Altered immunoreactivity of islet amyloid polypeptide (IAPP) may reflect major modifications of the IAPP molecule in amyloidogenesis

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Summary We have developed a mouse monoclonal antibody against rat/mouse islet amyloid polypeptide (IAPP). The antibody recognises an epitope in the N-terminal part of the molecule, which is conserved between different species. The antibody immunohistochemically labelled beta cells in normal islets of most different mammalian species including man and in one avian species. Previous immunohistochemical studies of human pancreatic tissue from individuals with non-insulin-dependent diabetes mellitus (NIDDM) have revealed a paradoxical and unexplained lack of IAPP immunoreactivity in beta cells close to amyloid in spite of the presence of IAPP mRNA. In contrast to these findings we show that the newly developed monoclonal IAPP antibody strongly labels such beta cells while islet amyloid deposits which are labelled by polyclonal antisera do not bind the monoclonal antibody. These findings with the polyclonal antisera and the monoclonal antibody indicate that IAPP undergoes one or several structural changes during the amyloidogenesis. Knowledge of these structural changes that may include abnormal folding or chemical modification of IAPP is probably important for the understanding of the amyloidogenesis and the pathogenesis of the islet lesion in NIDDM. [Diabetologia (1997) 40: 793–801]

Keywords Islet amyloid polypeptide, monoclonal antibody, non-insulin-dependent diabetes mellitus, immunohistochemistry, deposits.

Islet amyloid polypeptide (IAPP; amylin) is a putative polypeptide hormone, in humans it is expressed mainly by the beta cells of the islets of Langerhans. It is co-stored with insulin in the secretory granules [1], where it is located in the outer, translucent zone [2]. IAPP is a 37-amino acid polypeptide with almost 50 % identity with the neuropeptide calcitonin gene-related peptide (CGRP) [3, 4]. Like CGRP, IAPP is C-terminally amidated and has a disulphide bridge close to its N-terminus. The sequence differences between the two peptides are mainly located in the central part of the molecules and there is a 10 amino acid segment (positions 20–29) which in humans is completely different from other species. However, the interspecies differences that exist in the IAPP amino acid sequence are also mainly located in the 20–29 segment [5–7].

Many groups have made antibodies to human or rat IAPP. The immunogen has been either full-length IAPP or segments of the molecule. Thus, rabbit antisera can be made against the 20–29 segment of human IAPP. Due to sequence differences between species in this part of the IAPP molecule, such antisera do not cross-react with rat or mouse IAPP in immunohistochemistry and it does not react with CGRP. On the other hand, it has turned out to be very difficult to obtain polyclonal antisera to human or rat/mouse full-length IAPP that do not cross-react with CGRP. Thus, almost all commercially available polyclonal IAPP antisera show significant cross-reaction with CGRP. The cross-reaction is problematic in immunohistochemistry since both polypeptides can

sometimes be found at identical locations. Thus, it has recently been shown in the rat that IAPP is a neuropeptide present in some CGRP-positive neurones [8]. Therefore we thought that a monoclonal antibody (MAb), not cross-reacting with CGRP, should be of great potential value. We have developed a murine MAb to IAPP which is useful for immunohistochemistry on paraffin-embedded tissue material. This antibody cross-reacts with IAPP in several different vertebrates including humans.

Differences in the 20–29 segment of IAPP between species seem to explain why only certain species such as human and cat develop amyloid in their islets [9]. However, the pathogenesis of islet amyloid in non-insulin-dependent diabetes mellitus (NIDDM) is not well understood. In addition to an amyloidogenic IAPP structure, over-production resulting in an abnormally high local IAPP concentration has been suggested to be of importance [10]. Experiments with transgenic mice carrying the human IAPP gene and highly over-expressing human IAPP [11–14], have shown that in addition other, so far undetermined factors are of importance in the amyloidogenesis and the beta-cell lesion in NIDDM. In a combined immunohistochemical and in situ hybridization study we found a strongly reduced IAPP immunoreactivity in spite of the presence of IAPP mRNA in beta cells close to islet amyloid deposits [15]. With the use of the newly developed monoclonal antibody to IAPP in an immunohistochemical study of tissue material from patients with and without NIDDM we got unexpected results that may shed some light on these earlier findings.

Materials and methods

Production of monoclonal antibody. MAbs were produced using standard protocols [16, 17]. Full-length, C-terminally amidated rat/mouse IAPP was synthesized by Multiple Peptide Systems (San Diego, Calif., USA). Analysis by mass spectrometry revealed the expected mass. IAPP was conjugated to keyhole limpet hemocyanin (KLH) (Sigma, Stockholm, Sweden) with the aid of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (Sigma) as described [18, 19]. Female BALB/c mice were immunized intraperitoneally with 100 μg KLH-IAPP in 0.5 mol/l NaCl, mixed with Freund's complete adjuvant (Difco Laboratories, Detroit, Mich., USA) for the first injection. For subsequent injections Freund's incomplete adjuvant was used once a week. Blood was taken orbitally at regular intervals, diluted 1:100–1:800 and checked immunohistochemically on the islets of mouse pancreatic sections for the presence of IAPP antibodies. Three positive mice were boostered, and 3 days later killed by cervical dislocation and the spleen removed. Splenocytes were fused at a proportion of 10:1 with mouse non-secreting myeloma cells (line SP2/0) using 50% polyethylene glycol 4000 (Boehringer Mannheim, Mannheim, Germany). Fused cells were incubated in RPMI 1640 medium (Gibco BRL, Täby, Sweden) containing 15% fetal bovine serum at +37°C and 5% CO_2 for 2 h, diluted to 5×10^5/ml in the same solution and then mixed with new-born rat thymocytes

(5×10^4/ml) and seeded into 96-well microtitre plates (Costar, Cambridge, Mass., USA), at 100 μl/well. After 24 h incubation, 100 μl 2 × HAT (Hypoxanthine, Aminopterin, Thymidine) selection medium was added to each well. One week later, half the medium was removed and replaced with the appropriate amount of 1 × HAT selection medium.

Screening for and production of monoclonal antibodies. Screening of the MAb-producing hybrids was performed by immunohistochemistry on formalin-fixed paraffin embedded normal human and rat pancreatic material. For this, 5 μm sections were deparaffinized and incubated with the respective undiluted supernatants at +4°C overnight. Antibodies were visualized by the avidin-biotin method (see below). One hybridoma (4A5) which reacted specifically with islets was selected for MAb production and detailed studies. The hybridoma cell line was subcloned three times by limiting dilution prior to final mass culture.

Characterization of the monoclonal antibody 4A5. The isotype of MAb 4A5 was determined by an isotyping kit (Inno-Lia Mouse MAb Isotyping, Innogenetics N.V., Zwijndrecht, Belgium) to be of IgG2b ϰ type. The specificity of the MAb was studied by slot immunobinding. Peptides (Fig.1) were dissolved in 0.1 mol/l sodium carbonate buffer, pH 9.6 and spotted (5 μg for each slot) on a hybridization transfer membrane (Hybond – C extra, Amersham, Stockholm, Sweden) using a MilliBlot slot blotter (Millipore, Bedford, Mass., USA). To prevent unspecific binding the membrane was blocked for 1 h at room temperature with 5% non-fat milk in 0.1 mol/l Tris HCl buffer, pH 7.2, containing 0.15 mol/l NaCl (TBS) and 0.1% Tween 20, and then incubated overnight at +4°C with 4A5 supernatant, diluted 1:200 or rabbit antiserum A110 to rat/mouse IAPP, diluted 1:2000 in TBS. After washing with three changes of TBS containing 0.1% Tween 20, the membrane was incubated for 2 h with horseradish peroxidase-conjugated rabbit anti-mouse or swine anti-rabbit Ig (Dakopatts, Copenhagen, Denmark), diluted 1:1000. The immunoreaction was visualized with an ECL Western blotting detection system (Amersham) according to the instructions provided by the manufacturer. The specificity of MAb 4A5 was also tested by preabsorption of 4A5 supernatant with a final concentration of 200 μg/ml synthetic peptides including full-length human and rat/mouse-IAPP, rat IAPP8–37, IAPP7–17, human and rat IAPP20–29, human IAPP26–37, human N- and C-terminal IAPP flanking peptides, human insulin and human α-CGRP.

Tissues. Paraffin-embedded human pancreatic material was available from the laboratory files and included normal human pancreatic tissue from patients undergoing surgery for pancreatic neoplasms and pancreatic tissue from individuals with and without NIDDM obtained at autopsy within 4–10 h after death. These specimens have been described previously [15]. Paraffin-embedded pancreatic tissue from different animal species was also available from the laboratory files.

Human and rat normal pancreatic tissue was immersion-fixed in 2% paraformaldehyde-0.5% glutaraldehyde in 0.1 mol/l sodium phosphate buffer pH 7.6 for 4 h and then embedded in Unicryl (Biocell, Cardiff, UK) at +4°C.

Immunohistochemistry. Polyclonal rabbit antiserum A110 against rat IAPP has been described [20] and used at a dilution of 1:2000. Guinea pig antiserum to insulin was from Dako (Copenhagen, Denmark) and used at a dilution of 1:1000. The MAb 4A5 was used as supernatant diluted 1:100–1:200. Immunohistochemical studies were performed using the avidin-biotin method [21]. Deparaffinized sections were pretreated

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             -10        1       10        20        30        40        50     Peptides        4A5
                                                                                               reaction
      NHLKATPIESHQVEKRKCNTATCATQRLANFLVHSSNNFGAILSSTNVGSNTYGKRNAVEVLKREPLNYLPL  h-proIAPP
                         *                                          *

      NHLKATPIESHQV                                                             h-N-IAPP          -
                  HQVEKRKCNT                                                    h-ps-1            -
                     KCNTATCATQRLANFLVHSSNNFGAILSSTNVGSNTY                      h-1-37            +
                     KCNTATCATQRLANFLVRSSNNLGPVLPPTNVGSNTY                      r-1-37            +
                          CATQRLANFLV                                           7-17             +
                       ATQRLANFLVRSSNNLGPVLPPTNVGSNTY                           r-8-37           +
                                    SNNLGPVLPP                                  r-20-29           -
                                    SNNFGAILSS                                  h-20-29           -
                                        ILSSTNVGSNTY                            h-26-37           -
                                           NTYGKRNAVE                           h-ps-2            -
                                              NAVEVLKREPLNYLPL                   h-C-IAPP          -
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Fig. 1. Summary of slot blot analysis and absorption test of the monoclonal antibody 4A5 against synthetic polypeptides corresponding to different parts of the human (h-) or rat/mouse (r-) proIAPP. Numbers indicate amino acid residues of mature IAPP the limits of which are indicated by asterisks. Sequences corresponding to parts of the flanking peptides are N-IAPP and C-IAPP and sequences containing the two processing sites of proIAPP are shown as ps-1 and ps-2. The consensus sequence for reaction with 4A5 corresponds to IAPP8–17. The major part of human preproIAPP is above

Fig. 2. Slot blot analysis of monoclonal antibody 4A5 and polyclonal rabbit antiserum A110 with different polypeptides (for explanation of abbreviations, see Fig. 1). Antiserum 110 reacts with CGRP in addition to IAPP-related polypeptides

with 0.3 % H_2O_2 in TBS for 30 min before incubation with the primary antibodies which was performed overnight at + 4 °C. After rinsing in TBS, the sections were incubated with biotinylated swine anti-rabbit immunoglobulin or goat anti-mouse immunoglobulin (Dakopatts, Copenhagen, Denmark), rinsed and the reaction was visualized with peroxidase-conjugated avidin followed by 3,3diaminobenzidine-tetrahydrochloride (DAB). For insulin detection, goat anti-guinea pig horseradish peroxidase-labelled antibodies (Dakopatts) was used. Negative control sections included replacement of the primary antibody with rabbit or mouse non-immune serum.

Initial immunohistochemical experiments with the MAb 4A5 gave inconclusive results. However, incubation of the sections in 0.01 mol/l sodium citrate buffer, pH 6.0, which was poured on hot (+ 95 °C) and then allowed to cool prior to the incubation with the MAb resulted in an even, strong and reproducible reaction. This pretreatment method was found to be superior to pretreatment with trypsin or microwaves.

Sections, adjacent to those used for immunohistochemistry were stained with alkaline Congo red [22] and examined for amyloid deposits in polarized light.

Immune electron microscopy. The MAb used for immune electron microscopy was concentrated two times with the aid of membrane affinity separation system for purification of IgG (Kemila, Sollentuna, Sweden) and performed on ultrathin Unicryl sections from rat and human pancreata, mounted on formvar-coated nickel grids as described [23]. The reaction was visualized with 10 nm immunogold conjugate electron microscopy goat anti-mouse IgG (BioCell, Cardiff, UK).

Peptides. Rat/mouse C-terminally amidated full-length IAPP was from Multiple Peptide Systems (San Diego, Calif., USA). Human α-CGRP was purchased from Bachem, Bubendorf, Switzerland. Human insulin was from Eli Lilly (Indianapolis, Ind., USA). Carboxy-terminally amidated peptides corresponding to parts of the human or rat/mouse IAPP, the processing sites of human proIAPP and the flanking peptides of human IAPP (Fig. 1) were synthesized by automatic solid-phase synthesis on a model 430A peptide synthesizer (Applied Biosystems, Foster City, Calif., USA). All peptides showed the expected molecular mass by mass spectrometry.
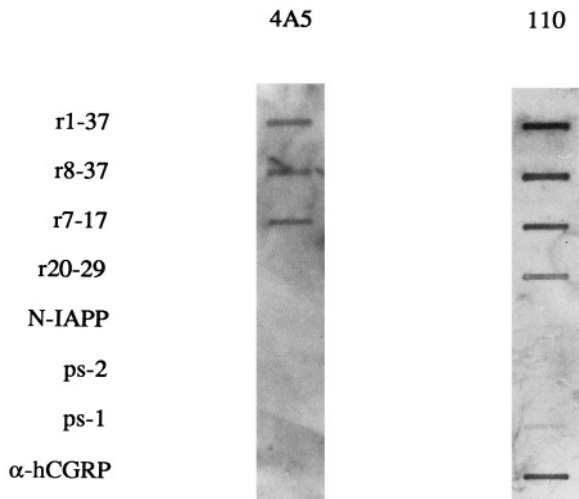
Results

Specificity of monoclonal antibody 4A5. In slot blot analysis (Figs. 1 and 2) with different peptides including rat IAPP1–37, rat IAPP8–37, rat IAPP7–17, rat IAPP20–29, human N- and C-terminal flanking peptides, peptides including the proIAPP processing sites, human α-CGRP and insulin, the MAb 4A5 showed a strong reaction with rat IAPP1–37, rat IAPP8–37 and rat IAPP7–17, but no reaction was seen with the other peptides. When MAb 4A5 was absorbed with the different peptides, only rat and human IAPP1–37, rat IAPP8–37 and rat IAPP7–17 completely abolished the immunohistochemical

Table 1. Immunoreaction of the monoclonal antibody 4A5 with pancreatic islets in different species. The amino acid sequences of IAPP8-17 and CGRP8-17 are also shown

IAPP8-17	Species	Ref.	4A5 immunoreactivity
A T Q R L T N F L T	Shrew	Unpublished	+
A T Q R L A N F L V	Human	[4]	+
A T Q R L A N F L V	Monkey	[35]	NT
A T Q R L A N F L I	Cat	[7]	+
A T Q R L A N F L I	Rabbit/hare	[20]	(+)
A T Q R L A N F L V	Dog	[35]	NT
A T Q R L A N F L L	Raccoon	[36]	NT
A T Q R L A N F L V	Rat/mouse	[5]	+
A T Q R L T N F L V	Guinea pig	[6]	+
A T Q R L T N F L V	Degu	[37]	−
A T Q R L A N F L V	Hamster	[5]	(+)
V T Q R L A D F L V	Chicken	[38]	+
T Q R L F L	Conserved in IAPP		
CGRP8-17			
V T H R L A G L L S	Human α and β	[39]	
V T H R L A G L L S	Rat α and β	[39]	
V T H R L A G L L S	Rabbit	[40]	
V T H R L A D F L S	Chicken	[41]	

+, Positive immunoreactivity; (+), weak immunoreactivity; −NT, not tested

reaction with normal rat islet tissue. The reactivity of MAb 4A5 is summarized in Figure 1. Absorption with the other peptides did not alter the immunoreactivity of MAb 4A5. A strong reactivity was obtained with rat IAPP1–37, rat IAPP8–37, rat IAPP7–17, rat IAPP20–29 and human α-CGRP when the rabbit polyclonal anti IAPP antiserum A110 was applied in slot blot experiments.

Fig. 3a, b. Consecutive sections of a normal human islet labelled with the monoclonal antibody to IAPP 4A5 **(a)** and the polyclonal antiserum A110 to IAPP **(b)**. The labelling pattern is the same in the two sections showing a labelling of islet beta cells. × 500
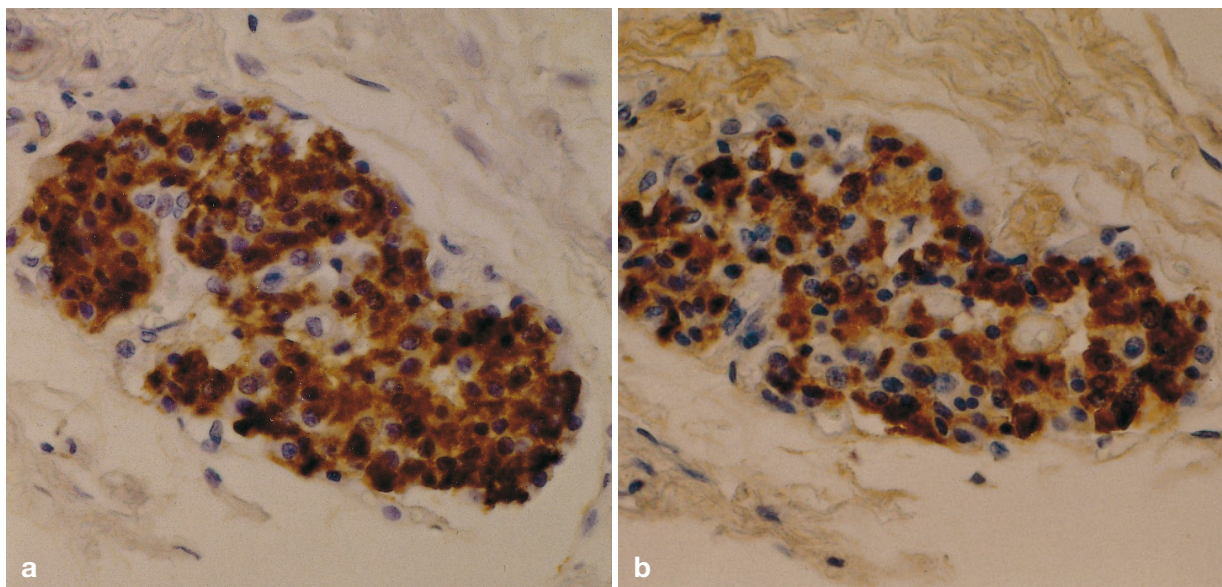
Immunohistochemical and immunocytochemical findings

Islets of Langerhans. Immunoreactivity with MAb 4A5 was found in a majority of cells in normal human and rat islets of Langerhans. Consecutive sections labelled with MAb 4A5 and antiserum to IAPP (A110) revealed a similar distribution pattern of cells demonstrating that the labelled cells were beta cells (Fig. 3).

The reactivity of MAb 4A5 with the islets of Langerhans in different animal species is shown in Table 1. As can be seen there is a broad cross reactivity of MAb 4A5 and beta cells of most studied species were labelled (Fig. 4). The intensity of the reaction varied and the islets of a few animals exhibited only very faint labelling. These include rabbit and hare
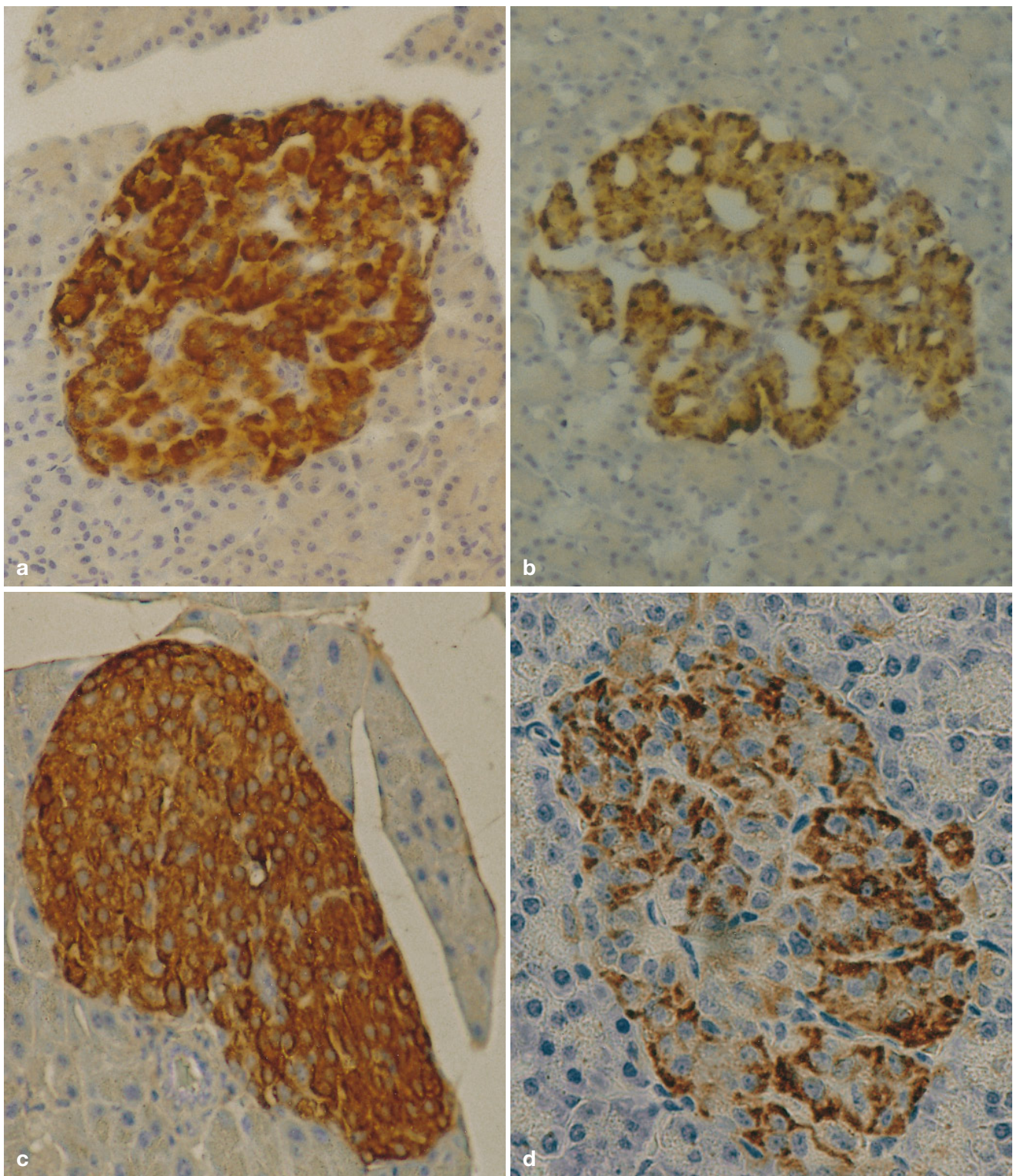
Fig. 4a–d. Pancreatic islets from different species labelled with the monoclonal antibody to IAPP 4A5: rat **(a)**, chicken **(b)**, mouse **(c)** and cat **(d)**. × 300 **(a)**, 350 **(b, c)** and 600 **(d)**

which seem to express comparably little IAPP in beta cells [20]. The immunoreactivity in avian pancreatic tissue was localized to the small insulin-containing islets while the large glucagon-containing islets did not show any reactivity.

Electron microscopically, MAb 4A5 immunoreactivity was limited to beta-cell secretory granules. In contrast to insulin immunoreactivity, MAb 4A5 labelling was predominantly limited to the outer translucent part of the beta-cell granules (Fig. 5). Compared
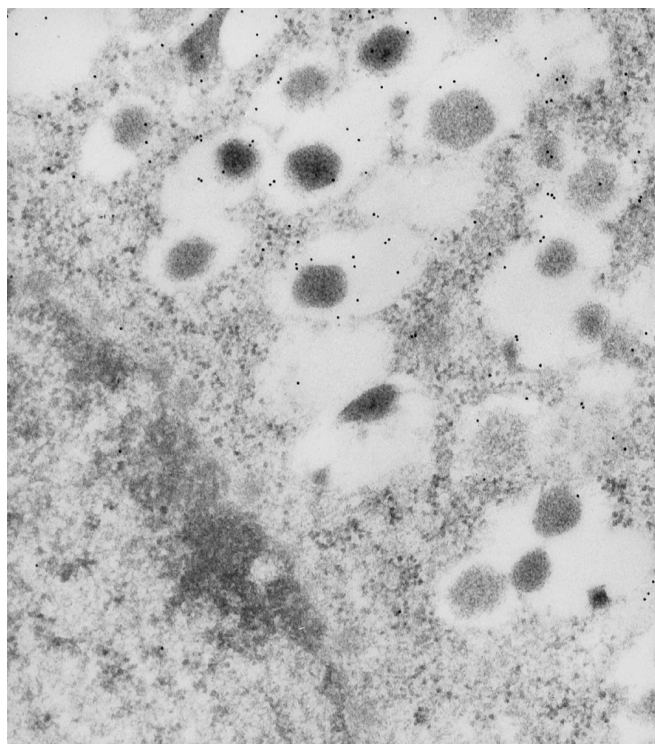
Fig. 5. Electron microscopy picture showing immunolabelling of normal human islet beta cell with the monoclonal antibody 4A5. The reaction is confined to the outer part of secretory granules where IAPP is stored. × 40 000

to labelling with antiserum to insulin and with polyclonal antiserum to IAPP the MAb 4A5 immunogold labelling was weak indicating that the antigenic epitope for MAb 4A5 is partly destroyed by the fixation and embedding procedure for electron microscopy.

IAPP-immunoreactivity in the pancreas of individuals with and without NIDDM. MAb 4A5 showed a strong immunolabelling of islet beta cells in the pancreata of patients with and without NIDDM (Tables 2 and 3; Fig. 6). Similar to what is seen in the normal

human pancreas with polyclonal IAPP antiserum [15] (Fig. 3), the reactivity varied somewhat between individual beta cells in a given islet. Thus, some beta cells were more strongly labelled than others. This phenomenon was obvious in pancreata of diabetic as well as of non-diabetic individuals. When the polyclonal antiserum A110 was used, little or no IAPP-immunoreactivity was seen in beta cells in islets with amyloid (Tables 2 and 3; Fig. 6) in accordance with previous reports [15, 24]. Interestingly, islet amyloid of diabetic and of non-diabetic individuals was almost completely devoid of reactivity with MAb 4A5 (Fig. 6) while the polyclonal antiserum A110 labelled islet amyloid strongly (Tables 2 and 3, Fig. 6). Pretreatment of the sections with hot citrate buffer did not alter the reactivity of beta cells or amyloid with antiserum A110. In order to study the possibility that formalin-fixation and paraffin embedding could change the reactivity with the antibodies, frozen sections of human pancreatic tissue with islet amyloid were labelled with MAb 4A5 and antiserum A110. However, identical results were obtained when compared to the paraffin-embedded material (not shown). Thus, pretreatment with hot citrate buffer was still necessary to obtain labelling of beta cells with MAb 4A5.

Discussion

We have developed a mouse MAb to rat/mouse IAPP. Immunohistochemically, the antibody shows reactivity with IAPP in different species but no cross-reactivity with CGRP was found in slot blot analysis. The epitope of the IAPP molecule could be determined to be situated between residues 8 and 17. This segment is within a conserved part of the IAPP molecule (Table 1) and differs in amino acid sequence from that of CGRP at some positions. The specificity to a conserved part of IAPP is in accordance with the wide cross reactivity between the

Table 2. Immunohistochemical reaction of islet beta cells and amyloid with monoclonal and polyclonal antibodies to IAPP in pancreatic tissue of individuals with non-insulin-dependent diabetes mellitus

Patient no.	Age (years)	Sex	Duration of diabetes (years)	Treatment	Percentage of islets with amyloid	MAb reacting with		PAb reacting with	
						Beta-cell	amyloid	Beta-cell	amyloid
861	83	Female	not known	Chl	27	+[a]	−	−[b]	+
1038	82	Male	16	Tol	89	2+	−	+[c]	+
72	65	Male	2	Chl	0	2+		2+	
479	68	Male	10	Chl	91	2+	−	2+[c]	+
1078	74	Male	8	Chl	97	2+	−	+	+
113	74	Female	15	Chl+Tol	98	2+	−	−[b]	+
80	78	Female	5	Chl	69	2+	−	+	+
298	74	Female	26	Ins	97	+	−	−	+

[a] Semiquantitative estimation of the strength of the immunohistochemical labelling of beta-cells (−; no labelling, +; weak to moderate labelling, 2+; strong labelling

[b] Only scattered IAPP-positive cells in islets with amyloid
[c] Very few islets with scattered IAPP-positive cells
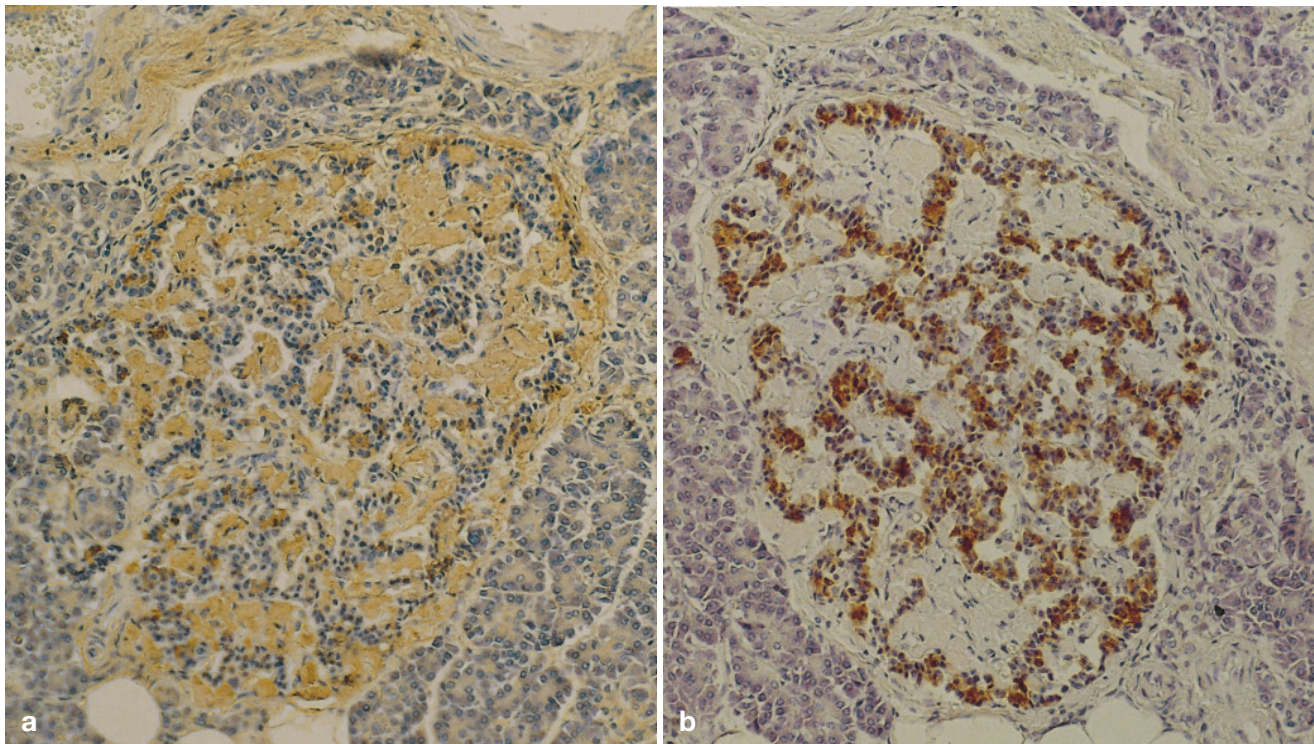Chl, Chlorpropamide; Tol, Tolbutamide; Ins, Insulin

Fig. 6a, b. Representative islet of Langerhans from a patient with NIDDM, labelled with the polyclonal antiserum A110 against IAPP **(a)**. Only few beta cells show a weak reaction while the amyloid is strongly labelled. The same islet labelled with the monoclonal antibody 4A5 against IAPP **(b)**. The beta cells are strongly positive while the amyloid is negative. × 200

tested mammalian and avian species found in the immunohistochemical study. Further studies with pancreata of reptiles have shown strong reactivity also in their islet beta cells (unpublished results).

Many MAbs demand frozen sections for their use in immunohistochemistry and therefore are of limited use. The method by which the MAb 4A5 was selected resulted in an antibody which reacts strongly with beta cells in pancreatic tissues that have been

processed for routine diagnostic purposes. This processing includes formalin fixation and paraffin embedding. The insensitivity to fixation and embedding in combination with the avidity of the antibody gives it a great potential for use in immunohistochemical studies as shown in the present paper.

The pattern of reactivity of islet beta cells and islet amyloid with rabbit polyclonal antiserum A116 to human IAPP and with anti-insulin antiserum has been described earlier [15]. Antiserum A116 shows the same reactivity with human islet tissue as antiserum A110, used in this study. Shortly, while normal beta cells label strongly with different polyclonal anti IAPP antisera, beta cells in islets which contain amyloid deposits lack or have very low IAPP immunoreactivity in spite of the presence of IAPP mRNA. This immunoreactivity pattern was seen also in the present study when we used the polyclonal antiserum

Table 3. Immunohistochemical reaction of islet beta cells and amyloid with monoclonal and polyclonal antibodies to IAPP in pancreatic tissue of non-diabetic individuals

Patient no.	Age (years)	Sex	Percentage of islets with amyloid	MAb reacting with		PAb reacting with	
				Beta-cell	amyloid	Beta-cell	amyloid
541	76	Male	15	+[a]	NT[b]	+	NT
903	67	Female	22	2+	−	2+	+
1069	80	Female	0	2+		2+	
1049	81	Male	9	2+	−	2+[c]	+
1029	78	Male	0	2+		2+	
1061	69	Male	6	2+	NT	2+	NT
608	71	Male	90	+	−	+[c]	+

[a] Semiquantitative estimation of the strength of the immunohistochemical labelling of beta-cells (−; no labelling, +; weak to moderate labelling, 2+; strong labelling

[b] NT; not tested due to small amount of amyloid, difficult to identify in immunolabelling
[c] Only scattered IAPP-positive cells in islets with amyloid

A110. The reactivity with antiserum to insulin is almost identical in beta cells of individuals with and without NIDDM [15, 24]. Islet amyloid is strongly IAPP immunoreactive (Fig. 6) but does not bind antibodies to insulin [24].

Several studies have shown that expression, storage and secretion of IAPP and insulin are normally parallel and at a fixed molar ratio [25, 26]. However, under certain pathophysiological conditions, a dissociation of the expression of the polypeptides may take place [27, 28]. The findings that islet beta cells in association with islet amyloid deposits lack or have weak IAPP immunoreactivity [24] in spite of retained insulin immunoreactivity and presence of IAPP mRNA [15], have never been explained but was believed to reflect increased secretion and diminished intracellular storage of IAPP. The strong immunoreactivity that MAb 4A5 showed with beta cells also in islets with a large amount of IAPP-amyloid was therefore unexpected but indicates that IAPP is synthesized and stored in these beta cells but probably in an abnormal form.

Of considerable interest was also the finding that the most concentrated form of IAPP, islet amyloid, did not react with MAb 4A5. These findings cannot be explained with certainty at present but several possibilities exist. Formation of amyloid fibrils from a native protein is believed to take part as an off-pathway aggregation by an intermediate at the folding-unfolding pathway [29, 30]. Although most of the three-dimensional structure of the folded protein is present, there are some discrete differences in the tertiary structure between the native protein and the protein in the amyloid fibril. A loss of a normally present antigenic epitope may therefore occur in the amyloid fibrillogenesis and could explain the lack of immunoreactivity of MAb 4A5 with IAPP in its amyloid form. The loss of immunoreactivity of the polyclonal antisera to IAPP with islet beta cells close to amyloid deposits is more difficult to explain with this hypothesis.

An alternative explanation to the altered immunoreactivity of IAPP in association with the amyloidogenesis is a modification of IAPP, yet to be shown. In addition to the main fibril protein, other components are present in all forms of amyloid. These components include the amyloid P-component, which is a glycoprotein, and proteoglycans, especially heparan sulphate proteoglycan (HSPG). HSPG is present in islet amyloid [31] and the amyloidogenesis may take place in close association with the basement membrane [32]. Non-enzymatic glycation has been implicated in the pathogenesis of Aβ-amyloid in Alzheimer's disease [33] and advanced glycation end products have been demonstrated immunohistochemically in islet amyloid in NIDDM (Bucala et al., unpublished data). Other post-translational modifications found in some amyloid materials include isomerization and racemization [34]. All these additional compounds and modifications which may be of great importance in the fibrillogenesis could also alter the antigenic properties of the IAPP molecule. Obviously further studies of the process of amyloid formation in the islets of Langerhans are highly indicated.

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