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Pancreatic β-cell responses to GLP-1 after near-normalization of blood glucose in patients with type 2 diabetes☆

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ABSTRACT

This study investigated the effects of strict glycaemic control on β-cell function in nine obese subjects with type 2 diabetes (T2DM), using graded glucose infusions together with infusions of saline or GLP-1 before (HbA_1c: 8.0 ± 0.4%) and after four weeks of near-normalization of blood glucose (BG) using insulin (mean diurnal BG: 6.4 ± 0.3 mmol/l; HbA_1c: 6.6 ± 0.3%). Nine matched healthy subjects acted as controls.
In controls, area-under-curve (AUC) for amylin, C-peptide and proinsulin were higher with GLP-1 than saline ($P<0.001$). The AUC amylin/C-peptide ratio was similar on both days, while AUC proinsulin/C-peptide ratio was higher with GLP-1 ($P=0.02$).
In the patients, amylin, C-peptide and proinsulin AUCs were unaltered by near-normoglycaemia per se. Proinsulin responses to GLP-1 were unchanged, but amylin and C-peptide AUCs increased ($P<0.05$) after insulin treatment, and AUC amylin/C-peptide ratios rose to control levels. Near-normoglycaemia tended to reduce AUC proinsulin/C-peptide ratio, which was significant ($P=0.04$) with GLP-1, but still higher than with saline ($P=0.004$).
In conclusion, amylin, C-peptide and proinsulin responses to glucose were unaffected by four weeks of near-normoglycaemia, whereas GLP-1 increased amylin and C-peptide secretion and amylin/C-peptide ratio. Near-normoglycaemia reduced proinsulin/C-peptide ratio during stimulation with GLP-1, suggesting that strict glycaemic control might ameliorate some of the disturbances in β-cell function characterizing T2DM.

1. Introduction

Studies on β-cell function in subjects with type 2 diabetes mellitus (T2DM) are limited by the fact that investigators cannot have direct access to the β-cell themselves. Therefore, our understanding of β-cell function is based upon indirect evidence obtained by measuring products of the β-cell which are secreted into circulation. Amylin, insulin, C-peptide and proinsulin (PI) are stored together in the secretory granules, and all four peptides are released into the circulation in response to β-cell secretagogues [1]. The β-cell is equipped with both regulated and constitutive pathways of secretion [2]. In glucose tolerant subjects, proinsulin is efficiently (>99%) sorted to the regulated secretory pathway, and is released primarily from secretory granules together with insulin and C-peptide [3]. Insulin and C-peptide are released in equimolar amounts, but because C-peptide, unlike insulin, does not undergo hepatic extraction, peripheral C-peptide concentrations are used as an index of insulin secretion [4]. Amylin is co-localized and co-secreted with insulin, but amylin has also been shown to be secreted via the constitutive secretory pathways in immature neonatal rat β-cells, as well as in human islets cultured in high glucose (14–16). A decreased plasma amylin concentration can be demonstrated in subjects with T2DM [5]. It is well recognized that the loss of glucose-induced insulin secretion in T2DM, manifested by the absent first-phase and decreased second-phase response, reflects multiple abnormalities in insulin secretion [6]. Thus, defective proinsulin processing, resulting in disproportionately elevated amounts of proinsulin in the secretory granule, has been proposed as a particular aspect of the β-cell dysfunction, and results in increased plasma proinsulin/insulin and proinsulin/C-peptide ratios [7]. One explanation for the increased proinsulin levels is that hyperglycaemia increases the demand for insulin, thereby forcing β-cells to secrete immature granules containing a higher concentration of proinsulin. This theory is supported by the fact that near-normalizing blood glucose (BG) for two weeks in poorly controlled T2DM patients, using an insulin pump, lowered proinsulin levels, both absolutely, as well as relatively to C-peptide [8].

Glucagon-like peptide 1 (GLP-1) is an incretin hormone secreted from intestinal L-cells in response to meal ingestion. In the pancreas, GLP-1 increases all steps in insulin biosynthesis, increases glucokinase and the glucose transporter GLUT2 mRNA levels in β-cells and

Abbreviation: GLP-1, glucagon-like peptide-1; T2DM, type 2 diabetes mellitus; BG, blood glucose; PG, plasma glucose.

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glucose-dependently stimulates meal-induced insulin secretion [9]. Animal studies have shown that GLP-1 stimulates β-cell proliferation and decreases β-cell apoptosis [10]. Furthermore, incretin-based therapies including both GLP-1 agonist and dipeptidyl peptidase-4 (DPP-4) inhibitors decrease the proinsulin/insulin ratio in T2DM, indicative of improved β-cell function [11].

There is considerable evidence that prolonged exposure of β-cells to high glucose levels in T2DM induces functional defects in insulin secretion in response to both glucose and non-glucose secretagogues [12]. Glucotoxicity is known to be partly reversible, since restoration of normoglycaemia with insulin in patients with T2DM improves β-cell function [8]. We have recently reported that 4 weeks of near-normoglycaemia did not change insulin response to glucose alone, but significantly improved GLP-1 potentiation of glucose-induced insulin secretion [13]. However, it is unclear whether restoration of normoglycaemia in T2DM affects amylin, C-peptide and proinsulin secretion during GLP-1 stimulation. Since amylin is co-secreted with insulin in response to β-cell stimulation, therapies that alter endogenous insulin secretion are likely to cause parallel changes in amylin secretion. Accordingly, treatment with GLP-1 increased expression and secretion of amylin in spontaneously diabetic rats [14]. The present study was designed to investigate whether a period of intensive insulin therapy, to normalize plasma glucose (PG), changes the pattern of amylin, proinsulin and C-peptide responses to glucose. Furthermore, we describe the acute effect of GLP-1 on the glucose-induced secretion of the three peptide hormones.

2. Materials and methods

2.1. Subjects

Detailed description of the demographic data and methods has been provided previously [13]. Briefly, nine patients with T2DM (six men) aged 53 ± 8 years (mean ± SD), duration of diabetes 5 ± 4 years (mean ± SD) with fasting PG 8.5 ± 4 mM (mean ± SD), and nine healthy subjects (six men, age; 55 ± 7 years (mean ± SD)) with fasting PG of 5.0 ± 0.3 mmol/l were studied. The BMI of the two groups was 31 ± 4 kg/m² and 31 ± 5 kg/m² (mean ± SD), HbA_{1c} 8.0 ± 0.4 and 5.4 ± 0.1% and fructosamine 316 ± 20 and 220 ± 8 uM, respectively. None of the healthy subjects had a family history of diabetes, and all had normal oral glucose tolerance (assessed by 75-g oral glucose tolerance tests). Neither patients nor healthy control subjects had islet cell autoantibodies or glutamate decarboxylase-65-autoantibodies.

The patients did not suffer from any overt diabetic complications or other somatic medical illnesses. Two of the patients were treated with diet only, six were treated with metformin and one was treated with metformin and sulfonylurea. Antidiabetic drugs were discontinued at least 3 weeks before each experiment. All studies were carried out at the Department of Endocrinology, Copenhagen University Hospital at Hvidovre, Copenhagen, Denmark, and all subjects agreed to participate after oral and written information. The ethics committee in Copenhagen Municipality approved the protocol, and the study was performed in accordance with the Helsinki Declaration II. The study is registered at http://www.clinicaltrials.gov.

The patients were investigated, as described below, before and after 4 weeks of insulin treatment. Glycaemic control before the start of insulin treatment was evaluated from blood glucose (BG) 7 point profiles carried out seven times daily for three days i.e., before and 1 1/2 h after each main meal and at 10 p.m. Additionally, HbA_1c and plasma fructosamine were measured.

2.2. Insulin treatment

To optimize glycaemic control, patients were treated with multiple insulin injections. Insulin therapy, including administration of a fast-acting insulin analog before each meal, as well as basal insulin coverage by intermediate-acting (NPH) insulin administered twice daily, was based on self-monitored BG using a One Touch Ultra meter (Life Scan). The targeted fasting (pre-breakfast) BG level was below 5 mmol/l (without incurring unacceptable risk of hypoglycaemia no patients experienced severe hypoglycaemia) and targeted postprandial BG level below 8 mmol/l [13].

2.3. Experimental protocol

The patients were investigated on separate days using graded glucose infusions together with infusion of GLP-1 (1 pmol kg^{-1} min^{-1}) or saline, before and after 4 weeks of insulin treatment, giving a total of four experimental days.

The evening before each investigation, patients were treated with Mixtard (30/70) (NovoNordisk, Denmark) at bedtime in order to obtain fasting BG between 6 and 10 mmol/l. No subject experienced BG levels of below 5.0 mmol/l on the evening and night before experiments, as evaluated from BG measurements at 10 p.m., 3 a.m. and 7 a.m. Subjects attended the ward at 8 a.m., after fasting (including abstinence from smoking) from 10:00 p.m. the evening before, and studies were performed with the subjects in a recumbent position. Intravenous catheters were placed in each forearm, one for blood sampling and one for infusion. The hand with the sampling catheter was heated. At the beginning of the experiment, patients received a continuous intravenous infusion of fast-acting insulin (Actrapid; NovoNordisk, Denmark) at a rate of 0.1 IU $kg^{-1}h^{-1}$. Once PG had reached 6 mmol/l, the infusion was stopped (time −60 min).

2.3.1. GLP-1 and glucose infusions

At time −30 min, an intravenous GLP-1 (1.0 pmol $kg^{-1}min^{-1}$; GLP-1 day) or saline (saline day) infusion was started using an automatic pump (Volumed μVP 5000, B. Braun; Melsungen, AG, Germany). A stepwise intravenous infusion of glucose (20% dextrose) was started at time 0 min at rates of 2, 4, 6, 8 and 12 mg/kg/min, with each infusion rate being maintained for 30 min. Blood samples were drawn at time 10, 20 and 30 min of each 30 min period for measurements of plasma insulin, C-peptide and glucose. Every 30 min throughout the study and at time −60, −30, −20, −10, and 0 min, blood samples were drawn for measurement of GLP-1, proinsulin, amylin and glucagon.

2.4. GLP-1

Synthetic GLP-1 (7–36) amide was purchased from PolyPeptide Laboratories (Wolfenbüttel, Germany), and the same lot number was used in all studies. The peptide, dissolved in sterilized water containing 2% human serum albumin (Human Albumin, Statens Serum Institute, Copenhagen, Denmark: guaranteed to be free of hepatitis B surface antigen, hepatitis C virus antibodies and human immunodeficiency virus antibodies), was subjected to sterile filtration, and appropriate amounts for each experimental subject were dispensed into glass ampoules and stored frozen (−20 °C) under sterile conditions until the day of the experiment. The peptide was demonstrated to be >97% pure and identical to the natural human peptide by HPLC, mass and sequence analysis.

2.5. Biochemical analysis

Blood samples for PG analysis were measured immediately by the glucose oxidase method on a Beckman glucose analyzer, (Ramcon, Denmark). Plasma C-peptide concentrations were measured by auto-DELPHIA automatic fluoroimmunoassay (Wallac, Inc.,Turku, Finland). Amylin was measured by ELISA with immunofluorescent detection (Linco). Total proinsulin was measured by a two-site ELISA [15]. Glucagon and total GLP-1 concentrations were measured after extraction of plasma with 70% ethanol (vol/vol, final concentration).