Bedtime Administration of NN2211, a Long-Acting GLP-1 Derivative, Substantially Reduces Fasting and Postprandial Glycemia in Type 2 Diabetes

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Glucagon-like peptide 1 (GLP-1) is a potent glucose-lowering agent of potential interest for the treatment of type 2 diabetes. To evaluate actions of NN2211, a long-acting GLP-1 derivative, we examined 11 patients with type 2 diabetes, age 59 ± 7 years (mean ± SD), BMI 28.9 ± 3.0 kg/m^2, HbA_{1c} 6.5 ± 0.6%, in a double-blind, placebo-controlled, crossover design. A single injection (10 μg/kg) of NN2211 was administered at 2300 h, and profiles of circulating insulin, C-peptide, glucose, and glucagon were monitored during the next 16.5 h. A standardized mixed meal was served at 1130 h. Efficacy analyses were performed for the fasting (7–8 h) and mealtime (1130–1530 h) periods. Insulin secretory rates (ISR) were estimated by C-peptide deconvolution analysis. Glucose pulse entrainment (6 mg · kg^{-1} · min^{-1} every 10 min) was evaluated by 1-min sampled measurements of insulin concentrations from 0930 to 1030 h and subsequent time series analysis of the insulin concentration profiles. All results are given as NN2211 versus placebo; statistical analyses were performed by analysis of variance. In the fasting state, plasma glucose was significantly reduced (6.9 ± 1.0 vs. 8.1 ± 1.0 mmol/l; P = 0.004), ISR was increased (179 ± 70 vs. 163 ± 66 pmol/min; P = 0.03), and plasma glucagon was unaltered (19 ± 4 vs. 20 ± 4 pg/ml; P = 0.17) by NN2211. Meal-related area under the curve $(AUC)_{1130–1530\ h}$ for glucose was markedly reduced (30.6 ± 2.4 vs. 39.9 ± 7.3 mmol · l^{-1} · h^{-1}; P < 0.001), ISR $AUC_{1130–1530\ h}$ was unchanged (118 ± 32 vs. 106 ± 27 nmol; P = 0.13), but the increment (relative to premeal values) was increased (65 ± 22 vs. 45 ± 11 nmol; P = 0.04). Glucagon $AUC_{1130–1530\ h}$ was suppressed (77 ± 18 vs. 82 ± 17 pmol · l^{-1} · h^{-1}; P = 0.04). Gastric emptying was significantly delayed as assessed by $AUC_{1130–1530\ h}$ of 3-ortho-methylglucose (400 ± 84 vs. 440 ± 70 mg · l^{-1} · h^{-1}; P = 0.02). During pulse entrainment, there was a tendency to increased high frequency regularity of insulin release as measured by a greater spectral power and autocorrelation coefficient (0.05 < P < 0.10). The pharmacokinetic profile of NN2211, as assessed by blood samplings for up to 63 h postdosing, was as follows: $T_{1/2}$ = 10.0 ± 3.5 h and T_{max} = 12.4 ± 1.7 h. Two patients experienced gastrointestinal side effects on the day of active treatment. In conclusion, the long-acting GLP-1 derivative NN2211 effectively reduces fasting as well as meal-related (~12 h postadministration) glycemia by modifying insulin secretion, delaying gastric emptying, and suppressing prandial glucagon secretion. *Diabetes* 51: 424–429, 2002

Patients with type 2 diabetes experience relative insulin deficiency as well as delayed and blunted meal-related insulin response (1). Glucagon excess contributes to the elevated fasting and postprandial glycemia (2). Because of a marked and glucose-dependent insulinotropic action and a restraining effect on glucagon release, glucagon like peptide-1 (GLP-1) is an obvious candidate for the treatment of type 2 diabetes (3). In addition, GLP-1 delays gastric emptying and reduces appetite in patients with type 2 diabetes also in nonemetic doses (4). Continuous intravenous infusion and repeated subcutaneous injections of GLP-1 (7-36 amide) effectively reduce fasting plasma glucose as well as meal-related glycemia (3,5). Even in patients with type 2 diabetes and secondary failure of sulfonylurea treatment, GLP-1 has been demonstrated to reduce glycemia effectively (6).

Increased β-cell function has been reported after overnight GLP-1 infusion, as measured by homeostasis model assessment (HOMA) and by first- and second-phase insulin secretion (7). The secretagogue effect is achieved by a concomitant increase of basal (nonpulsatile) and high-frequency pulsatile insulin release in patients with type 2 diabetes (8). In a study of ultradian insulin pulsatility, GLP-1 was reported to restore glucose entrainment in individuals with impaired glucose tolerance (9), indicating preferential effects on the coordination of insulin release.

GLP-1 is rapidly cleaved by dipeptidyl-peptidase IV after both intravenous and subcutaneous injections, and the development of long-acting derivatives is needed for clinical use. NN2211 is an acylated GLP-1 derivative with prolonged action due to a combination of albumin binding, metabolic stability, and slow release from the injection site. NN2211 has shown a favorable pharmacokinetic

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ApEn, approximate entropy; AUC, area under the curve; ELISA, enzyme-linked immunosorbent assay; GLP-1, glucagon-like peptide 1; HOMA, homeostasis model assessment; ISR, insulin secretory rates.

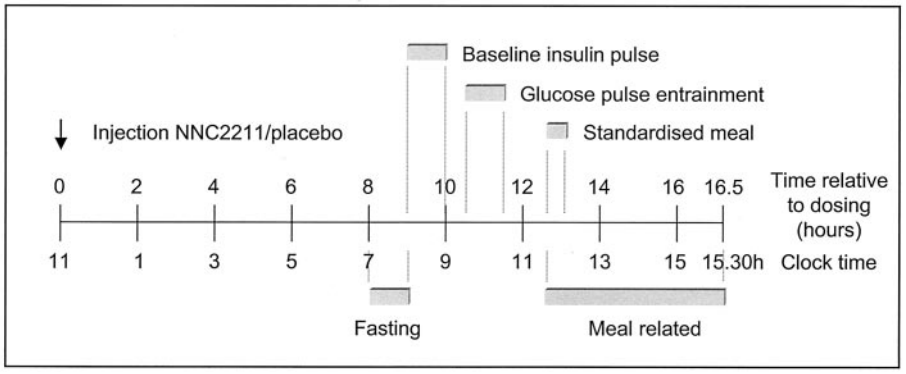

FIG. 1. Flow sheet illustrating the procedures on the study days. See text for details.

profile in nondiabetic humans, indicating a possibility of once-daily dosing in humans (10). Recent investigations in animals with experimental diabetes indicated that NN2211 increases β-cell mass, which might contribute to the long-term efficacy of GLP-1 (11).

In the present study, we assessed the effect of a single subcutaneous injection of NN2211 on fasting and meal-related circulating concentrations of glucose and glucagon, ISR, gastric emptying, and baseline and glucose-entrained high-frequency insulin release in a group of individuals with type 2 diabetes.

RESEARCH DESIGN AND METHODS

The protocol was approved by the local ethics committee and performed in accordance with the Helsinki declaration. Eleven patients (six men and five women) with type 2 diabetes were examined in a double-blind, placebo-controlled, randomized, crossover trial. Their age (mean ± SD) was 59 ± 7 years, BMI was 28.9 ± 3.0 kg/m^2, HbA_{1c} was 6.5 ± 0.6% (measured by high-performance liquid chromatography; normal range, 4.5–6.3%), and duration of diabetes was 2.7 ± 3.2 years (range 2 months to 12 years). Before study entry, four patients were treated with diet alone, and seven were additionally treated with oral hypoglycemic agents (sulfonylurea, $n = 2$; metformin, $n = 5$). Treatment with oral hypoglycemic agents was paused for 2 days before the study days.

The patients were asked to eat their usual dinner at 1800 h on the day before the study days and abstain from eating thereafter. They arrived at the clinic at 2200 h, and catheters were inserted in both antecubital veins for blood sampling and infusion purposes. At 2300 h, 10 μg/kg body wt NN2211 or placebo was injected subcutaneously into the abdomen using NovoPen 1.5 with NovoFine G30 8-mm needles as dispensing device. During the night, the patients remained asleep and blood samples were withdrawn every second hour. On the next morning, blood was sampled at 0700 and 0800 h for analysis of fasting values. From 0800 to 0900 h, the baseline high frequency pattern of insulin secretion was evaluated by a method previously described (12). In brief, 1.5 ml blood was collected every minute over a 10-s period preceded by withdrawing two times the dead space of the cannula via a three-way stopcock. Thereafter, isotonic saline was infused (1 ml/min) until the next sample was drawn. Samples were centrifuged, and serum samples were stored at −20°C until analysis. At 0900 h, pulsatile glucose infusion was initiated at a rate of 6 mg/kg over 1 min every 10 min succeeded by a 9-min pause. From 0930 to 1030 h, blood was sampled every minute for the analysis of glucose-entrained high-frequency insulin pulsatility.

At 1130 h, a standard meal of 2,500 kJ (52% carbohydrate, 29% fat, and 19% protein) was served. Patients were encouraged to eat the entire meal over the next 20 min. Four grams of 3-ortho-methyl-glucose (3-OMG) in 50 ml water was added to assess gastric emptying rate. Blood samples were collected every 15–30 min for the next 4 h for the measurements of glucose, insulin, C-peptide, and 3-OMG. A flowsheet of the procedures on study days is shown in Fig. 1.

Assays. All biochemical analyses were performed in duplicate. Plasma glucose was measured immediately on a Beckman glucose analyzer (Beckman, Palo Alto, CA) using the glucose oxidase technique. All other blood samples were stored at −20°C and analyzed within a month. 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 (DAKO Diagnostics). This assay has an interassay coefficient of variation (in triplicate) of 3%. Samples for glucagon measurements were collected in tubes with aprotinin/EDTA solution in an ice bath and centrifuged immediately thereafter. Quantitation was performed by a radioimmunoassay (GL-32K; Linco Diagnostics, St. Charles, MO). 3-OMG was analyzed by liquid chromatography tandem mass spectrometry. NN2211 concentrations were analyzed by ELISA using a monoclonal antibody against GLP-1/NN2211 as a capture antibody and another monoclonal antibody specific for the NH_2-terminal part of GLP-1/NN2211 for detection. Before this analysis, samples were incubated at 37°C to remove endogenous GLP-1, whereas NN2211 was stable toward this incubation.

Statistical analysis. Fasting data are presented as the mean of values at 0700 and 0800 h. ISR were estimated by mathematical analysis (deconvolution) of peripheral C-peptide concentrations using a two-compartment model, as described by Polonsky (13) and Eaton (14) and the standard C-peptide kinetic parameters published by Van Cauter et al. (15). This model allows accurate estimation of ISR also under non–steady-state conditions (13).

Baseline insulin pulsatility was quantified by deconvolution analysis in terms of insulin secretory burst mass, insulin secretory burst amplitude, interpulse interval, and basal (nonpulsatile) insulin secretion based on the 1-min serum insulin measurements. Initially, pulses were defined by cluster analysis identifying clusters of data points significantly elevated compared with nearby troughs (16). Subsequently, deconvolution was performed by an iterative multiparameter technique under the empirically validated assumptions that *1*) insulin is secreted in a finite number of bursts with *2*) individual amplitudes, *3*) a common half-duration superimposed on a basal time-invariant secretory rate, and *4*) a bi-exponential disappearance rate of insulin ($t_{1/2}$ = 2.8 and 5 min, fractional slow compartment 28%) (17).

Regularity of the insulin release process was evaluated by approximate entropy (ApEn) (18). ApEn measures the likelihood that runs of patterns repeat within a time series. A precise mathematical definition is given by Pincus (18). ApEn is a family of parameters dependent on the choice of the input parameters m (the length of the pattern) and r (the tolerance range within which values are considered equal) and should be compared only when applied to time series of equal length, as we do here. By application of a small r value (e.g., $r = 0.2 \times$ the overall series SD), ApEn evaluates fine (sub-) patterns in the time series, whereas a larger r value (e.g., $r = 1.0 \times$ SD) is applied to evaluate more coarse patterns (19). A larger absolute value of ApEn indicates a higher degree of process randomness. ApEn is stable to noise within the tolerance range r. To evaluate both the fine and the more coarse patterns in the time series, we calculated ApEn using $r = 0.2 \times$ SD and $r = 1.0 \times$ SD.

Glucose pulse entrainment was assessed by spectral analysis and autocorrelation analysis. In the spectral analysis, a Tukey window of 25 data points was used, and spectra were normalized assuming that the total variance in each time series was 100%. This enables comparison of spectral estimates despite the different absolute values of insulin. The values of the normalized spectra at t = 10 min during NN2211 and placebo were compared statistically. Autocorrelation analysis was performed without previous smoothing. The correlation coefficient at time lag 10 min was calculated using SPSS version 9.0 (20).

To eliminate effects of nonstationarity in the data, ApEn, spectral analysis, and autocorrelation analysis were performed on the residuals after subtraction of an 11-point centered unweighted moving average process (20).

Area under the curve (AUC) of hormones and glucose after meal stimula-

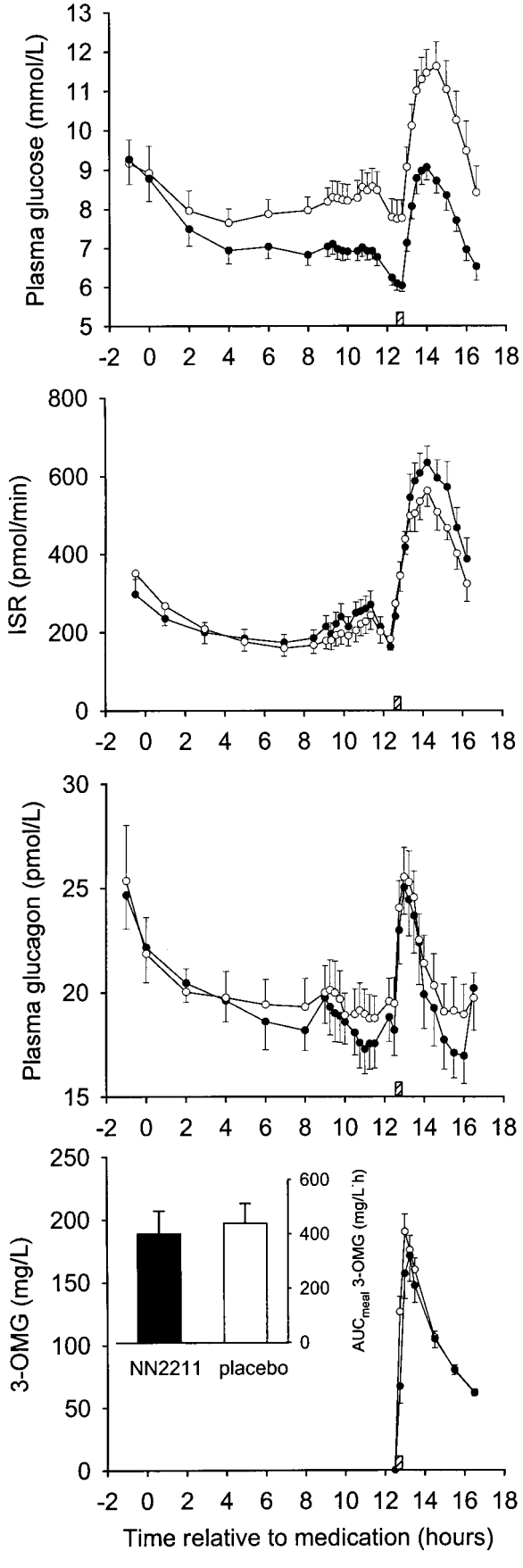

FIG. 2. Profiles of plasma glucose concentrations, ISR, plasma glucagon, and 3-OMG concentrations after a single injection of NN2211. Fasting and meal-related glucose was significantly reduced. The meal-related increment of ISR was increased, and the meal-related glucagon value was reduced. The profiles of insulin and C-peptide paralleled that of ISR. The insert in the bottom panel illustrates the delayed gastric emptying as measured by decreased AUC of 3-OMG. Data are mean ± SE.

tion was calculated by applying the trapezoidal rule. Because of preprandial differences, both absolute and incremental areas were calculated.

Adverse events, including ability to finish the meal, were recorded at the treatment days. All efficacy end points were analyzed using an analysis of variance model accounting for sequence of treatment, random subject effect, visit, and treatment. Differences were considered significant at $P < 0.05$. All results are given as NN2211 versus placebo.

RESULTS

Circulating concentrations (Fig. 2 and Table 1). Profiles of plasma glucose, insulin secretion, plasma glucagon, and rate of gastric emptying are shown in Fig. 2. Nonfasting plasma glucose was similar (8.8 ± 1.9 vs. 8.9 ± 2.3 mmol/l) at the time of NN2211 injection and declined gradually overnight. After NN2211 administration, plasma glucose was significantly lower throughout the study day compared with placebo, resulting in substantially lower fasting plasma glucose (6.9 ± 1.0 vs. 8.1 ± 1.0 mmol/l; $P < 0.01$) and premeal value (6.2 ± 0.7 vs. 7.7 ± 1.5 mmol/l; $P < 0.01$). After the mixed meal, both absolute and incremental AUC for glucose were markedly and significantly reduced by 23 and 27%, respectively, by NN2211 treatment. In the fasting condition, ISR was slightly but significantly elevated after NN2211 administration. The meal-related ISR was equal during the two treatment days, as measured by absolute AUC, whereas the meal-induced increment of insulin secretion was significantly elevated at the day of NN2211 treatment. Meal-related plasma glucagon was significantly suppressed on the day of active treatment, whereas increments were comparable. Gastric emptying was significantly delayed as measured by AUC of 3-OMG during the prandial period, and the 3-OMG peak was 15 min retarded after NN2211 treatment.

HOMA. Insulin secretory capacity as measured by HOMA was increased by ~50% after NN2211 treatment (0.56 ± 0.16 vs. 0.36 ± 0.12; $P < 0.001$), whereas insulin sensitivity remained unchanged (3.0 ± 1.3 vs. 3.1 ± 1.7; $P = 0.6$).

High-frequency insulin pulsatility. Results of deconvolution analysis during baseline assessment and glucose pulse induction are given in Table 2. At baseline, nonpulsatile insulin secretion was increased (8.1 ± 3.3 vs. 6.5 ± 3.0 pmol · l^{-1} · min^{-1}; $P = 0.04$). There was no change in insulin secretory burst mass, amplitude, or frequency. The fraction of total insulin delivered in pulses was unchanged at baseline (38 vs. 34%; NS) and during glucose pulse induction (35 vs. 34%; NS), reflecting unchanged insulin pulse mass, basal secretion, and interpulse interval.

Regularity of baseline serum insulin concentration time series, as assessed by ApEn, was unchanged (Table 2). During glucose pulse induction, there was a tendency toward increased entrainment on the day of NN2211 treatment as assessed by spectral analysis (spectral power, 7.3 ± 3.9 vs. 4.9 ± 2.5; $P = 0.095$) and autocorrelation analysis (autocorrelation coefficient, 0.16 ± 0.13 vs. 0.11 ± 0.17; $P = 0.066$).

Pharmacokinetic properties. The pharmacokinetic properties of NN2211 were evaluated by 11-point profiles for up to 63 h postdosing (Fig. 3). In all patients, measur-

TABLE 1
Fasting and meal-related results

	NN2211	Placebo	*P*
Fasting values			
plasma glucose (mmol/l)	6.9 ± 1.0	8.1 ± 1.0	<0.01
insulin secretory rate (pmol/min)	179 ± 70	163 ± 66	0.03
glucagon (pmol/l)	19 ± 4	20 ± 4	NS
Homeostasis model assessment			
HOMA-B	0.56 ± 0.16	0.36 ± 0.12	<0.001
HOMA-S	3.0 ± 1.3	3.1 ± 1.7	NS
Meal-related $AUC_{1130\text{-}1530h}$			
plasma glucose ($mmol \cdot l^{-1} \cdot h^{-1}$)	30.6 ± 2.4	39.9 ± 7.3	<0.001
plasma glucose ($mmol \cdot l^{-1} \cdot h^{-1}$) increment	7.0 ± 2.6	9.6 ± 4.5	0.04
insulin secretory rate (nmol)	118 ± 32	106 ± 27	NS
insulin secretory rate (nmol) increment	65 ± 22	45 ± 11	0.03
glucagon ($pmol \cdot l^{-1} \cdot h^{-1}$)	77 ± 18	82 ± 17	0.04
glucagon ($pmol \cdot l^{-1} \cdot h^{-1}$) increment	6 ± 10	6 ± 10	NS
3-OMG ($mg \cdot l^{-1} \cdot h^{-1}$)	400 ± 84	440 ± 70	0.02

Mean fasting values and HOMA were calculated from samples obtained at 7 h and 8 h. The meal response was calculated as integrated AUC in absolute values and as increments relative to premeal values (mean of 1115 h and 1130 h) for glucose, ISR, glucagon, and 3-OMG. NS, not significant.

able levels of NN2211 were detected 60 min after injection. The half-life of NN2211 was 10 ± 4 h, and the t_{max} was 12 ± 2 h. There was an interindividual variation in the total AUC and in the peak value of NN2211 of 34 and 41%, respectively.

Adverse events. No hypoglycemic episodes occurred during the study. Two patients experienced nausea on the day of NN2211 treatment. In one patient, this was mild, and the patient was able to finish the meal. In the other patient, nausea was of moderate severity, and she was not able to eat the meal. This patient was subsequently excluded from the meal-related calculations. The two patients who experienced gastrointestinal side effects had the highest peak plasma concentrations of NN2211.

DISCUSSION

In the present study, we investigated the acute effect of a single dose of NN2211, a long-acting GLP-1 derivative, on fasting and meal-related glycemia. GLP-1 is a potent insulin secretagogue (21) that, in addition, suppresses glucagon secretion (3,5). A reduced postprandial GLP-1 response may play a role in the inappropriate insulin and glucagon secretion in type 2 diabetes (22). The latter action could be important inasmuch as relative glucagon excess contributes to postprandial hyperglycemia in patients with type 2 diabetes by increasing glucose appearance (2,23). Although controversial, postprandial hyperglycemia may be an independent risk factor for the development of diabetic complications (24). A prompt and adequate insulin response to a meal challenge is required to maintain prandial glucose homeostasis.

In the present study, fasting plasma glucose was significantly reduced by 1.2 mmol/l to an average of 6.9 mmol/l. The hypoglycemic effect in the fasting condition was associated with increased insulin secretion. We found no change in glucagon concentration in the fasting state.

Glucose excursion after ingestion of a standard mixed meal, as measured by the AUC for glucose (absolute and incremental) and the peak glucose concentration, was significantly reduced. This effect was probably due to delayed gastric emptying and a suppression of glucagon secretion. The glucagon suppression might in fact be underestimated as a result of reduced glycemia on the day of active treatment. The absolute postprandial rate of

TABLE 2
Deconvolution and regularity analysis

	NN2211	Placebo	*P*
Deconvolution analysis—baseline period			
insulin secretory burst mass ($pmol \cdot l^{-1} \cdot pulse^{-1}$)	24 ± 14	22 ± 14	NS
insulin secretory burst amplitude ($pmol \cdot l^{-1} \cdot min^{-1}$)	10.3 ± 5.5	9.1 ± 5.3	NS
basal insulin secretion ($pmol \cdot l^{-1} \cdot min^{-1}$)	8.1 ± 3.3	6.5 ± 3.0	0.04
interpulse interval (minutes)	5.8 ± 0.7	5.8 ± 1.0	NS
Baseline insulin pulse regularity			
ApEn ($m = 1$, $r = 0.2 \times SD$)	1.41 ± 0.07	1.38 ± 0.11	NS
ApEn ($m = 1$, $r = 1.0 \times SD$)	0.65 ± 0.07	0.65 ± 0.08	NS
Glucose pulse entrainment			
spectral power	7.3 ± 3.9	4.9 ± 2.3	NS (0.095)
autocorrelation coefficient	0.16 ± 0.13	0.11 ± 0.17	NS (0.066)
ApEn ($m = 1$, $r = 0.2 \times SD$)	1.36 ± 0.07	1.36 ± 0.05	NS
ApEn ($m = 1$, $r = 1.0 \times SD$)	0.64 ± 0.08	0.65 ± 0.07	NS

Results from time series analysis of insulin concentration time series obtained during baseline conditions and during glucose pulse entrainment with 6 mg/kg every 10 min. NS, not significant, if $0.1 > P > 0.05$ the value is given in brackets.

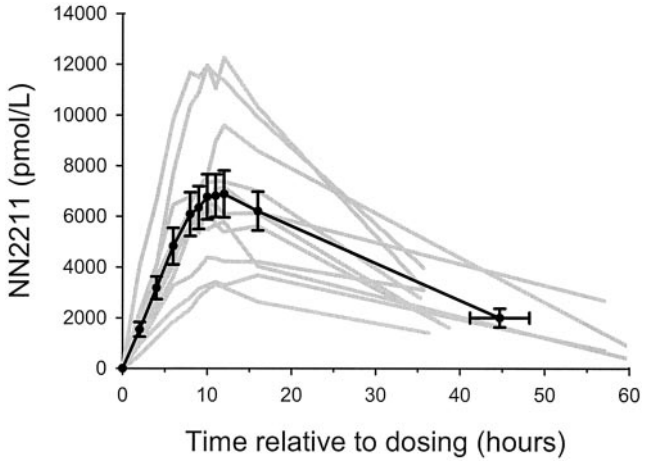

FIG. 3. Concentration profiles of NN2211 on the day of active treatment. The first 10 data points were obtained by sampling at fixed time intervals; the last data point was obtained between 35 and 63 h post dosing. Gray lines indicate individual concentration profiles; black line indicates mean ± SE.

insulin secretion was only slightly and insignificantly increased, however, on the basis of a substantially reduced plasma glucose level. The meal-induced increment of ISR, however, was almost 50% higher after NN2211 treatment. These results can be explained be the pronounced glucose dependence of GLP-1 action (25). This is supported by the 50% increase in insulin secretory capacity as measured by HOMA. Because steady state of insulin and glucose is not achieved after a single dose of NN2211, data based on HOMA analysis should be taken with some caution. NN2211 thus exhibits several of the favorable actions previously demonstrated for the native peptide: increased insulin secretion, suppressed glucagon secretion, and delayed gastric emptying. Whether satiety was independently affected (26) was not assessed.

Previous studies of GLP-1 action demonstrated normalization of fasting plasma glucose in populations of patients with type 2 diabetes comparable with the cohort studied here (3,5). In those studies, native GLP-1 was administered via recurrent subcutaneous injection or continuous infusion. NN2211 reduced the fasting plasma glucose concentration to 6.9 mmol/l, thus not achieving euglycemia. The in vitro potency of NN2211 is equal to that of native GLP-1 (27). Because NN2211 binds strongly to albumin, the plasma concentration of NN2211 cannot be directly compared with GLP-1 concentrations in studies involving administration of the native peptide. Furthermore, because a single dose of NN2211 was used, no information about the in vivo potency of the compound can be ascertained. It is worth noting, however, that the total 24-h dose of NN2211 given in the present study on molar basis is similar to the 24-h doses of GLP-1 administered in other studies (28). The onset of action of NN2211 is expected to be relatively slow because of its slowly increasing concentration. As the concentration increases and glucose slowly decreases, the stimulation of insulin secretion falls as a result of the glucose-dependent nature of NN2211's action. In contrast, when administered intravenously, the concentration of GLP-1 immediately reaches a high level and gives rise to a large increase in insulin secretion upon the first dose (28). Accordingly, additional studies are needed to define the clinical efficacy of NN2211 and the adverse event profile of the drug.

The quantitative importance of an induced delay in gastric emptying on glucose homeostasis has not been clarified. Numerous methods have been used to measure gastric emptying (29). In the present study, we assessed gastric emptying by serving a solution of 3-OMG with the meal. The method is simple and noninvasive and quantifies the absorption of a glucose derivative metabolized equal to glucose. The delayed gastric emptying is appropriate to reduce postprandial hyperglycemia. Prolonged gastric fullness, however, might contribute to adverse advents such as nausea, and indeed the patient with the worst severe nausea was the one with the most marked delay in gastric emptying. The two patients who experienced nausea had the highest serum concentrations of the GLP-1 derivative. Gastrointestinal side effects might thus be avoided by dose reduction or individual dose titration.

High-frequency oscillatory insulin release is likely to play a role in peripheral insulin action (30,31). Repeated low-dose glucose injections have recently been demonstrated to enforce endogenous insulin pulsatility in healthy individuals but not in patients with type 2 diabetes (32,33). We therefore performed analysis for baseline as well as glucose-entrained insulin pulsatility. We found no change in baseline insulin release regularity as measured by ApEn. Previous studies of native GLP-1 and another insulin secretagogue, gliclazide, gave similar results, and it has been suggested that intervention in the early diabetic or prediabetic state is necessary to prevent the deterioration of baseline insulin release regularity (8,34). Deconvolution analysis of baseline insulin time series revealed no substantial changes in the distribution of basal versus pulsatile insulin release consistent with findings in previous studies in patients with type 2 diabetes (8,35). No change in baseline insulin burst mass was detected, whereas this was increased in the aforementioned studies. This is likely to reflect, however, that the overall increase of insulin release upon GLP-1 treatment was higher in those studies rather than pharmacodynamic differences between NN2211 and the native hormone.

Glucose-entrained insulin release was evaluated by repeated punctuated intravenous glucose infusions and subsequent analysis of insulin concentration time series by spectral and autocorrelation analysis. Injection of NN2211 tended to enhance entrainment, albeit not significantly. Whether evident entrainment would emerge with long-term dosing is not known. In this regard, GLP-1 treatment of individuals with impaired glucose tolerance but not of patients with diabetes enhanced glucose entrainment of ultradian insulin release (9). The better entrainment might be due to an improved glucose sensing of the β-cell, thus enabling the β-cell to respond even to the minor glucose excursions induced by the study procedure (25).

Pharmacokinetic properties of NN2211 were evaluated by 11-point profiles for up to 63 h postdosing. The plasma half-life was ~10 h, and t_{max} was ~12 h postdosing, which is similar to the findings in healthy humans (10). Bedtime administration thus results in maximal plasma concentrations during the daytime, and once-daily dosing seems to be possible.

In summary, we report that a single injection of the

GLP-1 derivative NN2211 results in a substantial decrease in fasting and postprandial plasma glucose concentrations in patients with type 2 diabetes. Increased insulin release, suppressed glucagon secretion, and delayed gastric emptying all contribute to this action. The drug was generally well-tolerated, and the pharmacokinetic properties should allow bedtime dosing. These data thus provide a basis for longer-term studies to define the clinical efficacy of this so far promising drug.

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