Only one other study has examined the effects of intravenous infusion of GLP-1 on gastric emptying in rats. GLP-1 at 20 pmol·kg⁻¹·min⁻¹ reduced gastric emptying of a liquid in rats by 30% (49), which is identical to that produced by GLP-1 at 17 pmol·kg⁻¹·min⁻¹ on saline emptying in the present study. Together, these results suggest that GLP-1 may inhibit food intake in part by inhibiting gastric emptying.

To further assess the role of gastric emptying in mediating the anorexic response to GLP-1, we determined the effects of 40-min intravenous infusions of GLP-1 on real feeding and

Fig. 4. Effects of 15-min intravenous infusions of GLP-1 on cumulative food intake in 14 rats. Rats that were normally fed received a 15-min jugular vein infusion of GLP-1 beginning 15 min before dark onset. Food intake was determined from continuous computer recordings of changes in food bowl weight. Values are means ± SE. *P < 0.05, †P < 0.01, compared with the vehicle-treated group.

Fig. 5. Effects of intravenous infusions of GLP-1 for 3 h (A) and 15 min (B) on change in cumulative food intake in 12–14 rats. Data are from those presented in Figs. 1 and 4. *P < 0.05, †P < 0.01, ‡P < 0.001, compared with the vehicle-treated group.
sham feeding of liquid food in food-deprived rats during the last 30 min of the infusion period. In the sham-feeding model, where ingested liquid rapidly drains through a gastric cannula, the negative feedback effect of gastric distention on food intake that can result from peptide-induced inhibition of gastric emptying, is eliminated. We found that GLP-1 inhibits real feeding and sham feeding with a similar potency and efficacy, and at doses that inhibit ingestion and gastric emptying of chow and gastric emptying of saline. Thus GLP-1 can inhibit food intake independently of its action to inhibit gastric emptying of liquids and solids. However, under normally fed conditions, GLP-1 potently and effectively inhibits gastric emptying, which may also inhibit food intake indirectly by promoting gastric distention.

Endogenous GLP-1 appears to play an essential role in reducing gastric emptying, food intake, and energy reserves. Systemic administration of the GLP-1 receptor antagonist exendin(9–39) to rats has been shown to block GLP-1-induced inhibition of gastric emptying and small intestinal motility, and to increase gastric emptying and intestinal motility after a meal (16, 49, 50). Acute and chronic intracerebroventricular administration of exendin(9–39) has also been shown to increase food intake, body weight, and adiposity in rats (25, 44, 51). Only one previous study has examined the effects of systemic administration of the GLP-1 receptor antagonist exendin(9–39) on food intake. In rats, bolus intraperitoneal injection of exendin(9–39) (6 and 15 nmol) had no effect on food intake when given alone, and increased, rather than decreased, the anorexic response to a gastric preload of carbohydrate, but not fat or protein (37). The ability of these exendin(9–39) doses to antagonize anorexic responses to GLP-1 administration was not assessed. We show here that intravenous infusion of exendin(9–39) at 1,700 pmol·kg\(^{-1}\)·min\(^{-1}\) completely blocks the inhibitory effects of GLP-1 infusion (17 pmol·kg\(^{-1}\)·min\(^{-1}\)) on feeding and gastric emptying in rats. Thus GLP-1-induced inhibition of food intake and gastric emptying in rats appears to be mediated by the GLP-1 receptor that can be blocked by exendin(9–39).

The mechanisms through which GLP-1 inhibits food intake and gastric emptying have not been established. If GLP-1 acts as a blood-borne signal from the gut to decrease food intake and gastric emptying, then it would be important to determine whether food intake is reduced by intravenous doses of GLP-1 that reproduce meal-induced increases in plasma GLP-1. Intraperitoneal administration of GLP-1 would not suffice, because GLP-1 could possibly affect gastrointestinal motility or feeding before being absorbed into the circulation, thus precluding the establishment of a clear association between plasma GLP-1 and a feeding or gastric-emptying response. Many of the human studies cited above provided evidence suggesting that postprandial increases in plasma GLP-1 are sufficient to decrease food intake and gastric emptying (54). In rats, intravenous infusion of GLP-1 at 10 pmol·kg\(^{-1}\)·min\(^{-1}\) has been reported to increase plasma GLP-1 to a degree comparable to that produced by food intake (49), and the present study indicates that this dose is sufficient to decrease food intake and gastric emptying in rats. However, a wide variability in basal and stimulated plasma GLP-1 values has been reported in these studies, which likely reflects differences in the antisera used in the radioimmunoassays and methods of...
plasma extraction. Furthermore, most of these studies did not report concentrations of specific molecular forms of GLP-1 in plasma or the ability of specific forms to decrease food intake. GLP-1 is derived from the proglucagon gene (11). On the basis of known posttranslational processes, various isoforms of GLP-1 likely to be produced and secreted include GLP-1(1–36)-amide, GLP-1(1–36)-glycine, GLP-1(1–36)-glycine-arginine-arginine, GLP-1(7–36)-amide (designated GLP-1), and GLP-1(7–36)-glycine. Each of these isoforms except for GLP-1(1–36)-glycine-arginine-arginine has been detected in plasma and in intestinal and pancreatic tissue (35, 53). Two of these isoforms [GLP-1(7–36)-amide, GLP-1(7–36)-glycine] appear to be equipotent in reducing gastric emptying (33) and in stimulating insulin secretion (36). Once in the blood, the ubiquitous enzyme dipeptidyl-peptidase IV then extensively and rapidly inactivates these forms by removing a dipeptide from the N-terminal end (20). Vilsboll and Holst (56) recently stated that “most published (plasma GLP-1) data show results from assays that do not discriminate between the intact and degraded forms and, therefore, do not reflect the concentrations of bioactive hormones.” Most of the assays used in these studies also used antisera specific for the C-terminal end of GLP-1(7–36) amide, which does not recognize the bioactive glycine-extended form of GLP-1. Thus it remains to be determined whether meal-induced changes in plasma levels of specific molecular forms of GLP-1 are sufficient either alone or when combined to inhibit food intake and gastric emptying.

There is substantial evidence to indicate that the anorexic effects of GLP-1 are centrally mediated. If circulating GLP-1

![Fig. 8. Effects of intravenous infusions of GLP-1 on ingestion of 15% aqueous sucrose in 10–15 rats with either gastric cannula closed (real feeding) or open (sham feeding). Food-deprived rats with either an open or closed gastric cannula received a 40-min jugular vein infusion of vehicle (0.15 M NaCl, 0.1% BSA) or GLP-1 at 5 pmol·kg⁻¹·min⁻¹ (A), 17 pmol·kg⁻¹·min⁻¹ (B), or 50 pmol·kg⁻¹·min⁻¹ (C). Ten minutes after infusion onset, rats were provided access to 15% sucrose for 30 min. Values are means ± SE. ‡P < 0.01, †P < 0.001, compared with the vehicle-treated group.](http://ajpregu.physiology.org/)

![Fig. 9. Effects of intravenous infusion of GLP-1 receptor antagonist exendin(9–39) [ex(9–39)] on GLP-1-induced inhibition of gastric emptying of saline in 11 rats (A), and ingestion of 15% aqueous sucrose in 15 rats (B). In gastric-emptying experiments, food-deprived rats received a 20-min intravenous infusion of vehicle (0.15 M NaCl, 0.1% BSA), GLP-1 (17 pmol·kg⁻¹·min⁻¹), or coadministration of GLP-1 (17 pmol·kg⁻¹·min⁻¹) and exendin(9–39) (1,700 pmol·kg⁻¹·min⁻¹). At 10 min after onset of infusion, 5 ml of saline containing phenol red was instilled intragastrically, followed by collection of gastric contents 10 min later. Each value represents the mean ± SE of the volume of saline emptied from the stomach. In feeding experiments, food-deprived rats received a 40-min intravenous infusion of vehicle (0.15 M NaCl, 0.1% BSA), GLP-1 (17 pmol·kg⁻¹·min⁻¹), or coadministration of GLP-1 (17 pmol·kg⁻¹·min⁻¹) and exendin(9–39) (1,700 pmol·kg⁻¹·min⁻¹) beginning 10 min before 30-min access to 15% sucrose. Values are means ± SE. ‡P < 0.001, compared with the vehicle-treated group.](http://ajpregu.physiology.org/)
acts directly within the brain to inhibit the brain feeding system, then GLP-1 receptors should exist in brain sites linked to control of food intake, circulating GLP-1 should have access to these sites, and localized injection of GLP-1 into these sites should be more potent than intravenous administration of GLP-1 in decreasing food intake. In rats, intracerebroventricular, as well as site-specific hypothalamic (paraventricular nucleus, dorsomedial nucleus, lateral hypothalamus, ventromedial hypothalamus) administration of GLP-1, appears to be more potent than systemic administration in reducing feeding (9, 23–25, 44, 51). High-affinity GLP-1 binding sites are located throughout the brain in regions with and without a blood-brain-barrier, including the hypothalamus (arcuate, paraventricular nucleus, supraoptic nucleus, dorsomedial nucleus), central amygdala, hippocampus, nucleus accumbens, NTS, dorsal vagal nucleus, area postrema, and subfornical organ (14, 28, 52). In rats, intracerebroventricular, paraventricular nucleus, and lateral hypothalamus injections of the GLP-1 receptor antagonist exendin(9–39) have also been reported to increase food intake (25, 44, 51). It is not clear whether the source of the endogenous GLP-1 for these receptors is in the brain or periphery (gut and/or pancreas). GLP-1 receptors in the subfornical organ and area postrema, circumventricular organs, which have a limited blood-brain barrier, are reported to be either accessible (34) or responsive (62) to circulating GLP-1. Systemically administered GLP-1 and the GLP-1 receptor agonist exendin-4 have also been reported to readily penetrate the blood-brain barrier (18, 19). On the other hand, GLP-1 is synthesized by discrete populations of neurons in the nucleus of the solitary tract and adjacent medullary regions, which send fibers to numerous brain regions linked to the control of food intake, including the area postrema, hypothalamus (arcuate, paraventricular nucleus, supraoptic nucleus, dorsomedial nucleus), nucleus accumbens, and hippocampus (15, 17, 22, 28). Gastric distension has also been reported to induce c-Fos in medullary neurons containing GLP-1 (58).

There is some evidence to suggest that GLP-1 actions may also be mediated by vagal sensory nerves. If circulating GLP-1 acts at GLP-1 receptors on vagal sensory nerves to inhibit food intake and gastric emptying, then it would be important to determine whether vagal sensory nerves contain GLP-1 receptors, physiological doses of GLP-1 increase afferent vagal activity, and vagal denervation attenuates the effects of physiological doses of GLP-1, as well as GLP-1 receptor blockade, on food intake and gastric emptying. Several studies have shown that in rodents 1) GLP-1 receptor gene expression occurs in vagal nodose ganglion cells, and GLP-1 evokes action potentials and increases cytosolic Ca2+ in these cells (31); 2) intraportal GLP-1 administration increases hepatic vagal afferent and pancreatic vagal efferent activities (30); 3) ganglionic blockade abolishes the stimulatory effect of intraportal GLP-1 on insulin secretion (5); and 4) capsaicin denervation of vagal sensory nerves attenuates GLP-1-induced inhibition of gastric emptying (16) and stimulation of insulin secretion (1). It remains to be determined whether afferent vagal denervation attenuates the effects of physiological doses of GLP-1 or GLP-1 receptor blockade on food intake and gastric emptying.

In summary, the present study demonstrates that intravenous infusions of GLP-1 potently inhibit food intake, gastric emptying, and sham feeding in a dose-dependent manner with a similar potency and efficacy. Thus GLP-1 may inhibit food intake, in part, by reducing gastric emptying, yet can inhibit food intake independently of its action to reduce gastric emptying. These results support the hypothesis that GLP-1 acts as a hormonal signal from the gut to the brain to inhibit gastric emptying, reduce meal size, and decrease meal frequency and/or increase the satiety ratio (postmeal interval per meal size). It remains to be determined whether intravenous doses of GLP-1 that reproduce postprandial increases in plasma GLP-1 are sufficient to inhibit food intake and gastric emptying.

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