Glucose-Dependent Action of Glucagon-Like Peptide-1(7-37) In Vivo During Short- or Long-Term Administration

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In vitro, truncated glucagon-like peptides [GLP-1(7-36)-amide and GLP-1(7-37)] increase insulin secretion in a glucose-dependent manner, and desensitization to the action of GLP-1(7-37) has been demonstrated acutely with high concentrations. The purpose of these studies was to evaluate the glucose dependency and threshold of GLP-1(7-37) action in normal rats and in a rat model of type II diabetes and to assess the effects of long-term administration in vivo. All studies were conducted in conscious catheterized rats. An intravenous (IV) infusion of GLP-1(7-37) at 0.5, 5, or 50 pmol/min/kg during the second hour of a 2-hour 11-mmol/L hyperglycemic clamp in Sprague-Dawley rats produced a dose-related enhancement of the glucose-induced increase in plasma insulin concentration. A 1-hour infusion of a submaximal dose of GLP-1(7-37) (5 pmol/min/kg IV) in fasted and fed Sprague-Dawley rats produced small transient increases in plasma insulin (incremental increases above basal, 72 ± 27 and 96 ± 28 pmol/L, respectively) and decreases in plasma glucose (to levels ≥ 5.2 mmol/L). Infusion of GLP-1(7-37) (5 pmol/min/kg IV) during a hyperglycemic clamp at two sequentially increasing concentrations of glucose, 11 and 17 mmol/L, produced incremental increases in insulin of 600 and 1,200 pmol/L, respectively, relative to levels in clamped control rats. Similarly, infusion of GLP-1(7-37) (5 pmol/min/kg IV) in hyperinsulinemic, hyperglycemic Zucker diabetic fatty (ZDF) rats produced a transitory increase in plasma insulin concentration and normalized the plasma glucose concentration. Infusion of GLP-1(7-37) (5 pmol/min/kg IV) for 6 hours in rats maintained at 11 mmol/L glucose resulted in a sustained approximately twofold enhancement of the plasma insulin concentration, suggesting no evidence of acute desensitization. In rats infused with GLP-1(7-37) for 5 days at 15 pmol/min/kg (osmotic minipump subcutaneously), there was a small increase in basal plasma insulin concentration and no effect on glucose. In response to a glucose infusion (to clamp plasma glucose at 11 mmol/L), rats infused with GLP-1(7-37) for 5 days had greater than 50% higher insulin concentrations than vehicle-infused rats. There was no effect of long-term GLP-1(7-37) treatment on food intake or pancreatic insulin content. These results demonstrate the glucose dependency of GLP-1(7-37) in vivo. The incremental insulin response to GLP-1(7-37) was increased with hyperglycemia, and the glucose threshold for GLP-1(7-37) action was approximately 5 mmol/L. These results also demonstrate that GLP-1(7-37) is active after many hours or days of sustained exposure.

TRUNCATED GLUCAGON-LIKE peptides [GLP-1(7-37) and GLP-1(7-36)-amide] are naturally occurring peptide products of the preproglucagon gene that are synthesized primarily in the intestine, with smaller amounts in the pancreas.[1-3] Several lines of evidence suggest that similar to the well-studied gut peptide, gastric inhibitory peptide, GLP-1(7-37) and GLP-1(7-36)-amide act physiologically as incretins, that is, endogenous substances that are released from the intestine into the bloodstream in response to food and stimulate insulin secretion.[4] GLP-1(7-37) and GLP-1(7-36)-amide concentrations increase in the circulation of humans after feeding a mixed meal or after oral administration of glucose.[2,5,6] GLP-1(7-37) and GLP-1(7-36)-amide are potent insulin secretagogues in isolated islets, perfused pancreas, and cultured pancreatic islet cells and exhibit comparable activity.[7-12] Insulin-secretagogue and glucose-lowering activity have been demonstrated in animal models and in humans.[3,5,13-21]

Truncated GLPs represent potential therapeutic agents for the treatment of type II diabetes. Gastric inhibitory peptide has less insulin-secretagogue activity than GLP-1(7-36)-amide in normal humans, and in patients with non–insulin-dependent diabetes mellitus there is a decreased response to gastric inhibitory peptide but not to GLP-1(7-36)-amide.[21] An additional aspect of GLP-1(7-37) and GLP-1(7-36)-amide action that makes them attractive therapeutic agents is that the insulin-secretagogue activity is glucose-dependent,[8-10,12] and they thus may have lower potential for producing hypoglycemic episodes relative to insulin injections or oral sulfonylurea therapy if the glucose threshold for GLP-1(7-37) and GLP-1(7-36)-amide action is not less than euglycemic levels as it is for sulfonylureas.[22] However, to achieve optimal control of blood glucose concentration, such agents may need to be present at elevated levels in the circulation for many hours of the day. Homologous desensitization of GLP-1(7-37) has been demonstrated acutely in vitro with high concentrations.[23] Hence, efficacy with long-term administration in vivo may diminish.

The activity of GLP-1(7-37) and GLP-1(7-36)-amide at matched (clamped) levels of hyperglycemia has not been extensively studied. Additionally, there are no reports of activity of GLP-1(7-37) and GLP-1(7-36)-amide after long-term administration. The present studies were conducted to evaluate the glucose dependency and glucose threshold of GLP-1(7-37) action in vivo and to assess its activity after long-term continuous administration. Studies were conducted evaluating the effect of GLP-1(7-37) on glucose and insulin in normal rats in the fasted, fed, and hyperglycemic state (using a hyperglycemic clamp) and in the Zucker diabetic fatty (ZDF) rat, an animal model of non–insulin-dependent diabetes mellitus that is hyperglycemic and hyperinsulinemic. Activity of GLP-1(7-37) was also tested during a 6-hour intravenous (IV) infusion and 5-day administration by subcutaneously implanted minipump.

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MATERIALS AND METHODS

Animals and Surgical Preparation

All experiments were conducted in conscious, catheterized, male Sprague-Dawley rats weighing 300 to 350 g (Charles River, Wilmington, MA) or ZDF (fa/fa) or lean (+/?) control rats (body weights, 280 to 320 and 220 to 260 g, respectively; Genetic Models, Indianapolis, IN). Animals were housed in an environmentally controlled room (21°C with a 12-hour light/dark cycle) with food (standard rodent chow) and water available ad libitum. For surgical implantation of catheters, rats were anesthetized with sodium pentobarbital 50 mg/kg and PE50 tubing was implanted in the left carotid artery and right jugular vein. Catheters were exteriorized at the back of the neck, filled with a polyvinylpyrrolidone solution in heparinized saline, flame-sealed, and secured with tape. Rats were housed individually and given 5 to 7 days to recover from the surgery. On the day of the experiment, catheters were untaped and flushed with normal saline. During the experiment, rats were allowed to move about freely.

Dose-Response of GLP-1(7-37) Action

To determine an effective dose of GLP-1(7-37) for enhancement of the glucose-induced increase in plasma insulin concentration in Sprague-Dawley rats, GLP-1(7-37) was infused at 0.5, 5, or 50 pmol/min/kg IV during the second hour of a 2-hour 11-mmol/L hyperglycemic clamp. Glucose (50% solution) was infused at a variable rate to maintain plasma glucose concentration at 11 mmol/L. Blood samples (40 μL) for glucose determination were taken every 5 minutes and samples (250 μL) for insulin determination were taken every 15 to 30 minutes from the carotid artery catheter. Replacement blood (6 mL whole rat blood obtained by decapitation was collected through gauze into tubes containing 4 mL saline and 1 mL heparin) was given (500 μL) via the carotid artery catheter every 30 minutes.

GLP-1(7-37) Administration in Fed and Fasted Sprague-Dawley Rats

Rats that were fasted had food removed at 4 to 5 PM the evening before the experiment. GLP-1(7-37) (5 pmol/min/kg) or vehicle (2% heat-inactivated serum plus 1% sodium acetate in normal saline) was infused via the jugular catheter for 60 minutes. Blood samples were taken from the carotid artery catheter at 0, 15, 30, and 60 minutes for plasma insulin and every 5 or 15 minutes for plasma glucose determinations. In a limited number of animals, a blood sample was taken into tubes containing EDTA and aprotinin at the end of GLP-1(7-37) infusion for analysis of GLP-1(7-37) concentration.

GLP-1(7-37) Administration During Hyperglycemia in Sprague-Dawley Rats

To evaluate further GLP-1(7-37) action during hyperglycemia, a hyperglycemic clamp at two sequentially increasing concentrations of glucose was performed. From 0 to 75 minutes, glucose was infused IV at a variable rate to maintain plasma glucose concentration at 11 mmol/L. From 75 to 120 minutes, the glucose infusion rate was increased to maintain plasma glucose at 17 mmol/L. GLP-1(7-37) (5 pmol/min/kg) or vehicle (2% heat-inactivated serum plus 1% sodium acetate in normal saline) was infused IV from 30 to 120 minutes. Other details of the clamp protocol were given earlier.

GLP-1(7-37) Administration in ZDF and Lean Control Rats

Fed ZDF and Zucker diabetic lean rats were infused for 2 hours with GLP-1(7-37) at 5 pmol/min/kg IV (because of limited availability of rats of this strain, vehicle-infused control groups were not possible in these studies). Blood samples were taken from the carotid artery catheter every 15 minutes for plasma glucose and at 0, 15, 30, 60, and 120 minutes for insulin determinations.

6-Hour Infusion of GLP-1(7-37) During an 11-mmol/L Hyperglycemic Clamp

Sprague-Dawley rats were infused with glucose IV at a variable rate for 7 hours to maintain plasma glucose concentration at 11 mmol/L. GLP-1(7-37) (5 pmol/min/kg) or vehicle (saline) was infused from 1 hour through 7 hours. Blood samples for glucose determination were taken every 10 minutes and samples for insulin determination were taken once per hour from the carotid artery catheter.

Long-Term Administration of GLP-1(7-37) by Minipump in Sprague-Dawley Rats

Alzet osmotic minipumps (Alza, Palo Alto, CA) containing GLP-1(7-37) (at a concentration to deliver 15 pmol/min/kg) or vehicle (0.1% Tween 80 in saline) were implanted subcutaneously at the time of catheterization surgery. On day 5, basal blood samples were obtained and a hyperglycemic clamp was initiated. Glucose was infused via the jugular vein catheter for 2 hours at a variable rate to maintain plasma glucose concentration at 11 mmol/L. Blood samples for determination of glucose were taken every 5 minutes and samples for determination of insulin were taken at 0, 15, 30, 90, and 120 minutes of the clamp. At the end of the experiment, pancreata were excised, immediately frozen in liquid nitrogen, and stored at −80°C until analysis. Insulin content was determined by radioimmunoassay (RIA) on neutralized acid-ethanol extracts of homogenized pancreas.

Analytical Methods

Plasma glucose level was determined either by the glucose oxidase method using a Beckman Glucose Analyzer 2 (Brea, CA) or spectrophotometrically (hexokinase and glucose-6-phosphate dehydrogenase) on an Abbott VP Supersystem (Abbott Park, IL). Plasma insulin and pancreatic insulin levels were determined using an insulin RIA kit (Binax, Portland, ME). Plasma GLP-1(7-37) concentration was determined on unextracted plasma by an RIA kit (Peninsula Laboratories, Belmont, CA). GLP-1(7-37) was obtained from Bachem (Torrance, CA).

Statistical Analysis

Paired and unpaired two-tailed t tests were used for statistical comparison of data. In the study involving multiple doses of GLP-1(7-37), regression analysis was used to determine dose-response significance.

RESULTS

Dose-Response of GLP-1(7-37) During Hyperglycemia in Sprague-Dawley Rats

To assess dose-response of GLP-1(7-37) under conditions of hyperglycemia, GLP-1(7-37) was infused during the second hour of an 11-mmol/L hyperglycemic clamp at doses of 0.5, 5, or 50 pmol/min/kg. GLP-1(7-37) produced a dose-related enhancement of the glucose-stimulated increase in plasma insulin concentration and an increased rate of glucose infusion (Table 1). In subsequent studies involving IV infusion, the submaximal dose of 5 pmol/min/kg was used.

Table 1. Plasma Insulin Concentration and Glucose Infusion Rate During an 11-mmol/L Hyperglycemic Clamp

Treatment Group	Basal Insulin (pmol/L)	Glucose Infusion Only, 60-Minute Insulin (pmol/L)	Glucose Infusion ± GLP-1(7-37), 75 to 120-Minute Insulin (pmol/L)	ΔInsulin (%)	ΔGlucose Infusion Rate (%)
Vehicle	185 ± 15	800 ± 160	935 ± 150	20 ± 6	3 ± 3
GLP-1(7-37), pmol/min/kg					
0.5	165 ± 15	700 ± 185	840 ± 180	24 ± 11	12 ± 8
5	210 ± 40	670 ± 80	1,120 ± 100	73 ± 17	44 ± 9
50	235 ± 40	660 ± 60	1,465 ± 260*	121 ± 35*	31 ± 11*

NOTE. Mean ± SEM (n = 3 to 5). Catheterized male Sprague-Dawley rats were infused with glucose at a variable rate for 120 minutes to maintain plasma glucose concentration at 11 mmol/L. Vehicle or GLP-1(7-37) was infused during the second hour of glucose infusion. The 75- to 120-minute insulin value was calculated by averaging insulin concentrations from samples taken at 75, 90, and 120 minutes. Glucose infusion rate was averaged over the last 30 minutes of the clamp and divided by the value at 60 minutes to obtain Δglucose infusion rate (glucose infusion rates at 60 minutes were 218 ± 15, 208 ± 11, 185 ± 10, and 190 ± 15 μmol/min/kg in rats infused with GLP-1(7-37) at 0, 0.5, 5, and 50 pmol/min/kg, mean ± SEM.

*Significant dose-dependent effect of GLP-1(7-37) by regression analysis ($P < .05$).

GLP-1(7-37) Administration in Fed and Fasted Sprague-Dawley Rats

Infusion of GLP-1(7-37) for 60 minutes produced a small transitory increase in plasma insulin concentration in fasted rats (from 100 ± 3 to 175 ± 30 pmol/L, $P < .05$) and fed rats (from 245 ± 40 to 340 ± 54, $P < .05$). In parallel with the increased insulin concentration, there was a slight transitory decrease in plasma glucose concentration (from 5.9 ± 0.1 to 5.3 ± 0.2 mmol/L in fasted rats, $P < .05$, and from 7.2 ± 0.2 to 5.8 ± 0.2 in fed rats, $P < .05$; Fig 1). GLP-1(7-37) concentration, measured in samples from a limited number of rats, was 180 pmol/L.

GLP-1(7-37) Administration During Hyperglycemia in Sprague-Dawley Rats

To examine further the glucose dependency of GLP-1(7-37), it was infused at 5 pmol/min/kg during a stepped 11/17-mmol/L hyperglycemic clamp. At both levels of glucose, GLP-1(7-37)–infused rats had approximately two-fold higher insulin concentrations relative to vehicle-infused rats. The incremental increases in insulin relative to levels in clamped control rats were 600 and 1,180 pmol/L at 11 and 17 mmol/L glucose, respectively (Table 2 and Fig 2). These increments were larger than those observed in fasted and fed rats (75- and 100-pmol/L increases in insulin, respectively, relative to basal values; Figs 1 and 2). The glucose infusion rate was higher in GLP-1(7-37)–infused rats at both levels of hyperglycemia (Table 2).

GLP-1(7-37) Administration in ZDF and Lean Control Rats

ZDF rats were hyperinsulinemic (800 ± 85 *v* 200 ± 20 pmol/L, $P < .05$) and modestly hyperglycemic (8.3 ± 0.4 *v* 6.8 ± 0.2 mmol/L, $P < .05$) relative to lean control rats. Infusion of GLP-1(7-37) in ZDF rats produced a transitory

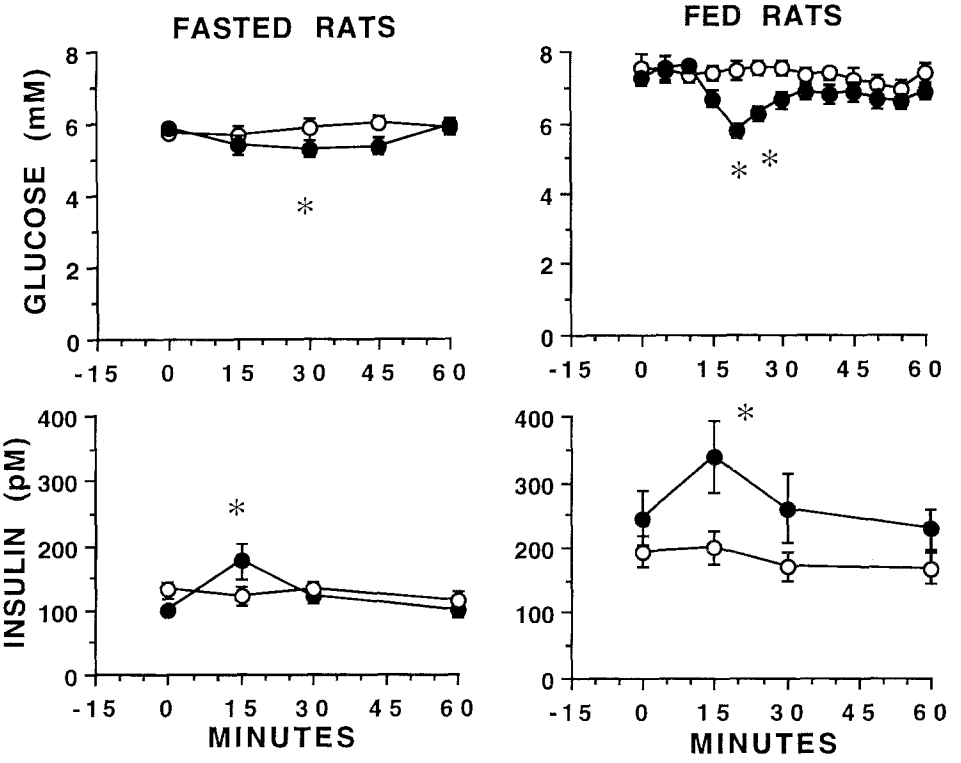

Fig 1. Plasma glucose and insulin concentrations in fasted and fed Sprague-Dawley rats infused with GLP-1(7-37) 5 pmol/min/kg IV (●) or vehicle (○) for 60 minutes. Mean ± SEM (n = 6). *Significantly different from basal value by paired *t* test ($P < .05$).

Table 2. Glucose Infusion Rate and Plasma Insulin Concentration in Sprague-Dawley Rats Infused With Vehicle or GLP-1(7-37) During an 11/17-mmol/L Hyperglycemic Clamp

Time (min)	Clamp Condition	Glucose Infusion Rate (μmol/min/kg)		Plasma Insulin Concentration (pmol/L)	
		Vehicle	GLP	Vehicle	GLP
30	11 mmol/L	143 ± 18	148 ± 5	655 ± 20	780 ± 60
60-75	11 mmol/L ± GLP	184 ± 20	281 ± 10*	700 ± 30	1,300 ± 100*
105-120	17 mmol/L ± GLP	331 ± 22	406 ± 13*	1,240 ± 125	2,420 ± 245*

NOTE. Mean ± SE (n = 5 to 6). Catheterized male Sprague-Dawley rats were infused with glucose at a variable rate to maintain plasma glucose at 11 (from 0 to 75 minutes) or 17 mmol/L (from 75 to 120 minutes). GLP-1(7-37) (5 pmol/min/kg) IV was infused from 60 to 120 minutes.
*Significant difference between GLP and corresponding vehicle value.

increase in plasma insulin concentration (which increased at the 15-minute time point only) and a sustained decrease in plasma glucose concentration (the lowest concentration was 5.5 mmol/L at 45 minutes of GLP-1(7-37) infusion). Plasma glucose concentration was similar to the level in lean rats throughout the 2 hours of GLP-1(7-37) infusion (Fig 3). In lean rats, there was a small decrease (5% to 15%, $P < .05$) in plasma glucose concentration relative to the basal level beginning at 15 minutes of GLP-1(7-37) infusion. There was no significant effect of GLP-1(7-37) infusion on plasma insulin concentration in lean rats. However, it is possible that there was a transitory increase in insulin level before the first sample taken at 15 minutes of GLP-1(7-37) infusion.

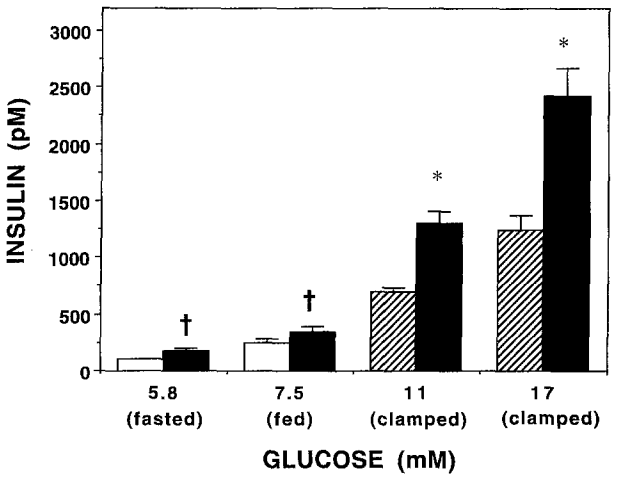

Fig 2. Insulin concentrations at different levels of glycemia in Sprague-Dawley rats infused with GLP-1(7-37) (■) or vehicle (▨) or in basal samples (□). Fasted or fed rats were infused with GLP-1(7-37) 5 pmol/min/kg for 1 hour. Data are from basal samples or samples taken at 15 minutes of GLP-1(7-37) infusion (for detailed time course, see Fig 1). Clamped rats were infused with glucose for 2 hours at a variable rate to maintain plasma glucose at sequentially increasing concentrations of 11 (from 0 to 75 minutes) or 17 mmol/L (from 75 to 120 minutes). During the 11/17-mmol/L hyperglycemic clamp, GLP-1(7-37) or vehicle was infused from 30 to 120 minutes. Data were calculated by averaging insulin values from samples taken at 60 and 75 minutes (11 mmol/L glucose) and 105 and 120 minutes (17 mmol/L glucose). Mean ± SEM (n = 6). †Significant effect of GLP-1(7-37) relative to corresponding basal value by paired *t* test ($P < .05$). *Significant effect of GLP-1(7-37) relative to corresponding control value by unpaired *t* test ($P < .05$).

6-Hour Infusion of GLP-1(7-37) During an 11-mmol/L Hyperglycemic Clamp

To assess whether GLP-1(7-37) could produce a sustained enhancement of glucose-stimulated insulin secretion in vivo, GLP-1(7-37) (5 pmol/min/kg IV) was infused for 6 hours during a 7-hour hyperglycemic clamp in Sprague-Dawley rats. GLP-1(7-37) infusion produced a sustained increase in plasma insulin concentration relative to levels in rats infused with vehicle (Fig 4). Plasma insulin concentration and glucose infusion rate were significantly higher in

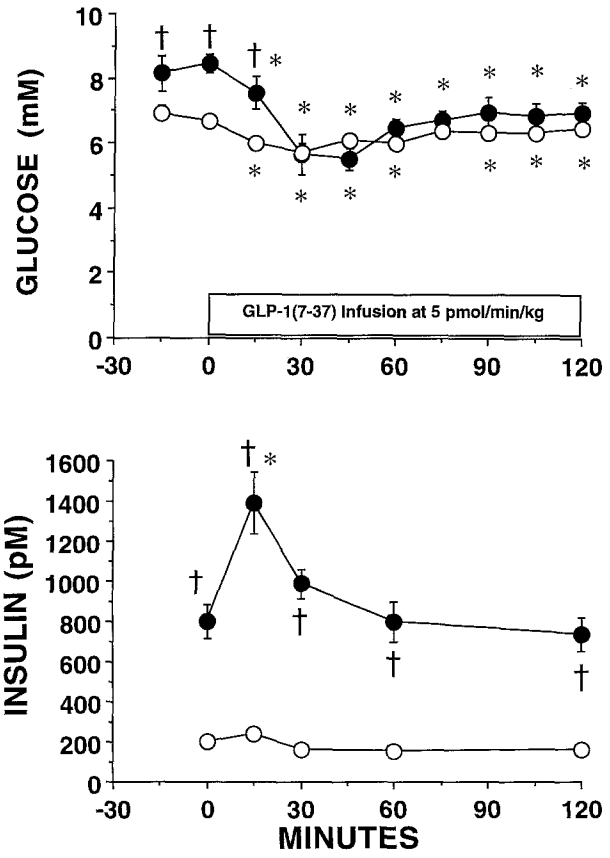

Fig 3. Plasma glucose and insulin concentrations in fed ZDF rats (●) and lean control rats (○) infused with GLP-1(7-37) 5 pmol/min/kg IV. Mean ± SEM (n = 6 to 8). Error bars that are not visible are encompassed within the symbol. *Significantly different from corresponding basal value by paired *t* test ($P < .05$). †Significantly different from corresponding value in lean rat ($P < .05$).

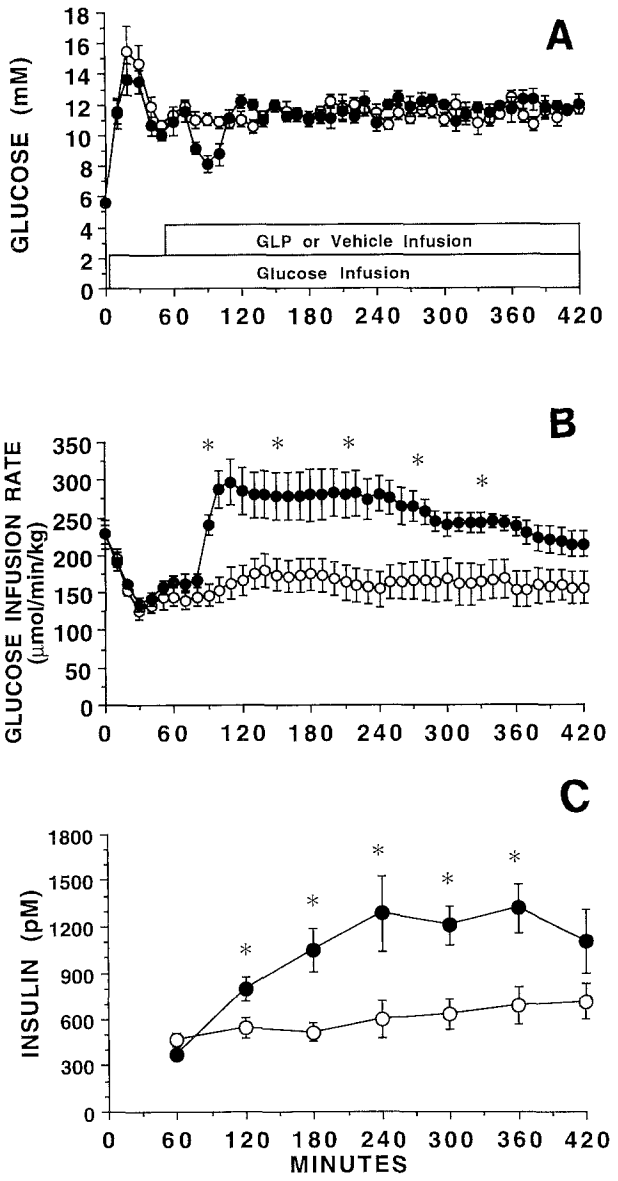

Fig 4. (A) Glucose concentration, (B) glucose infusion rate, and (C) plasma insulin concentration in Sprague-Dawley rats infused with GLP-1(7-37) 5 pmol/min/kg IV (●) or vehicle (○) for 6 hours beginning at 1 hour of a 7-hour hyperglycemic (11 mmol/L) clamp. Mean ± SEM (n = 5 to 6). *$P < .05$ v controls (for glucose infusion rate, values over each 60 minutes were averaged).

the GLP-1(7-37)–infused group for the first 5 hours of GLP-1(7-37) infusion.

Long-Term Administration of GLP-1(7-37) by Minipump in Sprague-Dawley Rats

Relative to control rats, basal plasma insulin levels were increased slightly in rats infused with GLP-1(7-37) for 5 days by minipump (240 ± 20 *v* 162 ± 5 pmol/L, $P < .05$), with no effect on plasma glucose concentration (7.7 *v* 7.5 mmol/L). During glucose infusion to maintain plasma glucose at 11 mmol/L, GLP-1(7-37)–treated rats had greater than 50% higher insulin concentrations than controls beginning at 60 minutes of glucose infusion (Fig 5). Consistent with the higher insulin level in GLP-1(7-37)–infused rats relative to controls, the glucose infusion rate during the 11-mmol/L hyperglycemic clamp was 33% ($P < .05$) higher. Long-term infusion of GLP-1(7-37) had no effect on 5-day food intake or pancreatic insulin content (Table 3). Additionally, there was no effect of GLP-1(7-37) administration on food intake on any day of the study (data not shown). In rats infused IV (5 pmol/min/kg) with infusate that was recovered from the minipumps and with glucose to maintain plasma glucose concentration at 11 mmol/L (for 2 hours), there was a 30% to 60% increase ($P < .05$) in insulin and a 34% increase ($P < .05$) in glucose infusion rate relative to clamped control rats infused with vehicle (data not shown). Hence, short-term administration of the minipump infusate produced an enhancement in the glucose-stimulated increase in insulin that was comparable to the long-term administration. Plasma GLP-1(7-37) concentrations were not measured in these studies, but it is likely that only a portion of the GLP-1(7-37) administered subcutaneously was absorbed,[24] and thus an IV dose of 5 pmol/min/kg is probably comparable to a 15-pmol/min/kg subcutaneous dose.

DISCUSSION

Several in vitro studies have demonstrated that the insulin-secretagogue activity of GLP-1(7-37) and GLP-1(7-36)-amide is glucose-dependent. In isolated islets and perfused pancreas, GLP-1(7-37) and GLP-1(7-36)-amide have no effect on insulin secretion at concentrations of glucose less than 2.8 mmol/L.[10,11] In pancreatic cell lines, a threshold for GLP-1(7-37)– and GLP-1(7-36)-amide–induced insulin secretion has also been demonstrated.[23] A variety of in vivo studies also suggest that the activity of GLP-1(7-37) and GLP-1(7-36)-amide is glucose-dependent; however, except in one study using a hyperglycemic clamp at one glucose concentration in humans,[21] the in vivo glucose dependency of GLP-1(7-37) and GLP-1(7-36)-amide has not been carefully assessed using matched

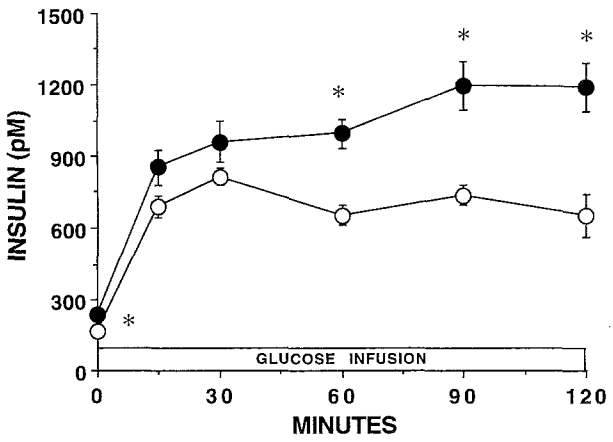

Fig 5. Basal and glucose-stimulated (11-mmol/L hyperglycemic clamp) insulin concentrations in Sprague-Dawley rats administered GLP-1(7-37) (15 pmol/min/kg subcutaneously) (●) or vehicle (○) by minipump for 5 days. Mean ± SEM (n = 8 to 9). *$P < .05$ v controls.

Table 3. Food Intake and Pancreatic Insulin Content in Sprague-Dawley Rats Administered GLP-1(7-37) or Vehicle for 5 Days by Minipump

Treatment Group	Food Intake (g/5 d)	Pancreatic Insulin Content (nmol/mg protein)
Vehicle	76 ± 4	390 ± 85
GLP-1(7-37)	74 ± 5	365 ± 30

NOTE. Mean ± SEM (n = 8 to 12).

plasma glucose concentrations. GLP-1(7-37) and GLP-1(7-36)-amide have been shown to decrease the glucose excursion after a meal or glucose challenge with plasma insulin concentrations that are lower, not different, or higher in GLP-1(7-37)– or GLP-1(7-36)-amide–treated subjects or animals relative to controls.[3,13-16,18-20] In other studies, GLP-1(7-36)-amide has been tested during a square-wave infusion of glucose, resulting in lower glucose concentrations with only slightly higher insulin concentrations relative to control.[17] In mice, high-dose GLP-1(7-36)-amide injections have been shown to increase basal insulin levels with no effect on glucose and to have an additive effect on glucose-stimulated increases in insulin.[3] Insulin-secretagogue activity and glucose dependence of GLP-1(7-37) and GLP-1(7-36)-amide in vivo have been difficult to discern because any decrease in plasma glucose concentration results in diminished GLP-1(7-37)– and GLP-1(7-36)-amide–induced insulin-secretagogue activity, and in studies involving oral glucose challenges, some of the effect of GLP-1(7-37) and GLP-1(7-36)-amide on plasma glucose may be due to an inhibitory effect on gastric emptying.[25] In the present studies, a submaximal dose of GLP-1(7-37) was tested at four different glucose concentrations (fasted 5.9 mmol/L, fed 7.2 mmol/L, clamped 11 mmol/L, and clamped 17 mmol/L) in normal rats and in a rat model of type II diabetes (8.3 mmol/L glucose). The approximate lower glucose concentration threshold for insulin-secretagogue activity appeared to be 5.3 mmol/L in fasted rats and 5.8 in both normal and diabetic fed rats. In diabetic rats infused with GLP-1(7-37), there was no evidence of insulin-secretagogue activity once plasma glucose concentration was normalized. In hyperglycemic clamp studies, the increase in insulin induced by GLP-1(7-37) was greater than in fed and fasted rats. Overall, the data demonstrate that the magnitude of insulin response was increased with the level of glycemia (Fig 2).

Because of its glucose-dependent secretagogue and glucose-lowering activity, GLP-1(7-37) may be useful as a therapeutic agent for the treatment of type II diabetes. However, sustained exposure may be needed for optimal control of blood glucose, since rebound hyperglycemia has been demonstrated in humans infused for short periods with GLP-1(7-37).[19] Acute homologous desensitization to GLP-1(7-37) has been demonstrated in insulinoma (HIT-T15) cells preperifused with GLP-1(7-37) 100 nmol/L for 5 minutes,[23] and this raises the possibility that tachyphylaxis may occur with long-term sustained exposure in vivo. The effects of long-term administration of GLP-1(7-37) and GLP-1(7-36)-amide have not been extensively studied in animal models or humans. Kawai and Ohashi[26] have reported that long-term (30-day) administration of GLP-1(7-36)-amide (by subcutaneous minipump) had no beneficial effect on oral glucose tolerance in normal rats and only a tendency for increased insulin and decreased glucose before and after an oral glucose challenge in diabetic rats, suggesting that desensitization had occurred. However, in perfused pancreata from long-term–infused rats, there was no evidence of impaired response to GLP-1(7-36)-amide. Additionally, they did not present data for acute effects of GLP-1(7-36)-amide on glucose tolerance. We have found that GLP-1(7-37) infusion in rats has no effect on glucose or insulin levels after a meal challenge (unpublished data, April-July, 1992), although it is reported to decrease glucose with no effect on insulin after an oral glucose tolerance test in rats.[16] In the present study, we have shown that GLP-1(7-37) is active in enhancing the glucose-induced increase in plasma insulin concentration for ≥ 5 hours when it is infused continuously during sustained hyperglycemia, suggesting that acute desensitization does not occur. Although plasma GLP-1(7-37) concentrations were not measured in these rats, in other studies this infusion rate of GLP-1(7-37) results in plasma concentrations of 180 pmol/L. It is possible that higher GLP-1(7-37) concentrations may induce desensitization. However, in human studies, low plasma concentrations of GLP-1(7-37) stimulate insulin secretion. Thus, it is unlikely that higher plasma concentrations of GLP-1(7-37) will be needed to obtain glucose-lowering activity in humans. In rats infused with GLP-1(7-37) for 5 days by minipump, there was a small increase in fed basal insulin concentration and a 50% to 80% enhancement of the glucose (given by IV infusion)-stimulated increase in plasma insulin concentration. Enhancement of the glucose-induced increase in insulin concentration was comparable to that seen with short-term infusion. The data suggest that the animals had not become completely desensitized to GLP-1(7-37). However, because we do not know the relative plasma concentrations for short- versus long-term administration, the possibility that some desensitization had occurred cannot be ruled out. The small increase in basal insulin in GLP-1(7-37)–infused rats, with no effect on glucose, suggests that some desensitization to insulin action may have occurred with long-term GLP-1(7-37) administration. However, relative to vehicle-infused rats, there was an increased glucose infusion rate during the hyperglycemic clamp comparable to the increased glucose infusion rate observed with short-term GLP-1(7-37) administration.

In addition to its insulin-secretagogue activity, GLP-1(7-37) and GLP-1(7-36)-amide have been shown to stimulate insulin biosynthesis, and therefore are considered to have insulinotropic activity.[7,27] They are also reported to have extrapancreatic effects, including inhibition of gastrin and gastric acid secretion and rate of gastric emptying.[25,28] There are GLP-1(7-37) and GLP-1(7-36)-amide receptors in various brain regions.[29] Central administration of truncated GLP in rats induces a decrease in food intake,[30] and studies with central injections of antibody suggest that GLP-1(7-37) and GLP-1(7-36)-amide may have a physiologic role in food intake.[31] In our studies, there was no

effect of long-term GLP-1(7-37) administration on food intake throughout the study. Thus, any potential intestinal or central effects did not impact food intake in this study. Based on pancreatic insulin content of rats treated with GLP-1(7-37) for 5 days, there did not appear to be an insulinotropic effect of GLP-1(7-37). However, because pancreatic insulin content does not necessarily reflect the rate of insulin biosynthesis, more detailed long-term dosing studies need to be performed to assess insulinotropic activity of GLP-1(7-37) and GLP-1(7-36)-amide in vivo.

In summary, the present studies demonstrate the glucose dependency of GLP-1(7-37) insulin-secretagogue activity in normal and diabetic rats and its euglycemic threshold of action. The long-term dosing studies indicate that in the rat, GLP-1(7-37) is active after many hours or days of sustained exposure.

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