Elimination of the Action of Glucagon-like Peptide 1 Causes an Impairment of Glucose Tolerance after Nutrient Ingestion by Healthy Baboons

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Abstract

Glucagon-like peptide 1 (GLP-1) is an insulinotropic hormone released after nutrient ingestion which is known to augment insulin secretion, inhibit glucagon release, and promote insulin-independent glucose disposition. To determine the overall effect of GLP-1 on glucose disposition after a meal we studied a group of healthy, conscious baboons before and after intragastric glucose administration during infusions of saline, and two treatments to eliminate the action of GLP-1: (*a*) exendin-[9-39] (Ex-9), a peptide receptor antagonist of GLP-1; or (*b*) an anti–GLP-1 mAb. Fasting concentrations of glucose were higher during infusion of Ex-9 than during saline (4.44±0.05 vs. 4.16±0.05 mM, $P < 0.01$), coincident with an elevation in the levels of circulating glucagon (96±10 vs. 59±3 ng/liter, $P < 0.02$). The postprandial glycemic excursions during administration of Ex-9 and mAb were greater than during the control studies (Ex-9 13.7±2.0 vs. saline 10.0±0.8 mM, $P = 0.07$; and mAb 13.6±1.2 vs. saline 10.6±0.9 mM, $P = 0.044$). The increments in insulin levels throughout the absorption of the glucose meal were not different for the experimental and control conditions, but the insulin response in the first 30 min after the glucose meal was diminished significantly during treatment with Ex-9 (Ex-9 761±139 vs. saline 1,089±166 pM, $P = 0.044$) and was delayed in three of the four animals given the neutralizing antibody (mAb 946±262 vs. saline 1,146±340 pM). Thus, elimination of the action of GLP-1 impaired the disposition of an intragastric glucose meal and this was at least partly attributable to diminished early insulin release. In addition to these postprandial effects, the concurrent elevation in fasting glucose and glucagon during GLP-1 antagonism suggests that GLP-1 may have a tonic inhibitory effect on glucagon output. These findings demonstrate the important role of GLP-1 in the assimilation of glucose absorbed from the gut. (*J. Clin. Invest.* 1996. 97: 133–138.) Key words: incretin hormone • enteroinsular axis • glucose tolerance • insulin secretion • glucagon

Introduction

Glucagon-like peptide 1(7-36)amide (GLP-1),[1] synthesized in and released from the mammalian intestinal tract, has a variety of actions consistent with a role in the regulation of carbohydrate metabolism (1, 2). GLP-1 is a potent insulinotropin (3, 4), and because its release is stimulated by ingested nutrients (5, 6), it has been proposed as a mediator of the incretin effect, namely the augmented insulin release by oral as compared with intravenous glucose (7–9). In addition, GLP-1 decreases glucagon secretion in vitro and in vivo (10, 11). These findings suggest that GLP-1 coordinates hormone secretion from the pancreatic islet in a manner that favors glucose anabolism. Studies in diabetic and healthy humans indicate that GLP-1 also promotes glucose disposition independent of islet hormone secretion (12, 13). In this context, transcription of the GLP-1 receptor gene in several nonislet tissues, including muscle, adipose tissue, and liver (14, 15), makes it plausible that GLP-1 has direct effects on glucose uptake and/or glucose production. Recent in vitro work demonstrating that GLP-1 increases glycogen synthesis in isolated hepatocytes and skeletal muscle supports this hypothesis (16, 17).

Although current information suggests that GLP-1 acts at multiple sites, the integrated effect of GLP-1 on carbohydrate metabolism has not been determined. This issue is important both in understanding normal and pathologic fuel metabolism. One of the classic paradigms of endocrinology has been the deduction of physiologic actions from the abnormalities observed in naturally occurring or induced hormone deficiency states. Recently, a GLP-1 receptor antagonist, exendin-[9-39] (Ex-9), has been shown to specifically block the actions of GLP-1 in tissue culture systems (18, 19). Two groups have used this peptide to inhibit the biologic effects of GLP-1 in rats before and for short periods after intraduodenal or oral glucose loads (20, 21). Insulin secretion was decreased and postprandial glycemia increased 30–45 min after the glucose load during treatment with Ex-9. Contemporaneous with these studies, we had independently initiated experiments to create functional deficiencies of GLP-1 in healthy conscious baboons to evaluate the role of this peptide in normal fuel homeostasis. We report herein the separate use of Ex-9 to block GLP-1 receptors, and a specific monoclonal antibody to immunoneutralize circulating GLP-1, to characterize the effects of elimination of this hormone on oral glucose tolerance.

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1. *Abbreviations used in this paper:* AIR_g, acute insulin response to glucose; Ex-9, exendin-[9-39]; GLP-1, glucagon-like peptide 1; IVGTT, intravenous glucose tolerance test; k_g, glucose disappearance constant; S_G, glucose effectiveness; S_I, insulin sensitivity index.

Methods

Materials. Ex-9 was synthesized by solid phase methods and purified by HPLC (18). Authenticity was determined by amino acid sequencing. A monoclonal antibody designated mAb 26.1 was generated from a hybridoma cell line that was a gift from Scios Nova Inc. (Mountain View, CA). This antibody specifically recognizes an epitope at the NH_2 terminus of GLP-1(7-36)amide (Jan Scardina, Scios Nova, personal communication). The immunoglobulin was partially purified by $(NH_4)_2SO_4$ precipitation, and the anti–GLP-1 antibody titer was determined. The GLP-1–immunoneutralizing capacity of mAb 26.1 was tested in rat islet monolayer cultures. 50 μg mAb 26.1 abolished the β cell stimulatory effect of 10^{-8} M GLP-1.

Animals. Five healthy male baboons, weighing 10–21 kg, were anesthetized with ketamine and halothane, and catheters were introduced into their femoral or subclavian venous system and into their stomach. The proximal ends of these catheters were tunneled subcutaneously and exited the skin on the animal's upper backs. The baboons were placed in jackets, and the catheters were passed through a tether which attached to the top of each animal's cage ($1 \times 1 \times 2$ m). The catheters eventuated in a swivel apparatus where the venous lines were connected to 150 mM saline and were accessed for blood sampling, and the gastric line was capped and available for infusions. This system permitted repeated studies in the baboons while they were awake and moving about their cages. The cages were housed in a room in the Regional Primate Research Center at the University of Washington. Animal care was provided by staff veterinarians and skilled animal technicians. Animal health was monitored by behavior, food intake, and intermittent measurements of electrolytes, blood cell counts, and blood cultures. The protocols were approved by the Institutional Animal Welfare Committee.

Protocols. Animals were studied after an overnight fast. In random order and on separate days, five baboons received continuous infusions of: (*a*) Ex-9 (150 nmol/kg/h) in 150 mM saline/0.1% HSA; or (*b*) saline/0.1% HSA, for the duration of the 450-min experiment. Three animals had the Ex-9 and the saline studies repeated three times each, and two animals twice each. At least 3 wk after the completion of the experiments with Ex-9 and saline, four of the animals were studied after receiving a 220-mg bolus of the anti–GLP-1 mAb; the fifth baboon had died of a surgical complication and could not be used in this protocol. Starting 20 min after the initiation of each treatment (Ex-9, mAb, or saline), basal blood samples were drawn every 2 min from −40 to 0 min. At 0 min an intragastric infusion of glucose (1.5 grams/kg) and D-xylose (0.5 gram) was given over 5 min. Postprandial blood samples were withdrawn periodically over the next 200 min. At 210 min a frequently sampled intravenous glucose tolerance test (IVGTT) was performed. The baboons received an intravenous infusion of glucose (3 grams/kg) followed 20 min later by an intravenous bolus of tolbutamide (125 mg/kg) and blood sampled for 210–390 min as previously described (22). The duration of each experiment was 7.5 h, and 90–100 ml of blood was removed from the animals on each day of study. The experiments were separated by at least 1 wk and no more than three experiments were performed on one animal per month.

Plasma analyses. Blood was collected into tubes containing heparin for analysis of glucose, insulin, and D-xylose, a benzamidine-based antiproteolytic cocktail (23) for glucagon measurement, and 0.5 M EDTA/500 KIU/ml aprotinin for assay of GLP-1. Samples were centrifuged immediately, and the plasma was removed and stored at −20°C. Plasma glucose was measured using a glucose oxidase method, and D-xylose by a colorimetric assay (24). Insulin and glucagon values were measured by previously described RIA (23, 25). GLP-1 was measured by RIA using antiserum 89390 (kindly provided by Dr. Jens Juul Holst, Paanum Institute, Copenhagen, Denmark), as described by Orskov et al. (26, 27), from ethanol extracts of plasma. Antiserum 89390 recognizes the COOH-terminal amidated arginine of GLP-1–related peptides and thus recognizes GLP-1(7-36)amide, the predominant bioactive moiety (27), but also the precursor GLP-1(1-36)amide (27), and the principal metabolite GLP-1(9-36)amide (28, 29). Ex-9 did not cross-react with either of the antisera used in the glucagon and GLP-1 RIAs. During the studies with the anti–GLP-1 mAb, plasma was extracted immediately into 70% ethanol to remove the circulating GLP-1–Ab complexes and to enable measurement of free GLP-1.

Data analyses. Fasting values were computed as the mean of the 21 premeal samples obtained in the Ex-9 and saline studies, and the 6 premeal samples taken after the bolus administration of mAb 26.1. The postprandial responses were defined by the incremental areas, above fasting levels, under the curves of glucose and insulin after the intragastric meal. These areas were calculated for each study by the trapezoidal method using values greater than fasting throughout the 180 min after intragastric glucose to calculate differences. Insulin increments after the glucose meal were also separated into 0–30- and 0–180-min time intervals to derive estimates of early and total insulin secretion throughout the meal disposition. The acute insulin response to glucose (AIR_g) was taken as the average incremental rise in insulin in the samples obtained from 2–10 min after the intravenous glucose bolus. The glucose disappearance constant (k_g) was calculated by determining the slope of the natural logarithm of the glucose values at 10, 12, 14, 16, and 19 min after the intravenous glucose. Insulin and glucose values obtained during the IVGTT were analyzed using the minimal model of glucose kinetics (30) to obtain the insulin sensitivity index (S_I) and glucose effectiveness (S_G).

The data from the multiple Ex-9 and saline studies were averaged for each animal. Comparisons of fasting, postprandial, and post-IVGTT values for the five animals receiving Ex-9 and the four animals given mAb 26.1 were made with their control (saline) studies using the *t* test for paired samples and the Wilcoxon signed ranks test; two-tailed analyses are reported in all cases. ANOVA for repeated measures was used to analyze GLP-1 levels before and after intragastric glucose in the different experimental groups. Data are presented as mean±SEM.

Results

Fasting concentrations of GLP-1 were similar on the days the baboons received Ex-9 or saline/HSA (5.0±0.5 vs. 6.1±0.5

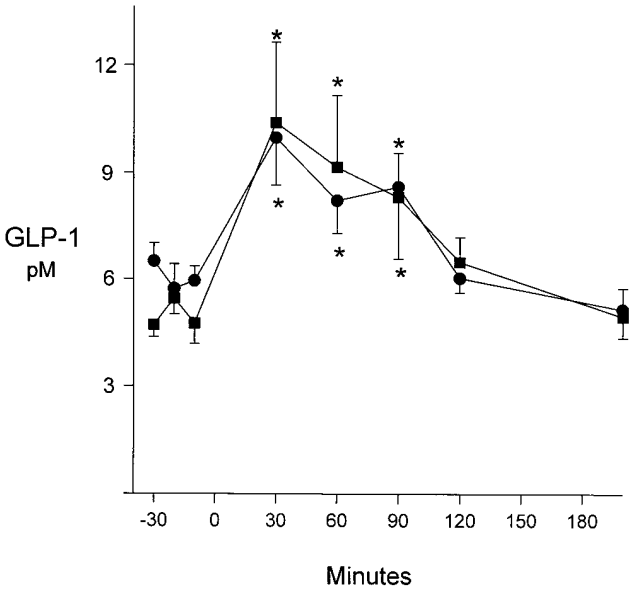

Figure 1. Mean concentrations±SEM of circulating GLP-1 before and after an intragastric glucose meal in five baboons treated with intravenous Ex-9 (*boxes*) or saline (*circles*). *$P < 0.05$ compared with fasting.

Table I. Fasting Concentrations of Insulin, Glucose, and Glucagon in Studies with Ex-9 and Saline Infusions

	Glucose	Insulin	Glucagon
	mM	*pM*	*ng/liter*
Saline	4.16±0.05	126±12	59±3
Ex-9	4.44±0.05*	102±12	96±10*

Results are expressed as mean±SEM. *$P < 0.02$ vs. saline. $n = 5$ pairs of studies.

pM). GLP-1 levels rose promptly after intragastric glucose in both sets of studies, remained significantly elevated for 90 min after the meal, and returned to preprandial concentrations by 120 min (Fig. 1). There were no differences in GLP-1 levels between the Ex-9 and saline experiments before or after glucose administration. Fasting GLP-1 concentrations in the four animals given mAb were slightly, but not statistically, higher than those during the saline studies in these animals (9.7±1.2 vs. 7.0±1.1, $P = 0.125$). Postprandial GLP-1 levels were not different than fasting after administration of mAb, with changes of 2.0±2.6, −0.4±1.9, and 0.1±1.8 pM at 30, 60, and 90 min, respectively. In contrast, GLP-1 concentrations in these baboons after intragastric glucose were significantly higher than fasting levels at 30, 60, and 90 min during saline infusion, with postprandial increments of 3.6±1.1, 2.5±0.8, and 2.8±1.1 pM ($P < 0.05$ at each time point). Thus, while there was a significant postprandial increment in GLP-1 with saline infusions, there was no detectable change in plasma GLP-1 in response to the glucose meal after mAb. This suggests that bioactive GLP-1(7-36)amide, the GLP-1 species released by nutrient stimulation (27), was removed from the circulation by the mAb.

During the infusion of Ex-9, mean fasting glucose concentrations were significantly higher in comparison with the controls (Table I). In addition, fasting glucagon levels were elevated when Ex-9 was infused. The fasting insulin concentrations were not different during the Ex-9 and control studies. Fasting insulin and glucose concentrations did not differ before and after the administration of mAb 26.1 to four baboons; glucagon concentrations were not measured in these studies.

After the glucose meal in the Ex-9 and saline studies the plasma glucose profiles rose to maximal levels in the first 40 min and returned to fasting values over ~ 120 min (Fig. 2 *A*). The postprandial glucose response was greater when the animals received Ex-9 compared with controls (Table II). Insulin

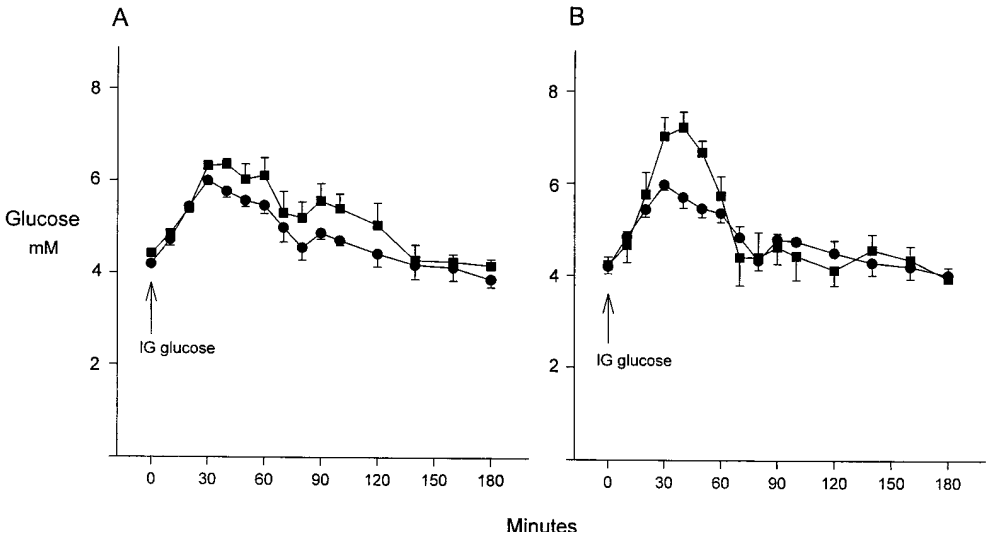

Figure 2. Mean levels±SEM of plasma glucose before and after a glucose meal in five baboons infused with saline (*circles*) or Ex-9 (*boxes*) (*A*) and in four baboons given anti–GLP-1 mAb 26.1 (*boxes*) or saline (*circles*) (*B*).

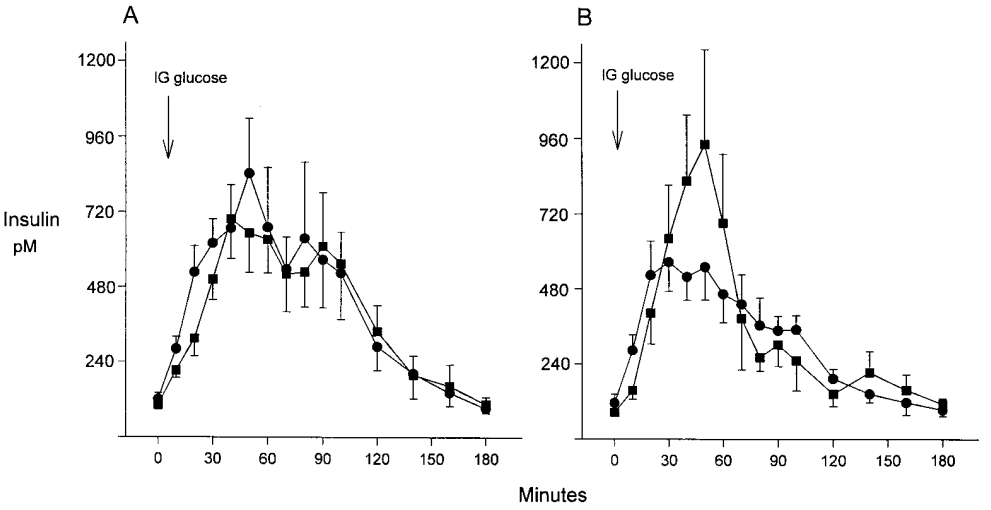

Figure 3. Mean plasma insulin concentrations± SEM before and after a glucose meal in five baboons treated with either Ex-9 (*boxes*) or saline (*circles*) (*A*) and in four baboons given anti–GLP-1 mAb 26.1 (*boxes*) or saline (*circles*) (*B*).

Table II. Postprandial Increments of Glucose and Insulin in Studies with Ex-9, mAb 26.1, and Controls (Saline)

	Glucose	Insulin (0–30)	Insulin (0–180)
	mM	*pM/30 min*	*pM/180 min*
Ex-9	13.7±2.0*	761±139‡	4270±1337
Control	10.0±0.8	1132±272	4952±1528
mAb	13.6±1.2‡	946±262	4302±1254
Control	10.6±0.9	1146±340	3648±1002

*$P = 0.07$, ‡$P < 0.05$ compared with controls.

Table III. Parameters of Intravenous Glucose Tolerance in Five Baboons Studied with and without Ex-9 and Four Baboons Studied with and without Anti–GLP-1 Ab (mAb)

	k_g	AIR_g	S_I	S_G
	%/min	*pM*	*$\times 10^{-5}$/min/pM*	*$\times 10^{-2}$/min*
Ex-9	2.45±0.03	48.6±5.7	13.5±6.8	3.9±0.8
Saline	2.54±0.1	49.4±5.9	9.1±4.1	4.7±0.7
mAb	2.69±0.3	46.5±12.1	10.4±3.8	4.2±0.6
Saline	2.68±0.1	49.5±7.0	10.0±5.2	4.5±0.3

Results are expressed as mean±SEM.

secretion followed a similar time course to glucose after the meal (Fig. 3 *A*), but in contrast to the differences in glycemic responses, the total incremental changes in insulin concentration were similar on the days the animals got Ex-9 and saline (Table II). However, the rise in insulin secretion was delayed when Ex-9 was administered as reflected in the smaller insulin increment in the first 30 min after the glucose meal (Table II).

After administration of the intragastric glucose in the paired mAb and control experiments, plasma glucose rose to peak levels within 30–40 min and returned to basal within 70 min (Fig. 2 *B*). The glycemic response was ~35% greater after immunoneutralization of GLP-1 than during the matched control experiments (Table II). Corresponding with this accentuated glycemic profile, the total insulin response was higher, but not significantly different, in the mAb versus control (Fig. 3 *B* and Table II). However, insulin release in the first 30 min after intragastric glucose was lower in three of the four baboons on the days they were immunoneutralized compared with control (Table II); the fourth animal had a very rapid rise in prandial glucose and a delayed insulin response was not detectable.

There were no differences in the plasma profiles of D-xylose between the Ex-9/control and mAb 26.1/control studies (Fig. 4), indicating that intestinal absorption of glucose was not significantly affected by the experimental conditions.

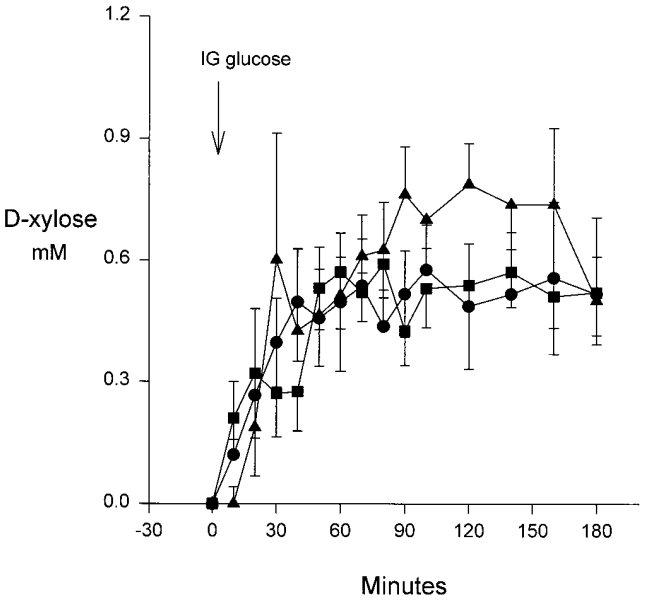

Figure 4. Plasma concentrations of D-xylose±SEM after an intragastric glucose/D-xylose meal in baboons during treatment with saline (*circles*), Ex-9 (*boxes*), or anti–GLP-1 mAb 26.1 (*triangles*).

The intravenous glucose tolerance (k_g) was not different from controls during the infusion of Ex-9 or after the administration of the monoclonal antibody (Table III). Similarly, AIR_g, S_I, and S_G did not differ among the experimental and control situations.

Discussion

These data are in keeping with an important role for GLP-1 in the disposition of glucose after eating. There were significant increases in postprandial glycemia during both competitive antagonism of GLP-1 receptors with Ex-9 and after immunoneutralization with mAb 26.1. We infer that circulating GLP-1-(7-36)amide was neutralized in the circulation by the mAb because it is the primary secreted GLP-1 peptide (27), and there was no change in the postprandial GLP immunoreactivity in these experiments. The presence of fasting amounts of GLP-1 immunoreactivity in the circulation after mAb treatment is likely due to other GLP-1 species, such as GLP-1(9-36) (28, 29), which are not recognized by mAb 26.1. We treated the animals with amounts of Ex-9 that were estimated to give a significant excess relative to circulating GLP-1. The concordance of our results, using two separate means to create functional GLP-1 deficiency, supports the conclusion that postprandial release of GLP-1 is an important factor regulating glucose disposition after nutrient ingestion. Moreover, the similarities in primate physiology make it likely that these observations are applicable to humans.

The alteration in intragastric glucose tolerance seen with both experimental methods to achieve functional GLP-1 deficiency could have been due to blunting of the insulinotropic effect of GLP-1 (3, 4), impairment of its extraislet action (12, 13), or a combination of the two. Based on this study it is not possible to distinguish between the relative contributions of these two putative mechanisms. Despite the greater glycemia with both Ex-9 and mAb, there were no quantitative differences in total postprandial insulin output between these experimental conditions and controls. It is likely that the comparable absolute amounts of insulin secreted reflect a compensatory β cell secretory response to the higher glucose levels occurring when GLP-1 activity was inhibited. Thus, in the absence of incretin-stimulated insulin secretion, postprandial glucose levels rose excessively, driving insulin release. This was best seen during immunoneutralization (Figs. 2 *B* and 3 *B*) when an accentu-

ated glucose excursion preceded a peak insulin release that was actually greater than that observed in the control studies.

Insulin release was delayed, as reflected by the lower insulin increments in the 30 min after intragastric glucose, in all five of the Ex-9 and three of four mAb experiments relative to controls. This is one potential explanation for the worsening of glucose tolerance during these studies. Insulin secretion in the first 30 min after oral glucose has been related to the acute insulin response to intravenous glucose (31, 32), and decrements in this early insulin response have long been used as a marker of insulin secretory abnormalities (32–34). The importance of the rate of insulin secretion in the regulation of postmeal glycemia has been emphasized frequently in the past (34–36) and is supported by our present results. Previous demonstrations of the rapid release of GLP-1 after meals (6, 37) are consistent with its acting in the early phases of nutrient disposition, and, taken together with the current findings, emphasize the contribution of GLP-1 to early insulin release in response to a meal. In vitro data indicate that GLP-1 induces glucose competence in β cells, heightening their response to increases in ambient glucose concentration (38). Thus, as has been reported for glucose-dependent insulinotropic polypeptide (GIP), GLP-1 may lower the threshold for glucose initiation of insulin secretion (39). It is reasonable to further propose that GLP-1 minimizes shifts in postprandial glycemia by amplifying the insulin response to absorbed nutrient stimuli.

Ex-9 has been used in two recent studies to block the action of GLP-1 during oral or intraduodenal glucose administration to rats (20, 21). In both of these reports, competitive antagonism of GLP-1 receptor binding caused an increased glycemic excursion in response to the carbohydrate meal and a significant reduction in total insulin release. The duration of the measurements was only for 30 and 45 min postcibum, so the decrease in insulin release was consistent with that seen in the 0–30-min postmeal in our baboons. It is possible that had these experiments been extended, the pattern of insulin release and the overall effect on glucose tolerance would have been similar to those reported here.

Competitive antagonism of GLP-1 with Ex-9 was associated with increased glucagon concentrations in the fasting state which coincided with a small increase in basal glucose concentrations, suggesting that the primary effect was an alteration of α cell output. Infusion of GLP-1 in physiologic amounts has been shown to decrease glucagon levels during hyperglycemic clamps (11), but the glucagonostatic effect of GLP-1 has not been shown at fasting concentrations. To our knowledge, this is the first demonstration of regulation of the islet by basal concentrations of a gastrointestinal hormone, and it raises the possibility that secretion of GLP-1 that occurs independently of acute nutrient ingestion may modulate release of an islet hormone without a concurrent change in substrate. Because we did not measure postprandial glucagon levels in these studies, we cannot comment on the possibility that augmented postmeal glucagon release contributed to the worsening of the glucose tolerance seen with the administration of Ex-9 and mAb. However, it is plausible that an alteration in α cell output caused by impaired GLP-1 action may have contributed to the higher glycemia in these studies.

There was no detectable effect of either Ex-9 or mAb on intravenous glucose tolerance. We have shown previously that, in healthy humans, infusion of GLP-1 to achieve either supraphysiologic or postprandial levels increases glucose disappearance and glucose effectiveness (13, 40). However, the IVGTTs in this study were performed at concentrations of GLP-1 that had returned to fasting levels (Fig. 1). Based on these data, it seems likely that the insulin-independent effect of GLP-1 to promote glucose disposition (12, 13, 40), either by stimulating glucose uptake or inhibiting hepatic glucose output, occurs only at the higher GLP-1 concentrations present after stimulated secretion. Furthermore, there was no evidence that Ex-9, nor mAb 26.1, has direct effects on insulin secretion or glucose disposition.

Previous work has indicated that GLP-1 may alter gastric emptying (41), an action that could contribute to its effect on glucose tolerance. It is unlikely that such an effect explains our results. First, the peak increase in plasma glucose after the meal was nearly identical in the Ex-9, mAb 26.1, and control studies, occurring at ~30–40 min postcibum. In addition, the plasma levels of D-xylose were not different among the control, Ex-9, and mAb conditions, suggesting that passage of the liquid meal, reflected in the absorption of this nonmetabolized sugar, was not increased when the GLP-1 effect was abolished. Despite these observations, we cannot conclude that GLP-1 does not play a role in gastric motility after eating. It is possible that had we interfered with the actions of GLP-1 after oral ingestion of a solid meal, and measured gastric motility directly, a difference from controls would have been seen.

Because GLP-1 affects several key sites in carbohydrate metabolism, there has been some enthusiasm for the therapeutic potential of this compound (42, 43). Although the present study examines physiologic effects of GLP-1, the results may have some applicability to the treatment of diabetes. It has been shown previously that subjects with type II diabetes are responsive to the insulinotropic action of GLP-1 (42, 43), so that delivery of this peptide before a meal might provoke the otherwise sluggish insulin response of these patients to ingested carbohydrate. Likewise, the action of GLP-1 to inhibit fasting glucagon secretion could be useful to restrain the high α cell secretion present in persons with type II diabetes (44). The data presented here also raise the question of whether or not abnormalities in GLP-1 secretion are involved in glucose intolerance. In one previous report, type II diabetic subjects were noted to have a higher GLP-1 response to ingested glucose than nondiabetic controls (45), but a subsequent study found no difference in the secretion of GLP-1 among similar groups (11). Little is known about the mechanisms triggering GLP-1 secretion, and although there is some variability in measured levels among individuals before and after meals naturally occurring GLP-1 deficiency states in humans have not been described. These important questions will require further study.

In summary, removal of the GLP-1 effect, either by competitive receptor blockade or immunoneutralization, causes a deterioration of intragastric glucose tolerance in nonhuman primates. This demonstrates that GLP-1 has a significant role in the disposition of glucose absorbed from the gut in a species closely related to humans. In addition, it appears that circulating GLP-1 has a regulatory effect on basal islet output of glucagon and consequently fasting glycemia. This finding raises the possibility that differences in basal and stimulated GLP-1 secretion among individuals may account for some of the variation in levels of glucose before, as well as after, eating. The results described here amplify the endocrine role of the gastrointestinal tract in fuel metabolism.

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