Glucagon-Like Peptide 1 Has a Physiological Role in the Control of Postprandial Glucose in Humans

Studies With the Antagonist Exendin 9-39

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Glucagon-like peptide 1(7-36) amide (GLP-1) is postulated to be the major physiological incretin in humans, but evidence is indirect. We report the first studies examining the physiological role of GLP-1 in the postprandial state in humans using the GLP-1 antagonist exendin 9-39. Exendin 9-39 completely blocked GLP-1–induced glucose-stimulated insulin release from perifused human islets of Langerhans. In healthy fasted volunteers, intravenous infusion of exendin 9-39 at 500 pmol · kg^{-1} · min^{-1} in the hyperglycemic state abolished the insulinotropic effect of a physiological dose of GLP-1 and fully reversed the glucose-lowering effect of GLP-1. Nine healthy subjects consumed a 150-g oral glucose tolerance test and were infused with 500 pmol · kg^{-1} · min^{-1} exendin 9-39 or saline. Exendin 9-39 increased the peak postprandial glucose level (exendin 9-39, 8.67 ± 0.35 vs. saline, 7.67 ± 0.35 mmol/l, $P \leq 0.005$) and increased postprandial plasma glucose incremental area under the curve by 35% (exendin 9-39, 152 ± 19 vs. saline, 113 ± 16 mmol · min · l^{-1}, $P \leq 0.05$). This could be explained as partly secondary to the blockade of glucose-induced suppression of glucagon and maybe also to an increased rate of gastric emptying. Thus, in humans exendin 9-39 acts as an antagonist of GLP-1 both in vitro and in vivo. When infused alone, exendin 9-39 causes a deterioration in postprandial glycemic control, suggesting that GLP-1 may be important for maintenance of normal postprandial glucose homeostasis in humans. *Diabetes* **48:86–93, 1999**

Glucagon-like peptide 1(7-36) amide (GLP-1), a product of posttranslational processing of the preproglucagon gene (1), is synthesized in intestinal L-cells (2,3). Plasma GLP-1 levels increase threefold after a meal in humans (4). When 0.5 pmol · kg · min^{-1} GLP-1, a concentration that mimics the postprandial rise, is infused into healthy human volunteers, the insulin response to intravenous glucose is potently increased and plasma glucose excursions are suppressed (4). The enhanced insulin response to oral over intravenous glucose is known as the incretin effect (5,6). Together with glucose-dependent insulinotropic polypeptide (GIP) (7), GLP-1 is thought to act as a physiological incretin in humans. In addition, GLP-1 has been shown to stimulate biosynthesis of insulin (8,9), suppress glucagon release (4), delay gastric emptying (10,11), and enhance peripheral glucose disposal (12,13). Furthermore, GLP-1 seems to be a central satiety factor in the rat (14) and has recently been suggested to promote satiety in humans (15).

GLP-1 enhances insulin secretion, whereas GIP has little or no effect in patients with type 2 diabetes (12,16). A clinical study of subcutaneous GLP-1 administered three times per day to such patients indicated that the glucose-lowering effect of GLP-1 was maintained for 3 weeks, and it could improve long-term parameters of glycemic control (17). Thus, a long-acting GLP-1 receptor agonist may be a therapeutic agent for type 2 diabetes in the future.

Evidence to support the importance of endogenous GLP-1 as an incretin in humans is indirect, relying on infusion of GLP-1 and GIP at doses that mimic postprandial levels (4,18). Exendin-4, isolated from the venom of the Gila monster (*Heloderma suspectum*), is an agonist at both the rat and human GLP-1 receptors (19–22). A fragment of exendin-4, exendin 9-39, has been found to be a specific antagonist of the GLP-1 receptor in vitro (21,23) and in vivo in the rat (24,25). We demonstrated that intravenous exendin 9-39 blocked GLP-1–induced insulin secretion but had no effect on the action of other insulinotropic agents in anesthetized fasted rats (24). In conscious rats trained to a once-daily feeding regime, exendin 9-39 alone blunted the rise in postprandial plasma insulin levels by 50%, resulting in an increase in glucose (24). In a separate study, in the rat, an intravenous bolus of exendin 9-39 before intraduodenal glucose reduced the insulin response by >50% (25). Exendin 9-39 has also been infused into baboons and caused an increase in postprandial glucose in response to intragastric glucose (26). A GLP-1 receptor knockout mouse is glucose intolerant, indicating the likely importance of GLP-1 in glycemic control in the mouse (27).

In contrast to the above, the minimal effect of intravenous GLP-1 on plasma insulin levels in the calf indicates that GLP-1 is not an important incretin in all species (28). We therefore set out to investigate whether exendin 9-39 blocks GLP-1–induced stimulation of insulin secretion by human

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AUC, area under the curve; CSS, steady-state concentration; exendin 9-39–LI, exendin 9-39–like immunoreactivity; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide 1(7-36) amide; HPLC, high-pressure liquid chromatography; MCR, metabolic clearance rate; OGTT, oral glucose tolerance test; PYY, peptide YY; RIA, radioimmunoassay; VD, apparent volume of distribution.

islets of Langerhans. We infused exendin 9-39 in humans to block the effects of a physiological dose of exogenous GLP-1 (previously reported in abstract form [29]) and infused this dose of exendin 9-39 with ingestion of an oral glucose tolerance test (OGTT) to assess the role of endogenous GLP-1 in postprandial glycemic control in humans.

RESEARCH DESIGN AND METHODS

In vitro studies

Collection, isolation, and perifusion of human pancreatic islets. After informed consent was obtained from next of kin, pancreases were removed from brain-dead, heart-beating organ donors. Islets were released by intraductal collagenase infusion and incubated at 37°C. The pancreases were disrupted using an automated digestion process (30). Islets were separated and maintained prior to perifusion as previously described (31). Briefly, a mixture of Krebs-Ringer bicarbonate buffer and CMRL-1066 culture media was used. Insulin release was measured from chambers containing 100 human islets, perifused with buffer at 0.4 ml/min. Each study was undertaken with a paired control and repeated six times. After a basal period with buffer containing 2.8 mmol/l glucose, samples were collected from 0 to 40 min before an increase in glucose concentration to 8.0 mmol/l, which was continued from 41 to 160 min.

Study 1: Blockade of exogenous GLP-1. The effect of GLP-1 (100 nmol/l), with or without exendin 9-39 (1 µmol/l), on insulin release was examined for 40 min (between 81 and 120 min) during perifusion with 8.0 mmol/l glucose.

Study 2: Blockade of exogenous GIP. To verify the specificity of exendin 9-39, the effect of GIP (100 nmol/l), with or without exendin 9-39 (2 µmol/l), was assessed as above.

Islet cell viability was verified in both experiments by perifusing with 2.8 mmol/l glucose from 161 to 200 min and with 20 mmol/l glucose from 201 to 220 min.

In vivo studies

Materials. GLP-1 and exendin 9-39 were synthesized using fluorene methoxy carbomyl chemistry on an Advanced Chemtech 396MPS peptide synthesizer. The products each comprised one major peak that was purified to homogeneity by reversed-phase high-pressure liquid chromatography (HPLC) on a C8 column (Phenomenex, Macclesfield, U.K.). Electrospray mass spectrometry was used to confirm the identity of the peptides. The Limulus Amoebocyte Lysate assay test for pyrogen was negative, and the peptides were sterile on culture.

Study 1: Blockade of exogenous GLP-1. We initially tested for possible side effects of exendin 9-39 starting at a dose of 0.1 pmol · kg^{-1} · min^{-1}. Then we performed pilot experiments at 1, 10, and 20 pmol · kg^{-1} · min^{-1}. To block the effects of 0.5 pmol · kg^{-1} · min^{-1} GLP-1, 50 pmol · kg^{-1} · min^{-1} exendin 9-39 was infused in six subjects using a protocol similar to that described below. This experiment showed partial blockade of the effect of GLP-1, and further studies with 100 and 250 pmol · kg^{-1} · min^{-1} predicted that a ratio of infusion rates of exendin 9-39 to GLP-1 of 1,000:1 was necessary to block the insulinotropic effect of GLP-1.

Six healthy subjects (four men and two women, age 25.5 ± 0.9 years [mean ± SE], BMI 23 ± 1 kg/m^2) participated in the study. Subjects gave informed written consent, and ethical approval was obtained from the local research ethics committee. Volunteers fasted before each study day. Subjects were taking no regular medication, had no allergies, and had no abnormalities on physical examination and electrocardiogram. They had no evidence of abnormal renal function and normal Hb, fasting plasma glucose, and insulin concentrations.

Each subject was studied on four occasions with at least 72 h between each study. On the morning of each study, a cannula was inserted into a large forearm vein for collection of blood, and two were inserted into veins in the opposite forearm, one for infusion of peptides and one for glucose. On the first study day, all subjects were infused with glucose and saline control to habituate them to the procedure and reduce any effects of anxiety. Thereafter, each subject had an infusion of glucose and saline (GLU), glucose and GLP-1 (GLP), or glucose, GLP-1, and exendin 9-39 (GLP + EX), in random order. All four studies were blinded to the subjects; the latter three were blinded to the investigators.

Infusion of 5 pmol · kg^{-1} · min^{-1} exendin 9-39 or saline control was started at 0 min. Ten minutes later, the rate was increased to 50 pmol · kg^{-1} · min^{-1}; 5 min later, to 250 pmol · kg^{-1} · min^{-1}; and 5 min later, to 500 pmol · kg^{-1} · min^{-1}. After the 5-min infusion of exendin 9-39 at the final dose, 0.5 pmol · kg^{-1} · min^{-1} GLP-1 or saline was infused and 25% glucose infused at 2 ml/min (0.5 g/min). Glucose infusion was terminated after 20 min and GLP-1 and exendin 9-39 after 55 min. Samples of GLP-1 and exendin 9-39 infusate were collected before initiation and after termination of the final infusion rate. Blood samples were collected 10 min before the start of exendin 9-39 infusion and for 140 min thereafter. The protocol was based on our original GLP-1 infusion studies (4). Subjects were attached to a cardiac monitor, and arterial blood pressure was measured regularly using a Critikon Dinamap vital signs indicator.

Study 2: Blockade of endogenous GLP-1. Nine healthy subjects (three men and six women, age 26.1 ± 0.8 years, BMI 22.4 ± 0.8 kg/m^2) participated in the study. Subjects gave informed written consent, and ethical approval was obtained. Volunteers underwent a screen as above and were fasted before each study day.

Subjects were studied twice with at least 72 h between each study. On the morning of the study, a cannula was inserted into a large forearm vein for collection of blood, and one was inserted into a vein in the opposite forearm for infusion of exendin 9-39 or saline. Subjects sat at a 45-degree angle throughout the studies. Subjects were infused with saline or exendin 9-39 in a randomized double-blind manner. Infusion of 5 pmol · kg^{-1} · min^{-1} exendin 9-39 or saline was started at –80 min. Ten minutes later, the rate was increased to 50 pmol · kg^{-1} · min^{-1}; and 10 min later, to 500 pmol · kg^{-1} · min^{-1}. A 150-g OGTT (600 ml Lucozade; SmithKline Beecham, Brentford, U.K.), was given at 0 min and consumed within 15 min. Exendin 9-39 or saline infusion continued for a further 70 min. Samples of exendin 9-39 infusate were collected before and after initiation and termination of the final infusion rate. Blood samples were collected 10 min before the start of exendin 9-39 infusion and for 210 min after the OGTT. Subjects were monitored as above.

Analytical methods. Blood was collected into heparinized tubes containing 5,000 KIU (0.2 ml) of aprotinin and centrifuged. Plasma was separated and stored at –20°C until analysis. Plasma glucose was measured using a BM/Hitachi 747 glucose analyzer (normal range 4.22–6.11 mmol/l). Plasma insulin, glucagon, GIP, and peptide YY (PYY) levels were measured by established radioimmunoassays (RIAs) (4,32–34). Plasma GLP-1 was measured using our RIA (4), but this was found to cross-react with exendin 9-39 at the levels found in the plasma. Thus, only plasma GLP-1 levels during infusions without exendin 9-39 are shown. Exendin 9-39 did not cross-react with any of the other assay antibodies up to concentrations of 1 µmol/l.

Exendin 9-39 assay. Exendin 9-39–like immunoreactivity (exendin 9-39–LI) was measured using an antiserum raised to synthetic exendin-4 conjugated with glutaraldehyde to bovine serum albumin at a dilution of 1:24,000. This antibody showed no cross-reactivity (<0.001%) with glucagon, GLP-1, or GLP-2. Synthetic exendin-4 was iodinated by the Bolton and Hunter method (35). The iodinated product was separated by reversed-phase HPLC using a C18 column on a 15–45% 90 min acetonitrile/water/0.05% trifluoroacetic acid gradient. It had a specific activity of 56 Bq/fmol, as determined by self-displacement in the assay. The assay standard was synthetic exendin 9-39 (1–100 fmol/tube). Assays were performed in a total volume of 0.8 ml phosphate buffer (0.06 mol/l, pH 7.4) containing 10 mmol/l EDTA, 8 mmol/l sodium azide, and 0.3% (wt/vol) bovine serum albumin. They were incubated for 4 days at 4°C before separation of free and antibody-bound peptide by dextran-coated charcoal (6 mg/tube). The assay could detect changes of 1.5 fmol/assay tube, and the inter- and intra-assay coefficients of variation (10 fmol addition) were 8.2 and 12.8%, respectively.

Calculations. The decay curve of exendin 9-39–LI concentrations (from study 2) was converted to natural logarithms and plotted against time. The resulting straight-line plot was used to derive the half-time of disappearance ($t_{1/2}$) for infused exendin 9-39–LI for each subject. The metabolic clearance rate (MCR) of exendin 9-39–LI was calculated for each volunteer from the steady-state concentration (CSS) (taken as the value at time 70) and the infusion rate at which this concentration was stable, where MCR = infusion rate/CSS. The apparent volume of distribution (VD) was calculated from the half-life and the steady-state clearance, where VD = MCR × $t_{1/2}$ × 1.44.

Statistical analysis. All results are presented as means ± SE. Rates of insulin release from the perifused pancreatic islets approximated a log-normal distribution. Comparisons of rates of insulin release in the perifusion studies within a set of islets were by paired t test of logarithmic data, and comparisons between sets of islets perifused together were by unpaired t test of logarithmic data.

In the in vivo studies, the incremental or decremental area under the curve (AUC) for each variable was calculated using the trapezoidal rule. For study 1, AUC was calculated from 25 min, when glucose and GLP-1 infusions started, to 140 min, when plasma levels of both had returned to baseline. Comparisons of data were by analysis of variance with post hoc Tukey's test.

For study 2, AUCs for postprandial glucose, insulin, glucagon, GIP, and PYY were calculated from 0 min, immediately before consumption of the OGTT, to 70 min, when the infusions ceased. To assess an effect on fasting compounds, AUC was calculated between –60 min, the time of initiation of the final infusion rate, and 0 min. Comparisons of AUCs, peak postprandial plasma glucose levels, and plasma glucagon levels between the exendin 9-39 and control studies were by paired t test.

RESULTS

In vitro studies

Blockade of exogenous GLP-1. Baseline insulin release from control islets was 2.6 ± 0.9 fmol · $islet^{-1}$ · min^{-1} during perifusion with 2.8 mmol/l glucose (Fig. 1*A*). Upon elevation of the

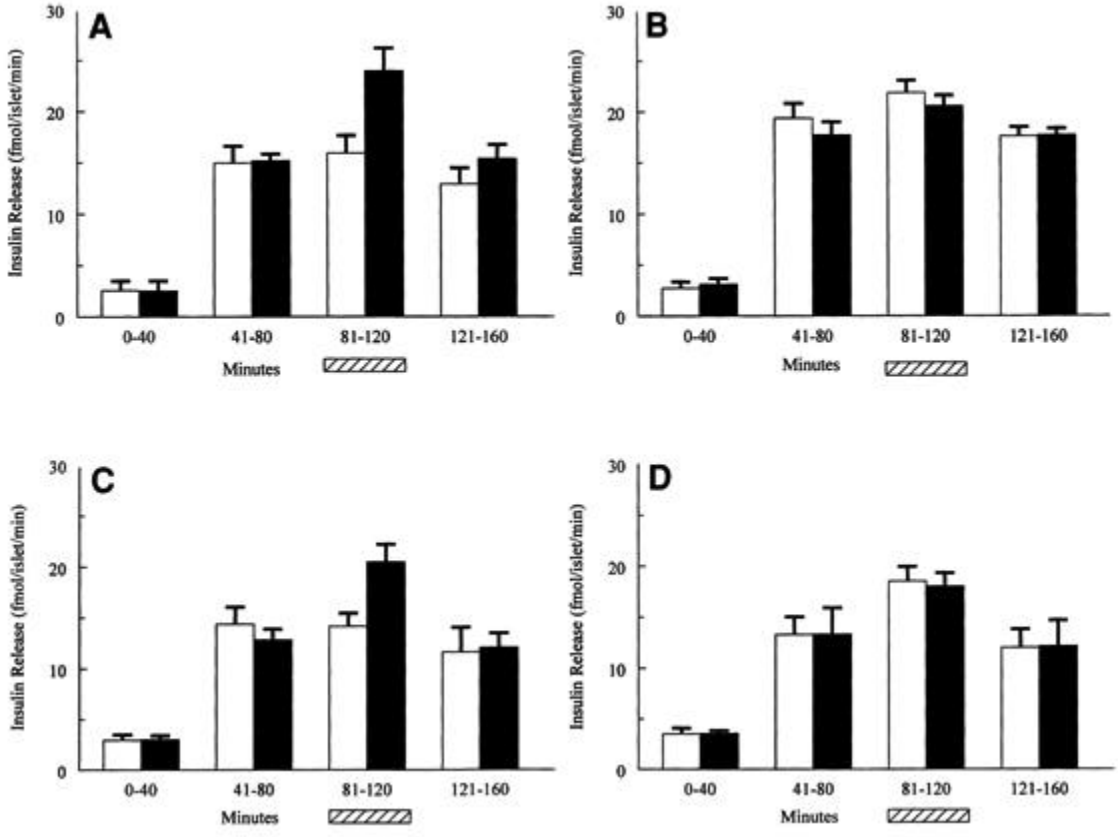

FIG. 1. Influence of glucose and added GLP-1 (*A*) or GLP-1 plus exendin 9-39 (*B*) on insulin release from perifused human islets of Langerhans (100 islets per perifusion). Influence of glucose and added GIP (*C*) or GIP and GIP plus exendin 9-39 (*D*) on insulin release from perifused human islets of Langerhans (100 islets per perifusion). The perifusate glucose concentration was 2.8 mmol/l from 0 to 40 min and 8 mmol/l from 41 to 160 min. *A*: GLP-1 (100 nmol/l) was added to treated chambers (■) from 81 to 120 min, and control chambers (□) were untreated. *B*: GLP-1 (100 nmol/l) plus exendin 9-39 (1 µmol/l) was added to treated chambers (■) from 81 to 120 min, and control chambers (□) were untreated. *C*: GIP (100 nmol/l) was added to treated chambers (■) from 81 to 120 min, and control chambers (□) were untreated. *D*: GIP (100 nmol/l) plus exendin 9-39 (2 µmol/l) was added to treated chambers (■) from 81 to 120 min, and GIP (100 nmol/l) was added to control chambers (□). Results are presented as means ± SE of insulin release (fmol · islet^{-1} · min^{-1}), collected as fractions every 4 min. ▨, period of peptide stimulation. n = 6 for each.

glucose concentration to 8.0 mmol/l, there was an increase to 15.2 ± 0.6 fmol · islet^{-1} · min^{-1} ($P \leq 0.01$). When GLP-1 (100 nmol/l) was added, insulin release from the treated islets increased by >50% compared with control islets (GLP-1, 24.0 ± 2.2 vs. control, 15.9 ± 1.7 fmol · islet^{-1} · min^{-1}, $P \leq 0.02$). After removal of GLP-1, insulin release from the treated islets decreased to match that of control islets (Fig. 1*A*). When exendin 9-39 (1 µmol/l) was added together with GLP-1 (100 nmol/l), no alteration in insulin release was observed compared with control islets (exendin 9-39 plus GLP-1, 20.5 ± 1.0 vs. control, 21.8 ± 1.2 fmol · islet^{-1} · min^{-1}, NS) (Fig. 1*B*).

Blockade of exogenous GIP. Baseline insulin release from control islets was 3.0 ± 0.5 fmol · islet^{-1} · min^{-1} during perifusion with 2.8 mmol/l glucose (Fig. 1*C*). Upon elevation of the glucose concentration to 8.0 mmol/l, there was an increase to 14.4 ± 1.7 fmol · islet^{-1} · min^{-1} ($P \leq 0.001$). When GIP (100 nmol/l) was added, insulin release from the treated islets increased by >40% compared with control islets (GIP, 20.5 ± 1.7 vs. control, 14.2 ± 1.3 fmol · islet^{-1} · min^{-1}, $P \leq 0.03$). After the removal of GIP, insulin release from the treated islets decreased to match that of control islets (Fig. 1*C*). There was no difference in the stimulation of insulin release caused by GIP (100 nmol/l) with or without exendin 9-39 (2 µmol/l) (exendin 9-39 plus GIP, 18 ± 1.3 vs. GIP, 18.5 ± 1.4 fmol · islet^{-1} · min^{-1}, NS) (Fig. 1*D*).

The viability of the islets in both studies was confirmed by the decrease in insulin secretion to basal levels in all sets of islets on reduction of perifusate glucose concentration to 2.8 mmol/l and by the increase well above the stimulated level with 20 mmol/l glucose perifusate (data not shown).

In vivo exendin 9-39 infusions. No subjects reported side effects from exendin 9-39 infusion.

Blockade of exogenous GLP-1. In the pilot study, exendin 9-39 infused at 50 pmol · kg^{-1} · min^{-1} caused a 21 ± 47% reduction in the increase in the AUC for insulin caused by 0.5 pmol · kg^{-1} · min^{-1} GLP-1 and caused a 21 ± 40% reduction in the GLP-1–induced decrease in the AUC for glucose (data not shown). In light of this data, we went on to infuse exendin 9-39 at 500 pmol · kg^{-1} · min^{-1}.

Plasma exendin 9-39–LI was undetectable in the fasted state before infusion. The measured infusion rate of exendin 9-39 was 218 ± 10 pmol · kg^{-1} · min^{-1}. Plasma exendin 9-39–LI levels peaked at the end of infusion at 53 ± 4 nmol/l (Fig. 2*A*). There was no difference in the plasma level of glucose or any hormones assayed between the first infusion of glucose alone and the second (data not shown). Pulse and blood

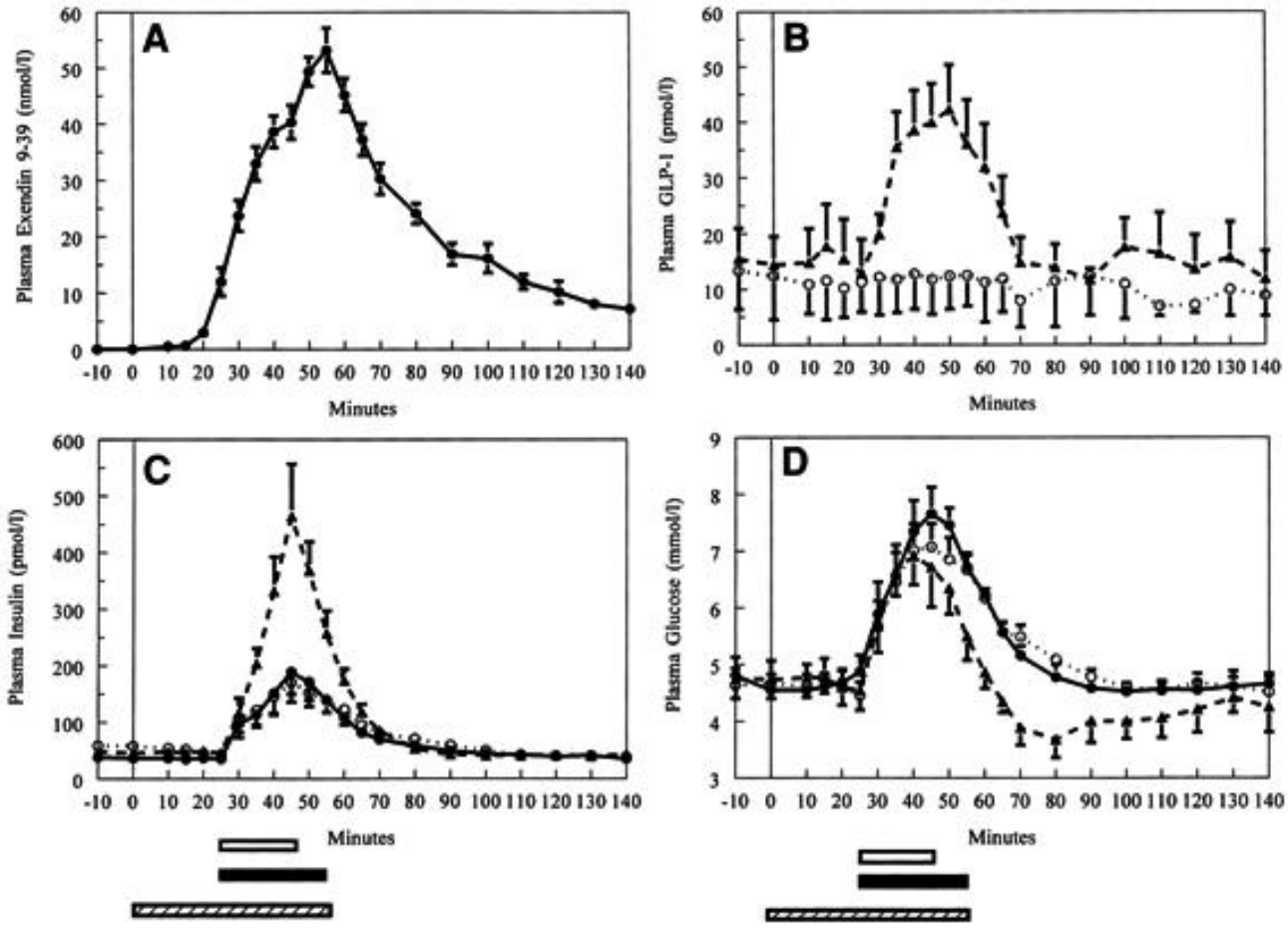

FIG. 2. Plasma exendin 9-39 levels (*A*), plasma GLP-1 levels (*B*), plasma insulin levels (*C*), and plasma glucose levels (*D*) in response to infusion of glucose alone (○), GLP-1 plus glucose (▲), and exendin 9-39 and GLP-1 plus glucose (●). Glucose (0.5 g/min) (▭) was infused from 25 to 45 min; GLP-1 (0.5 pmol · kg^{-1} · min^{-1}) (▬) was infused from 25 to 55 min; and exendin 9-39 (▨) was infused from 0 to 10 min (5 pmol · kg^{-1} · min^{-1}), 10 to 15 min (50 pmol · kg^{-1} · min^{-1}), 15 to 20 min (250 pmol · kg^{-1} · min^{-1}), and 20 to 55 min (500 pmol · kg^{-1} · min^{-1}). Results are presented as means ± SE. *n* = 6 healthy volunteers.

pressure were unaffected by any of the infusions. The measured actual infusion rate of GLP-1 was not different between the GLP and the GLP + EX infusions (GLP, 0.25 ± 0.04 vs. GLP + EX, 0.24 ± 0.04 pmol · kg^{-1} · min^{-1}, NS). Plasma GLP-1 concentrations in response to GLP-1 infusion rose from 14.8 ± 6.1 to 42.3 ± 8.1 pmol/l, similar to previously observed postprandial levels (4). Infusion of glucose alone did not affect plasma GLP-1 concentrations (Fig. 2*B*).

Infusion of GLP-1 with glucose caused a doubling of plasma insulin compared with glucose alone, (AUC 25–140 GLP, 8.6 ± 1.5 vs. GLU, 4.3 ± 0.5 nmol · min · l^{-1}, $P \leq 0.02$). Addition of exendin 9-39 abolished the insulinotropic effect of GLP-1 (AUC 25–140 GLP + EX, 4.8 ± 0.6 vs. GLU, 4.3 ± 0.5 nmol · min · l^{-1}, NS) (Fig. 2*C*).

Infusion of GLP-1 with glucose considerably decreased the glucose rise compared with glucose alone (AUC 25–140 GLP, 2.0 ± 8.4 vs. GLU, 83 ± 23 mmol · min · l^{-1}, $P \leq 0.01$). Addition of exendin 9-39 completely reversed the decrease in glucose rise caused by GLP-1 (AUC 25–140 GLP + EX, 82 ± 15 vs. GLU, 83 ± 23 mmol · min · l^{-1}, NS) (Fig. 2*D*).

Blockade of endogenous GLP-1. Exendin 9-39 had no effect on pulse or blood pressure. Ingestion of the OGTT produced similar basal and peak GLP-1 concentrations (12.6 ± 0.7 and 48.5 ± 4.3 pmol/l) (Fig. 3*A*), as infusion of GLP-1 produced in study 1 (14.8 ± 6.1 and 42.3 ± 8.1 pmol/l).

Exendin 9-39 increased the peak postprandial glucose level (exendin 9-39, 8.67 ± 0.35 vs. saline, 7.67 ± 0.35 mmol/l, $P \leq 0.005$). Postprandial glucose excursions, as measured by the AUC from 0 min to the end of the exendin 9-39 infusion at 70 min, were increased by 35% by GLP-1 receptor blockade (exendin 9-39, 152 ± 19 vs. saline, 113 ± 16 mmol · min · l^{-1}, $P \leq 0.05$) (Fig. 3*B*). After cessation of exendin 9-39 infusion, the plasma glucose level in response to exendin 9-39 dropped to that of the control. Analysis of the effect of exendin 9-39 on plasma glucose in the fasted state revealed that exendin 9-39 increased plasma glucose levels compared with control (exendin 9-39, 3.8 ± 2.0 vs. saline, –2.0 ± 2.6 mmol · min · l^{-1}, $P \leq 0.05$) (Fig, 3*C*).

Plasma insulin levels showed a tendency to increase in response to exendin 9-39 infusion, but this did not reach statistical significance (exendin 9-39, 40.4 ± 8.5 vs. saline, 32.7 ± 5.6 nmol · min · l^{-1}, NS) (Fig. 3*D*). There was no difference in the AUC for plasma insulin levels in the fasted state between the two infusions.

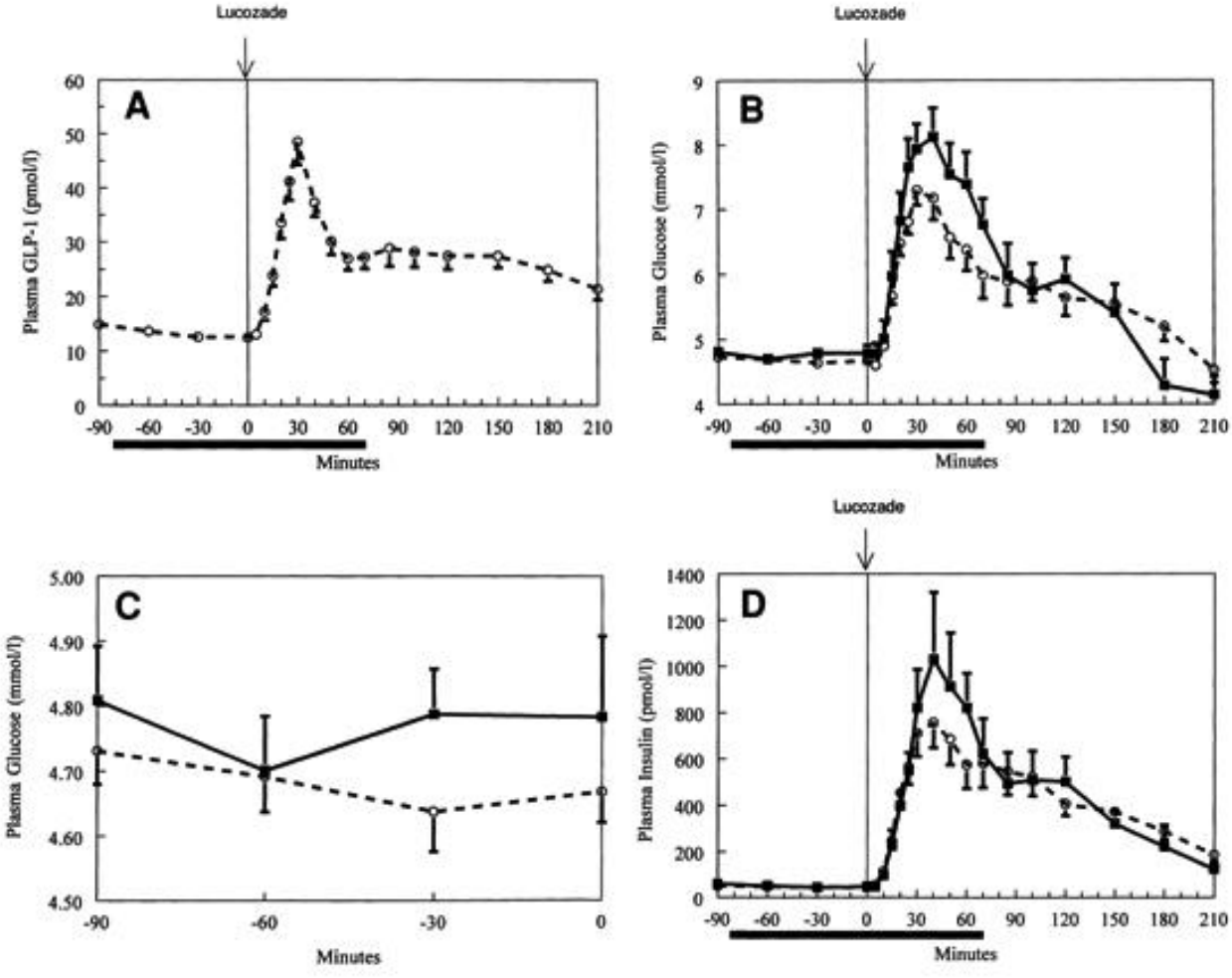

FIG. 3. Plasma GLP-1 levels (*A*), plasma glucose levels (*B*), and plasma insulin levels (*D*) in response to ingestion of 600 ml Lucozade at 0 min, with infusion of exendin 9-39 (■) or saline (○). Exendin 9-39 (▬) was infused from –80 to –70 min (5 pmol · kg^{-1} · min^{-1}), –70 to –60 min (50 pmol · kg^{-1} · min^{-1}), and –60 to +70 min (500 pmol · kg^{-1} · min^{-1}). *C*: Plasma glucose levels in the fasted state, enlarged from *A*. Results are presented as means ± SE. *n* = 9 healthy volunteers.

Although there was a tendency for blockade of glucose-induced suppression of glucagon, this was not significantly different as measured by the AUC (exendin 9-39, 39.9 ± 74.3 vs. saline, –123.7 ± 51.5 pmol · min · l^{-1}, P = 0.12) (Fig. 4*A*). Comparison of individual time points showed a significant difference between the two infusions from 20 min to the end of the experiment. Plasma glucagon levels were unaltered by exendin 9-39 in the fasted state (exendin 9-39, –64 ± 39.1 pmol · min · l^{-1} vs. saline, –44.8 ± 31.8 nmol · min · l^{-1}, NS).

Infusion of exendin 9-39 caused plasma PYY levels to increase 2.5-fold (exendin 9-39, 1,178 ± 133 vs. saline, 471 ± 129 pmol · min · l^{-1}, $P \leq 0.002$) (Fig. 4*B*).

In response to exendin 9-39 infusion, plasma GIP levels were increased by 27% (exendin 9-39, 8.5 ± 1.1 vs. saline, 6.7 ± 1.1 nmol · min · l^{-1}, $P \leq 0.01$) (Fig. 4*C*).

Exendin 9-39 pharmacokinetics. Plasma exendin 9-39–LI levels in response to infusion of exendin 9-39 are shown in Fig. 5. Fast protein liquid chromatography of plasma after exogenous exendin 9-39 infusion showed only a single form in the expected position for exendin 9-39 itself (data not shown). The measured infusion rate of exendin 9-39 was 186 ± 25 pmol · kg^{-1} · min^{-1}. The plateau level of exendin 9-39–LI was 72 ± 6 nmol/l. The course of exendin 9-39–LI disappearance followed first-order kinetics. The plasma half-life of exendin 9-39–LI in humans was calculated to be 33 ± 4 min. The mean MCR was 2.3 ± 0.3 ml · kg^{-1} · min^{-1}, and the apparent volume of distribution was 111 ± 16 ml/kg.

DISCUSSION

GLP-1 potently stimulated insulin release from perifused human pancreatic islets in the hyperglycemic state, as previously demonstrated (36). We have now shown that exendin 9-39 completely blocks this GLP-1–stimulated insulin release from human islets.

Exendin 9-39 was infused into 28 healthy subjects, and no adverse effects were observed in any subject. We infused GLP-1 at a concentration of 0.5 pmol · kg^{-1} · min^{-1}, identical to that used in our original report (4). It was again found to mimic the physiological postprandial levels found using our RIA, increase plasma insulin, and suppress glucose levels similar to our earlier study (4). Using a similar protocol, increasing doses of exendin 9-39 were studied, but they produced either partial or no blockade below the dose reported here. Exendin 9-39, at a dose of 500 pmol · kg^{-1} · min^{-1}, appeared to completely block the GLP-1–induced insulin secretion and consequent fall in plasma glucose levels in the hyperglycemic state.

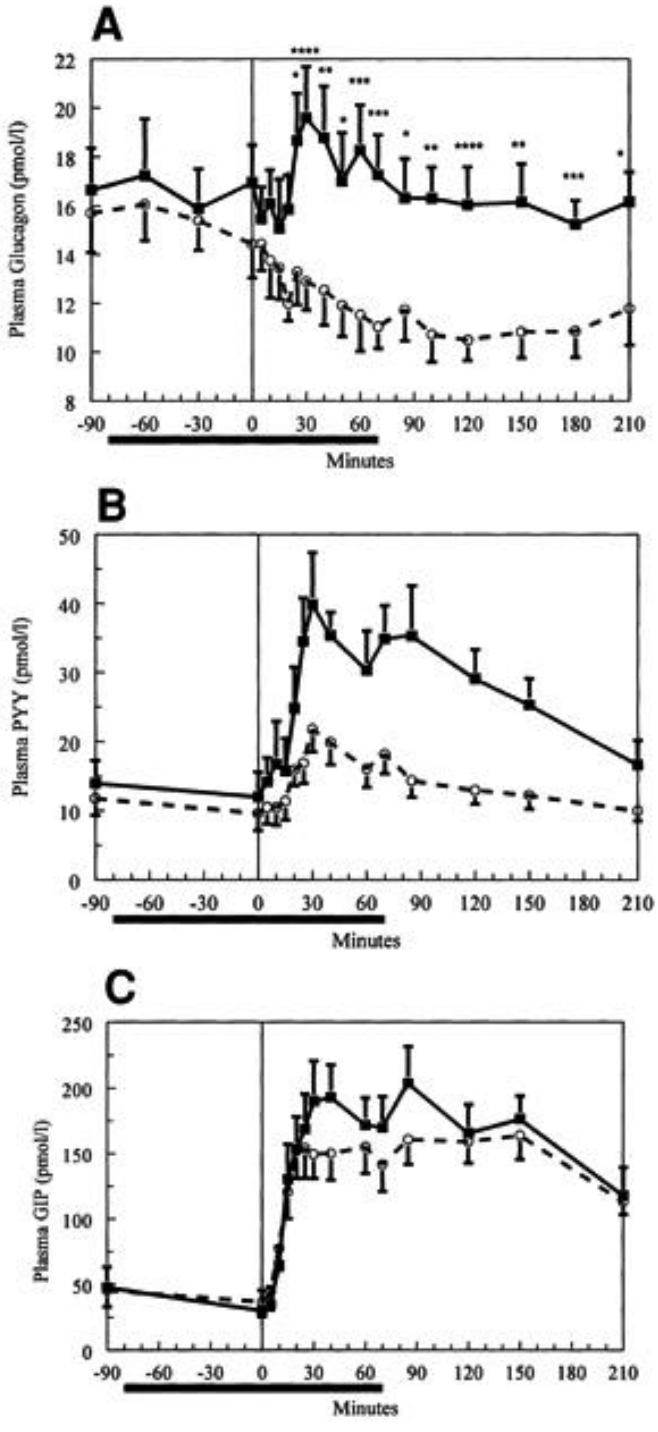

FIG. 4. Plasma glucagon levels (A), plasma PYY levels (B), and plasma GIP levels (C) in response to ingestion of 600 ml Lucozade at 0 min, with infusion of exendin 9-39 (■) or saline (○). Exendin 9-39 (▬) was infused from –80 to –70 min (5 pmol · kg^{-1} · min^{-1}), –70 to –60 min (50 pmol · kg^{-1} · min^{-1}), and –60 to 70 min (500 pmol · kg^{-1} · min^{-1}). Results are presented as means ± SE. n = 9 healthy volunteers. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.005$, **** $P \leq 0.001$, paired t test exendin 9-39 vs. saline.

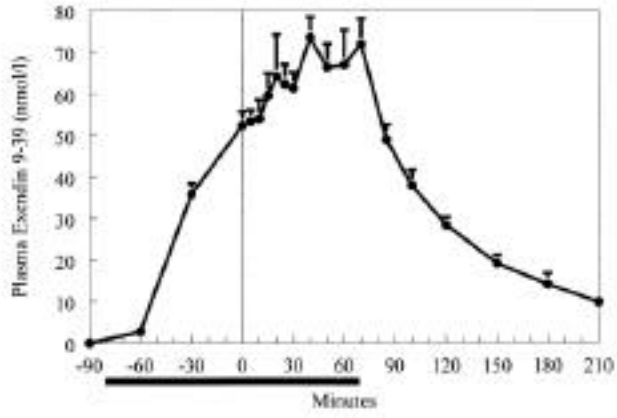

FIG. 5. Plasma exendin 9-39–LI levels. The course of exendin 9-39–LI disappearance followed first-order kinetics, allowing calculation of pharmacokinetics. Exendin 9-39 (▬) was infused from –80 to –70 min (5 pmol · kg^{-1} · min^{-1}), –70 to 60 min (50 pmol · kg^{-1} · min^{-1}), and –60 to +70 min (500 pmol · kg^{-1} · min^{-1}).

A sensitive and specific RIA for exendin 9-39 has been developed to identify the pharmacokinetics of this peptide in humans. The MCR of exendin 9-39–LI at 2.3 ml · kg^{-1} · min^{-1} is similar in magnitude to the normal glomerular filtration rate. The volume of distribution is a little over double the total-body water volume and does not suggest that exendin 9-39 is extensively tissue bound. No endogenous plasma exendin 9-39–LI was detectable by RIA before the onset of infusion.

Our results demonstrate that exendin 9-39 can antagonize the action of GLP-1 in humans in vivo, but high concentrations are required. The blockade is apparently less potent than in the rat, in which exendin 9-39 is effective at 30–100 times the concentration of GLP-1 (24,25). Poorer binding to the human GLP-1 receptor, or even altered post-receptor coupling, could be responsible. In study 1, exendin 9-39-LI levels did not plateau, presumably because of the long plasma exendin 9-39 half-life; thus, it is possible that a dose lower than 500 pmol · kg^{-1} · min^{-1} would be effective if given for a longer duration. In the baboon, blockade of the effect of GLP-1 could only be achieved at 150 nmol · kg^{-1} · h^{-1}, five times the concentration that we have found to effectively block GLP-1 in humans (26). Recent data from Schirra et al. (37) demonstrated blockade of the effect of GLP-1 in humans at the same ratio of exendin 9-39 to GLP-1 reported here.

It is possible that exendin 9-39 also blocks the GIP receptor in rat (38) and humans (39,40). In the rat, 1 µmol/l exendin 9-39 inhibits binding of GIP to its receptor by 39%, but this concentration of exendin 9-39 was completely unable to antagonize 10 nmol/l GIP-stimulated cAMP production (38). Further, there is little evidence of functional antagonism of the GIP receptor. Similar results were found in both reports of exendin 9-39 infusion in vivo. Exendin 9-39 infused at a concentration that completely blocked the insulinotropic effect of exogenous GLP-1 had no effect on the insulinotropic effect of GIP (24,25). Exendin 9-39 binds with very low affinity to the cloned human GIP receptor (39,40). When the human GIP receptor was transfected into Chinese hamster lung fibroblasts, exendin 9-39 could prevent cAMP formation by cells exposed to 0.6 nmol/l GIP but with an IC_{50} of 4.5 µmol/l, a concentration nearly 10,000 times as great, and complete blockade was not seen at 10 µmol/l (39). We have demonstrated that a concentration of exendin 9-39 double that which completely blocks the effect of GLP-1 on perifused human islets has no effect on GIP-induced insulin secretion.

Thus, it would seem unlikely that the concentrations of exendin 9-39 used here in vivo would affect the GIP component of the incretin effect. It has been shown that exendin 9-39 infused at a dose of 300 pmol · kg^{-1} · min^{-1} in humans is completely unable to block the effect of infused GIP (37).

The peak plasma GLP-1 level in response to an OGTT was not different from the peak plasma GLP-1 level with infusion of 0.5 pmol · kg^{-1} · min^{-1} GLP-1. Blockade of endogenous GLP-1 released in response to an OGTT, for palatability given in the form of Lucozade, caused a deterioration in postprandial glycemic control. This suggests that in humans, GLP-1 physiologically regulates postprandial glucose. Interpretation of the effect of exendin 9-39 on plasma insulin levels is difficult. Blockade of GLP-1–induced insulin secretion would be expected to decrease plasma insulin, which would in turn cause an increase in plasma glucose. However, the increase in plasma glucose would itself stimulate further insulin secretion. The relationship between plasma glucose and plasma insulin levels is not linear. Thus, it is well known that a small increase in glucose induced by a meal causes a relatively large increase in plasma insulin levels. The increase in glucose induced by exendin 9-39 did not increase insulin; thus, the insulin response to the prevailing glucose levels appears to be inadequate, which may imply that the GLP-1–induced insulin secretion has been attenuated or even blocked. It is noteworthy that the effect of exendin 9-39 on postprandial glucose and insulin found in this study is very similar to that found when exendin 9-39 was infused in the baboon (26).

Oral glucose ingestion reduces plasma glucagon levels, as was found here. Antagonism of the GLP-1 receptor blocked this glucagon suppression and even appeared to lead to an increase in plasma glucagon levels in response to glucose. Thus, the worsening of postprandial glycemic control may be partly related to an increase in plasma glucagon levels. Speculatively, our data may indicate that the normal suppression of glucagon induced by ingestion of glucose is not via an effect of glucose (41,42), insulin (43,44), or somatostatin (45), as previously surmised, but could be either directly or indirectly an effect of GLP-1.

Plasma PYY levels were also measured. Plasma PYY levels have been shown to be increased by an increase in the rate of gastric emptying (46), and PYY itself delays gastric emptying (47). Similarly, a delay in gastric emptying can decrease plasma PYY levels (46). Exendin 9-39 infusion caused a profound increase in plasma PYY during the course of the infusion. This might be explained by an increased rate of gastric emptying induced by blockade of GLP-1, as GLP-1 is known to delay gastric emptying (10,11). Any increased rate of gastric emptying, if present with exendin 9-39, would contribute to deterioration in glycemic control. Interestingly, a recent study demonstrated a decrease in the insulin response to duodenal glucose infusion with exendin 9-39, as well as an enhancement of antral and duodenal contractions (48).

GIP has been reported to be an incretin hormone in humans (7). Exendin 9-39 infusion caused a 27% increase in plasma GIP levels during the course of the infusion. The reason for this response is at present unknown, although this could also be secondary to an increased rate of gastric emptying. The increased levels of GIP would certainly be expected to increase plasma insulin and decrease plasma glucose levels, and could, speculatively, partly compensate for the blockade of GLP-1 decreasing the anticipated impaired glycemic control.

Exendin 9-39 infusion caused a small increase in the fasting plasma glucose level. There is no evidence that this increase is via an effect on plasma glucagon or insulin. A similar increase in fasting plasma glucose levels was seen both in the GLP-1 receptor knockout mouse (27) and when exendin 9-39 was infused in the baboon (26). The only other studies of the effects of exendin 9-39 in humans utilized a hyperglycemic clamp (37) or duodenal glucose infusion (48). In these studies, the effect of exendin 9-39 in the fasted state was examined, and the authors found a similar small but significant increase in fasting plasma glucose levels. In the human and baboon studies, the increase in fasting glucose appears to be at least partly secondary to an increase in glucagon. The increase in fasting glucose in the knockout mouse and the increase found here do not appear to be secondary to glucagon and may instead be due to blockade of the purported enhanced peripheral glucose disposal of GLP-1 (12,13).

Although GLP-1 is probably not the only incretin, these studies indicate for the first time that endogenous GLP-1 has a physiological role in humans. The first clinical trials of GLP-1 for the treatment of type 2 diabetes are now underway (49,17). Our present study demonstrating the role of endogenous GLP-1 in the regulation of fasting and postprandial glucose indicates that the GLP-1 receptor is a potential target for therapy of type 2 diabetes.

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