Systemic Administration of the Long-Acting GLP-1 Derivative NN2211 Induces Lasting and Reversible Weight Loss in Both Normal and Obese Rats

Philip J. Larsen,1,2 Christian Fledelius,3 Lotte Bjerre Knudsen,4 and Mads Tang-Christensen1

Postprandial release of the incretin glucagon-like peptide-1 (GLP-1) has been suggested to act as an endogenous satiety factor in humans. In rats, however, the evidence for this is equivocal probably because of very high endogenous activity of the GLP-1 degrading enzyme dipeptidyl peptidase-IV. In the present study, we show that intravenously administered GLP-1 (100 and 500 μg/kg) decreases food intake for 60 min in hungry rats. This effect is pharmacologically specific as it is inhibited by previous administration of 100 μg/kg exendin(9-39), and biologically inactive GLP-1(1-37) had no effect on food intake when administered alone (500 μg/kg). Acute intravenous administration of GLP-1 also caused dose-dependent inhibition of water intake, and this effect was equally well abolished by previous administration of exendin(9-39). A profound increase in diuresis was observed after intravenous administration of both 100 and 500 μg/kg GLP-1. Using a novel long-acting injectable GLP-1 derivative, NN2211, the acute and subchronic anorectic potentials of GLP-1 and derivatives were studied in both normal rats and rats made obese by neonatal monosodium glutamate treatment (MSG). We showed previously that MSG-treated animals are insensitive to the anorectic effects of centrally administered GLP-1(7-37). Both normal and MSG-lesioned rats were randomly assigned to groups to receive NN2211 or vehicle. A single bolus injection of NN2211 caused profound dose-dependent inhibition of overnight food and water intake and increased diuresis in both normal and MSG-treated rats. Subchronic multiple dosing of NN2211 (200 μg/kg) twice daily for 10 days to normal and MSG-treated rats caused profound inhibition of food intake. The marked decrease in food intake was accompanied by reduced body weight in both groups, which at its lowest stabilized at ~85% of initial body weight. Initial excursions in water intake and diuresis were transient as they were normalized within a few days of treatment. Lowered plasma levels of triglycerides and leptin were observed during NN2211 treatment in both normal and MSG-treated obese rats.

In a subsequent study, a 7-day NN2211 treatment period of normal rats ended with measurement of energy expenditure (EE) and body composition determined by indirect calorimetry and dual energy X-ray absorptiometry, respectively. Compared with vehicle-treated rats, NN2211 and pair-fed rats decreased their total EE corresponding to the observed weight loss, such that EE per weight unit of lean body mass was unaffected. Despite its initial impact on body fluid balance, NN2211 had no debilitating effects on body water homeostasis as confirmed by analysis of body composition, plasma electrolytes, and hematocrit. This is in contrast to pair-fed animals, which displayed homeoconcentration and tendency toward increased percentage of fat mass. The present series of experiments show that GLP-1 is fully capable of inhibiting food intake in rats via a peripherally accessible site. The loss in body weight is accompanied by decreased levels of circulating leptin indicative of loss of body fat. The profound weight loss caused by NN2211 treatment was without detrimental effects on body water homeostasis. Thus, long-acting GLP-1 derivatives may prove efficient as weight-reducing therapeutic agents for overweight patients with type 2 diabetes. Diabetes 50:2530–2539, 2001

Peripheral administration of glucagon-like peptide-1 (GLP-1) acutely affects food intake in humans, but the underlying mechanisms that decrease food intake concomitant with earlier onset of subjective sensation of fullness are not fully understood (1–4). The anorectic effects of continuous intravenous administration of GLP-1 are present in individuals who are lean or obese or have type 2 diabetes (1,3,4), suggesting that the observed effects are part of a physiologically relevant meal-terminating system. Results from similar experiments in rats have been ambiguous, probably because of high activity of GLP-1 degrading enzyme dipeptidyl peptidase-IV (DPP-IV) (5,6). Thus, early experiments were unable to demonstrate peripheral effects of intraperitoneal GLP-1 injections on feeding behavior (7,8), whereas later experiments have shown significant but short-lasting anorectic effects of subcutaneous administration of GLP-1 (9). Anorexia induced by peripheral administration of GLP-1 involves vagal control of gastric motility (10,11).

Central administration of 1–3 μg of GLP-1 specifically inhibits food intake in rats via a hypothalamic site that is sensitive to neonatal monosodium glutamate (MSG) lesioning (12). However, central administration of slightly
higher doses of GLP-1 leads to taste aversion, but because this latter effect is unaffected by MSG treatment, it further
stresses the specificity of the central GLP-1–induced anorexia (12). In addition, it is possible to elicit anorexia
without concomitant taste aversion if GLP-1 is injected directly into the hypothalamic paraventricular nucleus
(13). Acute injections of both GLP-1 and NN2211 exert profound adipsia and diuresis. These effects on body water
homeostasis could potentially hamper long-term treatment of patients with type 2 diabetes with GLP-1 agonists, and
the potential anorectic effects of these agonists may be accompanied with debilitating affects on body water
homeostasis.

Given that peripheral administration of GLP-1 affects food intake in humans, we decided to study further the
anorectic potential of this peptide in the laboratory rat. Dose-response studies investigating the effect of intravenous
administration of GLP-1(7-37) or a novel long-acting acylated GLP-1 derivative NN2211 (14) on food intake
were performed. In continuation of acute pharmacological studies, we examined the effect on food intake and body
weight of twice daily subcutaneous administration of NN2211 for 10 days followed by a 5-day recovery period.
To study the impact of 7 days of NN2211 treatment on energy expenditure (EE) and body composition, we studied
normal rats by indirect calorimetry and subsequently subjected them to dual energy X-ray absorptiometry
(DEXA) scanning. Before, during, and after treatment, blood biochemical markers of energy and fluid homeosta-
sis metabolic state were monitored.

RESEARCH DESIGN AND METHODS

Animals. All experiments were carried out on male Wistar rats. Normal male rats arrived at the animal unit 1 day before timed labor (E21). Neonatal Wistar pups received a number of subcutaneous injections with MSG (L-glutamic acid, G-1626; 4 mg/g body wt; Sigma, Vallensbaek Strand, Denmark) dissolved in sterile phosphate-buffered saline (50 mmol/l, pH 7.4) to a concentration of either 0.1 or 1.0 mg/ml. Solutions were always made fresh before use and stored at 4°C in sterile tubes. Material from several batches of NN2211 was used, and corrections for impurity were always performed. Subcutaneous injections were administered using standard 1-ml syringes equipped with 25-G needles.

Experiment 1: single dose of GLP-1. Sixteen adult male Wistar rats were equipped with jugular intravenous catheters (Department of Pharmacology, The Panum Institute, University of Copenhagen). Catheters were implanted under Avertin (tribromoethanol, 200 mg/kg) anesthesia in the right jugular vein with the tip aiming at the right atrium. After placement, the catheter was connected to a subcutaneous saline reservoir through which the intravenous fluid was delivered. Catheter patency was secured by instillation of heparinized (1,000 IU/mL) sterile isotonic saline into the catheter before closure with a metal rod. After 7 days of postoperative recovery, animals were housed individually in standard metabolic cages (Techniplast, Gazzada, Italy) and acclimatized over a period of 7 days to a 5-h restricted feeding scheme with access to food from 8:00 a.m. to 1 p.m. and water ad libitum.

Seven days after initiation of the restricted feeding scheme, animals were assayed to a random cross-over dosing paradigm. Five minutes before presentation of food, animals received an intravenous injection of GLP-1 (5, 100, or 500 μg/kg). Statistical analysis of intergroup treatment variation was carried out using factorial analysis of variance (ANOVA) followed by Scheffe post hoc analysis.

Experiment 2: single dose of NN2211. Normal adult male Wistar rats (n = 16) and MSG-treated rats (n = 16) were housed individually in metabolic cages with free access to a rat diet and water. Animals were kept on a 12:12 light:dark cycle, and the effect of NN2211 on nighttime food intake was assessed by injecting animals subcutaneously 2–3 h before lights out. Animals were left without food and water in the period from dosing to the onset of darkness. Three doses of NN2211 and vehicle were tested in a random crossover experiment (10, 50, and 200 μg/kg). Food and water intake and diuresis were monitored every 30 min for the first 2 h after onset of nighttime (lights off) and finally 12 h later at lights on (t0). All measurements were done in complete darkness with the assistance of night vision goggles (Bausch and Lomb, Rochester, NY). The minimum interval between experiments was 48 h. Statistical analysis of intergroup treatment variation was carried out using factorial ANOVA followed by Scheffe post hoc analysis.

Experiment 3: continuous administration of NN2211. Beginning 1 week before the first dose was administered, normal adult male Wistar rats (n = 16) and MSG-treated rats (n = 16) were housed individually in metabolic cages with free access to a rat diet and water. Animals were kept on a 12:12 light:dark cycle, and the chronic effect of two daily subcutaneous injec-
tions of NN2211 or vehicle on body weight, food and water intake, diuresis, and feces excretion was monitored. All parameters were measured between 9 and 11 a.m. while animals received their morning dose. On the basis of daily food intake and diuresis data, we calculated energy expenditure, diuresis, and fluid homeostasis. Comparisons of plasma samples were analyzed using factorial ANOVA followed by Bonferroni correction for multiple comparison. Biochemical data from plasma samples were analyzed using factorial ANOVA followed by Fisher’s or Scheffe’s post hoc analysis.

Experiment 4: effect of subchronic NN2211 treatment on energy expenditure and body composition. Twenty-four 12-week-old male rats were used in this study. The rats were housed individually in a temperature (23°C) and light-controlled environment (12:12 light:dark cycle; lights on 7:00 a.m. and light off 7:00 p.m.) with free access to food and water for at least 7 days before experimentation. The rats were stratified into three groups (G1, G2, and G3) according to weight 3 days before study start (n = 7 per group). The rats in G1 and G3 were treated with vehicle, and the rats in G2 were treated with NN2211 (200 μg/kg b.i.d.). The rats in G1 and G2 had free access to food and water during the 7-day treatment period, whereas the rats in G2 were “single” pair fed after the rats in G2. Body weight and food and water intake were recorded daily. By the end of the study (day 7), oxygen consumption and body composition were determined by indirect calorimetry and DEXA, respectively. Likewise, blood samples were collected for determination of hematocrit as well as for plasma

DIABETES, VOL. 50, NOVEMBER 2001 2531
Indirect calorimetry. was determined on defrosted carcasses. in plastic bags at By the end of the study (day 7), the rats were killed. The carcasses were stored coef

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1.0 coef

ment settings used were as follows: a scan speed of 40 mm/s, a resolution of

1.0 × 1.0 mm, and automatic/manual histogram width estimation. The coefficient of variation as assessed by 10 repeated measurements (with repositioning of the rat between each measurement) was 3.48, 3.17, and 3.73% for bone mineral content, lean tissue mass, and fat tissue mass, respectively. By the end of the study (day 7), the rats were killed. The carcasses were stored in plastic bags at −20°C before determination of body composition; which was determined on defrosted carcasses.

Indirect calorimetry. Oxygen consumption, CO2 production, EE, and the respiratory exchange ratio (RER) were determined by indirect calorimetry (Oxymax System; Columbus Instruments, Columbus, OH). The rats (n = 1 per chamber) were placed in airtight acrylic chambers (10.5 l). Oxygen and CO2 concentrations in the chamber in- and outlet gas were determined simultaneously every 20.25 min over a period of 4.4 h. Instrument settings used were as follows: a gas flow rate of 1.86 l/min, settle time of 90 s, measure time of 40 s, and system recalibration for each eight-chamber measuring cycle. By the end of the study (day 7), the nonfasted rats were subjected to indirect calorimetry (from 8:00 a.m. to 1:00 p.m.). In contrast to the previous 6 days, the nonfasted rats did not receive NN2211 between 7:30 and 8:30 before indirect calorimetry. On the day of indirect calorimetry, the rats received treatment at 9:30—after four pretreatment measurements. The rats had no access to food or water during their stay in the acrylic chamber. The indirect calorimetric measurements were performed over 3 days. As a “positive” instrument control, every day included a reference animal “treated” with the EE increasing compound 2,4-dinitrophenol (DNP, Sigma). On the basis of the measurements of O2 and CO2 in the chamber in- and outlet gas, estimates of O2 consumption, CO2 production, EE, and RER were calculated.

Blood sampling and biochemical assays

Blood sampling. Orbital blood samples were obtained by puncture of the orbital venous plexus with glass capillary tubes. Samples were taken in standard heparinized EDTA (0.18 mol/l) glass tubes (Vacutainer) to which aprotinin (1,500 KIE/ml) and bacitracin (5%) were added. After sampling, the tubes were kept on ice before being centrifuged (4°C at 5,000g for 10 min), and the resulting plasma was stored at −80°C before being analyzed. Trunk blood was obtained by decapitating animals and sampling into heparinized (~500 IU/tube) glass tubes to which aprotinin (1,500 KIE/ml) and bacitracin (5%) were added. Glycerol and FFA concentrations were determined in EDTA (0.18 mol/l) plasma containing 1% NaF (wt/vol).

Plasma glucose. Plasma glucose was measured on a standard COBAS analyzer (Toxicology Projects & Planning, Novo Nordisk, Copenhagen, Denmark).

Plasma leptin. Plasma leptin was measured using a commercially available mouse leptin enzyme-linked immunosorbent assay kit (Crystal Chemical, Chicago, IL), showing >95% cross-reactivity to rat leptin.

Plasma biochemistry. Orbital blood samples (days 0, 7, and 14) were taken from rats that were receiving 100 μg/kg b.i.d. NN2211 and a set of corresponding vehicle-treated animals. Plasma values of sodium, TG, cholesterol, creatinine, carbamide, and total protein were measured on a standard COBAS analyzer. FFA and glycerol were measured on a standard Hitachi Automatic analyzer.

Plasma potassium. Orbital blood samples (days 0, 7, and 14) were taken from rats that were receiving 200 μg/kg b.i.d. NN2211 and a set of corresponding vehicle-treated animals. Blood was collected in heparinized glass tubes (Vacutainer), and the potassium content in resulting plasma was measured potentiometrically with an ion selective probe on a standard COBAS analyzer.

RESULTS

Experiment 1

Effects of a single dose of GLP-1 on feeding behavior. Acute intravenous administration of high doses of GLP-1 (100 and 500 μg/animal) decreased food intake at 30 and 60 min after onset of feeding period in rats that were kept on a restricted feeding scheme (Fig. 1). The anorectic effect of the highest dose of GLP-1 (500 μg/animal) was completely abolished by previous administration of 100 μg of exendin(9-39) (6.1 ± 0.4 vs. 5.9 ± 0.3 g). Also, the biologically inactive peptide GLP-1(1-37) (500 μg/animal) had no acute effect on food intake (6.1 ± 0.4 vs. 6.2 ± 0.3 g). Water intake was similarly decreased in rats that were receiving an intravenous injection of GLP-1, and the pharmacological characteristics of water consumption mirrored that of feeding.

Intravenous administration of GLP-1 also affected diuresis. Water excretion was markedly and dose-dependently increased in rats that were receiving intravenous bolus injections of GLP-1 (100 and 500 μg/animal; Fig. 2). The effect displayed pharmacological specificity in as much it was not elicited by 500 μg/animal GLP-1(1-37). However, an attempt to antagonize the diuretic effect of 500 μg of GLP-1 with exendin(9-39) (100 μg/animal) was only partially successful. Thus, exendin(9-39) completely abolished anorectic GLP-1 actions, whereas the diuretic actions of 500 μg GLP-1 were only partially abolished (Fig. 2).

Experiment 2

Effects of a single dose of NN2211 on feeding behavior in rats. The acute effects of three doses of NN2211 on nighttime feeding were tested in a random crossover experiment (10, 50, and 200 μg/kg). The dose dependence of NN2211 on overnight food intake in normal rats is illustrated in Fig. 3. Two hours after onset of the feeding
session, 200 μg/kg NN2211 significantly inhibited food intake (3.8 ± 0.3 vs. 5.2 ± 0.3 g of food). The following morning (t_{720}), both 50 and 200 μg/kg significantly inhib-

Effects of a single dose of NN2211 on water intake and diuresis. In the same experiment, water intake and diuresis was monitored (Fig. 3). Both 50 and 200 μg/kg NN2211 significantly inhibited overnight (t_{720}) water intake in normal and MSG-treated rats (vehicle: 32.8 ± 2.0; 200 μg/kg: 21.1 ± 1.1; 200 mg/kg: 11.5 ± 2.5 g of food) as well as in MSG-treated rats (control: 12.5 ± 1.9; 50 μg/kg: 10.0 ± 1.0; 200 μg/kg: 5.8 ± 0.7 g of food).

Experiment 3
Effect of subchronic administration of NN2211 on food intake and body weight. Two daily injections of NN2211 to adult male Wistar rats dose-dependently decreased body weight over the entire 10-day treatment period with significant differences obtained with the 200 μg/kg b.i.d. dosing regimen between treatment days 7 and 14 (Fig. 4). Loss in body weight was preceded by decreased food and water intake and increased diuresis (Fig. 5). Food intake was significantly lower during the initial 3 days of treatment for normal animals that were treated with 100 μg/kg b.i.d. (data not shown). Thereafter, the anorectic effect of this low dose was no longer statistically significant. In animals that were treated with 200 μg/kg b.i.d., food intake was significantly lower throughout the 10-day treatment period. After cessation of the treatment, food intake normalized within a few days and body weight

FIG. 4. Subchronic administration of NN2211 dose-dependently decreases body weight in both normal adult male Wistar rats and MSG-treated rats. Animals received two daily subcutaneous injections of NN2211 (100 or 200 μg/kg) or vehicle for 10 days followed by a 5-day recovery period. Body weight was monitored each morning. Values are mean ± SE (n = 5–8). *, significant difference from relevant vehicle-treated control as determined by ANOVA followed by Bonferroni multiple comparison post hoc analysis.

FIG. 3. Single subcutaneous injections of NN2211 dose-dependently inhibit food and water in both normal and MSG-treated rats. Dose-response curves for 720 min of food and water intake as well as diuresis in normal and MSG-treated rats receiving a single subcutaneous dose of NN2211. *P < 0.05 versus vehicle (ANOVA followed by Scheffe’s post hoc analysis); †P < 0.05 versus 10 μg/kg NN2211 (ANOVA followed by Scheffe’s post hoc analysis); ‡P < 0.05 versus 50 mg/kg NN2211 followed by Scheffe’s post hoc analysis).
FIG. 5. The effect of subchronic administration of 200 μg/kg NN2211 to normal male Wistar rats (circles) or MSG-treated rats (boxes) on food and water intake as well as diuresis and feces excretion. Open symbols represent vehicle-treated animals; closed symbols represent NN2211-treated animals. Shaded areas reflect baseline values obtained from all animals in the group throughout the week before onset of treatment.
TABLE 1  
NN2211 treatment (200 μg/kg BID) exerts no adverse effects on plasma glucose, potassium, sodium, and plasma variables that reflect renal function (creatinine, carbamide, and protein)

<table>
<thead>
<tr>
<th>Treatment values (day 7)</th>
<th>Normal vehicle (n = 8)</th>
<th>Normal NN2211 (n = 8)</th>
<th>MSG vehicle (n = 4)</th>
<th>MSG NN2211 (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/l)</td>
<td>6.7 ± 0.5</td>
<td>6.9 ± 0.2</td>
<td>6.1 ± 1.0</td>
<td>6.7 ± 0.3</td>
</tr>
<tr>
<td>Potassium (K⁺) (mmol/l)</td>
<td>4.6 ± 0.3</td>
<td>4.2 ± 0.1</td>
<td>4.3 ± 0.2</td>
<td>4.1 ± 0.1</td>
</tr>
<tr>
<td>Sodium (Na⁺) (mmol/l)</td>
<td>136.5 ± 0.4</td>
<td>136.9 ± 0.6</td>
<td>138.2 ± 0.5</td>
<td>139.0 ± 0.3</td>
</tr>
<tr>
<td>Creatinine (mmol/l)</td>
<td>87.6 ± 1.7</td>
<td>75.6 ± 2.4</td>
<td>76.3 ± 3</td>
<td>77.5 ± 1.9</td>
</tr>
<tr>
<td>Carbamide (mmol/l)</td>
<td>8.4 ± 0.2</td>
<td>8.9 ± 0.5</td>
<td>7.3 ± 0.3</td>
<td>8.5 ± 0.3</td>
</tr>
<tr>
<td>Protein (g/l)</td>
<td>64.0 ± 1.2</td>
<td>63.4 ± 1.2</td>
<td>65.4 ± 1.0</td>
<td>64.1 ± 0.6</td>
</tr>
</tbody>
</table>

Recovery-phase values (day 14)

| Glucose (mmol/l)         | 6.8 ± 0.1             | 6.4 ± 0.2             | 5.7 ± 1.0           | 6.2 ± 0.2           |
| Potassium (K⁺) (mmol/l)  | 5.2 ± 0.5             | 4.6 ± 0.3             | 4.3 ± 0.4           | 5.0 ± 0.1           |
| Sodium (Na⁺) (mmol/l)    | 139.5 ± 0.6           | 138.7 ± 0.4           | 141.4 ± 0.7         | 140.2 ± 1.2         |
| Creatinine (mmol/l)      | 85.3 ± 1.2            | 81.9 ± 1.4            | 85.0 ± 1.1          | 90.4 ± 3.9          |
| Carbamide (mmol/l)       | 7.9 ± 0.2             | 8.0 ± 0.1             | 7.9 ± 0.3           | 7.3 ± 0.2           |
| Protein (g/l)            | 67.2 ± 1.2            | 65.0 ± 0.6            | 66.3 ± 0.7          | 64.3 ± 0.7          |

Data are means ± SD.

gradually increased toward that of vehicle-treated animals (Fig. 5).

Similar effects of NN2211 treatment on food intake were seen in obese MSG-treated rats. Thus, two daily injections of 200 μg/kg NN2211 significantly decreased body weight throughout the 10-day treatment period, whereas the 100 μg/kg b.i.d. dosing regimen displayed a trend toward weight reduction without reaching statistical significance. Also, food intake was significantly lower in MSG-treated animals that were treated with 200 μg/kg b.i.d. NN2211.

**Effect of subchronic administration of NN2211 on water intake, diuresis, and feces excretion.** In contrast to food intake, water intake was lower in NN2211-treated animals only at the initial days of treatment, because from day 4 onward, NN2211-treated groups displayed considerably higher water intake than corresponding vehicle-treated groups (Fig. 5). However, these effects were not statistically significant and had no impact on long-term body fluid homeostasis (see below).

In normal rats that were receiving 100 μg/kg b.i.d. NN2211, increased diuresis was accompanied by increased water intake from treatment day 2 onward to cessation of dosing (data not shown). In animals that were receiving 200 μg/kg b.i.d., however, fluid homeostasis was severely affected during the first 2 days of dosing, because a state of very low water intake coexisted with markedly increased diuresis (Fig. 5). For normal rats that were treated with 200 μg/kg b.i.d., apparent fluid balance with water consumption and diuresis at levels similar to vehicle-treated animals occurred from day 4 onward. Despite marked increasing effects on urinary water excretion, plasma sodium and potassium remained unaffected in animals that were treated with 200 μg/kg b.i.d. NN2211 (Table 1). Also, plasma variables reflecting renal function (creatinine, carbamide, and total protein) were unaffected by NN2211 treatment (Table 1). Feces excretion was followed in normal animals that were receiving 200 μg/kg b.i.d. and was observed to decrease during administration of NN2211 coincident with the decrease in food intake (Fig. 5).

In MSG-treated rats, NN2211 treatment (both 100 and 200 μg/kg b.i.d.) induced a statistically significant reduction of water intake and increased diuresis, leading to an initial negative water balance (Fig. 5). The compensatory water homeostatic mechanisms were clearly more undulating in MSG-treated animals that were treated with 200 μg/kg b.i.d. because they went through a phase characterized by polyuria and hyperdipsia from day 3 to day 6 before full compensation was obtained (Fig. 5). In the recovery phase after cessation of the treatment, the slightly lower water intake that accompanied lower food intake at the end of the NN2211 treatment period rapidly returned to control levels. As with normal animals, MSG-treated animals’ plasma electrolyte levels were unaffected by NN2211 treatment both during and after cessation of drug administration (Table 1).

**Experiment 4**

**Effect of subchronic administration of NN2211 on EE, body composition, food intake, and body weight.** As seen in experiment 3, 7 days of NN2211 treatment significantly lowered body weight (373.3 ± 14.7 vs. 417.3 ± 15.3 g), and the body weights in the pair-fed group were reduced similarly (378.5 ± 10.5). After 7 days of treatment, EE was considerably lower in both NN2211 and pair-fed animals (control 1,775 ± 39; pair fed 1,634 ± 49; NN2211 1,641 ± 27 kcal/h; n = 6–7, average of 3-h measurements). However, when EE was expressed as oxygen consumption per kilogram of body mass, no such differences were seen throughout the observation period (Fig. 6). Also, the RER was unaffected by 7 days of NN2211 treatment (Fig. 7). In contrast, pair-fed animals displayed a switch toward lipid metabolism, probably reflecting that these animals had been starved for a longer period of time before the onset of experiment.

Body composition analysis was carried out using a DEXA scanner specialized for small animals. Treatment with NN2211 reduced body weight by affecting both lean and adipose tissue mass, although the loss of fat mass did not quite reach statistical significance (Table 2). However, the percentages of lean and adipose tissue mass of total body weight changed in neither NN2211-treated nor pair-fed animals. The hydration status after 7 days of treatment was further assessed by measuring the hematocrit, and pair-fed animals displayed significant hemococoncentration.
in comparison to both ad libitum–fed controls and NN2211-treated animals (ad libitum 45.6 ± 0.8; NN2211 47.0 ± 0.9; pair-fed 49.1 ± 0.9% n = 7).

Effect of subchronic administration of NN2211 on plasma glucose and lipids. A number of biochemical plasma variables were assessed in animals that were subjected to either 7 or 10 days of NN2211 treatment (experiments 3 and 4). Data obtained from experiment 3 showed no effect of NN2211 on glucose and total cholesterol (Tables 1 and 3). However, subchronic administration of NN2211 lowered plasma TG levels in both normal and MSG-treated rats, probably a direct consequence of lowered food intake (Table 1). Blood obtained from animals in experiment 4 was analyzed for cholesterol, FFA, and glycerol. As in experiment 3, total cholesterol was unaffected but animals that were treated with NN2211 (200 μg/kg b.i.d.) displayed a tendency toward lower plasma levels of FFA in comparison with both pair-fed and ad libitum–fed control animals (NN2211, 136 ± 16; pair-fed, 195 ± 18; FFA, 192 ± 43 nmol/l; n = 6–7).

Because of different sampling techniques required for potassium analysis (heparinized and not EDTA tubes), this parameter was measured together with leptin in animals that were receiving 200 μg/kg b.i.d. Plasma leptin levels decreased in both normal and MSG-treated animals during NN2211 treatment (200 μg/kg b.i.d.; Fig. 8).

DISCUSSION
In contrast to earlier beliefs (7,8), intravenous administration of native GLP-1 significantly decreased food and water intake in rats via a peripherally accessible site. However, the current use of much higher doses of GLP-1 may well explain this discrepancy (500 μg/animal instead of 10 μg/animal). GLP-1 is primarily degraded by the circulating protease DPP-IV (EC 3.4.14.5), and activity levels of this circulating enzyme are very high in the rat compared with other mammals such as pigs and humans (5,15–17). Thus, plasma half-life of GLP-1 in rats is very short.

Like native GLP-1, peripheral administration of a single dose of NN2211 significantly inhibited nighttime food intake in rats. The effect was not readily recognized during the initial 120 min of the dark phase, probably reflecting protracted pharmacokinetic mobilization from subcutaneous injection depot as demonstrated previously in female rats (18). Pharmacokinetic characterization of this GLP-1 derivative in humans revealed plasma half-lives of active GLP-1 derivative of ~14 h, which is considerably longer than the half-life of endogenous GLP-1 (1.2 h) (19). Preliminary studies of NN2211 half-life in the rat have shown somewhat shorter values (single subcutaneous bolus: t1/2 = 4 h), probably because of aforementioned high activity levels of DPP-IV (L.B.K., unpublished observations).

The site of the anorectic action elicited by peripheral administration of GLP-1/NN2211 is different from the site that elicits anorexia in response to intracerebroventricular administration of GLP-1, i.e., it does not involve MSG-sensitive parts of the hypothalamus (12). However, participation of both central and peripheral sites in GLP-1–induced anorexia should be considered because a recent study showed that radiolabeled GLP-1 readily gains access to the central nervous system (18). The nucleus of the solitary tract is adjacent to the blood-brain barrier–free area postrema, and a number of studies have shown that peripheral administration of neuropeptides not only labels the area postrema but also diffuses into the adjacent regions (20). Such a mechanism is supported by our recent observations from rats that were implanted with GLP-1–synthesizing tumors in which anorexia develops irrespective of subdiaphragmatic vagal transection (P.J.L., unpublished observations). Thus, it seems likely that the anorectic effect of peripheral GLP-1 is mediated via a peripherally accessible site located either in the brainstem or on vagal afferents. In rats, the inhibitory actions of GLP-1 on gastric motility is mediated via the vagus nerve (11), but also direct inhibition of gastrin secretion as well as stimulation of somatostatin release may affect gastric emptying (21,22). Obviously, delayed gastric emptying coun-

![Figure 6](image-url)  
**FIG. 6.** Oxygen consumption was determined by indirect calorimetry by the end of the study (day 7). The rats received treatment at time point 0 (10:00 A.M.). The rats had no access to food or water while being subjected to indirect calorimetry. On each day of experimentation, an animal treated with the chemical uncoupler 2,4-dinitrophenol (DNP) was included as a “positive” instrument. The results are expressed as mean ± SE.

![Figure 7](image-url)  
**FIG. 7.** The RER was determined by indirect calorimetry by the end of the study (day 7). The rats received treatment at time point 0 (10:00 A.M.). The rats had no access to food or water while being subjected to indirect calorimetry. On each day of experimentation, an animal treated with the chemical uncoupler 2,4-dinitrophenol (DNP) was included as a “positive” instrument. The results are expressed as mean ± SE.
teracts excessive meal-related glucose excursions with con-
sequent beneficial glucose homeostatic effects. Decreased 
gastric motility may also be part of a premature inhibition of 
further ingestion as it constitutes a prandial satiety 
signal mediated via vagal stretch receptive nerve fibers.

A full analysis of NN2211 effects on the feeding pattern 
(meal size, intermeal interval, etc.) was not carried out, 
but preliminary data suggest that acute as well as sub-
chronic peripheral administration of NN2211 reduces meal 
size similar to what is known for other gastrointestinal 
hormones, such as CCK (22,23; P.J.L., unpublished obser-
vations). It has been suggested that signals that modify 
food intake via diminishing meal size are both direct and 
indirect (23). Direct signals are elicited when nutrients 
gain contact to various segments of the gastrointestinal 
tract, whereas indirect signals arise from other sources 
from GLP-1 receptor null mutant mice (GLP-1R 
(23)). Presentations of endogenous GLP-1 receptors 
function in regulation of satiety are incorrect, because 
these mice do not display an obese phenotype 
from GLP-1 receptor null mutant mice (GLP-1R 
null mutant mice). There is evidence that two 
signals, it seems plausible that peripherally released GLP-1 
represents a role in the hypothalamic paraventricular nucleus (13,30).

The regulatory role of both intestinal and central ner-
vous system GLP-1 as either hormonal or neurotransmitter 
mediators of satiety has been questioned by observations 
from GLP-1 receptor null mutant mice (GLP-1R−/−) 
because these mice do not display an obese phenotype 
(31). However, initial statements about the lack of GLP-1R 
function in regulation of satiety are incorrect, because 
further evidence that GLP-1 has a role as an acute short-
term mediator of satiety has actually been gained from 
studies on GLP-1R−/− mice. Careful analysis of the data 
presented by Scrocchi et al. (32) clearly demonstrates that 
GLP-1R−/− mice terminate the initial feeding period of 
the dark phase significantly later than wild-type animals, 
resulting in increased food intake during the initial 4 h of 
the dark phase. In GLP-1R−/− mice, other postprandial 
satiety factors probably take over the meal-terminating 
role of GLP-1 with resulting unaffected total caloric intake. 
So far, no loss of function mutations in the human GLP-1 
receptor has been reported, but the therapeutically inter-
esting question is whether long-term activation of periph-
erally accessible GLP-1 receptors constitutes a potential 
weight-reducing principle.

Using a novel long-acting GLP-1 derivative, NN2211, we

| TABLE 2 |
| Body composition |

<table>
<thead>
<tr>
<th></th>
<th>Ad libitum–fed vehicle</th>
<th>Pair-fed vehicle</th>
<th>NN2211</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>417.3 ± 15.3</td>
<td>378.5 ± 10.6†</td>
<td>373.3 ± 14.7†</td>
</tr>
<tr>
<td>BMC (g)</td>
<td>10.2 ± 0.8</td>
<td>10.2 ± 0.3</td>
<td>9.8 ± 0.6</td>
</tr>
<tr>
<td>Bone area (cm²)</td>
<td>70.4 ± 6.4</td>
<td>75.1 ± 2.0</td>
<td>68.7 ± 3.0</td>
</tr>
<tr>
<td>BMD (g/cm³)</td>
<td>0.145 ± 0.009</td>
<td>0.137 ± 0.004</td>
<td>0.143 ± 0.002</td>
</tr>
<tr>
<td>Lean tissue mass (g)</td>
<td>372.3 ± 18.9</td>
<td>321.2 ± 8.2†</td>
<td>339.6 ± 17.4†</td>
</tr>
<tr>
<td>Lean tissue mass (% of BW)</td>
<td>89.2 ± 2.5</td>
<td>84.9 ± 2.5ª</td>
<td>91.0 ± 3.5</td>
</tr>
<tr>
<td>Fat tissue mass (g)</td>
<td>35.5 ± 9.0</td>
<td>45.9 ± 9.7</td>
<td>24.6 ± 13.1</td>
</tr>
<tr>
<td>Fat tissue mass (% of BW)</td>
<td>8.5 ± 2.2</td>
<td>12.1 ± 2.3ª</td>
<td>6.5 ± 3.3</td>
</tr>
</tbody>
</table>

Data are mean ± SD. Body composition was determined by DEXA at the end of the study (day 7). The results are given as absolute values (grams) and as % of body weight (BW). †P < 0.05 versus ad libitum–fed vehicle; ªP < 0.01 versus ad libitum–fed vehicle.

| TABLE 3 |
| Plasma lipids: NN2211 treatment (100 µg/kg b.i.d.) reduces plasma triglyceride levels in both normal lean Wistar and MSG-treated rats |

<table>
<thead>
<tr>
<th>Treatment values (day 7)</th>
<th>Normal vehicle</th>
<th>Normal NN2211</th>
<th>MSG vehicle</th>
<th>MSG NN2211</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>1.98 ± 0.1</td>
<td>1.90 ± 0.1</td>
<td>2.07 ± 0.1</td>
<td>1.81 ± 0.8</td>
</tr>
<tr>
<td>Triglyceride (mmol/l)</td>
<td>1.58 ± 0.1</td>
<td>1.21 ± 0.3†</td>
<td>2.27 ± 0.3†</td>
<td>1.49 ± 0.1*</td>
</tr>
<tr>
<td>Recovery phase values (day 14)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>1.80 ± 0.2</td>
<td>1.98 ± 0.1</td>
<td>2.14 ± 0.1</td>
<td>1.98 ± 0.1</td>
</tr>
<tr>
<td>Triglyceride (mmol/l)</td>
<td>1.62 ± 0.2</td>
<td>1.29 ± 0.1</td>
<td>1.93 ± 0.2†</td>
<td>1.89 ± 0.2†</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SE (n = 8 in all groups). *P < 0.05 versus control within the group (ANOVA followed by Fisher’s post hoc analysis); †P < 0.05 versus respective non-MSG control (ANOVA followed by Fisher’s post hoc analysis).
A number of studies have investigated the potential anorectic effects of short-term intravenous GLP-1 infusion to human volunteers (<24 h). Both nonobese and obese humans respond to GLP-1 infusion by reducing their caloric intake without concomitant presence of gastrointestinal discomfort or increased equivalents of malaise (1,3). Subchronic administration of relatively high doses of the GLP-1 analogue exendin-4 to diabetic rodents decreases food intake and lowers body weight (33–35). Greig et al. (33) included a control group of normal-weight nondiabetic mice, in which both food intake and body weight remained unaffected by administration of 24 mmol/kg per day, suggesting that the anorectic effect may depend on increased plasma glucose levels or impaired insulin sensitivity. GLP-1–induced anorexia is not mediated via pancreatic insulin secretion because normal rats displayed full sensitivity to NN2211 during euglycemia, when GLP-1 has no insulinotrophic action. Extensive studies with NN2211 have shown that this derivative as native GLP-1 is incapable of causing severe hypoglycemia (36). Also, MSG-treated rats were fully sensitive to the anorectic action of NN2211 despite their well-known decreased peripheral insulin sensitivity (37,38). It is interesting to note that both circulating and postprandial levels of active GLP-1(7-36)amide are decreased in obese humans (39). In particular, obese individuals seem to have a selective attenuation of carbohydrate-induced postprandial GLP-1 secretion, and this defect may be related to the dyslipidemia experienced by most obese individuals because the degree of impaired GLP-1 release is tightly correlated to the circulating levels of nonesterified fatty acids (40). Therefore, acute regulation of feeding behavior in obese individuals may involve a weaker-than-normal postprandial GLP-1 satiety signal. In conjunction with carbohydrate ingestion, insulin-induced leptin secretion is markedly increased in comparison to levels seen during fat ingestion (41). Thus, lower-than-normal postprandial GLP-1 release in obese individuals may lead to an insufficient insulin release, causing both impaired glucose tolerance and lower leptin secretion.

In conclusion, we showed that peripheral administration of GLP-1 and a long-acting derivative thereof, NN2211, induce anorexia together with lowered water intake and increased diuresis. Furthermore, NN2211 confers lasting and reversible anorexia with accompanying weight loss and reduction of body adiposity. As seen for many other anorectic agents, NN2211 causes a moderate drop in total EE proportional to the induced weight loss. However, no change in substrate utilization was seen. Despite initial effects on water intake and diuresis, NN2211 administration has no debilitating effects on body water homeostasis.

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