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Role of the Vagus Nerve in Mediating Proximal Nutrient-Induced Glucagon-Like Peptide-1 Secretion*

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ABSTRACT

Plasma levels of glucagon-like peptide-1 (GLP-1) rise rapidly after nutrient ingestion, suggesting the existence of a proximal gut signal regulating GLP-1 release from the L cells of the distal small intestine. Glucose-dependent insulinotropic peptide (GIP) has been shown to be one such proximal signal; however, the dependence of GIP on gastrin-releasing peptide, a neuromodulator, suggested a role for the nervous system in this proximal-distal loop. Investigations into the nature of this proximal signal were therefore conducted in an *in situ* model of the rat gastrointestinal system. Infusions of corn oil into a 10-cm segment of duodenum that was isolated by loose ligation (to ensure that the luminal contents did not progress to the ileal L cell) increased the secretion of GLP-1 in parallel with that of gut glucagon-like immunoreactivity (gGLI; $r = 0.85$; $P < 0.05$). Infusion of fat into a transected segment of duodenum also significantly raised gGLI secretion compared with saline infusion, reaching a peak value of 132 ± 37 pg/ml above basal ($P < 0.05$). However, peak secretion was significantly delayed when the gut was transected compared with that after ligation alone (19 ± 4 *vs.* 6 ± 1 min, respectively; $P < 0.05$). Furthermore, bilateral subdiaphragmatic vagotomy in conjunction with gut transection completely abolished the fat-induced rise in gGLI secretion ($P < 0.001$). Consistent with a role for the vagus in the regulation of the L cell, stimulation of the distal end of the celiac branch of the subdiaphragmatic vagus nerve significantly stimulated the secretion of gGLI to a level of 71 ± 14 pg/ml above basal ($P < 0.05$). As found previously, supraphysiological infusion of GIP significantly increased gGLI secretion in control animals by 123 ± 32 pg/ml ($P < 0.05$); this was not prevented by hepatic branch vagotomy (96 ± 25 pg/ml; $P < 0.05$). In contrast, although infusion of GIP at physiological levels into sham-vagotomized animals also increased gGLI secretion, by 40 ± 6 pg/ml ($P < 0.05$), selective hepatic branch vagotomy abolished GIP-induced gGLI secretion ($P < 0.05$). The results of these experiments therefore demonstrate that the secretion of GLP-1 and gGLI from the ileal L cell in response to fat is regulated by a complex neuroendocrine loop, involving the enteric nervous system, the afferent and efferent vagus nerves, as well as the duodenal hormone GIP. (*Endocrinology* **140:** 1687–1694, 1999)

PROGLUCAGON is cleaved by tissue-specific prohormone convertases to the proglucagon-derived peptides (PGDPs) (1). The major pancreatic product of proglucagon processing is glucagon, whereas the intestinal products include glicentin, oxyntomodulin, glucagon-like peptide-1 (GLP-1), and GLP-2. Although GLP-1 possesses inhibitory action in terms of gastric emptying (2), its main effect is its role as an incretin, stimulating the secretion of insulin in a glucose-dependent manner (2–4). Other metabolic effects of GLP-1 include suppression of glucagon secretion (2), enhancement of glucose disposal (5), and inhibition of central feeding behavior (6). These pleiotropic actions of GLP-1, therefore, offer great potential for treatment of the insulin resistance and relative insulin insufficiency that define type II diabetes mellitus (7).

The release of GLP-1 and the other PGDPs from ileal L cells is under complex regulation. Agents that can directly stimulate the secretion of GLP-1 have been analyzed in various models of the intestinal L cell, including a primary cell culture of fetal rat intestinal cells (8, 9), a murine intestinal endocrine cell line (10), and a perfused model of the rat ileum (11, 12). The major secretagogues identified using these models include glucose-dependent insulinotropic peptide (GIP), gastrin-releasing peptide (GRP), calcitonin gene-related peptide, and agonists of acetylcholine. Fatty acids, when applied directly to fetal rat intestinal cell cultures, also stimulate GLP-1 secretion (9).

In the *in vivo* setting, GLP-1 is rapidly released from ileal L cells upon the ingestion of a mixed meal (13, 14) or by the infusion of fat directly into the duodenum (15, 16). Indeed, plasma levels of GLP-1 peak within 5–15 min of nutrient administration despite the fact that nutrients do not reach the ileum within this time frame (17). Furthermore, in patients with ileostomies to divert nutrient flow from the ileum, GLP-1 levels after ingestion of a mixed meal are similar to those observed in individuals with an intact gastrointestinal tract (13). These observations are not consistent with the idea that nutrients act directly on ileal L cells to stimulate the release of GLP-1, at least during the early phase of secretion. Therefore, it has been postulated that the secretion of GLP-1 is under the regulation of a proximal-distal loop, relaying information about nutrient and, in particular, fat ingestion from the proximal duodenum to the distal site of GLP-1-containing L cells (15, 16). Consistent with this concept, we have demonstrated that the duodenal peptide GIP, which is secreted in response to fat ingestion (14, 16), is an endocrine mediator of this proximal-distal loop in rats (16). However, recent data have demonstrated that the secretion of GLP-1 induced by duodenal fat can be completely inhibited by infusion of an antagonist for the neuropeptide GRP (18). These findings suggest that physiological doses of GIP act through the nervous system (either vagal or myenteric) to

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indirectly stimulate GLP-1 secretion, rather than acting directly at the level of the L cell.

The present investigation further examined the possible existence of a neural component of the proximal-distal loop regulating secretion of GLP-1 in the rat. Specifically, the intrinsic and extrinsic nervous systems were analyzed with respect to their ability to affect duodenal fat-induced GLP-1 secretion. In addition, the humoral mechanism by which nutrients in the proximal duodenum signal the distal L cell was reevaluated by examining the potential interaction of GIP with the vagus nerve.

Materials and Methods

Surgery

After fasting for 18–24 h, male Wistar rats weighing 337 ± 6 g (Charles River Canada, Inc., St. Constant, Canada) were lightly anesthetized with halothane and given an ip injection of sodium pentobarbital (60 mg/kg) to achieve a surgical plane of anesthesia. The carotid artery was cannulated to allow for arterial blood sampling. A maximum of nine samples were collected from each experimental animal. Blood was collected into 10% (vol/vol) Trasylol (5000 kalikrein inactivating units/ml; Bayer, Inc., Etobicoke, Canada)-EDTA (12 mg/ml)-diprotin A (34 μg/ml; Calbiochem, La Jolla, CA). Plasma was collected and stored at −20 C until time of assay, and the RBC were resuspended in an equal volume of heparinized saline and reinfused via a cannula in the jugular vein. To ensure that the experimental procedures were not stimulating a counterregulatory response, corticosterone levels (Diagnostic Products Corp., Los Angeles, CA) were detected in 50 μl plasma from all animals (n = 42) upon initiation (585 ± 25 ng/ml) and completion (552 ± 18 ng/ml) of blood sampling and were not significantly different.

Model of the proximal distal loop

To separate indirect from direct effects of nutrients on the ileal L cell, the proximal-distal loop governing GLP-1 secretion was modeled by infusing nutrients into a 10-cm segment of proximal duodenum (15, 16, 18). The duodenum was cannulated above the common bile duct and isolated from the remaining gut by loose ligation 10 cm distal to the site of the duodenal catheter. This ligation ensures that nutrients do not progress to the distal portions of the small intestine to directly stimulate GLP-1 secretion from the ileal L cells, but permits retention of all neurovascular connections. Blood was collected at −10 and 0 min to ascertain basal levels of secretion, and manipulation of the gastrointestinal tract was performed in the interval between these two time points. At 0 min, 3–4 ml fat in the form of corn oil (56% polyunsaturated fatty acids, 32% monounsaturated fatty acids, and 12% saturated fatty acids; Sunfresh Ltd., Toronto, Canada) were infused into the proximal duodenal segment, and blood samples were collected every 5–10 minutes thereafter up to 60 min.

Vagotomy

To ascertain the role of vagal innervation in the regulation of GLP-1 secretion, a series of vagotomies was performed before the start of experimental sampling in the interval between the first and second basal blood samplings. The procedures included 1) left cervical vagotomy, 2) bilateral subdiaphragmatic vagotomy, and 3) selective hepatic branch vagotomy. The bilateral subdiaphragmatic vagotomy was performed by transection of the esophagus, including the accompanying vagal trunks.

Vagal stimulation

The vagal stimulation protocol was based on parameters used in a similar study of vagally induced bombesin-like immunoreactivity release (19). Briefly, the celiac branch of the subdiaphragmatic vagus nerve was transected 30 min before the commencement of the experimental procedure. During this interval the distal portion of the celiac branch was placed over a bipolar stimulating electrode connected to a Grass stimulator (Grass Instruments, Quincey, MA). This preparation was then immersed in paraffin oil throughout the experiment. The electrical stimulation was begun at 0 min and lasted for 15 min at a voltage of 10 V and a frequency of 20 Hz [preliminary studies with lower frequencies did not significantly affect gut glucagon-like immunoreactivity (gGLI) secretion]. Blood was collected at 5, 10, and 15 min during the stimulation period and then every 10 min throughout the remainder of the experimental time course.

GIP infusions

Human GIP (Bachem California, Inc., Torrance, CA) was administered as a bolus via the jugular vein followed by a maintenance infusion through the femoral vein for 40 min and then a 20-min recovery period. Blood samples were collected every 10 min. GIP was infused at either a high (supraphysiological; 715 ng/kg bolus plus 1070 ng/kg·h infusion) or a low (physiological; 143 ng/kg bolus plus 214 ng/kg·h infusion) dose. The physiological dose of GIP was based on that used in a previous study, which was found to mimic levels of GIP produced by the ingestion of a fat meal (16). The supraphysiological dose of GIP was 5 times greater than the physiological dose.

Assays

In correlation experiments, RIA for GLI (glicentin/oxyntomodulin/glucagon) was carried out using 0.1 ml plasma with antiserum K4023 (Biospacific, Emeryville, CA), whereas RIA for immunoreactive glucagon (glucagon) used 0.2 ml plasma with antiserum 04A (Dr. R. H. Unger, Dallas, TX). The plasma levels of gGLI were obtained by subtraction of immunoreactive glucagon from GLI (15, 16, 18). Immunoreactive GLP-$1^{(x\text{-}36NH2)}$ was detected by RIA using an antiserum directed against the carboxyl-terminus of GLP-$1^{7\text{–}36NH2}$ (Affinity Research Products, Nottingham, UK). Before RIA for GLP-$1^{(x\text{-}36NH2)}$, 1 ml plasma was diluted with 2 ml 1% trifluoroacetic acid (pH 2.5 with diethylamine) and purified by passage through a C_{18} Sep-Pak cartridge (Waters Corp., Milford, MA). Peptides were eluted with 3 ml 80% isopropanol/0.1% trifluoroacetic acid, and the eluate was dried *in vacuo* to make a single sample for RIA (20). This procedure resulted in a recovery rate of 53.9 ± 6% when spiked plasma samples were analyzed. Immunoreactive GIP levels were determined using 0.1 ml plasma in a human GIP RIA kit (Peninsula Laboratories, Inc., Belmont, CA).

Data analysis

Peptide secretion is expressed as the change from basal values. All data are expressed as the mean ± SEM. Statistical significance between experimental groups was assessed by ANOVA using n-1 *post-hoc* custom hypothesis tests with Statistical Analysis System Software (SAS Institute, Inc., Cary, NC). Comparisons of plasma levels of hormones between basal levels and subsequent time points within an experimental group were made using repeated measures ANOVA. Significance was determined at the $P < 0.05$ level in these comparisons. Where single determinations were made, paired Student's *t* test was performed. Some data were $\log_{10}$ transformed before analysis.

Results

Correlation of GLP-1 and gGLI secretion

The large amount of plasma required for assay of GLP-1 (1 ml/determination) limited repeated sampling over a prolonged period of time. As the proglucagon molecule is cleaved in the intestinal L cell to produce glicentin/oxyntomodulin and GLP-1 in a 1:1 relationship, the secretion of gGLI has been used to monitor the secretion of GLP-1 indirectly, at least *in vitro* (9). To determine the relationship between gGLI and GLP-1 levels in the rat *in vivo*, plasma levels of gGLI and GLP-1 were determined in the same animals under basal conditions and in response to administration of fat into the duodenum (Fig. 1). gGLI levels were highly correlated with those of GLP-1 ($r = 0.85$; $P < 0.05$; $n = 3$). As the gGLI assay requires only 300 μl plasma, compared

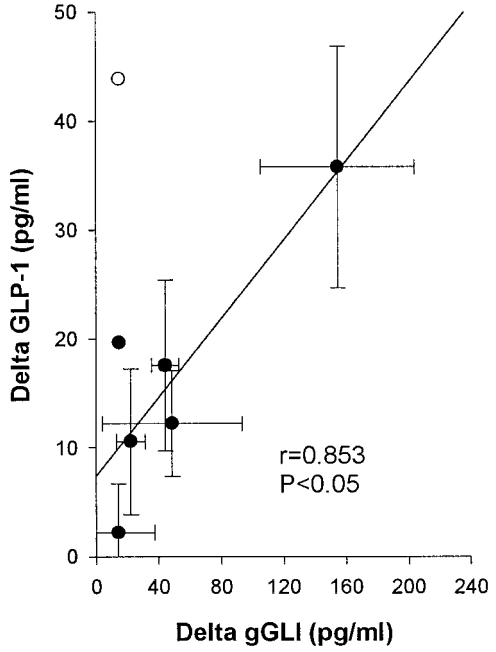

FIG. 1. Correlation of GLP-1$^{(x\text{-}36NH2)}$ and gGLI RIAs. The change in GLP-1 levels after corn oil challenge of the proximal-distal loop model (n = 3) were corrected for GLP-1 assay recovery rate and plotted as a function of the change in gGLI levels in the same plasma samples. The *open circle* indicates an outlier in the triplicate for GLP-1 at that level of gGLI. This outlier was not included in the regression analysis.

with 1 ml for the GLP-1 RIA, gGLI was used as a measure of GLP-1 secretion in all subsequent studies.

Effect of left cervical vagotomy on basal levels of gGLI

The mean basal level of gGLI was 193 ± 12 pg/ml (n = 36) in anesthetized rats. To assess whether the vagus nerve has a global effect on basal levels of gGLI, a left cervical vagotomy was performed in unstimulated animals. The right cervical vagus nerve was left intact to maintain cardiorespiratory function. Left cervical vagotomy reduced the mean basal level of gGLI from 205 ± 11 pg/ml in control animals to 113 ± 9 pg/ml ($P < 0.001$; n = 6). This finding therefore suggested that the vagus nerve plays a role in modulating basal gGLI secretion. Levels of plasma glucose and glucagon are shown in Table 1 and were not affected by left cervical vagotomy.

Corn oil infusion in the model of the proximal-distal loop

To confirm that the presence of fat in the proximal duodenum can signal the GLP-1-containing L cells in the ileum, the model of the proximal-distal loop was employed (15, 16). Infusion of 3–4 ml saline into a ligated segment of duodenum did not significantly alter the secretion of gGLI from basal levels (Fig. 2). In contrast, infusion of 3–4 ml fat in the form of corn oil induced a rapid rise in gGLI secretion ($P < 0.05$ at 5 min), which was maintained throughout the remainder of the experimental time course ($P < 0.001$ *vs.* saline infusion; n = 5). The role of the enteric nervous system in mediating this proximal signal to the ileal L cells was investigated by transection of the gastrointestinal tract at a site immediately distal to the isolated segment of duodenum before the infusion of corn oil. This procedure did not reduce the gGLI secretion induced by duodenal fat, as the infusion of corn oil elicited a significant rise in gGLI levels compared with that in saline controls ($P < 0.05$; n = 6; Fig. 2). The peak change in gGLI from basal was 132 ± 37 pg/ml ($P < 0.05$; n = 6), and

TABLE 1a. Changes in plasma glucose over time with left cervical vagotomy and celiac vagal stimulation

Time (min)	Control	Left cervical vagotomy	Time (min)	Celiac vagal stimulation
Basal	86 ± 2	92 ± 5	Basal	111 ± 4
0	0 ± 0	0 ± 0	0	0 ± 0
10	7 ± 4	11 ± 7	5	7 ± 5
20	11 ± 5	14 ± 8	10	5 ± 3
30	29 ± 8	22 ± 9	15	12 ± 7
40	26 ± 8	24 ± 10	25	16 ± 5
50	29 ± 9	21 ± 11	35	29 ± 7
60	28 ± 9	19 ± 12	45	35 ± 6

Means and SEMs are expressed in units of milligrams per dl and represent changes from basal levels. No significant differences were observed from control animals.

TABLE 1b. Changes in plasma immunoreactive glucagon over time with left cervical vagotomy and celiac vagal stimulation

Time (min)	Control	Left cervical vagotomy	Time (min)	Celiac vagal stimulation
Basal	105 ± 14	106 ± 13	Basal	135 ± 23
0	0 ± 0	0 ± 0	0	0 ± 0
10	−11 ± 20	11 ± 14	5	7 ± 18
20	−16 ± 15	23 ± 13	10	2 ± 5
30	22 ± 17	41 ± 20	15	40 ± 16
40	22 ± 16	59 ± 38	25	15 ± 20
50	72 ± 42	120 ± 68	35	24 ± 22
60	111 ± 72	211 ± 145	45	40 ± 21

Means and SEMs are expressed in units of picograms per ml and represent changes from basal levels. No significant differences were observed from control animals.

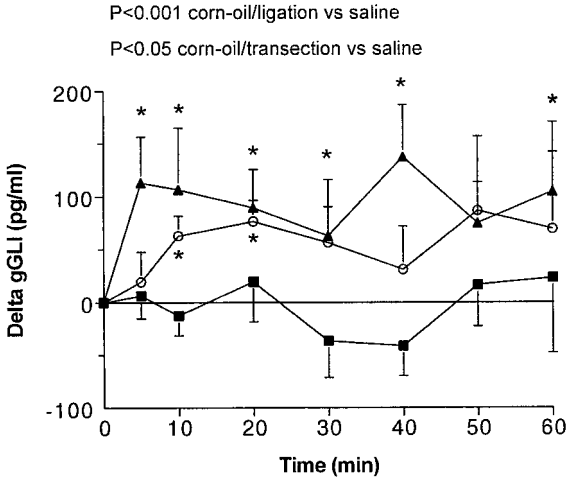

FIG. 2. Change in plasma levels of gGLI in response to 3–4 ml corn oil infused into the proximal segment of the duodenum, isolated by loose ligation (*closed triangles*; n = 5) or gut transection distal to the proximal segment (*open circles*; n = 6). Changes in plasma levels of gGLI in response to 3–4 ml saline are shown by the *closed squares* (n = 5). Differences between groups are represented by the *P* values in the legend. Differences from basal levels within each individual experimental group are indicated (*, $P < 0.05$ *vs.* basal).

this was not significantly different from that achieved by the infusion of corn oil into the ligated duodenal segment. However, a significant difference was observed in the timing of the corn oil-induced response of gGLI secretion in the two protocols, such that the fat-induced rise in gGLI levels was significantly delayed by transection of the gut compared with the animals undergoing gut ligation ($P < 0.05$ for corn oil/ligation *vs.* corn oil/transection at 5 min). Indeed, the peak change in gGLI secretion occurred at 19 ± 4 min in the transected enteric nervous system group compared with 6 ± 1 min in the ligated group ($P < 0.05$).

To investigate the possibility that extrinsic vagal fibers mediate the proximal signal to the ileal L cells, bilateral subdiaphragmatic vagotomies were performed in addition to gut transection, before the administration of fat (Fig. 3). In contrast to the response found in rats with an intact vagus, corn oil infusion into the proximal duodenum of vagotomized rats completely failed to stimulate gGLI secretion ($P < 0.001$ *vs.* intact vagus; n = 5). Changes from basal levels of plasma glucose and glucagon are shown in Table 2.

Effect of vagal stimulation on gGLI secretion

Electrical stimulation of the celiac branch of the vagus nerve was performed in unstimulated animals to determine whether the efferent vagus nerve mediates the signal from the proximal duodenum to the distal L cells (Fig. 4). The celiac branch of the vagus nerve was transected, and the distal end was stimulated for a 15-min period. Electrical stimulation using 10 V and a frequency of 20 Hz resulted in a significant rise in gGLI levels, which reached a peak of 71 ± 14 pg/ml above basal at the 10 min point ($P < 0.05$; n = 5). gGLI levels returned to basal during the final phase of the stimulation period, but, surprisingly, increased again to significance during the recovery period. No significant effect of celiac branch stimulation on plasma glucose and glucagon levels was detected compared with those in the control group (Table 1).

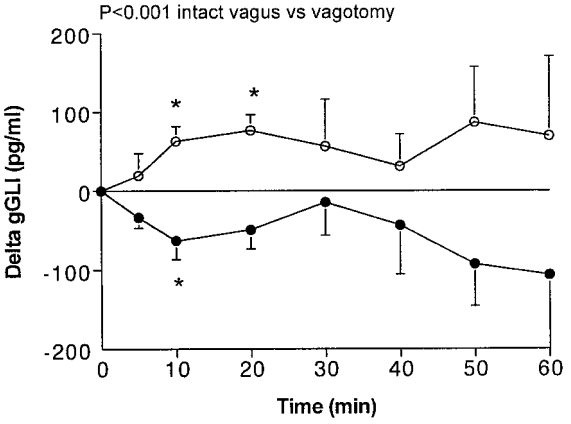

FIG. 3. Effect of subdiaphragmatic vagotomy on changes in plasma level of gGLI in response to duodenal corn oil. Changes in gGLI induced by corn oil infusion in gut-transected control animals (*open circles*; as also shown in Fig. 2; n = 6) and subdiaphragmatic vagotomized animals (*closed circles*; n = 5) are represented. Differences between groups are represented by the *P* values in the legend. Differences from basal levels within each individual experimental group are indicated (*, $P < 0.05$ *vs.* basal).

TABLE 2a. Changes in plasma glucose over time with duodenal saline or corn oil administration

Time (min)	Duodenal saline: gut transection	Duodenal corn oil infusions		
		Gut ligation[a]	Gut transection	Subdiaphragmatic vagotomy
Basal	127 ± 16	121 ± 6	121 ± 9	110 ± 6
0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
10	12 ± 4	29 ± 19	39 ± 8	27 ± 11
20	13 ± 6	33 ± 6	42 ± 12	25 ± 12
30	12 ± 9	25 ± 13	38 ± 14	35 ± 18
40	12 ± 20	44 ± 7	34 ± 14	25 ± 14
50	35 ± 20	54 ± 16	33 ± 14	24 ± 23
60	55 ± 29	52 ± 17	25 ± 18	27 ± 31

Means and SEMs are expressed in units of milligrams per dl and represent changes from basal levels.
[a] $P < 0.05$ *vs.* duodenal saline control group.

TABLE 2b. Changes in plasma immunoreactive glucagon over time with duodenal saline or corn oil administration

Time (min)	Duodenal saline: gut transection	Duodenal corn oil infusions		
		Gut ligation	Gut transection[a]	Subdiaphragmatic vagotomy
Basal	96 ± 19	111 ± 10	152 ± 39	112 ± 16
0	0 ± 0	0 ± 0	0 ± 0	0 ± 0[b]
10	−3 ± 13	−24 ± 8	42 ± 21	33 ± 30
20	−2 ± 18	−17 ± 6	50 ± 25	5 ± 8
30	−1 ± 20	−18 ± 10	157 ± 89	5 ± 12
40	−3 ± 30	10 ± 11	152 ± 51	171 ± 158
50	27 ± 5	23 ± 20	218 ± 49	235 ± 214
60	20 ± 28	54 ± 27	337 ± 45	252 ± 214

Means and SEMs are expressed in units of picograms per ml and represent changes from basal levels. Subdiaphragmatic vagotomy increased basal levels of IRG by 41 ± 9 pg/ml. This value was set at zero and taken as the reference for subsequent changes in this group.
[a] $P < 0.001$ *vs.* gut ligation group.
[b] $P < 0.05$ *vs.* basal level in subdiaphragmatic vagotomy group.

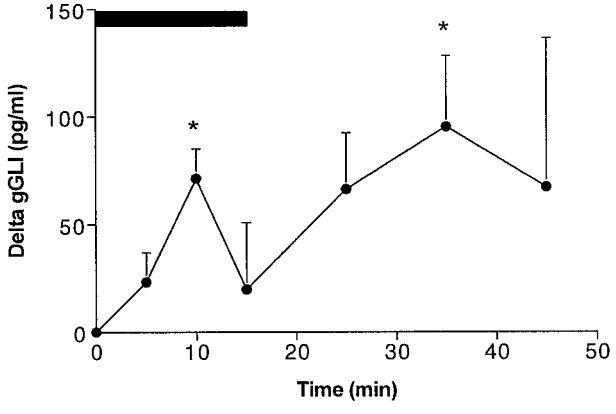

FIG. 4. Responses of plasma gGLI to electrical stimulation of the distal end of the celiac branch of the vagus nerve. Electrical stimulation consisted of 10 V at 20 Hz for a stimulation period of 15 min. Differences from basal are indicated (*, $P < 0.05$ *vs.* basal).

Effect of high GIP infusion with or without hepatic branch vagotomy

To determine the relative contribution of the vagus nerve to the proximal-distal loop with respect to that of the humoral component mediated by GIP (16), infusions of GIP at varying doses were performed in animals with or without a hepatic branch vagotomy (Fig. 5). Infusion of GIP at a sup-

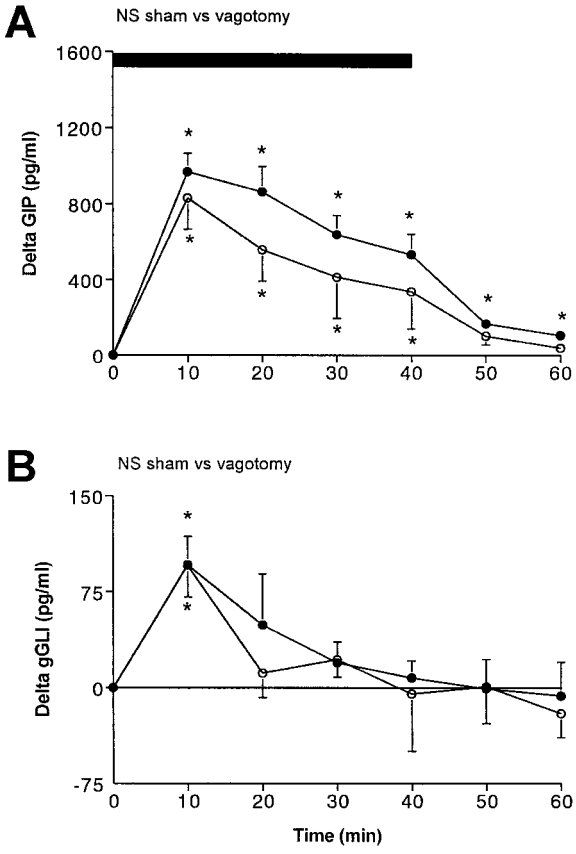

FIG. 5. Changes in plasma GIP (A) and gGLI (B) levels in response to supraphysiological infusions of GIP into control (sham hepatic branch vagotomized animals; *closed circles*; n = 7) or hepatic branch vagotomized animals (*open circles*; n = 6). The 40-min infusion period (*solid bar*) was followed by a 20-min recovery period. Differences between groups are represented by the *P* values in the legend. Differences from basal levels within each individual experimental group are indicated (*, $P < 0.05$ *vs.* basal).

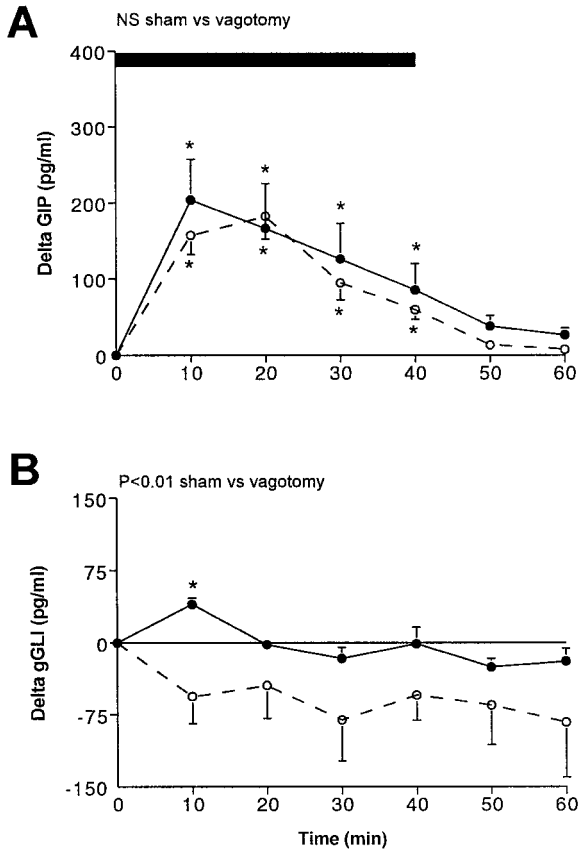

FIG. 6. Changes in plasma GIP (A) and gGLI (B) levels in response to physiological infusions of GIP into control (sham hepatic branch vagotomized animals; *closed circles*; n = 4) or hepatic branch vagotomized animals (*open circles*; n = 6). The 40-min infusion period (*solid bar*) was followed by a 20-min recovery period. Differences between groups are represented by the *P* values in the legend. Differences from basal levels within each individual experimental group are indicated (*, $P < 0.05$ *vs.* basal).

raphysiological dose resulted in rapidly elevated GIP levels that were not significantly different between the control and hepatic branch vagotomized animals. Peak immunoreactive GIP levels reached 1030 ± 121 pg/ml above basal ($P < 0.05$, n = 7) in the control group at 13 ± 2 min and returned toward basal during the subsequent infusion and recovery periods. These elevations in GIP levels resulted in significant increases in gGLI in control as well as vagotomized animals. The peak of gGLI above basal induced by the supraphysiological GIP infusion in the control group was 123 ± 32 pg/ml ($P < 0.05$; n = 7), and this was not significantly different from the gGLI response achieved by supraphysiological GIP infusion in the hepatic branch vagotomized animals (96 ± 25 pg/ml; $P < 0.05$; n = 6). Interestingly, GIP-induced gGLI secretion rapidly returned to baseline within 20 min of reaching peak levels despite elevated concentrations of GIP in both control and vagotomized groups.

Effect of low GIP infusion with or without hepatic branch vagotomy

The effects of physiological increases in GIP levels on gGLI secretion were also examined in the presence or absence of intact vagal innervation. The infusion of the physiological dose of GIP into control animals resulted in a peak change of 223 ± 43 pg/ml above basal at 13 ± 3 min ($P < 0.05$; n = 4). The level of GIP achieved by the physiological infusion of GIP in the vagotomized group was not significantly different from that in the control group, reaching a maximum of 169 ± 30 pg/ml above basal at 12 ± 2 min ($P < 0.05$; n = 5; Fig. 6). Furthermore, the GIP levels achieved by the infusion were significantly decreased compared with those after the supraphysiological dose of GIP ($P < 0.001$). In control animals, the physiological dose of GIP led to a small, but significant, elevation in gGLI secretion, reaching a peak of 40 ± 7 pg/ml above basal levels ($P < 0.01$; n = 4) and rapidly returning to basal levels by the 20 min point. In contrast, hepatic branch vagotomy completely abolished the rise in gGLI secretion induced by the physiological infusion of GIP ($P < 0.05$ *vs.* controls). Changes in plasma glucose and glucagon produced by the infusions of GIP are demonstrated in Table 3.

Discussion

Due to its numerous actions on metabolic processes, GLP-1 holds great promise as a treatment for type II diabetes mel-

TABLE 3a. Changes in plasma glucose over time with supraphysiological or physiological administration of GIP in sham- or hepatic branch-vagotomized animals

Time (min)	Supraphysiological GIP infusion		Physiological GIP infusion	
	Sham vagotomy	Hepatic vagotomy	Sham vagotomy	Hepatic vagotomy
Basal	94 ± 6	100 ± 4	104 ± 5	103 ± 7
0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
10	17 ± 7	11 ± 8	16 ± 8	17 ± 4
20	−8 ± 14	2 ± 6	5 ± 5	7 ± 2
30	−0 ± 7	−1 ± 5	6 ± 7	2 ± 1
40	−3 ± 3	−4 ± 4	1 ± 7	4 ± 4
50	−2 ± 5	0 ± 9	4 ± 8	6 ± 3
60	7 ± 7	10 ± 10	12 ± 11	11 ± 2

Means and SEMs for plasma glucose are expressed in units of milligrams per dl and represent changes from basal levels.

TABLE 3b. Changes in plasma immunoreactive glucagon over time with supraphysiological or physiological administration of GIP in sham- or hepatic branch-vagotomized animals

Time (min)	Supraphysiological GIP infusion		Physiological GIP infusion	
	Sham vagotomy	Hepatic vagotomy	Sham vagotomy[a]	Hepatic vagotomy[b]
Basal	67 ± 9	102 ± 21	95 ± 20	112 ± 7
0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
10	54 ± 10	38 ± 14	22 ± 5	32 ± 11
20	23 ± 7	25 ± 17	5 ± 2	18 ± 10
30	23 ± 5	19 ± 14	−0 ± 7	28 ± 12
40	41 ± 7	30 ± 18	10 ± 4	30 ± 17
50	31 ± 7	23 ± 10	11 ± 8	38 ± 16
60	23 ± 8	57 ± 22	35 ± 15	22 ± 32

Means and SEMs are expressed in units of picograms per ml and represent changes from basal levels.

[a] $P < 0.05$ *vs.* sham vagotomy with supraphysiological GIP infusion.

[b] $P < 0.01$ *vs.* sham vagotomy with physiological GIP infusion.

litus. Therefore, it is imperative that the factors that modulate the secretion of GLP-1 are understood. The results of the present study further this knowledge by demonstrating that a neuroendocrine loop exists that acts to stimulate the ileal L cells when nutrients are present in the proximal duodenum. The neural component of this proximal-distal loop has been demonstrated to involve the vagus nerve, whereas the humoral component has previously been described to be mediated by the duodenal hormone GIP (16). Furthermore, these two limbs of the proximal-distal loop have now been shown to interact within the physiological range to induce a rapid secretory response from the ileal L cell.

To investigate the secretion of GLP-1 *in vivo*, a method to evaluate GLP-1 levels over a prolonged period of time is necessary. The major limitation inherent to the RIA of GLP-1 is the large plasma volume that is necessary to accurately measure GLP-1 levels in rats. The results of the present study demonstrate that changes in the plasma levels of GLP-1 in rats *in vivo* are highly correlated with those of gGLI, a finding that is not surprising, as glicentin/oxyntomodulin and GLP-1 are cleaved from proglucagon in a one to one relationship (1). Furthermore, plasma levels of GLP-2, which is also cleaved from proglucagon in a one to one relationship with glicentin/oxyntomodulin, has recently been shown to be highly correlated with gGLI levels in the rat (21). Similar findings have been made for gGLI, GLP-1, and GLP-2 using *in vitro* models of the intestinal L cell (9, 22) (Brubaker, P. L., unpublished data). The results obtained for any one of these peptides may therefore be extrapolated to include all of the other intestinal PGDPs, at least in the rat.

To investigate the hypothesis that a neural component of the proximal-distal loop operates in conjunction with the previously described endocrine-based loop (15, 16), the vagus nerve was examined for global effects on basal GLP-1 secretion. This nerve represents the major parasympathetic innervation of the proximal portion of the gastrointestinal tract and is known to influence the secretion of other gut-derived hormones, including GRP (23) and secretin (24). Left cervical vagotomy significantly depressed the basal secretion of gGLI to approximately 56% of basal levels in control rats. This finding demonstrates a tonic stimulatory role for the left cervical vagus nerve in regulating secretion of GLP-1 from the L cell.

Nutrients such as fat can exert effects on GLP-1 secretion directly by stimulating the L cells at their luminal surface (9) or can have indirect effects on the L cell by exerting their influence through an intermediate messenger such as GIP. This duodenal hormone has previously been shown to stimulate secretion of GLP-1 in response to fat ingestion (16). The direct and indirect effects of fat can be separated *in vivo* by ensuring that the progress of nutrients is impeded before they can reach the site of the majority of the GLP-1-containing L cells within the ileum. This was accomplished in our model by applying a ligature loosely around the gastrointestinal wall at a position approximately 10 cm downstream from the duodenal site of infusion. Saline infusion into the proximal isolated segment did not induce secretion of GLP-1 from the L cells, indicating that the effects of fat on GLP-1 secretion are specific to this nutrient and are not due to distension of the duodenal wall or the mere presence of a fluid in the lumen of the duodenum. Consistent with the results of previous studies (15, 16), infusion of fat into the duodenum induced a rapid and prolonged rise in gGLI secretion despite the fact that this fat did not reach the ileum at any time during the experimental time course. Furthermore, the secretion of GLP-1 induced by duodenal fat cannot be explained by the presence of a few hypersensitive L cells in this region, because the response to fat is completely abolished when all sections of the intestine distal to the duodenum are removed (18). However, the rapid response of the L cell to duodenal fat (~5 min) as well as the finding that this response is dependent on the neuropeptide GRP (18) led to the current hypothesis that in addition to GIP, a component of the nervous system may also be involved in mediating the proximal nutrient signal to the distal L cell.

To explore the possibility that fibers within the submucosal or myenteric nervous plexus may play a role in the rapid L cell response to fat ingestion, possible contributions of the enteric nervous system were abolished by transection of the gastrointestinal tract before the infusion of corn oil. This procedure did not prevent gGLI secretion compared with the control group, thereby indicating that the enteric nervous system is not responsible for relaying information about nutrient ingestion to the ileal L cells. However, an element contained within the walls of the gastrointestinal

tract may be necessary for maintaining the appropriate timing of the response, as transection of the gut wall caused a marked delay in the peak of gGLI secretion. One possible explanation for this finding is that local afferents of vagal fibers may have been transected in this procedure. Local afferents are extensions of vagal afferents that divide from the main afferent nerve and supply a target organ directly without synapsing with higher centers (25). These have been shown to contain calcitonin gene-related peptide, which is a potent stimulator of gGLI secretion *in vitro* (8). In the present scenario, such afferents could possibly branch from vagal afferents that innervate the duodenal region and travel to the ileum within the walls of the gastrointestinal tract to exert effects on gGLI secretion in a reflex manner. As the main vagal nerves are not affected by the gut wall transection, this might explain why the gGLI response was only delayed rather than being totally abolished in these studies.

Consistent with a major role for the vagus nerve in mediating the proximal-distal loop, bilateral subdiaphragmatic vagotomy performed in addition to gut transection completely abolished the fat-induced rise in gGLI secretion. Two scenarios or a combination of the two may provide the reason why bilateral subdiaphragmatic vagotomy totally attenuated the fat-induced rise in gGLI secretion. The first deals with the possibility that transection of the subdiaphragmatic vagus nerve has rendered higher brain centers insensitive to sensory messages from the duodenum. The vagus nerve is known to contain many afferent fibers arising from the proximal gastrointestinal tract and has been previously shown to mediate both nutrient and hormonal signals from the gut in response to nutrient administration (24, 26, 27). In fact, the vagus nerve has been shown to express receptors that are able to differentiate between distinct classes of ingested lipid (28). This is an important finding, considering that both fat and GLP-1 are potent inhibitors of gastric motility (2, 29). Thus, activation of vagal afferents by ingested lipid may induce the secretion of GLP-1, thereby augmenting the enterogastrone effects of fat. Another possibility that may explain the reduced secretion of gGLI in response to bilateral subdiaphragmatic vagotomy is that the efferent mechanisms that act to stimulate GLP-1 secretion were disrupted by the vagotomy. It has been previously suggested that the parasympathetic nervous system is involved in the modulation of L cell secretion (30), and indeed, the L cell is stimulated by muscarinic agonists *in vitro* (8, 10). Thus, disruption of afferent and/or efferent vagal fibers in the rat results in a loss of the rapid L cell response to ingested nutrients. Consistent with this finding, it has recently been reported that administration of atropine abolishes the GLP-1 response to an oral glucose tolerance test in humans, although the effects of cholinergic blockade to inhibit gastric emptying were not taken into account in this study (30).

To differentiate between the afferent and efferent signals mediating GLP-1 secretion, the celiac branch of the vagus nerve was directly stimulated, resulting in significant stimulation of gGLI secretion at the 10 min point. The delay observed in the secretion of gGLI induced by electrical stimulation was very similar to that observed in the experiments involving fat-induced secretion in gut-transected animals. These findings lend further support to the idea of a role for the enteric nervous system in mediating a component of the proximal-distal loop, as this pathway was not affected by the electrical stimulation. The delay may also occur due to involvement of a peptidergic mediator in the efferent signaling pathway, as the effects of these neuromodulators are known to be preceded by long latent periods (31). Such a possibility is also supported by recent experiments showing that infusion of an antagonist to the GRP receptor completely abolishes proximal fat-induced gGLI secretion despite the fact that GIP levels remained elevated (18). A role for GRP in the signaling process at a level downstream of GIP was therefore indicated. GRP is localized extensively in the myenteric plexus (32) and potently stimulates the secretion of GLP-1 (8, 11, 12). Therefore, GRP is a likely candidate neuropeptide that may be released upon electrical stimulation of the celiac branch of the vagus nerve. It should be noted that the delayed secretion of gGLI in response to electrical stimulation is not dependent upon release of GIP from the duodenum, as vagal stimulation has previously been shown to have no effect on the GIP-containing K cell (33). The mechanism underlying the rebound secretion of gGLI during the recovery period remains to be elucidated. However, GLP-1 secretion is known to be pulsatile, with a frequency of approximately one pulse every 10–15 min. This pattern of secretion has been proposed to be regulated by the parasympathetic nervous system (30). Thus, it is possible that the initial vagal stimulation stimulating the release of gGLI in the present study coordinated the dispersed L cells in the distal small intestine, leading to an integrated rebound pulse of gGLI secretion during the recovery period.

The finding that a GRP receptor antagonist completely inhibits gGLI secretion in the presence of elevated GIP levels suggested that the neural and humoral arms of the proximal-distal loop may be integrated in the regulation of the L cell. This hypothesis was examined by experiments in which GIP was infused at different doses into animals that possessed intact or disrupted vagal innervation at the level of the hepatic afferent. When infused at supraphysiological doses, GIP stimulated gGLI secretion significantly in both control and vagotomized animals; however, a vagal dependence of GIP became evident when GIP was infused at physiological doses. This finding indicates that in the normal rat, the hepatic branch of the subdiaphragmatic vagus nerve mediates the stimulatory effect of GIP on the L cell. Consistent with this finding, both cholecystokinin (27) and secretin (24) have been reported to act on the exocrine pancreas through vagal afferents when administered at physiological doses, exerting direct effects only when infused at supraphysiological concentrations. Vagal sensory endings in the duodenum do not make contact with epithelial cells, but terminate in close association with the lamina propria (34). Thus, GIP-containing cells may function as taste cells by sampling the duodenal contents and, in turn, activating vagal afferents to stimulate GLP-1 secretion.

In conclusion, the secretion of GLP-1 and gGLI from the rat ileal L cell is regulated by a complex proximal-distal loop that involves both endocrine and neural factors (Fig. 7). Fat is sensed in the duodenum by luminal K cells, which secrete GIP in response to fat and glucose. GIP exerts its effects on the ileal L cell in two ways depending on the concentration

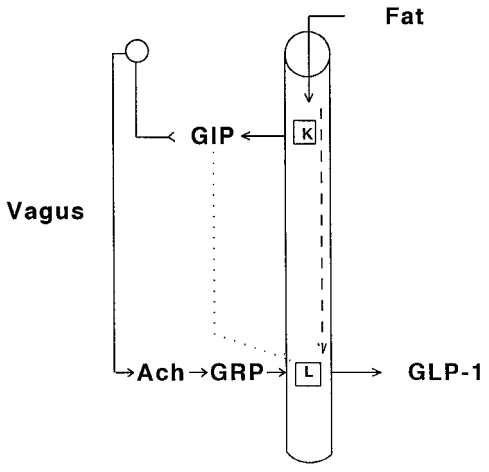

FIG. 7. Overview of the proposed elements of the proximal-distal neuroendocrine loop governing GLP-1 secretion from the rat ileal L cell. Fat in the lumen of the duodenum stimulates the release of GIP from the K cells, which, in turn, activates the L cell indirectly through vagal afferent pathways. The proximal signal is then mediated by vagal efferent pathways present in the celiac branch of the subdiaphragmatic vagus nerve that are thought to synapse with GRPergic neurons present in the myenteric plexus. At supraphysiological concentrations, GIP may also act directly through receptors on the L cell (*dotted line*). Mechanisms that stimulate early secretion may also be mediated through the enteric nervous system in the walls of the gastrointestinal tract (*dashed line*).

achieved. Physiological levels of GIP act through vagal afferent pathways to stimulate the L cell indirectly. This stimulation is carried to the L cell by efferent pathways located in the celiac branch of the vagus nerve and is thought to involve GRP. GIP can also stimulate the L cell directly at higher levels. Finally, a component of the enteric nervous system also appears to be responsible for the early stimulation of GLP-1 secretion within this loop. The secretion of GLP-1 from the distal L cell is, therefore, intimately connected with the presence of nutrients in the proximal duodenum through an interaction of neural and endocrine pathways. As knowledge is gained concerning the functioning of the neuroendocrine loop, examination of the function of this axis can be made in disease states, such as type II diabetes mellitus.

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