Islet amyloid polypeptide amide causes peripheral insulin resistance in vivo in dogs

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Summary. Islet amyloid polypeptide is a 37 amino acid hormone-like peptide which is the major protein component of islet amyloid deposits commonly found in patients with Type 2 (non-insulin-dependent) diabetes mellitus. Recent studies indicate that a physiologically active form of this peptide appears to be carboxyamided and secreted from the insulin-producing beta cell. In order to clarify the possible in vivo actions of islet amyloid polypeptide, we have studied the effects of synthesized islet amyloid polypeptide-amide on peripheral glucose utilization by performing hyperinsulinaemic euglycaemic glucose clamp studies on dogs. Exogenously administered islet amyloid polypeptide-amide (an infusion

from 1.0 to 100 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, over 2 h) inhibited the insulin-stimulated glucose disposal rate in a dose dependent manner. Twenty-five $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ of islet amyloid polypeptide-amide infused via a peripheral vein significantly lowered the glucose disposal rate by 20% (from 17.4 ± 1.7 to 14.4 ± 1.7 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $n = 5$, $p < 0.01$). These findings suggest that islet amyloid polypeptide-amide causes peripheral insulin resistance in vivo.

Key words: Islet amyloid polypeptide, amylin, amidation, in vivo effect, insulin resistance, diabetes mellitus.

Islet amyloid polypeptide (IAPP/amylin) is a 37 amino acid peptide, which is the major protein component of islet amyloid deposits, as shown by Westermarck et al. [1] and Cooper et al. [2]. This peptide appears to be a normal product of the insulin-producing beta cell [2, 3]. Recently Sanke et al. [4] identified and characterized a cDNA encoding the human IAPP. The nucleotide sequence of the clone suggests that a physiologically active form of IAPP is released from its precursor by proteolytic processing and is secreted by the beta cell in a carboxyamided form. These observations suggest that IAPP may be a normal islet hormone.

Recent studies suggest that IAPP may be involved in the pathogenesis of Type 2 (non-insulin-dependent) diabetes mellitus, Cooper et al. reported that both native IAPP extracted from human diabetic pancreas and purified by HPLC [5] and synthesized IAPP-carboxylate (IAPP-COOH) [6] cause resistance to insulin in the rat skeletal muscle in vitro. In order to clarify the possible in vivo action of IAPP, the effects of synthesized human IAPP-amide (IAPP-NH₂) on insulin-stimulated glucose utilization were studied on dogs using the hyperinsulinaemic euglycaemic glucose clamp technique (h-EGCT) [7].

Materials and methods

Synthesis of IAPP-NH₂ and IAPP-COOH

Human IAPP-NH₂ and IAPP-COOH were assembled by using an Applied Biosystems model 430A peptide synthesizer [(software version 1.40) Applied Biosystems, Foster City, Calif., USA] with HMBA-resin (for C-terminal amide) and Pam-resin (for C-terminal carboxylic acid) as the solid support. The final peptide resins were treated with anhydrous hydrogen fluoride in the presence of 20% p-cresol at -5°C for 60 min. After evaporation of excess hydrogen fluoride, the crude peptides were precipitated with ether and extracted with trifluoroacetic acid to filter off the solid supports. The crude products containing sulfhydryl groups were treated with K₃Fe(CN)₆ at a concentration of 0.1 mmol/l in aqueous 1 mol/l NH₄OAc containing 8 mol/l urea for 30 min. The crude products were then purified by preparative reverse-phase HPLC. The homogeneity of the final product was confirmed by analytical HPLC, capillary electrophoresis and amino acid analysis.

Hyperinsulinaemic euglycaemic glucose clamp test

Seven mongrel dogs weighing from 9 to 12 kg were subjected to h-EGCT under pentobarbital anaesthesia after an overnight fast. Human insulin (Novo Industry, Copenhagen, Denmark) at a rate of

Table 1. Dose dependent effect of IAPP-NH₂ on glucose disposal rate in hyperinsulinaemic euglycaemic glucose clamp study

IAPP-NH ₂ Infusion rate ($\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$)	Change of glucose disposal rate (%)		
	Before	During	After
1.0 ($n = 1$) ^a	100	100.4	
2.5 ($n = 1$) ^b	100	100.2	
10.0 ($n = 1$) ^a	100	93.9	
25.0 ($n = 1$) ^b	100	84.2	
50.0 ($n = 1$) ^a	100	79.9	
100.0 ($n = 1$) ^b	100	73.3	
2.5 ($n = 3$)	100	101.0 $\pm$ 3.8 NS	104.8 $\pm$ 5.1 NS
25.0 ($n = 5$)	100	81.9 $\pm$ 3.4 ^c	91.5 $\pm$ 6.4 NS

^{a,b} experiment performed on the same dog. Significance of the difference from basal (before): ^c $p < 0.01$, NS; not significant.

Before, during, after: last 30 min of every 120 min period before, during and after IAPP-NH₂ infusion, respectively

5 mU $\cdot$ kg⁻¹ $\cdot$ min⁻¹ was infused through a peripheral vein. Arterialized venous blood glucose concentrations were measured every 2 min by glucose oxidase method using Glucose MONITOR GM-1320 (Kyoto Dai-ichi Kagaku Co. Ltd., Kyoto, Japan). Glucose (25% solution) was infused via a peripheral vein to maintain the fasting blood glucose level and the glucose disposal rate (GDR) [7] was calculated. IAPP-NH₂ (1 to 100 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) or IAPP-COOH (25 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) was infused over 2 h through a peripheral vein 120 min after the start of h-EGCT.

Statistical Analysis

The data are shown as mean $\pm$ SEM. The statistical analysis was done by the paired Student's *t*-test and a *p* value less than 0.05 was considered significant.

Results

Sufficiently high and constant plasma insulin concentration (206 $\pm$ 6.7 $\mu\text{U/ml}$) was achieved by the i. v. infusion of 5 mU $\cdot$ kg⁻¹ $\cdot$ min⁻¹ insulin. A constant GDR was obtained 90 min after the start of h-EGCT, and it continued for at least 7 h without IAPP infusion.

The mean percent change of GDR for the last 30 min of every 120 min period before, during and after IAPP-NH₂ infusion is shown in Table 1. For a preliminary study, several doses of IAPP-NH₂ were infused in two dogs (upper panel of Table 1). The IAPP-NH₂ infusion lowered the GDR in a dose dependent manner. A decrease of more than 15% in the GDR was noted at an infusion rate of 25 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ or more. When 25 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ IAPP-NH₂ was infused over 2 h, the GDR began to fall 30 min after the start of the infusion. Obvious decline of the GDR was observed through the latter half of the infusion, and continued for about 80 min after discontinuation of the infusion (Fig. 1). A significant decline in the GDR was noted during the 25 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ IAPP-NH₂ infusion (from 17.7 $\pm$ 1.7 to 14.4 $\pm$ 1.7 mg $\cdot$ kg⁻¹ $\cdot$ min⁻¹, $n = 5$, $p < 0.01$). However, the GDR was not changed by the 2.5 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ IAPP-NH₂ infusion (lower panel of Table 1).

In one dog the same dose (25 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) of IAPP-NH₂ or IAPP-COOH was infused via a peripheral vein

(Fig. 1). The decline in the GDR induced by IAPP-COOH was markedly reduced compared with that induced by IAPP-NH₂. When the same dose of IAPP-NH₂ was infused through the portal vein in the same dog, the decline in the GDR was also reduced (Fig. 1).

Discussion

Islet amyloid deposits are found in the pancreas of > 90% of patients with Type 2 diabetes mellitus [3]. Their possible role in the development of Type 2 diabetes mellitus has been disputed. In 1987, an amyloid fibril was extracted and characterized from the islet amyloid deposits. However, the C-terminal residue of this novel peptide (IAPP [1] / Amylin [2]) was not reported to be Tyr-amide. Using native IAPP extracted from human diabetic pancreas, Leighton and Cooper [5] reported that 1×10^{-9} mol/l native IAPP inhibits both basal and insulin-stimulated rates of glycogen synthesis in stripped rat soleus muscle in vitro. The same result was obtained by Cooper et al. [6] using 1.2×10^{-7} mol/l synthesized IAPP-COOH. Recent studies, however, indicate that a physiologically active form of IAPP should be carboxyamidated [4]. The present study was thus designed to examine the effects of synthesized IAPP-NH₂ on insulin sensitivity in the peripheral tissue by employing the h-EGCT. This study clarified an in vivo action of exogenously administered IAPP-NH₂. That is, IAPP-NH₂ inhibited insulin-stimulated glucose metabolism. It was also discovered that IAPP-COOH has less effect than IAPP-NH₂ (Fig. 1). This finding may account for the much higher activity observed by Leighton and Cooper [5] in experiments with native IAPP, as opposed to material synthesized with free acid C-terminus

Fig. 1. Percent changes in blood glucose and glucose disposal rate during hyperinsulinaemic euglycaemic glucose clamp technique with 25 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ islet amyloid polypeptide (IAPP)-NH₂ or IAPP-COOH infusion performed on the same dog. Closed circle with solid line: IAPP-NH₂ into peripheral vein, open circle with solid line: IAPP-NH₂ into portal vein, and open triangle with dashed line: IAPP-COOH into peripheral vein

(IAPP-COOH) [6], if the native IAPP is carboxamidated.

Calcitonin gene-related peptide which has partial amino acid sequence identity with IAPP has been reported to cause both hepatic and peripheral insulin resistance [8]. Thus, the possibility arises that the decline of GDR during IAPP-NH₂ infusion is due to the increased hepatic glucose output induced by IAPP-NH₂, although the peripheral insulin concentration in all h-EGCTs reached about 200 µU/ml which is thought to be enough to suppress hepatic glucose output [7, 9]. However, we have evidence that IAPP-NH₂ (10^{-7} – 10^{-12} mol/l) does not stimulate hepatic glucose output in rat liver perfusion systems (data not shown). IAPP-NH₂ thus may cause insulin resistance in the peripheral tissue, although the observed effects could conceivably be mediated through alterations in blood flow and/or the sympathetic nervous system.

Our result, obtained in vivo, is consistent with that observed in vitro by Leighton and Cooper [5] and by Cooper et al. [6], although the peripheral concentration of infused IAPP-NH₂ could not be determined in this study. The significant inhibitory effect of IAPP-NH₂ was noted at the infusion rate of 25 µg·kg⁻¹·h⁻¹. While the effect could not be seen at the infusion rate of 2.5 µg·kg⁻¹·h⁻¹. These results will allow us to re-evaluate whether the inhibitory effect of IAPP-NH₂ is due to a physiological or pharmacological action once a sensitive and specific assay to measure the plasma IAPP-NH₂ concentration has been developed. Interpretation of our results must take into account the fact that chemically synthesized human IAPP was used on dogs. The homogeneity was checked by analytical reverse phase HPLC, capillary electrophoresis and amino acid analysis, but not by first atomic bombardment mass spectrometry.

The inhibitory effect of IAPP-NH₂ on insulin-stimulated glucose metabolism was reduced when it was infused via the portal vein (Fig. 1). This fact suggests that the liver may play some important role in the action/degradation of IAPP-NH₂. From such a point of view, further investigation is necessary to clarify the physiological action of IAPP-NH₂.

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