Glucagon-like peptide-1 inhibits gastric emptying via vagal afferent-mediated central mechanisms

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İmeryüz, Neşe, Berrak Ç. Yeğen, Ayhan Bozkurt, Tamer Coşkun, Maria L. Villanueva-Peñacarrillo, and Nefise B. Ulusoy. Glucagon-like peptide-1 inhibits gastric emptying via vagal afferent-mediated central mechanisms. *Am. J. Physiol.* 273 (*Gastrointest. Liver Physiol.* 36): G920–G927, 1997.—Exogenous administration of glucagon-like peptide-1-(7—36) amide (GLP-1), an insulinotropic hormone, inhibits gastric emptying and acid secretion in humans. The role of GLP-1 as a regulator of gastric function is elusive. In gastric fistula rats, vagal afferent denervation and peripheral administration of the GLP-1 receptor antagonist exendin-(9—39) amide enhanced emptying of a glucose meal, whereas intracerebroventricular exendin was ineffective. The rate of saline emptying was attenuated by peripheral as well as by central administration of GLP-1, and pretreatment with exendin by the respective routes reversed the inhibition by GLP-1. Vagal afferent denervation abolished the central and peripheral action of GLP-1 on gastric emptying. Neither peripheral cholinergic nor adrenergic blockade altered the delay of methyl cellulose meal emptying by intracisternal GLP-1 injection. Acid secretion in conscious pylorus-ligated rats was inhibited by intracisternal GLP-1 administration, whereas systemic GLP-1 was ineffective. These results support the notion that GLP-1 receptors participate in the central and peripheral regulation of gastric function. Furthermore, vagal afferent nerves mediate the inhibitory action of GLP-1 on gastric motor function. GLP-1 may be a candidate brain-gut peptide that acts as a physiological modulator of gastric function.

acid secretion; glucose; feeding behavior; exendin

GLUCAGON-LIKE peptide-1-(7—36) amide (GLP-1) is a member of an extended family of bioactive peptides, including glucagon, glucose-dependent insulinotropic peptide (GIP), secretin, and vasoactive intestinal polypeptide, all of which have closely related amino acid sequences and can stimulate insulin secretion in addition to a variety of other actions (4). In response to nutrient ingestion, GLP-1 and GIP are released into circulation from the intestinal mucosal endocrine cells, and both peptides are considered to play a role in the enteroinsular axis because of their ability to enhance insulin secretion (13, 30). GLP-1 aroused recent interest for being a potential therapeutic agent in noninsulin-dependent diabetic subjects due to its "blood glucose normalizing effect" (8).

In addition to its insulinotropic action, exogenously administered GLP-1 inhibits gastric emptying of nonnutrient (1) and nutrient liquid meals (14, 34). Furthermore, GLP-1 attenuates meal-induced antral propagated contractions and enhances pyloric tone (1, 22). Together with peptide YY, GLP-1 is proposed to participate in the "ileal brake" mechanism (32), a term originally coined to refer to inhibition of intestinal motor activity in response to nutrients in the distal gut. It is unknown whether endogenously released GLP-1 is a physiological modulator of gastric motor activity.

Another effect of exogenously administered GLP-1 on gastric function is the inhibition of pentagastrin (15, 23, 34)- and meal-stimulated (34) gastric acid secretion in humans. Although speculative, the inhibitory action of GLP-1 on acid secretion may be related to its ability to stimulate somatostatin release (11).

The mammalian brain stem (2), particularly the nucleus of the solitary tract (9) and the hypothalamus (2), expresses the GLP-1 gene and possesses GLP-1 binding sites (5, 28). Furthermore, the cloned brain GLP-1 receptor is structurally identical to the peripheral GLP-1 receptors (31). Recently, it was reported that centrally administered GLP-1 inhibits feeding (26, 27, 29) and drinking behavior and results in stimulation of diuresis and natriuresis in the rat (26). The distribution of GLP-1 and its receptors in the central nervous system together with recent functional evidence (26, 27, 29) suggest that this peptide may be a central neurotransmitter that modulates visceral functions. Yet the physiological significance of central GLP-1 is elusive, and it is unknown whether the inhibitory actions of GLP-1 on gastric function are mediated centrally.

Exendin-(9—39) is a COOH-terminal fragment of exendin-4, a bioactive peptide isolated from *Heloderma suspectum* venom that shares 50% structural homology with GLP-1 (3). The in vitro (6) and in vivo (13, 26, 30) actions of GLP-1 are antagonized by exendin-(9—39), making exendin a valuable tool for studying the biological actions of GLP-1.

The present study was undertaken to investigate the role of GLP-1 as a potential peripheral and central regulator of gastric emptying. It was also our aim to study the effect of GLP-1 on regulating gastric acid secretion.

MATERIALS AND METHODS

Animals

Adult female Sprague-Dawley rats weighing 170–250 g were housed individually in a light- and temperature-controlled room on a 12:12-h light-dark cycle, where the temperature (22 ± 2°C) and relative humidity (65–70%) were kept constant. The animals were fed a standard pellet lab chow, and food was withdrawn overnight before preparative

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surgery and emptying experiments, but access to water was allowed ad libitum. Experiments were designed considering accepted ethical standards for animal research.

Surgery

Under ether anesthesia, fasted rats were fitted with stainless steel Gregory cannulas in the body of the stomach, using aseptic procedures, as previously described (7). Animals were allowed at least 3 wk to recover from the operation and were housed individually.

Three weeks after implantation of the gastric cannula, a group of rats was anesthetized (100 mg/kg ketamine and 0.75 mg/kg chlorpromazine ip), and each rat was placed on a stereotaxic instrument (Stoelting Lab standard stereotaxic instrument). The rats were fitted with stainless steel cerebroventricular guide cannulas (22-gauge; Plastic Products, Roanoke, VA) inserted into the right lateral cerebral ventricle (1.1 mm caudal and 1.5 mm lateral to the bregma, 3.2 mm ventral to the surface of the skull) according to the atlas of Paxinos and Watson (17a). The cannula was held in place by dental acrylic cement anchored around three stainless steel screws. Three days were allowed before starting the emptying experiments. After each experiment, correct placement of the cannula was verified by injection of methylene blue and brain section.

A group of rats without gastric cannulas received intracisternal injection under light ether anesthesia. The head was fixed in a stereotaxic device, and the neck was flexed to expose the occipital region. The needle of a Hamilton syringe was inserted to puncture the occipital membrane. The withdrawal of cerebrospinal fluid into the syringe indicated the accuracy of the injection site.

Measurement of Gastric Emptying

Gastric fistula rats. The rate of gastric emptying was examined using methods described previously (7). Trained rats were fasted overnight and lightly restrained in Bollman-type cages. The stomach was flushed with warm saline until clean. Test meals of 3 ml containing phenol red (PR; 60 mg/l) as a nonabsorbable dilution marker were instilled into the gastric fistula. The osmolality of solutions was adjusted to 300 mosmol/kgH_2O. Routinely, a period of at least 30 min was allowed between emptying tests. The emptying of physiological saline and glucose (5.41% wt/vol) was studied in a random order. Glucose emptying was further studied after preloading with 3 ml of glucose for 10 min. Gastric emptying was determined from the volume and PR concentrations recovered, as previously described (7).

Methyl cellulose. The method first described by Scarpignato et al. (21) to examine the emptying of methyl cellulose was used. Methyl cellulose was dispersed in water with continuous stirring, and PR (50 mg/100 ml) was added. A volume of 1.5 ml of methyl cellulose was given by gavage through a polyethylene tube. Gastric emptying was determined 30 min after administration of the meal. Gastric emptying was calculated according to the following formula: % gastric emptying = (1 − amount of PR recovered from the test stomach / average amount of PR recovered from standard stomachs) × 100.

Measurement of Gastric Acid Output and Gastric Secretory Volume

Gastric secretory volume and acid output measurements were performed on rats without cannulas by the pyloric ligation method under ether anesthesia (35). After the rats recovered from anesthesia and were conscious, we allowed 2 h for acid to collect. After the rats were decapitated, we opened the cardia and obtained the gastric contents by opening the greater curvature of the stomach. After volume measurement, the collected specimen was titrated with 0.01 N NaOH.

Administration of Drugs

The solutions of GLP-1 and exendin-(9—39) amide (Peninsula Laboratories Europe, Merseyside, UK) were injected either subcutaneously or intracerebroventricularly and intracisternally in 5-μl volumes using a Hamilton syringe, or both peptides were dissolved in bovine serum albumin (BSA; GLP-1 in 1% and exendin in 0.1%) at pH 7.0 warmed to 37°C. Either vehicle (BSA) or GLP-1 was administered subcutaneously (1.5–500 pmol/kg), intracerebroventricularly (75–150 fmol/rat), or intracisternally (3–30 pmol/rat) before the emptying experiment commenced (30, 10, or 0 min, respectively). When exendin preceded GLP-1, a 10-min (for intracerebroventricular injections) or a 15-min (for subcutaneous injections) interval was given between the peptides.

In one group of rats, atropine methyl nitrate (1 mg/kg ip; Sigma) or bretylium tosylate (15 mg/kg ip; American Reagent Laboratories) was administered 5 min before intracisternal GLP-1 and methyl cellulose emptying were measured.

Perineural Application of Capsaicin to the Vagus Nerve

On the day of gastric cannula placement, one group of rats was anesthetized (100 mg/kg ketamine and 0.75 mg/kg chlorpromazine ip) and pretreated with atropine sulfate (2 mg/kg ip) to decrease the acute effects of capsaicin on the respiratory and cardiovascular systems. A 1% solution of capsaicin (Sigma) or vehicle (10% Tween 80 in oil; sham denervation) was applied to each vagus nerve in turn for 30 min. The total dose of capsaicin applied in each rat did not exceed 1 mg. After application, the area was rinsed with sterile saline. Animals were used in the emptying experiments 3 wk after the treatment. Before the experiment, capsaicin- and vehicle-treated rats were tested for impaired chemosensitivity by an eye-wiping test. In capsaicin-treated animals, the corneal afferents were no longer sensitive to a solution of 1% NH_4OH (18).

Food Intake

Animals were deprived of food for 24 h, and 70 g of preweighed rat chow were delivered immediately after subcutaneous (6 and 120 pmol/kg) or intracerebroventricular (75 fmol/rat) injections of GLP-1 or the vehicle at 1000, and the intake in the following 1 or 24 h was determined. Pellets and spillage were collected and weighed at the end of feeding periods.

Measurement of Blood Glucose

Capillary blood samples were obtained from the tails of rats treated with either vehicle or GLP-1 (6–500 pmol/kg sc or 75 fmol/rat icv; 30 or 10 min, respectively) or after (5, 30, and 60 min) intragastric glucose instillation. Blood glucose levels were measured by glucofilms (Bayer Diagnostics) using a glucometer (Ames 5499, Bayer Diagnostics).

GLP-1 Assay

The animals were treated with either vehicle or GLP-1 (6 and 10 pmol/kg sc or 75 fmol/rat icv; 30 or 10 min, respectively) before they were decapitated for trunk blood. Some rats were decapitated 5 min after intragastric glucose instillation, preceded by either the vehicle or exendin (6 pmol/kg sc) treatment. Blood samples were collected in Eppendorf tubes

containing 100 μl of 1 mmol/ml EDTA (Sigma) and 100 μl of 5,000 U/ml aprotonin (Trasylol, Bayer), and the plasma samples were stored at −70°C until they were assayed for GLP-1. Plasma GLP-1 was assayed as described previously (16). Briefly, plasma samples were extracted in 70% ethanol (vol/vol, final concentration). GLP-1 content in the extracts was assayed in duplicate by radioimmunoassay, using synthetic GLP-1-(7—36) amide (Peninsula Laboratories, Belmont, CA) and the anti-GLP-1 2135 (gift from Dr. Holst; final dilution, 1:50,000), which equally react with either GLP-1-(1—36) amide or GLP-1-(7—36) amide. The detection limit of the assay and recovery factor were 0.075 pmol/ml and 60%, respectively.

Statistics

The results are expressed as means ± SE. Analysis of variance was used for multiple comparisons followed by Dunnet's test. Student's *t*-test and Mann-Whitney *U*-tests were used for comparison of paired results. Differences were considered statistically significant if $P < 0.05$.

RESULTS

Effect of Central and Peripheral Administration of GLP-1 on Gastric Emptying Rate

Administration of GLP-1 at doses of 6 and 120 pmol/kg significantly delayed gastric emptying of saline (2.35 ± 0.06 and 2.43 ± 0.12 ml/5 min) compared with the vehicle-pretreated group (2.75 ± 0.04 ml/5 min). However, the response to doses of 3 and 500 pmol/kg (2.57 ± 0.05 and 2.90 ± 0.11 ml/5 min, respectively) were not different from the response to vehicle (Fig. 1*A*). The GLP-1 receptor antagonist exendin per se did not have any effect on the gastric emptying rate of saline at the subcutaneous doses of 3 and 6 pmol/kg, but the same doses abolished (2.63 ± 0.1 and 2.98 ± 0.07 ml/5 min, respectively) the inhibitory effect of GLP-1 (6 pmol/kg sc; 2.35 ± 0.06 ml/5 min) (Fig. 1*B*).

Gastric emptying of saline was also delayed by central GLP-1 administration (Fig. 2). When given intracerebroventricularly (75 and 150 fmol/rat), GLP-1 inhibited the emptying of saline (2.30 ± 0.09 and 2.32 ± 0.09 ml/5 min) ($P < 0.01$). When tested with the methyl cellulose emptying method, a significant delay in the gastric emptying was obtained at doses of 3, 10, and 30 pmol/rat GLP-1 given intracisternally (56.47 ± 5.47%, 22.92 ± 5.08%, and 7.85 ± 1.41%; $P < 0.001$) compared with the vehicle group (Table 1).

The gastric inhibitory effect of intracerebroventricular GLP-1 was reversed by the central and peripheral administration of exendin (Fig. 2), whereas neither the central (1 pmol/rat icv; ~5 pmol/kg) nor the subcutaneous (3 and 6 pmol/kg) injection of exendin alone had any effect.

Glucose (2.34 ± 0.06 ml/5 min; $n = 17$) and glucose after a preload (1.98 ± 0.06 ml/5 min; $n = 17$) significantly delayed the gastric emptying rate with respect to saline emptying (2.85 ± 0.02; $n = 13$; $P < 0.001$). Exendin, at the same dose (6 pmol/kg sc) that reversed the delaying effect of GLP-1, completely reversed the inhibitory effect of glucose on gastric emptying (2.64 ± 0.12 ml/5 min) and partially reversed that of glucose after preload (2.26 ± 0.12 ml/5 min; Fig. 3*B*). Central

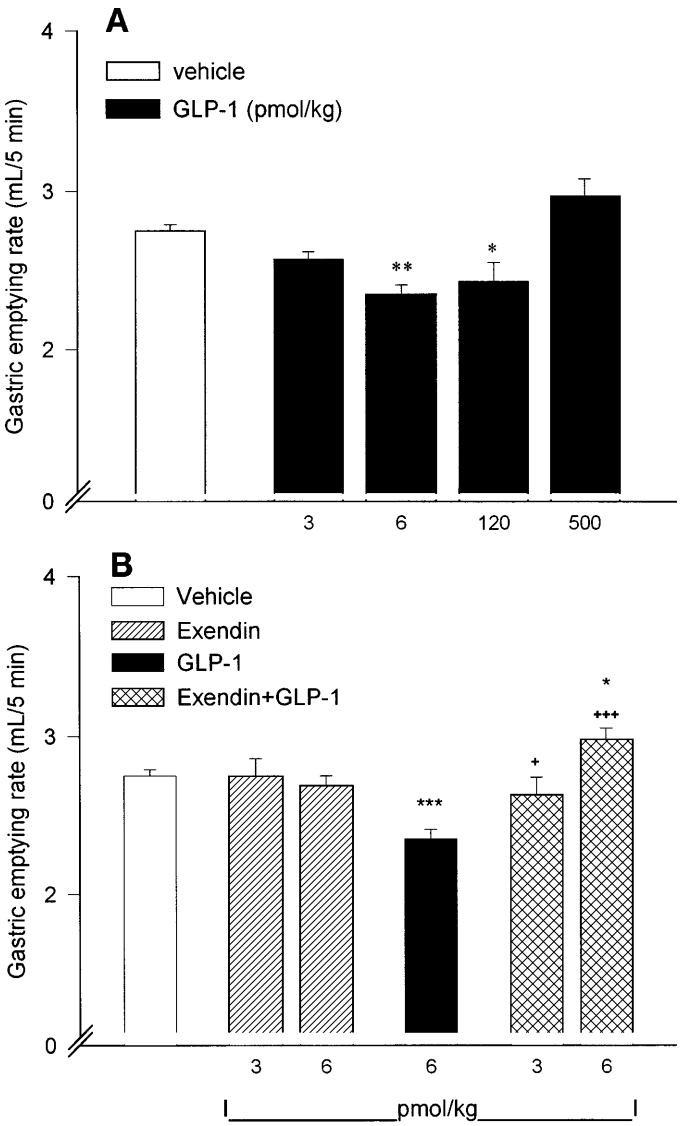

Fig. 1. Inhibition of gastric emptying rate of saline after systemic administration of glucagon-like peptide-1-(7—36) amide (GLP-1) (3–500 pmol/kg sc; $n = 7$–9) (*A*) and reversal of GLP-1-induced inhibition by systemic administration of exendin (3 and 6 pmol/kg sc; $n = 5$) (*B*). Exendin administration alone ($n = 5$–8) or vehicle ($n = 14$) had no significant effect on gastric emptying rate. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared with vehicle treatment; $^{+}P < 0.05$, $^{+++}P < 0.001$, compared with 6 pmol/kg GLP-1 treatment alone.

administration of the GLP-1 antagonist had no significant effect on glucose-induced inhibition of gastric emptying at 75 fmol/rat (glucose, 2.40 ± 0.09 ml/5 min; glucose preload, 1.61 ± 0.06 ml/5 min) and 1,000 fmol/rat (glucose 2.41 ± 0.06 ml/5 min; glucose preload, 1.80 ± 0.04 ml/5 min) (Fig. 3*B*).

Effect of Perineural Capsaicin on Delayed Gastric Emptying Rate Induced by GLP-1 or Glucose

The inhibitory effect of GLP-1 (6 pmol/kg sc and 75 fmol/rat icv) on the gastric emptying rate of saline was not observed in capsaicin-treated rats ($P < 0.05$–0.001; Fig. 4). Likewise, in perineurally capsaicin-treated rats, glucose emptied at a rate (2.86 ± 0.07 ml/5 min) similar to that of saline (Fig. 5). However, the slow

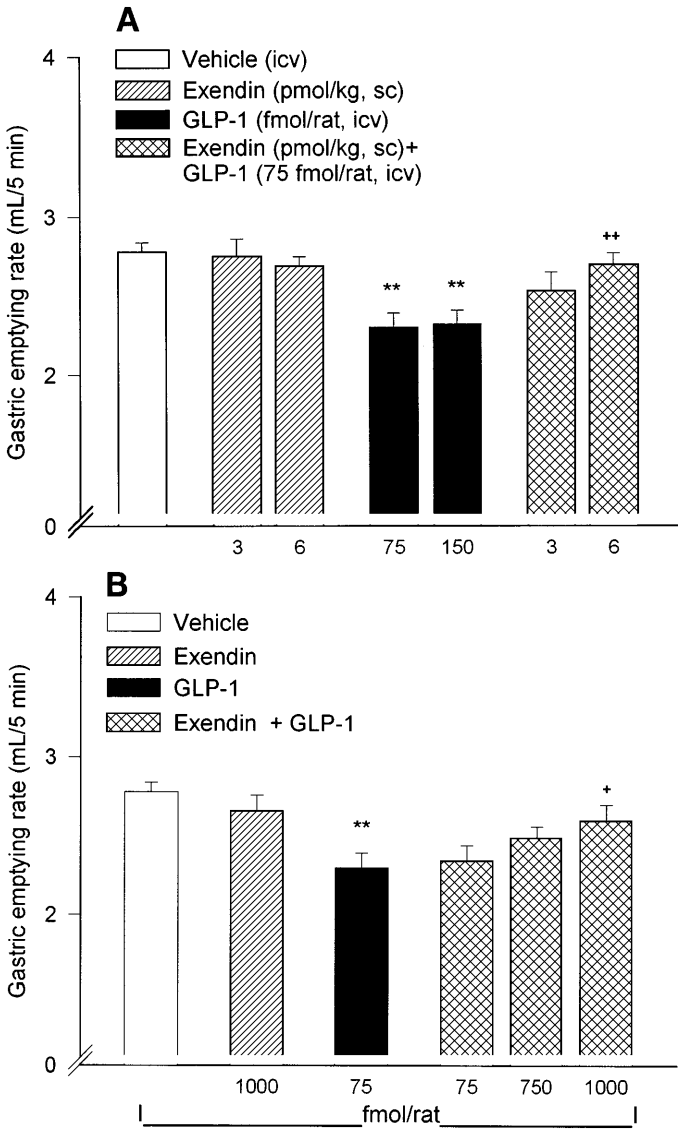

Fig. 2. Inhibition of gastric emptying rate of saline after intracerebroventricular administration of GLP-1 (75 and 150 fmol/rat; $n = 7–12$) and reversal of GLP-1-induced inhibition by systemic administration of exendin with 6 pmol/kg ($n = 9$) but not with 3 pmol/kg ($n = 10$) (*A*), or by intracerebroventricular administration of exendin with 1,000 fmol/rat ($n = 11$) but not with 750 fmol/rat ($n = 7$) and 75 fmol/rat ($n = 6$) (*B*). $^{**}P < 0.01$, compared with vehicle treatment; $^{++}P < 0.01$, compared with 75 fmol/rat GLP-1 treatment alone.

emptying rate induced by glucose after a preload was not affected by capsaicin.

Involvement of Muscarinic and Adrenergic Receptors in GLP-1-Induced Inhibition of Gastric Emptying

Neither atropine methyl nitrate nor bretylium tosylate had any significant effects on GLP-1-induced inhibition of gastric emptying (Table 1).

Effect of Central and Peripheral Administration of GLP-1 on Gastric Secretory Function

Central administration of GLP-1 (10 pmol/rat ic) decreased gastric secretory volume (1.24 ± 0.35 ml/2 h) and acid output (167.82 ± 62.99 μmol/2 h) measured by the pyloric ligation method compared with the vehicle-treated group (4.67 ± 0.19 ml/2 h and 800.69 ± 65.6 μmol/2 h; $P < 0.001$), whereas the same dose given subcutaneously had no significant effect (Fig. 6).

Effect of GLP-1 on Food Intake

GLP-1 had no significant effect either on 1-h or 24-h food intake in 24-h fasted rats injected subcutaneously at 6 and 120 pmol/kg and intracerebroventricularly at 75 fmol/rat (Table 2).

Effect of GLP-1 on Blood Glucose Level

Blood glucose levels were monitored in GLP-1- or vehicle-injected or sham- or vagal-denervated rats. GLP-1 did not alter the blood glucose level significantly when given either systemically (6 and 500 pmol/kg sc) or centrally (75 fmol/rat icv) (data not shown). Vagal afferent-denervated rats had an early hyperglycemia ($P < 0.001$) after the administration of a glucose test meal (134.3 ± 16.0 mg/100 ml at 30 min, 76.33 ± 7.45 mg/100 ml at 60 min) with respect to sham-denervated rats (83.8 ± 6.4 mg/100 ml at 30 min; 145.8 ± 44.7 mg/100 ml at 60 min).

Effect of Exogenous GLP-1 Administration and Intragastric Glucose on Plasma GLP Levels

Exogenous GLP-1 given subcutaneously (6 and 10 pmol/kg; 97.94 ± 14.25 and 94.52 ± 14.25 pmol/l) or intracerebroventricularly (75 fmol/rat; 91.52 ± 16.55 pmol/l) did not elevate plasma GLP-1 concentration significantly compared with vehicle-treated animals (71.53 ± 32.45 pmol/l). GLP-1 levels were not altered by intragastric glucose instillation when preceded by either the vehicle (86.57 ± 31.08 pmol/l) or exendin (6 pmol/kg sc; 97.85 ± 25.82 pmol/l).

DISCUSSION

The results of the present study suggest that GLP-1 plays a role in the regulation of gastric emptying. Our findings provide evidence for the involvement of central

Table 1. *Effect of GLP-1 on gastric emptying rate assessed by methyl cellulose method*

Study Groups	Gastric Emptying, %
Control (vehicle sc)	80.61 ± 5.12
GLP-1 (3 pmol/kg sc)	86.04 ± 3.30
GLP-1 (10 pmol/rat sc)	81.54 ± 5.12
Control (vehicle ic)	82.92 ± 4.76
GLP-1 (3 pmol/rat ic)	56.47 ± 5.47†
GLP-1 (10 pmol/rat ic)	22.92 ± 5.08‡
GLP-1 (30 pmol/rat ic)	7.85 ± 1.41*
Atropine (1 mg/kg ip)	41.91 ± 10.08†
Atropine + GLP-1 (10 pmol/rat ic)	18.47 ± 11.51†
Bretylium tosylate (15 mg/kg ip)	65.75 ± 6.81
Bretylium tosylate + GLP-1 (10 pmol/rat ic)	26.65 ± 8.93†§

Values are means ± SE; each group consists of 4–6 rats. Either vehicle (bovine serum albumin) or glucagon-like peptide-1-(7–36) amide (GLP-1) was administered subcutaneously or intracisternally immediately before emptying experiment commenced. $^{*}P < 0.05$, $^{\dagger}P < 0.01$, $^{\ddagger}P < 0.0001$ compared with vehicle; $^{\S}P < 0.001$ compared with bretylium alone.

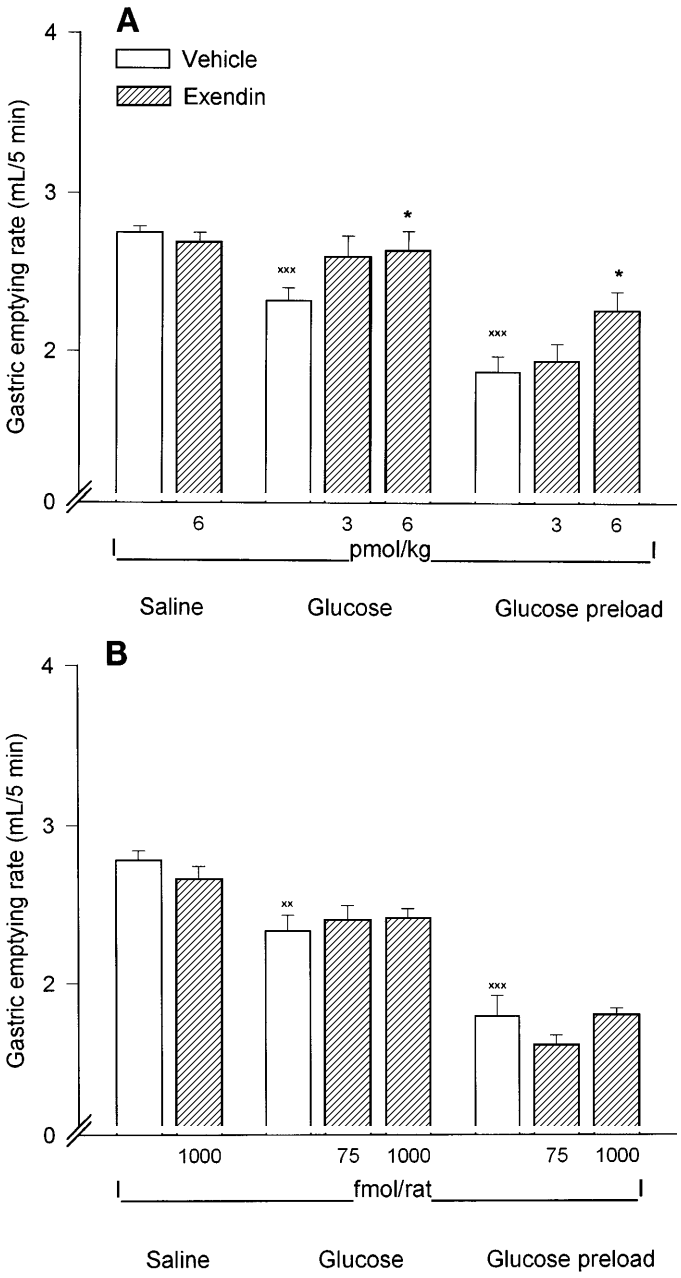

Fig. 3. Reversal of glucose-induced inhibition of gastric emptying rate by systemic exendin administration (6 pmol/kg sc; $n = 9$) (*A*) but not with central exendin pretreatment (75 or 1,000 pmol/rat icv; $n = 6–9$) (*B*). Lower dose of exendin (3 pmol/kg sc; $n = 11$) had no significant effect on gastric emptying rate of any glucose solution. $^{*}P < 0.05$, compared with vehicle treatment; $^{xx}P < 0.01$, $^{xxx}P < 0.0001$, compared with saline.

nervous system and capsaicin-sensitive vagal afferent nerves in the inhibitory action of GLP-1 on gastric function.

Inhibition of gastric motor activity by glucose meals was previously demonstrated to be partially mediated by a vagal afferent pathway (19). However, the role of endogenously released GLP-1 as an inhibitory regulator of gastric emptying has not been previously investigated. In the present study, the gastric inhibitory effect of a glucose meal with and without glucose preload was attenuated and abolished, respectively, by administration of the GLP-1 antagonist exendin-(9—39). Vagal afferent denervation also abolished the glucose-induced inhibition but did not alter glucose preload emptying. These findings demonstrate that endogenous GLP-1 and its receptors play a role in the glucose-induced inhibition of gastric emptying, and the action of GLP-1 is probably mediated by vagal afferents. Glucose preload is likely to activate inhibitory mechanisms other than vagal afferents.

Intracerebroventricular exendin-(9—39) administration, at the threshold dose that reversed the central action of GLP-1 on gastric emptying of a nonnutrient meal, did not alter the inhibition induced by a glucose meal. This suggests that under our experimental conditions central GLP-1 receptors do not appear to play a

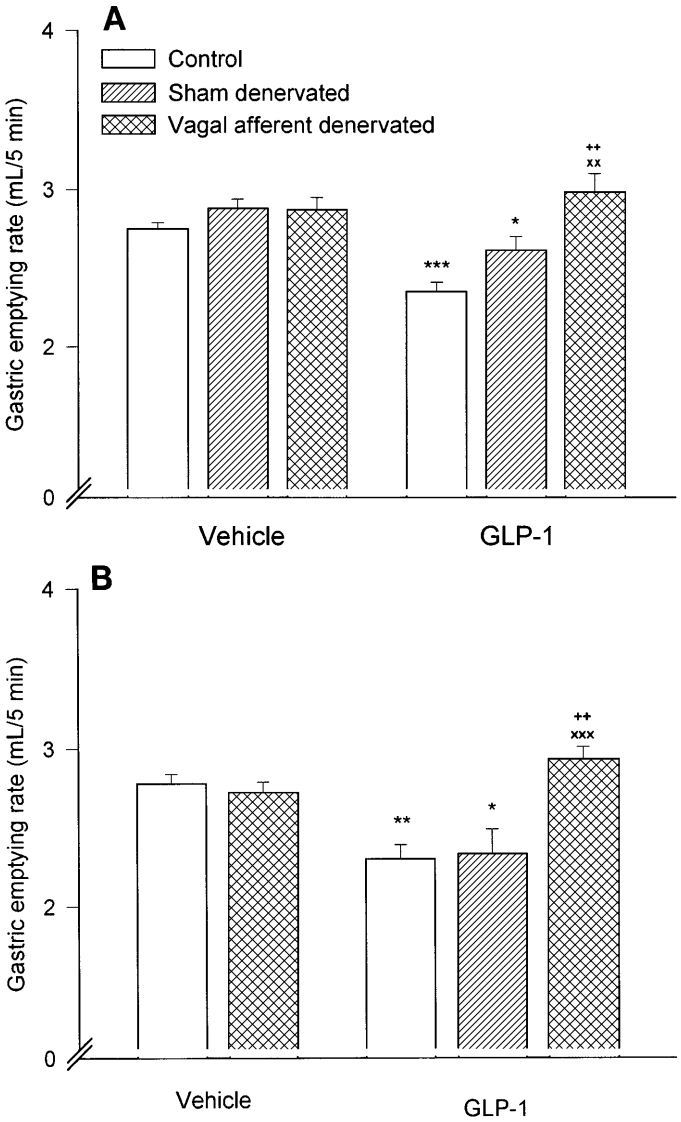

Fig. 4. Effect of perivagal capsaicin application on delayed gastric emptying rate of saline induced by systemic GLP-1 administration (6 pmol/kg sc; $n = 5–9$) (*A*) and central GLP-1 administration (75 fmol/rat icv; $n = 5–12$) (*B*). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.0001$, compared with vehicle; $^{++}P < 0.01$, compared with sham-denervated rats; $^{xxx}P < 0.001$, $^{xx}P < 0.01$, compared with normal controls.

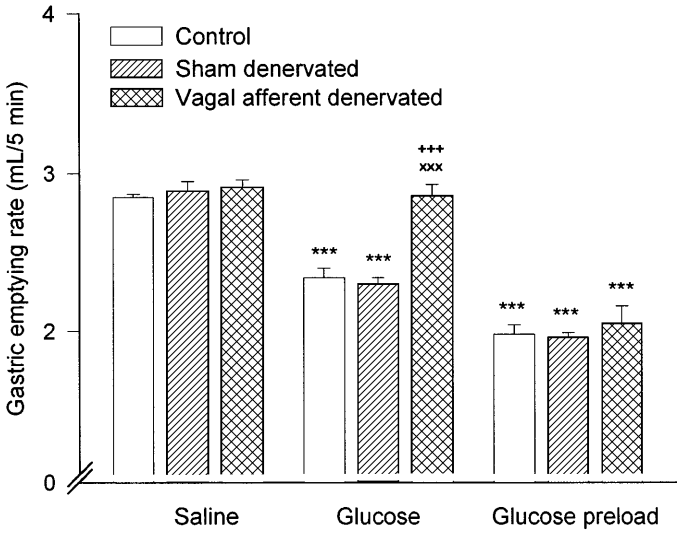

Fig. 5. Effect of perivagal capsaicin application on delayed gastric emptying rate induced by intragastric glucose administration (n = 6–14). $^{***}P < 0.0001$, compared with saline; $^{+++}P < 0.0001$, compared with sham-denervated rats; $^{xxx}P < 0.0001$, compared with normal controls.

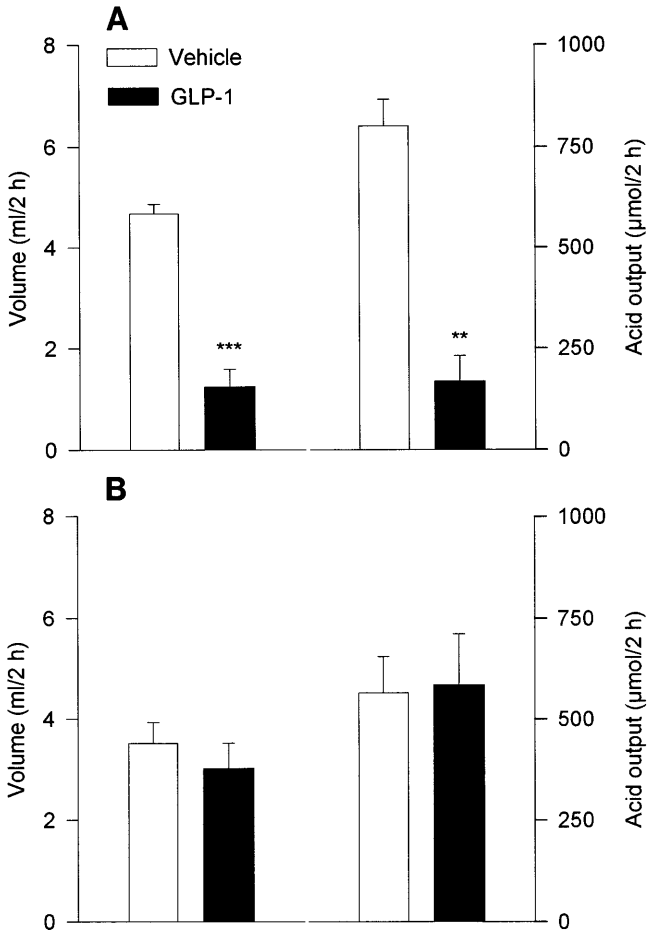

Fig. 6. Inhibition of gastric acid secretion with central GLP-1 administration (10 pmol/rat ic; n = 4–6) (A) but not with systemic GLP-1 administration (10 pmol/rat sc; n = 5–6) (B). $^{**}P < 0.001$, $^{***}P < 0.0001$, compared with vehicle.

Table 2. *Mean consumption of rat chow in 1-h and 24-h periods in rats treated with either vehicle or GLP-1*

Food Intake	g/1 h	g/24 h
Vehicle (sc)	2.44 ± 0.46	24.64 ± 1.08
GLP-1 (6 pmol/kg sc)	NT	22.75 ± 1.31
GLP-1 (120 pmol/kg sc)	2.06 ± 0.32	26.22 ± 1.53
Vehicle (icv)	NT	34.95 ± 1.38
GLP-1 (75 fmol/rat icv)	NT	34.94 ± 0.55

Values are means ± SE; each group consists of 6–8 rats. NT, not tested.

role in the gastric emptying of glucose. However, we have not tested higher exendin-(9—39) doses, which might be effective in reversing the inhibition by a glucose meal. Besides, the glucose meal may be activating various central or peripheral neuroendocrine responses involving other peptides along with GLP-1.

The plasma level of GLP-1 after the instillation of a glucose meal was not significantly higher compared with the level in the fasting state. Due to our experimental design, plasma might have been sampled at an early time in the course of plasma GLP-1 rise or the released GLP-1 might have undergone rapid metabolization (10). In addition, there is the possibility of local release of GLP-1 in the upper gut in response to glucose acting via a paracrine pathway to inhibit gastric emptying.

The regulation of gastric emptying of nonnutrient meals is mainly mediated by gastroduodenal mechanoreceptors and sensory afferent pathways (19). Subcutaneous exendin administration in intact rats and GLP-1 administration in rats with vagal afferent denervation did not alter the rate of gastric emptying of saline. However, GLP-1 in doses ranging from 6 to 300 pmol/kg significantly delayed gastric emptying, and exendin-(9—39) reversed the inhibitory effect of GLP-1. These results suggest that GLP-1 inhibits gastric emptying via vagal afferents and by interacting with its specific receptors. The finding of unchanged plasma GLP-1 levels after subcutaneous administration might have resulted from rapid degradation of the peptide (10). The GLP-1 doses higher than 500 pmol/kg were ineffective in inhibiting saline emptying. Supraphysiological doses of GLP-1 may lower blood glucose concentration (8), a factor that may enhance gastric emptying (25). However, blood glucose concentrations were not appreciably altered by administration of GLP-1 at the given doses. Thus it may be postulated that higher doses of GLP-1 may activate other mechanisms that override the delaying effect.

In the present study, intracerebroventricular GLP-1 doses that were ~13- to 20-fold lower than the peripheral inhibitory doses delayed gastric emptying of saline in gastric fistula rats. The magnitude of inhibition was comparable whether GLP-1 was administered systemically or centrally. The action of GLP-1 was reversed by central administration of exendin-(9—39), demonstrating a role for central GLP-1 in modulating gastric emptying. Sensory vagal denervation abolished this central inhibitory effect of GLP-1. This finding supports

the notion that the brain sites that mediate the inhibitory action of GLP-1 depend on intact vagal afferent input. The threshold dose of GLP-1 that inhibited gastric emptying was ~3,000- to 10,000-fold lower than the reported threshold doses that suppress feeding behavior (26, 27, 29). Additionally, we have not observed suppression of feeding behavior in rats centrally treated with GLP-1 at doses that inhibit gastric emptying. These results suggest that GLP-1-mediated central mechanisms that regulate gastric emptying and food intake are not alike.

The central inhibitory action of GLP-1 on gastric emptying is further supported by the dose-dependent delay of the methyl cellulose emptying with intracisternal GLP-1 administration; however, the inhibitory threshold dose with this route was higher than the one with intracerebroventricular administration. The reason for this finding may be due to different experimental designs and/or different brain sites affected by GLP-1.

Interestingly, subcutaneous administration of exendin-(9—39) abolished the inhibitory effect of GLP-1 administered intracerebroventricularly. It is possible that systemic exendin reverses the central inhibitory action of GLP-1 by acting peripherally. Recently, it was demonstrated in rats that peripherally administered GLP-1 gains access to the area postrema and subfornical organ, which possess GLP-1 binding sites (5, 17). These brain sites are among the circumventricular organs that lack a blood-brain barrier (12). Therefore, another explanation for the antagonism by exendin is the possibility of the peptide antagonist gaining access to the blood-brain barrier-free sites to inhibit the central action of GLP-1. Acute peripheral cholinergic blockade with atropine methyl nitrate and adrenergic blockade with bretylium tosylate did not alter the inhibitory action of GLP-1 administered intracisternally, demonstrating that peripheral cholinergic and adrenergic pathways are not considerably involved in the central inhibitory action of GLP-1 on nonnutrient meal emptying.

The inhibitory action of GLP-1 on gastric emptying might have been secondary to stimulation of acid secretion. In rat parietal cells, GLP-1 stimulates adenosine 3′,5′-cyclic monophosphate production and H^+ secretion (24). To determine whether central GLP-1 plays a role in the regulation of gastric acid secretion, we measured acid secretion in pylorus-ligated conscious rats, using an inhibitory dose of GLP-1 on gastric emptying. Intracisternally administered GLP-1 profoundly inhibited acid secretion in the conscious rat. The specificity of central inhibitory action of GLP-1 was supported by our finding of unaltered acid secretion by systemic administration of GLP-1 at a dose that is effective centrally. In accordance with our results, parenteral administration of GLP-1 was found to be ineffective in altering gastric acid secretion in the anesthetized rat (24). The role of neural pathways in the GLP-1-induced inhibition of gastric acid secretion was also emphasized by a previous study in which sham feeding-induced acid secretion was partially inhibited by exogenous GLP-1 administration (33). Taken together, these findings imply that central mechanisms have a predominant role in the GLP-1-induced inhibition of gastric acid secretion.

Our results indicate that GLP-1 may be a physiological central and peripheral modulator of gastric function. The dorsal vagal complex is the primary brain stem site that integrates gastrointestinal visceral information from vagal and spinal afferents as well as from the descending projections of higher centers in the process of programming gastric function (20). Our results related to the central inhibitory action of GLP-1 on gastric function, together with the previous demonstration of GLP-1, its gene expression (2, 9), and receptors (5, 28) in the dorsal vagal complex and the other brain sites that interact with this center, strongly suggest that endogenous central GLP-1 modulates gastric function. In support of this assumption is the previous finding of increased neural activity induced by central administration of GLP-1 in the aforementioned brain regions (27, 29). On the other hand, the peripheral action of GLP-1 on gastric emptying seems to be mediated by the vagal afferents and possibly by the blood-brain barrier-free brain site, the area postrema, which receives direct information from the gastrointestinal tract via vagal afferents and which has projections to and from the nucleus of the solitary tract and hypothalamic paraventricular nucleus (12, 20).

The effects of GLP-1 described in the present study are compatible with the notion that this peptide acts within the brain and also peripherally to initiate and coordinate gastric secretory and motor responses. Thus GLP-1 may be a candidate brain-gut peptide that acts as a physiological modulator of gastric function.

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