Virchows Archiv A Pathol Anat (1991) 418:411–417

Virchows Archiv A
Pathological Anatomy and Histopathology

Islet amyloid polypeptide in insulinoma and in the islets of the pancreas of non-diabetic and diabetic subjects

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Received March 6, 1990 / Accepted December 6, 1990

Summary. Amyloid deposition is a common pathological feature in insulinoma and in the islets of the pancreas in type-2 diabetic patients. The present immunohistochemical study revealed that normal B-cells, insulinoma, and amyloid deposits in insulinoma and diabetic pancreatic islets were commonly immunoreactive with antiserum to C-terminal synthetic tetradecapeptide of human islet amyloid polypeptide (IAPP) (24–37). Amyloid fibrils in insulinoma were also positive to IAPP by immunoelectron microscopy. A high level of IAPP was detected in the plasma and tissue of a insulinoma patient by radioimmunoassay suggesting that amyloid deposition in insulinoma is due to overproduction of IAPP. Amyloid deposits immunoreactive to IAPP were also seen in all diabetic pancreatic islets, but in no non-diabetic islets. There was much amyloid deposition in the islets of severe diabetics, whose B-cells demonstrated decreased immunoreactivities for IAPP and insulin. The IAPP content of the pancreas was 649.0 and 847.7 pg/mg wet weight in each of two diabetic patients, and 1034.6 and 1447.7 pg/mg wet weight in two non-diabetic patients. The present study revealed that IAPP is a bioactive peptide secreted from islet B-cells and are amyloidogenic peptide concerned in diabetogenensis and/or the progression of type-2 diabetes mellitus.

Key words: Islet amyloid polypeptide – Insulinoma – Pancreatic islet – Diabetes mellitus – Immunohistochemistry

Introduction

Deposition of amyloid fibrils is a common pathological feature in the pancreatic islets of type-2 diabetic patients (Westmark and Wilander 1978). It is also seen in insulin-treated diabetic patients, except for those of juvenile-type diabetes (Maloy et al. 1981). This deposition has therefore been considered to play a pathogenetic role in type-2 diabetes mellitus, but similar deposition of amyloid fibrils has also been frequently found in insulinoma (Westmark et al. 1977; Liu et al. 1985).

Recently, the peptide that forms the amyloid fibrils in the pancreatic islets of diabetic patients and insulinoma has been isolated and found to be composed of 37 amino acids. It is termed islet amyloid polypeptide (IAPP) (Westmark et al. 1987a–c), diabetes-associated polypeptide (Clark et al. 1987; Cooper et al. 1987), and also amylin (Cooper et al. 1988). Several biological activities of the peptide have been reported; inhibition of insulin release (Kogire et al. 1989; Ohsawa et al. 1989), non-inhibition of insulin release (Pettersson and Ahren 1990) and inhibition of both basal and insulin-stimulated glycogen synthesis in skeletal muscle in vitro (Leighton and Cooper 1988). In vivo and in vitro effects of amylin and amylin-amide on calcium metabolism have been observed in rats and rabbits (Datta et al. 1989). Isolation and characterization of cDNA clones encoding this peptide and its chromosomal localization have also been reported (Sanke et al. 1988, 1989). Thus, the peptide may have some pathological role in the aetiology and/or progression of type-2 diabetes mellitus and insulinoma and may also function in the systemic and/or local metabolism of nutrients, even in normal subjects.

In the present study, we synthesized C-terminal tetradecapeptide (24–37) of human IAPP and produced antibody by immunizing rabbits with this peptide, we also examined insulinoma by immunohistochemical and immunoelectron microscopic methods, using this antiserum. The radioimmunoassay (RIA) for IAPP using the antiserum has been established previously (Nakazato et al. 1989; Mitsukawa et al. 1990). The plasma and tissue content of IAPP were then measured in a patient with insulinoma. The pancreases of non-diabetic and diabetic subjects were also examined by RIA and immunohistochemistry.

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Table 1. The immunoreactivities of tumour cells and deposits of amyloid in insulinomas using antisera against insulin and IAPP, and reactivity by Congo red staining

Case	Age	Sex	Tumour cells		Amyloid		Congo red	IRI at fasting (μU/ml)
			Antisera against		Antisera against			
			Insulin	IAPP	Insulin	IAPP		
1	16	M	−~+++	−~+++	−	+	+	71.8
2	49	F	+++	−~+	−	+	+	42.0
3	57	F	++	−~+	−	+	+	27.3
4	33	F	++	−~+	N	N	N	30.0
5	47	F	++~+++	−	N	N	N	−
6	33	F	+	++	N	N	N	21.6
7	44	F	+~+++	−~++	N	N	N	−
8	56	M	++	−~++	N	N	N	24.0
9	51	F	++	−~+	N	N	N	36.5

IAPP, islet amyloid polypeptide; IRI, plasma level of immunoreactive insulin; +, ++, +++, relative immunoreactivity, negative to strong immunoreactivity; "+++" corresponds to that of normal islet cell; N, absence of amyloid deposit

Table 2. Immunoreactivities of B-cells and amyloid deposits for IAPP and tissue content of IAPP in diabetic cases 1–5, and non-diabetic patients cases 6–10

Case	Age at death (years)	Sex	Immunoreactivity				Tissue content of IAPP (pg/mg wet weight)	Type of DM and duration, treatment, diabetic complication, pathology of pancreas and other comments
			B-cell		Amyloid			
			Antisera against					
			Insulin	IAPP	Insulin	IAPP		
1	52	M	+	±	− P=45.3%	+	649.0	NIDDM 7y, HCC, LC, I Chr. pancreatitis with slight fibrotic change and fatty infiltration, triopathy (+), hyalinization of islets, arteriolosclerosis
2	59	M	++	+	− P=0.8%	+	847.7	NIDDM >1y, CHF, MI, O, triopathy (−), hyalinization of islets, arteriolosclerosis
3	55	M	+	±	− P=86.7%	+	ND	NIDDM 6y, Esophageal Ca., O. triopathy (−), hyalinization of islets, arteriolosclerosis
4	77	M	+	±	− P=49.2%	+	ND	NIDDM 13y, Cholangio Ca., O, triopathy (−), hyalinization of islets, arteriolosclerosis
5	74	M	++	±	− P=40.6%	+	ND	NIDDM 20y, acute subdural hemorrhage, I, nephropathy (+), hyalinization of islets, arteriolosclerosis, focal fat necrosis
6	30	M	++	++	Absent		1034.6	Subarachnoid haemorrhage, normal pancreas
7	35	M	++	++	Absent		604.0	Malignant lymphoma, chemotherapy, IVH, focal fat necrosis
8	40	M	++	++	Absent		553.4	Malignant lymphoma, chemotherapy, IVH, normal pancreas
9	47	M	++	++	Absent		704.2	Malignant lymphoma, chemotherapy, slight infiltration of lymphoma cells
10	64	M	++	++	Absent		1447.7	Cerebral haemorrhage, normal pancreas

IAPP, islet amyloid polypeptide; DM, diabetes mellitus; M, male; −, ±, +, ++, comparative immunoreactivity, − negative or markedly decreased, ± weak, + positive, ++ strong; NIDDM, non-insulin dependent diabetes mellitus; HCC hepatocellular caricinoma; LC, liver cirrhosis; I, insulin therapy; triopathy, diabetic neuropathy, retinopathy and nephropathy; CHF, congestive heart failure, MI, myocardial infarction; O, oral hypoglycemic agent, IVH, intravenous hyperalimentation; ND, not done; P, percentage of islets with amyloid deposits immunoreactive for IAPP (mean number of randomly counted islets: 139.4)

Materials and methods

The tetradecapeptide with C-terminal tyrosine amide corresponding to the (24–37) sequence of human IAPP was synthesized using a peptide synthesizer and purified by using reverse-phase high-performance liquid chromatography (HPLC). Antiserum was obtained by immunizing New Zealand white rabbits with intracutaneous and subcutaneous injections of peptide-conjugated bovine thyroglobulin. The antiserum did not cross-react with human calcitonin gene-related peptide, human insulin, human glucagon, human somatostatin or human pancreatic polypeptide in our RIA. Details of the characterization of the antiserum have previously been published (Nakazato et al. 1989; Mitsukawa et al. 1990).

The following antisera were also used for immunohistochemistry: antiserum against insulin (Peninsula Lab., St Helen's, UK), and antisera against insulin, glucagon, somatostatin, pancreatic polypeptide (PP), gastrin and vasoactive intestinal peptide (VIP) (Biogenex Lab., Calif.).

Tumour tissue was resected from the pancreas of a 16-year-old boy (Table 1, case 1). He was diagnosed as having insulinoma clinically by the presence of Whipple's triad and repeated attacks of unconsciousness and convulsions due to hypoglycaemia for about

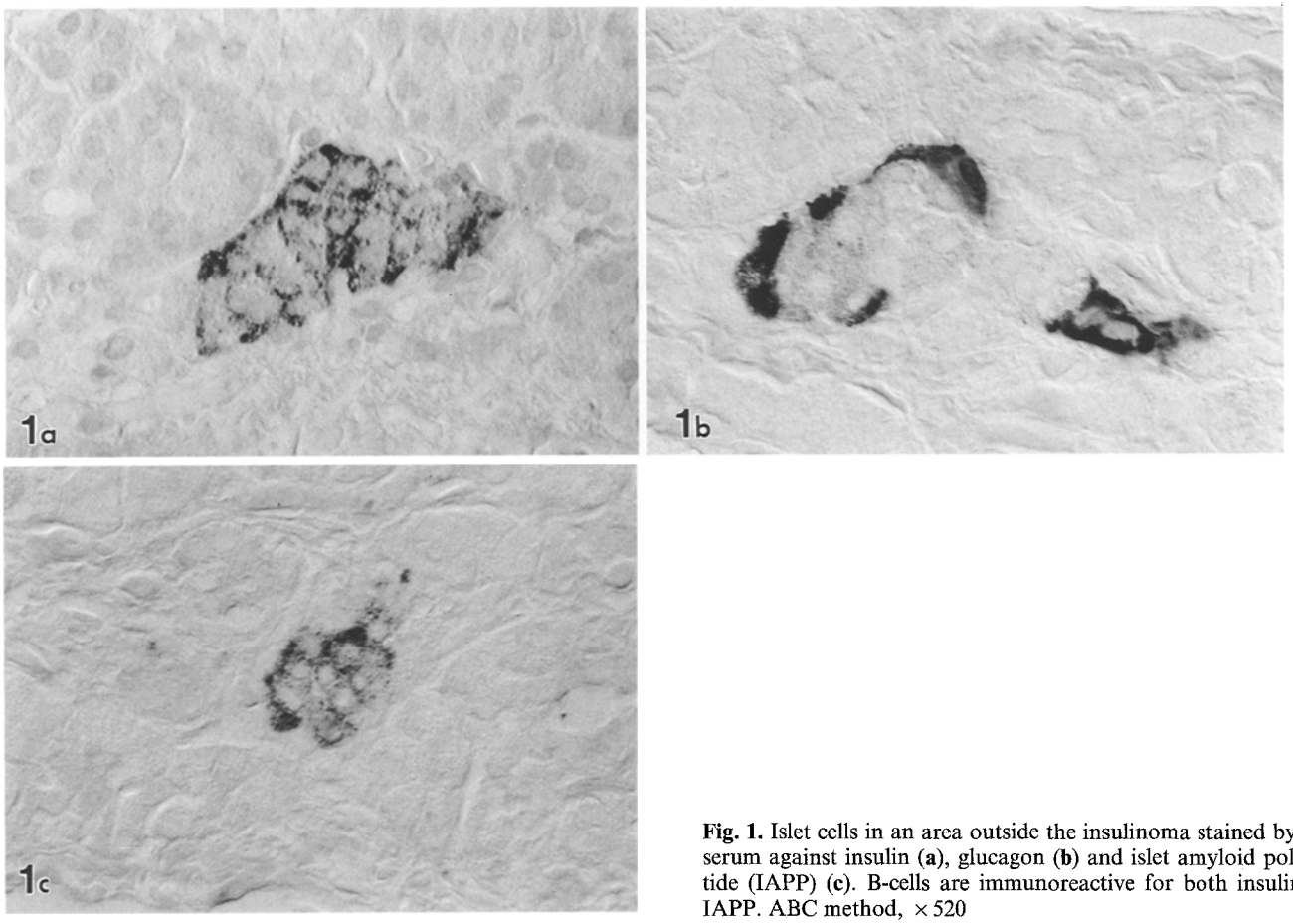

Fig. 1. Islet cells in an area outside the insulinoma stained by antiserum against insulin (**a**), glucagon (**b**) and islet amyloid polypeptide (IAPP) (**c**). B-cells are immunoreactive for both insulin and IAPP. ABC method, × 520

12 months. His fasting plasma glucose was 23 mg/dl, serum immunoreactive insulin (IRI) was 71 μU/ml, and C-peptide was 6.6 ng/ml after 15 h starvation. An intense vascular blush in a solitary tumour measuring approximately 10 mm in diameter was found in the head of the pancreas by coeliac angiography. The resected tumour, weighing 1.05 g, was cut into several pieces, fixed in 10% formalin and embedded in paraffin for routine pathological examination. Sections of approximately 3 μm thickness were cut from this block, stained by haematoxylin and eosin or Congo red and used for further immunohistochemistry. A block for immunoelectron microscopy was immediately cut into a 1-mm cube, immersed in Zamboni's fixative (2% paraformaldehyde, 0.2% picric acid in 0.1 M phosphate buffered saline adjusted to pH 7.2) at 4° C overnight, dehydrated in a graded ethanol series, and embedded in Epon 812. Finally, thin sections were cut with glass knives using an LKB ultratome. The remaining tissue was prepared for RIA immediately after resection.

Specimens from an additional eight cases of insulinoma, two with and six without amyloid deposition (Table 1), were also prepared for immunohistochemistry. All the cases had been clinically and pathologically diagnosed as insulinoma.

Paraffin sections of the pancreases of five patients with non-insulin dependent diabetes mellitus (NIDDM) and five non-diabetic patients (Table 2) were also prepared for immunohistochemistry. The tissue content of IAPP was determined in two patient with NIDDM and five non-diabetic patients, as described previously.

Paraffin sections of approximately 3 μm thickness were deparaffinized in xylene for immunohistochemistry and rehydrated through a graded series of ethanol to 0.01 M phosphate buffered saline adjusted to pH 7.4. Immunohistochemical staining was performed by an avidin biotin peroxidase complex (ABC) method (Toshimori et al. 1988, 1990). Some specimens were stained using an ABC alkaline phosphatase (ABC-AP) kit (Vectastain; Vector Lab., Calif.). The first antisera, at a dilution of 1/2000–4000, were reacted for 2 h at room temperature or overnight at 4° C in a moist chamber. The specimens were finally counterstained by methyl green for light microscopic examination. Congo red staining was performed on alternate sections.

For immunoelectron microscopy thin sections mounted on nickel grids were stained by an immunogold staining (IGS) method (Toshimori et al. 1988). The first antisera used for light microscopic immunohistochemistry were also used in the same dilution and reacted for 45 min at room temperature. The specimens were counterstained by uranyl acetate and lead citrate, and examined by a JEOL 200CX electron microscope.

The blood and tissue of the patient with insulinoma (case 1) were prepared for RIA immediately after sampling. Tissue content in the pancreases of non-diabetic and diabetic patients (Table 2) was measured immediately after resection of the tissue at autopsy, as for the insulinoma case. The methodology of the RIA for IAPP has been previously described (Nakazato et al. 1989; Mitsukawa et al. 1990).

Results

The insulinoma of case 1 was enucleated from the head of pancreas at surgery. Only one apparently normal islet was included in the tissue preparation. Cells in the central region of this normal islet stained for insulin (Fig. 1a), and those in the peripheral region stained for glucagon as previousy observed in normal islets (Fig. 1b). Cells immunoreactive to IAPP were noted at

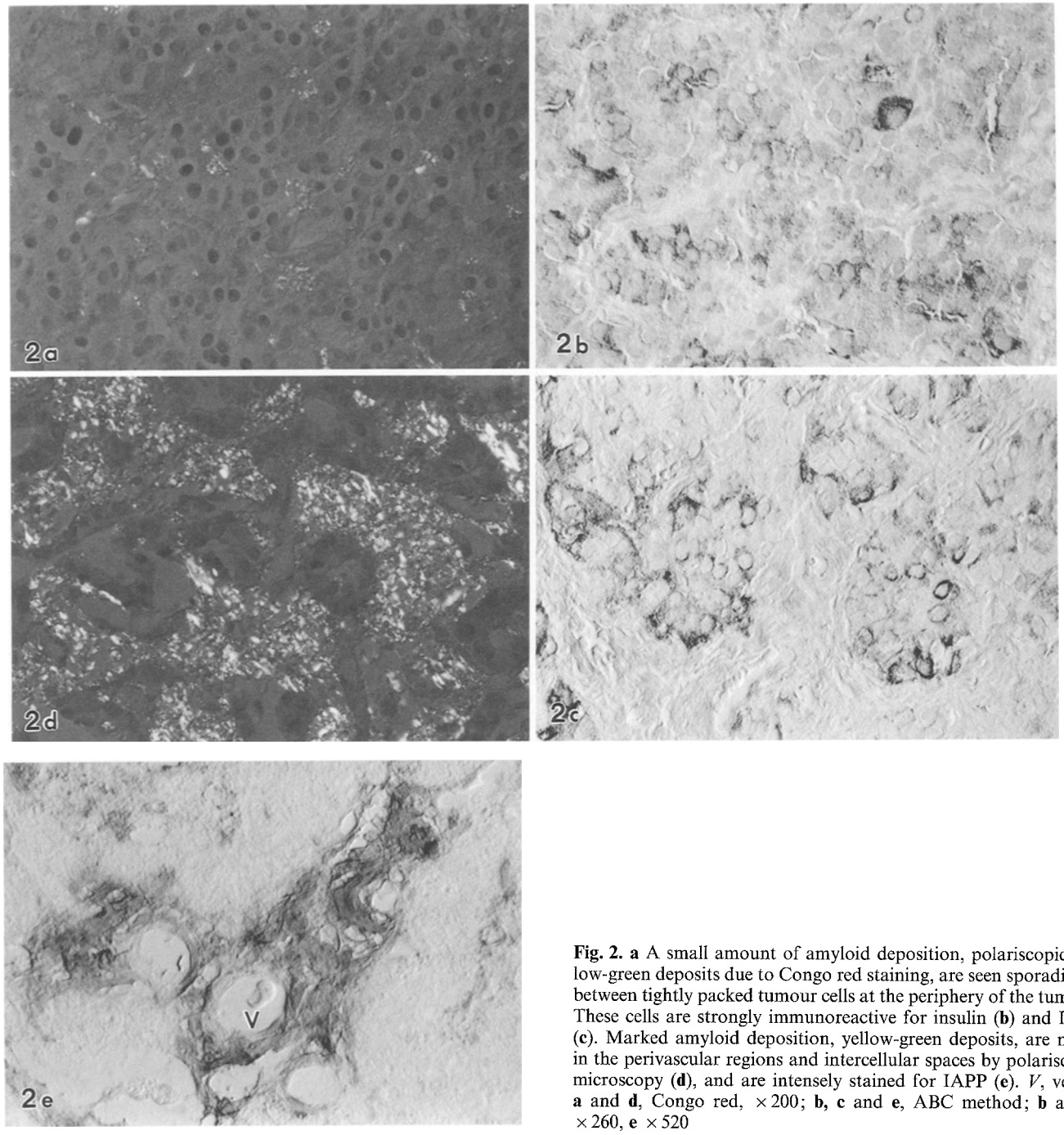

Fig. 2. a A small amount of amyloid deposition, polariscopic yellow-green deposits due to Congo red staining, are seen sporadically between tightly packed tumour cells at the periphery of the tumour. These cells are strongly immunoreactive for insulin (**b**) and IAPP (**c**). Marked amyloid deposition, yellow-green deposits, are noted in the perivascular regions and intercellular spaces by polariscopic microscopy (**d**), and are intensely stained for IAPP (**e**). *V*, vessel. **a** and **d**, Congo red, ×200; **b, c** and **e**, ABC method; **b** and **c** ×260, **e** ×520

the central region of the islet (Fig. 1c). Most of the cells that were immunoreactive for insulin in Fig. 1a also stained for IAPP by double staining of the same preparation (not shown).

Tightly packed tumour cells present a few milimetres beneath the capsule, where a small amount of amyloid deposition was noted with Congo red staining (Fig. 2a), were remarkably immunoreactive for both insulin and IAPP (Fig. 2b, c). These cells were commonly immunoreactive for both peptide by double immunostaining (not shown). The number of immunoreactive cells and their immunoreactivity for insulin gradually decreased from the periphery towards the centre (not shown). In the central region of the tumour, a large amount of amorphous amyloid between the dispersed tumour cells and surrounding vessels was stained by Congo red (Fig. 2d). These deposits stained very strongly for IAPP, whereas the tumour cells showed only faint immunoreactivity to IAPP (Fig. 2e). Both the tumour cells and amyloid deposits were immunohistochemically negative for glucagon, somatostatin, gastrin, PP and VIP (not shown).

The immunoreactivity to IAPP was blocked by preabsorption of the antiserum with an excess of the antigen. Positive immunostaining did not occur after replacement of the antisera with normal serum.

Deposits of amyloid fibrils were seen in the extracellu-

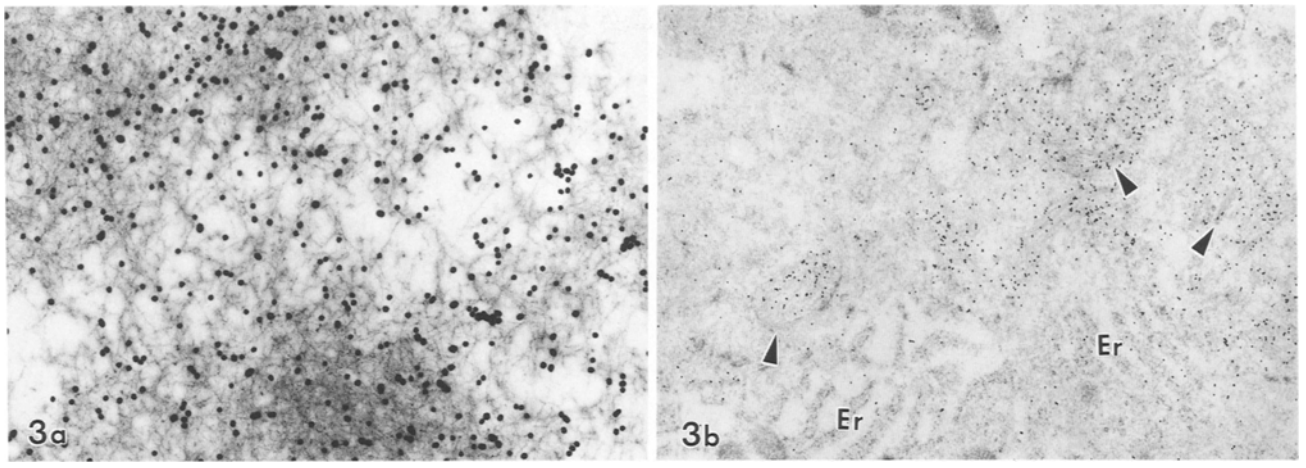

Fig. 3. a Immunoelectron micrograph showing deposits of amyloid fibrils labelled by gold particles. **b** Fibrillar elements immunoreactive for IAPP can be seen in the cytoplasm (*arrowheads*). Endoplasmic reticulum (*Er*). IGS method, **a** ×34000, **b** ×9600

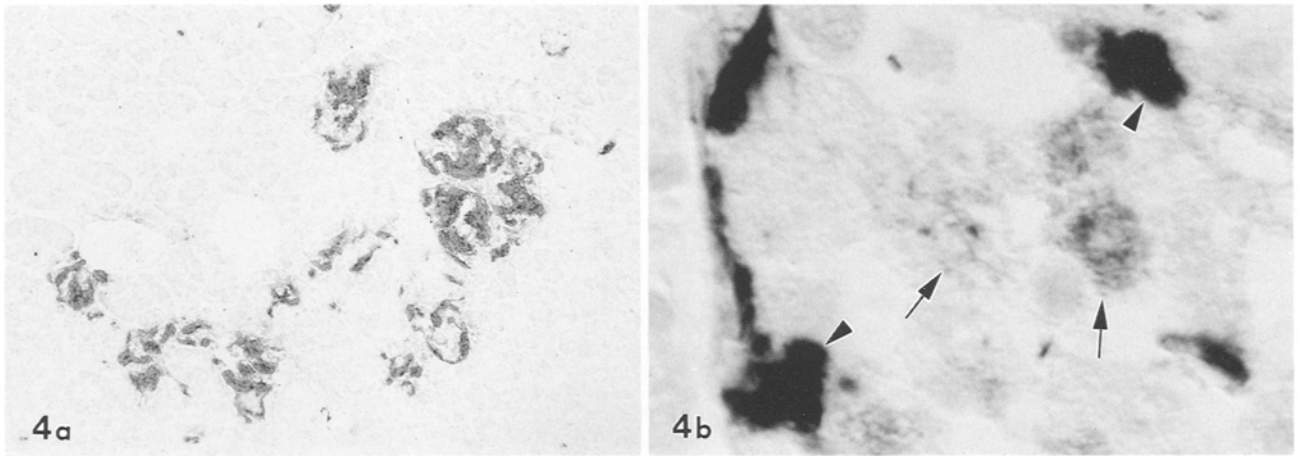

Fig. 4. a Amorphous amyloid deposits immunoreactive for IAPP are seen occupying the islets, and non intact B-cells are seen. **b** Immunoreactivity for IAPP is seen in both amyloid deposits (*arrowheads*) and in B-cells, which is remarkably decreased (*arrows*). ABC method, **a** ×135, **b** ×1100

lar space and the spaces surrounding small vessels and capillaries, and were labelled with gold particles by the IGS method for IAPP by immunoelectron microscopy (Fig. 3a). Intracellular deposition of fibrils was also found in the tumour cells (Fig. 3b). The immunoreactive fibrils were occasionally seen infiltrating into narrow intercellular spaces between capillary endothelial cells (not shown).

Amyloid deposits immunoreactive to IAPP were seen in three of nine cases of insulinoma. The deposition was most marked in case 1. The results are summarized in Table 1. The immunoreactivities of tumour cells for insulin or IAPP varied between cases and between cells, and the immunoreactivities for insulin was generally stronger than those of IAPP, except for case 6.

Pancreatic islets of non-diabetic patients strongly stained for both insulin and IAPP (not shown). B-cells were commonly immunoreactive for both peptides by double immunostaining of the same section (not shown). IAPP-immunoreactive cells did not corresponding with the cells immunoreactive for glucagon, PP or somatostatin (not shown).

Amyloid deposits in pancreatic islets of diabetic patients showed amorphous pink staining with haematoxylin and eosin, and yellow-green staining with Congo red using polariscopic light microscopy. The deposits were definitely immunoreactive for IAPP (Fig. 4a, b), whereas the immunoreactivity of B-cells for IAPP was generally decreased in diabetic islets compared with those in the non-diabetic (Fig. 4b). The results are summarized in Table 2. Amyloid depositions was present in 0.8–86.7% of islets (mean: 139.4 islets in five diabetic cases).

Plasma levels and tissue content of IAPP and other peptides in the patient with insulinoma (case 1) are shown in Table 3. Pre- and postoperative plasma levels of IAPP were 56.0 pg/ml and 26.4 pg/ml, respectively. The tissue content of IAPP was 23.7 ng/mg wet weight, and HPLC showed a single peak corresponding to IAPP. The tissue contents in the pancreas of each patients examined with NIDDM was 649.0 abd 847.7 pg/mg wet

Table 3. Plasma level of islet amyloid polypeptide (IAPP), insulin (IRI), C-peptide (CPR) and glucose, and tissue level of IAPP of a patient with insulinoma, case 1

	IAPP (pg/ml)	IRI (μU/ml)	CPR (ng/dl)	Glucose (mg/dl)
Plasma				
before operation	56.0	37.94	3.3	41
after operation	26.4	11.54	1.0	104
normal subjects[a]	13.5±4.8 (n=10, mean±SD)			
Tissue	23.66 ng/mg wet weight			

[a] Nakazato et al. 1989

weight, and that of five non-diabetic patients ranged from 553.4 to 1447.7 pg/mg wet weight, as shown in Table 2. Three cases (nos. 7–9) with a relatively low pancreatic IAPP contents were administered anticancer agents for malignant lymphoma.

Discussion

The present study demonstrated that normal islet B-cells, insulinoma cells, and amyloid deposits (amyloid fibrils) in both insulinoma and the pancreas of type-2 diabetic patients were commonly immunoreactive to IAPP. In addition, high levels of IAPP content in both the plasma (4 times higher than in normals) and tissue were demonstrated in a patient with insulinoma, the first reported case giving evidence of an increase in a substance causing amyloid deposition.

Our preliminary data revealed parallel response of plasma glucose, IAPP and IRI to 75 g oral glucose load before and after operation in the patient with insulinoma and also in normal subjects (Mitsukawa et al. 1990). Co-secretion of IAPP and insulin is supported by these dynamic studies in loading tests and morphological evidence obtained by immunoelectron miroscopy using antiserum against IAPP, which revealed localization of IAPP and insulin in the same secretory granules in normal B-cells (Johnson et al. 1988; Clark et al. 1989; Lukinius et al. 1989). However, the mechanism of deposition of amyloid fibrils in insulinoma and the islets of diabetic patients has still not been determined in detail. Amino acid residues, IAPP (20–29), especially IAPP (25–36), may have amyloidogenic properties (Betsholtz et al. 1990). Clark et al. (1989) suggested abnormal processing in IAPP production within B-cells resulting in islet amyloid deposition. However, Johnson et al. (1989) reported a possible linkage between progressive amyloid deposition and increased IAPP production preceeding the development of overt diabetes mellitus in cats. Insulinoma is considered to be a model of increased production and hypersecretion of the peptides. As demonstrated in the present study, amyloid fibrils immunoreactive for IAPP were found in both the cytoplasm and extracellular space of the tumour cells and also in the perivascular space including the intercellular spaces between endothelial cells in insulinoma. These findingngs suggest the possibility of both intracellular and extraclellular pre- and post-secretional formation of amyloid fibrils.

The biological activity of IAPP has not yet been clarified, either in the clinical setting or in laboratory studies. The deposition of amyloid would be expected to affect biosynthesis and/or release of the peptides from islets (Clark et al. 1988). However, it is not clear histopathologically whether such deposition of amyloid fibrils in diabetic islets is simply the outcome of pathological and/or biological changes in the islets, or the cause of decreasing abiltity of insulin secretion by occupying islets with amyloid during development of diabetes mellitus. The role and biological activity of IAPP including interaction between IAPP and insulin requires to be clarified.

The content of IAPP of pancreatic tissue in the two diabetic patients (cases 1 and 2) was 649.0 and 847.7 pg/mg wet weight. The former value was obtained in a poorly controlled patient with severe diabetic complications and amyloid deposition was present in 45.3% (78/172) of islets. The latter was obtained in a well-controlled patient, and amyloid deposition was seen in only 0.8% (17/158) of islets. The immunoreactivity in B-cells of the islets for insulin was as strong as in non-diabetic patients with relatively decreased immunoreactivity for IAPP. However, it is not clear whether such levels of IAPP reflect a pathological change in the diabetic pancreases since modifications by therapy could not be excluded. Pancreatic tissue content of IAPP in patients with malignant lymphoma was generally low. These patients were given anticancer agents and intravenous hyperalimentation, and these treatments possibly influenced the production and metabolism of IAPP. The content of IAPP in the normal human pancreas is still not known, although two non-diabetic patients with sudden death or acute death who received minimal treatment had values of 1034.6 (case 6) and 1447.7 (case 10) pg/mg wet weight. These values are similar to the IAPP content of rat pancreas, 1082.57±68.01 pg/ml (328.5±25.0 pmol/g) wet weight (Asai et al. 1990).

Acknowledgements. This research was supported by a Grant-in Aid for Scientific Research (C) to H. Toshimori from the Ministry of Education, Science and Culture of Japan (no. 051570649) and a Grant from Uehara Memorial Foundation. The authors wish to thank Dr. K. Toshimori, Department of Anatomy, Miyazaki Medical College, for his technical advice, and Professor M. Khono and A. Sumiyoshi, Department of Pathology, Miyazaki Medical College, for the pathological materials.

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