Peptides 88 (2017) 74–79

Contents lists available at ScienceDirect

Peptides

journal homepage: www.elsevier.com/locate/peptides

Glucagon increases insulin levels by stimulating insulin secretion without effect on insulin clearance in mice

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ARTICLE INFO

Article history:
Received 25 August 2016
Received in revised form
28 November 2016
Accepted 20 December 2016
Available online 21 December 2016

Keywords:
Glucagon
Insulin secretion
Insulin clearance
Population analysis

ABSTRACT

Circulating insulin is dependent on a balance between insulin appearance through secretion and insulin clearance. However, to what extent changes in insulin clearance contribute to the increased insulin levels after glucagon administration is not known. This study therefore assessed and quantified any potential effect of glucagon on insulin kinetics in mice. Prehepatic insulin secretion in mice was first estimated following glucose (0.35 g/kg i.v.) and following glucose plus glucagon (10 μg/kg i.v.) using deconvolution of plasma C-peptide concentrations. Plasma concentrations of glucose, insulin, and glucagon were then measured simultaneously in individual mice following glucose alone or glucose plus glucagon (pre dose and at 1, 5, 10, 20 min post). Using the previously determined insulin secretion profiles and the insulin concentration-time measurements, a population modeling analysis was applied to estimate the one-compartment kinetics of insulin disposition with and without glucagon. Glucagon with glucose significantly enhanced prehepatic insulin secretion (Cmax and $AUC_{0\text{-}20}$) compared to that with glucose alone ($p < 0.0001$). From the modeling analysis, the population mean and between-animal SD of insulin clearance was 6.4 ± 0.34 mL/min for glucose alone and 5.8 ± 1.5 mL/min for glucagon plus glucose, with no significant effect of glucagon on mean insulin clearance. Therefore, we conclude that the enhancement of circulating insulin after glucagon administration is solely due to stimulated insulin secretion.

1. Introduction

The chronically elevated blood glucose concentrations in type 2 diabetes (T2D) result mainly from insulin resistance in peripheral tissues in association with decreased β-cell function [1]. In addition, elevated concentrations of blood glucagon are also associated with increased insulin resistance and elevated glucose concentrations both in normal subjects and in T2D [2,3], and, therefore, glucagon also seems important for the pathophysiology of T2D.

A well-known effect of glucagon is to stimulate insulin secretion from the islet beta cells, which raises insulin concentrations [4]. There is, however, also a suggestion that glucagon may alter insulin kinetics. Thus, in healthy human subjects who underwent hyperglycemic clamp [5], insulin clearance was lower during greater stimulation of insulin secretion at higher glucose-clamp levels, suggesting that hyperglucagonemia may have an indirect influence on insulin clearance by inducing hyperglycemia and hyperinsulinemia. Furthermore, studies using rat skeletal muscle homogenates have shown that glucagon inhibits insulin-degrading enzymes (IDE) [6,7]. These studies are suggestive of a possible role of glucagon in altering insulin clearance which may contribute to increased circulating insulin after glucagon administration.

However, whether glucagon actually affects insulin kinetics is not known. Therefore, the present study was designed to quantify any effects of glucagon on insulin's disposition in a mouse model. Toward this end, prehepatic insulin secretion rate following intravenous glucose tolerance test (IVGTT) was determined both with and without exogenous glucagon, via C-peptide deconvolution methods. Then, using glucose, insulin and glucagon measured simultaneously following IVGTT, a modeling analysis was applied to estimate the clearance of insulin with and without exogenous glucagon.

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http://dx.doi.org/10.1016/j.peptides.2016.12.012
0196-9781/

2. Materials and methods

2.1. Animals

All experiments were approved by the regional ethics committee in Lund, Sweden and conducted in agreement with the policy. Female C57BL/6J mice, weighing 22.9 ± 1.2 g (range 19.7–24.8 g), were purchased from Taconic (Skensved, Denmark). Eight to ten week old mice were used in the study. Mice were maintained in a temperature-controlled room (22°C) on a light-dark cycle of 12 h each. Mice were fed a standard pellet diet (Lactamin, Stockholm, Sweden) and tap water ad libitum.

2.2. Experiments

After 4 weeks of acclimatization, mice were divided into two groups: a control cohort (n = 15, weight 22.2 ± 1.5 g) and a glucagon cohort (n = 15, weight 21.9 ± 1.2 g). The mice were anaesthetized with an intraperitoneal injection of midazolam (0.14 mg/mouse, Dormicum; Hoffman-La Roche, Basel, Switzerland) and a combination of fluanisone (0.9 mg/mouse) and fentanyl (0.02 mg/mouse; Hypnorm; Janssen, Beerse, Belgium). A basal blood sample was taken from the retrobulbar, intraorbital, capillary plexus in heparinised tubes, followed by rapid intravenous injection (volume 10 μL) of D-glucose (0.35 g/kg) or D-glucose (0.35 g/kg) together with glucagon (10 μg/kg) into a tail vein. Since our study was the first of its kind to assess and quantify the effects of glucagon on insulin dynamics, we wanted to conduct experiments in a controlled setting. Our experiments were designed in an effort to mimic hyperglucagonemia observed in diabetic animal models or diabetic patients with glucagon injection. Plasma glucose, glucagon, and insulin were also measured in each animal at 1, 5, 10, and 20 min after the i.v. administration of glucose alone or glucagon plus glucose. In a separate set of experiments, human C-peptide (dissolved in saline; Sigma, St Louis, MO, USA) was given intravenously (i.v.) over 3 s in a tail vein at the dose of 3.0 nmol/kg in 15 mice (weight 20.8 ± 1.1 g). Blood samples were taken at 1, 5, 10, 20, 30 and 50 min after the i.v. administration for measurement of plasma human C-peptide. All plasma samples were separated by centrifugation immediately and stored at −20°C until analysis.

2.3. Assays

Plasma insulin and glucagon were analyzed with sandwich immunoassay techniques (ELISA; Mercodia, Uppsala, Sweden) using double monoclonal antibodies. Mouse C-peptide was determined with a double-antibody radioimmunoassay using guinea pig anti-rat C-peptide antibody (cross-reacts to 100% with mouse C-peptide), rat C-peptide standard and ^{125}I-labelled rat C-peptide as tracer (Mercodia). Human C-peptide was determined by a double-antibody radioimmunoassay using guinea-pig anti-human C-peptide antibodies, ^{125}I-labelled human C-peptide and, as standard, human C-peptide (Merck Millipore, Darmstadt, Germany). Glucose was measured by the glucose oxidase technique and glucagon was determined by RIA (Millipore, Billerica, USA).

2.4. Assessment of C-peptide kinetics

The disposition of human C-peptide in the mouse was described by a two-compartment model, where the two compartments represent rapidly and more slowly equilibrating spaces, with irreversible loss occurring from the former as described previously [8]. In the model diagram presented in Fig. 1A, the parameters V1 and V2 (L) are the distribution volumes of the two spaces, while CLd and CLt (L/min) represent the distributional and total clearance rates. The C-peptide concentration-time data from all mice studied (3.0 nmol/kg (n = 15)) were analyzed simultaneously using a hierarchical, nonlinear mixed effects modeling approach. This population analysis yields estimates of the mean and inter-animal variability for the four model parameters. The hierarchical analysis was performed using the MLEM algorithm in the ADAPT software (version 5) [9].

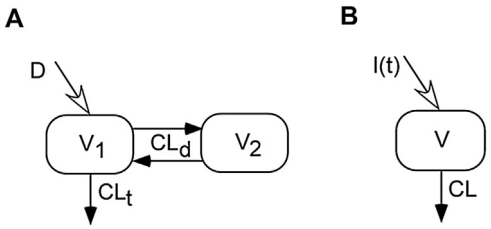

Fig. 1. (A) Two-compartment model of C-peptide kinetics and (B) one-compartment model of insulin kinetics.

2.5. Assessment of insulin secretion

β-cell insulin secretion was reconstructed via model-based deconvolution using the measured plasma C-peptide concentration profiles following IVGTT with (n = 15) and without (n = 15) glucagon administration from a previously reported study [10]. The kinetic parameters of the two-compartment used in the deconvolution were set at their mean values obtained from the human C-peptide kinetic study above. The C-peptide concentration-time profiles were pooled and a single set of prehepatic insulin secretion rates was estimated by maximum likelihood estimation using ADAPT [9], including the endogenous pre-IVGTT secretion rate (population analysis was attempted but led to large uncertainties in estimated parameters). The overall insulin delivery profile (*I(t)* pmol/min) was constructed as the piece-wise collection of the individual sample period delivery rates determined by deconvolution.

2.6. Non-compartmental analysis (NCA)

The area under the concentration versus time curve (AUC) from 0 to 20 min ($AUC_{0\text{-}20}$) and Cmax of glucose, insulin, and glucagon time course measurements in each animal was calculated using the NCA application in the ADAPT software [9]. Glucose elimination rate (λ, min^{-1}) was calculated based on the glucose concentration values from time 5–20 min [10].

2.7. Population analysis for insulin kinetics and statistical inference

For the insulin study, a one-compartment model (Fig. 1B) was used to describe insulin kinetics following IVGTT as in previous studies [11]. The respective insulin secretion profile, *I(t)*, determined by deconvolution of plasma C-peptide data as described above, was used as input for all mice in the cohort. For each of the two groups, the data from all mice were pooled and analyzed simultaneously using a hierarchical modeling analysis (MLEM algorithm in the ADAPT software (version 5) [9]). The parameters of the insulin kinetic model (clearance —CL (mL/min) and distribution volume-V (mL)) were assumed to follow a log-normal distribution and the errors associated with the measured insulin were assumed to be normally distributed with standard deviation linearly related to insulin (proportional and additive terms). Model selection was based on the resulting values of the Akaike Information Criterion (AIC, Ref. [9]), goodness-of-fit residual analysis and on the plausibility of the results. Differences in plasma C-peptide or insulin concentrations and insulin model parameter estimates (*e.g.*, CL) between cohorts were assessed using a *t* test.