Evaluation of β-Cell Secretory Capacity Using Glucagon-Like Peptide 1

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OBJECTIVE — β-Cell secretory capacity is often evaluated with a glucagon test or a meal test. However, glucagon-like peptide 1 (GLP-1) is the most insulinotropic hormone known, and the effect is preserved in type 2 diabetic patients.

RESEARCH DESIGN AND METHODS — We first compared the effects of intravenous bolus injections of 2.5, 5, 15, and 25 nmol GLP-1 with glucagon (1 mg intravenous) and a standard meal (566 kcal) in 6 type 2 diabetic patients and 6 matched control subjects. Next, we studied another 6 patients and 6 control subjects and, in addition to the above procedure, performed a combined glucose plus GLP-1 stimulation, where plasma glucose was increased to 15 mmol/l before injection of 2.5 nmol GLP-1. Finally, we compared the insulin response to glucose plus GLP-1 stimulation with that observed during a hyperglycemic arginine clamp (30 mmol/l) in 8 patients and 8 control subjects.

RESULTS — Peak insulin and C-peptide concentrations were similar after the meal, after 2.5 nmol GLP-1, and after glucagon. Side effects were less with GLP-1 than with glucagon. Peak insulin and C-peptide concentrations were as follows (C-peptide concentrations are given in parentheses): for patients ($n = 12$): meal, 277 ± 42 pmol/l (2,181 ± 261 pmol/l); GLP-1 (2.5 nmol), 390 ± 74 pmol/l (2,144 ± 254 pmol/l); glucagon, 329 ± 50 pmol/l (1,780 ± 160 pmol/l); glucose plus GLP-1, 465 ± 87 pmol/l (2,384 ± 2,99 pmol/l); for control subjects ($n = 12$): meal, 543 ± 89 pmol/l (2,873 ± 210 pmol/l); GLP-1, 356 ± 51 pmol/l (2,001 ± 130 pmol/l); glucagon, 420 ± 61 pmol/l (1,995 ± 99 pmol/l); glucose plus GLP-1, 1,412 ± 187 pmol/l (4,391 ± 416 pmol/l). Peak insulin and C-peptide concentrations during the hyperglycemic arginine clamp and during glucose plus GLP-1 injection were as follows: for patients: 475 ± 141 pmol/l (2,295 ± 379 pmol/l) and 816 ± 268 pmol/l (3,043 ± 508 pmol/l), respectively; for control subjects: 1,403 ± 308 pmol/l (4,053 ± 533 pmol/l) and 2,384 ± 452 pmol/l (6,047 ± 652 pmol/l), respectively.

CONCLUSIONS — GLP-1 (2.5 nmol = 9 μg) elicits similar secretory responses to 1 mg glucagon (but has fewer side effects) and a standard meal. Additional elevation of plasma glucose to 15 mmol/l did not enhance the response further. The incremental response was similar to that elicited by arginine, but hyperglycemia had an additional effect on the response to arginine.

Evaluation of β-cell function is of interest in relation to classification of patients. The exact β-cell mass cannot be measured directly. As a surrogate, the glucagon test has gained wide acceptance as a measure of β-cell function during daily life because the plasma C-peptide concentration 6 min after 1 mg intravenous glucagon has been shown, in most cases, to correspond to the maximal C-peptide concentration after a standard meal (1,2). The meal test may be inconvenient for patients because of the short time frame usually allowed for ingestion of the meal. Estimation of maximal secretory capacity has been made using the technically demanding and long-lasting hyperglycemic clamp with an infusion of 5 g L-arginine (3). Because glucagon-like peptide 1 (GLP-1) is the most potent insulinotropic hormone known and has been shown to strongly stimulate insulin secretion in patients with type 2 diabetes (4,5), we designed this study to compare the insulin response to GLP-1 with that observed after other common tests of β-cell function.

RESEARCH DESIGN AND METHODS

Subjects
Part 1 of the study. The first group of subjects consisted of 6 type 2 diabetic patients (4 men and 2 women, mean age 56 years [range 48–67], BMI 31.1 kg/m² [27–38], and HbA$_{1c}$ 9.6% [7.0–12.5]) and 6 healthy subjects individually matched for sex, age, and BMI (age 56 years [51–70], BMI 31.6 kg/m² [26–37 kg/m²], and HbA$_{1c}$ 5.5% [5.2–5.8]).

Part 2 of the study. The patient group was extended to include a further 6 type 2 diabetic men (age 59 years [49–69], BMI 30.0 kg/m² [26–35], and HbA$_{1c}$ 8.9% [8.1–10]) and another 6 matched healthy men (age 57 years [50–64], BMI 30.4 kg/m² [28–34], and HbA$_{1c}$ 5.7% [5.5–6]), so that the group now comprised a total of 12 type 2 diabetic patients and 12 matched healthy subjects. There were 7 patients treated with diet alone, whereas 5 were treated with diet and oral antidiabetic drugs (sulfonylureas and/or biguanides). There were 6 patients who had a history of hypertension and were treated with thiazides, ACE inhibitors, and/or calcium antagonists.

Part 3 of the study. Eight type 2 diabetic patients (7 men and 1 woman, age 55 years [49–69], BMI 30.9 kg/m² [27–35], and HbA$_{1c}$ 7.6% [6.3–8.6]) and 8 healthy subjects (age 55 years [51–64], BMI 31.1 kg/m² [25–38], and HbA$_{1c}$ 5.4% [5.0–6.0]) participated. There were 4 patients treated with diet alone, whereas 4 were treated with diet

and oral antidiabetic drugs (sulfonylureas and/or biguanides). There were 5 patients who had a history of hypertension and were treated with thiazides, ACE inhibitors, and/or calcium antagonists. All type 2 diabetic patients were diagnosed according to the criteria of the National Diabetes Data Group (4). None of the patients had impaired renal function (normal serum creatinine levels [<130 μmol/l] and no microalbuminuria), proliferative retinopathy, or impaired liver function. None of the healthy subjects had a family history of diabetes, and all had a normal oral glucose tolerance test. All agreed to participate by oral and written consent. The study was approved by the Copenhagen County Ethical Committee on 16 May 1997, and the study was conducted according to the principles of the Helsinki Declaration.

Protocol

All oral antidiabetic drugs were discontinued 72 h before the study. After an overnight fast (from 10:00 P.M.), the subjects were examined in the recumbent position with 2 cannulae inserted into the cubital veins: 1 for injection of GLP-1, glucagon, L-arginine, and/or glucose and 1 for blood sampling.

Part 1 of the study. All participants were examined on 6 separate days in a randomized order with a meal test, an intravenous bolus injection of glucagon (1 mg), or different doses of GLP-1 (2.5, 5, 15, or 25 nmol). For the meal test, venous blood was drawn 15, 10, and 0 min before and 15, 30, 45, 60, 75, 90, 120, 150, and 180 min after ingestion of a standard breakfast meal. The meal comprised 566 kcal (2,370 kJ) and was composed of 34% fat, 47% carbohydrate, and 19% protein. For intravenous glucagon or intravenous GLP-1, venous blood was sampled 15, 10, and 0 min before and 2, 3, 4, 6, 8, 10, 15, 20, 30, and 45 after intravenous bolus of 1 mg (287 nmol) biosynthetic glucagon (GlucaGen; Novo Nordisk, Bagsvaerd, Denmark) or the 4 different doses of GLP-1. Synthetic GLP-1(7-36) amide was purchased from Peninsula Europe (Meyerside, U.K.). The peptide was dissolved in sterilized water containing 2% human serum albumin (Human Albumin; Statens Serum Institute, Copenhagen, Denmark; guaranteed to be free of hepatitis B surface antigen, hepatitis C virus antibodies, and human immunodeficiency virus antibodies) and subjected to sterile filtration. Appropriate amounts of peptide for each experimental subject were dispensed into glass ampoules and stored

frozen under sterile conditions until the day of the experiment.

Part 2 of the study. β-Cell function was examined on 3 different days. In a randomized order, a standard meal test, an intravenous glucagon test (1 mg), and a GLP-1 bolus injection of 2.5 nmol GLP-1 were performed. In addition, in type 2 diabetic patients with fasting plasma glucose (FPG) <15 mmol/l (9 of the 12 patients) and in all healthy subjects, a combined glucose plus GLP-1 stimulation was performed. At time 0 (0 min), 50% glucose (wt/vol) was infused for 1 min to increase the plasma glucose to 15 mmol/l (calculated as [15 mmol/l –fasting plasma glucose] × 35 mg glucose × weight in kilograms), and 3 min later, 2.5 nmol GLP-1 was injected as a bolus for 2 min. GLP-1 is metabolized rapidly after intravenous injection (5,6) and might therefore be cleaved before a full effect on the β-cell is elicited, leading to a submaximal response. To examine the effect of a more lasting elevation of plasma GLP-1, 8 of the type 2 diabetic patients and 7 healthy subjects also received a subcutaneous injection of GLP-1 (1.5 nmol GLP-1/kg body wt injected into the periumbilical region) (7). Fifteen minutes later, plasma glucose was elevated to 15 mmol/l by intravenous glucose administration (50% wt/vol), as described above. Venous blood was sampled 15, 10, and 0 min before and 10, 20, 30, 40, 50, 60, 70, 80, and 90 min after GLP-1 administration. The results of this experiment were compared with the combined intravenous glucose plus GLP-1 stimulation in the same 8 patients and 7 healthy subjects (in 2 of the 8 patients, a GLP-1 injection without previous administration of glucose was performed instead of a combined glucose plus GLP-1 stimulation because FPG was 15 mmol/l).

Part 3 of the study. On 2 different days, in a randomized order, a combined glucose plus GLP-1 stimulation or a hyperglycemic clamp with injection of 5 g L-arginine monohydrochloride was performed to estimate maximal secretory capacity. During the hyperglycemic clamp, glucose (50% wt/vol) was injected at time 0 to increase the plasma glucose to 30 mmol/l (calculated as [30 mmol/l – FPG] × 35 mg glucose × weight in kilograms). Plasma glucose was kept at 30 mmol/l by continuous infusion of glucose, which was adjusted according to a bedside measurement of plasma glucose every 5 min. At 45 min, 5 g L-arginine monohydrochloride

was injected as a bolus for 30 s. Blood was sampled 15, 10, and 0 min before and 5, 10, 15, 20, 25, 30, 35, 40, 45, 47, 48, 49, 51, 53, 55, 60, 65, 70, 75, and 90 min after elevation of plasma glucose. The L-arginine was dissolved in 50 ml of sterilized water, dispensed into glass ampoules and stored at 4°C until the day of the experiment. Blood was sampled in fluoride tubes for measurement of glucose and in chilled EDTA tubes with aprotinin for peptide analyses (500 kallikrein inhibitory units/ml blood; Trasylol, Bayer, Leverkusen, Germany). Tubes were immediately cooled on ice and centrifuged within 20 min at 4°C, and plasma was stored at −20°C until analysis. During the experiments, all participants were interrogated about side effects, such as altered well-being, sweating, and nausea.

Analysis

Plasma glucose concentrations were measured during the experiments by a glucose oxidase method using a glucose analyzer (Yellow Springs Instrument Model 23 A; Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin concentrations were measured using commercial enzyme-linked immunosorbent assay kits (Dako, Copenhagen, Denmark). The sensitivity of the assay is ∼3 pmol/l, and the intra- and interassay coefficients of variation are 4–10% at 39–1.240 pmol/l. C-peptide concentrations were determined by radioimmunoassay (RIA), as described by Heding et al. (8), using the polyclonal antibody M1230 (9). The seldom found proinsulin conversion intermediate form des(64,65)-proinsulin cross-reacts strongly (126%), whereas the predominant forms of proinsulin-like immunoreactivity [des (31,32)] and intact proinsulin cross-react 13–15% relative to C-peptide (100%). The detection limit is ∼60 pmol/l, the intra-assay coefficient of variation is 5%, and the interassay coefficient of variation is 7.3%. Plasma samples were assayed for GLP-1 immunoreactivity using RIAs specific for each terminus of the GLP-1 molecule (10,11). Catalyzed by the ubiquitous enzyme dipeptidyl peptidase IV, GLP-1 is cleaved almost immediately to form an inactive NH_2-terminally truncated molecule, GLP(9-36) (5,6). Therefore, the NH_2-terminal assay (10) measures the concentration of intact surviving GLP-1, whereas the C-terminal assay (11) measures the sum of the intact peptide plus the primary metabolite.

Statistical analysis and calculations

All results are presented as the mean ± SEM. The significance of the difference between the different tests within the groups are calculated by repeated-measures analysis of variance (ANOVA) for parametric data followed by a Bonferroni test for multiple comparisons. Significance of difference between the patient and control group was calculated by a Student's *t* test, as appropriate. The level of statistical significance was set at $P < 0.05$.

RESULTS

Part 1 of the study

Insulin and C-peptide concentrations are shown in Fig. 1. Peak insulin and C-peptide concentrations occurred 6–10 min after intravenous injections of GLP-1 or glucagon and 90 min (patients) and 30–90 min (healthy subjects) after the meal (meal data not shown). The mean of individual peak insulin and C-peptide (given in parentheses) concentrations for type 2 diabetic patients were 286 ± 47 pmol/l (1,771 ± 237 pmol/l) after 2.5 nmol GLP-1; 318 ± 32 pmol/l (1,970 ± 172 pmol/l) after 5 nmol GLP-1; 348 ± 39 pmol/l (2,049 ± 169 pmol/l) after 15 nmol GLP-1; 360 ± 29 pmol/l (2,195 ± 164 pmol/l) after 25 nmol GLP-1; 265 ± 45 pmol/l (1,643 ± 178 pmol/l) after glucagon; and 197 ± 31 pmol/l (1,735 ± 218 pmol/l) after the meal. For healthy subjects, the corresponding results were 270 ± 18 (1,788 ± 110), 340 ± 67 (1,716 ± 254), 343 ± 57 (1,769 ± 215), 359 ± 60 (2,144 ± 248), 360 ± 39 (1,874 ± 158), and 386 ± 61 (2,398 ± 265) pmol/l, respectively. Peak insulin and C-peptide concentrations after 2.5 nmol GLP-1, the standard meal test, and 1 mg glucagon were not significantly different when individual peak concentrations were compared ($P = 0.059$, ANOVA). In the type 2 diabetic patients, insulin ($P = 0.0033$) and C-peptide ($P = 0.0006$) responses were higher with the high doses of GLP-1 (repeated-measures ANOVA). Similar results were obtained when values at a fixed sampling time (e.g., 6 min after infusion) were compared. Post hoc comparisons showed significant differences between 15 and 25 nmol GLP-1 versus the meal test ($P < 0.05$ and $P < 0.01$, respectively) with respect to insulin and between 2.5 and 25 nmol GLP ($P < 0.05$), 15 and 25 nmol GLP-1 versus glucagon ($P < 0.05$ and $P < 0.01$, respectively), and 25 nmol GLP-1 versus the meal test ($P < 0.05$) for C-peptide. Healthy sub-
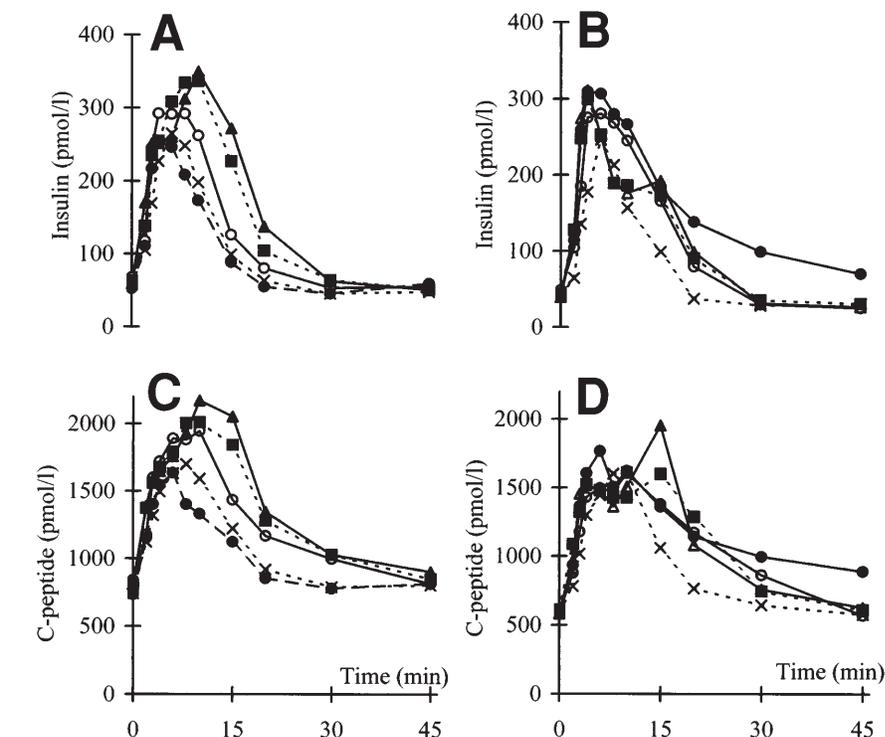
jects showed no significant differences in their responses on the 6 different days (insulin, $P = 0.57$; C-peptide, $P = 0.12$).

Basal plasma GLP-1 concentrations were between 4 and 10 pmol/l (both COOH- and NH_2-terminal), and basal concentrations of intact GLP-1 were reached again 10–30 min after intravenous injection of the 4 different GLP-1 doses. Peak plasma GLP-1 concentrations increased linearly with increasing doses of GLP-1 and were similar for type 2 diabetic patients and healthy subjects. The peak concentrations for total GLP-1 (COOH-terminal) were 357 ± 56, 647 ± 141, 1,978 ± 276, and 3,435 ± 331 pmol/l after 2.5, 5, 15, and 25 nmol GLP-1, respectively. Corresponding results for the intact GLP-1 peptide (NH_2-terminal) were 69 ± 17, 156 ± 44, 703 ± 77, and 1,070 ± 117 pmol/l, respectively.

There was no significant difference between side effects reported by the patient group or control group. Of the participants administered 2.5 nmol GLP-1, 42% complained of reduced well-being, 50% of nausea, and 17% of profuse sweating. The corresponding results in the participants administered 5 nmol GLP-1 were 42, 33, and 17%, respectively. With the glucagon

test, 83% of the participants complained of reduced well-being, 33% of profuse sweating, and 75% of nausea. In the patients given 15 and 25 nmol GLP-1, the frequency of side effects increased to 100% of the participants complaining of reduced well-being, 83 and 67%, respectively, of profuse sweating, and 67 and 83%, respectively, of nausea. The meal was tolerated well by all participants. For any GLP-1 dose, there was a significant plasma glucose–lowering effect in both the diabetic subjects (mean FPG on the 4 experimental days; range 11–12.6 mmol/l), in whom plasma glucose was reduced by 0.8–1.4 mmol/l (no significant difference between doses), and the healthy subjects (FPG 5.3–5.5 mmol/l), in whom plasma glucose was reduced by 1.0–1.3 mmol/l (no significant difference between doses). Mean FPG at the days of the glucagon test and meal test was 10.8 and 11.4 mmol/l, respectively, for the type 2 diabetic patients and 5.3 and 5.6 mmol/l, respectively, for the healthy subjects.

Part 2 of the study

The mean insulin and C-peptide concentrations from the second part of the study

Figure 1—*Mean plasma insulin and C-peptide concentrations in type 2 diabetic patients (n = 6; A and C) and healthy subjects (n = 6; B and D) after intravenous bolus injections of 2.5 nmol (×), 5 nmol (○), 15 nmol (■), and 25 nmol GLP-1 (▲), and 1 mg glucagon (●) in part 1 of the study.*
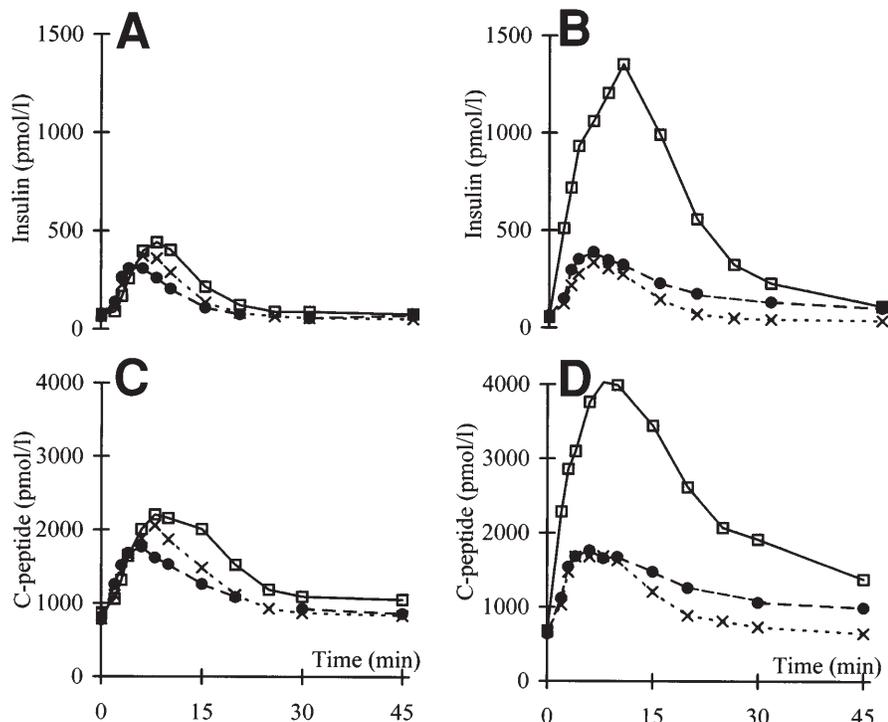
Figure 2—*Mean plasma insulin and C-peptide concentrations in type 2 diabetic patients (*n = 12;* A and* C*) and healthy subjects (*n = 12;* B and* D*) after intravenous bolus injections of 2.5 nmol GLP-1 (✕) and 1 mg glucagon (●) or after intravenous injection of 2.5 nmol GLP-1 plus glucose (□) in an amount sufficient to elevate plasma glucose to 15 mmol/l (see text).*

are shown in Fig. 2. Peak insulin and C-peptide (given in parentheses) concentrations for type 2 diabetic patients were 390 ± 74 pmol/l (2,144 ± 254 pmol/l) after 2.5 nmol GLP-1; 465 ± 87 pmol/l (2,384 ± 299 pmol/l) after combined glucose plus GLP-1 stimulation; 329 ± 50 pmol/l (1,780 ± 160 pmol/l) after glucagon; and 277 ± 42 pmol/l (2,181 ± 261 pmol/l) after the meal. For healthy subjects, the corresponding results were 356 ± 51 (2,001 ± 130), 1,412 ± 187 (4,391 ± 416), 420 ± 61 (1,995 ± 99), and 543 ± 89 (2,873 ± 210) pmol/l, respectively. The peak values were similar for 2.5 nmol GLP-1, the standard mixed meal, and the glucagon test (except for C-peptide responses for healthy subjects administered 2.5 nmol GLP-1 vs. the meal test [$P < 0.05$]). With the combined glucose plus GLP-1 stimulation, an increased insulin and C-peptide response was seen as compared with 2.5 nmol GLP-1 alone for healthy subjects ($P < 0.001$) (NS for patients [$n = 9$]). There was no significant difference between side effects reported by the patient group and the control group. Fewer side effects were reported in the second part of the study, with 54% of the par-

ticipants complaining of reduced well-being, 25% of profuse sweating, and 46% of nausea with the glucagon test compared with 42, 13, and 29%, respectively, with 2.5 nmol GLP-1. During the combined glucose plus GLP-1 stimulation, 19% of the patients complained of both reduced well-being and nausea, and only 10% complained of profuse sweating, which was significantly lower than the frequency of side effects reported during the glucagon test ($P < 0.05$). Peak insulin and C-peptide (given in parentheses) concentrations for type 2 diabetic patients were 382 ± 84 pmol/l (2,477 ± 299 pmol/l) after subcutaneous administration of 1.5 nmol GLP-1/kg at a glucose concentration of 15 mmol/l and 407 ± 103 pmol/l (2,093 ± 274 pmol/l) with the combined glucose plus GLP-1 stimulation (NS). For healthy subjects, the corresponding results were 1,323 ± 175 pmol/l (4,916 ± 664 pmol/l) and 1,161 ± 264 pmol/l (3,880 ± 610 pmol/l), respectively ($P > 0.05$ for peak insulin; $P = 0.046$ for C-peptide). Mean FPG on the 5 different experimental days was between 10.2 and 11.2 mmol/l for diabetic patients and between 5.3 and 5.5 mmol/l for healthy subjects.

Part 3 of the study
Plasma insulin and C-peptide concentrations from the third part of the study are shown in Fig. 3. Peak insulin and C-peptide concentrations occurred 6–10 min after termination of GLP-1 bolus injection during the combined glucose plus GLP-1 stimulation and 4 min after arginine injection during the hyperglycemic clamp. Mean insulin and C-peptide (given in parentheses) concentrations for type 2 diabetic patients were 63 ± 11 pmol/l (811 ± 111 pmol/l) at the time of GLP-1 injection and 189 ± 46 pmol/l (1,682 ± 280 pmol/l) after a 45-min hyperglycemic clamp immediately before injection of 5 g L-arginine. For healthy subjects, the corresponding results were 61 ± 14 pmol/l (6,89 ± 58 pmol/l) and 463 ± 126 pmol/l (2,657 ± 307 pmol/l), respectively. Incremental insulin and C-peptide (given in parentheses) responses calculated as the difference between the concentration at the time of GLP-1 or arginine injection and the peak response were 411 ± 130 pmol/l (1,483 ± 309 pmol/l) during the combined glucose plus GLP-1 stimulation and 628 ± 226 pmol/l (1,360 ± 250 pmol/l) during the hyperglycemic clamp for type 2 diabetic patients ($P = 0.19$ for incremental insulin; $P = 0.63$ for C-peptide). For healthy subjects, the corresponding results were 1,342 ± 302 pmol/l (3,364 ± 502 pmol/l) and 1,921 ± 338 pmol/l (3391 ± 388 pmol/l), respectively ($P = 0.008$ for incremental insulin; $P = 0.92$ for C-peptide). The absolute mean peak insulin and C-peptide (given in parentheses) concentrations for type 2 diabetic patients were 475 ± 141 pmol/l (2,295 ± 379 pmol/l) during the glucose plus GLP-1 stimulation and 816 ± 268 pmol/l (3,043 ± 508 pmol/l) during the hyperglycemic clamp ($P = 0.09$ for mean peak insulin; $P = 0.02$ for C-peptide). For healthy subjects, the corresponding results were 1,403 ± 308 pmol/l (4,053 ± 533 pmol/l) and 2,384 ± 452 pmol/l (6,047 ± 652 pmol/l), respectively ($P = 0.003$ for mean peak insulin; $P = 0.0003$ for C-peptide). Mean FPG was 8.9 mmol/l on the day of the combined glucose plus GLP-1 stimulation and 9.2 mmol/l on the day of the hyperglycemic clamp for the type 2 diabetic patients. The corresponding results for healthy subjects were 5.5 and 5.6 mmol/l, respectively.

CONCLUSIONS — The intestinal incretin hormone GLP-1 is the most potent stimulus known for β-cell secretion (12).

Furthermore, it also has been demonstrated to be remarkably effective in patients with type 2 diabetes (13). Thus, an intravenous infusion of GLP-1 into a group of patients with moderate type 2 diabetes during the conditions of a hyperglycemic clamp maintained at 8–9 mmol/l resulted in insulin and C-peptide responses of similar magnitude to those observed in a control group of healthy subjects (13). Further, in patients with long-standing disease and secondary failure of oral antidiabetic drugs, an infusion of GLP-1 caused insulin secretion that was sufficient, together with the simultaneous inhibition of glucagon secretion, to normalize blood glucose (14). Theoretically, the β-cell secretory capacity depends on *1*) the total β-cell mass, *2*) the sensitivity of the individual cells to the applied stimulus, and *3*) the secretory capacity of the individual cells. In diabetes, all of the 3 parameters may be impaired. In type 2 diabetes particularly, the sensitivity towards glucose is impaired (15–17), and it is therefore important to choose a stimulus for which β-cell sensitivity is best preserved. GLP-1 could be such a stimulus. In this investigation, we therefore compared β-cell secretory responses with GLP-1 in various doses and modes of administration to the response to a meal, to glucagon, and to arginine injected during a hyperglycemic clamp. In the dose-response part of the study, we found that similar peak insulin and C-peptide concentrations were obtained with a standard meal, 2.5 nmol GLP-1, and 1 mg glucagon; however, GLP-1 had fewer side effects than glucagon. Significantly greater responses were obtained with the higher doses of GLP-1; therefore, maximal responses to a single injection of GLP-1 may require a higher dose than the 2.5 nmol dose used in this study. On the other hand, an increasing number of patients reported side effects with the higher doses. In the normal subjects, similar responses were obtained with all doses. Interestingly, the absolute responses to either stimulus were virtually identical to those of the patients, confirming the observation that the insulinotropic effect of GLP-1 is widely preserved in type 2 diabetes (13). The finding that a dose-response relationship existed for the patients but not for the healthy subjects suggests that the β-cell sensitivity to GLP-1 may be reduced in the patients. The responses to glucagon were similar, indicating that glucagon is as efficient as GLP-1 as a stimulus for β-cell secretion (but much less potent and with more side effects). The results obtained in the extended groups of patients and healthy
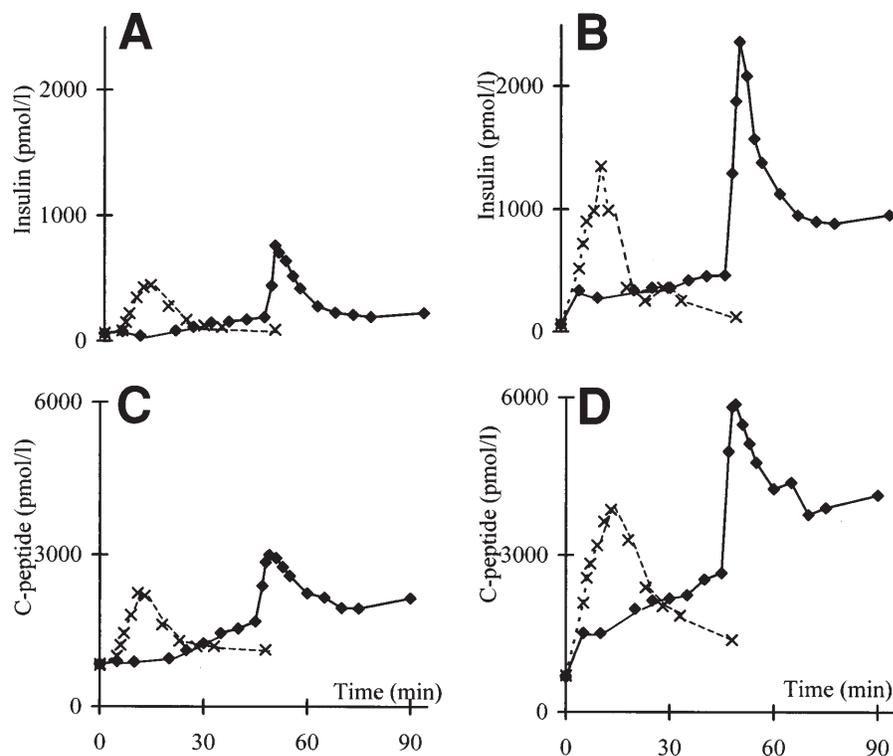

Figure 3—*Mean plasma insulin and C-peptide concentrations in type 2 diabetic patients (*n = 8;* A and* C*) and healthy subjects (*n = 8;* B and* D*) after intravenous injection of 2.5 nmol GLP-1 plus glucose in an amount sufficient to elevate plasma glucose to 15 mmol/l (✗) and during a 30 mmol/l hyperglycemic clamp with arginine (◆). Arginine (5 g) was injected intravenously as a bolus at 45 min.*

subjects were similar to those obtained in the dose-response study.

In the dose-response study, as expected, all doses of GLP-1 lowered plasma glucose concentrations, whereas increases were observed with both the meal test and the glucagon test. Thus, because of a smaller glucose signal to the β-cell, the effect of GLP-1 might have been underestimated because hyperglycemia potentiates the β-cell response to most insulin secretagogues, although less so in type 2 diabetic patients (3,18–20). Therefore, in part 2 of the study, we tested the effect of hyperglycemia on the β-cell response to GLP-1. Here, a pronounced difference between healthy subjects and patients emerged in that the secretory responses increased almost 4-fold in the healthy subjects, whereas only a minor increase was observed in the patients. On one hand, this presumably illustrates the glucose insensitivity of the diabetic β-cell; on the other hand, it might indicate that GLP-1 in a dose of 2.5 nmol is indeed capable of eliciting a β-cell response that is near maximal in the diabetic patients. Increasing the duration of

GLP-1 stimulation by subcutaneous rather than intravenous injection did not increase the peak responses further. The insulin and C-peptide response during the combined glucose plus GLP-1 stimulation would suggest that the β-cell secretory capacity is impaired to ∼25% in this group of type 2 diabetic patients compared with the β-cell secretory capacity of the healthy subjects.

To evaluate the relationship between the combined glucose plus GLP-1 stimulation and the maximal secretory capacity, we compared the responses of the combined test with those obtained using a hyperglycemic clamp plus arginine, as described by Ward et al. (3). The incremental insulin and C-peptide responses were similar for diabetic patients, but both the plasma insulin and C-peptide concentrations increased during the 30 mmol/l glucose clamp, so that the absolute β-cell response was greater with arginine than with GLP-1. The priming effect of the β-cell during the 45-min hyperglycemic clamp may explain the higher absolute insulin and C-peptide responses during the arginine clamp. Thus, even in patients with type 2

diabetes, the maximal secretory rate of the β-cell can only be elicited with a combination of high glucose concentrations (i.e., much higher than the patients' daily glucose levels) and an additional potent secretagogue, which could be either GLP-1 or arginine. However, the patients' capacity to secrete an amount of insulin, as elicited by physiological stimuli, such as ingestion of a mixed meal, may be gauged rapidly and conveniently and with little discomfort for the patients administered as little as 2.5 nmol GLP-1 intravenously. This test should be useful when administration of insulin therapy is considered in patients with type 2 diabetes. In addition, the test may be useful in judging residual β-cell capacity in patients with type 1 diabetes. However, further experiments are required to evaluate a GLP-1–based test.

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