Secretion of the Intestinotrophic Hormone Glucagon-like Peptide 2 Is Differentially Regulated by Nutrients in Humans

QIANG XIAO, * ROBIN P. BOUHEY, † DANIEL J. DRUCKER, ‡§ and PATRICIA L. BRUBAKER * ‡

Departments of *Physiology and † Medicine and § Banting and Best Diabetes Centre, University of Toronto and the Toronto Hospital, Toronto, Ontario, Canada

See editorial on page 261.

Background & Aims: Glucagon-like peptide 21-33 (GLP-21-33), an intestinally derived hormone, stimulates growth in rodent small and large bowel. To explore the physiology of GLP-21-33 secretion, we measured plasma GLP-2 levels in 6 healthy male volunteers, before and after test meals. Methods: Blood samples were collected over 24 hours with the subjects consuming a normal, solid mixed diet (2500 kcal) and for 4 hours after liquid test meals (400 kcal/300 mL) composed of carbohydrate, fat, or protein. All studies commenced at 9 am. Plasma was extracted and analyzed in radioimmunoassays for N-terminal immunoreactive GLP-2 (N-IR-GLP-2; measures bioactive GLP-21-33) as well as total IR-GLP-2 (T-IR-GLP-2), which includes GLP-21-33, GLP-23-33 (an inactive degradation product of GLP-21-33), and the pancreatic major proglucagon fragment (an inactive precursor that contains GLP-2). Basal and nutrient-stimulated plasma samples were also analyzed by high-performance liquid chromatography to determine the levels of GLP-21-33 and GLP-23-33. Results: N-IR-GLP-2 levels were increased 2.0 ± 0.2- to 2.8 ± 0.5-fold 40 minutes after each mixed meal (P < 0.05-0.01) and returned to basal overnight, whereas T-IR-GLP-2 levels were increased 1.3 ± 0.1-fold 40 minutes after breakfast only (P < 0.05). After ingestion of carbohydrate or fat alone, plasma N-IR-GLP-2 concentrations increased by 5.6 ± 2.0- and 2.7 ± 0.6-fold within 1 hour (P < 0.05). High-performance liquid chromatography analysis showed a relative increase in the levels of GLP-21-33 compared with GLP-23-33 (P < 0.05). Ingestion of the protein meal did not alter N-IR-GLP-2 levels, whereas T-IR-GLP-2 was increased by fat and protein (by 1.7 ± 0.2-fold for each, P < 0.01) but not by carbohydrate. Conclusions: These results show that secretion of GLP-21-33 from the intestine is regulated in a nutrient-dependent manner in normal humans.

In mammals, tissue-specific posttranslational processing of proglucagon by the prohormone convertases gives rise to different proglucagon-derived peptides in the pancreatic A and intestinal L cell.1-3 The major products of proglucagon processing in the intestine are glucagon-like peptide (GLP)-1 and GLP-21-33, whereas glucagon and the biologically inactive major proglucagon fragment (MPGF), which contains both GLP-1 and GLP-2, are generated in the pancreas. An additional form of GLP-2 is also found in the circulation, GLP-23-33, which represents the product of dipeptidylpeptidase IV (DP IV)-mediated inactivation of GLP-21-33 (Figure 1).4-6

Both glucagon and GLP-1 are well established as regulators of glucose homeostasis.7,8 It is only recently, however, that a biological role for GLP-2 as an intestinal growth factor has been reported.9 Administration of GLP-2 to rodents significantly increases small intestinal weight, through stimulation of crypt cell proliferation and inhibition of villus apoptosis, resulting in enhancement of villus height.5,9,10-12 GLP-2 also stimulates mucosal proliferation in the large bowel, and studies to date suggest that the actions of GLP-2 are specific to the gastrointestinal tract.9,11,12 Importantly, intestinal growth induced by GLP-2 appears to represent functionally normal, differentiated intestine, with significant increases in brush border enzyme activities and nutrient absorption after GLP-2 administration.13-15 In the short term, GLP-2 has also been reported to enhance glucose transporter activity14 as well as to exert inhibitory effects on gastric motility.15

More recent findings have indicated a potential role for GLP-2 in the treatment of intestinal insufficiency because GLP-2 therapy prevents total parenteral nutrition (TPN)-induced atrophy of the small bowel in rats.17 GLP-2 also reduces the severity of colonic injury in a murine model of experimental colitis12 and enhances the rate and magnitude of the intestinal adaptive response to massive small bowel resection in rats.15

Abbreviations used in this paper: DP IV, dipeptidylpeptidase IV; GLP-21-33, glucagon-like peptide 21-33; HPLC, high-performance liquid chromatography; IR, immunoreactive; MPGF, major proglucagon fragment; TFA, trifluoroacetic acid.

© 1999 by the American Gastroenterological Association 0016-5085/99/$10.00.
Figure 1. Schematic representation of different GLP-2-containing peptides. The binding sites for antisera UTH7 (circles) and #92160 (arrowhead) are indicated.

The establishment of a biological action for GLP-2 prompted the present investigation into the physiological regulation of GLP-2 secretion. We measured the changes in circulating GLP-2 levels over 24 hours with a normal diet. The GLP-2 response to the individual nutrient components of a meal in healthy humans was also assessed. Because determination of GLP-2-133 levels in the plasma by radioimmunoassay (RIA) is confounded by the presence of cross-reacting antigenic sites in GLP-233 and MGP (Figure 1), site-specific RIA and high-performance liquid chromatography (HPLC) were used throughout this study to permit differentiation of the different molecular forms of GLP-2 in the circulation.

Materials and Methods

Sample Collection

Six healthy male students aged 23 ± 1 years with a body mass index of 24 ± 1 kg/m² participated in the study. After an overnight fast, an intravenous needle was inserted into an antecubital vein and was kept patent by a slow infusion of normal saline. Blood samples were taken at 10-60-minute intervals over 24 hours with the subjects consuming a normal diet or over 4 hours after one of the three liquid test meals.

The 24-hour diet consisted of 2500 kcal, with 51.3%, 29.3%, and 19.5% of the calories derived from carbohydrate, fat, and protein, respectively, as follows: 9 AM breakfast (549 kcal: 100.0 g carbohydrate, 9.6 g fat, and 16.5 g protein); 11 AM snack (168 kcal: 24.8 g carbohydrate, 6.5 g fat, and 3.6 g protein); 1 PM lunch (582 kcal: 74.3 g carbohydrate, 22.7 g fat, and 23.8 g protein); 3 PM snack (135 kcal: 9.0 g carbohydrate, 8.3 g fat, and 6.1 g protein); 6 PM dinner (742 kcal: 77.4 g carbohydrate, 20.9 g fat, and 62.2 g protein); and 10 PM snack (351 kcal: 41.7 g carbohydrate, 15.0 g fat, and 12.1 g protein). Each meal was consumed over a 15-minute period, and each snack over 5 minutes.

The three liquid breakfasts, given at 9 AM in random order, were each composed of 400 kcal in a total volume of 300 mL, as follows: (1) carbohydrate (5 g dextrose added to 145 g Glucodex, 100 g Rougier Inc., Montreal, Quebec, Canada); (2) fat (35% whipping cream); and (3) protein (95 g Promod whey protein, Ross Products, Montreal, Quebec, Canada). All meals were stored at 4°C before serving, and a small amount of vanilla flavoring with no caloric value was added to the fat and protein meals. Each meal was consumed over a 5-minute period.

All blood samples (6 mL per sample) were collected into a 10% volume of Trasylol-EDTA-Diprotin A (5000 kallikrein-inhibitory units/mL; Miles Canada, Etobicoke, Canada). 1.2 mg/mL 0.1 mmol/L (an inhibitor of DP IV activity; Sigma Chemical Co., St. Louis, MO), and plasma was collected and stored in −70°C until extraction. All blood samples were obtained with informed consent under protocols approved by the Human Ethics Committee at the Toronto Hospital (Toronto, Ontario, Canada).

Peptide Extraction and RIA

The day before RIA, plasma samples were acidified by addition of two volumes of 1% trifluoroacetic acid (TFA; pH adjusted to 2.5 with diethylamine), and peptides were extracted by passage through a C18 SepPak cartridge (Waters Associates, Milford, MA). After washing with 0.1% TFA, the adsorbed peptides were eluted with 80% isopropanol containing 0.1% TFA. Recovery of GLP-2 with this extraction technique is 84% ± 17%. Alliquots of peptide eluates were then dried under vacuum for RIA.

RIA for GLP-2 were performed using antisera UTH7 or #92160, as described previously.4,13 Antisera UTH7 has no cross-reaction with GLP-1, glucagon, or any other structurally related peptide but recognizes the midsequence of GLP-2 (amino acids 25-30) and, thus, cross-reacts equally with intact GLP-2-133, GLP-233, and MGP (Figure 1). Therefore, immunoreactive (IR) GLP-2 detected using antisera UTH7 reflects total levels of GLP-2 in the plasma (T-IR-GLP-2). Antisera #92160 (a generous gift from Dr. J. J. Holst, Copenhagen, Denmark) recognizes the N-terminus of GLP-2-33 (N-IR-GLP-2), but does not cross-react with GLP-233 or any other related peptide.4,13 All samples from a given experiment were assayed together within each RIA. The intra-assay variations were 3.2% and 5.8%, and the concentrations at 50% inhibition of binding were 125 and 200 pg/tube for the UTH7 and #92160 RIA, respectively. The sensitivity of both assays was 10 pg/tube.

Reverse-Phase HPLC

All samples were subjected to SepPak extraction before HPLC, as described above. HPLC was performed using a Waters system with a C18 μBondapak column (Waters Associates). The solvent systems used were (A) 0.1% TFA in water and (B) 0.1% TFA in acetonitrile. GLP-2-133 and GLP-233 in plasma were separated using a gradient of 30%-60% solvent B over 45 minutes, followed by a 10-minute purge with 99% solvent B. The solvent flow rate was 1.5 mL/min, and 18-second fractions were collected.4 All fractions were dried under vacuum before RIA using antisera UTH7. Peptide recovery was close to 100% using this procedure.
Data Analysis

All data are presented as mean ± SEM. Statistical differences between basal and peak values of IR-GLP-2 were determined by the paired Student t test. Basal values were defined as the lowest GLP-2 concentration taken at the time of feeding, whereas peak values were taken as the highest GLP-2 concentrations within 60 minutes of feeding.

Results

24-Hour Study

Plasma levels of N-IR-GLP-2 increased significantly after each meal to 277% ± 45%, 195% ± 23%, and 228% ± 33% of premeal values within 40 minutes of ingestion of mixed nutrients at breakfast (P < 0.05), lunch (P < 0.01), and dinner (P < 0.05), respectively (Figure 2). N-IR-GLP-2 levels were not elevated in response to ingestion of any of the snacks. T-IR-GLP-2 levels also increased after ingestion of breakfast to 132% ± 10% of basal values (P < 0.05); however, no other meal or snack significantly elevated circulating T-IR-GLP-2 concentrations. The levels of both N-IR-GLP-2 and T-IR-GLP-2 returned to basal overnight.

Specific Nutrient Challenges

N-IR-GLP-2 levels started to increase within 10 minutes of carbohydrate ingestion, reaching 560% ± 199% (P < 0.05) of basal levels within 45 minutes and then declining to basal levels by 150 minutes (Figure 3). Similarly, fat ingestion also increased plasma concentrations of N-IR-GLP-2 to 274% ± 60% (P < 0.05) of controls by the 60-minute time point, followed by a gradual decline (Figure 4). A later plateau in GLP-2 secretion was also seen in response to these meals, at 90–120 minutes for the carbohydrate (P < 0.05) and at 150 minutes for the fat (P < 0.05) breakfasts. In contrast, ingestion of an isocaloric protein meal did not affect circulating levels of N-IR-GLP-2 (Figure 5).

The liquid carbohydrate breakfast did not affect levels of T-IR-GLP-2 in the circulation, whereas ingestion of fat induced a biphasic release of T-IR-GLP-2, with peaks at 169% ± 20% (P < 0.001) and 196% ± 45% of controls, at 45 and 90 minutes, respectively (Figures 3 and 4). The protein meal also increased secretion of T-IR-GLP-2 to 166% ± 15% of controls (P < 0.01) at 45 minutes (Figure 5).

Reverse-Phase HPLC

Basal, fasting plasma samples contained both GLP-21-33 and GLP-22-33, in a ratio of 1:2.2 ± 0.3 (Figure 6). Analysis of peak areas after ingestion of either carbohydrate or fat showed a 343% ± 145% increase in the levels of GLP-21-33 but only a 149% ± 23% increase in GLP-22-33 levels, thereby decreasing the ratio of GLP-21-33 to GLP-22-33 to 1:1.2 ± 0.3 (P < 0.05).

Discussion

The intestinotrop peptide GLP-21-33 is synthesized and secreted by the L cell of the distal ileum and
Figure 4. (A) Changes in plasma concentrations of N-IR-GLP-2 (●) and T-IR-GLP-2 (▲) over a 4-hour period with a 400-kcal (300 mL) fat breakfast in normal fasted human volunteers (n = 4–6). All studies started at 9 AM. Plasma samples were extracted before RIA. (B) Individual N-IR-GLP-2 responses, with different symbols used for each volunteer (as in Figure 3).

Figure 5. (A) Changes in plasma concentrations of N-IR-GLP-2 (●) and T-IR-GLP-2 (▲) over a 4-hour period with a 400-kcal (300 mL) protein breakfast in normal fasted human volunteers (n = 6). All studies started at 9 AM. Plasma samples were extracted before RIA. (B) Individual N-IR-GLP-2 responses, with different symbols used for each volunteer (as in Figure 3).

Figure 6. HPLC profiles of fasting (basal) and peak (stimulated) plasma samples (2 mL each) from normal fasted human volunteers fed a 400-kcal (300 mL) fat breakfast. The profiles shown are representative of 2 fat and 2 carbohydrate meals. Plasma samples were extracted before HPLC, and fractions were subjected to RIA for T-IR-GLP-2.

Although both GLP-21-33 and GLP-23-33 were found to be increased in response to feeding in these studies, there was a relatively greater increase in the levels of GLP-21-33 during the first hour. We have previously shown the...
presence of both GLP-21-33 and GLP-23-33 in plasma from fasting and fed humans, with approximately equal amounts of the two peptides present in the fed state, whereas other studies investigating the secretion of GLP-2 in humans have not differentiated between these two forms of GLP-2.2 The increase in GLP-21-33 levels in humans fed either carbohydrate or fat is presumably due to increased release from the L cell before degradation of the peptide by DP IV. GLP-21-33 levels in the circulation of rats are regulated via both DP IV-mediated degradation and renal clearance4,5,25, thus the subsequent decline in levels of N-IR-GLP-2 is probably mediated by one or both of these mechanisms.

N-IR-GLP-2 levels were found to increase after each of the mixed meals in the 24-hour study and in response to the carbohydrate and fat, but not the protein, breakfasts. These findings indicate a stimulatory effect of glucose and fat on the intestinal L cell, consistent with previous observations for the secretion of GLP-1 and PYY in humans.19-21,23 Direct stimulatory effects of both glucose and long-chain monounsaturated fatty acids have previously been reported for the rat L cell,26,27 in keeping with our findings in the human study. Although no effect of the 400 kcal protein (whey) meal was observed in the present study, or in a study on GLP-1 release in response to 115 kcal of homogenized turkey,9 other studies have found a small GLP-1 response to protein (375 kcal of grilled turkey) or amino acid (100 kcal) ingestion in humans,20,21 and a recent report has indicated that the rat intestinal L cell is sensitive to peptones in vitro.28 Because the sources and amounts of protein administered in each study differ, the significance of any protein effect on the L cell remains to be clearly established.

The results of this study suggest that the L cell may be sensitive to caloric loading, in addition to the type of nutrient ingested. Solid meals and liquid carbohydrate and fat breakfasts >400 kcal all stimulated secretion of N-IR-GLP-2 within 1 hour of ingestion, whereas none of the <400 kcal snacks induced a response. Previous studies by Schirra et al.29 have shown that a caloric load of >1.4 kcal/min into the duodenum is required for secretion of GLP-1 in normal humans. How this mechanism is mediated in humans is unclear; however, studies in rats have indicated that the L cell is regulated by nutrient ingestion via two distinct mechanisms, with early indirect effects of nutrients in the proximal gut being transmitted to the distal L cell through both the duodenal hormone glucose-dependent insulinotropic hormone and the vagus nerve.25,30,31 Regulation of PYY secretion by ingested nutrients has similarly been reported to involve both the extrinsic nervous system and cholecystokinin (CCK) in dogs.32 These findings therefore suggest that not only a minimum caloric load may be required to be present in the duodenum to stimulate the early phase of GLP-2 release as observed in the present study, but also that this pathway may be activated by carbohydrate and fat but not by protein. The mechanisms underlying the regulation of GLP-2 secretion in human subjects remain to be elucidated, but likely involve the vagus nerve and/or relevant duodenal hormones.

In contrast to the early and indirect effects of nutrients on GLP-2 secretion, later effects of nutrients (e.g., 1–3 hours after nutrient ingestion) have been proposed to be exerted directly at the level of the L cell, after passage of those nutrients from the proximal small bowel to the distal ileum.28,27 Consistent with this suggestion, a second smaller peak of GLP-2 release was observed in the volunteers fed carbohydrate or fat (at approximately 1.5–2.5 hours), as has also been observed for the secretion of GLP-1.19,20

Although the luminal regulation of GLP-2 secretion from the distal intestine is similar to that of other ileal peptides (e.g., PYY), it differs from that reported for several proximal intestinal hormones, including CCK. CCK release is stimulated by mixed meals, with long-chain fatty acids and protein being the most potent nutrients.33 However, like the early phase of carbohydrate- and fat-induced GLP-2 release, peptone-stimulated CCK release also seems to be indirect, involving both submucosal enteric neurons and a CCK-releasing peptide.34 Thus, the regulation of hormone secretion from enteroendocrine cells seems to be complex, with both direct and indirect effects of nutrients being exerted on the cells, often in a temporally dependent fashion.

Changes in T-IR-GLP-2 were found to be distinct from those of N-IR-GLP-2, consistent with the presence of large amounts of pancreatic MPFG in the circulation.4,22 Pancreatic glucagon release is known to be inhibited by glucose and stimulated by fat and protein,35 and these changes presumably occur in parallel with those of MPFG, which is coordinately released from proglucagon with glucagon.1,22 Because N-IR-GLP-2 levels were more than one quarter those of T-IR-GLP-2 in the present study, these findings emphasize the need for site-specific RIAs and/or HPLC analysis of the molecular forms of GLP-2 in any analyses of GLP-2 secretion in vivo.

The discovery that GLP-2 is a locally synthesized endogenous regulator of intestinal mucosal growth in rodents prompted us to carry out the present studies that indicate human GLP-2 is secreted in a nutrient-dependent manner. Indeed, TPN-fed rats develop mucosal atrophy of the small bowel that is prevented by intravenous coadministration of GLP-2 with the TPN infusate.17 These findings have potential clinical implica-
tions for understanding the importance of enteral nutrition and subsequent stimulation of trophic factors that maintain the structural and functional integrity of the human mucosal epithelium. Current treatment strategies for patients with intestinal inflammation or ulceration, or for patients who may be fasted for prolonged periods of time because of intercurrent illness or surgery, involve parenteral replacement of nutrition requirements via intravenous feeding. In such patients, the lack of enteral nutrition may be associated with relative GLP-2 deficiency, leading to structural and functional changes in the mucosal epithelium that may predispose, in some patients, to an increased risk of sepsis due to increased mucosal permeability. A subset of these patients may theoretically benefit from concomitant therapeutic strategies designed to either enhance endogenous GLP-2 production or replace GLP-2 exogenously. Similarly, the design of nutrient formulations for patients receiving enteral tube feedings may be optimized by consideration of the impact of specific nutrients on stimulation of endogenous trophic factors, such as GLP-2, that are important for mucosal epithelial function and integrity.

In summary, the results of this study show that secretion of the potent intestinal peptide, GLP-2, from the intestinal L cell is regulated by ingestion of carbohydrate, fat, or mixed nutrients in normal humans. Both indirect and direct effects of nutrients seem to be involved in the regulation of the L cell, which may assist in the development of specific strategies to enhance GLP-2 secretion in patients with compromised intestinal function.

References


Received January 21, 1999. Accepted March 30, 1999.

Address requests for reprints to: Patricia L. Brubaker, Ph.D., Room 3366, Medical Sciences Building, University of Toronto, 1 Kings College Circle, Toronto, Ontario M5S 1A6, Canada. e-mail: p.brubaker@utoronto.ca; fax: (416) 978-2593.

Supported by grants from Allelix Biopharmaceuticals, Inc., Mississauga, Ontario, Canada (to P.L.B.) and the Medical Research Council of Canada (to P.L.B. and D.J.D.).

Dr. Drucker is a Scientist of the Medical Research Council of Canada and is a consultant to Allelix Biopharmaceuticals, Inc.

The authors thank J. Peterson for excellent technical assistance, C. Miller (The Toronto Hospital, Toronto, ON, Canada) for dietary advice, and J. J. Holst (Copenhagen, Denmark) for the gift of antiserum #92160.