ANTIDIABETOGENIC EFFECT OF GLUCAGON-LIKE PEPTIDE-1 (7–36)AMIDE IN NORMAL SUBJECTS AND PATIENTS WITH DIABETES MELLITUS

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Abstract *Background.* Glucagon-like peptide-1 (7–36)amide (glucagon-like insulinotropic peptide, or GLIP) is a gastrointestinal peptide that potentiates the release of insulin in physiologic concentrations. Its effects in patients with diabetes mellitus are not known.

Methods. We compared the effect of an infusion of GLIP that raised plasma concentrations of GLIP twofold with the effect of an infusion of saline, on the meal-related release of insulin, glucagon, and somatostatin in eight normal subjects, nine obese patients with non-insulin-dependent diabetes mellitus (NIDDM), and eight patients with insulin-dependent diabetes mellitus (IDDM). The blood glucose concentrations in the patients with diabetes were controlled by a closed-loop insulin-infusion system (artificial pancreas) during the infusion of each agent, allowing measurement of the meal-related requirement for exogenous insulin. In the patients with IDDM, normoglycemic-clamp studies were performed during the infusions of GLIP and saline to determine the effect of GLIP on insulin sensitivity.

Results. In the normal subjects, the infusion of GLIP significantly lowered the meal-related increases in the blood glucose concentration ($P<0.01$) and the plasma concentrations of insulin and glucagon ($P<0.05$ for both comparisons). The insulinogenic index (the ratio of insulin to glucose) increased almost 10-fold, indicating that GLIP had an insulinotropic effect. In the patients with NIDDM, the infusion of GLIP reduced the mean (±SE) calculated isoglycemic meal-related requirement for insulin from 17.4±2.8 to 2.0±0.5 U ($P<0.001$), so that the integrated area under the curve for plasma free insulin was decreased ($P<0.05$) in spite of the stimulation of insulin release. In the patients with IDDM, the GLIP infusion decreased the calculated isoglycemic meal-related insulin requirement from 9.4±1.5 to 4.7±1.4 U. The peptide decreased glucagon and somatostatin release in both groups of patients. In the normoglycemic-clamp studies in the patients with IDDM, the GLIP infusion significantly increased glucose utilization (saline vs. GLIP, 7.2±0.5 vs. 8.6±0.4 mg per kilogram of body weight per minute; $P<0.01$).

Conclusions. GLIP has an antidiabetogenic effect, and it may therefore be useful in the treatment of patients with NIDDM. (N Engl J Med 1992;326:1316-22.)

THE existence of a chemical excitant of the endocrine pancreas was suggested as early as 1906.[1] This idea gained support in 1930, when it was demonstrated that the intravenous injection of crude secretin caused hypoglycemia in dogs by stimulating the endocrine pancreas.[2] This implied that the crude preparation of secretin contained an intestinal factor — "incretin" — that was able to stimulate the endocrine pancreas. The concept of incretin was clearly outlined by Creutzfeldt in 1979.[3] Incretin was defined as an endocrine transmitter that is produced in the gastrointestinal tract, is released by food intake (especially of carbohydrates), and stimulates insulin secretion in the presence of plasma peptide concentrations not exceeding those reached after meals. Glucose-dependent insulinotropic polypeptide is believed to have an important role in the mediation of this signal between the intestine and the pancreatic B cells after eating,[4] and it has therefore become a strong candidate for an incretin.[3] However, supraphysiologic levels of glucose-dependent insulinotropic polypeptide are needed to potentiate insulin secretion,[5] and amplification of the insulin response to oral glucose is partially preserved in the presence of antibodies to glucose-dependent insulinotropic polypeptide.[6] Therefore, it seems that there must be incretins other than glucose-dependent insulinotropic polypeptide.[7,8]

Glucagon-like peptide-1 (GLP-1) is a fragment of the proglucagon molecule.[9] This peptide has no metabolic effect in mammals. However, two shorter forms of GLP-1 — GLP-1 (7–37) and GLP-1 (7–36)amide — exert strong insulinotropic effects in vitro[10,11] and in vivo.[12,13] Since GLP-1 (7–36)amide, the naturally occurring form in humans,[14] is released during a meal[12,15] and after oral glucose administration[16] and potentiates glucose-induced insulin release,[12] this truncated form of GLP-1 may be an important incretin.[8,11] The peptide could thus be of potential value in the treatment of diabetes. Therefore, we investigated the effect of GLP-1 (7–36)amide, hereafter referred to as GLIP (glucagon-like insulinotropic peptide), on the release of hormones from islet cells in normal subjects and patients with non-insulin-dependent diabetes mellitus (NIDDM). In addition, we determined the effect of GLIP on the need for insulin after a standard meal in these patients. Since the peptide markedly decreased the insulin requirement and inhibited glucagon secretion in the patients with NIDDM, we extended the study to patients with insulin-dependent diabetes mellitus (IDDM).

METHODS

Study Subjects

The study protocols were approved by the Ethics Committee of the Karolinska Hospital, and all subjects gave written informed consent. Twenty-five subjects (eight normal subjects, nine patients with NIDDM, and eight patients with IDDM) participated in the study; their characteristics are shown in Table 1. The patients with diabetes were recruited from among those attending an outpatient

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Table 1. Characteristics of the Three Study Groups.*

Characteristic	Normal Subjects (N = 8)	Patients with NIDDM (N = 9)	Patients with IDDM (N = 8)
Sex (M/F)	5/3	6/3	6/2
Age (yr)			
Mean	52±12	57±8	36±14
Range	29–63	44–67	21–49
Body-mass index†			
Mean	25.4±2.3	32.9±8.4	23.7±2.0
Range	22.3–28.4	22.8–44.8	21.7–29.0
Duration of diabetes (yr)			
Mean		9.5±4.8	23.0±13.0
Range		5–21	5–35
Hemoglobin A_{1c} (%)‡			
Mean		8.5±3.3	6.2±0.9
Range		6.1–12.0	5.6–7.0

*Plus–minus values are means ±SD.

†The ratio of the weight in kilograms to the square of the height in meters.

‡Normal value, <5.7 percent.

clinic, and their illness fulfilled the criteria for NIDDM and IDDM described by the National Diabetes Data Group.[17] None of the patients had impaired renal function, autonomic neuropathy, or proliferative retinopathy, and all had normal liver function. The patients with IDDM had undetectable plasma C-peptide concentrations at base-line evaluation and after oral glucose administration. All 17 patients were being treated with NPH insulin and regular insulin. They were instructed to follow a standard diet for patients with diabetes for at least two weeks before the study and during the study. The normal subjects continued to follow their usual diet. The injections of NPH insulin were stopped 24 hours before the studies, and blood glucose concentrations were controlled with subcutaneous injections of regular insulin.

After each subject had fasted overnight, three cannulas were inserted at 7:30 a.m. on the day of each study. One cannula was placed in an antecubital vein and used to sample blood intermittently for hormone assays. The cannula was flushed with saline after each sampling. A second cannula, inserted retrogradely in a dorsal vein of the hand, was used for continuous monitoring of blood glucose concentrations. The venous blood was arterialized by heating the forearm and hand in a thermoregulated sleeve (Kanthal Medical Heating, Stockholm) at 45°C.[18] The third cannula was inserted in the contralateral antecubital vein and was used for all infusions. From approximately 8 a.m. to the end of the study, the patients (but not the normal subjects) were connected to a Biostator (Miles, Diagnostic Division, Elkhart, Ind.), a closed-loop insulin-infusion system (artificial pancreas), and received insulin intravenously to keep their basal and postprandial blood glucose concentrations normal. The target range for blood glucose concentrations was 4 to 5 mmol per liter under basal conditions and 6 to 7 mmol per liter after the study meal. The experiments were started 30 minutes after normoglycemia was achieved, which was a mean (±SE) of 58±5 minutes after connection to the Biostator in the patients with IDDM and 109±16 minutes in the patients with NIDDM, or 60 minutes after insertion of the cannulas in the normal subjects. The latter were not connected to the Biostator and received no insulin, but were otherwise studied in the same way. An infusion of saline or GLIP (Peninsula Laboratories, St. Helens, Merseyside, United Kingdom) at a rate of 0.75 pmol per kilogram of body weight per minute was then started and continued for 3½ hours. The two studies were performed in a random order 6 to 28 days apart. At time zero all participants were given a standard lunch, which they ate within 15 minutes while sitting in bed. The meal consisted of boiled potatoes, boiled beef, cooked carrots, a glass of milk containing 0.5 percent butterfat, and a slice of bread made from a mixture of wheat and rye flours; 28, 26, and 46 percent of the energy from this lunch were derived from protein, fat, and carbohydrates, respectively. Blood samples were obtained at −30, 0, 15, 30, 90, 120, 150, and 180 minutes.

In the patients with IDDM, insulin sensitivity was measured during hyperinsulinemic–normoglycemic-clamp studies performed after short-term normalization of the blood glucose concentration.[19] Insulin (0.8 mU per kilogram per minute) was infused for four hours, with or without GLIP (0.75 pmol per kilogram per minute). These experiments were conducted in random order, 14 to 28 days apart. The blood glucose concentration was kept at 4.7 mmol per liter. Glucose utilization was calculated during the last three hours of the insulin infusion.

Assays

Blood samples were collected in plastic tubes containing EDTA (0.048 ml, 0.34 M) and aprotinin (Trasylol containing 1000 IU of kallikrein inhibitor; Bayer, Leverkusen, Germany) and immediately placed on ice. The samples were centrifuged at 4°C, and the plasma was frozen at −20°C.

Blood glucose concentrations were measured according to the glucose oxidase method.[20] Hemoglobin A_{1c} was measured by isoelectric focusing.[21] Plasma C-peptide concentrations were determined by radioimmunoassay with commercially available kits (Novo Research Institute, Bagsværd, Denmark). The intraassay coefficient of variation was 6 percent, and the interassay coefficient of variation was 7 percent; the cross-reactivity of proinsulin in this assay was 75 percent. In the normal subjects, plasma insulin was measured by radioimmunoassay in which the intraassay coefficient of variation was 5 percent, the interassay variation was 10 percent, and the cross-reactivity of proinsulin was 80 percent. There was no cross-reactivity between C peptide and insulin in these two assays. In the patients, plasma free insulin was measured after insulin-antibody–insulin complexes were precipitated with polyethylene glycol.[22] Plasma glucagon was measured by radioimmunoassay with the antibody 30K.[23] The intraassay coefficient of variation was 5 percent, and the interassay variation was 14 percent; the lower limit of detection was 50 ng per liter. Somatostatin was measured in acid ethanol extracts of plasma[24,25] by radioimmunoassay with tyrosine-1 somatostatin (kindly provided by Dr. A. Arimura, Tulane University, New Orleans) labeled with iodine-125, synthetic somatostatin as the assay standard, and somatostatin-14 antibody produced in our laboratory.[26] The limit of detection was 0.32 pmol per assay tube. The interassay coefficient of variation was 7 percent, the intraassay variation 5 percent, and the recovery 84 to 91 percent.

GLIP was measured in extracts of plasma by radioimmunoassay with synthetic GLP-1 (PG (78–107) amide, code 7168; Peninsula Laboratories) as the assay standard, antiserum 2135 (final dilution, 1:150,000), and synthetic GLP-1 labeled with iodine-125 according to the stoichiometric chloramine-T method and purified by reverse-phase high-performance liquid chromatography on a Vydac C-18 column (Separations Group, London) for 100 minutes with a 30 to 50 percent gradient of acetonitrile in water (Grade S. Rathburn Chemicals, Walkerburn, United Kingdom). The antiserum used cross-reacts with equal strength with all peptides containing the GLP-1 sequence, regardless of the presence or absence of amino-terminal or carboxy-terminal extensions. The antiserum against GLP-1 did not cross-react with glucagon or secretin. The limit of detection of the assay in plasma was 5 pmol per liter. The intraassay coefficient of variation was 8 percent, and the interassay coefficient 16 percent. GLIP was extracted from plasma with the use of 70 percent ethanol (vol/vol, final concentration). The supernatant was dried in a vacuum centrifuge (Heto, Hilleröd, Denmark) and redissolved in veronal buffer (20 mM, pH 8.4) containing 0.1 percent bovine serum albumin (A-7034, Sigma Chemical, St. Louis) and thimerosal (0.6 mM). All plasma extracts were assayed in duplicate. The mean (±SD) recovery of GLIP added to plasma before extraction was 75±8 percent.[16]

The results of the assays for somatostatin and GLIP were corrected for losses that occurred during extractions. All samples from each participant were analyzed at the same time.

Statistical Analysis

Results are expressed as means ±SE unless otherwise indicated. Testing for significant differences was carried out with Student's t-test for paired data. Comparisons between groups of subjects were performed with Student's unpaired t-test or the Mann–Whitney

U test. The blood glucose and plasma hormonal patterns during the preprandial period were examined by analysis of variance for repeated measurements. The meal-related responses of blood glucose and plasma hormones were calculated as the integrated increments above the basal value that were recorded from 30 minutes before the meal to 180 minutes after the meal. The insulinogenic index (the ratio of insulin to glucose) was calculated by dividing the incremental values for plasma insulin by the increment in blood glucose during the postprandial period (from 0 to 180 minutes).

Insulin requirements were derived from the Biostator readings; the same algorithm was used in all experiments. Since blood glucose concentrations measured after the meal were lower after GLIP administration than after saline administration, the insulin requirements were overestimated. Therefore, the isoglycemic meal-related insulin requirement was calculated as previously described[27] by adjusting the Biostator constant for insulin sensitivity (VAR) — i.e., $VAR_1 \rightarrow VAR_c$ — to attain identical glycemic responses to meals ($G_1 = G_2$) after administration of the doses of insulin (I_1 and I_2) by the Biostator, as follows:

$$\left.\begin{aligned} G_1 &\xrightarrow{VAR_1} I_1 \\ G_2 &\xrightarrow{VAR_1} I_2 \end{aligned}\right\} \text{by Biostator measurement;}$$

$$\left.\begin{aligned} G_1 &\xrightarrow{VAR_c} I_2 \\ G_2 &\xrightarrow{VAR_c} I_R \end{aligned}\right\} \text{by calculation.}$$

G_1 denotes the glycemic response to the standard lunch in the control experiments with the function VAR_1, and I_1 the insulin requirement for this control meal. G_2 denotes the glycemic response to meals during GLIP administration, and I_2 the insulin requirement for these meals. VAR_c was determined by mathematical manipulation of the computer program in order to fit I_2 to G_1. Then, with the newly derived function VAR_c we replayed G_2 responses and calculated insulin requirement I_R, the isoglycemic meal-related insulin requirement. Hence, the isoglycemic meal-related insulin requirement reflects the comparison of insulin requirements in different experiments in which the postprandial glycemic response was assumed to be identical.

Results

Response to the Standard Meal

In the normal subjects, infusion of GLIP for 30 minutes before the meal increased plasma insulin concentrations and decreased blood glucose concentrations ($P<0.05$), but plasma glucagon concentrations did not change (Fig. 1). The infusion of GLIP lowered postprandial blood glucose concentrations and plasma insulin and glucagon concentrations (Fig. 1 and Table 2). However, the decrease in blood glucose concentrations was more pronounced than that in plasma insulin concentrations, as reflected by a mean (±SE) increase in the insulinogenic index during the postprandial period (from 25.0±30 to 262.2±32, $P<0.001$), suggesting that GLIP had an insulinotropic effect during the postprandial period. The infusion of GLIP had no significant effect on basal and postprandial plasma somatostatin concentrations (Table 2).

In the patients with NIDDM, normoglycemia (5.0±0.2 mmol per liter) was achieved before the test meal by the closed-loop, insulin-infusion system (Fig. 2). The infusion of GLIP increased fasting plasma C-peptide concentrations ($P<0.01$); accordingly,

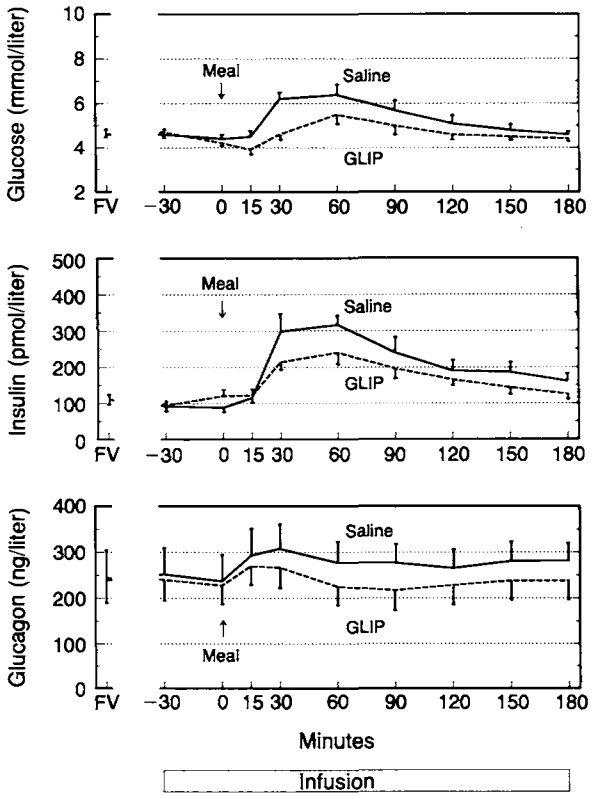

Figure 1. Effects of Infusion of GLIP or Saline on Mean (±SE) Postprandial Concentrations of Blood Glucose and Plasma Insulin and Glucagon in Eight Normal Subjects.
FV denotes fasting value. The rate of infusion was 0.75 pmol per kilogram of body weight per minute.

the decrease in fasting blood glucose concentrations during the 30 minutes before the meal was more pronounced during the GLIP infusion than during the saline infusion ($P<0.05$). The GLIP infusion significantly reduced the degree of postprandial hyperglycemia (Table 2). During the saline infusion, 17.4±2.8 U of insulin had to be administered during the meal in order to normalize the blood glucose concentration, whereas during the infusion of GLIP the insulin requirement decreased to 10.1±1.4 U (Table 3.) The insulin-sparing effect of GLIP became even more evident when the isoglycemic meal-related insulin requirement was calculated (2.0±0.5 U). This marked decrease in the requirement for exogenously administered insulin was reflected by the decrease in the integrated area under the curve for plasma free insulin (from 37.1±13.1 to 6.6±5.3 nmol per liter per 210 minutes), although the release of endogenous insulin in response to the meal was markedly enhanced by GLIP, as reflected by the higher plasma C-peptide responses (7.4±3.5 vs. 25.4±9.8 nmol per liter per 210 minutes). The infusion of GLIP also significantly suppressed glucagon and somatostatin release.

In the patients with IDDM, the infusion of GLIP decreased the postprandial increase in the blood glucose and plasma free insulin concentrations (Fig. 3). Furthermore, GLIP lowered the meal-related require-

Table 2. Integrated Blood Glucose and Plasma Hormone Responses to a Meal during Infusions of GLIP and Saline, According to Study Group.*

VARIABLE	NORMAL SUBJECTS			PATIENTS WITH NIDDM			PATIENTS WITH IDDM		
	SALINE	GLIP	P VALUE	SALINE	GLIP	P VALUE	SALINE	GLIP	P VALUE
Blood glucose (mmol/liter/210 min)	153.8±41.2	9.2±29.9	<0.01	133.0±34.6	3.3±20.4	<0.01	132.3±37.5	64.2±35.9	<0.01
Plasma insulin† (nmol/liter/210 min)	22.8±3.6	14.8±2.4	<0.05	37.1±13.1	6.6±5.3	<0.05	4.2±4.7	0.7±0.7	<0.01
Plasma C peptide (nmol/liter/210 min)	182.9±30.2	145.1±32.9	<0.05	7.4±3.5	25.4±9.8	<0.05	—	—	—
Plasma glucagon (μg/liter/210 min)	5.0±3.1	−0.6±3.0	NS	26.9±6.2	10.5±5.1	<0.005	14.4±1.9	1.4±2.2	<0.005
Plasma somatostatin (nmol/liter/210 min)	1.2±0.3	0.7±0.2	NS	0.9±0.1	0.4±0.1	<0.005	1.2±0.2	0.7±0.2	<0.01

*Plus–minus values are means ±SE and represent the area under the response curve for incremental values recorded from 30 minutes before infusion to 180 minutes after the start of infusion. P values indicate the significance of the difference between the responses to saline and the responses to GLIP.

†Total insulin was measured in the normal subjects and free insulin in the patients.

ment for exogenous insulin (from 9.5±1.4 to 5.4±0.3 U) and the calculated isoglycemic meal-related insulin requirement (from 9.4±1.5 to 4.7±1.4 U) (Table 3), in addition to decreasing glucagon and somatostatin release (Table 2).

The plasma GLIP concentrations at the initiation of the experiment as well as at 30 minutes before the meal and at time zero were higher ($P<0.05$) in the patients with NIDDM than in the fasting normal subjects and the patients with IDDM (Fig. 4). The meal increased plasma GLIP concentrations in the normal subjects and both groups of patients. The patients with NIDDM had a slightly greater increase than did the normal subjects (59.8±11.1 vs. 33.9±7.0 pmol per liter, $P<0.05$). In the patients with IDDM, the plasma GLIP concentration in response to the meal was 32.9±5.3 pmol per liter (a value not significantly different from that in the normal subjects). The infusion of GLIP increased plasma GLIP concentrations approximately twofold during the postprandial period in the normal subjects, the patients with NIDDM, and the patients with IDDM (76.7±9.6, 124.5±12.8, and 91.5±6.3 nmol per liter, respectively).

Response to Normoglycemic Clamping

During the clamp studies in the patients with IDDM, the steady-state concentrations of plasma free insulin increased to 473±53 pmol per liter during the saline infusion and to 479±58 pmol per liter during the GLIP infusion. The blood glucose concentrations were kept at 4.8±0.2 mmol per liter during the saline infusion and at 4.7±0.2 mmol per liter during the GLIP infusion (Fig. 5). The infusion of GLIP significantly increased glucose utilization, as compared with the infusion of saline (saline vs. GLIP, 7.2±0.5 vs. 8.6±0.4 mg per kilogram per minute, from 60 to 240 minutes; $P<0.01$).

DISCUSSION

We found that GLIP stimulates insulin release, inhibits glucagon release, and improves insulin sensitivity. Apart from insulin, glucagon and somatostatin are also known to be involved in carbohydrate metabolism. Glucagon increases hepatic glucose production by stimulating both glycogenolysis[28] and gluconeogenesis,[29] whereas somatostatin inhibits the secretion of both insulin and glucagon[30] and prolongs the absorption of nutrients by decreasing the motility of the small intestine.[31,32] The effects of GLIP were similar in

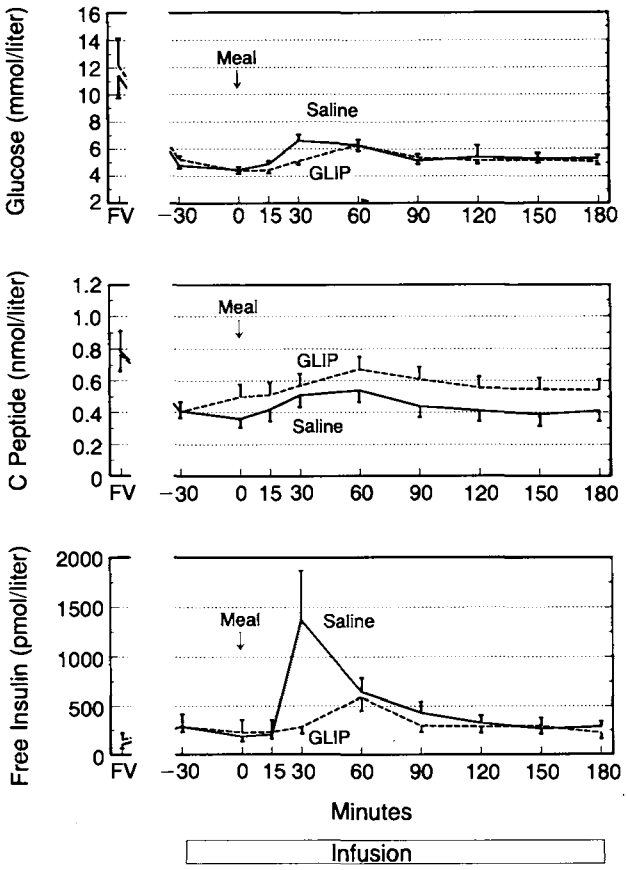

Figure 2. Effects of Infusion of GLIP or Saline on Mean (±SE) Postprandial Concentrations of Blood Glucose and Plasma C Peptide and Free Insulin in Nine Patients with NIDDM.
FV denotes fasting value before Biostator treatment.

Table 3. Requirements for Exogenous Insulin in Response to a Meal and Isoglycemic Meal-Related Insulin Requirement in the Patients with Diabetes.*

VARIABLE	PATIENTS WITH NIDDM			PATIENTS WITH IDDM		
	SALINE	GLIP	P VALUE	SALINE	GLIP	P VALUE
Insulin requirement	17.4±2.8	10.1±1.4	<0.005	9.5±1.4	5.4±0.3	<0.005
Isoglycemic meal-related insulin requirement	17.4±2.8	2.0±0.5	<0.005	9.4±1.5	4.7±1.4	<0.01

*Plus–minus values are means ±SE. P values indicate the significance of the difference between the responses to saline and the responses to GLIP.

all three study groups. The experiments were performed with a Biostator so that blood glucose concentrations could be kept similar during both the saline and GLIP infusions. However, because of the antidiabetogenic effects of the peptide, the postprandial blood glucose concentrations were lower during GLIP administration. The decrease in the plasma glucagon concentration during the infusion of GLIP was probably due to a direct effect of the GLIP rather than to changes in the prevailing blood glucose concentration. This conclusion is supported by the finding that prolonged infusion of GLIP inhibits basal glucagon release in vitro in isolated perfused pancreatic tissue from pigs[33] and rats.[34] The inhibitory effect of GLP-1 (7–37) on glucagon release may occur through a paracrine mechanism.[35]

In a study of patients who had undergone pancreatectomy and had poor glycemic control, a mixed meal did not stimulate somatostatin release, but the institution of glycemic control with a Biostator restored the somatostatin response to the meal.[36] This finding indicates that the meal-related release of somatostatin originates mainly in the gastrointestinal tract. Therefore, the inhibition of somatostatin release during the infusion of GLIP in our studies was probably due to decreased intestinal release of somatostatin. It is unlikely that it was due to direct inhibition of pancreatic D-cell secretion by GLIP, since the release of somatostatin by islet cells in vitro is enhanced by perfusion with GLP-1 (7–37).[33-35] Alternatively, GLIP may decrease the transit of nutrients, in turn suppressing somatostatin release.

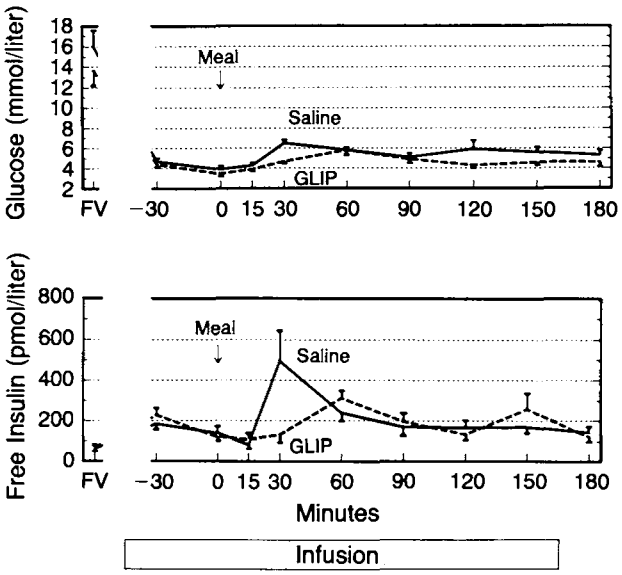

Figure 3. Effects of GLIP or Saline on Mean (±SE) Postprandial Concentrations of Blood Glucose and Plasma Free Insulin in Eight Patients with IDDM.
FV denotes fasting value before Biostator treatment.

The infusions of GLIP attenuated the postprandial increase in blood glucose concentrations, suggesting that the peptide may prolong the transit time of nutrients in the gastrointestinal tract. GLIP is known to prolong gastric emptying,[37] but its effect on transit time in the intestine has not been studied.

In the normoglycemic-clamp studies, plasma concentrations of free insulin were raised to about 470 pmol per liter. At this insulin concentration, hepatic glucose production is thought to be almost totally suppressed in normal subjects and patients with IDDM.[38,39] In these experiments, however, the plasma specific activity of the labeled glucose was not constant, so that the suppressive effect of insulin on hepatic glucose production may have been overestimated.[40] Therefore, it is not clear whether the improvement in insulin sensitivity that occurred during the infusion of GLIP was due to hepatic or extrahepatic factors.

In the patients with NIDDM, the infusion of GLIP decreased the calculated isoglycemic meal-related insulin requirement substantially. In this group, the decrease was only partially due to enhanced endogenous insulin secretion, since the infusion of GLIP decreased the plasma concentrations of free insulin by 82 percent. Therefore, in addition to potentiation of insulin secretion, a decrease in glucagon release and improvement in insulin sensitivity contributed to the antidiabetogenic effect of GLIP. The evidence that the antidiabetogenic effect of GLIP is not mediated solely by stimulation of insulin secretion is further supported by the finding that during the GLIP infusion the calculated isoglycemic meal-related insulin requirement decreased by 61 percent in the patients with IDDM.

Under non–steady-state conditions, C-peptide clearance varies widely.[41] Therefore, it is difficult to quantify the insulinogenic effect of the GLIP on the basis of C-peptide responses to a meal. It is also possible that GLIP alters the clearance rate of C peptide or insulin.

In our previous study,[16] patients with NIDDM who had elevated fasting blood glucose concentrations had increased fasting plasma GLIP concentrations as well as postprandial increases in the response to oral glucose. As demonstrated in our present study, short-term correction of hyperglycemia did not lead to

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