Chronic treatment with exendin(9–39)amide indicates a minor role for endogenous glucagon-like peptide-1 in metabolic abnormalities of obesity-related diabetes in *ob/ob* mice

B D Green, N Irwin, V A Gault, C J Bailey¹, F P M O'Harte and P R Flatt

School of Biomedical Sciences, University of Ulster, Coleraine BT52 1SA, Northern Ireland, United Kingdom

¹School of Pharmaceutical and Biological Sciences, Aston University, Birmingham, UK

(Requests for offprints should be addressed to B D Green; Email: b.green@ulster.ac.uk)

Abstract

Glucagon-like peptide-1 (GLP-1) is a potent insulinotropic hormone proposed to play a role in both the pathophysiology and treatment of type 2 diabetes. This study has employed the GLP-1 receptor antagonist, exendin-4(9–39)amide (Ex(9–39)) to evaluate the role of endogenous GLP-1 in genetic obesity-related diabetes and related metabolic abnormalities using *ob/ob* and normal mice. Acute *in vivo* antagonistic potency of Ex(9–39) was confirmed in *ob/ob* mice by blockade of the insulin-releasing and anti-hyperglycaemic actions of intraperitoneal GLP-1. In longer term studies, *ob/ob* mice were given once daily injections of Ex(9–39) or vehicle for 11 days. Feeding activity, body weight, and both basal and glucose-stimulated insulin secretion were not significantly affected

by chronic Ex(9–39) treatment. However, significantly elevated basal glucose concentrations and impaired glucose tolerance were evident at 11 days. These disturbances in glucose homeostasis were independent of changes of insulin sensitivity and reversed by discontinuation of the Ex(9–39) for 9 days. Similar treatment of normal mice did not affect any of the parameters measured. These findings illustrate the physiological extrapancreatic glucose-lowering actions of GLP-1 in *ob/ob* mice and suggest that the endogenous hormone plays a minor role in the metabolic abnormalities associated with obesity-related diabetes.

Journal of Endocrinology (2005) **185**, 307–317

Introduction

The incretin hormone glucagon-like peptide-1 (GLP-1) has potent insulinotropic effects on pancreatic β -cells, and further promotes glucose lowering by enhancing glucose uptake and glyconeogenesis in peripheral tissues (Valverde *et al.* 1994, Villaneuva-Penacarrillo *et al.* 1994, O'Harte *et al.* 1997). In addition, GLP-1 is reported to significantly reduce food consumption through a central effect and by inhibition of gastric emptying, which ultimately generates weight loss (Turton *et al.* 1996, Larsen *et al.* 2001). Consequently, GLP-1 has been put forward as a potential drug candidate for type 2 diabetes mellitus, and GLP-1 analogues are currently undergoing clinical trials (Holz & Chepurny 2003).

Speculation about GLP-1 has brought renewed interest in the related peptide, exendin-4(1–39)amide (Ex(1–39)) (Eng *et al.* 1992) which binds and activates the GLP-1 receptor (Göke *et al.* 1993, Thorens *et al.* 1993). Ex(1–39) is 39 amino acid peptide produced in the salivary glands of *Heloderma suspectum* (the Gila monster) and which shares

similar insulinotropic and glucose-lowering actions with GLP-1 (Greig *et al.* 1999, Young *et al.* 1999). Unlike GLP-1, which is rapidly degraded, Ex(1–39) is resistant to the action of dipeptidyl peptidase IV (DPP IV) and consequently has a prolonged biological action (Elahi *et al.* 1999). Chronic administration of Ex(1–39) improves glucose control in diabetic *db/db* mice (Greig *et al.* 1999), and reduces food intake and weight gain in Zucker fatty rats (Szayna *et al.* 2000). An analogue of Ex(1–39), AC2933, is currently being tested as an anti-diabetic drug (Holz & Chepurny 2003). However, the fact that Ex(1–39) is not a human physiological hormone and may trigger the immune system is something which should not be overlooked.

Interestingly, a truncated form of Ex(1–39), exendin(9–39)amide (Ex(9–39)), serves as a strong antagonist of the GLP-1 receptor, blocking the cellular and metabolic effects of GLP-1 (Göke *et al.* 1993, Thorens *et al.* 1993, Kolligs *et al.* 1995, Green *et al.* 2004). Acute studies have used Ex(9–39) to determine the contribution of GLP-1 to the incretin effect in normal animals (Kolligs *et al.* 1995,

Figure 1 Acute effects of Ex(9–39) on GLP-1-induced glucose lowering and insulin release in *ob/ob* mice. Concentrations of (A) plasma glucose and (B) insulin prior to, and 15, 30 and 60 min after intraperitoneal administration of glucose alone (18 mmol/l per kg) or in combination with 25 nmol/kg GLP-1, 25 nmol/kg GLP-1 plus equimolar GLP-1 or 25 nmol/kg GLP-1 plus equimolar Ex(9–39). The time of injection is indicated by the arrows. Plasma glucose and plasma insulin AUC values for 0–60 min after injection are shown. Values are means $\pm$ S.E.M. for eight mice. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ compared with glucose alone. $\Delta P < 0.05$, $\Delta\Delta P < 0.01$ and $\Delta\Delta\Delta P < 0.001$ compared with GLP-1 plus GLP-1.

Tseng *et al.* 1999). These studies have revealed that GLP-1 is an important physiological insulin-releasing hormone, contributing 32–60% to the overall incretin effect.

The risk of developing type 2 diabetes, a disease clinically characterised by elevated blood glucose, impaired glucose tolerance, hyperglycaemia and insulin resistance, is greatly increased in obese individuals (Kolterman *et al.* 1981, Bogardus *et al.* 1985, Golay & Felber 1994). The role of the enteroinsular axis and GLP-1 in obesity-related diabetes remains uncertain (Ranganath *et al.* 1996, Flint *et al.* 2001, Vilsboll *et al.* 2003). However, recent opinion is that GLP-1 secretion may be disturbed, whereas action on pancreatic β -cells is normal (Mannucci *et al.* 2000, Vilsboll *et al.* 2001).

In this investigation Ex(9–39) was employed to molecularly disrupt the action of GLP-1 in adult obese diabetic (*ob/ob*) mice to assess the involvement of endogenous GLP-1 in the established hyperphagia, hyperinsulinaemia, glucose intolerance and related metabolic abnormalities of

this commonly employed model of type 2 diabetes (Bailey & Flatt 2003). Ex(9–39) was administered once daily for 11 days and the effects on feeding activity, body weight, glucose tolerance, pancreatic β -cell function and insulin sensitivity were recorded. These measurements were repeated after a 9-day recovery period to evaluate the effects of alleviation of GLP-1 receptor antagonism. Comparative studies were conducted in normal mice.

Materials and Methods

Reagents

Ex(9–39) was a gift from Dr Andrew Young (Amylin Corporation, San Diego, CA, USA). Na¹²⁵I for iodination of insulin was obtained from Amersham International plc (Amersham, Bucks, UK). Bovine insulin, dextran-T70 and activated charcoal were obtained from Sigma (Poole, Dorset, UK). All other chemicals used were of the utmost purity available.

Figure 2 Food intake, body weight and plasma glucose and insulin concentrations of *ob/ob* mice receiving 11 daily injections of either saline or Ex(9–39). (A) Body weight, (B) food intake, (C) plasma glucose and (D) plasma insulin concentrations were measured for 5 days prior to, for 11 days during (indicated by solid bars) and for 9 days after treatment with saline or Ex(9–39) (25 nmol/kg body weight). Values are means $\pm$ S.E.M. for eight mice. * $P < 0.05$ compared with saline group.

Animals

The genetic background and characteristics of the *ob/ob* (*Lep^{ob}/Lep^{ob}*) mouse colony and normal control homozygous (+/+) mice have been outlined elsewhere (Bailey *et al.* 1982). Briefly, heterozygous C57BL/6J *ob/+* breeding pairs from the Jackson Laboratory, Bar Harbor, Maine, USA were obtained by the Institute of Animal Genetics, University of Edinburgh in 1957 and out-crossed to non-inbred local strains: JH for higher litter size and CRL for higher growth rate. Heterozygous breeding pairs from this stock were obtained by Aston University in 1966 where they have been maintained in a closed non-inbred colony. The Aston colony of (*ob/ob*) mice display hyperphagia, marked hyperinsulinaemia, islet hypertrophy and moderate hyperglycaemia (Bailey & Flatt 1995).

Animals, aged 15–19 weeks, were housed individually in an air-conditioned room at $22 \pm 2^\circ\text{C}$ with a 12 h light:12 h darkness cycle. Drinking water and a standard

rodent maintenance diet (Trouw Nutrition Ltd, Cheshire, UK) were freely available. All animal experiments were carried out in accordance with the UK Animals (Scientific Procedures) Act 1986. No adverse effects were observed following administration of saline or Ex(9–39).

Acute effects of Ex(9–39) on GLP-1-mediated glucose lowering and insulin release

To confirm that Ex(9–39) acted as a functional GLP-1 receptor antagonist in this group of *ob/ob* mice, acute experiments were performed. Fasted (18 h) *ob/ob* mice were administered intraperitoneally with glucose alone (18 mmol/kg body weight) or in combination with 25 nmol/kg GLP-1 or 25 nmol/kg GLP-1 plus an equivalent dose of either GLP-1 or Ex(9–39). Plasma glucose and insulin levels were measured in blood samples taken prior to and at 15, 30 and 60 min after injection.

Figure 3 Food intake, body weight and plasma glucose and insulin concentrations of normal mice receiving 11 daily injections of either saline or Ex(9–39). (A) Body weight, (B) food intake, (C) plasma glucose and (D) plasma insulin concentrations were measured for 5 days prior to, for 11 days during (indicated by solid bars) and for 9 days after treatment with saline or Ex(9–39) (25 nmol/kg body weight). Values are means $\pm$ S.E.M. for eight mice.

Long-term effects of Ex(9–39) on metabolism

Groups of *ob/ob* mice and normal control mice received once daily subcutaneous injections (1700 h) of either saline (0.9% (w/v) NaCl) or Ex(9–39) (25 nmol/kg in saline) over an 11-day period. The animals were also monitored for 9 days after the cessation of treatment. Food intake and body weight were recorded daily. Blood samples were collected on days 0, 1, 3, 7, 11, 14 and 20 (0900 h) from the cut tail tips of conscious mice. Glucose tolerance (18 mmol/kg, intraperitoneally), meal tolerance (15-min refeeding after an 18-h fast) and insulin sensitivity (50 U/kg, intraperitoneally) tests were conducted on day 11 and day 20. Blood samples were collected at the times indicated in the figures into chilled fluoride/heparin-coated microcentrifuge tubes (Sarstedt, Nümbrecht, Germany) and centrifuged (30s at 13 000g) using a Beckman microcentrifuge (Beckman Instruments, Palo Alto, CA, USA). The resulting plasma was then aliquoted into fresh Eppendorf tubes and stored at -20°C prior to analysis.

Analyses

Plasma glucose was assayed by an automated glucose oxidase procedure using a Beckman glucose analyser II (Stevens 1971). Plasma insulin was assayed by a modified dextran–charcoal radioimmunoassay (Flatt & Bailey 1981). *In vivo* data were compared using ANOVA, followed by the Student–Newman–Keuls post hoc test. Area under the curve (AUC) analysis employed the trapezoidal rule (Burington 1973). Groups of data from both were considered to be significantly different if $P < 0.05$.

Results

Acute Ex(9–39) antagonism of GLP-1 action

Figure 1 shows the plasma glucose and insulin responses of *ob/ob* mice to glucose alone or in combination with either GLP-1 plus equipotent GLP-1 or Ex(9–39). As expected, administration of GLP-1 markedly decreased the glycaemic excursion (Fig. 1A) and enhanced insulin concentrations (Fig. 1B) compared with glucose alone.

Figure 4 Long-term effects of Ex(9–39) treatment on glucose and insulin responses to intraperitoneal glucose administration in *ob/ob* mice. (A) Plasma glucose and (B) plasma insulin concentrations were measured prior to and at intervals after intraperitoneal administration of glucose (18 mmol/kg body weight) after 11 days of treatment with Ex(9–39). The time of injection is indicated by the arrows. Plasma glucose and plasma insulin AUC values for 0–60 min after injection are shown. Values are means $\pm$ S.E.M. for eight mice. * $P < 0.05$ compared with saline.

These actions of GLP-1 were substantially blocked by the established GLP-1-receptor antagonist, Ex(9–39) (Fig. 1A and B).

Long-term effects of Ex(9–39)

Figure 2 shows the effects of long-term Ex(9–39) treatment on body weight, food intake and plasma concentrations of glucose and insulin in *ob/ob* mice. Ex(9–39) had no significant effect on either body weight or food intake (Fig. 2A and B). However, on day 11 of treatment basal plasma glucose levels were significantly raised in mice treated with Ex(9–39), compared with those treated with saline ($P < 0.05$; Fig. 2C). Cessation of treatment returned glucose to pretreatment levels. No significant changes in plasma insulin levels were noted during or after the treatment period, although there appeared to be a trend towards decreased concentrations with Ex(9–39) (Fig. 2D). Treatment of normal mice with Ex(9–39) did not affect any of the parameters measured (Fig. 3).

Long-term effects of Ex(9–39) treatment on glucose tolerance

Figure 4 shows the effects of intraperitoneal glucose administration (18 mmol/kg) on glucose and insulin levels in *ob/ob* mice treated for 11 days with either saline or Ex(9–39). Glucose levels in Ex(9–39)-treated mice were significantly elevated 0, 15, 30 and 60 min after injection compared with mice treated with saline alone (Fig. 4A). This was confirmed by a significantly increased AUC value ($P < 0.05$; Fig. 4A). No significant changes in glucose-mediated insulin release were noted in mice treated with Ex(9–39) compared with those treated with saline (Fig. 4B). As shown in Fig. 5, no significant changes in plasma glucose or insulin levels were evident in *ob/ob* mice 9 days after cessation of Ex(9–39)-treatment. Treatment of normal mice with Ex(9–39) for 11 days did not significantly affect glycaemic or insulin responses to intraperitoneal glucose (Fig. 6). Responses of normal mice were identical 9 days following cessation of treatment (data not shown).

Figure 5 Reversal of long-term effects of Ex(9–39) treatment on glucose and insulin responses to intraperitoneal glucose in *ob/ob* mice. (A) Plasma glucose and (B) plasma insulin concentrations were measured prior to and at intervals after intraperitoneal administration of glucose (18 mmol/kg body weight) 9 days after cessation of Ex(9–39) treatment. The time of injection is indicated by the arrows. Plasma glucose and plasma insulin AUC values for 0–60 min after injection are shown. Values are means $\pm$ S.E.M. for eight mice.

Long-term effects of Ex(9–39) treatment on metabolic response to feeding

Figure 7 shows the glucose and insulin responses of fasted (*ob/ob*) mice to feeding after 11 days of treatment with Ex(9–39). An allowed period of 15 min of feeding caused significant rises in both plasma glucose (Fig. 7A) and insulin (Fig. 7B). However, responses in saline-treated mice and Ex(9–39)-treated mice were not significantly different. Food intake during the 15-min period was consistent with these data, showing no significant difference in saline-treated (0.5 ± 0.1 g/mouse per 15 min) and Ex(9–39)-treated (0.6 ± 0.1 g/mouse per 15 min) mice. Similarly 11 days of treatment with Ex(9–39) did not affect glucose or insulin responses to feeding in normal mice (Fig. 8). Food intake was 0.5 ± 0.1 g/mouse per 15 min and 0.5 ± 0.1 g/mouse per 15 min for control and Ex(9–39) respectively.

Long-term effects of Ex(9–39) treatment on insulin sensitivity

Figure 9 shows the effect of intraperitoneal administration of insulin on glucose levels in *ob/ob* mice (Fig. 9A) and

normal mice (Fig. 9B) following 11 days of treatment with Ex(9–39). No significant changes in insulin-induced glucose lowering were observed in animals receiving treatment or 9 days following the cessation of treatment (data not shown).

Discussion

Ex(1–39) is a 39 amino acid peptide isolated from *Heterodermia suspectum* (Gila monster) venom (Eng *et al.* 1992). It later transpired that Ex(1–39) was a highly potent agonist of the GLP-1 receptor (Thorens *et al.* 1993). Furthermore, a shortened form of this peptide, Ex(9–39), possessed significant antagonistic activity for this receptor (Göke *et al.* 1993, Thorens *et al.* 1993). These findings have been confirmed many times in acute tests involving rat GLP-1 receptors, humans (Schirra *et al.* 1998, Edwards *et al.* 1999) and various non-diabetic and diabetic animal models (Kolligs *et al.* 1995, Wang *et al.* 1995, Gault *et al.* 2003, Green *et al.* 2004).

Although many studies have demonstrated acute antagonistic actions of Ex(9–39), the chronic effects of Ex(9–39)

Figure 6 Long-term effects of Ex(9–39) treatment on glucose and insulin responses to intraperitoneal glucose administration in normal mice. (A) Plasma glucose and (B) plasma insulin concentrations were measured prior to and at intervals after intraperitoneal administration of glucose (18 mmol/kg body weight) after 11 days treatment with Ex(9–39). The time of injection is indicated by the arrows. Plasma glucose and plasma insulin AUC values for 0–60 min after injection are shown. Values are means $\pm$ S.E.M. for eight mice.

administration on insulin secretion and glucose homeostasis in animal models with established obesity-related diabetes such as *ob/ob* mice have not been evaluated. Given the beneficial effects of chronic administration of the GLP-1 receptor agonist, Ex(1–39), in *db/db* mice (Greig *et al.* 1999, Szayna *et al.* 2000), plus the role of GLP-1 in the enteroinsular axis, we anticipated that chronic administration of Ex(9–39) would have quite profound effects in *ob/ob* mice. This animal model is characterised by a less severe form of diabetes than *db/db* mice due to metabolic compensation through islet hypertrophy and marked hyperinsulinaemia (Bailey & Flatt 2003). In initial studies, we confirmed that this mutant displayed prominent insulin secretory and anti-hyperglycaemic responses to GLP-1 as described elsewhere (Green *et al.* 2004). Furthermore, administration of Ex(9–39) reduced the acute glucose-lowering action of GLP-1 by 70%, while the insulin-releasing action of GLP-1 was almost completely abolished (reduced by 95%). These results corroborated the findings of earlier investigations (Kolligs *et al.* 1995, Schirra *et al.* 1998, Edwards *et al.* 1999, Tseng *et al.* 1999, Green *et al.* 2004).

Administration of Ex(9–39) once daily for 11 days to *ob/ob* mice had no effect on feeding activity or body weight. Since GLP-1 is believed to exert satiety effects which can be reversed by Ex(9–39) in other species (Schick *et al.* 2003), this suggests that endogenous circu-

lating GLP-1 has no such role in *ob/ob* mice. However, the present study did not note an inhibitory effect of Ex(9–39) on feeding in normal mice. Consistent with a limited physiological role for circulating GLP-1 in *ob/ob* mice, basal and glucose-stimulated insulin secretion were not significantly changed by chronic Ex(9–39) treatment. However, small impairments in glucose homeostasis were noted, namely elevated basal glucose and impaired glucose tolerance after 11 days. These effects were independent of changes in insulin sensitivity and reversed by discontinuation of Ex(9–39) for 9 days.

In contrast to *ob/ob* mice, administration of Ex(9–39) to normal mice for 11 days was without affect on glucose homeostasis or any of the other parameters measured. This showed that the modest effects in *ob/ob* mice cannot be directly attributed to leptin deficiency. These general observations have parallels with GLP-1 receptor knockout (GLP-1R $-/-$) mice which exhibit only a modest intolerance to glucose (Scrocchi *et al.* 1996, Flamez *et al.* 1998, Scrocchi & Drucker 1998). In these transgenic animals it appears likely that there was a compensatory increase in the glucose-dependent insulinotropic polypeptide (GIP) arm of the enteroinsular axis from early life (Pederson *et al.* 1998). Although a similar adaptive response might have been induced very rapidly by the present 11-day administration of Ex(9–39), there is presently no commercial assay to specifically measure active

Figure 7 Long-term effects of Ex(9–39) treatment on glucose and insulin responses to feeding in *ob/ob* mice. Following the 11-day treatment period mice were fasted (18 h) overnight. At 0900 h, free access to food was allowed for 15 min (indicated by the solid bars) and (A) plasma glucose and (B) plasma insulin concentrations were measured. Plasma glucose and plasma insulin AUC values for 0–105 min after feeding are shown. Values are means $\pm$ S.E.M. for eight mice.

GIP(1–42) in small plasma volumes for mice. However, it is notable that the adverse effects on glucose homeostasis in *ob/ob* mice were not matched by any significant changes in insulin secretion. This supports the view that GLP-1 not only facilitates glucose lowering through glucose-dependent insulin release but also by a range of additional extrapancreatic effects (Valverde *et al.* 1994, Villanueva-Penacarrillo *et al.* 1994, O'Harte *et al.* 1997).

Although rather little is known about concentrations of 'active' GLP-1 in the circulation and intestines of *ob/ob* mice, there are no appreciable differences compared with normal mice (Mooney *et al.* 2002, Anini & Brubaker 2003). Indeed, plasma concentrations of GLP-1 appear to be decreased generally in obesity due to insensitivity to the normal stimulatory effects of leptin (Ranganath *et al.* 1996, Anini & Brubaker 2003). Interestingly, higher levels of DPP IV activity have been reported in *ob/ob* mice compared with lean controls (Ruter *et al.* 2004), but the significance of this observation needs clarification. In sharp contrast, early studies indicate that the sister incretin hormone, GIP, is present in both the plasma and intestines of *ob/ob* mice at remarkably raised concentrations (Flatt

et al. 1983, 1984). Taken together, these observations suggest a relatively minor role for circulating GLP-1 in the metabolic abnormalities of *ob/ob* mice. In contrast, GIP appears to be the major physiological component of the enteroinsular axis in these animals, as evidenced by acute blockade of GIP action with the receptor antagonist (Pro³)GIP (Gault *et al.* 2003). The established effects of GIP on insulin secretion and adipose tissue metabolism (Yip & Wolfe 2000) also indicate that markedly elevated GIP levels in this mutant contribute substantially to the characteristic hyperinsulinaemia, fat disposition and insulin resistance (Bailey & Flatt 2003).

In conclusion, this study has shown that prolonged antagonism of circulating GLP-1 in obese diabetic (*ob/ob*) mice with Ex(9–39) caused a slight impairment of glucose homeostasis without appreciable effects on food intake, pancreatic β -cell function or insulin sensitivity. No significant effects were observed in normal mice. These observations indicate a minor role for endogenous GLP-1 in the metabolic abnormalities of *ob/ob* mice and suggest that elevated concentrations of GIP make a major contribution to the obesity-related diabetes syndrome.

Figure 8 Long-term effects of Ex(9–39) treatment on glucose and insulin responses to feeding in normal mice. Following the 11-day treatment period mice were fasted (18 h) overnight. At 0900 h, free access to food was allowed for 15 min (indicated by the solid bars) and (A) plasma glucose and (B) plasma insulin concentrations were measured. Plasma glucose and plasma insulin AUC values for 0–105 min after feeding are shown. Values are means $\pm$ S.E.M. for eight mice.

Figure 9 Insulin sensitivity of (A) *ob/ob* mice and (B) normal mice chronically treated for 11 days with Ex(9–39). The time of injection is indicated by the arrows. Plasma glucose concentrations were measured prior to and at intervals after intraperitoneal administration of insulin (50U/kg body weight). Corresponding AUC values (0–60 min) for plasma glucose show overall effects to exogenous insulin administration.

Acknowledgements

These studies were supported by the Department of Health and Personal Social Services for Northern Ireland and the University of Ulster Strategy Research Funding. The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

References

- Anini Y & Brubaker PL 2003 Role of leptin in the regulation of glucagon-like peptide-1 secretion. *Diabetes* **52** 252–259.
- Bailey CJ & Flatt PR 1995 Development of antidiabetic drugs. In *Drugs, Diet and Disease: Mechanistic Approaches to Diabetes*, pp 279–326. Eds C Ioanides & PR Flatt. London: Ellis Horwood.
- Bailey CJ & Flatt PR 2003 Animal syndromes resembling type 2 diabetes. In *Textbook of Diabetes*, edn 3, pp 25.1–25.30. Eds JC Pickup & G Williams. Oxford: Blackwell Scientific Publications.
- Bailey CJ, Flatt PR & Atkins TW 1982 Influence of genetic background and age on the expression of the obese hyperglycaemic syndrome in Aston *ob/ob* mice. *International Journal of Obesity* **6** 11–21.
- Bogardus C, Lillioja S, Mott DM, Hollenbeck C & Reaven G 1985 Relationship between degree of obesity and *in vivo* insulin action in man. *American Journal of Physiology – Endocrinology and Metabolism* **248** E286–E291.
- Burington RS 1973 *Handbook of Mathematical Tables and Formulas*. New York: McGraw-Hill.
- Edwards CM, Todd JF, Mahmoudi M, Wang Z, Wang RM, Ghatti MA & Bloom SR 1999 Glucagon-like peptide 1 has a physiological role in the control of postprandial glucose in humans: studies with the antagonist exendin 9–39. *Diabetes* **48** 86–93.
- Elahi D, Clocquet A & Egan JM 1999 Exendin-4 is insulinotropic in non-diabetic and diabetic subjects. *Diabetologia* **42** 148A.
- Eng J, Kleinman WA, Singh L, Singh G & Raufman JP 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. *Journal of Biological Chemistry* **267** 7402–7405.
- Flamez D, Van Breusegem A, Scrocchi LA, Quartier E, Pipeleers D, Drucker DJ & Schuit F 1998 Mouse pancreatic beta-cells exhibit preserved glucose competence after disruption of the glucagon-like peptide-1 receptor gene. *Diabetes* **47** 646–652.
- Flatt PR & Bailey CJ 1981 Abnormal plasma glucose and insulin responses in heterozygous lean (*ob/+*) mice. *Diabetologia* **20** 573–577.
- Flatt PR, Bailey CJ, Kwasowski P, Swanston-Flatt SK & Marks V 1983 Abnormalities of GIP in spontaneous syndromes of obesity and diabetes in mice. *Diabetes* **32** 433–435.
- Flatt PR, Bailey CJ, Kwasowski P, Page P & Marks V 1984 Plasma immunoreactive gastric inhibitory polypeptide in obese hyperglycaemic (*ob/ob*) mice. *Journal of Endocrinology* **101** 249–256.
- Flint A, Raben A, Ersboll AK, Holst JJ & Astrup A 2001 The effect of physiological levels of glucagon-like peptide-1 on appetite, gastric emptying, energy and substrate metabolism in obesity. *International Journal of Obesity and Related Metabolic Disorders* **25** 781–792.
- Gault VA, Flatt CJ, Harriott P, Green BD & O'Harte FPM 2003 Effects of the novel (Pro²)GIP antagonist and exendin (9–39) amide GIP and GLP-1 induced cyclic AMP generation, insulin secretion and postprandial insulin release in obese diabetic (*ob/ob*) mice: evidence that GIP is the major physiological incretin. *Diabetologia* **46** 222–230.
- Göke R, Fehmann HC, Linn T, Schmidt H, Krause M, Eng J & Göke B 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. *Journal of Biological Chemistry* **268** 19650–19655.
- Golay A & Felber JP 1994 Evolution from obesity to diabetes. *Diabetes Metabolism* **20** 3–14.
- Green BD, Mooney MH, Gault VA, Irwin N, Bailey CJ, Harriott P, Greer B, Flatt PR & O'Harte FPM 2004 Lys⁹ for Glu⁹ substitution in glucagon-like peptide-1(7–36)amide (GLP-1) confers DPP IV resistance with cellular and metabolic actions similar to those of established antagonists GLP-1(9–36)amide and exendin (9–39). *Metabolism* **53** 252–259.
- Greig NH, Holloway HW, De Ore KA, Jani D, Wang Y, Zhou J, Garant MJ & Egan JM 1999 Once daily injection of exendin-4 to diabetic mice achieves long-term beneficial effects on blood glucose concentrations. *Diabetologia* **42** 45–50.
- Holz GG & Chepurmy OG 2003 Glucagon-like peptide-1 synthetic analogs: new therapeutic agents for use in the treatment of diabetes mellitus. *Current Medicinal Chemistry* **10** 2471–2483.
- Kolligs F, Fehmann HC, Göke R & Göke B 1995 Reduction of the incretin effect in rats by the glucagon-like peptide 1 receptor antagonist exendin (9–39) amide. *Diabetes* **44** 16–19.
- Kolterman OG, Gray RS, Griffin J, Burstein P, Insel J, Scarlett JA & Olefsky JM 1981 Receptor and postreceptor defects contribute to the insulin resistance in non-insulin-dependent diabetes mellitus. *Journal of Clinical Investigation* **68** 957–969.
- Larsen PJ, Fledelius C, Knudsen LB & Tang-Christensen M 2001 Systemic administration of the long-acting GLP-1 derivative NN2211 induces lasting and reversible weight loss in both normal and obese rats. *Diabetes* **50** 2530–2539.
- Mannucci E, Ognibene A, Cremasco F, Bardini G, Mencucci A, Pierazzuoli E, Ciani S, Fanelli A, Messeri G & Rotella CM 2000 Glucagon-like peptide (GLP)-1 and leptin concentrations in obese patients with Type 2 diabetes mellitus. *Diabetic Medicine* **10** 713–719.
- Mooney MH, Abdel-Wahab YHA, McKillop AM, O'Harte FPM & Flatt PR 2002 Evaluation of glycated glucagon-like peptide-1(7–36)amide in intestinal tissues of normal and diabetic animal models. *Biochimica et Biophysica Acta* **1569** 75–80.
- O'Harte FPM, Gray AM, Abdel-Wahab YHA & Flatt PR 1997 Effects of non-glycated and glycated glucagon-like peptide-1(7–36) amide on glucose metabolism in isolated mouse abdominal muscle. *Peptides* **18** 1327–1333.
- Pederson RA, Satkunarajah M, McIntosh CH, Scrocchi LA, Flamez D, Schuit F, Drucker DJ & Wheeler MB 1998 Enhanced glucose-dependent insulinotropic polypeptide secretion and insulinotropic action in glucagon-like peptide 1 receptor – / – mice. *Diabetes* **47** 1046–1052.
- Ranganath LR, Beety JM, Morgan LM, Wright JW, Howland R & Marks V 1996 Attenuated GLP-1 secretion in obesity: cause or consequence? *Gut* **38** 916–919.
- Ruter J, Hoffmann T, Demuth HU, Moschansky P, Klapp BF & Hildebrandt M 2004 Evidence for an interaction between leptin, T cell costimulatory antigens CD28, CTLA-4 and CD26 (dipeptidyl peptidase IV) in BCG-induced immune responses of leptin- and leptin receptor-deficient mice. *Biological Chemistry* **385** 537–541.
- Schick RR, Zimmermann JP, vom Walde T & Schusdziaara V 2003 Peptides that regulate food intake: glucagon-like peptide 1-(7–36) amide acts at lateral and medial hypothalamic sites to suppress feeding in rats. *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology* **284** R1427–R1435.
- Schirra J, Sturm K, Leicht P, Arnold R, Göke B & Katschinski M 1998 Exendin(9–39)amide is an antagonist of glucagon-like peptide-1(7–36)amide in humans. *Journal of Clinical Investigation* **101** 1421–1430.
- Scrocchi LA & Drucker DJ 1998 Effects of aging and a high fat diet on body weight and glucose tolerance in glucagon-like peptide-1 receptor – / – mice. *Endocrinology* **139** 3127–3132.

- Scrocchi LA, Brown TJ, MaClusky N, Brubaker PL, Auerbach AB, Joyner AL & Drucker DJ 1996 Glucose intolerance but normal satiety in mice with a null mutation in the glucagon-like peptide 1 receptor gene. *Nature Medicine* **2** 1254–1258.
- Stevens JF 1971 Determination of glucose by an automatic analyser. *Clinica Chimica Acta* **32** 199–201.
- Szayna M, Doyle ME, Betkey JA, Holloway HW, Spencer RG, Greig NH & Egan JM 2000 Exendin-4 decelerates food intake, weight gain, and fat deposition in Zucker rats. *Endocrinology* **141** 1936–1941.
- Thorens B, Porret A, Buhler L, Deng SP, Morel P & Widmann C 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.
- Tseng CC, Zhang XY & Wolfe MM 1999 Effect of GIP and GLP-1 antagonists on insulin release in the rat. *American Journal of Physiology – Endocrinology and Metabolism* **276** E1049–E1054.
- Turton MD, O'Shea D, Gunn I, Beak SA, Edwards CM, Meeran K, Choi SJ, Taylor GM, Heath MM, Lambert PD, Wilding JP, Smith DM, Ghatei MA, Herbert J & Bloom SR 1996 A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature* **379** 69–72.
- Valverde I, Morales M, Clemente F, Lopez-Delgado MI, Delgado E, Perea A & Villanueva-Peñacarrillo ML 1994 Glucagon-like peptide 1: a potent glycogenic hormone. *FEBS Letters* **349** 313–316.
- Villanueva-Peñacarrillo ML, Alcantara AI, Clemente F, Delgado E & Valverde I 1994 Potent glycogenic effect of GLP-1(7–36)amide in rat skeletal muscle. *Diabetologia* **37** 1163–1166.
- Vilsboll T, Krarup T, Deacon CF, Madsbad S & Holst JJ 2001 Reduced postprandial concentrations of intact biologically active glucagon-like peptide 1 in type 2 diabetic patients. *Diabetes* **50** 609–613.
- Vilsboll T, Krarup T, Sonne J, Madsbad S, Volund A, Juul AG & Holst JJ 2003 Incretin secretion in relation to meal size and body weight in healthy subjects and people with type 1 and type 2 diabetes mellitus. *Journal of Clinical Endocrinology and Metabolism* **88** 2706–2713.
- Wang Z, Wang RM, Owji AA, Smith DM, Ghatei MA & Bloom SR 1995 Glucagon-like peptide-1 is a physiological incretin in rat. *Journal of Clinical Investigation* **95** 417–421.
- Yip RG & Wolfe MM 2000 GIP biology and fat metabolism. *Life Sciences* **66** 91–103.
- Young AA, Gedulin BR, Bhavsar S, Bodkin N, Jodka C, Hansen B & Denaro M 1999 Glucose-lowering and insulin-sensitizing actions of exendin-4. Studies in obese diabetic (*ob/ob*, *db/db*) mice, diabetic fatty Zucker rats, and diabetic rhesus monkeys (*Macaca mulatto*). *Diabetes* **48** 1026–1034.

Received 5 January 2005

Accepted 1 February 2005