Islet amyloid polypeptide-like immunoreactivity in the islet B cells of Type 2 (non-insulin-dependent) diabetic and non-diabetic individuals

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Summary. A novel peptide, islet amyloid polypeptide (IAPP), with structural resemblance to calcitonin gene-related peptide has recently been purified from amyloid deposits in an insulinoma and from islets of Langerhans. By immunohistochemical methods, using antisera to a synthetic undecapeptide of IAPP and to insulin, we show that freshly fixed islet B cells in man, guinea pig, rat, mouse and hamster exhibit strong IAPP-immunoreactivity while A cells are unreactive. In human autopsy material, all of 11 non-diabetic individuals had IAPP immunoreactivity of the islets. In comparison 8 of the 13 patients with Type 2 (non-insulin-dependent) diabetes had no IAPP immunoreactive cells. The proportion of islet cells having IAPP immunoreactivity exceeded 10% in only 1 of the 5 remaining diabetic patients while in all 13 patients substantially more than 10% of the islet cells contained immunoreactive insulin. IAPP-positive amyloid deposits were found in 20–99% of the islets in 12 of the Type 2 diabetic pat-

ients while 6 of 11 non-diabetic subjects had amyloid in 3–11% of their islets. In islets with IAPP-immunoreactive amyloid, very few IAPP-cells were seen despite a strong reaction of the B cells with antiserum to insulin. This study shows that IAPP is a normal islet B cell component and that IAPP immunoreactivity in B cells is diminished in Type 2 diabetes while IAPP is deposited as amyloid fibrils in the islets of Langerhans. Although the function of IAPP is unknown, its occurrence in the islet B cells and its structural relation to calcitonin gene-related peptide makes a hormonal nature probable. The present study indicates an altered expression or metabolic fate of IAPP in Type 2 diabetes.

Key words: Islet amyloid polypeptide, Type 2 (non-insulin-dependent) diabetes, insulin secretion, immunohistochemistry, islet B cell.

Type 2 (non-insulin-dependent) diabetes mellitus is a world-wide and prevalent disease whose pathogenesis is poorly understood. Deficient insulin release following a glucose stimulus is characteristic but peripheral insulin resistance has also been discussed as a primary defect [1, 2].

While the total islet mass is drastically reduced in Type 1 (insulin-dependent) diabetes, it is only moderately reduced in Type 2 diabetes [3–6]. The mass of B cells in Type 2 diabetes is about 50% as compared to normal controls [5, 7, 8], but the reduction in volume of B cells alone cannot explain the diabetic state.

Deposition of a hyaline material in the islets of Langerhans in some cases of diabetes mellitus was described in 1900 [9]. Later, the hyaline deposits were shown to have the typical properties of amyloid, and are now regarded as a form of localised amyloidosis [10]. Subsequently, islet amyloid deposits were also found to be common in aging non-diabetic persons [7, 8, 11, 12]. Quantitative data, however, indicate that the number of affected islets as well as the amounts of amyloid in the islets are generally much larger in Type 2 diabetes than in non-diabetic controls [7, 8]. Even though infiltration of amyloid in the islets is associated

with a loss of B cells [7, 8] and may also interfere with the diffusion of glucose and hormones, impairment of the islet function due to the deposition of amyloid may not be the only alteration involved in the pathogenesis of diabetes.

The close association of amyloid in islets to B cells [13] and the occurrence of amyloid in insulin-producing tumours has provided strong evidence that this type of amyloid is a product of B cells. It has therefore been suspected that amyloid in islets and insulinomas is derived from insulin or proinsulin. However, we recently found that the major protein in this type of amyloid is a previously unknown polypeptide which we identified as insulinoma or islet amyloid polypeptide (IAPP) [14–16]. Although unique, the amino acid sequence of IAPP has about 50% identity to the neuropeptide calcitonin gene-related peptide (CGRP) [15]. This clarification of the nature of islet amyloid may also provide new insight regarding the pathophysiology of the islet derangements in Type 2 diabetes.

The aim of the present investigation was to study the occurrence and distribution of IAPP immunoreactive cells in the pancreas of human and several animal species, and to determine whether there is altered

IAPP immunoreactivity in the islets of individuals with Type 2 diabetes.

Material and methods

Tissues

Pancreatic tissue from three human patients undergoing pancreatic resection due to carcinoma was used for the study of IAPP immunoreactivity in normal islets. These patients had no signs of metabolic disease and the morphology of the pancreatic tissue outside the tumour was normal. The specimens were immediately fixed in Bouin's solution and 4% formaldehyde, and embedded in paraffin. Specimens of pancreas, fixed in the same way, were also obtained from mouse, rat, guinea pig and hamster.

For the semiquantitative study (see below), pancreatic specimens from human patients were obtained at autopsies performed within 12 h after death. The pancreas was removed immediately after the abdomen was opened, and pieces of the pancreatic head, body and tail were fixed in Bouin's solution and 4% formaldehyde solution and were then embedded in paraffin.

Antisera

An undecapeptide, with the sequence Cys-Ala-Thr-Gln-Arg-Leu-Ala-Asn-Phe-Leu-Val, corresponding to positions 7–17 of human IAPP [14, 15], was synthesised (Cambridge Research Biochemicals Ltd., Harston, Cambridge, UK) and linked to key hole limpet hemocyanin. Antisera were raised in a guinea pig (MA 50) and a rabbit (AA 82) by intracutaneous and subcutaneous injection of the conjugate (0.3 mg) in Freund's complete adjuvant, followed by weekly injections of equal amounts of the IAPP conjugate in Freund's incomplete adjuvant. Serum was collected from the animals beginning at one week after the fourth immunisation. Guinea pig antiserum to insulin was obtained from Dr. L. Wide (Uppsala), and rabbit antiserum to rat CGRP was purchased from Milab (Malmö, Sweden).

Immunohistochemistry

Immunohistochemical studies were performed using the peroxidase-antiperoxidase (PAP) and biotin-avidin (ABC) (Vector Laboratories, Burlingame, Calif, USA) methods [17]. Deparaffinised sections of pancreas were initially incubated with the primary antisera in serial (1:50 to 1:1,600) dilutions overnight at room temperature. The PAP and the ABC techniques were subsequently performed with the primary antisera MA 50 and AA 82 diluted 1:200 and 1:600 respectively. The insulin antiserum was diluted 1:1000 and the CGRP antiserum 1:800. The reaction was visualised with 3,3-diaminobenzidine-tetrahydrochloride (DAB). In control experiments, the antisera were replaced by normal sera from guinea pig or rabbit. The specificity of the IAPP antiserum was also studied by absorption with the synthetic undecapeptide and with synthetic human insulin ("Humulin", Kabi-Vitrum, Stockholm, Sweden). For absorption, 100 μg of substrate per ml of diluted serum was used. A sequential immunostaining and silver impregnation method, described in detail previously [18], was used for identification of the islet cells reacting with the IAPP antiserum. In other experiments, consecutive sections were stained with Grimelius' silver stain and immunostained for IAPP and for insulin.

Semiquantitative study of IAPP in Type 2 diabetes mellitus

Details regarding the two groups of patients included in this study are shown in Tables 1 and 2. In the diabetic group, only patients with clinically confirmed overt Type 2 diabetes were included. The control group included subjects that had no clinical signs of diabetes and also had negative urine glucose tests.

Only pancreatic tissues without obvious autolysis, and with islets reacting with the anti insulin antiserum, were included in the study. Of the 33 patients from whom pancreatic tissue had been collected at autopsy, 9 were rejected, 7 due to weak or no insulin reactivity in the islets and one due to autolysis. One other patient had systemic amyloidosis, making quantification of islet amyloid impossible. Of the 24 remaining patients, 13 had Type 2 diabetes mellitus while 11 had no signs of this disease (Table 1 and 2).

Adjacent sections of pancreatic tail were used from each patient for staining with alkaline Congo red, and with the PAP method for the demonstration of insulin and IAPP. Only the rabbit antiserum AA 82 was used for this study. The percentage of islets containing any amyloid was determined in the Congo red stained sections using a polarisation microscope. All islets in the sections, irrespective of size, were included. The proportion of immunoreactive islet cells in entire sections stained for either insulin or IAPP were estimated using a semiquantitative scale: 0 = no reactive cells, 1 = < 10%, 2 = 10–50%, and 3 = > 50% immunoreactive cells respectively.

Results

IAPP distribution in normal human and animal pancreas

Human pancreas obtained at surgery. Fixation with Bouin's solution was superior to formalin with regard to IAPP immunoreactivity when the guinea pig antiserum MA 50 was used while the rabbit antiserum AA 82 reacted equally good with either formalin or Bouin-fixed material. A majority of the human islet cells showed a strong reaction with either IAPP antiserum. The intensity of cytoplasmic immunoreactivity was uniform from cell to cell. The staining pattern observed with IAPP antiserum was the same as with the anti insulin antiserum although the latter generally gave a stronger reaction (Fig. 1). Reactivity with the anti IAPP antiserum was confirmed to be limited to the B cells using the consecutively immunostained sections and the silver impregnation method. Argyrophilic cells (mainly A cells) were unreactive. A few islets contained only a few scattered IAPP cells, and by the sequential staining procedure these islets were demonstrated to consist mainly of A cells. In all normal pancreatic specimens, IAPP-positive cells without obvious connection with the islets were found among the exocrine acini. Occasional single IAPP immunoreactive cells were also present in the exocrine ducts. Absorption of the anti IAPP antiserum with the synthetic peptide and omission of the primary antiserum abolished the reaction with islet cells while absorption with insulin had no effect on the reactivity. Immunostaining with the CGRP antiserum resulted in a weak reaction with only a few islet cells. No further characterisation of these cells was performed.

Animal pancreas. IAPP immunoreactive cells were observed in the islets of Langerhans of the rat, mouse, guinea pig and hamster. In all animals, a majority of islet cells were reactive, giving an intense reaction similar to that seen in normal human islets. The IAPP-positive cells in the rat, mouse, guinea pig and hamster

Table 1. Percentage of islets with amyloid and estimated number of cells reacting with antisera to insulin and to IAPP in sections of the pancreatic tail of patients with Type 2 (non-insulin-dependent) diabetes

Patient no.	Sex	Age at death (years)	Cause of death	Duration of diabetes (years)	Treatment	Percentage of islets with amyloid	Immunoreactive cells with antiserum to:	
							Insulin	IAPP
1	M	81	Myocardial infarction	3	Unknown oral antidiabetic drug	42	3+	1+
2	M	60	Pulmonary emboly	Unknown	Chlorpropamide+ insulin for short periods	0	2+	1+
3	F	70	Cardiac failure	7	Chlorpropamide+ insulin for short periods	30	3+	1+
4	F	69	VOC and cardiac failure	10	Chlorpropamide	91	3+	0
5	M	70	Myocardial infarction	10	Chlorpropamide	74	2+	0
6	F	73	Cerebral haemorrhage	Unknown	Unknown oral antidiabetic drug	74	2+	1+
7	F	72	Cirrhosis and carcinoma of the liver	6	Chlorpropamide	90	3+	0
8	F	76	Myocardial infarction	12	Glibenclamide	68	3+	0
9	F	70	Myocardial infarction	10	Chlorpropamide+ phenformin	53	3+	0
10	F	70	Mammary carcinoma with metastases	Unknown	Diet	20	2+	0
11	M	63	Myocardial infarction	20	Insulin	99	2+	0
12	F	77	Lactic acidosis	10	Glibenclamide+ phenformin	98	3+	0
13	F	81	Cardiac insufficiency	Unknown	Unknown	53	3+	2+

$0 = $ no, $1+ = <10\%$, $2+ = 10$–50%, and $3+ = >50\%$ immunoreactive islet cells, respectively

Table 2. Percentage of islets with amyloid and estimated number of cells reacting with antisera to insulin and to IAPP in sections of the pancreatic tail of patients without signs of diabetes

Patient no.	Sex	Age at death (years)	Cause of death	Percentage of islets with amyloid	Immunoreactive cells with antiserum to:	
					Insulin	IAPP
1	F	81	Myocardial infarction	0	3+	3+
2	M	88	Cardiac failure	5	3+	1+
3	M	79	Myocardial infarction	9	3+	3+
4	M	58	Chronic glomerulonephritis with uraemia	11	3+	3+
5	M	76	Cardiac failure	0	3+	3+
6	F	56	VOC and cardiac failure	5	3+	1+
7	F	60	Myelogenic leukaemia+ cerebral haemorrhage	0	3+	3+
8	M	59	Myocardial infarction	0	3+	2+
9	F	90	Myocardial infarction	3	3+	3+
10	M	73	Pulmonary tuberculosis with respiratory insufficiency	4	3+	3+
11	M	83	Myocardial infarction	0	3+	2+

$0 = $ no, $1+ = <10\%$, $2+ = 10$–50%, and $3+ = >50\%$ immunoreactive islet cells, respectively

were confirmed to be B cells based on the consecutive sections stained with the argyrophilic method (Fig. 2). Immunostaining with anti CGRP antiserum resembled that with the anti IAPP antiserum in the guinea pig, hamster and mouse. In rat islets, however, a few cells were strongly reactive while a major cell population gave a weaker reaction.

Semiquantitative estimation of IAPP reactive cells in diabetic and non-diabetic adults

Occurrence of islet amyloid. As seen in Table 1, all but one of the 13 diabetic patients had many islets with amyloid. The degree of amyloid infiltration was usually extensive. The amyloid had the typical staining
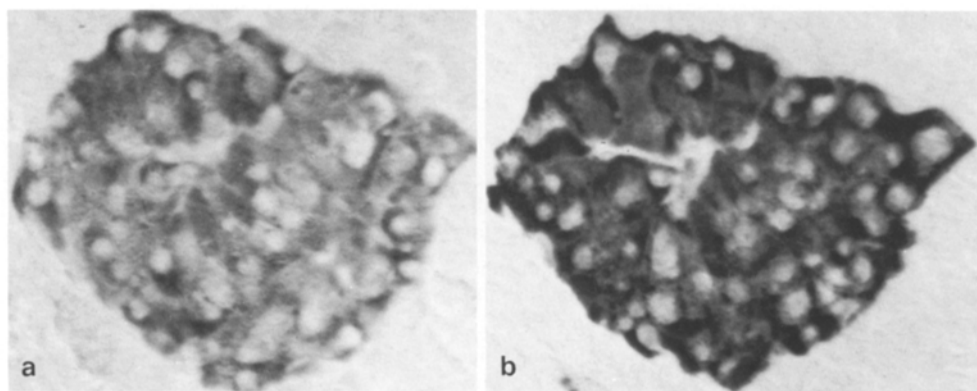
Fig. 1 a-b. Consecutively cut sections of human normal islet of Langerhans, immunohistochemically stained for **a** IAPP and **b** insulin. The distribution of the immunoreactivity is virtually identical. ×370
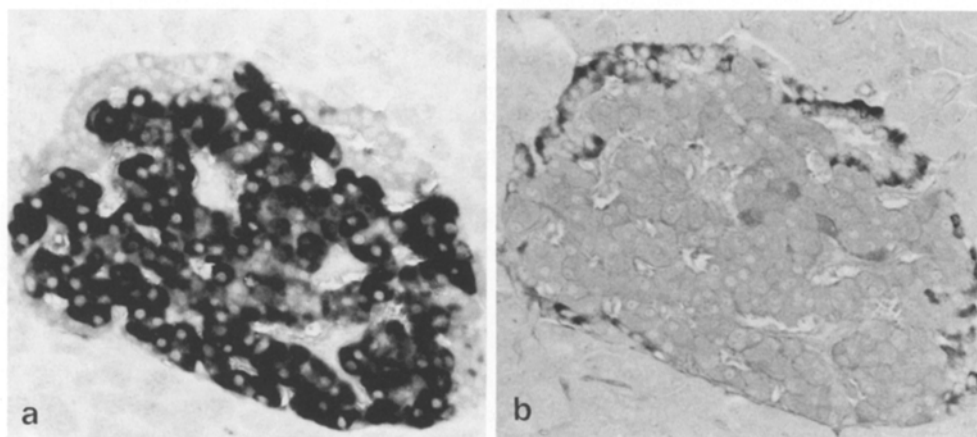

Fig. 2a, b. Consecutively cut sections of rat islet of Langerhans, immunohistochemically stained for **a** IAPP and **b** with the silver impregnation of Grimelius. The argyrophilic A cells do not react with antiserum to IAPP. ×220
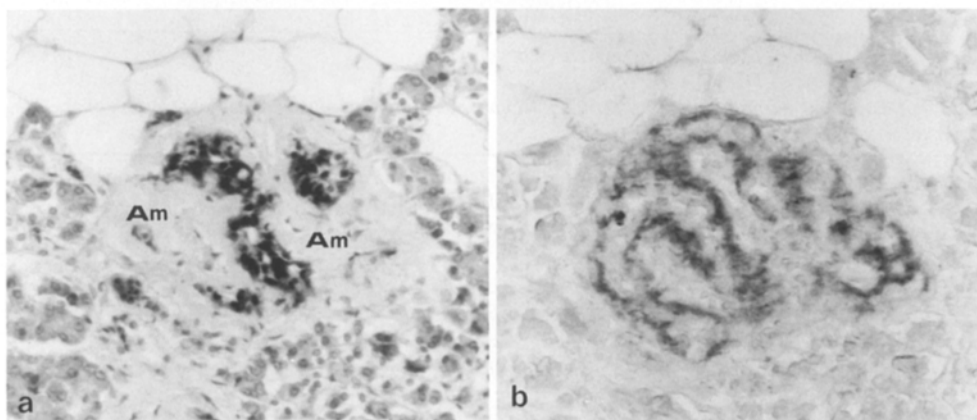

Fig. 3a, b. Immunohistochemically stained islet of Langerhans from a patient with Type 2 diabetes. **a** The islet B cells, but not the amyloid (Am), exhibit a strong reaction with antiserum to insulin. **b** When antiserum to IAPP is used, the amyloid reacts, while the B cells remain unstained. ×250

properties and distribution as previously described (6–12). Six of the 11 non-diabetic patients also had deposits of amyloid in the islets (Table 2). However, fewer islets were affected and the amounts of amyloid in the individual islets were generally smaller as compared to diabetic patients.

Insulin immunoreactivity. In all pancreatic specimens from both groups of patients, a significant proportion of cells in the islets of Langerhans exhibited insulin immunoreactivity. The immunostaining for insulin was always strong and no certain difference in the intensity of reaction was detected between the B cells of the diabetic and non-diabetic groups. However, quantitative

differences were apparent with respect to the number of insulin reactive cells in islets of the diabetic and non-diabetic groups. Four of the 13 diabetic patients had islets with only 10–50% of the cells giving insulin immunoreactivity (Table 1). B cells close to amyloid deposits were as strongly stained as B cells in apparently normal islets. The amyloid remained unstained in all sections (Fig. 3).

IAPP immunoreactivity. The percentage of islet cells with IAPP immunoreactivity in the non-diabetic group is given in Table 2. All of the 11 non-diabetic individuals had islet IAPP immunoreactivity, and a majority of the islet cells reacted with the IAPP antiserum in 7 of
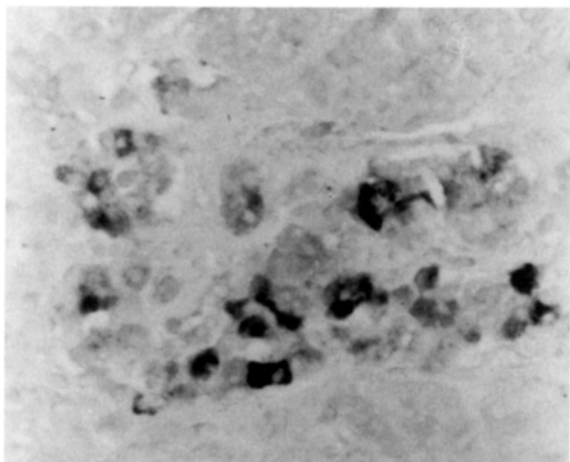
Fig. 4. Type 2 diabetic patients without islet amyloid. There is a remarkable reduction of the number of B cells that react immunohisto-chemically with antiserum to IAPP. ×400

the 11 patients. In these patients, the staining intensity in different cells was fairly uniform. These results are in sharp contrast to those of the diabetic patients (Table 1). Islets from only 5 of the 13 diabetic patients contained any IAPP-positive cells, mainly (4 of 5 patients) having less than 10% of cells with IAPP immunoreactivity. In those islets where IAPP immunoreactivity was found, the staining intensity varied greatly with some cells exhibiting very strong reaction while others were only weakly positive. In both diabetic and non-diabetic patients, islets with amyloid usually contained no IAPP-positive cells in spite of a strong insulin immunoreactivity in the B cells (Fig. 3). In only one patient (no. 13, Table 1) IAPP-positive cells were found together with amyloid deposits. Islet amyloid of both diabetic and non-diabetic patients reacted strongly with the antiserum to IAPP (Fig. 3).

Discussion

Deposition of amyloid, although not pathognomonic, is the single most typical alteration in the islets of Langerhans in Type 2 diabetes mellitus. The obvious lack of interest in this substance by scientists working in the diabetes field may seem strange but is probably partly related to the fact that experimentally-induced diabetic animals do not develop islet amyloid.

The fibril proteins of amyloid localised to one organ or tissue are believed to be synthesised close to the deposits [19]. Previous electron microscopic studies of the amyloid of the islet of Langerhans have indicated that the islet B cells are likely to be responsible for the production of this type of amyloid [13]. Since the amyloid in the C cell tumour of the thyroid consists of a calcitonin-related protein, probably procalcitonin [20], it has seemed logical to assume that islet amyloid contains an insulin- or proinsulin-related protein. In fact, amyloid-like fibrils are easily formed synthetically by

insulin [21, 22] and a weak insulin B-chain reactivity has been demonstrated in islet amyloid [23]. On the other hand, islet amyloid does not react immunohisto-chemically with antibodies to insulin [23]. In a recent study, we showed that the amyloid fibrils in an insulin-producing pancreatic tumour mainly consisted of a previously unknown polypeptide which we designated insulinoma or islet amyloid peptide, IAPP [14]. Subsequently we have shown that IAPP is a major constituent of islet amyloid peptide, IAPP [14]. Subsequently we have shown that IAPP is a major constituent of islet amyloid in man and cat [15, 16]. This composition of islet amyloid has recently been confirmed [24]. IAPP, at least in its amyloid forms, consists of 37 amino acid residues and has almost 50% identity with the neuropeptide CGRP [15].

The present study has shown IAPP immunoreactivity in the B cells of apparently normal human pancreatic islets and also in B cells of rat, mouse, guinea pig and hamster. These results indicate a wide occurrence of the peptide in several mammalian species. Since IAPP structurally resembles CGRP, it is of interest that CGRP immunoreactivity previously has been described in islet cells of the rat and mouse [25], a finding also confirmed in the present study. It cannot be ruled out that some of these findings are due to a cross reactivity of CGRP antibodies with IAPP although the presence of both polypeptides is also possible. It should be noted that CGRP antibodies labelled only a few human islet cells weakly and did not bind to islet amyloid, although such immunoreactivity recently has been reported [24].

Most of the diabetic human patients in this study had advanced islet amyloid deposits in contrast to the material of non-diabetic patients. Islet amyloid deposits in non-diabetic patients were present but to a much lesser extent. The fact that islet amyloid occurs in many non-diabetic elderly patients, however, may also indicate some abnormality in their islets. The reduction of the number of IAPP-immunoreactive cells in the islets of some non-diabetic patients may support this theory. Furthermore, the most obvious reduction of IAPP immunoreactive cells was found in two patients (no.'s 2 and 6, Table 2) in whom islet amyloid occurred.

As compared to non-diabetic patients, there was a pronounced reduction of IAPP-reactive cells in the islets of Type 2 diabetic patients. Some (but much smaller) reduction of the number of insulin-containing cells was also evident. Islets with amyloid, both in diabetic and non-diabetic patients, usually did not contain any IAPP immunoreactive cells.

Our data indicating decreased number of IAPP immunoreactive cells in Type 2 diabetic patients is based on evaluation of human pancreatic tissue obtained from routine autopsies. Certain limitations are obviously inherent to the use of autopsy material but they were considered in the experimental design and inter-

pretation of results in the present investigation. Surgically resected human pancreata from three patients without clinical evidence of metabolic disease were immediately fixed and used as positive controls for IAPP and insulin immunoreactivity. Only pancreatic tissues without evidence of autolysis and with insulin immunoreactivity were used for the semiquantitative study. Although variations with respect to death-to-autopsy time interval and length of tissue fixation are inevitable with the tissue specimens used in this study, the variables in the two groups should be random and thus should not significantly affect the comparison of the islet tissue from diabetic patients with that from the non-diabetic patients. Prospective studies utilising cats provide an opportunity to perform studies like this under more optimal conditions [26].

These findings suggest that there is an altered production or metabolism of IAPP in Type 2 diabetes that is in some way linked to the formation of islet amyloid. The lack of cellular IAPP in islets with amyloid may appear contradictory to this scenario, but formation of amyloid from IAPP or a related IAPP-precursor is likely a progressive process that may eventually be associated with IAPP depletion.

Since IAPP is apparently localised to the islet B cells, a decrease in the number of these cells due to any cause would be reflected by a deficit of IAPP. This is certainly most relevant in juvenile (Type 1) diabetes, where B cells are virtually totally destroyed. The pathogenic importance of decreased IAPP in either form of diabetes is not yet known, and new studies must be initiated to determine the function of IAPP in normal humans and animals.

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