GLP-1 secretion is enhanced directly in the ileum but indirectly in the duodenum by a newly identified potent stimulator, zein hydrolysate, in rats

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Hira T, Mochida T, Miyashita K, Hara H. GLP-1 secretion is enhanced directly in the ileum but indirectly in the duodenum by a newly identified potent stimulator, zein hydrolysate, in rats. Am J Physiol Gastrointest Liver Physiol 297: G663–G671, 2009. First published August 6, 2009; doi:10.1152/ajpgi.90635.2008.—Glucagon-like peptide-1 (GLP-1) is released from enteroendocrine cells (L cells) in response to food ingestion. The mechanism by which dietary peptides stimulate GLP-1 secretion in the gut is unknown. In the present study, we found that a hydrolysate prepared from zein, a major corn protein (ZeinH), strongly stimulates GLP-1 secretion in enteroendocrine GLUTag cells. Stimulatory mechanisms of GLP-1 secretion induced by ZeinH were investigated in the rat small intestine under anesthesia. Blood was collected through a portal catheter before and after ZeinH administration into different sites of the small intestine. The duodenal, jejunal, and ileal administration of ZeinH induced dose-dependent increases in portal GLP-1 concentration. GLP-1 secretion in response to the ileal administration of ZeinH was higher than that in the duodenal or jejunal administration. Capsaicin treatment on esophageal vagal trunks abolished the GLP-1 secretion induced by duodenal ZeinH but did not affect the secretion induced by jejunal or ileal ZeinH. These results suggest that ZeinH in the jejunum or ileum directly stimulates GLP-1 secretion but duodenal ZeinH indirectly stimulates GLP-1 secretion via the vagal afferent nerve. A direct blood sampling method from the duodenal vein and ileal mesenteric vein revealed that ZeinH administered into the ligated duodenal loop enhanced GLP-1 concentration in the ileal mesenteric vein but not in the duodenal vein. This confirmed that ZeinH in the duodenum induces GLP-1 secretion from L cells located in the ileum by an indirect mechanism. These results indicate that a potent GLP-1-stimulating peptide, ZeinH, induces GLP-1 secretion by direct and indirect mechanisms in the rat intestine.

glucagon-like peptide-1; GLUTag cells; dietary peptide; zein

GLUCAGON-LIKE PEPTIDE-1 (GLP-1) is one of the incretin hormones that stimulate glucose-dependent insulin secretion and pancreatic β-cell proliferation (4, 9); GLP-1 also inhibits gastric emptying and food intake. In addition, a recent study demonstrated that GLP-1 reduces lipid absorption in rats (27). These actions of GLP-1 could present great benefits for the treatment of type-II diabetes mellitus. Stable GLP-1 analogs and dipeptidyl peptidase-IV (DPP-IV) inhibitors that protect active GLP-1 from cleavage by DPP-IV have been developed for clinical use (10).

Glucose and fatty acids are potent stimulants of GLP-1 release from enteroendocrine L cells in the distal intestine in response to food ingestion (1, 14). Previous studies have demonstrated that fatty acids stimulate GLP-1 secretion via direct and indirect mechanisms (30, 31, 32). At the cellular level, studies using murine GLP-1-producing enteroendocrine cell lines show that sodium-dependent glucose cotransporters (15) and sweet taste receptor T1R3 (23) mediate glucose-induced GLP-1 secretion, and fatty acid receptors GPR40 (12) and GPR120 (18) mediate fatty acid-induced GLP-1 secretion. However, GLP-1 secretion in response to luminal dietary proteins or peptides has been studied less.

In humans, GLP-1 secretion is stimulated by whey preload (16). Peptones induce GLP-1 secretion in rats (11) and enteroendocrine cell lines (7). Several studies have also shown the involvement of GLP-1 in dietary protein-induced satiety (2, 37). However, the mechanism by which dietary proteins or peptides induce GLP-1 secretion in vivo remains to be investigated. An in vitro study showed that ERK and MAPK pathways are involved in peptone-induced GLP-1 secretion in the human enteroendocrine cell line NCI-H716 (29).

The purpose of the present study is to clarify the stimulating pathways for GLP-1 secretion by luminal peptides in the rat intestine. We screened several protein hydrolysates to find a strong stimulant of GLP-1 secretion from an enteroendocrine cell line, then administered potent hydrolysate into the duodenal, jejunal, and ileal lumen of rats to compare the sensitivities to the hydrolysate. We also investigated the signaling pathways involved in the local GLP-1 secretion at the tissue level induced by the hydrolysate in rats.

Since GLP-1 has potential in the treatment or prevention of type-II diabetes, a greater understanding of GLP-1 secretion mechanisms would be advantageous to the development of preventive or treatment strategies against obesity or diabetes via the manipulation of GLP-1 secretion.

MATERIALS AND METHODS

Materials

Cell culture consumables (Dulbecco’s modified Eagle’s medium, fetal bovine serum, penicillin-streptomycin, and trypsin-EDTA solution) were purchased from Invitrogen (Carlsbad, CA). Other reagents were purchased from Wako (Osaka, Japan) unless specified. Bovine serum albumin (BSA), albumin egg hydrolysate (AEH), and meat hydrolysate (MHY) were purchased from Sigma (St. Louis, MO).

Zein hydrolysate (ZeinH) was prepared as follows: Zein (Tokyo Chemical Industry, Tokyo, Japan) (50 g) was suspended in deionized water (500 ml) and the pH was adjusted to 7.0. The suspension was shaken for 60 min at 55°C with the addition of papain (250 mg, Papain F; Asahi Food and Health Care, Tokyo, Japan) and was treated in boiling water for 20 min to stop the enzyme reaction. After centrifugation and filtration (0.2-μm pore size), the supernatant was lyophi-
lized as ZeinH. Country bean peptone (CBP), which was found to strongly stimulate cholecystokinin (CCK) secretion from the enteroneuronal cell line STC-1 in our previous study, was prepared as described previously (35). In brief, country beans (Dolichos lablab) were immersed in water and boiled, and the husk was removed. The beans were then homogenized in phosphate buffer at pH 1.9, and pepsin was added and stirred for 10 min at 37°C. After neutralization and desalting, the supernatant was lyophilized to obtain CBP. Peptide content was estimated by Lowry’s assay using BSA as a standard, and molecular size was analyzed by fast protein liquid chromatography (AKTA explorer 10S and Superdex Peptide 10/300 GL column, GE Healthcare Bio-Sciences AB, Uppsala, Sweden). ZeinH and CBP had peptide content of 65.5 and 60.7% as determined by Lowry’s protein assay and an average molecular weight of 1,600 and 2,900 Da, respectively. AEH and MHY had peptide content of 69 and 80%, respectively, but both hydrolysates mainly consist of peptides with molecular weights of <1,200 Da (8).

Cell Experiment (Experiment 1): Study of GLP-1 Secretion From GLUTag Cells

GLUTag cells (a gift from Dr. D. J. Drucker, University of Toronto, Toronto, Canada) were grown in Dulbecco’s modified Eagle’s medium (Invitrogen, cat. no. 12100-038) supplemented with 10% fetal bovine serum, 50 IU/ml penicillin, and 500 μg/ml streptomycin in a humidified 5% CO2 atmosphere at 37°C. Cells were routinely subcultured by trypsinization upon reaching 80–90% confluence.

GLUTag cells were grown in 48-well culture plates at a density of 1.25 × 105 cells/well for 2–3 days until they reached 80–90% confluence. Cells were washed twice with HEPES buffer (140 mM NaCl, 4.5 mM KCl, 20 mM HEPES, 1.2 mM CaCl2, 1.2 mM MgCl2, and 10 mM β-glucose, pH 7.4) to remove the culture media and exposed to test agents dissolved in the same buffer for 60 min at 37°C.

Supernatants were collected from the wells and centrifuged at 800 g for 5 min at 4°C to remove remaining cells, then stored at −50°C until the GLP-1 concentration was measured with a commercial enzyme immunoassay (ELIA) kit (Yanaihara Institute, Shizuoka, Japan). The GLP-1 assay detects both intact GLP-1(7-36) and metabolite GLP-1(7-36) with 100 and 95.6% cross-reactivity, respectively. Cross-reactivities for GLP-1(1-37), GLP-1(1-36)amide, and GLP-1(7-37) are <0.1, 0.3 and <0.1%, respectively. The minimum detection limit of the assay is 60 pM for GLP-1(7-36) amide. Intra- and interassay coefficients for the rat plasma are 5.36–6.60 and 5.51–18.87%, respectively.

Animal Experiments (Experiments 2–5)

Male Sprague-Dawley rats (7 wk old) were purchased from Japan SLC (Hamamatsu, Japan) and were fed with semipurified diets containing 25% casein based on AIN-93G (28) for 3–7 days. After a 24-h fast, in situ experiments were carried out under anesthesia with ketamine (80 mg/kg body wt, Ketalar, Daiichi Sankyo, Tokyo, Japan) mixed with xylazine (12 mg/kg, MP Biomedicals, Irvine, CA). During the experiment, additional ketamine (200 mg/kg body wt, Ketalar, Daiichi Sankyo, Tokyo, Japan) was injected to keep the rats anesthetized, and body temperature was maintained with a heating pad.

Blood samples (100 μl) for GLP-1 measurement were drawn into a syringe containing aprotinin (final concentration at 200 KIU/ml, Wako) and heparin (final concentration at 50 IU/ml, Ajinomoto, Tokyo, Japan) through a portal catheter (experiments 2–4) or directly from the mesenteric veins (experiment 5). Plasma was separated from blood samples by centrifugation at 2,500 g for 15 min at 4°C and then frozen at −80°C until GLP-1 measurements were taken. Plasma GLP-1 concentrations were measured with a GLP-1 ELISA kit as described above. Because secreted GLP-1 (active GLP-1) is immediately degraded by DPP-IV, changes in active GLP-1 are affected by both GLP-1 secretion and DPP-IV activity in the plasma at that time of blood collection. Therefore changes in total GLP-1 level accurately reflect actual GLP-1 secretion, and changes in total GLP-1 level from a basal state (ΔGLP-1) are presented in results in the animal experiments.

The experiments were performed in a temperature-controlled room maintained at 23 ± 2°C with a 12-h light-dark cycle (0800–2000, light period). The study was approved by the Hokkaido University Animal Committee, and the animals were maintained in accordance with the guidelines for the care and use of laboratory animals of Hokkaido University.

Experiment 2: effects of duodenal or ileal administration of ZeinH on GLP-1 secretion in anesthetized rats. A middle abdominal incision was made in rats under anesthesia. The small tip (7–8 mm) of a polyethylene catheter (SP 28; ID 0.4 mm, OD 0.8 mm; Natsume Seisakusho, Tokyo, Japan) connected to a silicone catheter (Silascon no. 00, ID 0.5 mm, OD 1.0 mm; Dow Corning, Kanagawa, Japan) was inserted into the portal vein. A basal (0 min) blood sample (100 μl) was drawn from the portal catheter, and the catheter was filled with saline containing heparin (50 IU/ml) between each blood sampling. Desalting water (2 ml) or test liquids (200, 900, 450, and 90 mg glucose in 2 ml) were then directly administered into the duodenum or ileum (45 cm distal to the ligament of Treitz). Portal blood was collected through the portal catheter at 15, 30, 60, 90, and 120 min after the administration.

Experiment 3: effects of ZeinH administration into the ligated duodenal, jejunal, and ileal loops on GLP-1 secretion. A catheter was inserted into the portal vein as described above. A ligated duodenal, jejunal, or ileal loop was prepared separately in each individual rat. The duodenal loop was prepared between the pylorus and the ligament of Treitz. The jejunal loop (20 cm in length) was prepared between 5 and 25 cm distal to the ligament of Treitz, and the ileal loop (20 cm in length) was prepared between 5 and 25 cm proximal to the cecum. The proximal and distal ends of the loops were ligated with a silk thread. After basal (0 min) blood collection, ZeinH (300 mg in 1 ml water) was directly injected into the loops. Portal blood was collected through the portal catheter at 15, 30, 60, 90, and 120 min after the administration. To examine osmotic effects of the liquid administered into the ligated intestinal loop, a 2.58% (wt/vol) NaCl solution with the same osmolality (892 mosM) as 300 mg/ml ZeinH was administered into the duodenal- and ileal-ligated loop, respectively. Portal blood was collected before (0 min), 30, and 60 min after administration. The osmolarity of samples was determined by the freezing point depression method (SRL, Tokyo, Japan).

Experiment 4: effects of vagal denervation on GLP-1 secretion induced by ZeinH in the ligated duodenal, jejunal, and ileal loops. Rats were treated with capsaicin (Sigma Chemical, St. Louis, MO) or the vehicle (olive oil-Tween 20 ratio = 8:2) as described previously (17). A small piece of gauze soaked with the capsaicin (Sigma Chemical, St. Louis, MO) solution (10 mg/ml, 0.2 ml/rat) or the vehicle (0.2 ml/rat) was applied to the abdominal vagal trunk for >30 min. During treatment with capsaicin or the vehicle, a portal vein catheter and the ligated duodenal, jejunal, or ileal loop were prepared as described above. After basal (0 min) blood collection, ZeinH (300 mg in 1 ml water) was directly injected into the loop. Portal blood was collected through the catheter before (0 min) and at 15, 30, 60, 90, and 120 min after the administration of ZeinH.

RT-PCR. RNA was isolated from the small and large intestinal mucosa using Trizol (Invitrogen). cDNA was prepared from 1 μg RNA by using Reversetranscriptase I reverse transcriptase (Wako) and subjected to PCR using primers based on the rat proglucagon mRNA sequence (GenBank accession no. NM_012707; forward primer 5’-cattcagagccatcact-3’, reverse primer 5’-ctctggtagcaggttac-3’) and rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA sequence (GenBank accession no. NM_017008, forward primer 5’-tccacaccgtgtttcg-3’, reverse primer 5’-gacacacaccgctg-3’). PCR conditions were 95°C for 5 min, then 30 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s. PCR products were separated...
by 1.5% agarose gel electrophoresis and visualized by ethidium bromide staining. Data was expressed as the relative band intensity of proglucagon against GAPDH.

Experiment 5: effects of duodenal ZeinH on local GLP-1 secretion into the duodenal vein and ileal mesenteric vein. A duodenal loop was prepared as described above. Blood samples were drawn directly from the duodenal vein and the ileal mesenteric vein from the same rats by using a syringe with a needle (Mjyector 29G × 1/2", Terumo, Tokyo, Japan) (23). ZeinH (300 mg in 1 ml water) was directly administered into the duodenal loop and blood was collected at 0, 30, and 60 min after the administration. The blood was drawn once from one of the branches of the duodenal vein or ileal mesenteric vein at each time point. At the next time point, blood was drawn from the branch next to the previously sampled branch.

Experiment 6: effects of various dietary peptides administered into the duodenum on ileal GLP-1 secretion. MHY and AEH were administered into the duodenal ligated-loop, as well as ZeinH (300 mg in 1 ml). The small tip (5 mm) of a polyethylene catheter (SP 10; ID 0.28 mm, OD 0.61 mm; Natsume Seisakusyo, Tokyo, Japan), connected to a silicone catheter (Silascon no. 00, ID 0.5 mm, OD 1.0 mm; Dow Corning, Kanagawa, Japan), was inserted into one of the ileal mesenteric veins. The blood was collected through the catheter before (0 min) and after (30, 60 min) the duodenal administration of peptides. The catheter was filled with saline containing heparin (50 IU/ml) between each blood sampling.

Statistical Analysis

Results are expressed as means ± SE. Statistical significance was assessed by one-way or two-way ANOVA, and significant differences among mean values were determined by the Tukey’s HSD post hoc test (P < 0.05).

RESULTS

Experiment 1: ZeinH Strongly Stimulates GLP-1 Secretion From GLUTag Cells

GLUTag cells were exposed to several protein hydrolysates, BSA, and depolarization stimuli (70 mM KCl). The unhydrolyzed protein BSA did not induce GLP-1 secretion. AEH and MHY were chosen because they are effective in inducing GLP-1 secretion from another enteroendocrine cell line, STC-1 (7). CBP was also found to stimulate CCK secretion from STC-1 cells in our previous study (35). At a concentration of 5 mg/ml, these hydrolysates induced only small increases in GLP-1 secretion (Fig. 1A). However, ZeinH prepared from the major corn protein zein induced significant (P < 0.01) and the highest increase in GLP-1 secretion in a dose-dependent manner (Fig. 1, A and B). From these results, we chose ZeinH for the following in situ experiments in rats.

Experiment 2: Administration of ZeinH Into the Duodenum or Ileum Induces Increase in Portal GLP-1 in Anesthetized Rats

To examine the effect of ZeinH on GLP-1 secretion in the rat intestine, water, glucose and ZeinH were administered into the duodenum or ileum of anesthetized rats. ΔGLP-1 are presented in Fig. 2, A and B, since changes after administrations of glucose or ZeinH are relatively small against basal GLP-1 level (Fig. 2, C and D), and basal GLP-1 levels varied (1.5–3.5 nM) among individual rats and separate experiments. The duodenal administration of water and glucose (90 mg) did not cause any significant changes in portal plasma GLP-1 concentration. In contrast, the duodenal administration of ZeinH (200–500 mg) induced a significant increase in GLP-1 concentration at 15–30 min (Fig. 2A).

The ileal administration of water, 90 mg glucose, and 200 mg ZeinH did not cause any statistically significant changes in GLP-1 concentration (Fig. 2B). However, the peak increase in GLP-1 induced by the administration of ileal glucose (0.51 ± 0.09 nM at 30 min) was significantly higher than that (0.03 ± 0.05 nM at 15 min) induced by the duodenal administration of glucose (P < 0.05 by Student’s t-test) (Fig. 2, A vs. B). Increase in GLP-1 by 200 mg ZeinH after the ileal administration (maximum ~0.4 nM) showed a trend to be higher than that after the duodenal administration (maximum ~0.3 nM) (Fig. 2, A vs. B).

The ileal administration of 500 mg ZeinH induced a significant and prolonged increase in portal GLP-1 concentration, and values after the ileal administration of 500 mg ZeinH were significantly higher than those after the duodenal administration at the same time point (P < 0.05 by Student’s t-test). Since the duodenum and ileum were not separated by ligation or segmentation in this experiment, ZeinH administered into the duodenum might reach to the lower segments of the intestine. Therefore, we could not determine whether GLP-1 secretion was stimulated in the duodenum or in distal parts of the intestine by duodenally instilled ZeinH.
Fig. 2. Changes in portal GLP-1 concentrations after the duodenal (A) and ileal (B) administration of water, glucose, and ZeinH solution in anesthetized rats. Portal blood was collected before (0 min) and after (15, 30, 60, 90, and 120 min) the administration of test liquids (2 ml) into the duodenum (A and C) or ileum (B and D). □ Water (n = 7 for A and C, n = 6 for B and D); ○ 90 mg (500 μl) glucose (n = 7 for A and C; n = 9 for B and D); ● 200 mg ZeinH (n = 7 for A, n = 10 for B); ○ 500 mg ZeinH (n = 7 for A and C, n = 9 for B and D). Values are plasma GLP-1 concentration (A and B) from the basal value (0 min); means ± SE. Two-way ANOVA P values for duodenal and ileal administration are all <0.05 for treatment, time, and treatment × time (A and B); the values for duodenal administration are 0.277, <0.05, <0.05; and the values for ileal administration are 0.54, <0.05, <0.05 for treatment, time, and treatment × time, respectively. +Significantly different from the value at 0 min in each group (Tukey’s test, P < 0.05). Plots at the same time point not sharing the same letter differ significantly between treatments (Tukey’s test, P < 0.05).

Experiment 3: Ileal Administration of ZeinH Induces Higher GLP-1 Secretion Than Does Duodenal or Jejunal Administration in Ligated Intestinal Loops of the Anesthetized Rats

ZeinH was administrated into ligated loops prepared in the duodenum, jejunum, or ileum to compare the GLP-1 secretory responses to locally administered ZeinH. The ileal administration of ZeinH induced higher increases in portal GLP-1 concentration than did the duodenal or jejunal administration of ZeinH (Fig. 3, A and B). Increments of GLP-1 level after the administrations of ZeinH were again relatively small against basal GLP-1 levels (Fig. 3B) as well as the results shown in Fig. 2. However, significant and similar increases by both the jejunal and duodenal administration were observed by expressing ΔGLP-1 from basal values (Fig. 3A). Therefore, ΔGLP-1 after luminal administrations of test liquids are presented in the following results. A NaCl solution with the same osmolarity as 300 mg/ml ZeinH did not induce significant GLP-1 secretion after the administration into the duodenal- or ileal-ligated loop (Fig. 3C), indicating that osmotic effects are not responsible for ZeinH-induced GLP-1 secretion.

We compared proglucagon mRNA expression by RT-PCR in the small and large intestinal regions. As GLP-1 peptide is translated from the proglucagon gene, proglucagon mRNA expression reflects GLP-1 expression. Consistent with the distribution of GLP-1-producing cells (13, 25), proglucagon mRNA expression gradually increased from the upper to lower intestine in rats used in the present study (Fig. 4). Duodenal proglucagon expression was significantly lower than both jejunal and ileal expression. The difference between GLP-1 secretion induced by the jejunal and ileal administration of ZeinH (Figs. 2 and 3) seems to reflect the distribution of GLP-1-producing enteroendocrine cells (L cells). Even though the proglucagon expression level in the duodenum appeared lower than that in the jejunum and the ileum, the duodenal administration of ZeinH induced a significant increase in GLP-1 secretion, similar to that of jejunal ZeinH-induced GLP-1 secretion.

Experiment 4: Duodenal Administration of ZeinH Induces GLP-1 Secretion Via a Capsaicin-Sensitive Pathway

The results from experiment 3 (Figs. 3 and 4) suggest that ZeinH administrated into the duodenum indirectly stimulates GLP-1 secretion from L cells in the distal intestine via some signaling pathways. A previous paper demonstrated the involvement of the vagus nerve in duodenal fat-induced GLP-1 secretion (32). To examine whether the vagus nerve mediates in ZeinH-induced GLP-1 secretion, ZeinH was administrated into ligated loops in anesthetized rats after treatment with capsaicin (0.2 mg/rat) or the vehicle (Fig. 5). Consistent with the results from Fig. 3, the ileal administration of ZeinH showed a trend to induce higher GLP-1 secretion than either the jejunal or ileal administration in vehicle-treated rats (one-way ANOVA; P value was 0.06 when values at 120 min in
vehicle-treated rats were compared). Capsaicin treatment did not affect GLP-1 secretion in response to the jejunal or ileal administration of ZeinH (Fig. 5, B and C). In contrast, GLP-1 secretion induced by duodenal ZeinH was abolished in capsaicin-treated rats (Fig. 5A). These results demonstrate that GLP-1 secretion induced by duodenal ZeinH is mediated by vagal afferent pathway.

**Experiment 6: Effects of Various Dietary Peptides Administered Into the Duodenum on Ileal GLP-1 Secretion**

MHY, AEH, and ZeinH were administered into the duodenum, and blood samples were collected from the catheter inserted into the ileal mesenteric vein. Cannulation into the duodenal vein was not possible in the experiment because the duodenal vein was too narrow for the cannulation. Only ZeinH induced a significant increase in GLP-1 level (Fig. 7). MHY and AEH showed a tendency to increase GLP-1 level, but those changes were not statistically significant.

**DISCUSSION**

The secretion of GLP-1 from enteroendocrine L cells is stimulated by luminal nutrients and neuroendocrine factors (6, 9). Fatty acids and glucose are well-known stimuli, and the mechanisms by which these nutrients induce GLP-1 secretion have been investigated in vivo (21, 22, 32) and in vitro (12, 15, 18, 23). However, little is known about dietary peptide-induced GLP-1 secretion. Peptones (AEH or MHY) were shown to induce GLP-1 secretion in the isolated vascularly perfused rat ileum (7, 11) and in enteroendocrine cell lines (STC-1 and NCI-H716 cells) (7, 29). In the present study, we found that a hydrolysate prepared from corn zein potently stimulates GLP-1 secretion in the enteroendocrine cell line GLUTag and that ZeinH directly and indirectly stimulates GLP-1 secretion from ileal L cells in rats in situ intestinal loop experiments.

We first compared the potential of several protein hydrolysates to stimulate GLP-1 secretion in the GLP-1-producing enteroendocrine cell line GLUTag (Fig. 1A). ZeinH prepared from corn zein induced the highest increase in GLP-1 secretion among the hydrolysates tested, including previously used commercial peptones known to stimulate GLP-1 secretion in vitro (11, 7, 29). For this reason, we used ZeinH in subsequent rat experiments. Zein protein has low digestibility due to its strong
hydrophobicity (19, 33); however, in vitro digestion products from zein possess antioxidant or angiotensin-converting enzyme-inhibiting activities (39, 40). In the present study, we found that in vitro digestion with pepsin and chymotrypsin liberates the active structures of zein to stimulate GLP-1 secretion from L cells. Further investigations are necessary to identify the active fragments in ZeinH and the intracellular signaling pathways by which GLP-1 secretion is stimulated. It was previously shown that AEH and MHY at 20–50 mg/ml induced significant GLP-1 secretion in STC-1 and NCI-H716 cells, respectively (7, 29), but both AEH and MHY at 5 mg/ml induced only slight increase in GLP-1 secretion in GLUTag cells in the present study. These differences are thought to be due to differences in the hydrolysate concentrations tested. CBP strongly stimulated CCK secretion in STC-1 cells in our previous study (35); however, CBP is not a strong stimulant of GLP-1 secretion from GLUTag cells. A possible explanation of these differences is that the sensitivity to peptides may differ between CCK-producing I cells and GLP-1-producing L cells.

ZeinH was administered directly into the duodenum or ileum to examine GLP-1-releasing activity in rats in experiment 2. Although ZeinH induced a significant increase in portal GLP-1 after both duodenal and ileal administration, the increase in GLP-1 level appeared higher and longer lasting after the ileal administration than after the duodenal administration of ZeinH (Fig. 2). This suggests site-dependent GLP-1 secretion in response to a peptide, ZeinH, as well as in response to other nutrients (glucose and fatty acids) (30). However, the intestinal tissue was not segmented in that experiment; we cannot exclude the possibility that duodenally administered ZeinH flowed into the ileal lumen induced GLP-1 secretion.

Glucose at 90 mg/2 ml (250 mM) administered into the duodenum and the ileum failed to induce significant increases in portal GLP-1 (Fig. 2). A previous paper demonstrated that ileal perfusion of 250 mM glucose induced GLP-1 secretion in a vascular perfusion system in rats (250–300 g body wt) (11). In that paper, the authors administered a total of 337.5 mg glucose in 30 min of perfusion. In another study (30), 375 mg glucose in the ileum but not in the duodenum was effective to induce GLP-1 secretion. Thus a higher dose of glucose than the present study may be necessary to stimulate GLP-1 secretion in our in situ experiment.

GLP-1 secretory responses to ZeinH administered into different parts of the intestine were further investigated by using ligated intestinal loops in experiment 3. As expected, ZeinH administered into the ileal loop induced the highest increase in GLP-1 secretion among the three regions (duodenum, jejunum, and ileum) (Fig. 3A). This seems to reflect the intestinal distribution of proglucagon gene expression, which is increased along with the axis from the proximal to the distal intestine (13, 25). However, GLP-1 secretion in response to the duodenal administration of ZeinH was similar to that in response to the jejunal administration, although duodenal proglucagon expression is apparently lower than ileal proglucagon expression (Fig. 4). These results suggest that ZeinH directly acts on L cells to stimulate GLP-1 secretion in the jejunum and the ileum but indirectly stimulates the secretion in the duodenum.

In the case of fatty acid-induced GLP-1 secretion, indirect stimulation has been proposed as a “proximal-distal enteroendocrine loop” (6, 32). In experiment 4, vagal afferent denervation by the capsaicin treatment completely abolished duodenal ZeinH-induced GLP-1 secretion, but the treatment did not affect GLP-1 secretion induced by the jejunal or ileal administration of ZeinH (Fig. 5). These results strongly support the hypothesis that dietary peptides in the jejunum and ileum directly stimulate GLP-1 secretion from enteroendocrine L cells, but the peptides in the duodenum indirectly stimulate GLP-1 secretion. A previous paper has shown that peptone induces glucose-dependent insulinotropic peptide (GIP) secretion in rats (38), suggesting that GIP (30), acetylcholine (2, 5), and gastrin-releasing peptide (31) may have roles in the dietary peptide-induced “indirect” GLP-1 secretion mediated by the afferent vagus, as well as in fat-induced indirect GLP-1 secretion. Experiments were carried out under ketamine-anaesthetized conditions in the present study. Ketamine has been used in several studies on intestinal motility. Previous studies demonstrate that ketamine does not influence the ileal cholinergic contractions induced by exogenous acetylcholine (20), and vagal activation is induced by luminal nutrients in ketamine-anaesthetized rats (34). The anesthesia may have little effect on neuronal activities of the intestine in the present study.

Direct blood collection from the duodenal vein and the ileal mesenteric vein enabled us to observe local GLP-1 secretion from the duodenum and ileum (experiment 5). Administration of ZeinH into the ligated duodenal loop induced an increase in GLP-1 levels in the ileal mesenteric vein, but not in the duodenal vein (Fig. 6). This result confirms that duodenal ZeinH-induced GLP-1 secretion from the ileal L cells occurs via an indirect mechanism. Although proglucagon mRNA expression was detectable in the duodenum, no increase in GLP-1 in the duodenal vein was observed. GLP-1-producing cells in the duodenum might possess only low sensitivity to dietary peptides or might not release enough GLP-1 to be detectable in the present experiment. Thus an important question arises for investigation in future studies as to whether the
same type of enteroendocrine cells, for example GLP-1-producing L cells, in different regions possess the same degree of sensitivity to specific nutrients. Neither MHY nor AEH increased significantly but showed a tendency to increase in GLP-1 in the ileal mesenteric vein after the duodenal administration of those peptides (Fig. 7). Those peptides might require a much higher amount to induce a significant increase in ileal GLP-1 as indicated in GLUTag cell experiment (Fig. 1). These results suggest that the indirect stimulation of GLP-1

![Fig. 6](image_url)

Fig. 6. Changes in plasma GLP-1 concentrations in the duodenal vein and ileal mesenteric vein after the administration of ZeinH into ligated duodenal loop. A ligated duodenal loop was prepared in anesthetized rats. Blood samples were collected from the duodenal vein (●, n = 8) and ileal mesenteric vein (○, n = 18) before and after duodenal ZeinH administration (300 mg in 1 ml water). One-way ANOVA P values were 0.56 and 0.023 for the duodenal vein and ileal mesenteric vein, respectively. *Significantly different from the value at 0 min (Tukey’s test, P < 0.05).

![Fig. 7](image_url)

Fig. 7. Changes in plasma GLP-1 concentrations in the ileal mesenteric vein after the administration of ZeinH, MHY, and AEH into ligated duodenal loop. A ligated duodenal loop was prepared in anesthetized rats. Blood samples were collected from the catheter inserted into the ileal mesenteric vein before (0 min) and after (30, 60 min) the duodenal administration (300 mg in 1 ml water) of ZeinH (●, n = 7), MHY (▲, n = 7), and AEH (■, n = 7). Two-way ANOVA P values were 0.27, <0.01, and 0.7 for treatment, time, and treatment × time, respectively. *Significantly different from the value at 0 min (Tukey’s test, P < 0.05).
GLP-1 secretion may not be specific only for ZeinH, but it relies on the potency or amount of the peptide to induce significant secretion of GLP-1.

GLP-1 secretion induced by dietary peptides has been demonstrated in previous papers, but the stimulatory mechanism was not well investigated. In the present study, we found that even though it requires relatively higher amount (300–500 mg/rat) than fatty acids (oleic acid: 150 mM × 3 ml = 127 mg/rat) (1), the peptide ZeinH is able to stimulate GLP-1 secretion by both direct and indirect mechanisms. In case of fasted rats, 6–10 g of diet (AIN-93G based diet containing 20–25% casein) was consumed within 1 h (3, 26). This amount of diet contains 1,500–2,000 mg casein as a protein source. Thus 300–500 mg of protein/peptide is possible to present in the intestinal lumen in the postprandial state. Present results suggest that such dual regulation (6) is common in nutrient-induced GLP-1 secretion in vivo. Understanding GLP-1 secretion mechanisms by dietary peptides provides opportunities to control GLP-1 secretion by orally ingested GLP-1-releasing peptides such as ZeinH for attenuating postprandial hyperglycemia in humans.

Local GLP-1 secretion was observed by direct collection of the blood from the ileal mesenteric vein (Fig. 6). We further developed a method for cannulation into the mesenteric vein (experiment 6). By using these methods, indirect GLP-1 secretion from the ileum induced by duodenal nutrient was demonstrated for the first time. The mesenteric cannulation method should be widely applicable for studying local secretion of gut hormones and absorption of luminal nutrients. However, this method is only applicable under anesthetized conditions at the present time.

The question remains as to the physiological relevance of the indirect stimulation of GLP-1 secretion by the duodenal dietary peptides. As it is unusual for mammals to ingest a single nutrient, the presence of dietary peptides in the duodenum may be recognized as a representative of a "meal" to signal the presence of carbohydrates and upcoming sugar absorption. Therefore, GLP-1 secretion stimulated by duodenal dietary peptides may help to prevent hyperglycemia via the immediate stimulation of insulin secretion from the pancreatic β-cells. GLP-1 also has roles in the inhibition of gastric emptying and suppression of food intake (4). The indirect stimulation of GLP-1 secretion by duodenal nutrients may also be involved in these functions.

In summary, we found that a hydrolysate prepared from corn zein (ZeinH) strongly stimulates GLP-1 secretion from the murine GLP-1-producing enteroendocrine cell line, GLUTag. ZeinH administered into the ileum induced higher GLP-1 secretion than that administered into the duodenum or jejunum in anesthetized rats. Treatment of the vagal afferent nerve with capsaicin abolished GLP-1 secretion induced by ZeinH instilled in the duodenum, but not in the jejunum or ileum. These results suggest that dietary peptides in the duodenum indirectly stimulate GLP-1 secretion via the vagal afferent pathway, but peptides in the jejunum and ileum directly act on L cells to stimulate GLP-1 secretion. Collection of blood directly from the duodenal vein and the ileal mesenteric vein confirmed that ZeinH in the duodenum induces GLP-1 secretion from ileal L cells via an indirect mechanism. These findings indicate that direct and indirect regulations of GLP-1 secretion mediate not only fat-induced GLP-1 secretion but also dietary peptide-induced GLP-1 secretion in the intestine.

GRANTS

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REFERENCES


