

Glucagon-like Peptide-1 Is a Physiological Incretin in Rat

Zhili Wang, Ren Ming Wang, Ali A. Owji, David M. Smith, Mohammad A. Ghatei, and Stephen R. Bloom

Division of Endocrinology, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 0NN, United Kingdom

Abstract

Glucagon-like peptide-1 7–36 amide (GLP-1) has been postulated to be the primary hormonal mediator of the enteroinsular axis but evidence has been indirect. The discovery of exendin (9–39), a GLP-1 receptor antagonist, allowed this to be further investigated. The IC_{50} for GLP-1 receptor binding, using RIN 5AH β -cell membranes, was found to be 0.36 nmol/l for GLP-1 and 3.44 nmol/l for exendin (9–39). There was no competition by exendin (9–39) at binding sites for glucagon or related peptides. In the anaesthetized fasted rat, insulin release after four doses of GLP-1 (0.1, 0.2, 0.3, and 0.4 nmol/kg) was tested by a 2-min intravenous infusion. Exendin (9–39) (1.5, 3.0, and 4.5 nmol/kg) was administered with GLP-1 0.3 nmol/kg, or saline, and only the highest dose fully inhibited insulin release. Exendin (9–39) at 4.5 nmol/kg had no effect on glucose, arginine, vasoactive intestinal peptide or glucose-dependent insulinotropic peptide stimulated insulin secretion. Postprandial insulin release was studied in conditioned conscious rats after a standard meal. Exendin (9–39) (0.5 nmol/kg) considerably reduced postprandial insulin concentrations, for example by 48% at 15 min (431 ± 21 pmol/l saline, 224 ± 32 pmol/l exendin, $P < 0.001$). Thus, GLP-1 appears to play a major role in the entero–insular axis. (*J. Clin. Invest.* 1995; 95:417–421.) **Key words:** insulin • glucose • glucagon • gastric inhibitory polypeptide • β -cell

Introduction

Glucose ingestion results in a greater elevation in plasma insulin levels than an equivalent plasma glucose concentration attained by the intravenous route. The augmented response to oral glucose defines the entero–insular axis, also referred to as the glucocretin effect. It is thought to be mediated in part by the release of gut hormones into the blood which potentiate glucose-induced β -cell insulin secretion (1–3). A number of gut peptides have been suggested as being insulinotropic, such as secre-

tin, gastrin-releasing peptide, vasoactive intestinal peptide (VIP),¹ and cholecystokinin (1–3). However, these peptides display their insulinotropic activity only at pharmacological plasma concentrations. The most likely physiological glucocretins that have been characterized are glucose-dependent insulinotropic polypeptide (GIP) (4, 5) and GLP-1 7–36NH₂ (GLP-1) (6–9).

GLP-1 is a member of an extended family of bioactive peptides, including glucagon, GIP, pituitary adenylate cyclase activating polypeptide (PACAP) and VIP, all of which have closely related amino acid sequences and can stimulate insulin secretion. The endocrine L cells of the intestine are the major site of synthesis of GLP-1, (10, 11), which is released principally in response to luminal carbohydrate and long chain fatty acids (12). In man, the infusion of GLP-1 to achieve the plasma GLP-1 concentrations seen after a meal, increases plasma insulin concentrations and decreases plasma glucagon and blood glucose (6). Thus, GLP-1 was proposed as one of the mediators of the enteroinsular axis in man (6).

Recently, two biologically active peptides, exendin 3 and 4, differing only at amino acid positions 2 and 3, have been isolated from helodermatidae venom (13, 14). Exendin 4 was found to bind avidly to the GLP-1 receptor and its fragment, exendin (9–39), to be an antagonist at this receptor (15, 16). The latter provided a potential tool to assess the importance of GLP-1 in the entero–insular axis. We determined the specificity and GLP-1 inhibitory ratio of exendin (9–39) using in vitro receptor studies. The in vivo action of exendin (9–39) on glucose-, arginine-, GIP-, and VIP-induced insulin release was examined in the anaesthetized rat. The effect of exendin (9–39) on postprandial insulin release was investigated in the conscious rat. Exendin (9–39) appeared an effective GLP-1 antagonist without action on arginine and glucose triggered insulin release or known insulin secretagogues other than GLP-1. A dramatic reduction of postprandial insulin release was seen in the conscious rat, strongly suggesting a physiological role for GLP-1 as a hormonal mediator of the entero–insular axis.

Methods

Receptor binding assays

Peptide iodination. All peptides were iodinated by the iodogen (1,3,4,6-tetrachloro-3 α ,6 α -diphenylglycoluril; Pierce, Rockford, IL) method (17). GLP-1 (3 nmol) (CRC, Harrow, UK) in 10 μ l of 0.2 M phos-

Address correspondence to Professor S. R. Bloom, Division of Endocrinology, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 0NN, U.K. Phone: 81-740-3242; FAX: 81-740-3142.

Received for publication 7 July 1994 and in revised form 12 October 1994.

J. Clin. Invest.

© The American Society for Clinical Investigation, Inc.

0021-9738/95/01/0417/05 \$2.00

Volume 95, January 1995, 417–421

1. **Abbreviations used in this paper:** GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1 (7–36) amide; IC_{50} , median inhibitory concentration; PACAP, pituitary adenylate cyclase activating peptide; VIP, vasoactive intestinal peptide.

phate buffer pH 7.2 was reacted with 10 µg of iodogen reagent and 37 MBq Na¹²⁵I (Amersham International, Little Chalfont, Amersham, Bucks, UK) for 2 min on ice. The reaction products were separated by HPLC on a C₁₈ reverse phase column (Waters Novapak; Millipore Corp., Milford, MA) using an 80 min 25–45% acetonitrile/water/0.05% trifluoroacetic acid gradient. Fractions showing binding were aliquoted, freeze-dried, and stored at –20°C.

Porcine glucagon (4 nmols) (Novo Nordisk Pharmaceutical Ltd, Crawley, West Sussex, UK) was iodinated as above. Porcine VIP (IAF, Laval, Quebec, Canada) and PACAP (Peptide Products, Southampton, Hants, UK) were iodinated by the same method with modifications as previously described (18, 19).

Membrane preparation. The glucose responsive β-cell line RIN 5AH was cultured in RPMI 1640 medium plus 5.5 mM glucose. Membranes were prepared by differential centrifugation as described previously (20). Briefly, osmotically lysed RIN 5AH cells or rat tissues were homogenised in ice-cold 50 mM Hepes pH 7.6, containing 0.25 M sucrose, 10 µg/ml soybean trypsin inhibitor, 0.5 µg/ml pepstatin, 0.25 µg/ml leupeptin and antipain, 0.1 mg/ml benzamidin, 0.1 mg/ml bacitracin, and 30 µg/ml aprotinin (all chemicals from Sigma, Poole, Dorset, UK). The homogenates were centrifuged at 1,500 g for 20 min at 4°C and the supernatants centrifuged at 100,000 g for 1 h at 4°C. The pellets were resuspended in 10 ml of the above buffer without sucrose and centrifuged at 100,000 g for 1 h at 4°C. The final pellets were resuspended to a protein concentration of 2–5 mg/ml, aliquoted and stored at –80°C.

Binding assays. GLP-1 binding assays were performed by modification of our previous methods (21). Briefly, ¹²⁵I-GLP-1 (750 Bq, specific activity = 8 Bq/fmol) was incubated with 200 µg of RIN 5AH membrane protein for 90 min at 22°C in a final volume of 0.5 ml of 25 mM Hepes pH 7.4 plus 2 mM MgCl₂, 0.1% bacitracin, 0.05% tween 20 and 1% bovine serum albumin (BSA). Non-specific binding was determined in the presence of 200 nM unlabeled GLP-1. Glucagon binding assays were performed by the method of Rodbell et al (22) using 100 µg of rat liver membrane protein and 1,000 Bq ¹²⁵I-glucagon in 0.5 ml of 20 mM Hepes pH 7.6, 1 mM EDTA, 0.02% bacitracin, 0.002% soybean trypsin inhibitor and 2.5% BSA. Incubations were for 20 min at 30°C. Nonspecific binding was determined in the presence of 1 µM unlabeled porcine glucagon. Binding of ¹²⁵I-VIP and ¹²⁵I-PACAP was assessed using previous methods (18, 19). Specific binding was defined as the difference between counts in the absence and presence of unlabeled peptide. Data from competition curves was analysed by nonlinear regression using Graph Pad Inplot 3.1 (Graphpad Software, San Diego, CA) to determine the median inhibitory concentration (IC₅₀).

Intravenous infusion of peptides in fasting anaesthetized rats

Male Wistar rats (200–250 g) were obtained from Bantin and Kingman (Hull, UK) and maintained on rat chow ad libitum. They were fasted from 17:00 on the day before study. On the study day general anaesthesia was induced by an intraperitoneal injection of 0.7 ml of an aqueous mixture of 1.25 mg/ml midazolam (Hypnovel, Roche, Welwyn Garden City, UK), 91 µg/ml fentanyl citrate and 2.5 mg/ml fluanisone (Hypnorm, Janssen, Oxford, UK), and maintained with further 0.25 ml doses. Cannulae (0.63 mm outer diameter; Portex, Hythe, UK) were placed in a femoral vein for infusion of peptides and in a femoral artery for blood sampling, and were kept patent with flushes of saline containing 50 U/ml heparin.

After a 20-min run-in period of a continuous infusion of saline, peptides were infused over 2 min in a solution of 1% glucose and 5% rat plasma in saline. Blood samples (0.5 ml) were taken at –5, 0, 2, 4, 6, 10, and 20 min. Blood glucose concentration was measured using Glucostix (Ames, Basingstoke, UK) and a Glucometer II (Ames). Plasma insulin concentrations were measured by radioimmunoassay, (23). The control experiment was identical to the peptide treated groups except that a test substance was not infused. A single administration was given to a single animal which was then killed.

Dose ranging study of GLP-1. GLP-1 was infused over 2 min as

above, at 0.1, 0.2, 0.3, and 0.4 nmol/kg. A dose response curve was plotted to gauge the approximate maximum effective dose.

Dose ranging study of exendin (9–39) with GLP-1. GLP-1 (0.3 nmol/kg) with exendin (9–39) (CRC, Harrow, UK) was infused over 2 min as above. Three different doses of exendin (9–39) were infused; 1.5, 3, and 4.5 nmol/kg. These doses were chosen as the RIN 5AH B-cells, GLP-1 receptor assay had shown a 10-fold lower affinity for exendin (9–39) than for GLP-1.

Effect of exendin (9–39) alone. Exendin (9–39) (4.5 nmol/kg) was infused over 2 min as above.

Interaction of exendin (9–39) with non-GLP-1 secretagogues. To determine the specificity of action of exendin (9–39); 50% glucose (0.8 g/kg), VIP (0.8 nmol/kg) or GIP (0.8 nmol/kg) were infused from 0 to 2 min, with and without exendin (9–39) (4.5 nmol/kg), as above. Arginine (375 mg/kg) was infused from 2 to 4 min after saline or exendin (9–39) (4.5 nmol/kg), which were infused from 0 to 2 min. Exendin (9–39) was pre-infused in this circumstance to prevent any possibility of the arginine reacting chemically with exendin (9–39).

Action of GLP-1 in trained conscious fed rats

In pilot studies untrained rats showed stress responses to injection which altered their feeding and therefore variably altered glucose and insulin levels. Male Wistar rats (200–250 g) were therefore trained for 10 d before experimentation. They were fasted from 17:00 to 09:00 and saline (0.3 ml) was injected subcutaneously at 09:00 before feeding. After injection of saline, rats were exposed to chow for 30 min and then the chow was removed. At the end of ten days the rats were accustomed to the injection and were conditioned to eating quickly (consuming 5 g standard chow, within 30 min). After fasting from 17:00 the night before rats were injected subcutaneously at 09:00 with either saline (control group, *n* = 40) or exendin (9–39) (0.5 nmol/kg, *n* = 20). This dose was chosen to mimic the ratio we had used in anesthetized animals taking into account the known naturally occurring postprandial concentration of GLP. After the injection 20 of the fasted control rats were killed (time minus 30 min). The exendin (9–39) treated group and the rest of the control group were exposed to food for 30 minutes, following which food was withdrawn, in an identical manner to the procedure carried out during training. 15 min and 45 min after the end of the meal 10 animals from each of the saline treated and exendin (9–39) treated groups were killed by intraperitoneal pentobarbitone overdose and blood rapidly collected by cardiac puncture. Measurements of plasma insulin, and blood glucose were made on the –30-, 15-, and 45-min blood samples. Plasma GLP-1 levels were measured in the 15-min samples.

Statistical analysis

All results are given as mean ± SEM. Statistical analysis was performed by one way analysis of variance for each peptide separately. The effect of the experimental peptides were compared to control rats using repeated measures ANOVA of the data (24). A *P* value of less than 0.05 was considered to be statistically significant.

Results

Receptor binding assay

Effects of GLP-1 and exendin (9–39) on ¹²⁵I-GLP binding in RIN 5AH membranes. Competition curves were constructed to determine the relative affinities of GLP-1 and its antagonist exendin (9–39) for GLP-1 receptors (Fig. 1). GLP-1 showed an IC₅₀ of 0.36 ± 0.04 nM for the GLP-1 receptor compared with an IC₅₀ of 3.44 ± 0.30 nM for exendin (9–39). Thus GLP-1 has an approximately 10-fold higher affinity than exendin (9–39) at this site.

Effects of exendin (9–39) on binding of peptides related to GLP-1. To investigate if exendin (9–39) was a specific antago-

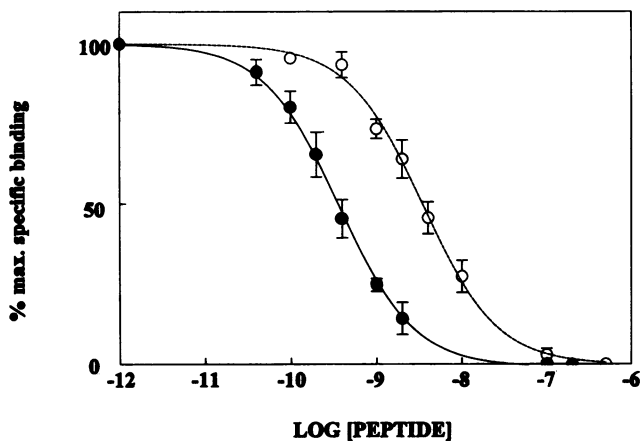


Figure 1. Competition of ^{125}I GLP-1 binding in RIN 5AH membranes by GLP-1 and exendin (9-39). Values are expressed as a percentage of maximal specific binding and are the mean of three experiments \pm SE with assays performed in triplicate. (●) GLP-1; (○) exendin (9-39).

nist for GLP-1 receptors, we examined its effect on binding of three related peptides which also increase insulin secretion from the β -cell, namely glucagon, VIP and PACAP. At concentrations of up to 10^{-6} M no competition by exendin (9-39) for binding sites of ^{125}I -labeled glucagon, VIP, or PACAP was observed (results not shown).

Fasted anaesthetized rats

Dose ranging study of GLP-1. There was no significant difference between baseline insulin levels in the five groups of animals. At 2 min after completion of the GLP-1 (0.1, 0.2, 0.3, and 0.4 nmol/kg) infusion there was a significant increase in plasma insulin (498 ± 48 , 925 ± 36 , 1784 ± 73 , and 2024 ± 47 pmol/l) as compared to control (214 ± 78 pmol/l, $P < 0.01$) (Fig. 2). The insulin levels remained elevated in a dose related manner at 4 and 6 minutes, but had returned to baseline levels by 10 min.

Dose ranging study of exendin (9-39) with GLP-1. There

was no significant difference between baseline insulin levels in the four groups of animals. When GLP-1 (0.3 nmol/kg) was infused in combination with three doses of exendin (9-39) (1.5 nmol/kg, 3.0 nmol/kg, 4.5 nmol/kg) there was a dramatic and highly significant dose-dependent decrease in insulin levels at 2 min (1288 ± 75 pmol/l, 416 ± 56 pmol/l, 233 ± 38 pmol/l, $P < 0.001$), compared with GLP-1 (0.3 nmol/kg) alone (1784 ± 73 pmol/l) (Fig. 2). This dose-dependent effect was similarly evident at 4 and 6 min.

Exendin (9-39) alone. There was no significant effect of exendin (9-39) (4.5 nmol/kg) on insulin levels when exendin (9-39) was infused alone (262 ± 48 pmol/l) compared with control (214 ± 35 pmol/l). The glucose concentrations did not alter.

Exendin (9-39) plus arginine or glucose. To investigate if there was any nonspecific effect of exendin (9-39) on β -cell function, arginine was infused with and without exendin (9-39). Arginine alone caused a significant increase in insulin concentration from a basal insulin value of 86 ± 41 to 1428 ± 178 pmol/l immediately after the infusion of arginine. The insulin concentration remained elevated for the following 16 min. When exendin (9-39) and arginine were infused there was no significant difference in the insulin response when compared to arginine alone, (peak insulin with exendin was 1440 ± 107 pmol/l compared with value above) (Fig. 3).

The glucose mediated increase in insulin peaked at 1346 ± 77 pmol/l compared with peak insulin level of 1399 ± 24 pmol/l with glucose plus exendin (9-39) (not significant).

Exendin plus peptide secretagogues (VIP, GIP). The co-administration of exendin (9-39) at 4.5 nmol/kg had no effect on the concentration or time course of the insulin response curve to GIP and VIP. Peak values at 2 min were, for GIP (0.8 nmol/kg) alone 1156 ± 27 pmol/l, GIP plus exendin (9-39) 1107 ± 38 pmol/l, VIP (0.8 nmol/kg) alone 969 ± 36 pmol/l, and VIP plus exendin (9-39) 961 ± 29 pmol/l (Fig. 2).

Conscious trained feeding rats

Blood glucose was significantly increased in the exendin (9-39) treated rats compared with the saline treated group at 15

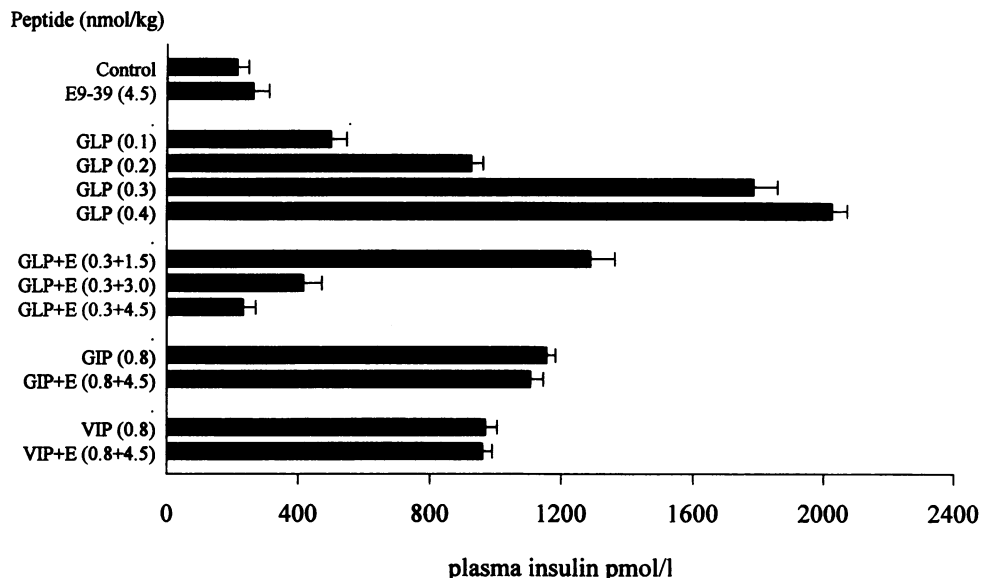


Figure 2. Insulin concentrations in fasted anaesthetized rats after 2-min peptide infusion. GLP-1 was infused in a dose ranging study at 0.1, 0.2, 0.3, and 0.4 nmol/kg, with the maximal insulin response seen at 0.4 nmol/kg. Exendin (9-39) was co-administered with GLP 0.3 nmol/kg in a dose ranging study at 1.5, 3.0, 4.5 nmol/kg with the maximal inhibitory dose seen at 4.5 nmol/kg. GIP and VIP, with or without co-administration of exendin (9-39) (4.5 nmol/kg), resulted in similar insulin concentrations.

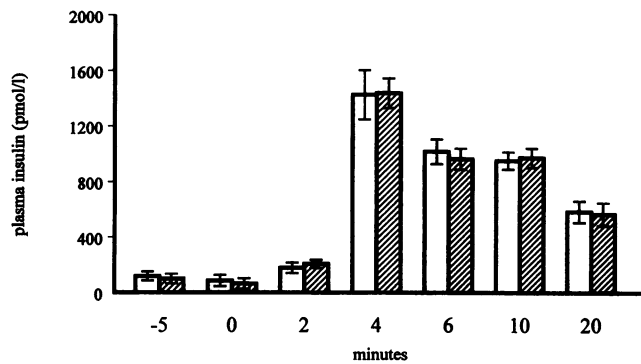


Figure 3. Insulin concentrations in fasted anaesthetized rats after a 2-min infusion of arginine with or without exendin (9–39). The insulin response to arginine 375 mg/kg (open bars), or iv exendin 4.5 nmol/kg plus arginine 375 mg/kg (hatched bars), was measured at –5, 0, 2, 4, 6, 10, and 20 min. There was no significant difference in the rise in plasma insulin concentrations.

min (9.9 ± 0.2 mmol/l vs. 8.6 ± 0.2 mmol/l, $P < 0.001$) and at 45 min (8.7 ± 0.19 mmol/l vs. 7.5 ± 0.1 mmol/l $P < 0.001$) (Fig. 4 a). The insulin level was significantly decreased in the exendin (9–39) treated group compared with the saline treated group at 15 min (224 ± 32 pmol/l vs 431 ± 21 pmol/l, $P < 0.001$) and at 45 min (208 ± 20 pmol/l vs 349 ± 38 pmol/l, $P < 0.01$) (Fig. 4 b). It should be noted that plasma GLP-1 levels at 15 min were unaffected by exendin (9–39) injection (control; 21.3 ± 2.3 pmol/l and exendin (9–39); 21.2 ± 5.0 pmol/l).

Discussion

The GLP-1 receptor antagonist exendin (9–39) allows investigation of the postulated role of GLP-1 in the entero–insular axis. The RIN 5AH cell GLP-1 receptor was used as a model of the islet β -cell GLP-1 receptor (15). Using specific receptor binding assays, GLP-1 exhibited an IC_{50} of 0.36 nM for its receptor, a concentration considered in the physiological range (0.2–0.6 nM) (6). Exendin (9–39) displaced GLP-1 from the receptor at $\sim 10\%$ of the potency of the natural ligand. Using exendin (9–39) concentrations 300 times greater than the IC_{50} for GLP-1 receptor binding there was no displacement of binding of other mooted insulin secretagogue receptors in the same peptide family, i.e., glucagon, VIP, and PACAP.

In the fasted anaesthetized rat model, exendin (9–39) alone had no effect on insulin levels, suggesting the absence of a partial agonist effect at the concentrations used here. It is possible that an agonist effect may become apparent at higher concentrations. However, exendin (9–39) had a dramatic effect on the action of GLP-1, completely abolishing the stimulation of insulin secretion at a concentration ratio of approximately 10:1. Exendin (9–39) did not influence glucose or arginine stimulated insulin secretion, suggesting that there was no non-specific effect on the β -cell. In the light of the known similarity of amino acid sequence of GIP and GLP-1 (25), and the proposed role of GIP in the entero–insular axis (4, 5) it was important to exclude any effect of exendin (9–39) on GIP-stimulated insulin secretion. Neither GIP nor VIP-stimulated insulin release was significantly influenced by concomitant administration of exendin (9–39), at a dose which completely abolished GLP-1 stimulated insulin release.

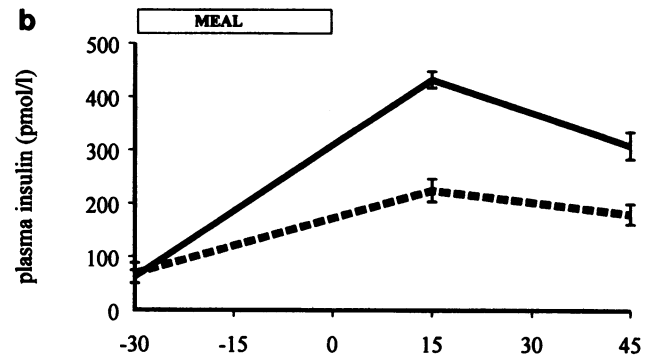
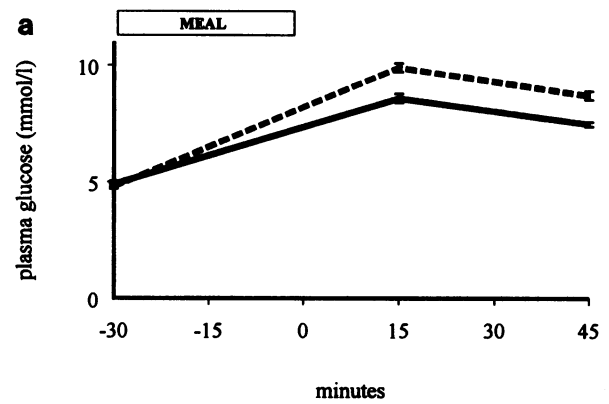


Figure 4. Insulin and glucose concentrations after a meal in conscious trained rats. All rats were injected with pre-meal subcutaneous saline for 10 d beforehand. On the day of the experiment exendin (9–39) treated animals (dotted line) were given pre-meal subcutaneous exendin (9–39) (4.5 nmol/kg) and control animals (solid line) received pre-meal subcutaneous saline. Glucose concentrations were significantly higher ($P < 0.001$) and insulin concentration were reduced in the exendin treated animals ($P < 0.001$ at 15 min and $P < 0.01$ at 45 min).

In the conscious trained rat exendin (9–39) treatment considerably reduced postprandial insulin concentrations compared with the saline treated group. Endogenous GLP-1 thus appears to contribute to the physiological release of insulin in the postprandial period. The lower insulin levels occurred in spite of the glucose concentrations in the exendin (9–39) treated rats being significantly higher than the control group. This tendency to glucose intolerance suggests GLP-1 may be required to maintain postprandial glucose concentrations at an optimal level.

GLP-1 has been shown to have three main physiological actions of relevance to postprandial glucose tolerance. It delays gastric emptying (26), inhibits glucagon release (6) and stimulates insulin release (6, 7, 27). The latter two actions depend on the ambient glucose concentration (7, 8, 28). The inhibition of glucagon is of importance when glucose is low (and perhaps in patients with diabetes who have abnormal glucagon release), and the stimulation of insulin release when glucose is elevated, as after a meal. In the conscious rat experiments reported here, the effect of exendin (9–39) would tend to accelerate gastric emptying and thereby enhance insulin release, for example by

means of a faster rise of glucose in the blood. While a larger glucose rise was seen, this was associated with a sustained reduction in insulin release, suggesting a predominant β -cell effect.

While there is no convincing evidence that postprandial GLP-1 is abnormal in patients with type II diabetes, GLP-1 may have therapeutic relevance. Trials of GLP-1 in the treatment of diabetes are under way and, at least acutely, GLP-1 appears to be effective at enhancing insulin release and significantly lowering blood glucose in patients with type II diabetes (27, 29).

Acknowledgments

We would like to thank P. Byfield for synthesis of GLP-1 and exendin (9-39) and A. Karlsen (Hagedorn Research Laboratory, Denmark) for the gift of the RIN 5AH cells.

This work was supported by grants from the Medical Research Council and the British Diabetic Association.

References

1. Unger, R. H., and A. M. Eisentraut. 1969. Entero-insular axis. *Arch. Intern. Med.* 123:261-266.
2. Ebert, R., and W. Creutzfeld. 1987. Gastrointestinal peptides and insulin secretion. *Diab. Metab. Rev.* 3:1-16.
3. Dupre, J. 1991. The endocrine pancreas. Raven Press, New York. 253 pp.
4. Dupre, J., S. A. Ross, D. Watson, and J. C. Brown. 1973. Stimulation of insulin secretion by gastric inhibitory polypeptide in man. *J. Clin. Endocrinol. & Metab.* 37:826-828.
5. Nauck, M., W. Schmidt, J. Ebert, J. Strietzel, P. Cantor, G. Hoffman, and W. Creutzfeldt. 1989. Insulinotropic properties of synthetic human gastric inhibitory peptide in man: interactions with glucose, phenylalanine, and cholecystokinin 8. *J. Clin. Endocrinol. & Metab.* 69:654-662.
6. Kreyman, B., G. Williams, M. A. Ghatei, and S. R. Bloom. 1987. Glucagon-like peptide-1 7-36: a physiological incretin in man. *Lancet.* 2:1300-1304.
7. Mojsov, S., G. C. Weir, and J. F. Habener. 1987. Insulinotropin: glucagon-like peptide I (7-37) co-encoded in the glucagon gene is a potent stimulator of insulin release in the perfused rat pancreas. *J. Clin. Invest.* 79:616-619.
8. Holst, J. J., C. Orskov, O. V. Nielsen, and T. W. Schwartz. 1987. Truncated glucagon-like peptide I, an insulin-releasing hormone from the distal gut. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 211:169-174.
9. Mojsov, S., M. G. Koczyński, and J. F. Habener. 1990. Both amidated and nonamidated forms of glucagon-like peptide I are synthesized in the rat intestine and the pancreas. *J. Biol. Chem.* 265:8001-8008.
10. Orskov, C., M. Bersani, A. H. Johnsen, P. Hojrup, and J. J. Holst. 1989. Complete sequences of glucagon-like peptide-1 from human and pig small intestine. *J. Biol. Chem.* 264:12826-12829.
11. Vardell, I. M., A. E. Bishop, K. L. Sikri, L. O. Uttenthal, S. R. Bloom, and J. M. Polak. 1985. Localization of glucagon-like peptide (GLP) immunoreactants in human gut and pancreas using light and electron microscopic immunocytochemistry. *J. Histochem. Cytochem.* 33:1080-1086.
12. D'Alesio, D., R. Thirlby, E. Laschansky, H. Zebroski, and J. Ensink. 1993. Response of tGLP-1 to nutrients in humans. *Digestion.* 54(6):377-379.
13. Eng, J., W. A. Kleinman, L. Singh, G. Singh, and J. P. Raufman. 1992. Isolation and characterization of exendin-4, an exendin-3 analogue, from *Heloderma suspectum* venom. Further evidence for an exendin receptor on dispersed acini from guinea pig pancreas. *J. Biol. Chem.* 267:7402-7405.
14. Malhotra, R., L. Singh, J. Eng, and J. P. Raufman. 1992. Exendin-4, a new peptide from *Heloderma suspectum* venom, potentiates cholecystokinin-induced amylase release from rat pancreatic acini. *Regul. Pept.* 41:149-156.
15. Thorens, B., A. Porret, L. Buhler, S. P. Deng, P. Morel, and C. Widmann. 1993. Cloning and functional expression of the human islet GLP-1 receptor. Demonstration that exendin-4 is an agonist and exendin-(9-39) an antagonist of the receptor. *Diabetes.* 42:1678-1682.
16. Goke, R., H. C. Fehmann, T. Linn, H. Schmidt, M. Krause, J. Eng, and B. Goke. 1993. Exendin-4 is a high potency agonist and truncated exendin-(9-39)-amide an antagonist at the glucagon-like peptide 1-(7-36)-amide receptor of insulin-secreting beta-cells. *J. Biol. Chem.* 268:19650-19655.
17. Fracker, P. J., and J. C. Speck. 1978. Protein and cell membrane iodinations with a sparingly soluble chloroamide, 1,3,4,6-tetrachloro-3 α ,6 α -diphenylglycoluril. *Biochem. Biophys. Res. Commun.* 80:849-857.
18. Suda, K., D. M. Smith, M. A. Ghatei, J. K. Murphy, and S. R. Bloom. 1991. Investigation and characterisation of receptors for PACAP (pituitary adenylate cyclase activating polypeptide) in human brain by radioligand binding and chemical cross-linking. *J. Clin. Endocrinol. & Metab.* 72:958-964.
19. Suda, K., D. M. Smith, M. A. Ghatei, and S. R. Bloom. 1992. Investigation of the interaction of VIP binding sites with VIP and PACAP in human brain. *Neurosci. Lett.* 137:19-23.
20. Bhogal, R., D. M. Smith, P. Purkiss, and S. R. Bloom. 1993. Molecular identification of binding sites for calcitonin gene-related peptide (CGRP) and islet amyloid polypeptide (IAPP) in mammalian lung: Species variation and binding of truncated CGRP and IAPP. *Endocrinology.* 133:2351-2361.
21. Kanse, S. M., B. Kreyman, M. A. Ghatei, and S. R. Bloom. 1988. Identification and characterisation of glucagon-like peptide-1 7-36 amide-binding sites in the rat brain and lung. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 241:209-212.
22. Rodbell, G., M. J. Krans, S. L. Pohl, and L. Birnbaumer. 1971. The glucagon-sensitive adenylyl cyclase system in plasma membranes of rat liver. III Binding of glucagon: method of assay and specificity. *J. Biol. Chem.* 246:1861-1871.
23. Albano, J. D. M., R. P. Ekins, G. Maritz, and R. C. Turner. 1972. A sensitive, precise radioimmunoassay of serum insulin relying on charcoal separation of bound and free moieties. *Acta Endocrinol.* 70:487-509.
24. Comparison of several groups. 1987. Armitage P, Berry G. Statistical Methods in Medical Research. 2nd ed. Blackwell, Oxford, England. 186-213.
25. Dockray, G. J. 1989. Handbook of Physiology. American Physiology Society, Bethesda, MD. 133 pp.
26. Wettergren, A., B. Schjoldager, P. E. Mortensen, J. Myhre, J. Christiansen, and J. J. Holst. 1993. Truncated GLP-1 (proglucagon 78-107-amide) inhibits gastric and pancreatic functions in man. *Dig. Dis. Sci.* 38:665-673.
27. Gutniak, M., C. Orskov, J. J. Holst, B. Ahren, and S. Efendic. 1992. Antidiabetogenic effect of glucagon-like peptide-1 (7-36)amide in normal subjects and patients with diabetes mellitus. *N. Engl. J. Med.* 326:1316-1322.
28. Fehmann, H. C., and J. F. Habener. 1992. Insulinotropic hormone glucagon-like peptide-1 (7-37) stimulation of proinsulin gene expression and proinsulin biosynthesis in insulinoma beta TC-1 cells. *Endocrinology.* 130:159-166.
29. Nauck, M. A., N. Kleine, C. Orskov, J. J. Holst, B. Willms, and W. Creutzfeldt. 1993. Normalization of fasting hyperglycaemia by exogenous glucagon-like peptide 1 (7-36 amide) in type 2 (non-insulin-dependent) diabetic patients. *Diabetologia.* 36:741-744.