Failure to establish islet amyloid polypeptide (amylin) as a circulating beta cell inhibiting hormone in man

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Summary. The presence of islet amyloid polypeptide in amyloid within pancreatic islet cells in Type 2 (non-insulin-dependent) diabetes, and its reported inhibition of glucose uptake by skeletal muscle *in vitro*, has prompted speculation concerning its role in the pathogenesis of diabetes. We investigated the effect of infused synthetic amidated human islet amyloid polypeptide (mol. wt. 3904, confirmed by mass spectroscopy) on intravenous glucose tolerance. Seven healthy, non-obese volunteers (age $\pm$ SD, 27 ± 4 years) were infused over 50 min with normal (0.9%) saline or islet amyloid polypeptide at $50 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$. After 20 min, a bolus of 0.5 g/kg glucose was given within 1 min and blood sampling continued for up to 60 min. Circulating concentrations of islet amyloid polypeptide reached at steady state were

$1130 \pm 90 \text{ pmol/l}$. The calculated half-life was $11.8 \pm 0.9 \text{ min}$, metabolic clearance rate $5.7 \pm 0.6 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ and apparent distribution space therefore $94 \pm 12 \text{ ml/kg}$. However, islet amyloid polypeptide was found to have no effect on the peak value reached, or the total area under the curve for plasma glucose, insulin or glucagon following intravenous glucose. This study suggests circulating islet amyloid polypeptide may not be an important influence on intravenous glucose tolerance in man.

Key words: Islet amyloid polypeptide, man, intravenous glucose tolerance test, Type 2 (non-insulin-dependent) diabetes mellitus, radioimmunoassay.

Islet (or insulinoma) amyloid polypeptide (IAPP), or amylin, a 37 amino acid peptide with 46% homology to calcitonin gene-related peptide (CGRP), has recently been purified from an insulinoma [1] and found to comprise the major component of the pancreatic amyloid deposits of Type 2 (non-insulin-dependent) diabetic patients [2]. IAPP has been co-localised with insulin in the beta cell secretory granule [3]. It opposes the action of insulin on isolated muscle [4] and inhibits insulin release from isolated rat islets *in vitro* [5].

These findings have prompted speculation concerning the possible role of IAPP in normal physiology and in the pathogenesis of Type 2 diabetes. We therefore investigated the effect of IAPP on the *in vivo* glucose tolerance in normal human volunteers.

Subjects and methods

Seven healthy, non-obese and fasting volunteers were studied, 3 male, 4 female, mean $\pm$ SD age 27 ± 4 years, body mass index $21.2 \pm 1.6 \text{ kg/m}^2$. The study protocol was approved by the Hammersmith Hospital ethical committee and informed written consent obtained from each subject. Infusions were carried out single-blind and

in random order. Subjects received either a control infusion of 0.9% NaCl (normal saline) (containing 10% plasma) or IAPP at a nominal rate of $50 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$. At least 7 days was left between each infusion.

Sterile, synthetic, human amidated IAPP (mol.wt. 3904, confirmed by mass spectroscopy, M-Scan Ltd., Silwood Parks, Berks., UK) was purchased from Bachem Inc. (Torrance, Calif., USA) and had > 99% purity by HPLC. The peptide was dissolved immediately before infusion in normal saline containing the subjects own plasma (10% by volume). After 20 min ("time 0") 20% dextrose (0.5 g/kg) was given within one min *i. v.* The infusion was continued for a further 30 min and blood sampling for up to 60 min after glucose administration.

Blood samples were collected into appropriate tubes for measurement of glucose, glucagon, insulin and IAPP. After centrifugation, plasma was stored immediately at -20°C until assay. A sample from the infusion line was also taken and frozen for estimation of actual concentration of IAPP administered.

Radioimmunoassay (RIA)

Established RIAs were used to measure plasma concentrations of insulin and glucagon. RIA of IAPP in the plasma samples and infusates was carried out in 0.8 ml 0.06 mol/l phosphate buffer, pH 7.4, containing 10 mmol/l EDTA, 8 mmol/l sodium azide, 1.0% (weight/volume) bovine serum albumin and 0.02% polyoxyethylene sodium

Fig. 1. The concentrations (mean $\pm$ SEM, $n = 7$) of glucose (top left), insulin (top right), glucagon (bottom left) and islet amyloid polypeptide IAPP (bottom, right) achieved by an i.v. glucose bolus (indicated by arrow) administered (at time 0 min) during a 50 min (from -20 to +30 min) saline (0.9% NaCl) infusion (○) and IAPP infusion (●) are shown

monolaureate (Tween 20, Sigma Chemical Co., St Louis, Mo., USA). The antibody, raised to human IAPP, was purchased from Peninsula Laboratories (St Helens, Merseyside, UK). IAPP was iodinated using Bolton and Hunter reagent and purified by HPLC. The tracer had a specific activity of 34 Bq/fmol. The assay standard was synthetic, human amidated IAPP. Samples were incubated with antibody for 24 h at 4 °C before addition of tracer (0.9 fmol/tube) and for a further 3 days before separation of free and antibody-bound peptide by dextran-coated charcoal (6 mg/tube). The assay had a detection limit of 5 fmol/assay tube. Inter- and intra-assay variations were less than 10%.

Glucose measurements were carried out on a glucose analyser (RA-1000, Technicon Instruments Co. Ltd., Basingstoke, UK) using Technicon Method No. SM4-014.3185. The assay had a coefficient of variation of 1.2%.

Statistical analysis

Results are expressed as mean $\pm$ SEM. Total areas under the curve (AUCs) were calculated, using parallelogram approximations, and these were then compared using Student's paired *t*-tests. Glucose disappearance rates following the i.v. glucose were expressed in terms of the K value [6].

Results (Fig. 1)

There were no adverse reactions to the infusions and no change in blood pressure or pulse rate.

Fasting plasma glucose levels were similar during both infusions. The glucose bolus caused a 4–5 fold increase in blood sugar within 3–5 min. Calculation of the clearance rate of glucose during the saline infusion gave a K value of 2.6 ± 0.3 , mean $\pm$ SEM, $n = 7$, and during IAPP infusion a K value of 2.4 ± 0.5 (normal K value > 1.2). The AUC for the glucose response was also unchanged: saline, AUC 449 ± 73 ; IAPP, AUC 485 ± 58 .

Plasma insulin rose from a fasting level of 17 ± 5 pmol/l to 470 ± 80 pmol/l 5 min after glucose administration.

During IAPP infusion, insulin rose during the same period from 20 ± 4 to 452 ± 111 pmol/l. The AUC was again unchanged: saline, AUC $11,803 \pm 1,825$; IAPP, AUC $12,726 \pm 2,983$.

Pancreatic glucagon was suppressed during the i.v. glucose tolerance test from a fasting concentration of 10.5 ± 1.8 pmol/l to 6.7 ± 1.4 at 10 min and during IAPP infusion from 14.3 ± 2.9 to 8.4 ± 1.9 pmol/l. There were no differences in the respective AUC.

IAPP concentrations from the infusion line indicated a peptide loss of $86 \pm 2\%$, presumably due to peptide aggregation or adsorption onto plastic surfaces. Mean plasma IAPP concentration for the last 30 min of infusion was 1330 ± 90 pmol/l. The calculated metabolic clearance rate was 5.7 ± 0.6 ml $\cdot$ kg $^{-1}$ $\cdot$ min $^{-1}$ and the half-time of decay from the incremental plateau was 11.8 ± 0.9 min. The apparent distribution space was therefore 94 ± 12 ml/kg [7]. Plasma IAPP-like immunoreactivity during the saline infusion was 20 ± 2 pmol/l, at the detection limit of the assay, and did not rise significantly following i.v. glucose infusion.

Discussion

Anatomical and experimental evidence has pointed to a possible role for IAPP not only in the regulation of pancreatic beta cell function, but also in the pathogenesis of diabetes. This might be by direct interference with insulin release [5], by contributing to the peripheral insulin resistance which is a prominent feature of Type 2 diabetes [4], or by inducing amyloid production which in turn may impair beta cell function [1, 2].

However, IAPP has not yet been shown to have any effect on carbohydrate metabolism in vivo. This study investigated the effect of high dose IAPP on i.v. glucose tolerance in healthy volunteers. Despite considerable losses, circulating plasma concentrations of IAPP in excess of 1 nmol/l were achieved and yet IAPP had no effect on glucose clearance or glucose-stimulated insulin and pancreatic glucagon responses.

One possible explanation is that biologically inactive amylin was being infused. These infusions were carried out using synthetic human, C-terminal amidated IAPP. This is presumed to be the biologically active moiety [8], although a non-amidated form has been isolated [1]. While we cannot be absolutely certain that the IAPP infused was identical to the native form, animal experiments using this same peptide have shown it to dramatically affect calcium metabolism [9], suggesting biological potency. It is also possible that the 37 amino acid IAPP is not the active form and that alternative intracellular processing may yield more potent forms (as in the case of glucagon-like peptide-1 [10]). However, the same consideration would apply to in vitro studies using synthetic IAPP which have shown bioactivity [5, 9].

Our findings do not preclude a role for IAPP as a local modulator of insulin secretion by the beta cell, in a paracrine or autocrine role, which might be of considerable importance in the pathophysiology of diabetes mellitus. However, the lack of any effect of very elevated IAPP lev-

els on i.v. glucose tolerance in man may indicate that it is unlikely to be acting as a circulating hormone to cause peripheral insulin resistance or influence insulin release.

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