Normalization of Insulin Responses to Glucose by Overnight Infusion of Glucagon-Like Peptide 1 (7-36) Amide in Patients with NIDDM

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Glucagon-like peptide 1 (GLP-1) is a natural enteric incretin hormone, which is a potent insulin secretogogue in vitro and in vivo in humans. Its effects on overnight glucose concentrations and the specific phases of insulin response to glucose and nonglucose secretogogues in subjects with NIDDM are not known. We compared the effects of overnight intravenous infusion of GLP-1 (7-36) amide with saline infusion, on overnight plasma concentrations of glucose, insulin, and glucagon in eight subjects with NIDDM. The effects on basal (fasting) β-cell function and insulin sensitivity were assessed using homeostasis model assessment (HOMA) and compared with seven age- and weight-matched nondiabetic control subjects. The GLP-1 infusion was continued, and the first- and second-phase insulin responses to a 2-h 13 mmol/l hyperglycemic clamp and the insulin response to a subsequent bolus of the nonglucose secretogogue, arginine, were measured. These were compared with similar measurements recorded after the overnight saline infusion and in the control subjects who were not receiving GLP-1. The effects on stimulated β-cell function of lowering plasma glucose per se were assessed by a separate overnight infusion of soluble insulin, the rate of which was adjusted to mimic the blood glucose profile achieved with GLP-1. Infusion of GLP-1 resulted in significant lowering of overnight plasma glucose concentrations compared with saline, with mean postabsorptive glucose concentrations (2400–0800) of 5.6 ± 0.8 and 7.8 ± 1.4 mmol/l, respectively ($P < 0.0002$). Basal β-cell function assessed by HOMA was improved from geometric mean (1 SD range), 45% β (24–85) to 91% β (55–151) by GLP-1 ($P < 0.0004$). First-phase incremental insulin response to glucose was improved by GLP-1 from 8 pmol/l (−8–33) to 116 pmol/l (12–438) ($P < 0.005$), second-phase insulin response to glucose from 136 pmol/l (53–352) to 1,156 pmol/l (357–3,748) ($P < 0.0002$), and incremental insulin response to arginine from 443 pmol/l (172–1,144) to 811 pmol/l (272–2,417) ($P < 0.002$). All responses on GLP-1 were not significantly different from nondiabetic control subjects. Reduction of overnight glucose by exogenous insulin did not improve any of the phases of stimulated β-cell function. Prolonged intravenous infusion of GLP-1 thus significantly lowered overnight glucose concentrations in subjects with NIDDM and improved both basal and stimulated β-cell function to nondiabetic levels. It may prove to be a useful agent in the reduction of hyperglycemia in NIDDM. *Diabetes* 45:1524–1530, 1996

β-cell dysfunction and impaired insulin sensitivity both contribute to NIDDM (1). The β-cell dysfunction is characterized by impairment of basal insulin secretion (2), of the first- (3) and second-phase (4) insulin responses to glucose, and of the insulin responses to nonglucose secretogogues (5). The deterioration of glucose control with time is predominantly due to progressive impairment of β-cell function (6). Sulfonylurea therapy enhances the β-cell function by approximately twofold (7), but this is usually insufficient to maintain near-normal glucose concentrations, either basally or after meals. Other therapeutic agents that can improve β-cell function are required.

The β-cell insulin response to a given increase in plasma glucose is greater when the glucose has been given orally rather than intravenously (8). This is due in part to secretion of gut hormones, termed incretins, which potentiate glucose-induced insulin secretion (9). The peptides that appear to make the greatest contribution are gastric inhibitory peptide (GIP) and specific truncated forms of glucagon-like peptide 1 (GLP-1) (10,11). The latter is derived from the pre-proglucagon gene by tissue-specific posttranslational processing and is secreted from the L-cells of the distal ileum and colon. The incretin forms of this peptide are GLP-1(7-37) and GLP-1 (7-36) amide, the latter naturally occurring in humans (12). For ease of expression, we refer to these active truncated peptides as GLP-1.

GLP-1 is a potent stimulator of insulin secretion in the perfused pancreas (13–15). Given as a 4-h intravenous infusion, the therapeutic potential of GLP-1 was demonstrated by the normalization of fasting plasma glucose concentrations in poorly controlled NIDDM subjects on

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% β, percentage β-cell function; CV, coefficient of variation; GIP, gastric inhibitory peptide; GLP-1, glucagon-like peptide 1; HOMA, homeostasis model assessment; % S, percentage insulin sensitivity.

TABLE 1
Clinical characteristics of the diabetic and control subjects

	Patients with diabetes	Control subjects
n	8	7
Age (years)	58.1 ± 10.1	56.3 ± 7.1
Sex (M/F)	6/2	3/4
Weight (kg)	95.0 ± 25.8	91.9 ± 14.1
BMI (kg/m^2)	31.3 ± 5.6	30.4 ± 2.9
Fasting plasma glucose (mmol/l)	9.1 ± 1.6	5.6 ± 0.3

Data are means ± SD.

maximal tablet therapy (16). Short (30- to 180-min) intravenous infusions (17,18) and premeal subcutaneous injections (19) of GLP-1 have reduced postprandial glucose excursions in NIDDM subjects, an effect likely to have been contributed to by GLP-1–induced delay in gastric emptying (20). In vitro (15,21,22) and in vivo (16) studies have suggested that the insulinotropic actions of GLP-1 are highly glucose dependent, i.e., greater at raised than normal glucose concentrations, presenting the possibility that GLP-1 therapy for NIDDM might be free of hypoglycemia, which often accompanies sulfonylurea or insulin therapy.

We undertook this study to determine the degree to which a 12-h continuous overnight infusion of GLP-1 would improve the postabsorptive fasting plasma glucose concentrations in NIDDM patients and enhance both basal insulin secretion and stimulated insulin secretory responses to a hyperglycemic clamp and intravenous injection of the nonglucose secretogogue arginine. The possibility that improvements in the insulin secretory responses might in part be due to reduction of fasting plasma glucose concentrations per se was assessed by a separate overnight insulin infusion designed to mimic the decrease in plasma glucose achieved with GLP-1 infusion.

RESEARCH DESIGN AND METHODS

Subjects. Eight patients with NIDDM who were maintaining their usual body weight were studied (Table 1), together with seven nondiabetic control subjects with similar age and obesity. The diabetic subjects had all met World Health Organization criteria for NIDDM at diagnosis and had been treated with either diet alone (n = 4), or with additional sulfonylurea (n = 2) or metformin (n = 2). The oral hypoglycemic agents were discontinued at least 3 weeks before entry into the study. The nondiabetic control subjects all had normal fasting glucose values and had normal glucose responses to a standard meal-tolerance test. The study was approved by the Central Oxford Research and Ethics Committee, and all subjects gave written informed consent.

Protocol. The diabetic subjects were admitted to an investigation ward at 2100 on three occasions, having finished their last meal by 1900. Two intravenous cannulae were inserted with local anesthetic. Samples were taken from the distal forearm, which was heated with a thermoregulated blanket to arterialize the blood samples. At 2200, after pre-infusion blood samples were drawn, intravenous infusions of GLP-1 (7-36) amide (Bachem, CA), placebo (0.9% NaCl), or soluble human insulin (Novo Nordisk, Peas Pottage, U.K.) in 0.9% NaCl—all containing 0.45% human serum albumin—were commenced. The infusions were given in randomized order, except that the GLP-1 infusion always preceded the insulin infusion.

GLP-1 was infused at a rate of 1.2 pmol · kg^{-1} · min^{-1}. During the insulin infusion, blood was drawn every 20 min for immediate measurement of glucose with a glucose analyzer (Yellow Springs, Hampshire, U.K.), and the infusion rate adjusted to mimic the blood glucose profile achieved in that subject during the GLP-1 infusion. On all three nights, blood was drawn hourly for laboratory plasma glucose measurement and every 2 h for other assays.

At 0700, subjects were moved to a clinical room for a hyperglycemic clamp. The saline and insulin infusions were discontinued 30 min before the clamp was commenced, and the GLP-1 infusion continued throughout (Fig. 1). After three basal blood samples at 5-min intervals, a glucose bolus was given to raise the blood glucose acutely to 13 mmol/l. It was then clamped at that level for 130 min. Blood glucose was measured every 2 min with a glucose analyzer (Yellow Springs) and the glucose infusion rate adjusted using an unbiased iterative computer program to assess the glucose requirements (23). At 120 min, an intravenous bolus of 5 g L-arginine (Sigma, Dorset, U.K.) was given over 25 s. Blood was drawn continuously by a peristaltic pump during the clamp and arginine infusion, and integrated samples for each time period were used for subsequent laboratory biochemical analyses.

Nondiabetic control subjects attended on one occasion after an overnight fast and underwent the identical hyperglycemic clamp/arginine infusion protocol, without GLP-1.

Biochemical assays. Blood samples were drawn into plastic tubes containing heparin for glucose and insulin samples, or heparin and aprotinin (Trasylol, 2,000 kallikrein inactivator units per milliliter blood; Bayer, Germany) for glucagon and GLP-1 samples and were immediately placed on ice. The samples were centrifuged at 4°C. Plasma glucose concentrations were measured fresh and plasma samples for other assays stored at -20°C. Plasma glucose was determined by a hexokinase method on a Cobas MIRA discrete analyzer (Roche Diagnostica, Herts, U.K.). Plasma specific insulin was assayed by a method that has no cross-reactivity with intact proinsulin. It was measured by two-site enzyme-linked immunosorbent assay, using monoclonal antibodies HUI-018 and OXI-005 (Novo Nordisk, A/S, Bagsvaerd, Denmark) (24). Calibrators (7.5 mU human monocomponent insulin, catalog number 7358210, Novo Nordisk, A/S) were diluted in insulin-free plasma prepared by charcoal extraction. Interassay coefficient of variation (CV) was 7.5% at 294 pmol/l. Pancreatic glucagon and GLP-1 concentrations were determined in ethanol-extracted plasma. Pancreatic glucagon was measured by competitive radioimmunoassay, using rabbit anti-porcine glucagon antibody YY89, specific for COOH-terminal of pancreatic glucagon (25), with charcoal separation. Interassay CV was 12.2% at 43 pmol/l. GLP-1 (7-36) amide was measured by competitive radioimmunoassay, using rabbit anti-synthetic GLP-1 (7-36) amide antibody R600-8 with charcoal separation. Interassay CV was 11% at 45 pmol/l.

Data analysis

Basal (fasting) β-cell function and insulin sensitivity. β-cell function and insulin sensitivity were assessed in the basal (fasting) state, in the diabetic and nondiabetic control subjects, by the relationship between fasting glucose and insulin concentrations, analyzed by homeostasis model assessment (HOMA) (26). This uses a computer model based on the known interactions of glucose and insulin in different organs, including the pancreas,

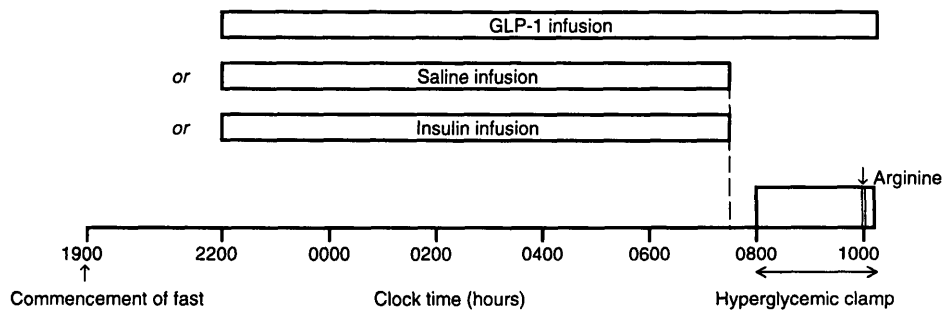

FIG. 1. Study protocol. Bars represent infusions on the three separate nights. GLP-1 infusion was continued throughout the night and subsequent hyperglycemic clamp. Saline and insulin infusions were stopped 30 min before the clamp.

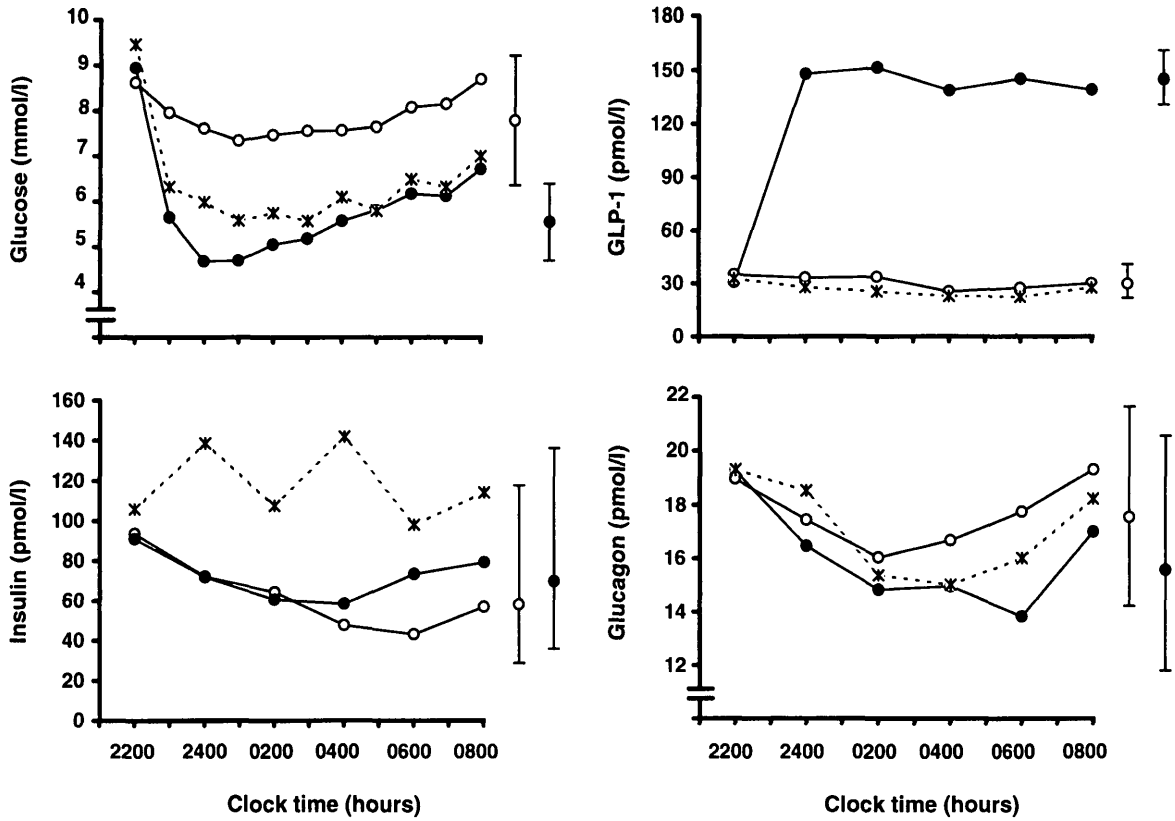

FIG. 2. Plasma glucose, specific insulin, GLP-1, and glucagon responses to overnight intravenous infusions of saline (○), GLP-1 (●), or soluble insulin (✳). Symbols with bars to the right of each figure indicate the mean ± SD (glucose) or geometric mean (1 SD range) (insulin, GLP-1, glucagon) postabsorptive levels from 2400 to 0800 on saline (○) and GLP-1 (●).

liver, and peripheral tissues. For each individual, the model determines the β-cell function (% β) and insulin sensitivity (% S) that uniquely predict the measured fasting glucose and insulin concentrations. The % β and % S are expressed relative to values in a lean nondiabetic reference population aged 18–25 years, assigned an arbitrary mean value of 100%.

Stimulated β-cell function. β-cell responses to insulin secretogogues were assessed by the first- and second-phase insulin responses to a hyperglycemic clamp at 13 mmol/l and the subsequent response, at that glucose concentration, to the nonglucose secretogogue, arginine. The incremental first-phase insulin response to glucose, over the first 12 min of the clamp, was calculated from the mean plasma insulin concentration obtained from continuous venous sampling, with subtraction of the mean basal insulin concentration obtained from three samples at −15, −10, and −5 min before the clamp. The second-phase insulin response was calculated as the mean insulin concentration from 90 to 120 min of the clamp obtained by continuous venous sampling. The incremental response to arginine was calculated as the mean insulin concentration over the 10 min after the arginine bolus minus the mean concentration over the preceding 10 min, both obtained by continuous venous sampling.

Statistical analysis. Results are expressed as geometric means (1 SD range), apart from glucose results, which are expressed as means ± SD. The overnight study data were analyzed using repeated measures analysis of variance in PROC GLM, using SAS (SAS Institute, Cary, NC). Basal and stimulated β-cell function and basal insulin sensitivity were analyzed using log-transformed data. For the first-phase insulin secretion, negative values for some of the diabetic subjects necessitated addition of a constant to all values before log transformation. The constant chosen was that which best produced a nonskewed distribution of the data. Paired *t* tests were used to compare therapies in the diabetic subjects and unpaired *t* tests to compare the diabetic subjects with the nondiabetic control subjects.

RESULTS

Overnight infusions. During the GLP-1 infusion, the plasma GLP-1 concentrations reached steady state within 2 h and remained stable thereafter (Fig. 2). The steady-state concentrations between 2400 and 0800, group geometric mean (1 SD range), were 145 pmol/l (131–161), approximately five times higher than on the saline infusion, 30 pmol/l (22–41). The GLP-1 concentrations during the insulin infusion night were not significantly different from the saline night.

During the night when the saline infusion was given, plasma glucose concentrations fell over the first 3 h from (mean ± SD) 8.6 ± 2.4 mmol/l at 2200, reaching a nadir of mean 7.4 ± 1.2 mmol/l at 0100 (Fig. 2). Thereafter, concentrations remained stable until around 0500 when they began to rise gradually, reaching a mean fasting (0800) concentration of 8.7 ± 2.1 mmol/l. During the GLP-1 infusion, glucose concentrations reached a nadir of mean 4.7 mmol/l (range 3.5–6.3) after 2-h infusion. From 0100, there was a gradual rise in glucose, reaching a mean fasting (0800) concentration of 6.7 ± 1.7 mmol/l, significantly lower than the fasting concentration on saline ($P < 0.0005$). The mean rate of rise of glucose from 2400 to 0800 during the GLP-1 infusion was 0.25 mmol · l^{-1} · h^{-1}, significantly greater than that during the saline infusion, 0.13 mmol · l^{-1} · h^{-1} ($P < 0.0004$). Despite this, mean glucose concentrations throughout the GLP-1 night remained lower than those during the saline night, with a mean postabsorptive glucose concentration from 2400 to 0800 on the GLP-1 night of 5.6 ± 0.8 mmol/l, compared with 7.8 ± 1.4 mmol/l on the saline night. With the variable insulin infusion, the glucose profile from 0100 was not significantly different from that achieved with the GLP-1 infusion.

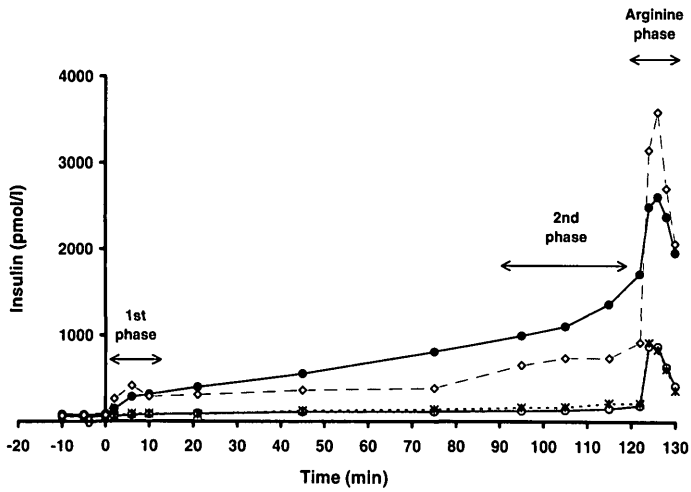

FIG. 3. Specific insulin profiles (geometric means) during hyperglycemic clamp at 13 mmol/l in diabetic subjects after overnight infusion of saline (—○—), overnight infusion of soluble insulin (—✕—), or during continued infusion of GLP-1 (—●—), compared with nondiabetic control subjects (—◇—). The clamp was commenced at time 0′ and the arginine administered at time 120′.

Specific insulin concentrations were not significantly different between the GLP-1 and saline infusions during the night. Fasting (0800) insulin concentrations during the GLP-1 infusion were, geometric mean (1 SD range), 79 pmol/l (41–154), significantly higher than after the saline infusion, 57 pmol/l (26–125) ($P < 0.05$). Insulin concentrations were, as expected, higher at some points during the night when insulin was administered by peripheral intravenous infusion. There was no significant difference in glucagon concentrations, during the night or fasting (0800), among the three infusions.

Basal β-cell function and insulin sensitivity. In the diabetic group after the saline infusion, the basal β-cell function at 0800, assessed by HOMA, was 45% β (24–85) (geometric mean, 1 SD range), less than in the similarly obese nondiabetic control subjects, 104% β (83–130) ($P < 0.007$) (Fig. 4). With infusion of GLP-1, the basal β-cell function in the diabetic group improved to 91% β (55–151) ($P < 0.0004$ compared with saline), not significantly different from the nondiabetic control group. All but one individual exhibited near normalization of % β with GLP-1 infusion (Fig. 4), the exception being a nonobese patient who had particularly severe impairment of basal β-cell function.

Basal insulin sensitivity determined by HOMA, at 0800, was similar in the nondiabetic control subjects and the diabetic subjects after saline (geometric means, 37 vs. 40% S, respectively) and did not change significantly in response to the GLP-1 infusion in the diabetic subjects, with geometric mean 31% S.

Stimulated β-cell function. Similar blood glucose concentrations were achieved between groups during the hyperglycemic clamps with concentrations (mean ± SD) over the last 10 min of the clamp of 12.8 ± 0.3 mmol/l in the nondiabetic control subjects and 13.1 ± 0.2, 13.2 ± 0.2, and 13.2 ± 0.2 mmol/l in the diabetic subjects on the saline, GLP-1, and insulin limbs of the study, respectively. The geometric mean insulin profiles during the hyperglycemic clamps are shown in Fig. 3 and the data for each individual for the specific phases of insulin secretion in Fig. 4. The incremental first-phase insulin response to glucose was impaired in the diabetic subjects after the saline infusion, 8 pmol/l (–8–33), compared with the nondiabetic control subjects, 255 pmol/l (145–430) ($P < 0.0001$). This was increased in the diabetic subjects by GLP-1 infusion to 116 pmol/l (12–438) ($P < 0.005$ compared with saline), not significantly different from nondiabetic control levels. The glucose bolus given to raise the blood glucose acutely to 13 mmol/l was greater during the GLP-1 infusion than after saline because of the difference in fasting glucose concentrations. After overnight insulin, however, the fasting glucose concentration in each individual was similar to that during GLP-1 infusion. A similar glucose bolus was therefore given after overnight insulin as during GLP-1 infusion, and the first-phase insulin response of 26 pmol/l (–3–77) was not significantly higher than that after saline infusion.

The second-phase insulin response to glucose was also impaired in the diabetic group after saline infusion, 136 pmol/l (53–352), compared with the nondiabetic subjects, 694 pmol/l (308–1,563) ($P < 0.004$). GLP-1 increased the second-phase insulin response in the diabetic subjects to 1,156 pmol/l (357–3,748), significantly higher than after the saline infusion ($P < 0.0002$) and not significantly different from nondiabetic control levels.

The incremental insulin response to arginine was impaired in the diabetic subjects after the saline infusion, 443 pmol/l (172–1,144), compared with nondiabetic control subjects, 1,724 pmol/l (1,153–2,576) ($P < 0.004$) and was improved by the GLP-1 infusion to 811 pmol/l (272–2,417) ($P < 0.002$ compared with saline), not significantly different from nondiabetic control levels.

The reduction of overnight fasting plasma glucose concentrations toward normal by exogenous insulin infusion did not increase either the first- or second-phase insulin responses to the hyperglycemic clamp or the insulin response to arginine.

DISCUSSION

The prolonged GLP-1 infusion significantly reduced mean plasma glucose concentrations throughout the night compared with a control saline infusion. The characteristically impaired first- and second-phase insulin responses to glucose in patients with NIDDM were enhanced to the normal range.

Although the GLP-1, saline, and insulin infusions were started at least 3 h after the last meal, the decrease in glucose concentrations over the first 3 h on the saline infusion night may in part have reflected postprandial changes. The overnight glucose concentrations on the saline infusion night remained stable until 0500 and thereafter rose gradually until 0800. This "dawn phenomenon" is well described (27) and is probably secondary to nocturnal increases in growth hormone secretion. The GLP-1 infusion reduced the plasma glucose to normal by 2 h in all subjects, but the glucose concentrations rose gradually thereafter. The rate of rise of glucose from 2400 to 0800 on the GLP-1 night was greater than the rise seen on the saline night, raising the possibility that GLP-1 may have been losing its efficacy overnight. Nevertheless, a

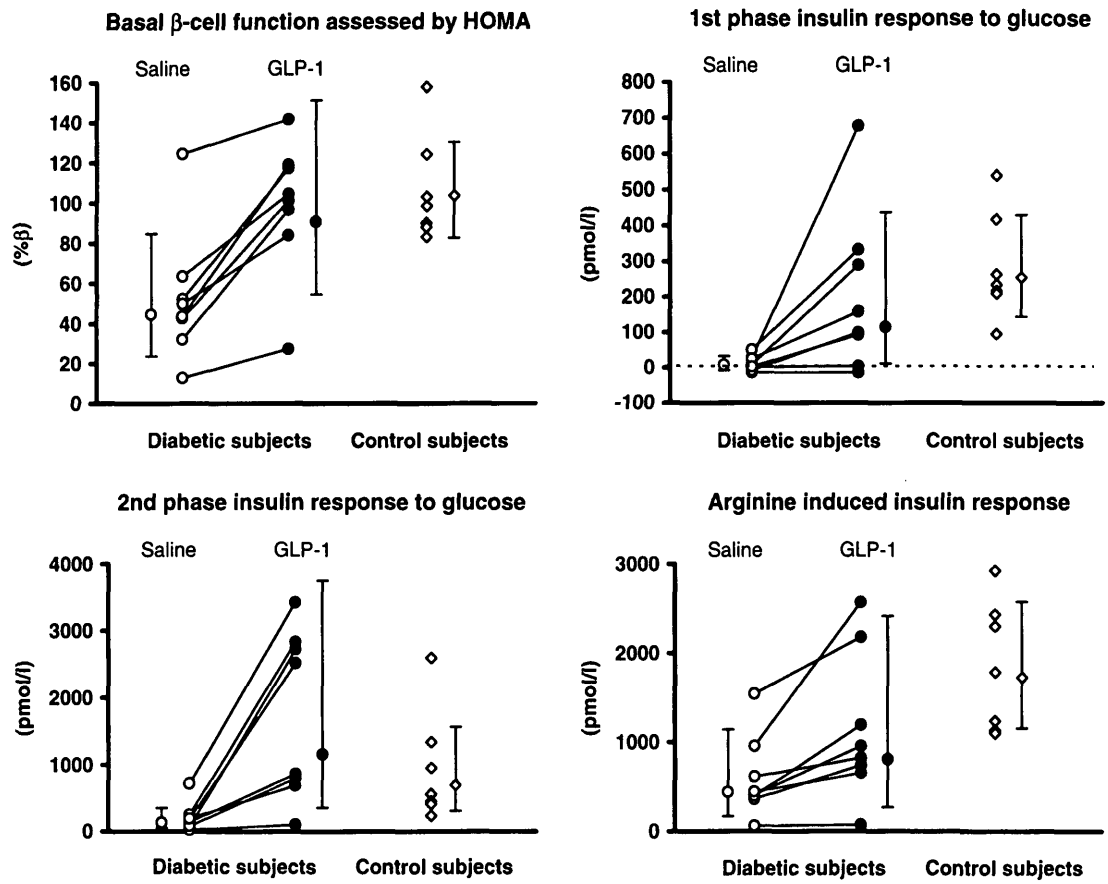

FIG. 4. Measurements of basal (0800) β-cell function and phases of stimulated insulin secretion in diabetic subjects after overnight infusion of saline (○) or during continued infusion of GLP-1 (●), compared with nondiabetic control subjects (◇). Symbols with bars indicate geometric mean (1 SD range).

marked reduction in overnight and fasting plasma glucose concentration was maintained with GLP-1.

A previous study of intravenous GLP-1 administration to subjects with NIDDM showed a temporary increase in plasma insulin concentrations, which returned to the initial values as the raised glucose concentrations reached near-normal levels (16). This is in keeping with the known glucose dependency of the insulinotropic action of GLP-1 (15,21,22). It is likely that the marked decrease in glucose concentrations over the first 2 h of the GLP-1 infusion in this study was similarly due to an initial increase in insulin concentrations, which was not detected, because no measurements were taken until 2 h after the commencement of the infusion, by which time the glucose concentrations were already normal. In the basal state, at 0800, the GLP-1–induced reduction in glucose concentrations was achieved by improvement in β-cell function, as shown by the higher insulin concentrations than those after the saline infusion. The basal β-cell function was assessed using HOMA of the insulin concentrations relative to the glucose concentrations (26) and showed that the GLP-1 infusion improved β-cell function to within the normal range in all but one subject, who had a particularly marked β-cell deficit. Detailed studies of the effect of GLP-1 on the characteristics of glucose-responsiveness of β-cells have not been done. Nevertheless, HOMA would detect improvements in β-cell function whether due to increase in V_{max} (maximal insulin response to glucose), decrease in K_m (half maximally stimulating glucose concentration), or a combination of the two. Subjects with NIDDM characteristically have impairment of basal (2) and stimulated (3–5) insulin secretion. Overnight infusion of GLP-1 significantly improved basal β-cell function, first- and second-phase insulin responses to glucose, and the insulin response to arginine, suggesting that the β-cell function may have been globally improved.

Previous studies have suggested that GLP-1 may improve insulin sensitivity (17). In our subjects, insulin sensitivity, assessed in the basal state (0800) by HOMA, was not significantly altered by GLP-1 infusion. The characteristics of the glucose-insulin feedback system are such that an improvement in insulin-induced peripheral glucose uptake would have resulted in lower basal plasma insulin levels, rather than the increase observed. Reduction in plasma glucagon concentrations by GLP-1 is thought to contribute to its ability to lower plasma glucose concentrations (16,17,28,29). We did not observe reduction in overnight plasma glucagon concentrations with GLP-1 infusion, possibly because normalization of the glucose concentrations by 2 h, when the first glucagon levels were measured, masked an initial reduction in glucagon.

The effects of GLP-1 on the β-cell are mediated via a specific receptor (30,31) that activates adenylate cyclase, resulting in increased cAMP levels. Activation of cAMP-dependent protein kinases, such as protein kinase A (10),

may increase insulin release via a number of mechanisms, including increases in intracellular calcium levels by increased calcium influx through voltage-dependent calcium channels (21,32,33) and mobilization of calcium from intracellular stores (34). cAMP-mediated mechanisms have also been shown to have a direct effect on the insulin secretory machinery (35). GLP-1 also stimulates insulin gene expression (36,37), probably via a cAMP-responsive element in the insulin gene promoter.

The improvement of β-cell responses by GLP-1 in patients with NIDDM might be due to the induction of supraphysiological intracellular cAMP concentrations that compensate, via the above mechanisms, for whatever defects in NIDDM account for the β-cell dysfunction. Alternatively, β-cell defects in NIDDM, which may even include reduced sensitivity to incretins (8,38), might result in low cAMP concentrations that were restored to normal by GLP-1 therapy in the patients studied. In addition, accumulation of glycogen in the β-cells secondary to prolonged hyperglycemia might downregulate β-cell responses to exogenous glucose (39) and GLP-1 may reverse this by the activation of β-cell glycogen phosphorylase via cAMP (40).

This study shows that continuous stimulation by GLP-1 in subjects with NIDDM has improved postabsorptive glucose concentrations over 10 h, compared with the previously studied 4 h, and has markedly improved β-cell function. Thus, GLP-1, which also delays gastric emptying (20), offers a potential therapy for NIDDM that may combine reduction in both fasting and postprandial hyperglycemia. In addition, the effect of GLP-1 on improvement in β-cell function might be additive to that of sulfonylureas, which act via a different mechanism by closing ATP-sensitive potassium channels in β-cells. The rising plasma glucose concentration during overnight GLP-1 administration raises the possibility that tachyphylaxis may ensue, although the insulin responses were still in the normal range after 10 h of GLP-1 infusion. Continued stimulation by GLP-1, or by an agonist, may have potential therapeutic relevance in NIDDM, particularly if GLP-1 therapy gives little risk of hypoglycemia due to the glucose dependency of its action.

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