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Localisation of islet amyloid polypeptide and its carboxy terminal flanking peptide in islets of diabetic man and monkey

A. Clark[1, 2], J. Lloyd[1, 2], A. Novials[3], J. C. Hutton[3] and J. F. Morris[2]

[1] Diabetes Research Laboratories, Radcliffe Infirmary, [2] Department of Human Anatomy, Oxford and
[3] Department of Clinical Biochemistry, Addenbrooke's Hospital, Cambridge, UK

Summary. Islet amyloid polypeptide is a normal constituent of islet Beta cells and is derived from a larger precursor by removal of flanking peptides at the carboxy (C) and amino (N) terminals. The role of these flanking peptides in the formation of amyloid in Type 2 (non-insulin-dependent) diabetes mellitus and in insulinomas is unknown. The C-terminal flanking peptide of islet amyloid polypeptide was localised by immunocytochemistry in human and monkey pancreatic islets from Type 2 diabetic and non-diabetic individuals by use of specific polyclonal antisera. Immunoreactivity for the C-terminal peptide was found in insulin-containing cells in both diabetic and non-diabetic tissue: no antibody binding was detected in islet amyloid of Type 2 diabetic man or of monkeys although a positive reaction occurred with antisera for islet amyloid polypeptide. The C-terminal peptide was localised by immunogold electron microscopy in the insulin granules in both diabetic and non-diabetic individuals but, unlike islet amyloid polypeptide, was not detected in lysosomes. The absence of immunoreactivity for the C-terminal peptide in amyloid suggests that incomplete cleavage of this flanking peptide from islet amyloid polypeptide is not a factor in the formation of islet amyloid.

Key words: Islet amyloid polypeptide, islet amyloid, Type 2 (non-insulin-dependent) diabetes mellitus, Beta cell, pancreas.

Islet amyloid is formed in Type 2 (non-insulin-dependent) diabetes mellitus and in insulinomas from a 37 amino acid peptide, islet amyloid polypeptide (IAPP) [1]. This peptide is a normal component of diabetic and non-diabetic Beta cells where it is located in insulin granules and lysosomes [2]. Analysis of the encoding cDNA suggests that IAPP is derived from a larger propeptide (89 amino acids in man) by proteolytic cleavage [3]. However, little is known about the processing pathway of IAPP in the Beta cell under normal conditions and in diabetes.

It is unlikely that amyloid develops as a result of genetically determined amino acid substitution of IAPP in Type 2 diabetes [4]. Abnormal cleavage of the propeptide may be involved since immunoreactivity for the N-terminal flanking peptide (pro-IAPP$_{18-30}$) has been identified in the amyloid deposits [5]. Alternatively, this small peptide may, like amyloid P component and glycated proteins, adhere to the previously formed fibrils. Involvement of the C-terminal flanking peptide. (C-IAPP) in islet amyloidosis was examined with immunocytochemistry in tissue from non-diabetic and diabetic man and monkey.

Materials and methods

Antisera production

The C-terminal flanking peptide of IAPP (20 amino acids) with the sequence Gly, Lys, Arg, Asn, Ala, Val, Glu, Val, Leu, Lys, Arg, Glu, Pro, Leu, Asn, Tyr, Leu, Pro, Leu, was synthesised by automatic solid phase synthesis (model 431 A, Applied Biosystems, Warrington, UK). An N-terminal cysteine was added to permit conjugation to a hapten for antibody production. C-IAPP was conjugated to keyhole limpet haemocyanin by use of a hetero-bifunctional linkage using m-maleimidobenzoyl-N-hydroxysuccinimide. Rabbits were immunised with the conjugated peptide in Freund's complete adjuvant. The resulting antisera cross-reacted with the non-conjugated peptide on ELISA plates coated with 2 μg/ml of the peptide. These antisera did not cross-react with IAPP$_{1-37}$ or IAPP. NH2$_{1-37}$ (2 μg/ml) on ELISA plates. The specificity of the antibody for immunohistochemistry was confirmed by preabsorption of the diluted antibody with 10 μg/ml of the unconjugated C-IAPP. No immunoreactivity was found after preabsorption.

Specimens

Postmortem pancreas specimens were examined from 14 Type 2 diabetic subjects with islet amyloid (aged 48–89 years), 10 non-diabetic patients (aged 40–78 years), two obese diabetic Macaca

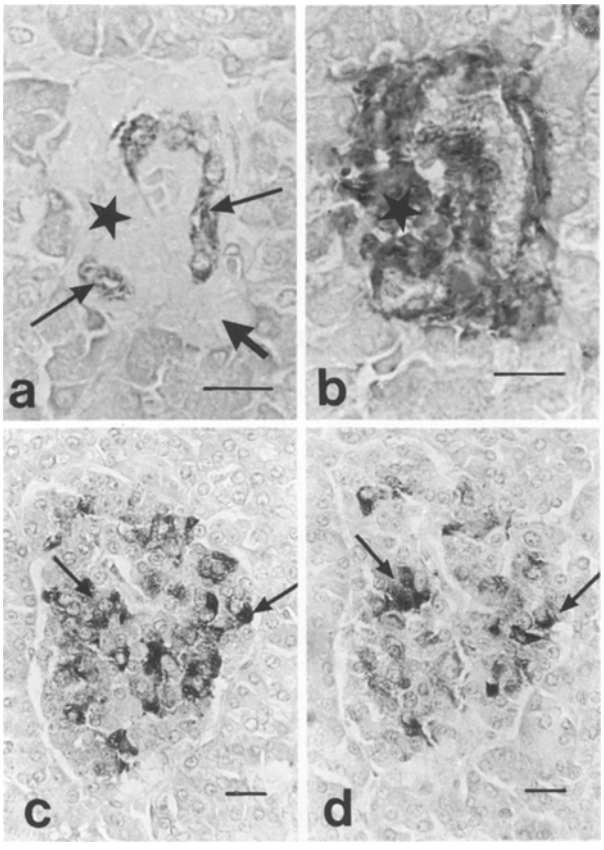

Fig. 1 a–d. Immunoreactivity for C-IAPP (islet amyloid polypeptide), and IAPP in pancreatic islets. (**a**), (**b**): Serial 5 μm sections from a Type 2 (non-insulin-dependent) diabetic subject: (**a**) immunoreactivity for C-IAPP is present in the few cells *(arrows)* that remain in this islet which is severely affected with amyloid but no labelling is present in the amyloid (*). (**b**) The serial section shows positive immunoreactivity for IAPP over the amyloid deposits (*). (**c**), (**d**) Serial sections of an islet of a non-diabetic subject (**c**) immunoperoxidase labelling of a small number of islet cells for C-IAPP. (**d**) Immunoreactivity for IAPP is in a similar population of islet cells *(arrows)*. Scale bar, 20 μm

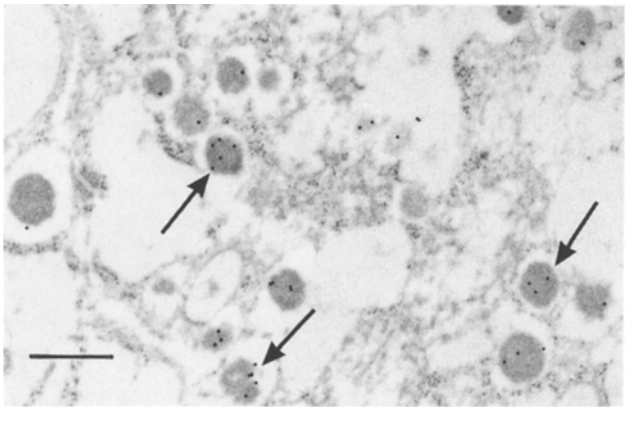

Fig. 2. Immunogold labelling of Beta cells in an islet from a non-diabetic monkey. Immunoreactivity is present over the insulin granules *(arrows)* Scale bar, 1.0 μm

nigra with islet amyloid (aged 15 years; fasting plasma glucose, FPG > 10 mmol/l) two non-diabetic Macaca nigra (aged 2 years, FPG < 6.0 mmol/l), and four non-diabetic Macaca fascicularis (aged 6 years; FPG < 6.0 mmol/l). Specimens for light microscopy were fixed in 10% formaldehyde in 0.9% sodium chloride, dehydrated and embedded in paraffin wax. Tissue for electron microscopy was fixed in 2% paraformaldehyde, 0.5% glutaraldehyde in 0.1 mol/l cacodylate buffer, pH 7.2, and embedded in LR gold resin (Taab Laboratories, Aldermaston, UK).

Immunohistochemistry

Immunoreactivity for C-IAPP, IAPP and insulin was examined light microscopically with immunoperoxidase and antisera to insulin (diluted 1:1000) raised in a guinea pig, or to $IAPP_{7-37}$ (diluted 1:1000) and C-IAPP (diluted 1:1000) raised in rabbits. Co-localisation of insulin and C-IAPP was examined by double labelling on the same section with fluorescein-conjugated (FITC) anti-guinea pig antiserum (Dako, High Wycombe, UK) (for insulin) and peroxidase conjugated anti-rabbit antiserum (Dako) (for C-IAPP). Immunoreactivity was detected electron microscopically by incubation of ultrathin sections mounted on nickel grids with primary antisera at 1:500 dilution (anti-IAPP and anti-C-IAPP) or 1:1000 (anti-insulin) followed by protein-A gold (ICN, High Wycombe, UK)

Results and Discussion

Immunoreactivity in islet amyloid. Immunoreactivity for C-IAPP was not detected in islet amyloid (Fig. 1 a) of any specimen of diabetic human or monkey pancreas even though a positive staining reaction for $IAPP_{7-37}$ was present (Fig. 1 b). This suggests that C-IAPP is not part of the structure of the amyloid fibrils. Although $IAPP_{1-37}$ was identified as the major constituent of amyloid in the extracted samples of pancreatic tissue [1], the presence of pro-IAPP (or other fragments of pro-IAPP) in amyloid could not be excluded. Incomplete processing of procalcitonin at both the C- and N-terminals has been implicated in the amyloidosis associated with medullary carcinomas of the thyroid [6, 7].

Immunoreactivity in islet cells. The C-terminal peptide was detected in islet cells of diabetic and non-diabetic individuals (Fig. 1 c) which were identified as Beta cells by double labelling experiments. The density of the staining reaction for C-IAPP was not uniform in all Beta cells (Fig. 1 c): a dense peroxidase reaction was present in a small number (approximately 30% of Beta cells) of Beta cells and a similar population of cells showed immunoreactivity for IAPP (Fig. 1 d). Electron microscopy demonstrated immunogold labelling of C-IAPP located over insulin granules in Beta cells of both diabetic and non-diabetic individuals (Fig. 2) but not over amyloid deposits in a diabetic monkey. Furthermore, no labelling for C-IAPP could be detected in lysosomes or lipofuscin bodies in Beta cells although these showed positive immunoreactivity for IAPP on serial ultrathin sections. As with light microscopy, no labelling for C-IAPP was found in glucagon-, pancreatic polypeptide- or somatostatin-containing cells.

The biosynthetic pathway for IAPP is unknown, but the presence of Lys Arg sequences suggests that proteolytic cleavage of pro-IAPP similar to that of proinsulin is involved [8]. Elevated levels of proinsulin have been found in patients with insulinomas and Type 2 diabetes mellitus and also in a subject known to have islet amyloid [9] implying that abnormal processing of Beta cell products could be implicated in amyloid formation in these conditions.

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Dr. A. Clark
Diabetes Research Laboratories
Radcliffe Infirmary
Woodstock Road
Oxford
UK