Original Paper

Hormone Research

Horm Res 1998;49:221–225

Received: December 4, 1996
Accepted after revision: October 13, 1997

Anath Shalev
Ronald Ninnis
Ulrich Keller

Departments of Medicine and Research, University Hospital Basel, Switzerland

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-D_2-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 pancratic β-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-

This work was supported by grants from the Swiss National Science Foundation (No. 32-39747.93) and the 'Wissenschaftliche Kredit', University Hospital Basel. A.S. is a recipient of grants from the Postgraduate Course in Experimental Medicine and Biology (Swiss National Science Foundation), from the 'Freiwillige Akademische Gesellschaft' and from ESPEN-Ajinomoto.

KARGER
Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0301–0163/98/0495–0221$15.00/0

This article is also accessible online at:
http://BioMedNet.com/karger

Dr. Anath Shaley
Department of Medicine
University Hospital of Basel, Petersgraben 4
CH–4031 Basel (Switzerland)
Tel. +41 61 265 25 25, Fax +41 61 361 53 51, E-Mail Shalev@ubaclu.unibas.ch

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/m^2 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-D_2-glucose. A priming dose of 6,6-D_2-glucose (16.6 μmol/kg; 98% enriched, sterile and pyrogen free, Mass Trace, Woburn, Mass., USA) was injected. Thereafter 6,6-D_2-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, Bagsvaeard, 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, Dübendorf, 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-D_2-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-D_2-glucose tracer/tracee ratio (TTR; D_2-labelled/unlabelled glucose) using a gas chromatograph-mass spectrometer (Hewlett Packard Mod. 5890/5970, Palo Alto, Calif., USA). The glucose derivative was (2,3,4,5,6)-pentakis-*O*-trimethylsilyl-*O*-methyloxime-*D*-glucose, and the masses used for the mass-spectrometric measurements of labelled and unlabelled glucose were 321 and 319, respectively [13]. Glucose rate of appearance equalled glucose rate of disappearance (Rd) since steady state conditions were present during baseline and during the last 30 min of the infusion period as D_2-glucose TTR did not change over time (ANOVA with repeated measures). Due to the enrichment of the variable glucose infusion, the TTR did not decrease over the whole period of time. Rd as a measure of peripheral glucose utilization was calculated by dividing the D_2-glucose infusion rate by the D_2-glucose TTR. Division of Rd by plasma glcuose concentration yielded the metabolic clearance rate of glucose (MCR). Hepatic glucose output (HGO) was computed by subtracting the total glucose infusion rate from the glucose rate of appearance [14]. D_2-background enrichments were subtracted from all corresponding plasma values. Plasma concentrations of insulin, proinsulin, C-peptide and glucagon were measured by radioimmunoassays [INSIK-5 (P2796), SORIN Biomedica, Saluggia, Italy; coefficient of variation, CV: 8.2%; human proinsulin, LINCO Research, St. Charles, Mo., USA; C-PEP-CT2, CIS Bio International, France (CV 5.8%; cross-reactivity with proinsulin 13%; no detectable cross-reactivity with GLP-1) and glucagon double antibody, Diagnostic Products, Bühlmann Laboratories, Basel, Switzerland (CV 8.7%), respectively]. The software STATVIEW (Macintosh) was used for statistical analyses. Effects of protocol and time were assessed by repeated measures ANOVA. Paired t tests were performed to compare differences between the two protocols. Results are expressed as means ± SEM.

Results

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 ($p < 0.02$). Rd (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-

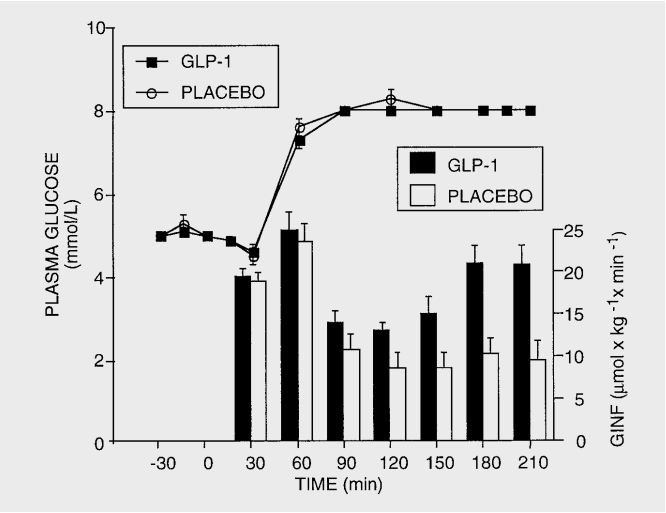

Fig. 1. Plasma glucose concentrations (lines) during baseline (–30 to 0 min) and during pancreatic and hyperglycemic clamping (30–210 min) in 8 healthy subjects receiving GLP-1 or saline infusion and the required glucose infusion rate (GINF; bars) for maintaining plasma glucose at 8 mmol/l. Means ± SEM; 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).

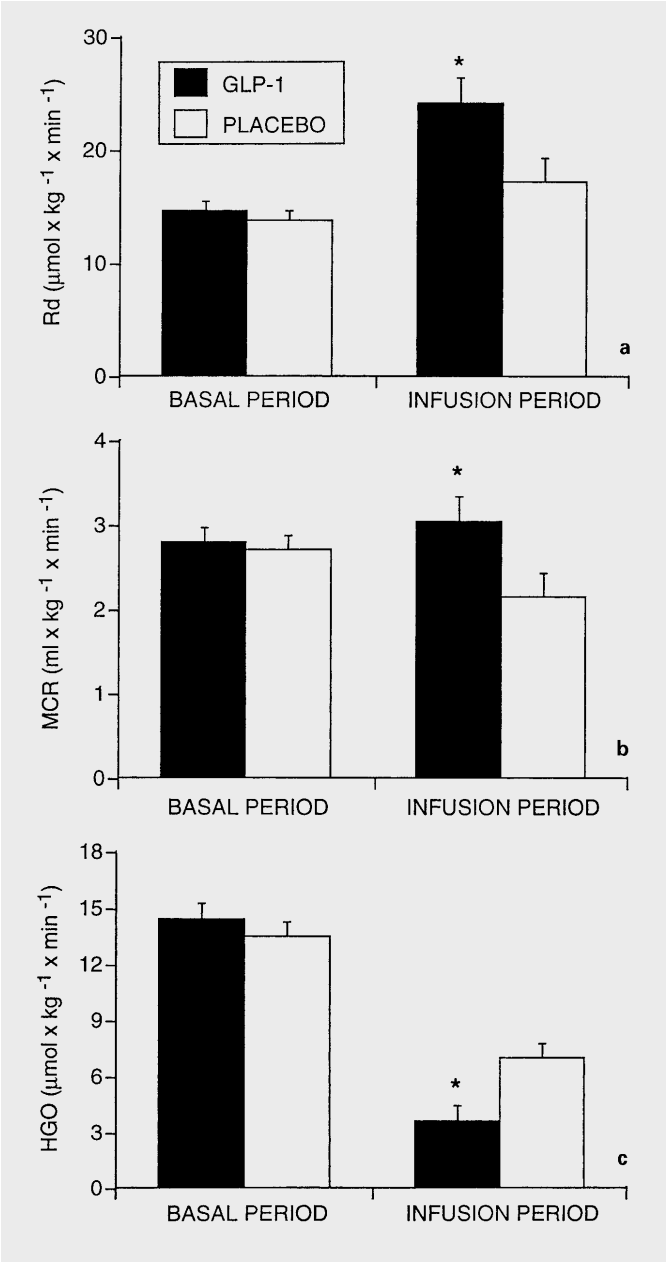

Fig. 2. Effects of GLP-1 or saline infusion on Rd (**a**), MCR (**b**) and HGO (**c**) in 8 healthy subjects, as assessed by 6,6-D_2-glucose kinetics during baseline (–30 to 0 min) and during the last 30 min of pancreatic and hyperglycemic clamping (180–210 min). Means ± SEM. * $p < 0.03$, GLP-1 vs. placebo.

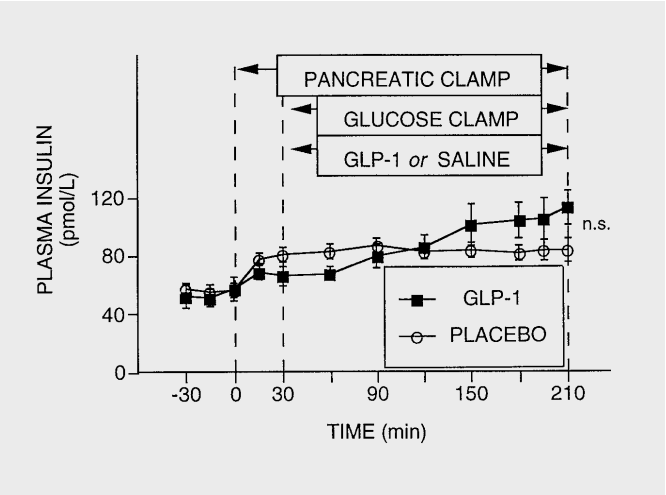

Fig. 3. Effects of GLP-1 or saline infusion on plasma concentrations of insulin in 8 healthy subjects (baseline –30 to 0 min; somatostatin infusion 0–210 min and hyperglycemic infusion period 30–210 min). Means ± SEM. GLP-1 vs. placebo: NS.

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 $\mu mol \times kg^{-1} \times min^{-1}$ 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.

Acknowledgments

We would like to thank Ms. S. Vosmeer, Ms. K. Dembinski and Mr. N. Sansano for excellent technical assistance.

References

1 Holst JJ, Orskov C, Nielsen OV, Schwartz TW: Truncated glucagon-like peptide I, an insulin-releasing hormone from the distal gut. FEBS Lett 1987;211:169–174.

2 Göke R, Wagner B, Fehmann HC, Göke B: Glucose-dependency of the insulin stimulatory effect of glucagon-like peptide-1 (7–36) amide on the rat pancreas. Res Exp Med (Berl) 1993; 193:97–103.

3 Fehmann HC, Habener JF: Insulinotropic hormone glucagon-like peptide-1 (7–37) stimulation of proinsulin gene expression and proinsulin biosynthesis in insulinoma beta TC-1 cells. Endocrinology 1992;130:159–166.

4 Hvidberg A, Nielsen MT, Hilsted J, Orskov C, Holst JJ: Effect of glucagon-like peptide-1 (proglucagon 78–107 amide) on hepatic glucose production in healthy man. Metabolism 1994; 43:104–108.

5 Nauck MA, Kleine N, Orskov C, Holst JJ, Willms B, Creutzfeldt W: Normalization of fasting hyperglycaemia by exogenous glucagon-like peptide 1 (7–36 amide) in type 2 (non-insulin-dependent) diabetic patients. Diabetologia 1993;36:741–744.

6 Gutniak M, Orskov C, Holst JJ, Ahren B, Efendic S: Antidiabetogenic effect of glucagon-like peptide-1 (7–36) amide in normal subjects and patients with diabetes mellitus. N Engl J Med 1992;326:1316–1322.

7 D'Alessio DA, Kahn SE, Leusner CR, Ensinck JW: Glucagon-like peptide 1 enhances glucose tolerance both by stimulation of insulin release and by increasing insulin-independent glucose disposal. J Clin Invest 1994;93:2263–2266.

8 Villanueva PM, Alcantara AI, Clemente F, Delgado E, Valverde I: Potent glycogenic effect of GLP-1 (7–36) amide in rat skeletal muscle. Diabetologia 1994;37:1163–1166.

9 Hood VL, Keller U, Haymond MW, Kury D: Systemic pH modifies ketone body production rates and lipolysis in humans. Am J Physiol 1990;259:E327–E334.

10 Abumrad NN, Rabin D, Diamond MP, Lacy WW: Use of heated superficial hand vein as alternative site for the measurement of amino acid concentrations and for the study of glucose and alanine kinetics in man. Metab Clin Exp 1981;30:936–940.

11 Molina JM, Baron AD, Edelman SV, Brechtel G, Wallace P, Olefsky JM: Use of a variable tracer infusion method to determine glucose turnover in humans. Am J Physiol 1990;258: E16–E23.

12 DeFronzo RA, Tobin JD, Andres R: Glucose clamp technique: A method for quantifying insulin secretion and resistance. Am J Physiol 1979;237:E214–W223.

13 Kuery D, Keller U: Trimethylsilyl-*O*-methyloxime derivatives for the measurements of (6,6-2H2)-D-glucose-enriched plasma samples by gas chromatography-mass spectrometry. J Chromatogr 1991;572:302–306.

14 Cobelli C, Toffolo G, Bier DM, Nosadini R: Models to interpret kinetic data in stable isotope tracer studies. Am J Physiol 1987;253: E551–E564.

15 Nauck MA, Heimesaat MM, Orskov C, Holst JJ, Ebert R, Creutzfeldt W: Preserved incretin activity of glucagon-like peptide 1 (7–36 amide) but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. J Clin Invest 1993;91:301–307.

16 Rizza RA, Mandarino LJ, Gerich JE: Dose-response characteristics for effects of insulin on production and utilization of glucose in man. Am J Physiol 1981;240:E630–E639.

17 Polonsky KS, Rubenstein AH: The kinetics and metabolism of insulin, proinsulin, and C-peptide; in DeGroot LJ (ed): Endocrinology. Philadelphia, Saunders, 1989, vol 2, pp 1304–1317.

18 Revers RR, Henry R, Schmeiser L, Kolterman O, Cohen R, Rubenstein A, Frank B, Galloway J, Olefsky JM: Biosynthetic human insulin and proinsulin have additive but not synergistic effects on total body glucose disposal. J Clin Endocrinol Metab 1984;58:1094–1098.