Effects of Glucagon-Like Peptide 1 (7–36 Amide) on Glucose Kinetics during Somatostatin-Induced Suppression of Insulin Secretion in Healthy Men

Key Words
Incretin
Pancreatic clamp
Glucose clamp
Peripheral glucose metabolism

Abstract
Glucagon-like peptide 1 (GLP-1) is known to stimulate insulin secretion and biosynthesis, but has also been shown to decrease insulin requirements in type 1 diabetic subjects suggesting insulin-independent effects. To assess whether GLP-1 exerts also direct effects on whole-body glucose metabolism, 6,6-D2-glucose kinetics were measured in 8 healthy volunteers receiving once GLP-1, once saline during hyperglycemic glucose clamping, while somatostatin with replacement amounts of insulin, glucagon and growth hormone was infused. Even though endogenous insulin secretion could not be blocked completely (increased plasma concentrations of C-peptide and proinsulin), somatostatin infusion resulted in stable insulin and glucagon plasma levels in both protocols (GLP-1 vs. placebo: NS). After 3 h of GLP-1 infusion, peripheral glucose disappearance significantly increased compared to placebo ($p < 0.03$) despite of somatostatin-induced suppression of insulin and glucagon secretion. Thus, GLP-1 infusion seems to have direct stimulatory effects on peripheral glucose metabolism in man.

Introduction
The incretin glucagon-like peptide 1, GLP-1 (7–36 amide) is known to stimulate insulin secretion from the pancreatic $\beta$-cell [1]. This insulinotropic activity is highly glucose dependent [2], suggesting no risk for hypoglycemia during administration of this peptide. In addition, GLP-1 activates proinsulin gene transcription, augments the rate of insulin gene expression and maintains the rate of insulin biosynthesis [3]. Administration of synthetic GLP-1 to healthy volunteers has been demonstrated to decrease hepatic glucose output [4]. In non-insulin-dependent (type 2) diabetic subjects, GLP-1 infusion has been shown to induce insulin and C-peptide secretion and to decrease glucagon release [5]. All these effects can easily be explained by an increase in plasma insulin concentrations caused by GLP-1 infusion. However, since GLP-1 administration was able to reduce insulin requirements in C-peptide-deficient, insulin-dependent (type 1) diabetic subjects [6], this peptide might also have an insulin-inde-
pendent activity of its own. This hypothesis is supported by the enhanced glucose effectiveness (insulin-independent glucose disposal) observed during GLP-1 infusion in healthy man [7] and by in vitro studies suggesting a glycogenic effect of GLP-1 in skeletal muscle [8].

The present study addressed the question whether GLP-1 infusion has also direct effects on whole-body glucose metabolism. To overcome GLP-1-induced hormonal changes, somatostatin was infused during all studies, while insulin, glucagon and growth hormone were replaced in basal amounts (pancreatic clamp) [9]. Glucose clamping was performed at 8 mmol/l taking into account the glucose dependence of GLP-1 effects and imitating mild hyperglycemia during restricted insulin availability.

**Subjects and Methods**

**Subjects**

Eight healthy male volunteers with a body mass index of 22 ± 1 kg/m² and a mean age of 25 ± 1 years gave their written informed consent to participate. For the last 2 weeks before the study, their body weight had been stable and they had not been on a diet or taking any drugs. The study protocol was approved by the Human Ethics Committee, Department of Medicine, University of Basel. Every volunteer was studied twice, with an interval of at least 1 week, once with GLP-1 and once with placebo (saline), in randomized order.

**Procedures**

At 8 a.m. after a 12-hour overnight fast, a Teflon cannula was inserted into an antecubital vein, and blood samples were obtained to assess background plasma enrichments of 6,6-D₂-glucose. A priming dose of 6,6-D₂-glucose (16.6 μmol/kg; 98% enriched, sterile and pyrogen free, Mass Trace, Woburn, Mass., USA) was injected. Thereafter, 6,6-D₂-glucose was infused at 0.22 μmol × kg⁻¹ × min⁻¹. A butterfly needle was inserted retrogradely into a dorsal hand vein for withdrawal of arterialized blood [10] while the hand was kept in a heated box at 57 °C. After a 2-hour equilibration period for the stable labeled isotope, blood samples were obtained during a 30-min baseline period. Thereafter, continuous infusions of somatostatin (90 ng × kg⁻¹ × min⁻¹; Stilamin, Serono, Aubonne, Switzerland), insulin (0.87 pmol × kg⁻¹ × min⁻¹; Actrapid HM, Novo, Bagsvaerd, Denmark), glucagon (0.2 ng × kg⁻¹ × min⁻¹; GlucaGen, Novo) and recombinant human growth hormone (6 ng × kg⁻¹ × min⁻¹; Genotropin, kindly provided by Pharmacia, Diübendorf, Switzerland) were started. This dose of somatostatin was at the limit of tolerance—nausea (without vomiting) occurred in 4 of 16 studies. After 30 min an infusion of synthetic GLP-1 (7–36 amide; 1.2 pmol × kg⁻¹ × min⁻¹; sterile and pyrogen free, net peptide content 91.68%, peptide purity >97%, Saxon Biochemicals, Hannover, Germany) or of placebo (NaCl 0.9% w/v) was commenced and continued for 180 min. This pharmacological infusion rate of GLP-1 has been used previously [5]. Immediately before administration, GLP-1, somatostatin and the other hormones were mixed in saline in 50-ml syringes with 2 ml of a subject’s own blood in order to prevent adsorption of the hormones to surfaces. During the infusion period hyperglycemia of 8 mmol/l was achieved and maintained by a glucose infusion (20% w/v). The infusion rate was frequently adjusted depending on the actual plasma glucose level. The variable glucose infusion was enriched to 1.5% with 6,6-D₂-glucose in order to prevent an underestimation of the rate of hepatic glucose production [11]. Plasma glucose concentrations were measured in 5- to 10-min intervals using a glucose oxidase method (Yellow Springs Glucose Analyzer model 23 AM, Yellow Springs, Ohio, USA) [12].

**Analyses and Calculations**

Plasma samples were analyzed for 6,6-D₂-glucose tracer/tracee ratio (TTR; D₂-labelled/unlabelled glucose) using a gas chromato-

**Glucose Kinetics**

During hyperglycemic clamping, plasma glucose concentrations increased rapidly and remained at 8.0 ± 0.1 mmol/l for the last 2 h in both protocols (fig. 1). The glucose infusion rates (GINF) required for maintaining plasma glucose at 8 mmol/l were 75% higher during the last 120 min of GLP-1 infusion than during placebo (p < 0.02). Rδ (fig. 2a) increased from 14.6 ± 0.9 during the basal period to 24.2 ± 2.2 μmol × kg⁻¹ × min⁻¹ during the last 30 min of GLP-1 infusion (infusion vs. basal period: p < 0.001) compared to placebo from 13.8 ± 0.8 to 17.2 ± 2.1 μmol × kg⁻¹ × min⁻¹ (infusion vs. basal peri-
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Fig. 1. Plasma glucose concentrations (lines) during baseline (–30 to 0 min) and during pancreatic and hyperglycemic clamping (30–210 min) in 8 healthy subjects receiving GLP-1 or saline infusion and the required glucose infusion rate (GINF; bars) for maintaining plasma glucose at 8 mmol/l. Means ± SEM; GLP-1 vs. placebo: plasma glucose levels (NS); GINF (p < 0.02).

od: NS; GLP-1 vs. placebo: p < 0.03). MCR (fig. 2b) augmented during GLP-1 administration from 2.81 ± 0.16 to 3.05 ± 0.29 ml × kg⁻¹ × min⁻¹. During placebo MCR slightly decreased from 2.70 ± 0.18 to 2.16 ± 0.27 ml × kg⁻¹ × min⁻¹ (GLP vs. placebo: basal: NS; clamp: p < 0.03). During the hyperglycemic clamp of the GLP-1 protocol, HGO decreased from baseline to 25 ± 5% (from 14.40 ± 0.94 to 3.61 ± 0.78 µmol × kg⁻¹ × min⁻¹; clamp vs. basal: p < 0.0001) and during placebo to 52 ± 6% (from 13.54 ± 0.85 to 7.01 ± 0.82 µmol × kg⁻¹ × min⁻¹; clamp vs. basal: p < 0.0001; GLP-1 vs. placebo: p < 0.01; fig. 2c).

Plasma Hormone Concentrations

Plasma insulin concentrations (fig. 3) remained at high basal level during the 210 min of combined infusion of somatostatin, insulin, glucagon and growth hormone. In spite of slightly increased levels at the end of the GLP-1 infusion, insulin concentrations were not significantly different between GLP-1 and placebo at any time during pancreatic clamping. However, during the 3-hour GLP-1 infusion, the plasma concentrations of C-peptide and proinsulin increased from 474 ± 33 (basal) to 634 ± 107 and from 7.3 ± 0.5 to 16.5 ± 2.5 pmol/l, respectively, whereas C-peptide and proinsulin levels decreased from basal 447 ± 46 to 65 ± 10 and from 6.1 ± 0.5 to 3.1 ± 0.3 pmol/l, respectively, during saline infusion (GLP-1 vs. saline: p < 0.002 and p < 0.03, respectively). Glucagon plasma levels remained unchanged by GLP-1 and saline infusion (15.9 ± 2.6 and 17.3 ± 1.5 pmol/l, respectively; GLP-1 vs. saline: NS).

Fig. 2. Effects of GLP-1 or saline infusion on Rd (a), MCR (b) and HGO (c) in 8 healthy subjects, as assessed by 6,6-D₂-glucose kinetics during baseline (–30 to 0 min) and during the last 30 min of pancreatic and hyperglycemic clamping (180–210 min). Means ± SEM. * p < 0.03, GLP-1 vs. placebo.
Discussion

In the present study, somatostatin infusion resulted in stable plasma glucagon levels throughout the experiment during both GLP-1 and saline infusion. Somatostatin also induced a marked suppression of insulin secretion (during GLP-1 infusion, plasma concentrations of insulin were 15 times lower than without somatostatin [15]).

Despite somatostatin-induced suppression of glucagon and insulin secretion, GLP-1 administration resulted in an increased rate of peripheral glucose disappearance suggesting, at least in part, a direct peripheral effect of GLP-1. A difference of 7 μmol × kg⁻¹ × min⁻¹ in Rd as found in the present study between GLP-1 and placebo has been observed only during an increase in plasma insulin concentrations of 180 pmol/l [16]. However, in the present study, measured plasma insulin levels differed by 30 pmol/l at the most between the two protocols. Although this amount of insulin could contribute to the observed changes in Rd, it is unlikely. Even to reduce the more insulin-sensitive HGO by 50% as demonstrated in the present study, an increase in plasma insulin of more than 50% would be necessary [16]. However, it is possible that increased insulin concentrations in the portal system, undetected in peripheral blood, were involved in this hepatic effect. The HGO decrease in the placebo group can be explained by the hyperglycemia present in both protocols.

C-peptide is known to be metabolically inactive [17], whereas proinsulin-mediated glucose disposal has been shown to be 7% that of insulin on a molar basis [18]. Consequently, the proinsulin increase during the GLP-1 infusion of 9.2 pmol/l would have the same effect as an increase in plasma insulin by 0.6 pmol/l, a rather negligible amount. Taken together, the increase in insulin precursors in the circulation cannot account for the observed changes in glucose metabolism.

The suggested direct effects of GLP-1 on peripheral tissue, i.e. skeletal muscle (Rd), are in agreement with in vitro experiments describing direct glycogenic effects of GLP-1 on rat skeletal muscle [8] and can explain, at least in part, the glucose-lowering effects of GLP-1 observed in type 1 diabetic patients [6].

In conclusion, the present results suggest that besides its known insulinotropic activity the incretin GLP-1 has metabolic effects influencing peripheral glucose metabolism in humans. Further studies in insulin-dependent C-peptide-deficient diabetic patients are necessary in order to confirm the therapeutical significance of these findings.

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References


