Mechanism of action of exenatide to reduce postprandial hyperglycemia in type 2 diabetes

Antonio Cervera, Estela Wajchberg, Apiradee Sriwijitkamol, Marianna Fernandez, Pengou Zuo, Curtis Triplitt, Nicolas Musi, Ralph A. DeFronzo, and Eugenio Cersosimo

Division of Diabetes, Department of Medicine, University of Texas Health Science Center at San Antonio, San Antonio, Texas

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Exenatide has comparable potency to GLP-1 (11) and is resistant to degradation by DPP-IV (12). Following a single subcutaneous injection, the peak plasma exenatide concentration is reached within 2 h, and the incretin can be measured in plasma for up to 10 h (12, 30). Exenatide improves glycemic control primarily by reducing postprandial hyperglycemia, with a modest effect to decrease the fasting plasma glucose concentration (24). However, the physiological mechanisms responsible for reducing postprandial hyperglycemia have yet to be elucidated and quantitated in humans. In the present study, we investigated the mechanisms (suppression of EGP, increased splanchnic glucose uptake, delayed gastric emptying, increased peripheral tissue glucose disposal) by which exenatide attenuates postprandial hyperglycemia in T2DM. The effect of exenatide on postprandial plasma triglyceride concentration, a risk factor for cardiovascular disease, also was evaluated.

SUBJECTS AND EXPERIMENTAL DESIGN

Subjects. Twelve T2DM subjects (9 males/3 females, age 44 ± 2 yr, BMI 34.1 ± 4.0 kg/m², Hb A1c 7.5 ± 1.5%, diabetes duration 6.6 ± 3.5 yr, negative GAD antibodies, and no diabetic complications) participated in three mixed-meal tolerance tests (MMTT). All subjects were in good general health as determined by medical history, physical examination, screening blood tests, urinalysis, and electrocardiogram. Diabetes was treated with metformin alone (n = 5), sulfonylurea (SU) alone (n = 6), or both (n = 1). Since results of all measured parameters were similar in SU-treated and metformin-treated subjects, data from all subjects have been combined for presentation. No subject was taking any other medication known to affect glucose or lipid metabolism. Body weight was stable (±3 lbs) in all participants for 6 mo prior to study. No subject participated in a heavy exercise program. All studies were carried at the Clinical Research Center (CRC) of the University of Texas Health Science Center at San Antonio (UTHSCSA). The study was approved by the Institutional Review Board of the UTHSCSA, and informed written consent was obtained from each patient before participation.

Study design. At screening, all subjects received dietary advice and remained on a weight-maintaining diet (50% carbohydrate, 30% protein, 20% fat) until study end. Patients continued to take their regular antidiabetic medication throughout the study, but not on the morning of MMTT.

MMTT with double tracer. All subjects participated in three 6-h MMTT performed 2–4 wk apart with iv intravenous saline infusion
during the meal [control (CON) study]; 2) intravenous exenatide (EXE; 0.05 µg/min) started 15 min before the meal and decreased to 0.025 µg/min 45 min after meal ingestion; and 3) intravenous exenatide plus intravenous glucagon (E+G) at a variable rate estimated to match the plasma glucagon level during saline control study in each subject. Studies 2 and 3 were performed in random order after study 1. Because of the difficulty in measuring portal insulin concentration, an intravenous insulin replacement study was not performed. For each study, subjects were admitted to the CRC at Texas Diabetes Institute at 0700 in the morning after a 10-h overnight fast. A catheter was inserted into an antecubital vein, and a primed (25 µCi × FPG/100) continuous (0.25 µCi/min) infusion of [3-3H]glucose was started 180 min prior to MMTT and maintained for the entire study duration (9 h). A second catheter was placed retrograde in a vein on the dorsum of the hand, which was placed in a heated box (70°C) for blood withdrawal. At the end of the isotope equilibration period (t = 0 min), a premeal urine sample was obtained. Blood samples were drawn at −30, −20, −10, −5, and 0 min for measurement of fasting plasma insulin, glucagon, triglyceride, acetalaminophen, and glucose concentration and glucose-specific activities. A saline infusion was begun at t = 0 min, and subjects ingested a standardized meal containing 75 g of oral glucose (Trutol), 25 g of fat, and 20 g of protein. The meal was prepared at the kitchen of the Texas Diabetes Institute under direct supervision of a dietitian. The solid component of the meal was made with eggs, cheese, margarine, and bread up to a total of 600 kcal. The oral glucose load was mixed with [1-14C]glucose, and acetalaminophen elixir 30 ml (1,000 mg) was added. Patients consumed the meal over 10–15 min, and plasma samples for determination of plasma glucose, insulin, glucagon, C-peptide, triglycerides, exendin-4, and acetalaminophen concentrations and [1-14C]- and [3H]glucose-specific activities were collected every 15–30 min until study end (t = 360 min). Plasma exendin-4 was measured for an additional 60 min (t = 420 min). Upon study termination, urine was collected, and volume and glucose concentration were measured.

Within 10–14 days, subjects returned for a repeat MMTT with intravenous EXE, which was started at t = −15 min at 0.05 µg/min for 45 min and then decreased to 0.025 µg/min until study end. These infusion rates were chosen to raise plasma exendin4 concentration to ~90–100 pg/ml, which is approximately one-half of the peak exendin4 concentration observed following a 5-µg subcutaneous injection (15). Within 10–14 days after completion of this study, subjects returned for a third study, during which exendin4 was infused as above with variable glucagon infusion (E+G) designed to match the plasma glucagon levels attained during the saline control study. Blood and urine samples were collected as described in study one.

Analytic determinations. Plasma glucose concentration was determined by the glucose oxidase method with a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA). Plasma insulin, C-peptide, and glucagon concentrations were determined by radioimmunooassay (Diagnetics Products, Los Angeles, CA). Plasma triglyceride concentration was measured by chromatography (Liposcience, Durham, NC), plasma acetalaminophen by a liquid chromatographic spectrometric assay (PPD Laboratories, Middleton, WI) and plasma exendin-4 by ELISA (Linco Research, St. Charles, MO). Plasma [3H]glucose and [14C]glucose radioactivity was determined on barium hydroxide-zinc sulfate-precipitated plasma extracts as previously described (8).

Calculations. Following an overnight fast, steady-state conditions prevailed, and rate of endogenous glucose appearance (Ra) equaled the rate of glucose disappearance (Rd) and was calculated as the ratio of the [3-3H]glucose infusion rate (dpm/min) ÷ steady-state plasma [3-3H]glucose specific activity (dpm/mg). After glucose ingestion, nonsteady conditions prevail and the total glucose Ra and Rd were computed from [3-3H]glucose data using a two-compartment model for the glucose system. Calculations were based on a total glucose distribution volume of 250 ml/kg, an initial glucose distribution volume of 65 ml/kg, and a clearance rate of glucose from the first compartment of 29 ml·kg−1·min−1. Urinary glucose loss (0–360 min) was subtracted from the total glucose Rd to obtain the rate of tissue glucose disposal following the mixed meal. The [1-14C]glucose data were used to calculate the Rd of oral glucose (RaO) as follows. The plasma [1-14C]glucose radioactivity was divided by the specific activity of the glucose drink to calculate the plasma “oral glucose concentration” that would be attained in the systemic circulation if the sole source of glucose were the oral load. The calculated “oral” glucose concentration and the [3-3H]glucose radioactivity then were used to compute the RdO in peripheral plasma. EGP was obtained as the difference between total Rd (RdT) and RdO (8).

Statistics. Results obtained for the glucose kinetic parameters and plasma hormone/substrate concentrations in each of the three MMTTs (CON vs. EXE vs. E+G) were compared using ANOVA with repeated measures. Statistically significant differences were confirmed by the Bonferroni test. Statistical significance was set at P < 0.05.

RESULTS

Fasting plasma glucose concentrations (122–146 mg/dl) were comparable in all three studies (Fig. 1A). Following the MMTT (CON), plasma glucose rose to a mean of 196 ± 9 mg/dl. During MMTT with EXE, the elevation in mean plasma glucose (127 ± 8 mg/dl) was markedly attenuated. During MMTT with E+G, the rise in mean plasma glucose (152 ± 7 mg/dl) was intermediate between CON and EXE studies (P < 0.001 for CON vs. EXE for and EXE vs. E+G). Plasma insulin concentration (Fig. 1B) rose from 52 ± 7 to a mean of 99 ± 12 pmol/l during saline infusion, and greater increases were observed with EXE (41 ± 6 to 179 ± 20) and E+G (45 ± 7 to 190 ± 17) (both P < 0.01 vs. CON). Fasting plasma C-peptide concentration increased from 3.5 ± 0.4 to a mean of 7.4 ± 0.8 pg/ml during MMTT with saline (CON) and rose fourfold with EXE (2.6 ± 0.7 to 12.4 ± 0.9) and E+G (4.4 ± 0.2 to 16.9 ± 0.8 pg/ml) (both P < 0.001).

During the CON study, there was an initial (0–120 min) paradoxical increase in plasma glucagon (Fig. 1C), followed by a ~10% decrease during the last 4 h. Consequently, mean plasma glucagon (0–360 min) remained unchanged (84 ± 5 vs. 79 ± 6 pg/ml). Exenatide was accompanied by an ~20% decrease in mean plasma glucagon (85 ± 5 to 67 ± 7 pg/ml, P < 0.05 vs. CON). In the E+G study, plasma glucagon (81 ± 4 vs. 74 ± 4 pg/ml, P > 0.20) was not significantly different from CON. During the CON study, fasting plasma triglyceride (136 ± 26 mg/dl) increased to a mean of 152 ± 28 after meal ingestion. During EXE (185 ± 25 to 180 ± 24 mg/dl) and E+G (169 ± 25 to 153 ± 18), plasma triglyceride concentration declined significantly compared with CON (both P < 0.05; Fig. 1D).

Plasma exendin-4 levels were undetectable during the CON study. During EXE, with or without glucagon, an initial plateau in plasma exenatide (75 ± 8 pg/ml) was reached ~15 min after the meal was consumed. This plateau closely approximated plasma exendin-4 levels in patients receiving a subcutaneous exenatide injection of 5 µg given 30 min before a meal (15). At study end, plasma exendin-4 levels had increased slightly to a new plateau between 90–100 pg/ml.

Glucose kinetics. In the CON study, the glucose RaT increased from 255 ± 30 mg/min at baseline to a mean of 379 ± 30 over the 360-min period after mixed-meal ingestion (Fig. 2A). RaT of glucose was completely attenuated during EXE infusion (231 ± 17 vs. 212 ± 6 mg/min) and markedly reduced by E+G (247 ± 23 to 271 ± 13 mg/min) (both P < 0.01 vs. CON).
and P < 0.05 EXE vs. E+G and vs. CON). The RaO (Fig. 2B) during EXE and E+G were equally reduced by ~50% over the 6-h postmeal period. RaO (0–360 min) in CON (129 ± 19 mg/min) was reduced to 59 ± 7 and 63 ± 9 mg/min in the EXE and E+G groups, respectively (both P < 0.01 vs. CON). The endogenous glucose Rg (Fig. 2C) remained unchanged (255 ± 30 vs. 249 ± 19 mg/min) during the CON study and was suppressed by ~40% with EXE (231 ± 11 to 142 ± 12 mg/min) and by ~20% during E+G (248 ± 15 to 209 ± 12 mg/min) (both P < 0.01 control, P < 0.01 EXE vs. E+G).

During the 6-h period following meal ingestion, ~36 g of the 75-g oral glucose load were retained in the splanchnic area and ~15 g appeared in the systemic circulation (P = NS vs. EXE, P < 0.01 vs. CON).

During the 6-h period following the mixed meal (Fig. 3), there was a 58% decrease in mean plasma acetaminophen concentration in EXE vs. CON (840 ± 135 vs. 1,995 ± 270 μg/ml, P < 0.001), indicating a significant inhibition of gastric emptying (22). Thus, of the 16-g increment in glucose retained within the splanchnic area during EXE vs. CON, 9 g (0.58 ± 16 g) could be accounted for by glucose retained within the stomach, i.e., delayed gastric emptying.

The glucose Rd (mean from 0–360 min) was 2.4 ± 0.3, 2.3 ± 0.2, and 2.3 ± 0.1 mg·kg⁻¹·min⁻¹ in the CON, EXE, and E+G groups, respectively. Urine glucose excretion during CON, EXE, and E+G was 0.20 ± 0.04, 0.06 ± 0.02, and 0.08 ± 0.04 mg·kg⁻¹·min⁻¹, respectively. To account for differences in plasma glucose concentration during the three studies, we calculated the tissue glucose metabolic clearance rate (MCR) [(Total Rd – urine glucose excretion) ÷ mean plasma glucose conc.]. Baseline tissue glucose MCR declined slightly in CON (1.68 ± 0.09 to 1.53 ± 0.08 mg·kg⁻¹·min⁻¹,
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P = NS) and increased slightly but not significantly in EXE (1.74 ± 0.13 to 1.87 ± 0.14 mg·kg⁻¹·min⁻¹) and E+G (1.77 ± 0.18 vs. 2.16 ± 0.38 mg·kg⁻¹·min⁻¹) studies. When tissue glucose MCR was expressed per increment in plasma insulin concentration, no significant differences between CON, EXE, and E+G (0.19 ± 0.03 to 0.09 ± 0.02, 0.25 ± 0.04 to 0.06 ± 0.01, 0.24 ± 0.03 to 0.07 ± 0.03 mg·kg⁻¹·min⁻¹ per μU/ml) were observed.

DISCUSSION

In the present study, an intravenous exenatide infusion, designed to approximate levels observed during a 5-μg exenatide subcutaneous injection, attenuated postprandial hyperglycemia in T2DM subjects by 1) suppressing endogenous (hepatic) glucose production and 2) decreasing the Ra of ingested glucose in the systemic circulation. Exenatide infusion was accompanied by an ~45% decrease in endogenous glucose release and by an ~50% reduction in the RaO in the systemic circulation over the 6-h postmeal period. Replacement of glucagon during exenatide infusion blunted by 56% (from 45 to 20%) the suppressive effect of exenatide on EGP during the 6-h MMTT. Thus, approximately one-third of the decline in plasma glucose concentration (Fig. 1A) following exenatide infusion during the mixed meal can be explained by the decline in EGP. Exenatide did not significantly alter the tissue MCR of glucose. Thus, the improvement in postprandial hyperglycemia cannot be explained by an increase in tissue sensitivity to insulin.

A reduction in the RaO in the systemic circulation also contributed to the decline in postprandial hyperglycemia following exenatide. The reduction in RaO reflects ingested glucose that is retained in the splanchnic area (liver plus gastrointestinal tissues) and glucose that is retained within the stomach (delayed gastric emptying). On the basis of the plasma acetaminophen levels, which closely reflect the liquid component of the meal (1), 58% of the glucose retained in the splanchnic area could be accounted for by slowing of gastric emptying. This is not unexpected, since previous experiments with GLP-1 analogs (10, 22) and with exenatide (1) have shown a close relationship between the plasma GLP-1 level and delayed gastric emptying. The plasma exendin-4 levels

Fig. 2. A: glucose kinetics during mixed-meal/dual-tracer study. In CON (■), total glucose rate of appearance (RaT) increased from 255 ± 30 (baseline) to a mean of 379 ± 30 mg/min over the 360-min period after the mixed meal. RaT was significantly attenuated during EXE (●; 231 ± 17 to 212 ± 6 mg/min) and during E+G (○; 247 ± 23 to 271 ± 13 mg/min), *P < 0.01 vs. CON, **P < 0.05 EXE vs. E+G. B: RaO of oral glucose (RaO). RaO during exenatide infusion with or without glucagon replacement was similarly reduced by ~50% over the 6-h postmeal period. Mean RaO was 109 ± 9 mg/min in CON, 64 ± 8 mg/min in EXE, and 43 ± 5 in E+G. *P < 0.001 vs. saline. C: RaE of endogenous glucose (RaE). RaE during the 6-h period of mixed-meal tolerance test (MMTT) did not change (255 ± 30 to 249 ± 19 mg/min) during CON and was suppressed by ~40% in EXE (231 ± 11 to 142 ± 12 mg/min). Glucagon infusion with exenatide reduced the decline in RaE by ~20% compared with exenatide infusion alone (248 ± 15 to 209 ± 12 mg/min) *P < 0.01 vs. CON, and **P < 0.05 vs. CON.

Fig. 3. Mean plasma acetaminophen concentration during the 8-h period following mixed meal ingestion during CON (■) and EXE (●) studies. There was a 58% decrease in mean plasma acetaminophen concentration during EXE (840 ± 135) vs. CON (1,995 ± 270) study. *P < 0.001.
achieved in the present study were between 75 and 100 pg/ml, which has been shown to significantly inhibit gastric emptying (1). If one assumes that the delay (58%) in gastric emptying (measured with acetaminophen) quantitatively reflects the amount of glucose retained within the stomach, then it can be estimated that, of the 16 g (EXE, 52 g – 36 g) and 24 g (E+G, 60 g – 36 g) of the ingested glucose load retained within the splanchnic tissues (liver + gastrointestinal), ~9 g is retained within the stomach and ~7 g is taken up by the splanchnic tissues during EXE, whereas ~14 g is retained within the stomach and ~10 g is taken up by the splanchnic tissues during E+G. It should be noted, however, that the marked delay in gastric emptying, i.e., retention of glucose within the stomach, would significantly underestimate any contribution of enhanced splanchnic (hepatic) glucose uptake to the reduction in postprandial hyperglycemia. Therefore, the possibility remains that GLP-1 could have an even greater effect to augment splanchnic (hepatic) glucose uptake following glucose ingestion (4, 5, 13).

During a mixed meal or oral glucose ingestion in healthy nondiabetic subjects, insulin secretion is stimulated and the resultant hyperinsulinemia inhibits endogenous (hepatic) glucose production (5). Consistent with previous publications (8, 9, 14, 22), we observed an initial paradoxical rise in EGP followed by a failure of suppression of EGP during the MMTT in T2DM subjects (Fig. 2). When exenatide was administered with the mixed meal, suppression of EGP was enhanced by 45%. The percent suppression of EGP in the saline control and exenatide studies was strongly and positively correlated (r = 0.48, P < 0.001) with the plasma insulin/glucagon ratio. When glucagon was replaced during exenatide infusion, suppression of EGP was blunted (20 vs. 45%; Fig. 2). Thus, approximately one-half (56%) of the improved suppression of EGP during exenatide infusion is attributable to inhibition of glucagon secretion. By subtraction, the other half (44%) of the enhanced suppression of EGP during exenatide infusion is explained by the stimulation of insulin secretion. Although the demonstration that “incretins” inhibit glucagon secretion is not novel (4, 28), the present investigation represents the first study that has provided quantitative information about the contributions of glucagon inhibition and insulin stimulation to the reduction in postprandial hyperglycemia following exenatide. These experiments demonstrate that even a modest inhibition of glucagon (~20% decrease in systemic plasma glucagon levels) is sufficient to account for approximately one-half of the postmeal suppression of hepatic glucose production. Furthermore, the decrease in plasma glucagon concentration and related suppression of EGP during exenatide infusion can account for about one-third of the decline in postprandial glucose levels (Fig. 1A). It should be mentioned that, because we did not have access to the portal circulation, our analysis is limited to tissues during EXE, whereas urinary glucose excretion was small in all three studies. The difference in the integrated plasma glucose AUC between the CON and exenatide studies was ~23 g over 6 h. Of this, 13 g can be explained by glucose retention within the splanchnic bed and ~4 g by enhanced suppression of EGP secondary to inhibition of glucagon secretion (i.e., differences in plasma glucose AUC between EXE and E+G studies). This leaves 6 g to be accounted for by enhanced suppression of EGP by insulin per se, independent of changes in glucagon. This agrees well with our earlier speculation about the impact of the increase in plasma insulin on the suppression of EGP and its contribution to the reduction in postprandial plasma glucose excursion. In the present study, exenatide infusion with and without glucagon was accompanied by a reduction in postmeal hypertriglyceridemia, as opposed to the rise in plasma triglyceride levels observed in the CON study (Fig. 1D). This is in agreement with previous results that demonstrated that intravenous GLP-1 administration abolished the postprandial rise in plasma triglyceride levels (22). Although the present study was not designed to examine the mechanism(s) responsible for the attenuated rise in plasma triglyceride levels, delayed gastric emptying, which retards the entry of nutrients into the duodenum, is an obvious contributing factor (29). Alternatively, GLP-1 may have a direct inhibitory effect on duodenal triglyceride absorption, potentially via inhibition of gastric lipases (26). The effect of exenatide infusion on postprandial plasma FFA levels was not examined in this study; however, other investigators have demonstrated that plasma FFA levels decrease during GLP-1 analog infusion (25). This is consistent with the known stimulation of insulin secretion by GLP-1 and
insulin-mediated inhibition of lipolysis (22). These favorable changes in circulating lipids are of considerable clinical interest, since excessive postprandial triglyceride elevation has been implicated as a risk factor for atherosclerotic cardiovascular complications in T2DM (24).

In summary, we have demonstrated that an infusion of exenatide, designed to simulate plasma exenatide levels observed in clinical practice, attenuates postprandial hyperglycemia by suppressing endogenous (hepatic) glucose production and decreasing the rate of ingested glucose appearance in the systemic circulation. Therapeutic plasma exenin-4 levels achieved in our studies were associated with a marked reduction in gastrointestinal glucose absorption, whereas splanchnic (hepatic) glucose uptake was enhanced modestly. Collectively, these two actions of exenatide (delayed gastric emptying and enhanced splanchnic glucose uptake) accounted about one-third of the decrease in postprandial hyperglycemia observed with exenatide. Inhibition of glucagon secretion by exenatide (which accounted for ~56% of the inhibition of endogenous glucose production following exenatide) explained about one-third of the decrease in postprandial hyperglycemia. By substitution, stimulation of insulin secretion by exenatide must account for the remaining suppression of endogenous glucose production and about one-third of the decline in postprandial plasma glucose levels. We conclude that, within the therapeutic range, exenatide exerts its major effect to reduce postprandial hyperglycemia by inhibiting hepatic glucose production (by stimulating insulin and inhibiting glucagon secretion), delaying gastric emptying, and augmenting splanchnic glucose uptake.

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DISCLOSURES

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