The GLP-1 Derivative NN2211 Restores β-Cell Sensitivity to Glucose in Type 2 Diabetic Patients After a Single Dose

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Glucagon-like peptide 1 (GLP-1) stimulates insulin secretion in a glucose-dependent manner, but its short half-life limits its therapeutic potential. We tested NN2211, a long-acting GLP-1 derivative, in 10 subjects with type 2 diabetes (means ± SD: age 63 ± 8 years, BMI 30.1 ± 4.2 kg/m^2, HbA_{1c} 6.5 ± 0.8%) in a randomized, double-blind, placebo-controlled, crossover study. A single injection (7.5 μg/kg) of NN2211 or placebo was administered 9 h before the study. β-cell sensitivity was assessed by a graded glucose infusion protocol, with glucose levels matched over the 5–12 mmol/l range. Insulin secretion rates (ISRs) were estimated by deconvolution of C-peptide levels. Findings were compared with those in 10 nondiabetic volunteers during the same glucose infusion protocol. In type 2 diabetic subjects, NN2211, in comparison with placebo, increased insulin and C-peptide levels, the ISR area under the curve (AUC) (1,130 ± 150 vs. 668 ± 106 pmol/kg; $P < 0.001$), and the slope of ISR versus plasma glucose (1.26 ± 0.36 vs. 0.54 ± 0.18 pmol · l[min^{-1} · mmol^{-1} · kg^{-1}]; $P < 0.014$), with values similar to those of nondiabetic control subjects (ISR AUC 1,206 ± 99; slope of ISR versus plasma glucose, 1.44 ± 0.18). The long-acting GLP-1 derivative, NN2211, restored β-cell responsiveness to physiological hyperglycemia in type 2 diabetic subjects. *Diabetes* 52:1786–1791, 2003

Glucagon-like peptide 1 (GLP-1) is a hormone that stimulates insulin secretion and simultaneously decreases glucagon secretion (1,2). The insulinotropic effect is glucose dependent. Because GLP-1 stimulates insulin secretion primarily at elevated glucose levels, it is possible that GLP-1 therapy of type 2 diabetes might present a low risk of hypoglycemia (3). GLP-1 might also decrease hepatic glucose production indirectly (4), delay gastric emptying, and suppress appetite in type 2 diabetic patients (5). This array of effects gives GLP-1 the potential to be an efficacious and safe glucose-lowering agent for type 2 diabetes. In addition, GLP-1 has been shown to stimulate the differentiation of islet progenitor cells into insulin-producing cells and may be important for β-cell neogenesis (6).

Short-term (12-h) infusion of GLP-1 as well as 6-week continuous subcutaneous infusion of GLP-1 has been shown to significantly improve insulin secretion in type 2 diabetic patients (7,8). However, native GLP-1 has a very short half-life because of its rapid degradation by dipeptidyl peptidase IV and, thus, is unlikely to be used as a therapeutic drug in diabetes treatment. NN2211 is an acylated derivative of GLP-1 with full agonistic activity at the GLP-1 receptor in vitro (9). NN2211 is slowly degraded because of a combination of albumin binding, metabolic stability, and gradual release from the injection site. Pharmacokinetic profiles in healthy volunteers and type 2 diabetic subjects have shown that NN2211 is suitable for once-daily injection (10–12). NN2211, in a single dose of 10 μg/kg, has been found to effectively reduce fasting and postprandial hyperglycemia, delay gastric emptying, and suppress prandial glucagon secretion in type 2 diabetic patients (12).

In the present study, we assessed the effect of a single subcutaneous injection of NN2211 on β-cell sensitivity to glucose using a graded glucose infusion protocol in a group of adults with type 2 diabetes. The trial was a randomized, double-blind, placebo-controlled crossover trial. The degree of improvement of β-cell function was also assessed by comparison with a control group of healthy volunteers of similar age who did not receive the drug.

RESEARCH DESIGN AND METHODS

Type 2 diabetic subjects. The protocol was approved by the University of Michigan Institutional Review Board and performed in accordance with the Declaration of Helsinki. All subjects gave their informed consent after the nature of the study was explained in detail to them. Type 2 diabetic subjects ($n = 10$; 6 men, 4 women) were evaluated in a randomized, double-blind, placebo-controlled, two-period crossover trial. Baseline clinical characteristics for diabetic and control subjects are summarized in Table 1. All diabetic subjects met American Diabetes Association (ADA) criteria for type 2 diabetes with a fasting plasma glucose ≥7.0 mmol/l or a 2-h plasma glucose ≥11.1 mmol/l, determined by oral glucose tolerance testing. Subjects were treated for a minimum of 2 months with diet therapy or 3 months of oral hypoglycemic monotherapy before entering the study. Of the 10 subjects, 9 were treated with oral hypoglycemic agents (sulfonylurea, $n = 4$; metformin, $n = 4$; α-glucosidase inhibitor, $n = 1$) and 1 was treated with diet alone. Treatment

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ADA, American Diabetes Association; AUC, area under the curve; ELISA, enzyme-linked immunosorbent assay; GCRC, General Clinical Research Center; GLP-1, glucagon-like peptide 1; ISR, insulin secretion rate.

TABLE 1
Clinical characteristics of diabetic and control subjects

	Diabetic subjects	Control subjects
n	10	10
Age (years)	62.9 ± 7.6	61.3 ± 7.3
Sex (male/female)	6/4	5/5
Weight (kg)	88.1 ± 18.9	75.2 ± 13.6
BMI (kg/m^2)	30.1 ± 4.2	26.0 ± 2.8
HbA_{1c} (%)	6.5 ± 0.8	5.1 ± 0.4
Duration of diabetes (years)	5.4 ± 5.7	—

Data are mean ± SD.

with a sulfonylurea was discontinued 1 week before study days and other oral antidiabetic agents were discontinued 1 day before study days.

Subjects ate their usual evening meal and then fasted after 20:00 on the day before study days. They arrived at the University of Michigan General Clinical Research Center (GCRC) at 20:00. Catheters were inserted in antecubital veins and contralateral hand/wrist veins for blood sampling and infusion purposes. At 23:00, 7.5 μg/kg of NN2211 or placebo were injected subcutaneously into the abdomen in random order using NovoPen 1.5 with NovoFine needles. There was a 3–6 week interval between dosing periods. Insulin was administered as a low-dosage (0.5–5.0 units/h) continuous infusion overnight to ensure baseline glucose levels were near normal and comparable before the graded glucose infusion protocol during each study day.

A graded glucose infusion protocol was initiated at 08:00 the following day. The aim of this procedure was to assess insulin secretion in response to gradually raising plasma glucose levels from ~5 to 12 mmol/l over 3 h. Subjects were studied in the supine position. The antecubital intravenous catheter was used for insulin and glucose infusions. The second intravenous catheter in a dorsal hand/wrist vein of the contralateral arm was placed into a warming box heated to 60°C to obtain arterialized blood samples for glucose and insulin. After an initial baseline sample was taken, a small intravenous dose of insulin (0.007–0.014 units/kg) was administered to lower the glucose level to ~5 mmol/l. The insulin was allowed to decay (20 min after bolus insulin), after which samples were drawn at 5-min intervals for 15 min to define baseline levels of glucose, insulin, and C-peptide. An intravenous infusion of 20% dextrose was then started. Every 5 min, samples were drawn for determination of insulin, C-peptide, and glucose. For each of the 5-min samples, plasma glucose was measured using a Beckman glucose analyzer (Beckman, Palo Alto, CA), and the glucose infusion rate was adjusted accordingly to gradually raise plasma glucose levels from 5 to 12 mmol/l over 3 h. Periodic additional blood samples were drawn for glucagon and NN2211 concentrations.

Normal control subjects. Healthy subjects (n = 10; 5 men, 5 women) who did not receive the trial medication also underwent the graded glucose infusion protocol to provide reference data on β-cell function. All control subjects met ADA criteria for normal glucose tolerance with a fasting plasma glucose ≤6.1 mmol/l and 2-h plasma glucose <7.8 mmol/l, as determined with an oral glucose tolerance test. Subjects were selected to approximate the demographic characteristics of the diabetic patients. As shown in Table 1, the diabetic and control subjects were well matched except for BMI, which was higher in diabetic subjects. The control subjects were admitted to the GCRC the morning after an overnight fast. The graded glucose infusion protocol was carried out as described for the diabetic patients, except control subjects did not receive the overnight insulin infusion or the trial medication.

Assays. Plasma glucose was measured immediately at bedside with a Beckman glucose analyzer using the glucose oxidase technique. HbA_{1c} was measured by high-performance liquid chromatography, with a normal range of 3.8–6.4%. All other blood samples were centrifuged and serum was stored at –20°C until analysis. Serum insulin was quantified using a highly specific and sensitive two-site enzyme-linked immunosorbent assay (ELISA) with an interassay coefficient of variation of 3% (DAKO Diagnostics, Cambridgeshire, U.K.). C-peptide was measured by a two-site monoclonal-based ELISA with an interassay coefficient of variation (in triplicate) of 3% (DAKO Diagnostics). Glucagon was assessed by radioimmunoassay (GL-32K; Linco Research, St. Charles, MO.). NN2211 concentrations were analyzed by ELISA using a capture monoclonal antibody against GLP-1/NN2211 and a detection monoclonal antibody specific for the NH_2-terminal portion of GLP-1/NN2211. Before pharmacokinetic analysis, the samples were incubated at 37°C to remove endogenous GLP-1, as NN2211 is stable with incubation. The mean accuracy (recovery) of the NN2211 assay has been reported as 102% (range 93–113%) (11).

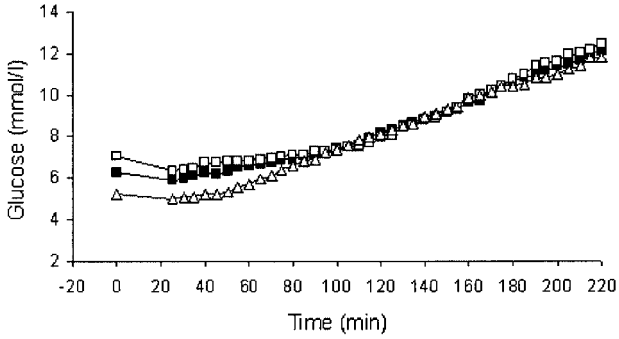

FIG. 1. Profile of plasma glucose concentrations during graded glucose infusion studies after a single injection of NN2211 (■) or placebo (□) given 9 h before the study in type 2 diabetic subjects. Results are compared with those of healthy control subjects (△) who did not receive the drug. Baseline glucose values were normalized by overnight insulin infusion in diabetic subjects. Glucose levels during variable rate glucose infusion begun at time 0 were well matched during the NN2211 and placebo studies in diabetic subjects and were also matched with values for control subjects from 80 to 220 min. Data are means ± SE, n = 10 for each group.

Statistical analysis. The primary objective of the efficacy analysis was to compare the effects of NN2211 versus placebo on β-cell responsiveness to graded glucose infusion as assessed by the insulin secretion rate (ISR) area under the curve (AUC) over the 40- to 220-min time interval of graded glucose infusion studies. The ISR during the graded glucose infusion protocol was derived by deconvolution of peripheral C-peptide concentrations and previously determined C-peptide kinetics (13,14). The total ISR over the 40- to 220-min time interval was estimated for each subject by calculating the AUC using the trapezoidal rule.

The secondary objectives of the efficacy analysis were to compare NN2211 with placebo with respect to the following: *1*) slope of the ISR versus plasma glucose level for each subject (estimated by a regression model of ISR on plasma glucose level), *2*) glucagon AUC over the 40- to 220-min time interval (calculated in a similar way to that of ISR AUC), and *3*) insulin clearance (mean ISR divided by mean insulin concentration).

Adverse events were recorded on the treatment days. All efficacy end points for NN2211 versus placebo were analyzed using an ANOVA model for the crossover design. Two-sided tests were performed with P = 0.05 as the level of significance. Results for diabetic subjects are given as NN2211 versus placebo.

RESULTS

Plasma glucose, insulin, and C-peptide levels. Profiles of plasma glucose, insulin, and C-peptide during graded glucose infusion studies after NN2211 or placebo administration in diabetic subjects are displayed in Figs. 1 and 2. Results are compared with those of healthy control subjects who did not receive the drug. Fasting plasma glucose levels in diabetic subjects at initiation of the graded glucose infusion protocol (9 h after dosing and overnight insulin infusion) were slightly lower with NN2211 than with placebo (6.28 ± 0.29 vs. 7.03 ± 0.39 mmol/l). However, glucose levels were well matched in diabetic subjects over the 40- to 220-min time interval of the graded glucose infusion protocols, which was included in ISR AUC determination, and were also matched with control subjects over the 80- to 220-min time interval. Variable glucose infusion rates were used to achieve the matched glucose levels during graded glucose infusion studies, with mean total glucose infusion rates of 233 ± 13 ml with NN2211 and 167 ± 11 ml with placebo in the diabetic subjects (P < 0.001) and 338 ± 16 ml in the control subjects.

Fasting levels of insulin (90 ± 41 vs. 70 ± 26 pmol/l) and C-peptide (0.8 ± 0.1 vs. 0.7 ± 0.1 nmol/l) were similar for NN2211 and placebo. Insulin and C-peptide levels signifi-

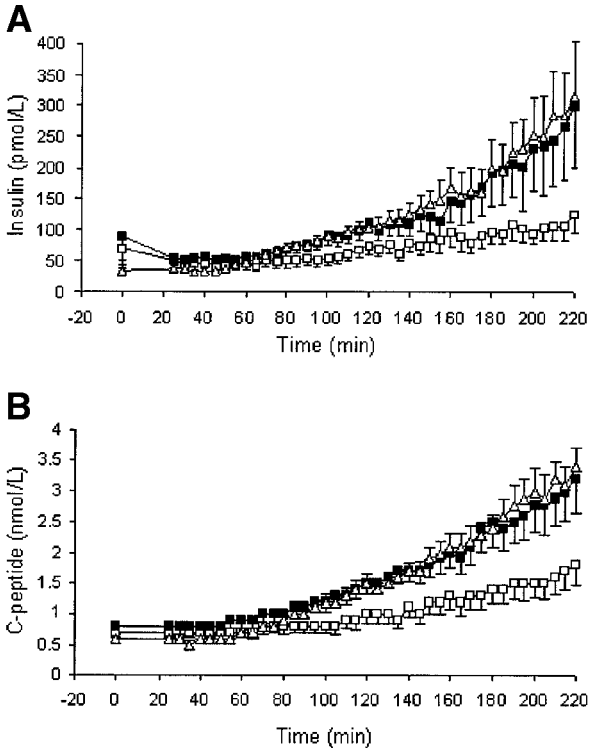

FIG. 2. Profiles of plasma insulin (*A*) and C-peptide (*B*) concentrations during graded glucose infusion studies after a single injection of NN2211 (■) or placebo (□) in type 2 diabetic subjects. Results are compared with those of healthy control subjects (△) who did not receive the drug. Insulin and C-peptide levels significantly increased in response to the graded hyperglycemic stimulus after NN2211 compared with after placebo ($P < 0.001$ for insulin AUC and C-peptide AUC) to values similar to those of healthy control subjects. Data are means ± SE, $n = 10$ for each group.

cantly increased in response to the graded hyperglycemic stimulus after NN2211 compared with placebo in diabetic subjects ($P < 0.0001$ for both insulin and C-peptide AUC). Values of insulin and C-peptide with NN2211 in diabetic subjects were similar to those of healthy control subjects who did not receive the drug.

Insulin secretion. As shown in Fig. 3, the ISR increased substantially with NN2211 administration compared with placebo during the graded glucose infusion studies over the glucose range 6–12 mmol/l, and was similar to values in healthy control subjects who did not receive the drug. The effects of NN2211 in enhancing insulin secretory response became evident after 40–60 min of glucose infusion, when the plasma glucose levels reached 6–7 mmol/l. The drug effect was increasingly apparent as plasma glucose levels increased further to 12 mmol/l, thus displaying the glucose-dependent action of NN2211. As summarized in Fig. 4, β-cell sensitivity to glucose as assessed by the ISR AUC was increased by ~70% with NN2211 treatment (1,130 ± 150 vs. 668 ± 106 pmol/kg; $P < 0.001$). The ISR AUC with NN2211 in diabetic subjects was similar to values in healthy control subjects who did not receive the drug (1,206 ± 99). The ISR AUC was an estimate of the total amount of insulin secreted per kilogram of body weight over the 40- to 220-min time interval of the graded glucose infusion studies. The effect of NN2211 on the slope of ISR versus plasma glucose level is summarized in Fig. 5. The slope of ISR versus plasma glucose level increased by ~133% with NN2211 (1.26 ± 0.36 vs. 0.54 ± 0.18 pmol · l[min^{-1}] · $mmol^{-1}$ · kg^{-1}]; $P < 0.014$). The slope of ISR versus plasma glucose level with NN2211 in diabetic subjects was comparable to the value in healthy control subjects (1.44 ± 0.18).

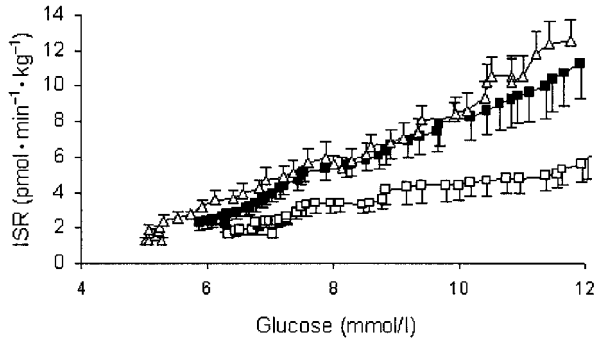

FIG. 3. Relation between ISR and plasma glucose levels during the graded glucose infusion protocol in type 2 diabetic subjects who received NN2211 (■) or placebo (□). ISR was derived by deconvolution of peripheral C-peptide concentrations. ISR was substantially increased with NN2211 compared with placebo over the glucose range 6–12 mmol/l and was similar to values in healthy control subjects (△) who did not receive the drug. Data are means ± SE, $n = 10$ for each group.

The higher glucose infusion rate with NN2211 versus placebo in diabetic subjects was compatible with the greater ISR after NN2211. However, the glucose infusion rate in diabetic subjects remained considerably less than that of healthy control subjects, whereas serum insulin curves were virtually superimposable. Thus, the diabetic subjects were likely to have a greater degree of insulin resistance compared with the healthy control subjects, despite similar insulin secretory responses in the two groups. This observation was compatible with reduced sensitivity to endogenously secreted insulin in the diabetic subjects compared with healthy control subjects.

Other measures. As shown in Fig. 6, glucagon levels were similar with NN2211 and placebo administration in the fasting state and did not change over time in the diabetic subjects. The glucagon AUC remained unchanged with NN2211 compared with placebo (14,665 ± 1,350 vs. 15,761 ± 1,788 pg · min^{-1} · ml^{-1}; $P = 0.24$). Plasma glucagon levels were higher in the fasting state and during graded glucose infusion studies in diabetic subjects compared with control subjects, although the glucagon AUC was not significantly different (11,827 ± 768 pg · ml^{-1} in healthy control subjects; $P = 0.11$). Insulin clearance (mean ISR divided by mean insulin concentration) was similar with NN2211 and placebo (0.06 ± 0.01 vs. 0.05 ± 0.01 l · min^{-1} · kg^{-1}; $P = 0.35$). The pharmacokinetic profile of NN2211 was evaluated by 10-point profiles for up to 17 h after dosing. The AUC of NN2211 plasma concentration from 0 to 17 h was 70,742 ± 19,256 pmol/l, the C_{max} of NN2211 was 5,884 ± 1,778 pmol/l, and the T_{max} of NN2211 was 13.1 ± 2.8 h. The $t_{1/2}$ was not determined because of the limited duration of postdosing profiling and because this parameter has been reported in previous studies with NN2211 in healthy ($t_{1/2}$ = 11–15 h) and diabetic subjects ($t_{1/2}$ = 10.0 ± 3.5 h) (10–12).

Adverse events. All diabetic and control subjects completed the study. As expected, no hypoglycemic events occurred. Only one diabetic subject experienced a gastro-

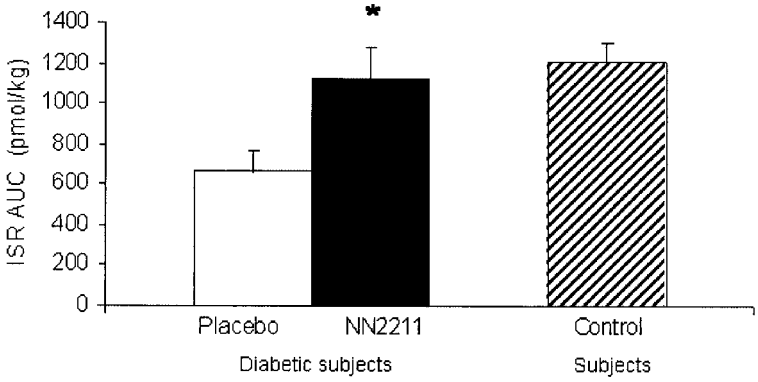

FIG. 4. ISR AUC during the 40- to 220-min time interval of graded glucose infusion studies in diabetic subjects treated with placebo (□) or NN2211 (■) and in control subjects (▨) who did not receive the drug. After NN2211 injection, the ISR AUC was significantly increased in diabetic subjects compared with after placebo (*$P < 0.001$) and was similar to that of control subjects. Data are means ± SE, $n = 10$ for each group.

intestinal side effect of mild diarrhea on the day of active treatment; that subject was able to complete the study protocol and had resolution of gastrointestinal symptoms before discharge. A second diabetic subject experienced mild headache on the day of active treatment; that subject also was able to complete the study and had resolution of the headache before initiation of the graded glucose infusion protocol.

DISCUSSION

In this study, we investigated the effect of a single dose of NN2211, a long-acting GLP-1 derivative, on β-cell responsiveness to physiological hyperglycemia in a group of adults with type 2 diabetes. GLP-1 is a potent glucose-dependent, insulinotropic hormone that is of potential interest for the treatment of type 2 diabetes. GLP-1 administered by continuous infusion and repeated subcutaneous injection has been shown to significantly reduce fasting and postprandial hyperglycemia in type 2 diabetic patients (2,3,5).

Continuous infusion of GLP-1 for 12 h has been shown to improve basal and stimulated β-cell function, as assessed by hyperglycemic clamp and arginine stimulation in type 2 diabetic patients (7). A 6-week continuous subcutaneous infusion of GLP-1 (compared with saline infusion) has been shown to significantly improve insulin secretion, as assessed by the hyperglycemic clamp method in 10 type 2 diabetic subjects, and also to decrease fasting glucose, HbA_{1c}, and fructosamine levels (8). In a recent study, GLP-1 infusion also increased insulin secretory response to graded glucose infusion in a dosage-dependent manner in type 2 diabetic and healthy control subjects (15). However, the rapid degradation of GLP-1 and the need for continuous infusion prevent the hormone's broader clinical use. In contrast, NN2211 is a slowly degraded GLP-1 analog that has been found to be suitable for once-daily dosing.

In the present study, a single dose of NN2211 (compared with placebo) significantly increased insulin and C-peptide levels and substantially improved the overall insulin secretory response to a controlled, gradual increase of glucose levels over a physiological range during the glucose infusion protocol in type 2 diabetic subjects. NN2211 was administered the night before study days to achieve maximum concentration of the drug during the graded glucose infusion protocol the following morning. One advantage of this study was the careful matching of glucose levels over time, which allowed quantitative comparisons of insulin secretion with placebo versus with NN2211, and also comparison with a nondiabetic control group over the same glucose range. In addition, insulin secretion was assessed in response to physiological postprandial glucose levels, which contrasts with the high nonphysiological glucose levels achieved with the hyperglycemic clamp method.

The effects of NN2211 in enhancing the insulin secretory response became evident after 40–60 min of glucose infusion when the plasma glucose levels reached 6–7 mmol/l. The drug effect was increasingly apparent as plasma glucose levels increased further to 12 mmol/l. The glucose level dependency of the effect of NN2211 on insulin secretion agrees with findings of GLP-1 studies in normal (6,17) and diabetic subjects (3). The lack of effect of NN2211 on insulin secretion at euglycemia indicates that this drug might not lead to inappropriate insulin secretion, which could limit the risk of hypoglycemia in

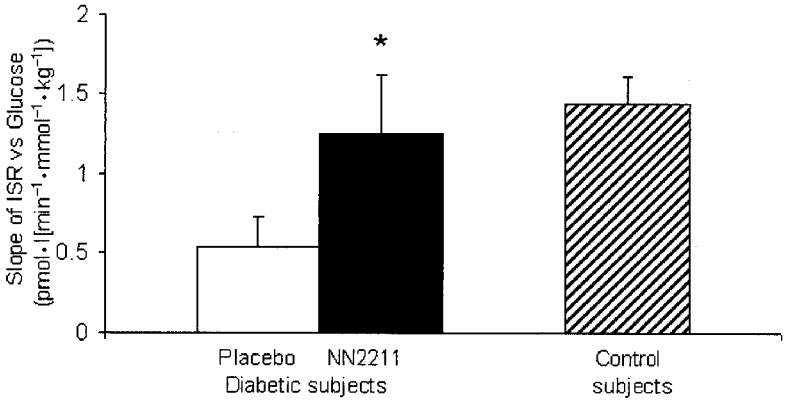

FIG. 5. Slope of ISR versus glucose during graded glucose infusion studies in diabetic subjects treated with placebo (□) or NN2211 (■) and in control subjects (▨) who did not receive the drug. After NN2211 injection, slope of ISR was significantly increased in diabetic subjects compared with after placebo (*$P < 0.014$) and was similar to that of control subjects. Data are means ± SE, $n = 10$ for each group.

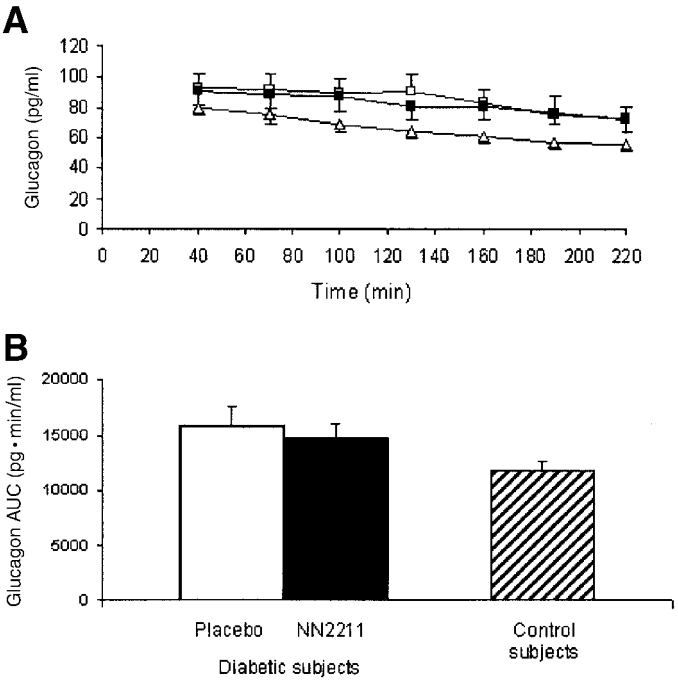

FIG. 6. Profile of plasma glucagon (*A*) and glucagon AUC (*B*) during graded glucose infusion studies. *A*: Plasma glucagon in diabetic subjects treated with placebo (□) or NN2211 (■) and in control subjects (△) who did not receive the drug. Plasma glucagon levels in diabetic subjects were similar with NN2211 and placebo and did not change over time. *B*: Glucagon AUC remained unchanged with NN2211 (■) compared with placebo (□) in diabetic subjects ($P = 0.24$). Plasma glucagon levels were higher in diabetic subjects compared with control subjects (▨), although glucagon AUC was not significantly different ($P = 0.11$). Data are means ± SE, $n = 10$ for each group.

type 2 diabetic patients. Administration of subcutaneous GLP-1 and intravenous glucose has been shown to induce reactive hypoglycemia in healthy volunteers, but not in type 2 diabetic subjects (18). However, long-term clinical intervention studies are needed to test this hypothesis.

The insulin and C-peptide levels and insulin secretory response achieved with NN2211 in diabetic subjects were remarkably similar to those of healthy control subjects with normal glucose tolerance who did not receive the drug. The restoration of insulin secretion dynamics has not been observed with oral hypoglycemic agents in type 2 diabetic patients (19,20). Thus, the dramatic improvement of insulin responses during graded hyperglycemia after NN2211 is encouraging. However, this comparison does not take into account the likely greater insulin resistance in diabetic subjects, given their higher BMI, increased fasting insulin levels, and decreased glucose infusion rates needed to achieve similar glucose and insulin levels during study. At a comparable level of insulin resistance, individuals with normal β-cells would likely secrete much more insulin in response to a challenge. Thus, despite the dramatic improvement of insulin secretion in the diabetic subjects after NN2211, it is likely that their β-cell function remained impaired.

Impairment of islet sensitivity to glucose is an early abnormality of β-cell function, as demonstrated in studies of families with maturity-onset diabetes of the young. Subjects who have glucokinase mutations with elevated fasting and postprandial glucose levels were found to have similar first-phase insulin response to intravenous glucose tolerance testing compared with nondiabetic control subjects (21). However, insulin secretion rates were 61% lower with graded glucose infusion studies in which glucose infusion rates were increased in a stepwise fashion. Our subjects with relatively well-controlled type 2 diabetes also had a very poor insulin secretion response to graded glucose infusion (see placebo values in Figs. 3–5 compared with those of nondiabetic control subjects).

In a previous study, a single injection of NN2211 was found to reduce fasting and postprandial hyperglycemia, suppress prandial glucagon secretion, and delay gastric emptying in type 2 diabetic patients (12). This study also evaluated insulin secretion in response to a standard meal. Postprandial insulin secretion was only slightly and insignificantly increased with NN2211; however, the interpretation of these findings is difficult as postprandial glucose levels were also substantially reduced with NN2211 compared with placebo. In contrast, in the current study, the insulin secretory response increased dramatically with NN2211 compared with placebo when patients were studied while glucose levels were carefully matched.

A limitation of this study was its short duration, in that the effect of only a single injection of NN2211 was tested. Clearly, a longer-term study is needed to define the effectiveness of NN2211 in enhancing insulin secretion in type 2 diabetic patients. Another limitation was the overall good glycemic control of the patients studied. The presence of residual β-cell function in these patients may have contributed to the observed dramatic effect of NN2211 (although β-cell function was grossly impaired in the absence of NN2211). The effectiveness of NN2211 on insulin secretion in patients with poorly controlled type 2 diabetes needs to be assessed. All of the diabetic subjects in the current study were treated with oral monotherapy, except one subject who was treated through diet. A possible carryover effect of sulfonylurea treatment on insulin secretion should have been limited by discontinuation of these agents 1 week before the studies. In addition, the metformin used by four subjects and the α-glucosidase inhibitor used by one subject were withheld 1 day before the studies; however, these agents are not known to have direct effects on β-cell function. In the current study, effects on fasting hyperglycemia could not be assessed, as diabetic subjects received an overnight insulin infusion to achieve comparable glucose levels before initiation of graded glucose infusion studies. We observed similar glucagon levels with intravenous glucose stimulation with NN2211 and placebo, in contrast to the meal-related suppression of glucagon levels seen with NN2211 (12) and GLP-1 (2). Because hyperglycemia tends to suppress glucagon secretion, the current protocol was not well suited to testing for a possible suppressive effect of NN2211 on glucagon secretion. In addition, because this study did not compare the effects of GLP-1 and NN2211, it cannot be concluded that NN2211 will be as efficacious as GLP-1 in all circumstances.

In summary, during controlled, matched hyperglycemia in patients with well-controlled type 2 diabetes, a single dose of NN2211 (compared with placebo) increased insulin and C-peptide levels and dramatically improved insulin secretory response to glucose, as assessed by the increased ISR AUC and the increased slope of ISR versus

plasma glucose level. NN2211 was well tolerated, without evidence of hypoglycemia or significant side effects, and the pharmacokinetic properties should allow convenient once-daily dosing.

We conclude that acute administration of the long-acting GLP-1 derivative, NN2211, restores β-cell responsiveness to physiological hyperglycemia in patients with type 2 diabetes. NN2211 has been shown to have the potential beneficial effects of improving insulin secretion, decreasing fasting and postprandial hyperglycemia, decreasing prandial glucagon secretion, and delaying gastric emptying. Long-term studies are needed to elucidate the full therapeutic potential of NN2211.

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