Lack of effect of exendin-4 and glucagon-like peptide-1-(7,36)-amide on insulin action in non-diabetic humans

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Abstract

Aims/hypothesis. The aim of this study was to determine whether rapid conversion to inactive and potentially antagonistic peptides could alter the response to GLP-1.

Methods. We evaluated the ability of exendin-4, a GLP-1 analogue resistant to degradation by dipeptidyl peptidase IV, to modulate insulin-induced stimulation of glucose uptake and suppression of glucose production in eight healthy subjects during infusion of GLP-1 ($1.2 \text{ pmol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$), exendin-4 ($0.12 \text{ pmol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$), or saline. Glucose was clamped at 5.3 mmol/l and insulin was infused to progressively increase insulin concentrations to about 65, 190 and 700 pmol/l , respectively. Endogenous insulin secretion was inhibited with somatostatin to ensure comparable portal insulin concentrations while glucagon and growth hormone were maintained at basal concentrations.

Results. Glucose, insulin, C-peptide, glucagon and growth hormone concentrations did not differ on the

three occasions. In contrast, cortisol concentrations were greater during both exendin-4 ($25.1\pm 4.4 \text{ mmol/l}$ per 7 h; $p<0.01$) and GLP-1, ($17.0\pm 2.0 \text{ mmol/l}$ 7 h; $p<0.05$) than saline ($13.5\pm 1.5 \text{ mmol/l}$ per 7 h). While insulin-induced stimulation of glucose disappearance at the highest insulin concentrations tended to be greater and insulin-induced suppression of glucose production lower in the presence of exendin-4 or GLP-1 than saline, the differences were not significant.

Conclusion/interpretation. Exendin-4 and GLP-1 increase cortisol secretion in human subjects. However, neither alters insulin action in non-diabetic human subjects. These data also suggest that the lack of an effect of GLP-1 on insulin action is not likely to be explained by rapid degradation to inactive or antagonistic peptides. [Diabetologia (2002) 45:1410–1415]

Keywords GLP-1, exendin-4, insulin action, glucose production, cortisol secretion.

GLP-1 (7–36) is actively being evaluated as a therapy for diabetes mellitus. GLP-1 (7–36) increases insulin secretion, decreases glucagon secretion and delays gastric emptying [1, 2, 3, 4, 5, 6, 7]. In vitro data suggest it might also enhance insulin action. Although controversial, GLP-1 receptors, to which Exendin-4

also binds [8], have been reported to be present in muscle [9, 10], fat [11, 12] and the liver [13]. GLP-1 (7–36) can increase glucose uptake in each of these tissues with its effects being additive to those of insulin [12, 14, 15, 16]. In contrast, results from in vivo studies have been less consistent. GLP-1 (7–36) has been reported to increase glucose uptake during hyperglycaemia and hyperinsulinaemia in diabetic rats [17], pancreatectomized dogs [18] and in Type I (insulin-dependent) diabetic patients [19, 20]. In contrast, GLP-1 (7–36) has been reported to have no effect on the insulin action in non-diabetic human subjects [21], in dogs [22] or Type II diabetic patients [23]. Interestingly, conflicting results have been obtained by several investigators [24] including ourselves [20].

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Abbreviations: GLP-1 (7–36), Glucagon-like peptide 1 (7–36); DPPIV, dipeptidyl peptidase IV; GIR, glucose infusion rate.

Several factors could account for these discordant results. A positive but small effect on insulin action has most consistently been observed in severely insulin deficient animals or Type I diabetic patients [18, 20]. The methods used to assess insulin action also have differed ranging from clamps, to frequently sampled intravenous glucose tolerance tests to prandial infusions of glucose [17, 18, 19, 20, 21, 22, 23]. We were intrigued by the possibility that the pattern of degradation of GLP-1 (7–36) also might have influenced the results of these studies. GLP-1 (7–36) is rapidly converted by dipeptidyl peptidase IV (DPP-IV) to GLP-1 (9–36). GLP-1 (9–36) is not only inactive but also has been shown in some assays systems [25] to be a competitive antagonist of GLP-1 (7–36). If GLP-1 (9–36) also is an antagonist in human subjects, then it is possible that assessment of the effects of GLP-1 (7–36) on insulin action is confounded by the presence of GLP-1 (9–36) in plasma, the amount of which could vary depending on disease state and duration of GLP-1 (7–36) infusion. We therefore hypothesized that a GLP-1 analogue that was resistant to degradation by DPP-IV would have a readily evident effect on insulin action that would exceed that observed during infusion of GLP-1 (7–36) alone.

Exendin-4 is a 39-amino acid peptide originally isolated from the salivary gland of the lizard *Heloderma horridum* [26, 27]. Exendin-4 shows sequence homology to GLP-1 but is resistant to the actions of DPP-IV and, consequently, has a longer circulating and biological half-life in humans [28]. Exendin-4 is approximately ten times more potent than GLP-1 (7–36), but at equipotent doses, has similar effects on insulin secretion, gastric emptying and satiety [29]. As with GLP-1 (7–36), preliminary studies indicate that administration of exendin-4 results in a substantial and sustained reduction in plasma glucose concentrations in people with Type II diabetes [29].

The experiments were undertaken to test the hypothesis that exendin-4 enhances insulin action in non-diabetic human subjects. We further hypothesized that the effects of exendin-4 on insulin action would be greater than those exerted by GLP-1 (7–36). To test these hypotheses, non-diabetic volunteers were studied on three occasions during infusions of exendin-4, GLP-1 (7–36) or saline. On each occasion, glucose was clamped at about 5.5 mmol/l while plasma insulin was progressively raised to concentrations that spanned the physiologic range.

Subjects and methods

Subjects. After approval by the Mayo Institutional Review Board, eight subjects with no personal or family history of diabetes gave informed written consent to participate in the study. The subjects' mean age was 28.3 ± 4.2 years, weight 72.5 ± 4.6 kg and body mass index 25.6 ± 1.3 kg/m². All subjects

were in good health and at a stable weight. None regularly engaged in vigorous physical exercise.

Experimental design. Seven subjects were studied on three occasions separated by at least one week. One subject was unable to complete the GLP-1 study day due to loss of an infusion site. All subjects were admitted to the Mayo Clinic General Clinical Research Center at 18.00 hours the evening before the study. Following ingestion of a standard 10 Kcal/kg meal (55% carbohydrate, 30% fat, and 15% protein), subjects fasted until the end of the study. On the morning of the study at 05.00 hours (–180 min), an 18-gauge cannula was inserted into the non-dominant forearm and a primed continuous infusion of [³-³H] glucose (12 μ Ci bolus, 0.12 μ Ci/min continuous; New England Nuclear, Boston, Mass., USA) was started and continued until the end of the study.

At 07.15 hours (–45 min), an 18-gauge cannula was inserted in a retrograde fashion into a dorsal hand vein. The hand was then placed in a heated box in order to obtain arterialized venous blood samples. At 08.00 hours (0 min), an infusion of either GLP-1 (1.2 pmol·kg^{–1}·min^{–1}), Exendin-4 (0.12 pmol·kg^{–1}·min^{–1}) or saline was started with the order of study being random. An intravenous infusion of somatostatin (120 ng·kg^{–1}·min^{–1}), human growth hormone (3 ng/kg/min), and glucagon (0.65 ng/kg/min) was started at the same time. Insulin was infused at a rate of 0.25 mU/kg/min from 0–180 min, 0.6 mU/kg/min from 181–300 min, and 2.0 mU/kg/min from 301–420 min. Glucose (containing [³-³H] glucose) was infused intravenously in amounts sufficient to maintain plasma glucose concentration at about 5.3 mmol/l. The rate of the basal intravenous [³-³H] glucose infusion was altered as follows in an effort to approximate the anticipated pattern of fall of glucose production and thereby maintain plasma glucose specific activity constant: (0 to 180 min: 50%, 181 to 300 min: 25%, 301 to 420 min, 15% [30].

Analytical techniques. Arterialized plasma samples were placed in ice, centrifuged at 4 °C, separated, and stored at –20 °C until assay. Plasma glucose concentrations were measured using a glucose oxidase method (Yellow Springs Instrument Co., Yellow Springs, Ohio, USA). Plasma insulin, cortisol and growth hormone concentrations were measured using a chemiluminescence assay with reagents obtained from Beckman (Access Assay, Beckman, Chaska, Minn., USA). Plasma glucagon and C-peptide concentrations were measured by radioimmunoassay (Linco Research, St. Louis, Mo., USA). Body composition was measured using dual-energy X-ray absorptiometry (DEXA scanner, Hologic, Waltham, Mass., USA). Plasma [³-³H] glucose specific activity was measured using liquid scintillation counting [31].

Calculations. Glucose appearance and disappearance were calculated using the steady state equations of Steele [32] using the actual tracer infusion rate for each interval. The volume of distribution of glucose was assumed to equal 200 ml/kg and the pool correction factor to equal 0.65. Endogenous glucose production was calculated by subtracting the glucose infusion rate from the tracer-determined rate of glucose appearance. All rates of infusion and turnover were expressed per kg of lean body mass.

Statistical analysis. Data in the text and figures are expressed as means $\pm$ SEM. All rates are expressed as μ mol per kilogram of lean body mass per min. In order to compare turnover under steady-state conditions from –30 to 0 min, 150 to 180 min, 270 to 300 min and 390 to 420 min, glucose disappearance and endogenous glucose production were calculated and used for statistical analysis. Repeated measures ANOVA was used to

Fig. 1. Plasma glucose, insulin and c-peptide concentrations observed during the study. *Open circles* represent values observed during saline infusion, *solid diamonds* represent values observed during GLP-1 infusion and *solid triangles* those values observed during exendin-4 infusion

Fig. 2. Plasma glucagon, growth hormone and cortisol concentrations observed during the study. *Open circles* represent values observed during saline infusion, *solid diamonds* represent values observed during GLP-1 infusion and *solid triangles* those values observed during exendin-4 infusion

test differences between groups. Student's *t* test was used post hoc to test the hypothesis that GLP-1 and exendin-4 increase insulin-induced suppression of endogenous glucose production and stimulation of glucose disappearance compared to saline infusion. A *p* value of less than 0.05 was considered to be statistically significant. In order to compare differences in hormone concentrations on the three study days, area under the curve of hormone concentration against time was calculated using the trapezoidal rule. Repeated measures ANOVA was used to test differences between study days.

Results

Plasma glucose, insulin and C-Peptide concentrations. Plasma glucose concentrations did not differ either prior to or during the hyperinsulinemic clamps on the three study days (Fig. 1). Insulin concentrations also did not differ on the three study days. Plasma insulin concentrations averaged respectively 68 ± 10 , 60 ± 6 , and 69 ± 5 pmol/l during the low dose insulin infusion, 201 ± 21 , 197 ± 14 , and 187 ± 13 pmol/l during the middle dose insulin infusion, and 733 ± 64 , 683 ± 44 , and 700 ± 55 pmol/l during the high dose insulin infusion on the GLP-1, exendin-4 and saline study days. Somatostatin resulted in prompt and comparable suppression of C-peptide on all the three study days.

Plasma glucagon, growth hormone and cortisol concentrations. Glucagon and growth hormone concentrations remained constant and equal on the three study days (Fig. 2, upper and middle panels). Basal cortisol concentrations also did not differ on the three study days. Plasma cortisol concentrations progressively fell on the saline study day, rose on the exendin-4 study day with an intermediate response being observed on the GLP-1 study day (Fig. 2, bottom panel). This resulted in higher ($p < 0.01$) plasma cortisol concentrations on the exendin-4 and GLP study days than on the saline study day (25.1 ± 0.4 vs 17.0 ± 0.2 vs 13.4 ± 0.1 mmol/l per 7 h). Plasma cortisol concentrations on the exendin-4 study day were higher ($p < 0.05$) than those on the GLP-1 study day.

Glucose infusion rates and glucose specific activity. The intravenous glucose infusion rate (GIR) necessary to maintain euglycaemia (Fig. 3, upper panel) when analysed using repeated measures ANOVA did not differ on the exendin-4, GLP-1 and saline study days during the low (15.6 ± 2.3 vs 12.5 ± 1.3 vs 11.2 ± 2.3 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$), middle (53.9 ± 5.4 vs 50.6 ± 5.0 vs 46.8 ± 4.1 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) and high (91.1 ± 5.4 vs 96.4 ± 6.0 vs 90.5 ± 3.8 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) insulin infusions.

Plasma [$3\text{-}^3\text{H}$] glucose specific activity was constant and equal on the three study days (Fig. 3, lower panel).

Fig. 3. Specific activity and intravenous glucose infusion rates necessary to maintain a constant plasma glucose concentration observed during the study. *Open circles* represent values observed during saline infusion, *solid diamonds* represent values observed during GLP-1 infusion and *solid triangles* those values observed during exendin-4 infusion

Glucose disappearance and endogenous glucose production. When analysed with repeated measures ANOVA, glucose disappearance did not differ on the exendin-4, GLP-1 and saline study days (Fig. 4) during the low (27.2 ± 3.4 vs 24.1 ± 1.4 vs 24.1 ± 3.2 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$), middle (57.7 ± 5.6 vs 62.7 ± 6.8 vs 52.3 ± 4.7 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$), or high (99.4 ± 6.7 vs 101.7 ± 5.1 vs 92.7 ± 4.1 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) dose insulin infusions.

Similarly repeated measures ANOVA indicated that endogenous glucose production also did not differ on the exendin-4, GLP-1 and saline study days (Fig. 5) during the low (7.4 ± 2.0 vs 8.9 ± 1.4 vs 5.8 ± 1.2 $\mu\text{mol}/\text{kg}/\text{min}$), middle (11.4 ± 1.1 vs 11.4 ± 2.1 vs 12.0 ± 1.3) or high (5.7 ± 2.6 vs 8.8 ± 4.2 vs 3.3 ± 1.2 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) dose insulin infusions, respectively.

Discussion

The effects of GLP-1 (7–36) on insulin action and glucose effectiveness have been a source of debate [6, 7]. In vitro studies have suggested that GLP-1 (7–36) alone or in combination with insulin can enhance liver, muscle and fat glucose uptake in isolated prepa-

Fig. 4. Rates of glucose disappearance observed during the study. *Open circles* represent values observed during saline infusion, *solid diamonds* represent values observed during GLP-1 infusion and *solid triangles* those values observed during exendin-4 infusion

Fig. 5. Rates of endogenous glucose production observed during the study. *Open circles* represent values observed during saline infusion, *solid diamonds* represent values ob-

served during GLP-1 infusion and *solid triangles* those values observed during exendin-4 infusion

rations of these tissues [9, 12, 14, 15, 16, 17]. We [23] and other investigators [21, 22, 24, 33, 34] have been unable to detect an effect of GLP-1 (7–36) on insulin action or glucose effectiveness in either non-diabetic or Type II diabetic subjects. Other studies [21, 24, 33] have reported that GLP-1 (7–36) does not alter insulin induced stimulation of glucose disposal in non-diabetic humans when GLP-1 (7–36) was infused for 3 h or less. In contrast, we observed that GLP-1 (7–36) increased glucose disappearance and impaired suppression of glucose production in people with Type I diabetes during the final hour of a 4 h hyperglycaemic and hyperinsulinaemic glucose clamp [20]. This led us to wonder whether a time-dependent generation of GLP-1 (7–36) degradation products could be responsible for these apparently conflicting results.

The present data argues against this explanation. The effects of GLP-1 (7–36) and a degradation-resistant analogue exendin-4, on insulin-induced stimulation of glucose disappearance and suppression of glucose production were essentially the same. Consistent with our prior observation in Type I diabetic subjects, post hoc analysis of our data indicated that glucose disappearance was slightly higher during the final hour of the GLP-1 (7–36) infusion than on the saline study day. The glucose infusion rates required to maintain euglycaemia also were slightly higher on the GLP-1 and exendin-4 study days. However, the differences were trivial. Therefore, while it is possible that these effects would become statistically significant if a larger number of subjects were studied, the present data taken in conjunction with the results of previous studies [20, 23] indicate that their biologic significance at most, will be small. Since the insulin infusions were sequential, we cannot distinguish between an effect of time or insulin dose. Perhaps more importantly, the effect on insulin action, if anything was less with exendin-4 arguing strongly against the possibility that generation of the GLP-1 receptor antagonist GLP-1 (9–36) obscured an otherwise more potent effect on insulin action. These data also suggest that both exendin-4 and GLP-1 primarily exert their glucose lowering effect in Type II diabetes by increasing insulin secretion, decreasing glucagon secretion and by delaying gastric emptying rather than by enhancing insulin-action.

We confirm that infusion of GLP-1 causes an increase in cortisol concentration [20, 23, 33]. Interestingly, this effect was even more prominent during infusion of exendin-4. Previous studies have shown that the GLP-1 stimulates both ACTH and cortisol implying a central site of action [33]. Since GLP-1 or exendin-4 can cause nausea, this could potentially contribute to increased ACTH concentrations. However, the increase in cortisol seems to be independent of nausea since this side effect only occurred on one of the GLP-1 and three of the exendin-4 study days. Furthermore nausea, when it occurred, was transient

and mild whereas the elevation of cortisol persisted throughout the 7 h of the experiment. Cortisol impairs insulin action in a time and dose-dependent manner [35]. Therefore, the increased cortisol concentrations could have offset the insulin-sensitizing effects of GLP-1 or exendin-4. If so, this will occur when these agents are used to treat diabetes since the doses used in these experiments are similar to those used in previous therapeutic trials.

In conclusion, insulin-induced stimulation of glucose uptake or suppression of glucose production were comparable during infusion of GLP and exendin-4 and differed minimally from that during infusion of saline. These data argue against a role of degradation of GLP by DPV IV peptidase in the modulation of GLP effects on insulin action. They lend further support to the concept that both of these agents lower blood glucose in people with Type II diabetes primarily by increasing insulin secretion, inhibiting glucagon secretion and delaying gastric emptying.

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