Effect of the GLP-1 Receptor Agonist Lixisenatide on Counter-regulatory Responses to Hypoglycemia in Subjects With Insulin-Treated Type 2 Diabetes

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OBJECTIVE

Counterregulatory responses are critical to prevent hypoglycemia in subjects with type 2 diabetes. This is particularly important in insulin-treated patients. This study explored the effect of the glucagon-like peptide 1 receptor agonist lixisenatide on the hormonal counterregulatory responses to insulin-induced hypoglycemia when added to basal insulin therapy in subjects with type 2 diabetes.

RESEARCH DESIGN AND METHODS

The study was a single-center, double-blind, randomized, placebo-controlled crossover study involving 18 subjects with type 2 diabetes (11 males) with a mean age of 55 years, diabetes duration of 12 years, HbA_{1c} level of 7.7%, fasting blood glucose (FBG) concentration of 9.7 mmol/L, and a BMI of 33 kg/m^2, who were treated with basal insulin (mean duration 7 years, daily dose 39 units/day) and metformin (mean daily dose 2.1 g). Subjects received treatment with lixisenatide or placebo for 6 weeks in random order, with a 4-week washout period in between. After 6 weeks of treatment, subjects underwent a two-step hyperinsulinemic hypoglycemic clamp at 3.5 and 2.8 mmol/L.

RESULTS

After 6 weeks of treatment, HbA_{1c} and FBG levels were lower after lixisenatide therapy than after placebo therapy. At the hypoglycemic level of 3.5 mmol/L, glucagon and epinephrine levels were significantly lower during lixisenatide treatment than during placebo treatment, whereas at 2.8 mmol/L glucagon and epinephrine levels did not differ between the subjects. Cortisol, pancreatic polypeptide, and norepinephrine levels did not differ significantly between the treatments.

CONCLUSIONS

Glucagon and epinephrine levels are reduced by lixisenatide at a concentration of 3.5 mmol/L, but their counterregulatory responses to deep hypoglycemia at a concentration of 2.8 mmol/L are sustained during treatment with lixisenatide in combination with basal insulin.

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In people with type 2 diabetes, the glucose sensing of the islet α-cells is impaired, which leads to glucagon hypersecretion at normal and elevated glucose levels, and impaired glucagon counterregulation during hypoglycemia (1–3). Reducing the hyperglucagonemia is therefore an important target for the treatment of diabetes (4). Hyperglucagonemia is targeted by glucagon-like peptide 1 (GLP-1) and, consequently, by the GLP-1 receptor agonists (1,5–8). However, preserving a functional glucose counterregulation is critically important for glucose-lowering treatment to prevent hypoglycemia (9,10). It has been shown previously in healthy volunteers (11,12) and in subjects with type 2 diabetes treated with oral agents (13) that GLP-1 or GLP-1 receptor agonists do not compromise glucagon counterregulation during hypoglycemia. However, because of the recent development in which GLP-1 receptor agonists are also used in combination with insulin therapy (14–19), it is of importance to determine the hormonal counterregulation during hypoglycemia under this condition also. We therefore explored whether the GLP-1 receptor agonist lixisenatide affects the glucagon, norepinephrine, epinephrine, cortisol, and pancreatic polypeptide (PP) responses to insulin-induced hypoglycemia when the GLP-1 receptor agonist is added to basal insulin in subjects with type 2 diabetes. Lixisenatide is a newly developed GLP-1 receptor agonist that is based on the structure of exendin-4 (20). It improves glycemia with low risk for hypoglycemia in monotherapy, in association with metformin, and in combination with insulin (21–24).

RESEARCH DESIGN AND METHODS

Study Population and Study Design

The study was a single-center, double-blind, placebo-controlled, crossover study in a total of 18 patients with type 2 diabetes treated with basal insulin plus metformin. The study was undertaken according to good clinical practice, approved by the Ethic Committee in Lund, Sweden, and registered at ClinicalTrials.gov (clinical trial reg. no. NCT02020629) and https://www.clinicaltrialsregister.eu/ (EudraCT2012-004959-36) databases. All subjects gave written consent to participate before the study, and the study was monitored by an external monitor.

Figure 1 illustrates the design of the study. Each patient attended a screening visit where the inclusion/exclusion criteria were assessed. The study population consisted of male and female (nonfertile or of child-bearing potential using a medically approved birth control method) patients with type 2 diabetes who were treated with basal insulin (insulin detemir, insulin glargine, or NPH insulin) plus metformin with HbA_{1c} at 7–10% (53–86 mmol/mol), aged >18 years. Exclusion criteria were treatment with antihyperglycemic agents apart from basal insulin and metformin; type 1 diabetes (including latent autoimmune diabetes in adults); pregnancy or lactation; a history of any secondary forms of diabetes; acute infections that may affect blood glucose control within 4 weeks prior to visit 1; a history of recent (<2 weeks) recurrent or severe hypoglycemic episodes or hypoglycemia unawareness; donation of ≥1 unit (500 mL) of blood; significant blood loss equaling at least 1 unit of blood within the past 2 weeks or a blood transfusion within the past 8 weeks; treatment with growth hormone and an oral or parenteral corticosteroid (>7 consecutive days of treatment) within 8 weeks prior to visit 1 and thereafter during the whole study period; use of other investigational drugs within 30 days prior to visit 1; abnormal laboratory findings at the time of screening, including amylase and/or lipase three or more times the upper limit of the normal laboratory range and P-calcitonin ≥20 pg/mL (5.9 pmol/L); personal or immediate family history of medullary thyroid cancer (MTC) or a genetic condition that predisposes to MTC (e.g., multiple endocrine neoplasia syndromes); a history of unexplained pancreatitis, chronic pancreatitis, pancreatectomy, stomach/gastric surgery, allergic reaction to any GLP-1 receptor agonist; or a clinically relevant history of gastrointestinal disease associated with prolonged nausea and vomiting, cardiovascular, hepatic, neurological, or endocrine disease, active malignant tumor, or other major systemic disease or patients with short life expectancy.

Eligible patients were randomized at a second visit and completed two treatment periods, receiving a different blinded study medication during each period (lixisenatide or placebo, in random

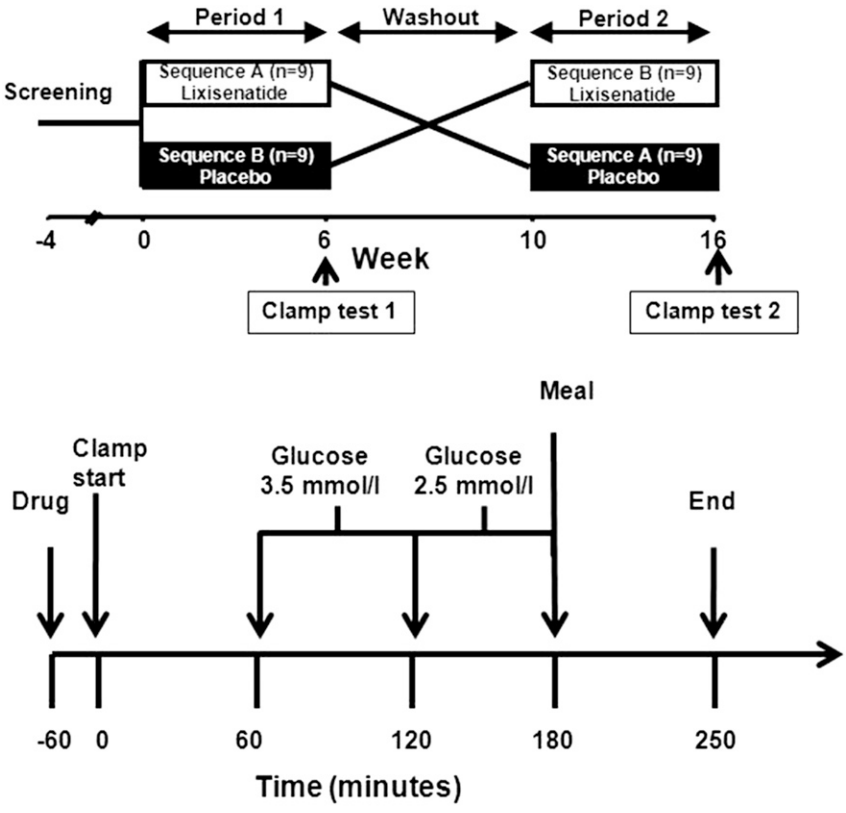

Figure 1—Schematic illustration of the study with the overall crossover design (top panel) and the clamp procedure (bottom panel).

order) in addition to continued treatment with their basal insulin and metformin. Baseline visits were performed prior to each of the two treatment periods, at which time the patient was assessed clinically and the blinded study medication was dispensed for the 6 weeks of treatment. After 6 weeks, a two-step hyperinsulinemic hypoglycemic clamp was performed. The blinded study medication was then discontinued, and a 4-week washout period before the next treatment period was started; after the second 6-week treatment period, the clamp study was repeated. Patients therefore received two treatments (lixisenatide and placebo) in a randomized order. Lixisenatide was administered at 10 μg daily, s.c., within 1 h before breakfast for 2 weeks, followed by the maintenance dose of 20 μg once daily. In patients with an HbA_{1c} level of <7.5% (<58 mmol/mol), the insulin dose was reduced by 20% at the time of the start of the study in order to limit the risk of hypoglycemia. After 1 week, the dose could be increased again at the investigator's discretion according to fasting blood glucose (FBG) levels. If hypoglycemia (cutoff definition 3.1 mmol/L) was confirmed during the treatment period, patients were requested to contact the study center for discussion regarding possible adjustment of the insulin dose. All randomized patients completed the study.

Clamp Procedure

Hyperinsulinemic hypoglycemic clamps were performed after 6 weeks of treatment with lixisenatide or placebo. Patients arrived at the study site in the morning after an overnight fast (no food or drink, except water, was allowed after 10:00 P.M. the preceding evening). Blinded study medication (lixisenatide or placebo) and the usual regular morning dose of metformin were taken 15 min before the start of the clamp procedure. The regular basal insulin dose was given the evening before the clamp, and those patients who were receiving twice-daily doses did not receive the basal insulin in the morning before the clamp. During the clamp procedure, patients received a primed 15-min infusion of insulin (Actrapid; Novo Nordisk, Bagsvaerd, Denmark) followed by insulin infusion at a constant rate to reduce blood glucose concentrations to 3.5 mmol/L and then to further reduce blood glucose levels to 2.8 mmol/L. The 15-min insulin priming infusion was 1.3 international units (IU)/m^2 if the FBG concentration was ≤5 mmol/L, 3.9 IU/m^2 if the FBG concentration was 5–7.5 mmol/L, 6.5 IU/m^2 if the FBG concentration was 7.5–10 mmol/L, 9.1 IU/m^2 if the FBG concentration was 10–15 mmol/L, and 13 IU/m^2 if the FBG concentration was >15 mmol/L. The constant infusion rates for the various fasting glucose levels were 3, 9, 18, 21, and 30 IU/m^2/h, respectively. The two target glucose levels were maintained for 30 min. Glucose (200 mg/mL) was infused at a variable rate (dependent on bedside blood glucose monitored every 5 min) that resulted in the desired glucose concentration. The insulin infusion was then stopped, and a standardized meal was given, consisting of chicken, potatoes, sauce, and green peas with 442 kcal (13% from fat, 36% from carbohydrate, and 51% from protein). Samples were obtained at prespecified time points throughout the clamp test for hormonal assays.

Adverse Events

Adverse events (AEs) were sought by nondirective questioning of the patient at each visit during the study. AEs were also detected when they were mentioned by the patient during or between visits or through physical examination, laboratory test, or other assessments. All AEs, including hypoglycemia, were recorded. Hypoglycemia was defined as either symptomatic hypoglycemia, confirmed hypoglycemia (blood glucose concentrations ≤3.1 mmol/L), or a hypoglycemia episode that required assistance for glucose control.

Laboratory Measurements

Cortisol, epinephrine, norepinephrine, HbA_{1c}, fasting plasma glucose, and safety laboratory assessments were measured by the Department of Clinical Chemistry (Skåne University Hospital, Malmö, Sweden). Samples for glucagon and PP were placed in chilled tubes containing EDTA (7.4 mmol/L) and aprotinin (500 kallikrein inhibitor units/mL; Novo Nordisk), and were immediately centrifuged at 4°C; plasma was frozen at −20°C until analysis. Glucagon and PP were analyzed at the Department of Clinical Sciences Lund (Lund University). Glucagon concentrations were analyzed with an ELISA (catalog #10–1271–01; Mercodia, Uppsala, Sweden). Glucagon was measured using the recently developed sandwich ELISA, using monoclonal antibodies against both to the C- and N-terminal regions or glucagon, which has been shown to have higher specificity and reliability than previously used methods (25). The assay is specific for pancreatic glucagon, and shows 4.4% cross-reactivity with oxyntomodulin and 0.8% cross-reactivity with glicentin, but with no other peptide. It has a detection limit of 1 pmol/L; the intra-assay coefficient of variation (CV) is 3.3–5.1%, and the interassay CV is 7.3–9.4% at various concentrations ranging from low to high. PP was determined with an ELISA (catalog #EZHPP40 K; Merck Millipore, Billerica, MA). The assay is specific for human PP and shows no cross-reactivity with neuropeptide Y, peptide YY, or any other gut hormone. It has a sensitivity of 12.3 pg/mL, an intra-assay CV of 3.3–5.0%, and an interassay CV of 4.4–9.8% at various concentrations ranging from low to high. Samples for the determination of norepinephrine and epinephrine levels were obtained in ice-chilled sodium-heparin tubes, and their concentrations were determined by high-performance liquid chromatography. Samples for the determination of cortisol were taken in sodium-lithium-heparin tubes, and its concentrations were determined with the Access Immunoassay System (Beckman Coulter, Fullerton, CA).

Data Analysis and Statistics

During the hypoglycemic clamp step, the change in the concentrations of analytes were calculated from time 60 to time 120 min (i.e., step 1 of hypoglycemia at 3.5 mmol/L glucose), from time 120 to time 180 min (i.e., step 2 of hypoglycemia at 2.8 mmol/L), and from time 60 to 180 min (i.e., steps 1 and 2 together). Analytes during the clamp were also evaluated by calculating the area under the curve (AUC) with the trapezoid method. Between-treatment differences in measured variables were estimated with a paired *t* test. There was no difference in responses to placebo or lixisenatide depending on the order sequence of treatment. Therefore, in the statistical analysis all sequences with lixisenatide and placebo, respectively, were analyzed together. A *P* value of <0.05 was considered significant.

RESULTS

Subjects

A total of 21 subjects were screened, and 18 patients (11 males, 7 females) were randomized. The mean (±SD) age of the randomized patients was 55 ± 12 years, the mean duration of diabetes was 11.7 ± 7.6 years, the mean duration of insulin therapy was 7.1 ± 5.3 years, the mean daily dose of insulin was 39 ± 22 IU, the mean daily dose of metformin was 2.1 ± 0.2 g, the mean body weight was 99 ± 17 kg, the mean BMI was 33 ± 5 kg/m^2, the mean HbA_{1c} concentration was 60.5 ± 3.0 mmol/mol (7.7 ± 0.3%), and the mean FBG was 9.7 ± 0.6 mmol/L. All 18 randomized subjects completed the study. There was no difference in baseline characteristics in patients starting therapy with either lixisenatide or placebo (data not shown).

Effects on HbA_{1c}, FBG, Body Weight, and Insulin Dose

Table 1 shows the baseline levels and effects of treatment in HbA_{1c} and FBG levels. HbA_{1c} level was significantly reduced by lixisenatide treatment, but not by placebo treatment. During the 6-week treatment with lixisenatide, the mean HbA_{1c} level was reduced by −0.5 ± 0.1% (−5.8 ± 1.2 mmol/mol, $P < 0.001$), whereas the mean HbA_{1c} level was reduced by −0.1 ± 0.1% (−1.0 ± 1.3 mmol/mol) with placebo treatment. The between-group difference was −0.4 ± 0.1% (−4.8 ± 1.0 mmol/mol, $P = 0.042$). Also, FBG level (between-group difference −1.6 ± 0.5 mmol/L, $P = 0.046$), body weight (between-group difference −1.1 ± 0.4 kg, $P = 0.043$), and daily insulin dose (between-group difference −2.6 ± 3.9 units, $P = 0.023$) were reduced with lixisenatide treatment compared with placebo treatment. There were similar changes resulting from lixisenatide treatment in the nine patients who had started with lixisenatide treatment as in the nine patients who had started with placebo treatment (data not shown). In those subjects who started with lixisenatide treatment, HbA_{1c} levels remained low for the 4-week washout period at 57.3 ± 4.3 mmol/mol (7.4 ± 0.4%) and FBG levels were at 9.0 ± 1.1 mmol/L at the start of the subsequent placebo treatment period. In those patients, HbA_{1c} level (from 57.3 ± 4.3 to 60.2 ± 4.6 mmol/mol [7.4 ± 0.4 to 7.7 ± 0.4%], $P = 0.015$) but not FBG level increased during the placebo period. There was no change in the daily dose of metformin during the study.

Table 1—Baseline and 6-week change in HbA_{1c} level, FBG level, body weight, and daily insulin dose during the study

	Lixisenatide	Placebo	*P* value[a]
Baseline HbA_{1c} (mmol/mol; IFCC)	61.4 ± 3.0	59.9 ± 2.8	0.548
Baseline HbA_{1c} (%; DCCT)	7.7 ± 0.3	7.6 ± 0.3	0.548
6-week HbA_{1c} (mmol/mol; IFCC)	55.6 ± 2.4	58.8 ± 2.9	0.020
6-week HbA_{1c} (%; DCCT)	7.3 ± 0.2	7.5 ± 0.3	0.020
Change in HbA_{1c} (mmol/mol; IFCC)	−5.8 ± 1.2 ($P < 0.001$)[b]	−1.0 ± 1.3 ($P = 0.432$)[b]	0.042
Change in HbA_{1c} (%; DCCT)	−0.5 ± 0.1 ($P < 0.001$)[b]	−0.1 ± 0.1 ($P = 0.432$)[b]	0.042
Baseline FBG (mmol/L)	9.7 ± 0.6	9.3 ± 0.7	0.547
6-week FBG (mmol/L)	8.3 ± 0.6	9.3 ± 0.7	0.023
Change in FBG (mmol/L)	−1.6 ± 0.4 ($P = 0.002$)[b]	0.0 ± 0.6 ($P = 0.986$)[b]	0.046
Baseline body weight (kg)	99.5 ± 4.6	99.8 ± 4.5	0.962
6-week body weight (kg)	97.7 ± 4.4	99.1 ± 4.4	0.823
Change in body weight (kg)	−1.7 ± 0.4 ($P < 0.001$)[b]	−0.6 ± 0.4 ($P = 0.095$)[b]	0.043
Baseline daily insulin dose (units)	39 ± 24	39 ± 25	0.934
6-week daily insulin dose (units)	37 ± 24	40 ± 24	0.684
Change in daily insulin dose (units)	−1.8 ± 4.1 ($P = 0.076$)[b]	0.8 ± 2.1 ($P = 0.135$)[b]	0.023

Data are reported as the mean ± SD, unless otherwise indicated. [a]*P* value for the probability level of random difference between the two treatments. [b]*P* value for the probability level of random difference before and after treatment within each treatment arm. DCCT, Diabetes Control and Complications Trial unit; IFCC, International Federation of Clinical Chemistry and Laboratory Medicine unit.

Hyperinsulinemic Hypoglycemic Clamp

Glucose Levels

After initiation of the clamp procedure, glucose levels were similarly reduced after lixisenatide and placebo treatment to reach the first steady state at 90 min, which was kept stable at 3.5 ± 0.1 mmol/L after lixisenatide treatment and 3.4 ± 0.1 mmol/L after placebo treatment for 30 min ($P = 0.61$) (Fig. 2 and Table 2). Then, at 120 min, the glucose infusion rate was reduced, which resulted in a further fall in the glucose level to the second steady state at 150 min, which was 2.8 ± 0.04 mmol/L after lixisenatide treatment and 2.9 ± 0.07 mmol/L after placebo treatment ($P = 0.34$), and kept stable for 30 min. The concentration of the glucose infused until 180 min was 0.83 ± 0.09 mmol/kg after treatment with lixisenatide and 0.90 ± 0.13 mmol/kg after placebo ($P = 0.59$). The total amount of insulin that was infused during the clamp procedure did not differ between lixisenatide treatment (46 ± 9 IU) and placebo treatment (51 ± 11 IU, $P = 0.65$).

At 180 min, insulin infusion was stopped, and a standardized 442-kcal mixed meal was given. Glucose levels then increased to 9.3 ± 0.6 mmol/L (lixisenatide) and 9.9 ± 0.5 mmol/L (placebo), respectively, after 60 min ($P = 0.363$).

Glucagon Levels

The fasting glucagon level was not different between the two treatments (Fig. 2 and Table 2). During the initial 60 min of the clamp, when glucose levels were gradually reduced from fasting to 3.5 mmol/L, glucagon levels were reduced in both groups, but more after lixisenatide treatment than after placebo treatment. This resulted in a significantly lower 60-min value for glucagon after lixisenatide treatment compared with placebo treatment ($P = 0.005$). Then, when a 3.5 mmol/L concentration of glucose was reached and clamped, glucagon levels increased after both treatments with no significant difference between the treatments ($P = 0.20$). Since glucagon levels were lower after lixisenatide treatment than after placebo treatment at 60 min and the

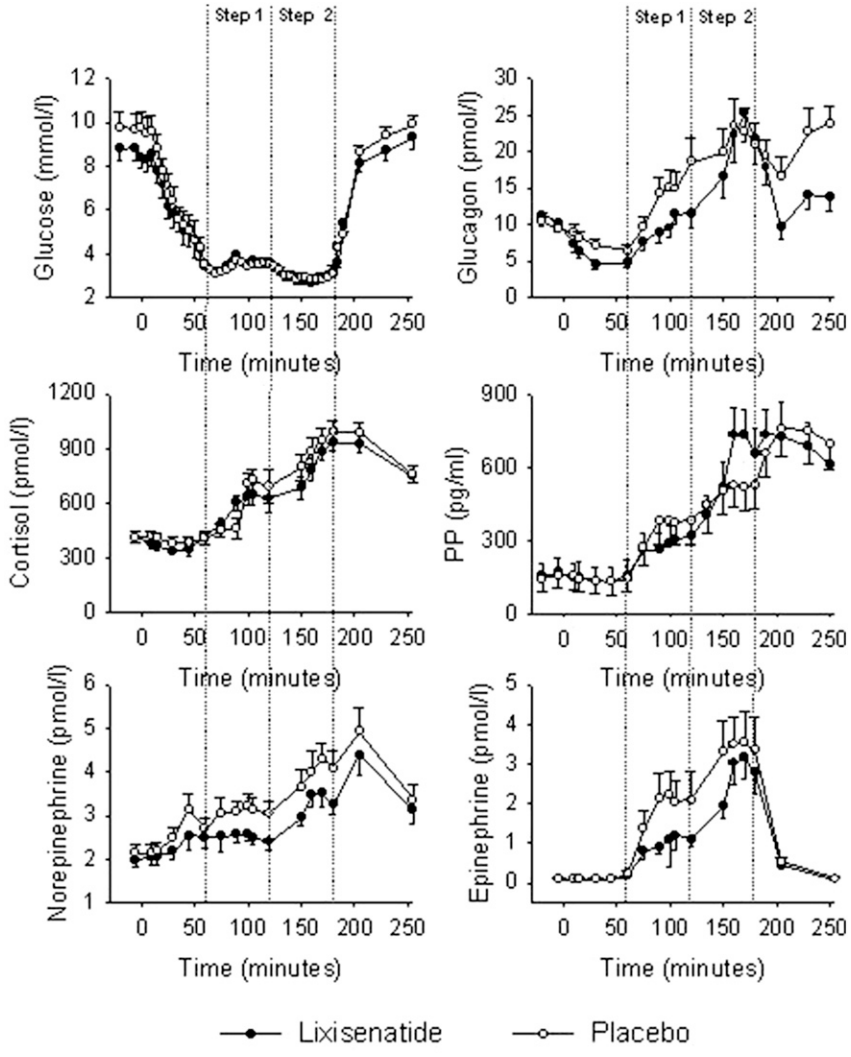

Figure 2—Glucose, glucagon, cortisol, PP, norepinephrine, and epinephrine levels during the hyperinsulinemic hypoglycemic clamp after 6 weeks of treatment with lixisenatide (filled symbols) or placebo (open symbols) in 18 patients with insulin-treated type 2 diabetes. Dotted lines show the time intervals for the two steps of the hypoglycemia clamp (3.5 and 2.8 mmol/L, respectively). Means ± SEM are shown.

glucagon increase achieved by reducing glucose to 3.5 mmol/L was not significantly different between treatments, glucagon levels were still significantly lower after lixisenatide treatment than after placebo treatment at 120 min (P = 0.045), as were the mean glucagon levels during the 60- to 120-min clamp period (P = 0.008) and the AUC for glucagon during minutes 60–120 (i.e., during the 3.5 mmol/L clamp) (P = 0.013). Then, when glucose levels were further reduced to 2.8 mmol/L in the second hypoglycemic step, glucagon levels increased further in both groups. This increase was significantly higher after lixisenatide treatment than after placebo treatment (P = 0.042). This resulted in glucagon levels at 180 min not being significantly different between the treatments and, also, the mean glucagon during the 120- to 180-min study period and the AUC for glucagon during minutes 120–180 not being significantly different between the treatments. Furthermore, the increase in glucagon levels or the AUC for glucagon during the entire clamp was not significantly different between the two treatments (Table 2). At 180 min, a standardized 442-kcal mixed meal was served; thereafter, glucagon levels were, again, lower after lixisenatide treatment than after placebo treatment, and the 255-min glucagon concentration, which ended the study, was 13.8 ± 1.9 pmol/L after lixisenatide treatment and 23.8 ± 2.3 pmol/L after placebo treatment ($P < 0.001$).

Cortisol, Norepinephrine, Epinephrine, and PP

Baseline levels and levels at 120 and 180 min of cortisol, norepinephrine, and PP did not differ between the treatments (Fig. 2 and Table 3). Therefore, the 120- and 180-min increases in cortisol, PP, or norepinephrine did not differ significantly between lixisenatide and placebo treatment, although there was a nonsignificant trend for lower norepinephrine levels at both 3.5 and 2.8 mmol/L glucose after lixisenatide treatment compared with placebo treatment (P = 0.112). Also baseline epinephrine levels did not differ between the treatments. However, the 120-min epinephrine levels were significantly lower after lixisenatide treatment than after placebo treatment (P = 0.021), and, therefore, the epinephrine response to reducing glucose concentration to 3.5 mmol/L was lower during lixisenatide treatment than during placebo treatment, as is evident both by the lower increase in levels (P = 0.022), the lower mean epinephrine levels (P = 0.023), and the AUC for epinephrine (P = 0.041) during the 3.5 mmol/L clamp (Table 3). Thereafter, the epinephrine response to reducing the glucose concentration to 2.8 mmol/L was not different between the groups, resulting in a nonsignificant difference in epinephrine levels at 180 min.

AEs

In total, 56 AEs were reported during the study, of which 52 occurred during treatment with study medication (29 with lixisenatide treatment [in 13 patients], 23 with placebo [in 12 patients]) and 4 occurred during the washout period between the treatments (3 in the lixisenatide/placebo treatment sequence and 1 in the placebo/lixisenatide treatment sequence). The overall AE profile during treatment with lixisenatide was similar to that during placebo administration, except that nausea was reported in seven patients during lixisenatide treatment and in two patients during placebo treatment. Mild hypoglycemia was reported in one patient during treatment with lixisenatide. No severe hypoglycemia (i.e., requiring third-party assistance) was reported. One severe AE was reported in a patient who had received the placebo and then had vertigo. The patient was admitted

Table 2—Glucagon levels and counterregulation during the hyperinsulinemic hypoglycemic clamp test in subjects with insulin-treated type 2 diabetes

	Lixisenatide	Placebo	*P* value
Glucagon level at 0 min (pmol/L)	10.7 ± 1.3	9.8 ± 1.2	0.18
Glucagon level at 60 min (pmol/L)	4.5 ± 0.7	6.0 ± 0.8	0.005
Glucagon level at 120 min (pmol/L)	11.2 ± 1.7	16.1 ± 2.6	0.045
ΔGlucagon at 3.5 mmol/L (60–120 min) (pmol/L)	6.7 ± 1.6	10.1 ± 2.3	0.20
Mean glucagon at 3.5 mmol/L (60–120 min) (pmol/L)	8.1 ± 1.1	11.4 ± 1.6	0.008
AUC glucagon at 3.5 mmol/L (60–120 min) (pmol/L · min)	471 ± 63	668 ± 91	0.013
Glucagon level at 180 min (pmol/L)	21.7 ± 3.4	21.1 ± 3.0	0.827
ΔGlucagon at 2.8 mmol/L (120–180 min) (pmol/L)	10.5 ± 2.3	4.9 ± 2.3	0.042
Mean glucagon at 2.8 mmol/L (min 120–180 min) (pmol/L)	19.2 ± 2.9	20.7 ± 2.9	0.63
AUC glucagon at 2.8 mmol/L (120–180 min) (pmol/L · min)	988 ± 147	1,116 ± 152	0.36
ΔGlucagon during whole clamp (60–180 min) (pmol/L)	17.2 ± 3.0	15.0 ± 2.5	0.45
AUC glucagon during whole clamp (60–180 min) (pmol/L · min)	1,460 ± 208	1,782 ± 29	0.12
Glucagon level at 240 min	13.8 ± 1.9	23.8 ± 2.3	<0.001

Data are reported as the mean ± SEM, unless otherwise indicated. Δ, change in level.

to the hospital overnight and during the night the vertigo subsided.

CONCLUSIONS

This study evaluated the hormonal counterregulatory responses to a two-step hypoglycemia clamp procedure in insulin- and metformin-treated patients with type 2 diabetes when lixisenatide or placebo was added. The study had a crossover design, and therefore all patients underwent treatment with both lixisenatide and placebo. The main finding was that *1*) the glucagon and epinephrine levels were reduced by lixisenatide at a glucose concentration of 3.5 mmol/L, whereas *2*) their levels at deep hypoglycemia at a glucose concentration of 2.8 mmol/L were not significantly different between the two treatments. Furthermore, as reported before, lixisenatide was also found to reduce HbA_{1c} levels, fasting glucose levels, and body weight compared with placebo when added to basal insulin therapy (21–24). There was also a slight reduction in the daily dose of insulin, and it was found that lixisenatide was well tolerated and that nausea was the most common AE, as was expected from this class of drugs.

Glucagon levels were reduced immediately after the administration of lixisenatide, which confirms the results of previous studies (26) that lixisenatide inhibits glucagon secretion. After this initial 60-min period, and while glucose levels were reduced to 3.5 mmol/L, glucagon levels were still lower during lixisenatide treatment than during placebo treatment, and although the increase in glucagon (glucagon counterregulation to mild hypoglycemia) was not significantly different between the two treatments, the levels of glucagon remained lower during lixisenatide treatment, which was also evident by a lower AUC for glucagon. This shows that at a hypoglycemic concentration of 3.5 mmol/L glucagon levels are still reduced by the administration of lixisenatide. In contrast, during the second hypoglycemic step, when the glucose concentration was further reduced to 2.8 mmol/L, the increase in glucagon was more pronounced during lixisenatide treatment than during placebo treatment, resulting in a similar glucagon level at the end of the 2.8 mmol/L hypoglycemic period. Overall, therefore, the study shows that lixisenatide treatment reduces glucagon levels at glucose levels down to ~3.5 mmol/L, but that the glucagon counterregulation to deep hypoglycemia is preserved during treatment with lixisenatide and, therefore, that the inhibition of glucagon secretion stops when glycemia reaches critically low levels.

A preserved glucagon counterregulation to hypoglycemia has previously been shown for native GLP-1 and the GLP-1 receptor agonist exenatide in 9 and 12 healthy volunteers, respectively (11,12), and for the GLP-1 receptor agonist albiglutide in subjects with type 2 diabetes who had been treated with oral agents (13). In these studies, the glucagon levels when glucose levels were 3.5 mmol/L were not different from those after placebo treatment, although glucose was not clamped at this level, which thus may be a difference from the results in the current study. This discrepancy in regard to 3.5 mmol/L glucagon levels among the different GLP-1 receptor agonists may be explained by different glucose thresholds for the glucagon response among the different GLP-1 receptor agonists, by different durations of clamping glucose levels, or by different glucagon characteristics in the different study populations. Although the explanation is important to establish, a most likely explanation is that the current study was performed in patients treated with basal insulin who in general have a longer duration of diabetes and also are given exogenous insulin, both of which are conditions that may reduce glucagon secretion. In addition, a so-called hypoglycemia-associated autonomic failure, due to recent antecedent hypoglycemia, may have attenuated a counterregulatory response in these patients (27). Since an add-on to basal insulin may become an important positioning of GLP-1 receptor agonists, there is, therefore, a need for head-to-head studies between different GLP-1 receptor agonists to examine glucagon counterregulation to mild hypoglycemia in this population.

The reason why the glucagon counterregulation to deep hypoglycemia is not reduced during treatment with lixisenatide, despite the well-known effect of lixisenatide of inhibiting glucagon secretion at hyperglycemia (27),

Table 3—Cortisol, PP, norepinephrine, and epinephrine levels and counterregulation during the hyperinsulinemic hypoglycemic clamp test in subjects with insulin-treated type 2 diabetes

	Cortisol (pmol/L or nmol/L · min)		PP (pg/mL or pg/mL · min)		Norepinephrine (pmol/L or pmol/L · min)		Epinephrine (pmol/L or pmol/L · min)	
	Lixisenatide	Placebo	Lixisenatide	Placebo	Lixisenatide	Placebo	Lixisenatide	Placebo
Level 0 min	411 ± 29	463 ± 32	171 ± 55	157 ± 55	2.0 ± 0.2	2.1 ± 0.2	0.09 ± 0.02	0.11 ± 0.03
Level 60 min	420 ± 28	404 ± 35	157 ± 63	145 ± 53	2.5 ± 0.2	2.7 ± 0.2	0.19 ± 0.06	0.23 ± 0.07
Level 120 min	629 ± 78	691 ± 89	321 ± 77	381 ± 96	2.4 ± 0.2	3.0 ± 0.3	1.19 ± 0.34	2.06 ± 0.54[a]
ΔAt 3.5 mmol/L (60–120 min)	219 ± 78	308 ± 85	163 ± 59	235 ± 79	0.0 ± 0.1	0.3 ± 0.2	0.99 ± 0.31	1.82 ± 0.53[a]
Mean level 60–120 min	558 ± 52	574 ± 60	247 ± 66	312 ± 77	2.4 ± 0.58	2.8 ± 0.23	0.78 ± 0.18	1.47 ± 0.39[a]
AUC at 3.5 mmol/L (60–120 min)	31.2 ± 2.7	32.2 ± 3.1	14.1 ± 4.0	15.8 ± 4.0	144 ± 12	173 ± 15	41.5 ± 11.5	68.7 ± 16.8[a]
Level at 180 min	931 ± 46	989 ± 64	660 ± 99	624 ± 96	3.3 ± 0.3	4.1 ± 0.4	2.77 ± 0.51	3.35 ± 0.83
ΔAt 2.8 mmol/L (120–180 min)	328 ± 34	287 ± 58	339 ± 88	243 ± 79	1.1 ± 0.23	1.0 ± 0.27	1.54 ± 0.34	1.29 ± 0.49
Mean level at 120–180 min	716 ± 104	804 ± 106	562 ± 88	482 ± 91	2.4 ± 0.30	3.9 ± 0.53	2.61 ± 0.45	3.15 ± 0.69
AUC at 2.8 mmol/L (120–180 min)	47.6 ± 4.1	50.1 ± 4.3	29.5 ± 4.8	27.2 ± 5.4	166 ± 14	215 ± 19	120 ± 24	162 ± 40
ΔAt whole clamp (60–180 min)	511 ± 51	684 ± 66	503 ± 88	479 ± 79	0.77 ± 0.21	1.4 ± 0.31	2.58 ± 0.49	3.11 ± 0.82

Data are reported as the mean ± SEM. Δ, change in level. Concentrations (pmol/L or pg/mL) refer to values taken at specific time points, whereas concentrations times minutes (nmol/L · min or pg/mL · min) refer to values for AUC. [a]Probability level of random difference between the two treatments at $P < 0.05$ or less (for details, see RESULTS).

is probably the glucose-dependent mechanism of action of GLP-1. It has thus been demonstrated both in healthy subjects (28) and in subjects with type 2 diabetes (29) that GLP-1 clearly suppresses glucagon secretion when glucose levels are elevated above fasting levels. However, when reducing glucose levels below fasting levels, this inhibitory effect on glucagon secretion vanishes, as was demonstrated in the hypoglycemic clamp study using exenatide (12). In that study, exenatide inhibited glucagon secretion at 4.0 mmol/L glucose but not at 3.2 mmol/L glucose, suggesting that the threshold of GLP-1 receptor activation to inhibit glucagon secretion is between these glucose levels. This is confirmed in the current study in insulin-treated patients. In fact, glucagon levels were significantly lower during treatment with lixisenatide compared with placebo when glucose levels were clamped at 3.5 mmol/L, but not at lower levels, which suggests that the inhibitory effect of lixisenatide treatment on glucagon secretion vanishes when glucose levels are reduced to ~3.5 mmol/L. Below this level, the hypoglycemia itself could increase glucagon levels as it normally does without inhibition by lixisenatide.

Several mechanisms are involved in glucagon secretion during hypoglycemia, and these include, besides a direct action of low glucose to stimulate glucagon secretion, the activation of the autonomic nervous system and the adrenal glands to increase levels of epinephrine (9,10,30). There is a different glucose threshold for these mediators; autonomic parasympathetic activation is important at a higher glucose level compared with circulating epinephrine and islet sympathetic activation, which become operative during deep hypoglycemia (30). We measured PP, which is a marker of autonomic nerve activation, mainly parasympathetic nerves (31), and we also determined norepinephrine and epinephrine levels to explore these counterregulatory factors after treatment with lixisenatide. We showed that all these variables increased during hypoglycemia, although the increase in norepinephrine was not evident until the deep hypoglycemia at 2.8 mmol/L, which is in line with the knowledge that sympathetic nerves are of relevance for hypoglycemia only in conditions of deep hypoglycemia (30). The hypoglycemia responses of cortisol, PP, and norepinephrine were not significantly different between lixisenatide and placebo treatment, although there was a nonsignificant trend that norepinephrine levels at both 3.5 and 2.8 mmol/L glucose were lower after lixisenatide treatment than after placebo treatment. In contrast, the epinephrine response to reducing glucose concentration to 3.5 mmol/L was significantly reduced by lixisenatide treatment, whereas the epinephrine response to further reduction in glucose concentration to 2.8 mmol/L was not different between the treatments. A normal epinephrine response to deep hypoglycemia by lixisenatide treatment is supported by studies on GLP-1 (11), exenatide (12), and albiglutide (13), whereas the finding that lixisenatide reduces epinephrine levels at glucose levels of 3.5 mmol/L contrasts with the normal response at this level by exenatide (12) and albiglutide (13), and is in contrast with an increase in epinephrine levels by exenatide during exercise (32). Again, whether this characteristic is due to differences between the GLP-1 receptor agonists, differences in clamp technique, or differences between healthy subjects and subjects with type 2 diabetes treated with oral agents or basal insulin remains to be studied.

A strength of the study is the crossover design, with each subject serving as his/her own matched control, whereas a limitation of the study is that it was only a 6-week study. Therefore, glucagon counterregulation to hypoglycemia after long-term treatment with the combination of lixisenatide and basal insulin remains to be established. Another limitation of the study is that the hormonal responses to hypoglycemia were determined after 30-min persistent and stable hypoglycemia, which is different from the clinical situation when there is an actual increase in circulating glucose levels by the counterregulation. However, the clamp technique is superior to quantify the counterregulation since the glucose level is stable (33) and therefore suited for the aim of this study to explore the glucagon counterregulation.

In summary, we conclude that in insulin-treated patients with type 2 diabetes at a glucose level of 3.5 mmol/L, lixisenatide reduces glucagon and epinephrine levels compared with placebo, whereas the glucagon and epinephrine counterregulation to deep hypoglycemia at 2.8 mmol/L is sustained.

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Sanofi had no role in the design or conduct of the study; in the collection, management, analysis, or interpretation of the data; in the preparation or review of approval of the article; or in the decision to submit the article for publication.

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