Subcutaneous glucagon-like peptide-1 (7-36) amide is insulinotropic and can cause hypoglycaemia in fasted healthy subjects

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ABSTRACT

1. Glucagon-like peptide-1 (7-36) amide (GLP-1) is a gut hormone released postprandially that stimulates insulin secretion, suppresses glucagon secretion and delays gastric emptying. The insulinotropic action of GLP-1 is more potent under hyperglycaemic conditions. Several published studies have indicated the therapeutic potential of subcutaneous GLP-1 in non-insulin-dependent (Type 2) diabetes mellitus.

2. We investigated whether subcutaneous GLP-1, at a dose shown to improve glycaemic control in early Type 2 diabetes, is insulinotropic at normal fasting glucose concentrations. A double-blind, randomized, crossover study of 10 healthy subjects injected with GLP-1 or saline subcutaneously after a 16 h fast was performed. The effect on cardiovascular parameters was also examined.

3. GLP-1 caused a near 5-fold rise in plasma insulin concentration. After treatment with GLP-1, circulating plasma glucose concentrations fell below the normal range in all subjects. One subject had symptoms of hypoglycaemia after GLP-1. A rise in pulse rate was found which correlated with the fall in plasma glucose concentration. An increase in blood pressure occurred with GLP-1 injection which was seen at the same time as the rise in plasma GLP-1 concentrations.

4. This study indicates that subcutaneous GLP-1 can override the normal homoeostatic mechanism maintaining fasting plasma glucose in man, and is also associated with an increase in blood pressure.

INTRODUCTION

Glucagon-like peptide-1 (7-36) amide (GLP-1) is a peptide product of the post-translational processing of the preproglucagon gene in the gut and central nervous system [1,2]. In the gut it is synthesized in and released from the L-cells of the ileal and colonic mucosa [3,4], and it circulates in high concentrations after a meal in man [5]. When GLP-1 is infused into healthy human volunteers to mimic the rise seen after a meal, the insulin response during intravenous glucose infusion is potently accentuated, while glucagon and glucose concentrations are suppressed [5]. The enhanced insulin response to oral compared with intravenous glucose is known as the incretin effect [6,7]. Together with gastric inhibitory polypeptide [8], GLP-1 acts a physiological incretin in man.

GLP-1 enhances insulin secretion in patients with
Type 2 diabetes mellitus, albeit less potently than in healthy volunteers. Gastric inhibitory polypeptide has little or no effect on insulin secretion in these patients [9–11]. In addition to its incretin effect, GLP-1 suppresses glucagon release [5], delays gastric emptying [12] and may enhance peripheral glucose disposal [9,13]. GLP-1 seems to be a central satiety factor in the rat, but no satiety effect is seen when administered peripherally at a physiological dose [14].

GLP-1 has been proposed as a potential treatment for Type 2 diabetes, and has been studied in subcutaneous [15–17] and buccal preparations [18]. We have recently performed the first chronic study of treatment of late-stage Type 2 diabetes with subcutaneous GLP-1 and found that the glucose lowering effect of GLP-1 was fully maintained for a period of 3 weeks, indicating agonism of the GLP-1 receptor as a potential treatment for Type 2 diabetes [19]. A further clinical study demonstrated that subcutaneous GLP-1 at a dose of 80 nmol had a greater beneficial effect in subjects with early Type 2 diabetes [20]. In its native form, GLP-1 is relatively short acting; however, a recent report demonstrated that pharmacological plasma levels of GLP-1 can be maintained for many hours when dissolved in a solution containing zinc chloride or protamine sulphate [21].

Other workers have found that the insulinotropic effect of GLP-1 is glucose dependent, a more potent effect occurring when plasma glucose is elevated [11]. Intravenous GLP-1 has been reported to stimulate insulin secretion marginally in the fasted state, but not to cause hypoglycaemia, the lowest single plasma glucose recorded in one study being 3.6 mmol/l [22]. The authors concluded that GLP-1 could be safely employed to reduce hyperglycaemia in patients with Type 2 diabetes, without the risk of hypoglycaemia [22]. A second study also indicated that intravenous GLP-1 could stimulate insulin secretion in the euglycaemic state, but since glucose was infused, no assessment of hypoglycaemia could be made [23]. Acute subcutaneous injection of GLP-1, up to a dose of 4.5 nmol/kg, has been reported to cause a small decrease in glucose and increase in insulin in the fasted state [16], but in this study an intravenous bolus of glucose was administered 30 min after injection of GLP-1. No study has examined the effects of subcutaneous GLP-1 on plasma glucose or insulin in the fasted state for more than 30 min. Chronic treatment with subcutaneous GLP-1 in patients with late-stage Type 2 diabetes was not found to induce hypoglycaemic events [19]; similarly, no patients suffered hypoglycaemia in our recent study with a higher dose of GLP-1 in subjects with early Type 2 diabetes [20], but in both studies subjects ate immediately after each injection.

In view of the potential of subcutaneous administration of a GLP-1 receptor agonist as a treatment for Type 2 diabetes, an understanding of its effect on stimulating insulin in the fasted state and/or causing hypoglycaemia is vital. Furthermore, while exogenous GLP-1 has been found to produce effects on the cardiovascular system in both neonatal [24] and calf [25] tissue, there are no data available of any cardiovascular effect of GLP-1 in man.

The present study was designed to investigate the hypoglycaemic and cardiovascular effects of subcutaneous GLP-1 in healthy volunteers in the fasting state.

**METHODS**

**Subjects**

Ten healthy subjects (five male and five female, age 25.5 ± 0.9 years, body mass index 23.4 ± 0.7 kg/m²) voluntarily participated in the study. The research was carried out in accordance with the Declaration of Helsinki (1989). All subjects gave informed written consent, and ethical approval was obtained from the Hammersmith Hospital Research Ethics Committee. Volunteers were asked to consume their normal evening meal between 17:00 and 18:00 hours on the evening before each study day, and fasted thereafter. Subjects were taking no regular medication, had no allergies, and had a normal physical examination and ECG. Renal function, haemoglobin, fasting plasma glucose and insulin concentrations were all within normal limits.

**Protocol**

The GLP-1 used in this study was synthesized using fmoc chemistry on an Advanced Chemtech 396MPS peptide synthesizer. The product comprised one major peak which was purified to homogeneity (> 98%) by reversed phase HPLC on a C8 column (Phenomenex, Macclesfield, U.K.). Electrospray mass spectrometry was used to confirm the identity of the peptide. The peptide content was 85%, as determined by quantitative amino acid analysis. The Limulus amoeocyte lysate assay test for pyrogen was negative, and the peptide was sterile on culture.

Each subject was studied on two occasions with at least 72 h between each study day. On the morning of the study (08:30–09:00 hours), a cannula was inserted into a large forearm vein for collection of blood. After 30 min a subcutaneous injection of GLP-1 (80 nmol dissolved in 1 ml of sterile physiological saline) or 1 ml of sterile physiological saline was given into the anterior abdominal wall. This dose was chosen as we found it to improve postprandial glucose control in a therapeutic study [20]. The study was double-blinded and the injections administered in random order by the same investigator on each occasion. Blood samples were collected 20 and 10 min before injection and 0, 10, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 80, 90, 100, 110 and 120 min after the injection which was given at time 0. Subjects were
Glucagon-like peptide-1 can cause hypoglycaemia.

**Figure 1** Plasma GLP-1 (A), insulin (B), glucose (C) and glucagon (D) concentrations in response to subcutaneous injection of GLP-1 (●), or saline (○). Injection was administered at 0 min. Data are means ± S.E.M. *P < 0.05, **P < 0.01, ***P < 0.005, ****P < 0.001, GLP-1 compared with saline.
attached to a cardiac monitor and arterial blood pressure was measured using a Critikon Dinamap vital signs indicator. Each subject was fed on cessation of the study.

**Analytical methods**

Blood was collected into heparinized tubes containing 0.3 mg (0.2 ml) of aprotinin, centrifuged immediately, and plasma separated and stored at $-20^\circ$C until analysis. Plasma glucose was measured using a BM/Hitachi 747 glucose analyser (normal fasting range 4.22–6.11 mmol/l). Plasma insulin, glucagon, somatostatin and GLP-1 were measured by established radioimmunoassays [5].

**Statistical analysis**

Data are presented as means ± S.E.M. Comparisons between treatment groups at each time point, measured as the change from baseline (time 0), were made by analysis of variance (ANOVA).

**RESULTS**

Plasma GLP-1 concentrations rose from $20 ± 3$ pmol/l at 0 min to a peak of $206 ± 43$ pmol/l at 20 min ($P < 0.005$ versus saline control), remaining increased to 120 min ($P < 0.05$ versus control) after GLP-1 injection (Figure 1A).

Plasma insulin concentrations rose from $21 ± 5$ pmol/l at 0 min to $98 ± 21$ pmol/l at 10 min ($P < 0.005$ versus saline control), remaining increased to 35 min ($P < 0.05$ versus control) after GLP-1 injection (Figure 1B).

Plasma glucose decreased from $4.72 ± 0.08$ mmol/l at 0 min to $3.46 ± 0.14$ mmol/l at 30 min ($P < 0.001$ versus saline control) after GLP-1 injection (Figure 1C). The mean of the nadir of plasma glucose was $3.28$ mmol/l (range 2.31–3.87 mmol/l). One subject experienced hypoglycaemic symptoms with GLP-1 injection, but none with saline. This subject had tachycardia (> 100 beats/min), was pale, confused and dizzy, with decreased mental ability (as measured by time to count backwards from 100 in sevens), and felt generally ill at ease.

There was a tendency for a decrease in plasma glucagon concentrations with GLP-1 injection followed by an increase as the plasma glucose concentrations fell below 3.5 mmol/l, but these changes were not statistically significant (Figure 1D). The plasma somatostatin concentrations were not altered by the treatment. Saline injection did not affect plasma GLP-1, insulin, glucose, glucagon or somatostatin concentrations.

Heart rate increased above basal levels from 10 to 50 min after GLP-1 injection, reaching a maximum difference of 10 beats/min at 40 min (GLP-1, 64 ± 2 beats/min; saline, 54 ± 2 beats/min, $P < 0.01$) (Figure 2A).

Mean blood pressure increased significantly at 10 and 20 min after GLP-1 injection, reaching a maximum difference of 6 mmHg at 20 min (GLP-1, 83 ± 5 mmHg; saline, 77 ± 4 mmHg, $P < 0.05$) (Figure 2B). Pulse pressure was unaltered by GLP-1 (pulse pressure at time 0, saline 48.4 ± 2.5 mmHg versus GLP-1 51.8 ± 3.7 mmHg; time +10, saline 51 ± 4.2 mmHg versus GLP-1 51.2 ± 2.5 mmHg; time +20, saline 47.5 ± 4.2 mmHg versus GLP-1 50.3 ± 3.4 mmHg, all non-significant).

**DISCUSSION**

Subcutaneous injection of 80 nmol GLP-1 increased plasma GLP-1 concentrations to approximately 10 times basal levels. Plasma insulin increased by an average of 5-fold after GLP-1. This insulinotropic effect was associated with a decrease in plasma glucose concentration below the lower limit of the normal range of our assay in all subjects. Three of the subjects had a sufficiently potent response to GLP-1 for plasma glucose
to decrease below 3 mmol/l; the lowest level recorded was 2.31 mmol/l. One subject became symptomatically hypoglycaemic, displaying adrenergic symptoms and signs, and also became neuroglycopenic. This subject had the lowest weight and thus the largest dose of GLP-1 per kg body weight. This effect has not been reported previously, but no study has looked at the effect of GLP-1 administered in this way in normoglycaemic conditions in healthy volunteers.

The increase in plasma insulin and decrease in plasma glucose concentrations was not accompanied by a significant change in plasma glucagon concentrations. GLP-1 has been shown to suppress glucagon slightly in the fasting state [22], and hypoglycaemia stimulates glucagon, thus, these two effects may have cancelled each other out. While plasma GLP-1 concentrations were highest there was a tendency for a decrease in plasma glucagon concentrations, followed by an increase as the plasma glucose concentrations fell below 3.5 mmol/l, but these changes were not statistically significant. Interestingly, the profile of the plasma glucagon concentrations is very similar to that found by Ritzel et al. [16] in the fasting state after GLP-1 injection before glucose infusion. In their study the differences in plasma glucagon concentrations were found to be significant.

GLP-1 has previously been shown to stimulate somatostatin secretion in animals [26], but infusion in humans has not previously demonstrated this effect [27]. We investigated whether there were any changes in plasma somatostatin concentrations in case these might also be influencing the insulin and glucagon responses. There was, however, no significant change with GLP-1 administration.

The rise in heart rate found in our study correlates with the drop in plasma glucose and this is likely to be secondary to adrenergic activation caused by the hypoglycaemia. The small and brief rise in mean arterial blood pressure is unexpected, and correlates more with the rise in insulin and GLP-1 than the drop in glucose. There is no change in plasma glucose at 10 min, when blood pressure is increased. In addition, the lack of effect of GLP-1 on pulse pressure argues against sympathetic nervous system activation being the cause of the increase in blood pressure. Exogenous administration of insulin, to mimic postprandial plasma insulin concentrations, has not been demonstrated to increase blood pressure in the young [28]. The peak plasma insulin concentration found here is well below postprandial levels. In addition, intravenous infusion of GLP-1 and glucose, resulting in a much greater peak in plasma insulin level but a lower peak in plasma GLP-1 level than in the present study, is not associated with an effect on blood pressure (C. M. B. Edwards, J. F. Todd, M. Mahmoudi, Z. Wang, R. M. Wang, M. A. Ghatei and S. R. Bloom, unpublished work). Since the increase in blood pressure coincides with the peak in GLP-1 levels, this suggests that GLP-1 increases blood pressure in man. GLP-1 in pharmacological doses has previously been shown to cause an increase in blood pressure and heart rate in the rat [24]; these effects were blocked by the specific GLP-1 antagonist, exendin 9-39 [29]. GLP-1 increased mean blood pressure without affecting pulse pressure as we have found here. In the calf, GLP-1 infusion is associated with a chronotropic effect without a change in blood pressure [25]. In both of the above studies the cardiovascular effects persisted after adrenergic and cholinergic blockade, indicating a potential direct effect. Furthermore, GLP-1 receptors are present in the heart in man [30]. Studies of the mechanism by which GLP-1 causes this increase in blood pressure in man will be of interest.

GLP-1 has been suggested as a novel treatment for Type 2 diabetes, and one potential advantage is its reported glucose dependence, and thus low risk of hypoglycaemia. We have shown that subcutaneous GLP-1 is insulinotropic and for the first time shown that it may cause hypoglycaemia in fasting healthy volunteers. However, the increase in plasma insulin concentrations produced by GLP-1 in this fasting study is much less than previously found under hyperglycaemic conditions [5], indicating that, as previously reported [11], the effect of GLP-1 is glucose dependent. GLP-1 is less insulinotropic in subjects with Type 2 diabetes than in normal volunteers, the former also having higher fasting glucose concentrations and thus less risk of hypoglycaemia. However, given the results of our recent study that patients with greater β-cell reserve and thus relatively normal fasting plasma glucose levels seem to be better targets for GLP-1 therapy, caution is needed regarding this potential side effect. The novel demonstration of an increase in blood pressure, presumably caused by GLP-1, may be a direct effect, and indicates that there might be a risk of hypertension should GLP-1 be used as a long-term therapeutic agent for Type 2 diabetes.

This study demonstrates that subcutaneous GLP-1 is insulinotropic in the fasted state, and is sufficiently potent to disturb the normally tight homoeostatic mechanism maintaining glucose in the fasted state.

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REFERENCES


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