Subcutaneous glucagon-like peptide-1 improves postprandial glycaemic control over a 3-week period in patients with early Type 2 diabetes

Jeannie F. TODD, C. Mark B. EDWARDS, Mohammad A. GHATEI, Hugh M. MATHER* and Stephen R. BLOOM
ICSM Endocrine Unit, Hammersmith Hospital, Du Cane Road, London W12 ONN, U.K., and *Endocrine Unit, Ealing Hospital NHS Trust, Uxbridge Road, Middlesex UB1 3HW, U.K.

A B S T R A C T

1. Glucagon-like peptide-1 (7-36) amide (GLP-1) is released into the circulation after meals and is the most potent physiological insulinotropic hormone in man. GLP-1 has the advantages over other therapeutic agents for Type 2 diabetes of also suppressing glucagon secretion and delaying gastric emptying. One of the initial abnormalities of Type 2 diabetes is the loss of the first-phase insulin response, leading to postprandial hyperglycaemia.

2. To investigate the therapeutic potential of GLP-1 in Type 2 diabetes, six patients were entered into a 6-week, double-blind crossover trial during which each received 3 weeks treatment with subcutaneous GLP-1 or saline, self-administered three times a day immediately before meals. A standard test meal was given at the beginning and end of each treatment period.

3. GLP-1 reduced plasma glucose area under the curve (AUC) after the standard test meal by 58% (AUC, 0–240 min: GLP-1 start of treatment, 196 ± 141 mmol·min^{-1}·l^{-1}; saline start of treatment, 469 ± 124 mmol·min^{-1}·l^{-1}; $F = 16.4$, $P < 0.05$). The plasma insulin excursions were significantly higher with GLP-1 compared with saline over the initial postprandial 30 min, the time period during which the GLP-1 concentration was considerably elevated. The plasma glucagon levels were significantly lower over the 240-min postprandial period with GLP-1 treatment. The beneficial effects of GLP-1 on plasma glucose, insulin and glucagon concentrations were fully maintained for the 3-week treatment period.

4. We have demonstrated a significant improvement in postprandial glycaemic control with subcutaneous GLP-1 treatment. GLP-1 improves glycaemic control partially by restoring the first-phase insulin response and suppressing glucagon and is a potential treatment for Type 2 diabetes.

INTRODUCTION

Glucagon-like peptide-1 (7-36) amide (GLP-1) is synthesized in the intestinal L-cells by tissue-specific post-translational processing of the preproglucagon gene [1,2]. Its circulating concentration increases after a meal in man [3,4]. The plasma concentration of GLP-1 rises from a fasting level of 15 pmol/l to a peak postprandial level of 40 pmol/l [3]. The rise in plasma insulin is approximately 3-fold higher when glucose is administered orally compared with intravenously [3]. The enhanced insulin response to oral over intravenous glucose is known as the incretin effect [3,4] and GLP-1 is proposed to be a physiological incretin in man [3]. The recently created GLP-1 receptor knockout mouse has impaired glucose tolerance, confirming the importance of this peptide in glucose homoeostasis [5].

GLP-1 has a number of other physiological actions

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Correspondence: Professor S. R. Bloom.

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that may be beneficial in the treatment of Type 2 diabetes. GLP-1 suppresses glucagon release [3], delays gastric emptying [6] and may enhance peripheral glucose disposal [7,8]. The ability of GLP-1 to stimulate insulin secretion has been reported to be dependent on an elevation of blood glucose, thus protecting against hypoglycaemia [9,10]. However, there is evidence that this is not a universal finding (C. M. B. Edwards, J. F. Todd, M. A. Ghatei and S. R. Bloom, unpublished work).

A therapeutic potential of GLP-1 has thus been proposed. Studies using single subcutaneous [11–13] or buccal preparations [14] have suggested this. We have previously performed the first chronic study of treatment of Type 2 diabetes with subcutaneous GLP-1, and found that the glucose-lowering effect of GLP-1 fully persisted for a period of 3 weeks [15]. However, this beneficial effect on glycaemic control was not accompanied by a rise in plasma insulin. These patients were poorly controlled on moderate to maximal doses of oral hypoglycaemic agents, suggesting poor β-cell reserve. Therefore, these patients may have been unable to further increase postprandial insulin release in response to GLP-1.

One of the initial abnormalities of Type 2 diabetes is the loss of the first-phase insulin response [16,17], leading to postprandial hyperglycaemia. In order to further investigate the therapeutic potential of GLP-1 in Type 2 diabetes and its ability to increase the postprandial insulin response, we have undertaken a 3-week trial of treatment with new subjects at an earlier stage of their disease using a higher dose of GLP-1. This dose of GLP-1 has previously been shown to be the highest dose tolerated without side effects (J. P. H. Wilding, M. A. Ghatei and S. R. Bloom, unpublished work).

METHODS

Subjects

Six patients (four male, two female) with uncomplicated Type 2 diabetes, controlled by diet or low doses of sulphonylureas, were recruited into the study from our diabetic clinics [age 39 ± 3.4 years, body mass index 32 ± 3.1 kg/m^2, duration of known diabetes 2.0 ± 0.6 years, HbA_{1c} 7.2 ± 0.4 % (normal range 4.3–5.6 %)]. The research was carried out in accordance with the Declaration of Helsinki (1989). All patients gave informed consent for participation in the study, which was approved by the Hammersmith Hospital Ethics Committee.

Protocol

The GLP-1 used in this study was synthesized using fluorenylmethoxycarbonyl (fmoc)-polyamide solid phase synthesis on an Advanced Chemtech 396MPS peptide synthesizer. The product comprised one peak whose homogeneity was confirmed by chromographic purification (Phenomenex, Macclesfield, U.K.). Electrospray mass spectrometry was used to confirm the identity of the peptide. The Limulus Amoebocyte Lysate assay test for pyrogen was negative, and the peptide was sterile on culture.

The patients followed a standard diabetic diet and continued their usual treatment throughout the study. The subjects were entered into a 6-week, double-blind crossover trial during which each received 3 weeks treatment with subcutaneous sterile synthetic human GLP-1 (80 nmol) or saline in random order, with a 1-week interval without treatment between each study period. After assessment of correct technique, the injections were self-administered subcutaneously into the anterior abdominal wall three times a day immediately before each meal. At the beginning and end of each 3-week treatment period, the patients were given a standard test meal after an overnight fast. The standard test meal consisted of two eggs, two slices of white bread, 25 g of Flora margarine and 300 ml of orange juice (2092 kJ, 51 % carbohydrate, 15 % protein and 34 % fat). The meal, which was consumed over 15 min, and subcutaneous GLP-1 or saline were given at time 0. Blood was taken at time -20, -10, 0, $+15$, $+30$, $+45$, $+60$, $+90$, $+120$, $+150$, $+180$, $+210$ and $+240$ min for measurement of glucose, insulin, glucagon, somatostatin and GLP-1.

Analytical methods

Blood samples were collected in tubes containing lithium heparin and 4000 KIU of aprotinin ('Trasylol®', Bayer, Haywards Heath, U.K.), centrifuged immediately at 4 °C and the plasma stored at -20 °C until assay. Glucose concentrations were measured with an automated glucose analyser [RA-1000, Technicon Instruments Co. Ltd., Basingstoke, U.K. (normal fasting range 3.5–5.0 mmol/l)]. Plasma insulin, glucagon, somatostatin and GLP-1 were measured by established sensitive and specific radioimmunoassays developed in this laboratory [3].

Statistical analysis

The data are presented as means $\pm$ S.E.M. Area under the curve (AUC) over baseline was calculated using the trapezoidal rule to assess the differences for the four different meals. All data were analysed statistically using a two-way repeated measures analysis of variance.

RESULTS

All injections were well tolerated and no objective or subjective side-effects were noted. GLP-1 increased postprandial plasma GLP-1 levels to approximately seven

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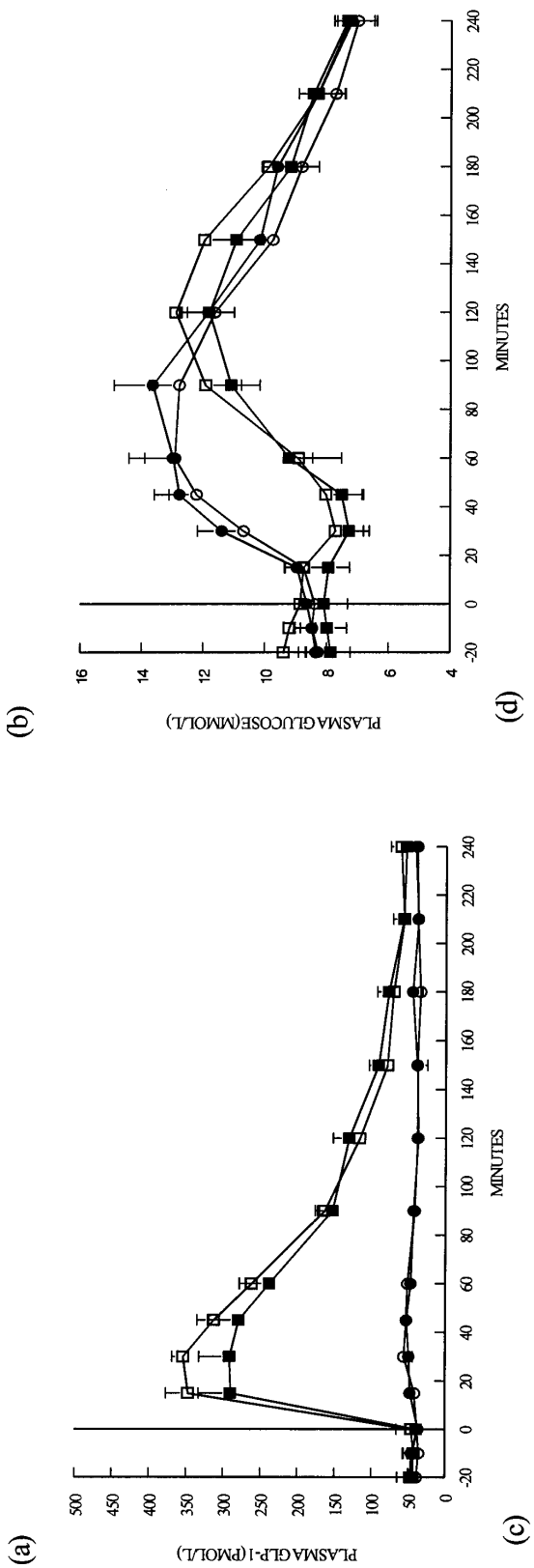

Figure 1 Effect of exogenous GLP-1 on plasma concentrations of (a) GLP-1, (b) glucose, (c) glucagon and (d) insulin after the standard test meals at the beginning and end of each treatment period

○, saline start; ●, saline end; □, GLP-1 start; ■, GLP-1 end.

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times those of control at 30 min (GLP-1 start of treatment, 354 ± 15 pmol/l; saline start of treatment, 56 ± 12 pmol/l), returning to baseline at 180 min (Figure 1a). Fasting plasma glucose levels were similar before each standard test meal (saline start of treatment, 8.4 ± 0.5 mmol/l; saline end of treatment, 8.7 ± 0.4 mmol/l; GLP-1 start of treatment, 8.9 ± 0.9 pmol/l; GLP-1 end of treatment, 8.1 ± 0.8 mmol/l; P not significant). There was a significant reduction in postprandial plasma glucose excursions after the test meals with GLP-1 compared with saline (Figure 1b). GLP-1 reduced plasma glucose AUC after the standard test meal by 58% (AUC, 0–240 min: GLP-1 start of treatment, 196 ± 141 mmol·min^{-1}·l^{-1}; saline start of treatment, 469 ± 124 mmol·min^{-1}·l^{-1}; $F = 16.4$, $P < 0.05$). Moreover, the rise in plasma glucose concentration after the meal was delayed for 60 min with GLP-1 (AUC, 0–60 min: GLP-1 start of treatment, −58.5 ± 12.2 mmol·min^{-1}·l^{-1}; saline start of treatment, 75.2 ± 26.4 mmol·min^{-1}·l^{-1}; $F = 15.9$, $P < 0.01$). In addition, the beneficial effect of GLP-1 on postprandial plasma glucose concentrations was maintained for the 3-week treatment period (AUC, 0–240 min: GLP-1 start of treatment, 196 ± 141 mmol·min^{-1}·l^{-1}; GLP-1 end of treatment, 234 ± 142 mmol·min^{-1}·l^{-1}; $P = 0.8$). Fasting plasma glucagon levels were similar before each standard test meal. The plasma glucagon levels were significantly lower over the 240-min postprandial period with GLP-1 treatment (AUC, 0–240 min: GLP-1 start of treatment, −1.5 ± 1.8 nmol·min^{-1}·l^{-1}; saline start of treatment, 3.5 ± 1.2 nmol·min^{-1}·l^{-1}; $F = 14.6$, $P < 0.05$) (Figure 1c). There was no escape from the potent glucagon-lowering effect of GLP-1 over 3 weeks (AUC, 0–240 min: GLP-1 start of treatment, −1.5 ± 18 pmol·min^{-1}·l^{-1}; GLP-1 end of treatment, −2.6 ± 1.5 pmol·min^{-1}·l^{-1}; $P = 0.47$).

The basal plasma insulin concentrations were not significantly different between the four study days (saline start of treatment, 128.6 ± 28.0 pmol/l; saline end of treatment, 130.2 ± 25.2 pmol/l; GLP-1 start of treatment, 155.1 ± 46.5 pmol/l; GLP-1 end of treatment, 95.0 ± 15.6 pmol/l; P not significant). There was no significant effect of GLP-1 on the plasma insulin levels over the 240-min postprandial period (AUC, 0–240 min: GLP-1 start of treatment, 59.5 ± 17.3 nmol·min^{-1}·l^{-1}; saline start of treatment, 55.5 ± 17.8 nmol·min^{-1}·l^{-1}; $P = 0.35$) (Figure 1d), but this was in the face of a 50% reduction in plasma glucose with GLP-1. However, the postprandial plasma insulin excursions were significantly higher with GLP-1 compared with saline controls over the initial 30-min post injection, the time period during which the GLP-1 concentration was considerably elevated (AUC, 0–30 min: GLP-1 start of treatment, 3.9 ± 1.0 nmol·min^{-1}·l^{-1}; saline start of treatment, 1.7 ± 0.5 nmol·min^{-1}·l^{-1}; $F = 15.9$, $P < 0.01$) (Figure 1d). There was no escape from the insulin-stimulating effect of GLP-1 over 3 weeks (AUC, 0–30 min: GLP-1 start of treatment, 3.9 ± 1.0 nmol·min^{-1}·l^{-1}; GLP-1 end of treatment, 3.3 ± 1.0 nmol·min^{-1}·l^{-1}; $P = 0.79$). Fructosamine levels were measured at the beginning and end of each treatment period and although there was a tendency to drop with GLP-1 treatment [saline start, 4.1 ± 0.2 mmol/l; saline end, 4.1 ± 0.4 mmol/l; GLP-1 start, 4.0 ± 0.4 mmol/l; GLP-1 end, 3.6 ± 0.2 mmol/l (normal range 2.2–2.8 mmol/l)], this did not reach statistical significance ($P = 0.46$). Similarly, plasma triacylglycerols also showed a tendency to drop with GLP-1 treatment but this did not reach statistical significance [saline start, 3.0 ± 0.6 mmol/l; saline end, 3.1 ± 0.9 mmol/l; GLP-1 start, 3.4 ± 1.1 mmol/l; GLP-1 end, 2.1 ± 0.2 mmol/l (normal range < 2.0 mmol/l); $P = 0.35$].

DISCUSSION

This is only the second clinical trial of chronic treatment with subcutaneous GLP-1 in patients with Type 2 diabetes. GLP-1 levels peaked at 30–45 min post injection and, for 45 min post injection, the postprandial plasma glucose rise was completely prevented. The beneficial effect of GLP-1 on postprandial glucose, insulin and glucagon levels was fully maintained over 3 weeks; hence there is no evidence for down-regulation of the effects of GLP-1 *in vivo* in man, in contrast to the effects observed *in vitro* [18,19]. Although the drop in fructosamine from 4.0 mmol/l to 3.6 mmol/l with GLP-1 was not statistically significant, the tendency to improve over the 3-week treatment period indicates that long-term treatment with GLP-1 might improve long-term glycaemic control.

In this study, the improvement in postprandial glycaemic control can be attributed to a number of beneficial effects of GLP-1. One of the initial abnormalities of Type 2 diabetes is the loss of the first-phase insulin response, leading to postprandial hyperglycaemia [16,17]. In this group of patients, improvement of plasma glucose levels by GLP-1 may have been mediated by a partial restoration of the first-phase insulin response. The pattern of plasma insulin concentrations seen here after GLP-1 is biphasic, unlike the normal uniphasic response seen in non-diabetic subjects. Both the early insulin-releasing effect of GLP-1 and the delay in gastric emptying caused by GLP-1 are likely to have created this biphasic pattern in insulin concentrations. It is also noteworthy that these subjects had the same plasma insulin response over the 240-min postprandial period in the face of a 50% reduction in plasma glucose, indicating a relative enhancement of insulin release in response to GLP-1. Infusion of GLP-1 has previously been shown to reduce the meal-related insulin requirements in patients with Type I diabetes suggesting the underlying mechanism is a delay in gastric emptying and/or enhanced

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insulin sensitivity, perhaps due to suppression of glucagon [8,20]. The improvement in postprandial glucose levels after GLP-1 in this study may have been due to an increase in insulin secretion and a suppression of glucagon secretion although delay in gastric emptying probably contributed, particularly given the dramatic decrease in plasma glucose between 15 and 90 min compared with the saline injections. The beneficial effect of GLP-1 is unlikely to be mediated via a change in circulating somatostatin levels as GLP-1 caused no alteration in plasma somatostatin concentrations in this study (results not shown).

A recent study has demonstrated that therapeutic plasma levels of GLP-1 in healthy volunteers can be achieved after taking a single specially developed buccal tablet [14]. Although the bioavailability of buccal GLP-1 is only 47% of that given subcutaneously, this study indicates another potential therapeutic route of administration. We have demonstrated a significant improvement in postprandial glycaemic control with GLP-1 that is fully maintained over 3 weeks in patients with early-stage Type 2 diabetes. The increase in insulin found in this study is in contrast to our previous study in which there was no change in plasma insulin concentrations in patients treated with a lower dose of GLP-1 at a more advanced stage of their disease [15]. Thus, it would appear that patients with greater β-cell reserve may be more likely to benefit from this kind of agent.

The beneficial effect on glycaemic control demonstrated in the present study occurred despite the short-lived elevation of plasma GLP-1 levels after the injection. Further studies using longer acting GLP-1 analogues are required to assess whether GLP-1 is to provide a new therapeutic avenue.

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REFERENCES

1 Varndell, I. M., Bishop, A. E., Sikri, K. L., Uttenthal, L. O., Bloom, S. R. and Polak, J. M. (1985) Localization of glucagon-like peptide (GLP) immunoreactants in human gut and pancreas using light and electron microscope immunohistochemistry. J. Histochem. Cytochem. **33**, 1080–1086
2 Mojsov, S., Heinrich, G., Wilson, I. B., Ravazzola, M., Orci, L. and Habener, J. F. (1986) Preproglucagon gene expression in pancreas and intestine diversifies at the level of post-translational processing. J. Biol. Chem. **261**, 11880–11889
3 Kreymann, B., Williams, G., Ghatei, M. A. and Bloom, S. R. (1987) Glucagon-like peptide-1 7-36: a physiological incretin in man. Lancet **2**, 1300–1304
4 Fehmann, H. C., Goke, R. and Goke, B. (1992) Glucagon-like peptide-1 (7-37)/(7-36) amide is new incretin. Mol. Cell. Endocrinol. **85**, C39–C44
5 Scrocchi, L. A., Brown, T. J., Maclusky, N. et al. (1996) Glucose intolerance but normal satiety in mice with a null mutation in the glucagon-like peptide-1 receptor gene. Nature Med. **2**, 1254–1258
6 Wettergren, A., Schjoldager, B., Mortensen, P. E., Myhre, J., Christiansen, J. and Holst, J. J. (1993) Truncated GLP-1 (proglucagon 78-107 amide) inhibits gastric and pancreatic functions in man. Dig. Dis. Sci. **38**, 665–673
7 D'Alessio, D. A., Kahn, S. E., Leusner, C. R. and Ensinck, J. W. (1994) Glucagon-like peptide enhances glucose tolerance both by stimulation of insulin release and by increasing insulin independent glucose disposal. J. Clin. Invest. **93**, 2263–2266
8 Gutniak, M. K., Ørskov, C., Holst, J. J., Ahren, B. and Efendic, S. (1992) Antidiabetogenic effect of glucagon-like peptide-1 (7-36) amide in normal subjects and patients with diabetes. N. Engl. J. Med. **326**, 1316–1322
9 Nathan, D. M., Schreiber, E., Fogel, H., Mojsov, S. and Habener, J. F. (1992) Insulinotropic action of glucagon-like peptide-1-(7-37) in diabetic and non-diabetic subjects. Diabetes Care **15**, 270–276
10 Holz, IV, G. G., Kuhtreiber, W. N. and Habener, J. F. (1993) Pancreatic beta-cells are rendered glucose-competent by the insulinotropic hormone glucagon-like peptide-1 (7-37). Nature (London) **361**, 362–365
11 Gutniak, M. K., Linde, B., Holst, J. J. and Efendic, S. (1994) Subcutaneous injection of the incretin hormone glucagon-like peptide-1 abolishes postprandial glycaemia in NIDDM. Diabetes Care **17**, 1039–1044
12 Ritzel, R., Ørskov, C., Holst, J. J. and Nauck, M. (1995) Pharmacokinetic, insulinotropic, and glucagonostatic properties of GLP-1 (7-36) amide after subcutaneous injection in healthy volunteers. Dose–response relationships. Diabetologia **38**, 720–725
13 Nauck, M. A., Wollschlager, D., Werner, J. et al. (1996) Effects of subcutaneous glucagon-like peptide 1 (GLP-1 (7-36) amide) in patients with NIDDM. Diabetologia **39**, 1546–1553
14 Gutniak, M. K., Larsson, H., Heiber, S. J., Juneskans, O. T., Holst, J. J. and Ahren, B. (1996) Potential therapeutic levels of glucagon-like peptide 1 achieved in humans by a buccal tablet. Diabetes Care **19**, 843–848
15 Todd, J. F., Wilding, J. P. H., Edwards, C. M. B., Khan, F. A., Ghatei, M. A. and Bloom, S. R. (1997) Glucagon-like peptide-1: a trial of treatment in non-insulin dependent diabetes mellitus. Eur. J. Clin. Invest. **27**, 533–536
16 Seltzer, H. S., Allen, E. W., Herron, A. L. and Brennan, M. T. (1967) Insulin secretion in response to glycaemic stimulus: relation of delayed initial insulin release to carbohydrate intolerance in mild diabetes mellitus. J. Clin. Invest. **46**, 323–335
17 Pfeifer, M. A., Halter, J. B. and Porte, Jr, D. (1981) Insulin secretion in diabetes mellitus. Am. J. Med. **70**, 579–588
18 Widmann, C., Dolci, W. and Thorens, B. (1996) Desensitization and phosphorylation of the glucagon-like peptide-1 (GLP-1) receptor by GLP-1 and 4-phorbol 12-myristate 13-acetate. Mol. Endocrinol. **10**, 62–75
19 Gromada, J., Dissing, S. and Rorsman, P. (1996) Desensitization of glucagon-like peptide-1 receptors in insulin-secreting beta TC3 cells: role of PKA-independent mechanisms. Br. J. Pharmacol. **118**, 769–775
20 Willms, B., Werner, J., Holst, J. J., Ørskov, C., Creutzfeldt, W. and Nauck, M. (1996) Gastric emptying, glucose responses and insulin secretion after liquid test meal: effects of exogenous glucagon-like peptide (GLP-1) (7-36) amide in patients with type-2 (non-insulin-dependent) diabetes mellitus. J. Clin. Endocrinol. Metab. **81**, 327–332

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