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ScienceDirect
Regulatory Peptides 137 (2006) 168–172
REGULATORY PEPTIDES
www.elsevier.com/locate/regpep

The elimination rates of intact GIP as well as its primary metabolite, GIP 3-42, are similar in type 2 diabetic patients and healthy subjects

Tina Vilsbøll [a,*], Henrik Agersø [b], Torsten Lauritsen [c], Carolyn F. Deacon [e], Kasper Aaboe [a], Sten Madsbad [d], Thure Krarup [a], Jens Juul Holst [e]

[a] Department of Internal Medicine F, Gentofte Hospital, Denmark
[b] Exploratory ADME, Novo Nordisk A/S, Denmark
[c] Department of Anaesthesiology, Rigshospitalet, Denmark
[d] Department of Endocrinology, Hvidovre Hospital, Denmark
[e] Department of Medical Physiology, The Panum Institute, Denmark

Received 3 March 2006; received in revised form 7 July 2006; accepted 13 July 2006
Available online 28 August 2006

Abstract

The incretin hormone, glucose-dependent insulinotropic polypeptide (GIP, previously known as gastric inhibitory polypeptide), is rapidly degraded to the biologically inactive metabolite GIP (3-42) in the circulation, but little is known about the kinetics of the intact hormone and the metabolite and whether differences exist between patients with type 2 diabetes mellitus and healthy subjects. We examined eight type 2 diabetic patients (six men, two women); mean (range) age: 59 (48–69) years; BMI: 31.6 (26.0–37.7) kg/m^2; HbA_{1C}: 9.0 (8.2–13.2) %; fasting plasma glucose (FPG): 10.0 (8.3–13.2) mmol/l and 8 healthy subjects matched for age, gender and BMI. An intravenous bolus injection of GIP (7.5 nmol) was given and venous blood samples were drawn the following 45 minutes. Peak concentrations of total GIP (intact+metabolite, mean±SEM) and intact GIP (in brackets) were 920±91 (442±52) pmol/l in the type 2 diabetic patients and 775±68 (424±30) pmol/l in the healthy subjects (NS). GIP was eliminated rapidly with the clearance rate for intact GIP being 2.3±0.2 l/min in the type 2 diabetic patients and 2.4±0.2 l/min in the healthy subjects (NS). The volumes of distributions were similar in the two groups and ranged from 8 to 21 l per subject. The primary metabolite, GIP 3-42, generated through the action of dipeptidyl peptidase IV (DPP-IV), was eliminated with a mean half-life of 17.5 and 20.5 min in patients and healthy subjects (NS). Conclusion: Elimination of GIP is similar in obese type 2 diabetic patients and matched healthy subjects. Differences in elimination of GIP and its primary metabolite, therefore, do not seem to contribute to the defective insulinotropic effect of GIP in type 2 diabetes.

Keywords: Glucose-dependent insulinotropic polypeptide; GIP; Glucagon-like peptide-1; GLP-1; Elimination; Incretin hormones

1. Introduction

Glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are intestinal incretin hormones which are released in response to ingestion of a mixed meal. Both incretin hormones contribute to the incretin effect, i.e. the potentiation of insulin release in response to ingestion of glucose or nutrients (as opposed to parenteral administration) [1,2]. Once secreted, GIP and GLP-1 are rapidly degraded in plasma by the enzyme dipeptidyl peptidase IV (DPP-IV) to form N-terminally truncated, biologically inactive peptides [3].

Abbreviations: GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; PG, plasma glucose; FPG, fasting plasma glucose; AUC, area under the curve from time 0 to infinity; DPP-IV, dipeptidyl peptidase IV; CL, clearance; C_{max}, the maximum observed plasma concentration; t_{max}, time to reach C_{max}; $t_{1/2}$, half-life; V_z, volume of distribution during the elimination phase; NS, not statistically significant.

* Corresponding author. Department of Internal Medicine F, Gentofte Hospital, Niels Andersens Vej 65, DK-2900 Hellerup, Denmark. Tel.: +45 40940825; fax: +45 39777661.
E-mail address: t.vilsboll@dadlnet.dk (T. Vilsbøll).

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doi:10.1016/j.regpep.2006.07.007

The intact peptides are inactivated in the capillaries of the gut and the liver and further degraded in the peripheral tissues, while the kidney is important for the final elimination of the metabolites [4,5]. Both hormones are currently considered for the treatment of type 2 diabetes because of their glucose lowering effect [6,7]. However, the therapeutic use of the peptides is still limited by their short in vivo half-lives. Different approaches are currently being evaluated to make use of the therapeutic potential of the incretin hormones: DPP-IV resistant analogues of GIP and GLP-1 have been synthesized to extend the in vivo half-life of the peptides [7], and inhibitors of the degrading enzyme DPP-IV have been generated to block the rapid degradation of endogenous GIP and GLP-1 [8].

Previous studies have shown that the incretin effect is abolished or severely reduced in patients with type 2 diabetes [9] and that the insulinotropic effect of GIP is severely reduced [10]. The postprandial responses of GIP in type 2 diabetic patients have been reported to be decreased [11–13], unaltered [14] or increased [15]. However, determined with specific assays, a smaller meal response is a significant finding in type 2 patients as compared to healthy subjects [12]. Decreased GIP plasma concentrations could be caused by an increased elimination of the peptide in type 2 diabetic patients compared to healthy subjects. Indeed, in a previous study, we found a shorter plasma half-life for GIP in patients with type 2 diabetes compared to matched healthy subjects [16]. The aim of the present investigation was to perform a detailed, mathematical kinetic analysis of GIP in type 2 diabetic patients and matched healthy subjects after intravenous administration of GIP and thereby elucidates whether an increased elimination of GIP contributes to the impaired incretin effect seen in type 2 diabetic patients. Since it has been reported that the primary metabolite, GIP (3-42) may act as an antagonist at the GIP receptor in mice [17], it was considered important to study its elimination as well.

2. Materials and methods

2.1. Participants

We studied 8 type 2 diabetic patients (six men, two women); mean (range) age: 59 (48–69) years; BMI: 31.6 (26.0–37.7) kg/m^2; HbA_{1C}: 9.0 (8.2–13.2) %; fasting plasma glucose (FPG): 10.0 (8.3–13.2) mmol/l; duration of diabetes: 42 (8–97 months), and 8 matched healthy subjects (six men, two women): age: 58 (51–70) years; BMI: 31.9 (26.4–37.9) kg/m^2; FPG: 5.6 (5.2–6.4) mmol/l; HbA_{1C}: 5.6 (5.2–6.0) %. Four patients were treated with diet alone while four were treated with diet and oral antidiabetics (biguanides and/or sulphonylureas). Five patients had a history of hypertension and were treated with thiazides or ACE-inhibitors. All diabetic patients were diagnosed according to the criteria of WHO [18,19]. All patients had normal renal function (serum creatinine levels < 130 μmol/l, albuminuria < 300 mg/day), no proliferative retinopathy or impaired liver function. None of the healthy subjects had a family history of diabetes and all had normal oral glucose tolerance test (OGTT). All agreed to participate and gave oral and written consent. The study was approved by the Copenhagen County Ethical Committee, and the study was conducted according to the principles of the Helsinki Declaration.

2.2. Experimental protocol

All oral antidiabetics were discontinued 3 days before the study. After an overnight fast (from 10 PM), the subjects were studied recumbent with two cannulas inserted into the cubital veins, one for injection of GIP and one for blood sampling. All participants were examined once and received an intravenous bolus injection of 7.5 nmol of GIP. Venous blood was sampled 15, 10 and 0 min before GIP administration and frequently during the following 48 min. Blood was sampled into chilled EDTA tubes (6 mmol/l) with aprotinin (500 KIU/ml blood; Trasylol, Bayer, Leverkusen, Germany) for hormone analyses. Tubes were immediately cooled on ice and centrifuged at 4 °C within 20 min. Studies in our laboratory have shown that this procedure effectively prevents DPP-IV mediated degradation of GIP [16]. Plasma was stored at minus 20 °C until analysis. Synthetic GIP was purchased from PolyPeptide Laboratories GmbH (Wolfenbüttel, Germany). The peptide was dissolved in sterilized water containing 2% human serum albumin (Human Albumin, Statens Serum Institute, Denmark, guaranteed to be free of hepatitis-B surface antigen, hepatitis-C virus antibodies and human immunodeficiency virus antibodies) and subjected to sterile filtration. Appropriate amounts of peptide for each experimental subject were dispensed into glass ampoules and stored frozen under sterile conditions until the day of the experiment. The peptide was demonstrated to be >97% pure and identical to the natural human peptides by HPLC-, mass-, and sequence analysis.

2.3. Analysis

Total GIP was measured using the C-terminally directed antiserum R65 [20,21], which reacts fully with intact GIP and the N-terminally truncated metabolite, GIP (3-42). The assay has a detection limit of less than 2 pmol/l and an intra-assay variation of approximately 6%. Intact, biologically active GIP was measured using a newly developed assay as described [12,16]. The assay is specific for the intact N-terminus of GIP, and cross-reacts less than 0.1% with GIP (3-42), or with the structurally related peptides GLP-1 (7-36)amide, GLP-1 (9-36)amide, GLP-2 (1-33), GLP-2 (3-33) or glucagon at concentrations of up to 100 nmol/l. Intra-assay variation was less than 6% and inter-assay variation was approximately 8 and 12% for 20 and 80 pmol/l standards, respectively. In some cases, in the fasting state, the value of N-terminal immunoreactivity exceeded the value of C-terminal immunoreactivity by a factor greater than might be expected because of the variation associated with the use of two assays of differing specificity. The explanation for this is unknown, but the following possibilities may contribute: 1) Presence of non-specific interfering plasma factors (which were not eliminated by prior extraction) having a greater effect in one assay compared to the other. 2) The presence of endogenous GIP metabolites, other than GIP (3-42), cross-reacting in one but not the other assay. 3) The presence of other endogenous factors