Effect of Intravenous Infusion of Exenatide (Synthetic Exendin-4) on Glucose-Dependent Insulin Secretion and Counterregulation During Hypoglycemia

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This study assessed whether glucose-dependent insulin secretion and overall counterregulatory response are preserved during hypoglycemia in the presence of exenatide. Twelve healthy fasted volunteers were randomized in a triple-blind crossover study to receive either intravenous exenatide (0.066 pmol · kg^{-1} · min^{-1}) or placebo during a 270-min stepwise hyperinsulinemic-hypoglycemic clamp (insulin infusion 0.8 mU · kg^{-1} · min^{-1}). Plasma glucose was clamped sequentially at 5.0 (0–120 min), 4.0 (120–180 min), 3.2 (180–240 min), and 2.7 mmol/l (240–270 min). At 270 min, insulin infusion was terminated and plasma glucose increased to ~3.2 mmol/l. The time to achieve plasma glucose ≥4 mmol/l thereafter was recorded. Insulin secretory rates (ISRs) and counterregulatory hormones were measured throughout. Glucose profiles were superimposable between the exenatide and placebo arms. In the presence of euglycemic hyperinsulinemia, ISRs in the exenatide arm were ~3.5-fold higher than in the placebo arm (353 ± 29 vs. 100 ± 29 pmol/min [least-square means ± SE]). However, ISRs declined similarly and rapidly at all hypoglycemic steps (≤4 mmol/l) in both groups. Glucagon was suppressed in the exenatide arm during euglycemia and higher than placebo during hypoglycemia. Plasma glucose recovery time was equivalent for both treatments. The areas under the concentration-time curve from 270 to 360 min for cortisol, epinephrine, norepinephrine, and growth hormone were similar between treatment arms. There were no differences in adverse events. In the presence of exenatide, there was a preserved, glucose-dependent insulin secretory response and counterregulatory response during hypoglycemia. *Diabetes* 53:2397–2403, 2004

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O.S. holds stock in Amylin.

FFA, free fatty acid; GLP, glucagon-like peptide; ISR, insulin secretory rate.

The pathogenesis of type 2 diabetes is characterized by peripheral insulin resistance and progressive failure of pancreatic β-cell function, ultimately resulting in deficient insulin secretion. Furthermore, an excessive glucagon secretion and an impaired incretin response to meals contribute to the metabolic derangement of the disease (1–4). Control of circulating glucose levels is rarely optimal, and many currently available therapies also have unfavorable side effects and restrictions, limiting the extent of their use (5–8). This emphasizes the need for novel antidiabetic agents.

Glucagon-like peptide (GLP)-1 is an incretin hormone secreted from the intestinal mucosa in response to meal ingestion. Physiological GLP-1 exhibits several glucoregulatory functions, such as glucose-dependent enhancement of insulin secretion, suppression of glucagon secretion, delayed gastric emptying, and reduction of food intake. It may even promote β-cell preservation and improved neogenesis (9). GLP-1 has an extremely short half-life in plasma.

Exenatide (synthetic exendin-4) is a 39–amino acid peptide incretin mimetic that demonstrates the above glucoregulatory actions of GLP-1 (10,11), and it has been shown to be a potent agonist to the GLP-1 receptor in vitro (12). Clinical studies in humans have demonstrated markedly improved glycemic control after exenatide was injected twice daily (13–15). Most notably, a reduction in HbA_{1c} of 1.1% was observed after only 28 days on the breakfast and dinner exenatide regimen (14). More recent data report a sustained significant reduction in HbA_{1c} after 6 months of exenatide treatment in subjects with type 2 diabetes (16). In the 28-day study, the most common adverse event observed with exenatide treatment was transient mild-to-moderate nausea (14). However, stimulation of insulin secretion, albeit glucose dependent, and concomitant suppression of glucagon by exenatide could hypothetically increase the risk of hypoglycemia. Consequently, the present study was undertaken to examine whether glucose-dependent insulin secretion and the overall counterregulatory response are preserved during hypoglycemia in the presence of exenatide.

RESEARCH DESIGN AND METHODS

The protocol was approved by the ethics committee of the County of Aarhus, Denmark, and all subjects provided written informed consent. This study was

conducted in accordance with the recommendations provided in the Declaration of Helsinki (1964), including all amendments up to and including the South Africa revision (1996).

Twelve healthy, Caucasian, male volunteers with no personal or immediate family history of diabetes were enrolled. Subjects were 27.5 ± 2.4 years of age (range 24–32) with a BMI of 23.3 ± 2.1 kg/m^2 (range 21.2–29.0) (means ± SD). No prior medications were reported. One subject in the placebo arm was withdrawn from the study due to moderate and transient elevation of liver enzymes secondary to mononucleosis.

This was a single-center, randomized, triple-blind, placebo-controlled, crossover study designed to assess the effects of a continuous intravenous infusion of exenatide on the counterregulatory response to hypoglycemia and the recovery of plasma glucose levels from hypoglycemia upon termination of the glucose clamp. It was also designed to assess the effects of exenatide on insulin secretion during euglycemia and various levels of hypoglycemia. Following an overnight (10-h) fast with water as desired, subjects received either intravenous exenatide (0.066 pmol · kg^{-1} · min^{-1}, 0.4 μg · kg^{-1} · day^{-1}) or placebo during a stepwise hypoglycemic clamp (0–270 min) and a subsequent recovery period (270–360 min). Subjects received a fixed-rate infusion of insulin (0.8 mU · kg^{-1} · min^{-1}), with glucose (20%) infused to maintain glycemic plateaus of 5 mmol/l (0–120 min), 4 mmol/l (120–180 min), and 3.2 mmol/l (180–240 min). Eventually, plasma glucose was clamped to a nadir of 2.7 mmol/l and then released at 270 min when insulin infusion was terminated. Glucose infusion was then adjusted to establish plasma glucose concentrations of ~3.2 mmol/l, at which point glucose infusion was terminated. The design has been described earlier in detail (17,18). Subsequently, the time to achieve ≥4 mmol/l plasma glucose was recorded. Circulating levels of insulin, C-peptide, free fatty acids (FFAs), and counterregulatory hormones were measured throughout the study. Visits were structured as two 3-day inpatient stays (days −1, 1, and 2) with a 4- to 6-week washout interval between crossover periods. Adverse events were recorded throughout the study.

Assays. Glucose, insulin, C-peptide, glucagon, epinephrine, norepinephrine, growth hormone, cortisol, and FFA were assayed by the Medical Research Laboratory, Aarhus University Hospital (Aarhus, Denmark). Plasma glucose was measured immediately after sampling on a Beckman glucose analyzer (Beckman, Palo Alto, CA) using the glucose oxidase technique. All other blood samples were stored at −20°C (C-peptide at −80°C) until assay. Serum insulin was quantified using a highly specific and sensitive two-site enzyme-linked immunosorbent assay with an intra-assay coefficient of variation of 2.1% (Dako Diagnostics, Cambridgeshire, U.K.). Serum C-peptide was measured by a two-site monoclonal-based enzyme-linked immunosorbent assay (Dako Diagnostics). This assay has an intra-assay coefficient of variation of 3.3% at the low end of the assay range and 5.7% at the high end of the range. Samples for glucagon were collected in tubes with trasylol/EDTA solution in an ice bath and centrifuged immediately thereafter. Quantitation was performed by radioimmunoassay (Linco Research, St. Charles, MO). The lower detection limit of this assay is 0.1 ng/l, and the sample is diluted if the concentration value exceeds 650 ng/l. The intra-assay coefficient of variation is 4–6.8%. The assay has no detectable cross-reactivity. Serum FFA was determined by a colorimetric method using a commercial kit (Wako Chemicals, Neuss, Germany). Serum growth hormone was analyzed with a two-site monoclonal immunofluorometric assay (Delfia, Wallac Oy, Finland). Serum cortisol was measured using a solid-phase time-resolved fluoroimmunoassay (Delfia), and, finally, plasma epinephrine and norepinephrine were determined by electrochemical detection after high-pressure liquid chromatography (19).

Plasma exenatide concentrations were measured by Amylin Pharmaceuticals (San Diego, CA) using an immunoenzymetric assay. Briefly, microtiter plates were coated with an anti-exenatide monoclonal antibody (Amylin Pharmaceuticals) at 4°C for 1–3 days. Nonspecific binding sites were blocked using 1% nonfat dried milk diluted in 0.05 mol/l carbonate buffer (pH 9.5). Samples were added to wells and incubated 1–2 h at room temperature. A biotinylated secondary monoclonal antibody (Amylin Pharmaceuticals) preincubated with streptavidin-alkaline phosphatase was added to each well and incubated at room temperature for 1–2 h. Wells were washed with Tris-buffered saline/Tween (0.05 mol/l Tris, 0.15 mol/l sodium chloride, 0.02% sodium azide, and 0.1% Tween-20) between incubation steps. The assay was developed using 50 μl of 0.1 mg/ml 4-methylumbelliferyl phosphate solution. The reaction was stopped using 0.1 mol/l sodium phosphate/1.5 mol/l sodium chloride. The fluorescent signal was measured using a microplate fluorometer (Dynatech, Chantilly, VA) and analyzed with Multicalc software version 2.0 (Wallac, Gaithersburg, MD) using the spline smoothed-fit option.

Calculations and statistics. Subjects were included in the evaluable population if they completed the infusion procedures on day 1 of both study periods. The area under the concentration time curve was computed using the linear trapezoidal method. The time-weighted mean concentration over the sampling period, C_{ave}, was calculated as area under the concentration time curve divided by the sampling period. C_{max} and C_{min} were defined as the highest and lowest observed concentrations, respectively. T_{max} was defined as the time to peak concentration. During hyperinsulinemic euglycemia, insulin sensitivity was calculated as the mean glucose infusion rate divided by the mean serum insulin concentration in the 90- to 120-min time interval. The exenatide treatment effects on mean insulin secretory rates (ISRs) during each glycemic step and on insulin sensitivity and plasma glucagon during euglycemia were examined by a mixed-effect model that included treatment (two levels), treatment sequence (two levels), and period (two levels) as fixed effects and subject-within-sequence as a random effect. The 95% CIs were derived from the least-square means and the SE means for each treatment. For all other biological parameters, mean values for each glycemic step were $\log_e$ transformed and then analyzed in the same manner as for ISR. SAS/STAT version 8.2 and WinNonlin version 3.1 software programs were used for statistical analyses. Data are reported as means ± SE unless otherwise noted.

ISRs were estimated by mathematical analysis of plasma C-peptide concentrations using a two-compartment model according to published methods (20). A noncommercial software written in the Java platform was supplied by a private source. GraphPad Prism version 4.0 for Windows (GraphPad Software, San Diego, CA) was used to find the best-fit curve for the exenatide pharmacokinetic profile.

RESULTS

Exenatide pharmacokinetic profile. Plasma exenatide concentrations rapidly increased for the first 2 h of infusion (Fig. 1*A*). Targeted therapeutic exenatide concentrations (≥50 pg/ml) were achieved by 1 h after the initiation of infusion. A steady-state plateau phase was achieved by ~120 min, and concentrations remained constant (~130 pg/ml) for the rest of the observation period.

Glucose profile. Plasma glucose profiles were superimposable between the exenatide and placebo treatment arms (Fig. 1*B*), reaching identical mean nadirs (C_{min}) of 2.8 mmol/l during the 240- to 270-min hypoglycemic interval. During the subsequent recovery interval (270–360 min), equivalent amounts of glucose were infused in both treatment arms. The least-square mean values for total glucose infused during the recovery interval were 12,328 ± 2,586 mg for the exenatide arm and 10,192 ± 2,586 mg for the placebo arm (95% CI 6,477–18,178 mg). Glucose recovery time (i.e., the time to achieve a plasma glucose concentration ≥4 mmol/l) was similar in both treatment arms (41 ± 16 min for exenatide vs. 42 ± 15 min for placebo [means ± SD]).

Insulin, C-peptide, and ISRs. Serum insulin concentrations were significantly higher in the exenatide arm than in the placebo arm during the 90- to 120-min euglycemic step (Fig. 2*A*), as evidenced by a geometric 95% CI for the difference of 1.05–1.34, with least-square geometric mean values of 413 ± 22 pmol/l in the exenatide arm and 348 ± 18 pmol/l in the placebo arm. Serum insulin concentrations were comparable in both arms during the hyperinsulinemic-hypoglycemic clamp (120–270 min), as well as during the recovery phase (270–360 min).

As shown in Fig. 2*B*, serum C-peptide concentrations more than doubled during the euglycemic clamp (0–120 min) in the exenatide arm, peaking at 120 min. In the presence of exenatide, C-peptide concentrations declined rapidly when glucose concentrations fell below 5 mmol/l. In contrast, serum C-peptide decreased gradually in the placebo study during the hyperinsulinemic clamp (0–270 min).

In the presence of euglycemic hyperinsulinemia (90–120 min), ISRs in the exenatide arm were 3.5-fold higher than placebo (353 ± 29 vs. 100 ± 29 pmol/min [least-square

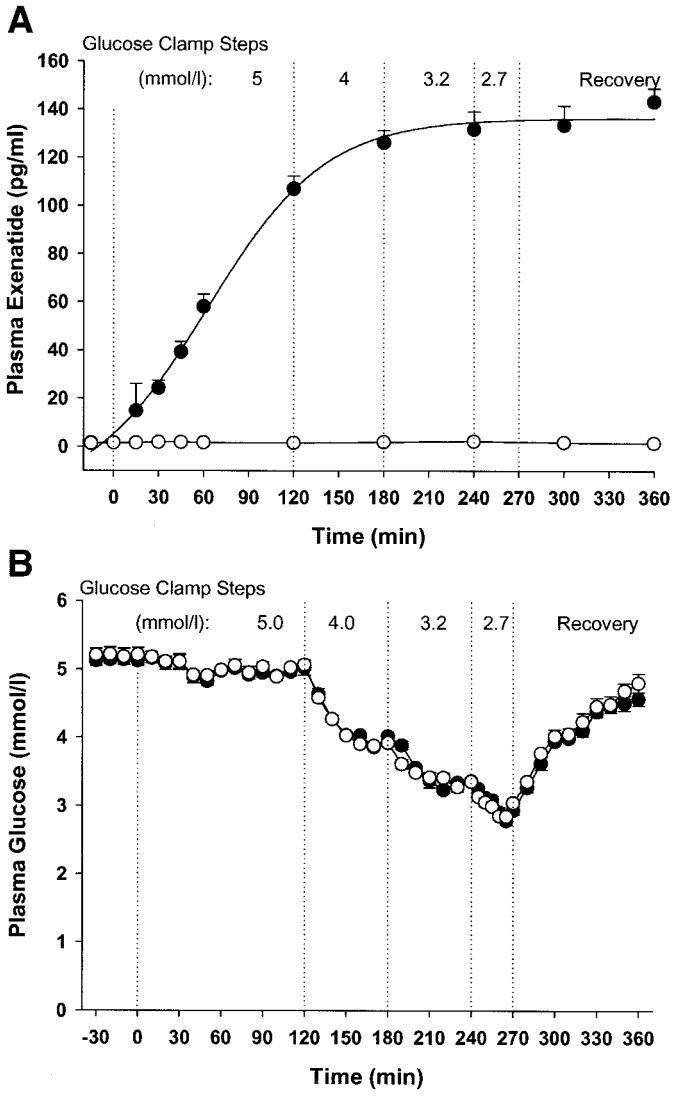

FIG. 1. ***A*: Exenatide pharmacokinetic profile. Exenatide pharmacokinetic curve fit to Boltzmann sigmoidal equation: Y = −21.5 + 157.8/{1+exp[(62.65 − X)/39.29]}; R^2 = 0.9965. *B*: Plasma glucose. Basal period: −30 to 0 min. Infusion of exenatide or placebo commenced at 0 min. Glycemic steps: 0–120 min, euglycemia with plasma glucose at ~5.0 mmol/l; 120–180 min, hypoglycemia with plasma glucose at ~4.0 mmol/l; 180–240 min, hypoglycemia with plasma glucose at ~3.2 mmol/l ending in nadir of ~2.8 mmol/l; 270–360 min, recovery phase. ○, placebo treatment arm; ●, exenatide treatment arm. Data are means ± SE; *n* = 11 per treatment arm.**

means ± SE] (Fig. 2*C*). During subsequent glycemic steps (plasma glucose ≤4 mmol/l), ISRs were similar in both groups and declined rapidly and progressively. Slight increases in ISRs were observed in both treatment arms during the recovery interval.

Counterregulatory hormones. During euglycemic hyperinsulinemia, plasma glucagon was reduced by ~50% in the exenatide arm (14.9 ± 7.3 ng/l) compared with the placebo arm (28.4 ± 14.0 ng/l) (Fig. 3). Plasma glucagon increased throughout the hypoglycemic steps in both treatment groups, with significantly lower glucagon concentrations with exenatide treatment at 4 mmol/l glucose (150–180 min: exenatide 26.9 ± 3.6 ng/l vs. placebo 39.1 ± 5.2 ng/l [geometric least-square means]; 95% CI ratios 0.53 and 0.89, respectively). With the progression of hypoglycemia, glucagon concentrations continued to increase, and significantly higher glucagon concentrations were observed in the exenatide treatment group than in the placebo group during the 240- to 270-min glycemic interval (exenatide 182.0 ± 29.0 ng/l vs. placebo 142.1 ± 22.6 ng/l; geometric 95% CI ratios 1.02 and 1.61, respectively) The geometric least-square mean C_{max} for plasma glucagon in the exenatide arm (212.1 ± 32.3 ng/l) was significantly higher compared with the placebo arm (165.7 ± 25.2 ng/l), as evidenced by geometric 95% CI ratios of 1.03 and 1.59. There was no difference between treatment arms during the recovery interval (270–360 min).

Serum cortisol concentrations rose progressively during the hypoglycemic steps in both treatment arms (Fig. 4*A*). There were transiently higher circulating cortisol levels in the exenatide arm during the transition from euglycemia to hypoglycemia (90–120 and 120–180 min). However, neither during the two final hypoglycemic steps (180–240 and 240–270 min) nor during the recovery interval did serum cortisol concentrations differ between the two treatment arms. There were no statistically significant differences in plasma epinephrine, norepinephrine, or serum growth hormone between treatment arms (Fig. 4*B*–*D*).

FFAs. There were no statistically significant differences in serum FFA concentrations between treatment arms. After the initiation of the hyperinsulinemic clamp, serum FFA levels were almost completely restrained, with a decline from the 0.3- to 0.4-mmol/l range to the 0.02- to 0.06-mmol/l range. However, during the recovery interval, serum FFAs in both arms showed an identical, pronounced, and rapid increase, eventually reaching the 0.8- to 1.0-mmol/l range by 360 min.

Insulin sensitivity. There was no statistically significant difference between insulin sensitivity during exenatide exposure (0.025 ± 0.0024 mg · kg^{-1} · min^{-1} per pmol/l) and placebo (0.027 ± 0.002 mg · kg^{-1} · min^{-1} per pmol/l) during the initial 120 min of infusion.

Adverse events. There were no serious or severe adverse events. There was one case of moderate nausea (8.3%) and two cases of mild-to-moderate headache (16.7%) in the placebo arm, and one case of mild nausea (8.3%) in the exenatide arm.

DISCUSSION

The primary objectives in the present study were to explore the glucose-dependent insulin secretion and to determine the counterregulatory hormonal response during hypoglycemia in humans acutely exposed to exenatide.

The infusion of exenatide at a constant rate resulted in predictable and reproducible plasma pharmacokinetics for exenatide that were characterized by achievement of steady state at ~120 min, with exenatide concentrations remaining within the targeted therapeutically relevant range for the remainder of the study.

During the hyperinsulinemic-euglycemic part of the study, a substantial increase in ISR (~3.5-fold) was observed during exenatide administration compared with the placebo arm, where a gradual decline was noted. Under these experimental conditions, exenatide offset the inhibitory effects of a pronounced hyperinsulinemia on the ability of β-cells to secrete insulin during normoglycemia. Very importantly, however, as soon as glycemia com-

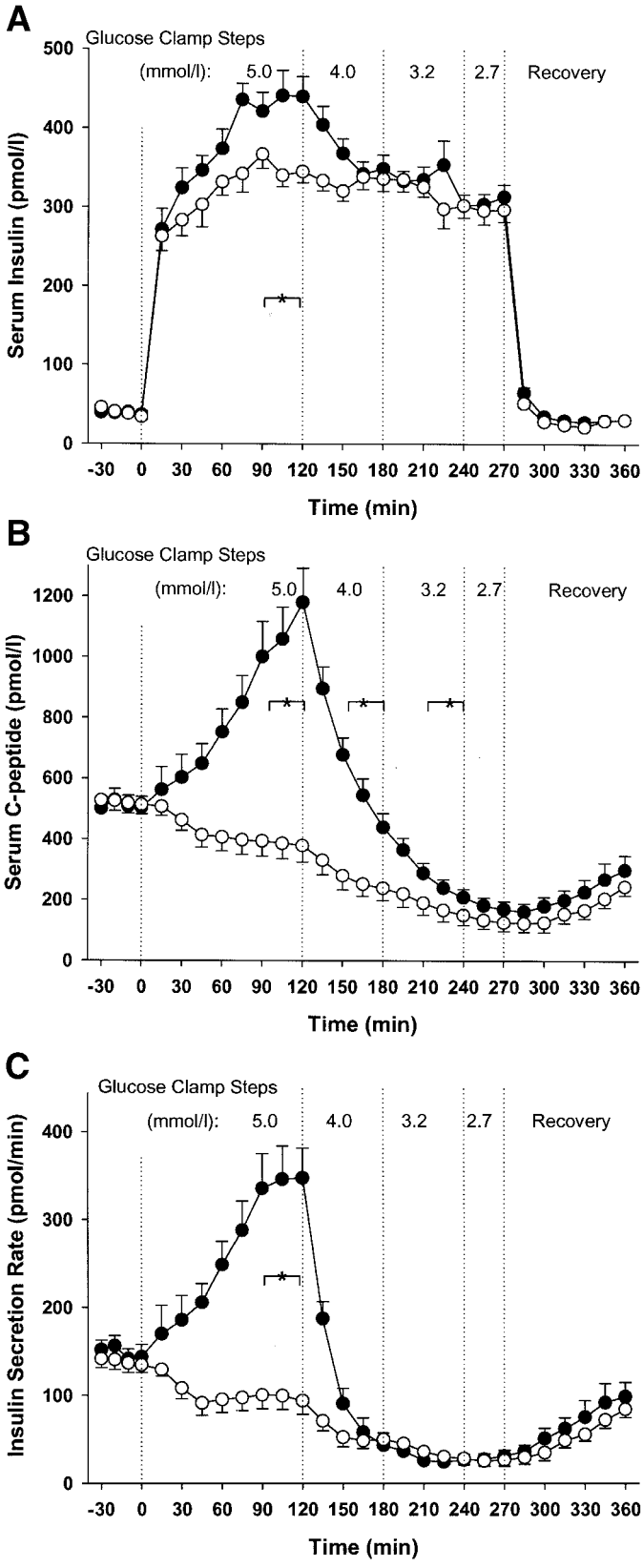

FIG. 2. Insulin secretory response. Basal period: −30 to 0 min. Infusion of exenatide or placebo commenced at 0 min. Glycemic steps: 0–120 min, euglycemia with plasma glucose at ~5.0 mmol/l; 120–180 min, hypoglycemia with plasma glucose at ~4.0 mmol/l; 180–240 min, hypoglycemia with plasma glucose at ~3.2 mmol/l ending in nadir of ~2.8 mmol/l; 270–360 min, recovery phase. ○, placebo treatment arm; ●, exenatide treatment arm. Data are means ± SE; $n = 11$ per treatment arm. *$P < 0.05$, exenatide vs. placebo during steady state of a glycemic interval.

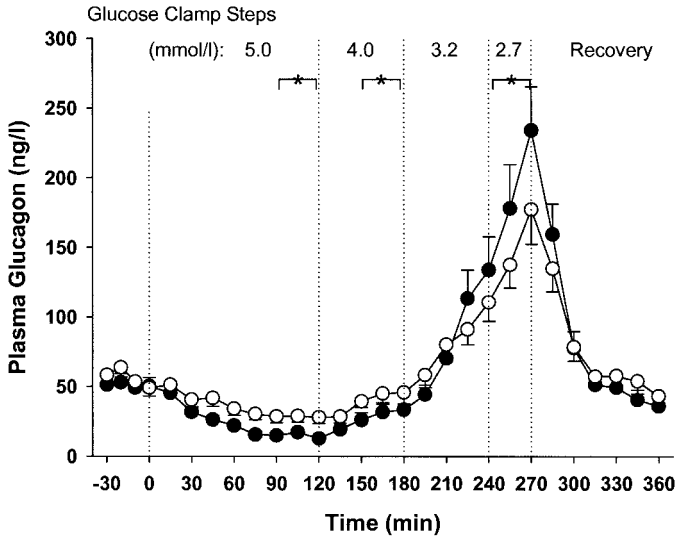

FIG. 3. Glucagon counterregulatory hormone response. Basal period: −30 to 0 min. Infusion of exenatide or placebo commenced at 0 min. Glycemic steps: 0–120 min, euglycemia with plasma glucose at ~5.0 mmol/l; 120–180 min, hypoglycemia with plasma glucose at ~4.0 mmol/l; 180–240 min, hypoglycemia with plasma glucose at ~3.2 mmol/l ending in nadir of ~2.8 mmol/l; 270–360 min, recovery phase. ○, placebo treatment arm; ●, exenatide treatment arm. Data are means ± SE; $n = 11$ per treatment arm. *$P < 0.05$, exenatide vs. placebo during steady state of a glycemic interval.

menced falling below 5 mmol/l, the ISR was markedly reduced and similar to ISR in the placebo arm, demonstrating the glucose dependence of the insulinotropic action of exenatide in the lower euglycemic-hypoglycemic range. Whether this abrupt suppression of exenatide-stimulated insulin secretion is attributable to a certain set point and/or to dynamics in glycemia cannot be assessed using this experimental design. It should be noted that our data on the effect of exenatide on ISR during hypoglycemia are similar to recent findings by Nauck et al. (21) investigating the effect of GLP-1 on ISR during hypoglycemia.

The counterregulatory hormonal response during exenatide administration is of considerable interest. During hyperinsulinemic euglycemia, plasma glucagon was, as expected, suppressed to a larger extent in the exenatide arm than in the placebo arm, due to the actions of exenatide on α-cell function during euglycemia. During marked hypoglycemia, glucagon secretion increased considerably in both treatment arms, with the peak concentration ~30% higher during exenatide administration. The mechanisms behind this are not clear. It should be emphasized that the plasma glucose concentrations during the two treatment arms were superimposible, with identical plasma glucose nadirs. It could be argued that the exenatide arm was exposed to greater insulinemia (0–120 min) than the placebo arm. However, convincing data have demonstrated that different levels of hyperinsulinemia before hypoglycemia do not alter the glucagon response (22). It is therefore tempting to speculate whether the initial suppression of glucagon secretion during exenatide administration allows for more glucagon secretory granules readily releasable during pronounced hypoglycemia. Nevertheless, the observation gives some evidence for a glucose-dependent glucagon secretion during exenatide administration in the hypoglycemic condition that is able to override the glucagonostatic effect of exenatide. In the

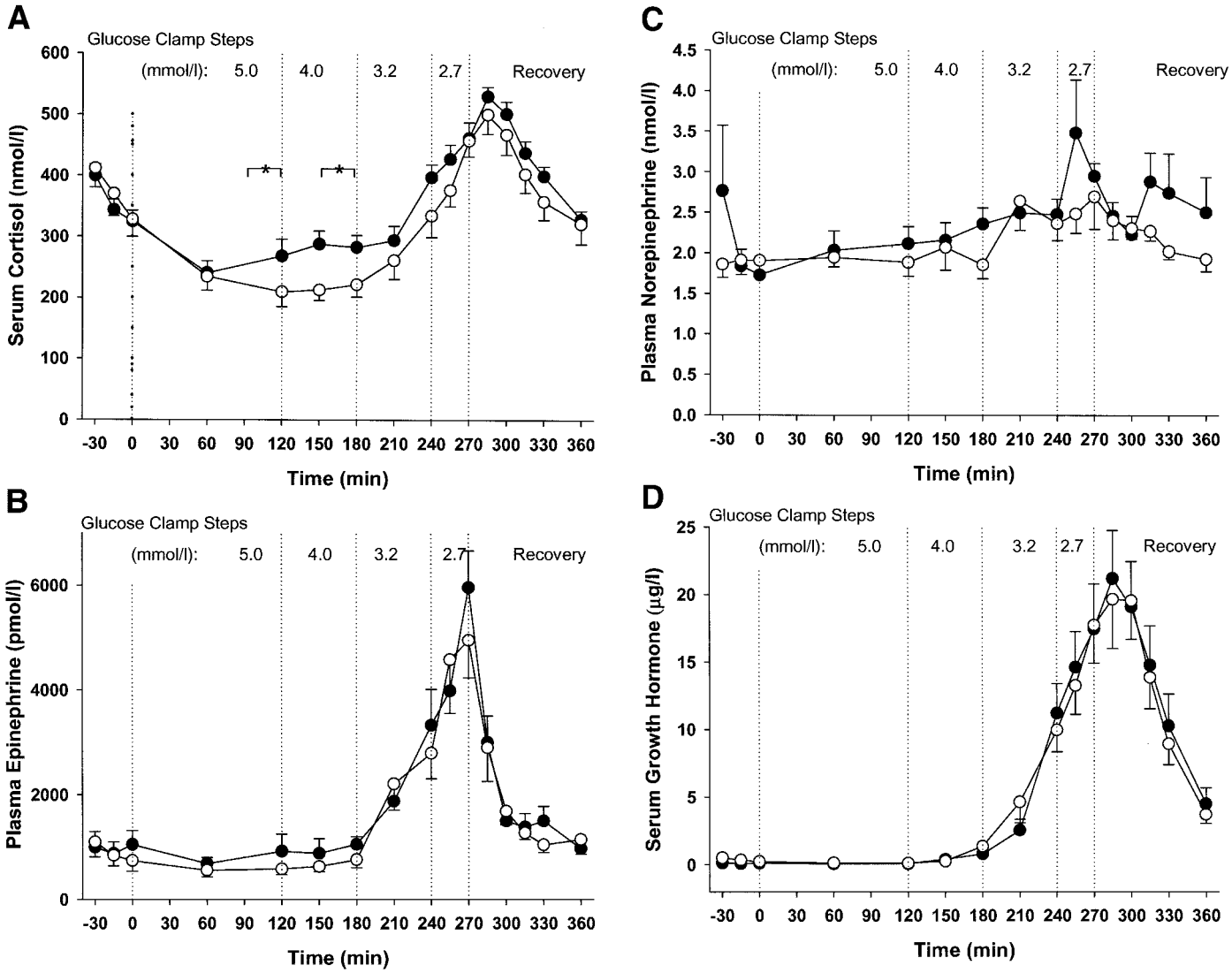

FIG. 4. Counterregulatory hormone response for cortisol (*A*), epinephrine (*B*), norepinephrine (*C*), and growth hormone (*D*). Basal period: −30 to 0 min. Infusion of exenatide or placebo commenced at 0 min. Glycemic steps: 0–120 min, euglycemia with plasma glucose at ~5.0 mmol/l; 120–180 min, hypoglycemia with plasma glucose at ~4.0 mmol/l; 180–240 min, hypoglycemia with plasma glucose at ~3.2 mmol/l ending in nadir of ~2.8 mmol/l; 270–360 min, recovery phase. ○, placebo treatment arm; ●, exenatide treatment arm. Data are means ± SE; $n = 11$ per treatment arm. *$P < 0.05$, exenatide vs. placebo during steady state of a glycemic interval.

aforementioned study by Nauck et al. (21), which had a comparable design, an initial reduction in plasma glucagon was observed in the GLP-1 arm during hyperinsulinemic euglycemia that was similar to our data. During marked hypoglycemia, however, glucagon secretion was not enhanced during GLP-1 administration compared with placebo. It is unlikely that the small variations in the experimental designs would explain the discrepancy in hypoglycemia-induced glucagon secretion during GLP-1 and exenatide administrations, though dosing of the two compounds may be difficult to compare. Thus, the altered glucagon may be ascribable to differences between the two peptides in their action on the α-cells and/or the somatostatin-secreting δ-cells.

The other counterregulatory hormones did not differ between exenatide and placebo during hypoglycemia. In particular, serum growth hormone concentrations were similar in the exenatide arm and the placebo arm, both with an average maximum at 285 min. Again, this is in contrast to the findings with GLP-1 of Nauck et al. (21), who demonstrated a diminished growth hormone response during acute GLP-1 infusion under hypoglycemic conditions. The same group has confirmed this attenuated growth hormone release pattern using a GLP-1 derivative in a preliminary publication (23). Whether this could play a significant role during long-standing hypoglycemia is not clear. The presence of GLP-1 receptors on somatotropic cells is a possibility, as previous reports have demonstrated that GLP-1 is able to modify hypothalamic and other pituitary functions (24,25). One may hypothesize differential binding to receptors between GLP-1 and exenatide or the presence of polymorphic receptors. Mechanisms operating via ghrelin are another possible scenario. An inverse relationship between ghrelin and GLP-1 has been recently proposed (26). In the current study, however, no difference in circulating ghrelin was observed between the exenatide and placebo arms (data not shown).

Finally, a small increase in serum cortisol was present during euglycemia in the exenatide compared with the placebo arms. This is consistent with previous observations during acute GLP-1 and exenatide administration, and the mechanisms are still uncertain (27,28).

The increased insulin concentrations and decreased glucagon levels at euglycemia may theoretically result in a larger glycogen accumulation in the liver during exenatide infusion compared with placebo. In light of this and of the augmented glucagon effect during profound hypoglycemia, one may have expected a shorter recovery time during exenatide treatment compared with placebo, but this was not observed. There may be several reasons: *1*) the sensitivity of this experimental design was not sufficient to detect small differences in the recovery time, *2*) the marked hyperinsulinemia was at a level that may have masked the opposing effects of differing levels of glucagon and potentially different amounts of releasable glycogen stores, and/or *3*) dynamics in portal glucagon concentrations are apparently more important than actual peripheral concentrations (29). Serum FFA concentrations were almost identical during both exenatide and placebo arms during the whole study. On balance, from the present study, it is difficult to speculate how the augmented glucagon response to hypoglycemia with exenatide may translate to patients with type 2 diabetes, keeping in mind that glucagon responses are commonly impaired in this patient population (30,31).

In summary, these data are consistent with a preserved glucose-dependent insulinotropic action during short-term intravenous infusion of exenatide during hypoglycemia. In addition, the overall counterregulatory response was unaltered. However, circulating plasma glucagon was augmented in the presence of pronounced hypoglycemia during exenatide administration. Our results obtained in healthy individuals may not necessarily apply to type 2 diabetic patients. Thus, studies in individuals with type 2 diabetes treated with exenatide for a longer period are needed to determine the clinical relevance of our observations.

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