Glycated IAPP shows a reduced inhibitory action on insulin secretion.

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Islet amyloid polypeptide (IAPP) also called amylin is a 37 amino acid long peptide co-secreted with insulin in a regulated manner from pancreatic β-cell in response to nutrient ingestion. IAPP was originally isolated from post-mortem pancreatic amyloid deposits from human NIDDM cases [1] and has been associated with the pathogenesis of amyloidosis [2]. IAPP is structurally similar to calcitonin gene related peptide (CGRP) sharing approximately 46% sequence identity [1]. It is thought that these two peptide hormones may act via the same specific receptors on target tissues [3]. CGRP has an inhibitory action on insulin secretion in rat islets in vitro [4] and in vivo [5]. Its addition to BRIN-BD11 cells has been demonstrated to have inhibitory actions on insulin secretion [6,7]. Hyperglycaemia resulting in glycation of proteins may contribute to the pathogenesis of diabetes. Glycation can result in alteration of biological activity of proteins and peptides including insulin [8,9]. Recent studies have demonstrated that pancreatic β-cell levels of glycated proinsulin and insulin are significantly raised in hyperglycaemic animal models of diabetes [10]. The initial aim of the present study was firstly to investigate the action of rat IAPP on insulin secretion in glucose-sensitive pancreatic BRIN-BD11 cells in culture. Secondly to structurally characterize a glycated IAPP analogue and examine its insulinotropic action in these cells.

Rat IAPP fractions (1 mg/ml, 100 μl) were prepared under hyperglycaemic conditions (220 mM glucose) at 37°C (pH 7.4, 10 mM sodium phosphate buffer, 887μl) with a 1000-fold molar excess (over IAPP) of NaBH₃CN (12.5 mM). The reaction was stopped after 24 h by addition of 0.5M acetic acid (30~1 molar excess (over IAPP) of NaBH₃CN (12.5 mM)). IAPP was characterized by plasma desorption mass spectrometry and non-glycated forms by HPLC. HPLC-purified glycated and non-glycated samples were prepared under acidification conditions and non-glycated forms by HPLC. HPLC-purified glycated IAPP was characterized by plasma desorption mass spectrometry (Mr 4866.3 Da) demonstrating that it contained a single glucitol adduct (i.e. monoglucalylated). Automated peptide sequence analysis utilizing on-line PTH derivatization yielded just 17 pmol PTH-Lys in cycle 1 compared to 1024 pmol PTH-Lys in each well and aliquots were stored at -20°C for measurement of insulin secretion.

The effect of glycated and non-glycated IAPP on insulin secretion was investigated using BRIN-BD11 cells. BRIN-BD11 cells were cultured in RPMI-1640 tissue culture medium containing 10% (v/v) fetal calf serum, 1% (v/v) antibiotics (100 U/ml penicillin, 0.1 mg/ml streptomycin) and 11.1 mM glucose. Insulin release from BRIN-BD11 cells was determined using cell monolayers. The cells were harvested with the aid of trypsin/EDTA (Gibco Life Technologies Ltd., Paisley, Strathclyde, UK), seeded into 24-multiwell plates (Nunc, Roskilde, Denmark) at a density of 2.5 x 10⁵ per well, and allowed to attach during overnight culture. Acute studies of insulin release were preceded by 40 min pre-incubation at 37°C in 10 mM Krebs Ringer bicarbonate buffer (115 mM NaCl, 4.7 mM KCl, 1.28 mM CaCl₂, 1.2 mM MgSO₄, 5.6 mM KCl, 2.6 mM KH₂PO₄, 12 mM MgSO₄, 10 mM NaHCO₃, 5.6 mM glucose) supplemented with 1.1 mM glucose. Test incubations were performed at 37°C using the same buffer supplemented with glucose, non-glycated or glycated IAPP as indicated in the Table. After 20 min incubation, the buffer was removed from each well and aliquots were stored at -20°C for measurement of insulin by RIA [11].

Results in Table 1 indicate that a range of IAPP concentrations (3x10⁻¹⁰ to 3x10⁻⁷ M) had no effect on acute (20 min) insulin release from pancreatic B-cells cultured at 5.6 mM glucose. However, under hyperglycaemic culture conditions (16.7 mM glucose) non-glycated IAPP (3x10⁻¹⁰ to 3x10⁻⁹ M) induced a significant 24–32% inhibition of insulin output compared to controls (glucose alone). Glycation of IAPP significantly reduces its inhibitory action on insulin secretion by 19–35% from 3x10⁻¹⁰ to 3x10⁻⁷ M at 16.7 mM glucose (Table 1).

These results indicate that BRIN-BD11 cells like normal pancreatic B-cells [7] are responsive to the inhibitory action of IAPP at least under hyperglycaemic conditions. These BRIN-BD11 cells probably possess specific receptors for IAPP or CGRP which mediate the inhibition of insulin secretion. Furthermore structural modification of IAPP by glycation at the amino terminus significantly reduces its inhibitory action on pancreatic β-cell insulin release in vitro.

### Table 1. Effect of non-glycated and glycated IAPP on insulin secretion from BRIN-BD11 cells

<table>
<thead>
<tr>
<th>Peptide conc. (M)</th>
<th>Insulin secretion (% of control)</th>
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<tbody>
<tr>
<td>0 (control)</td>
<td>100.0 ± 5.9</td>
</tr>
<tr>
<td>3x10⁻¹⁰</td>
<td>105.3 ± 8.9</td>
</tr>
<tr>
<td>3x10⁻⁹</td>
<td>107.1 ± 15.9</td>
</tr>
<tr>
<td>3x10⁻⁸</td>
<td>109.0 ± 25.9</td>
</tr>
<tr>
<td>3x10⁻⁷</td>
<td>107.1 ± 15.9</td>
</tr>
</tbody>
</table>

*p<0.05 compared to control; **p<0.05, ***p<0.01 compared to non-glycated IAPP at the same glucose concentration (Student’s unpaired t-test).