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Review

Islet amyloid and type 2 diabetes: from molecular misfolding to islet pathophysiology

Emma T.A.S. Jaikaran, Anne Clark *

Diabetes Research Laboratories, Oxford Centre for Diabetes, Endocrinology and Metabolism, Radcliffe Infirmary, Woodstock Road, Oxford, UK

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Abstract

Islet amyloid polypeptide (IAPP, amylin) is secreted from pancreatic islet β-cells and converted to amyloid deposits in type 2 diabetes. Conversion from soluble monomer, IAPP 1–37, to β-sheet fibrils involves changes in the molecular conformation, cellular biochemistry and diabetes-related factors. In addition to the recognised amyloidogenic region, human IAPP (hIAPP) 20–29, the peptides human or rat IAPP 30–37 and 8–20, assume β-conformation and form fibrils. These three amyloidogenic regions of hIAPP can be modelled as a folding intermediate with an intramolecular β-sheet. A hypothesis is proposed for co-secretion of proIAPP with proinsulin in diabetes and formation of a 'nidus' adjacent to islet capillaries for subsequent accumulation of secreted IAPP to form the deposit. Although intracellular fibrils have been identified in experimental systems, extracellular deposition predominates in animal models and man. Extensive fibril accumulations replace islet cells. The molecular species of IAPP that is cytotoxic remains controversial. However, since fibrils form invaginations in cell membranes, small non-toxic IAPP fibrillar or amorphous accumulations could affect β-cell stimulus-secretion coupling. The level of production of hIAPP is important but not a primary factor in islet amyloidosis; there is little evidence for inappropriate IAPP hypersecretion in type 2 diabetes and amyloid formation is generated in transgenic mice overexpressing the gene for human IAPP only against a background of obesity. Animal models of islet amyloidosis suggest that diabetes is induced by the deposits whereas in man, fibril formation appears to result from diabetes-associated islet dysfunction. Islet secretory failure results from progressive amyloidosis which provides a target for new therapeutic interventions.

Keywords: Amyloid; Diabetes; Islet amyloid polypeptide; Islet; Fibril

1. Introduction

The first description of amyloid deposits in pancreatic islets of a diabetic subject were made more than 100 years ago [1]. This is one of the most common pathological features of diabetes, which affects more than 150 million individuals worldwide [2]. The causal factors for the many pathophysiological factors that contribute to type 2 diabetes are largely unknown; these include both the relationship of islet amyloid to the disease processes in diabetes as well as the causative factors for conversion of the component peptide to insoluble fibrils. Improved understanding of pancreatic islet function in diabetes and development of biophysical methods to investigate protein folding processes have resulted in new ap-

* Corresponding author. Fax: +44-1865-72-38-84.
E-mail address: anne.clark@drl.ox.ac.uk (A. Clark).

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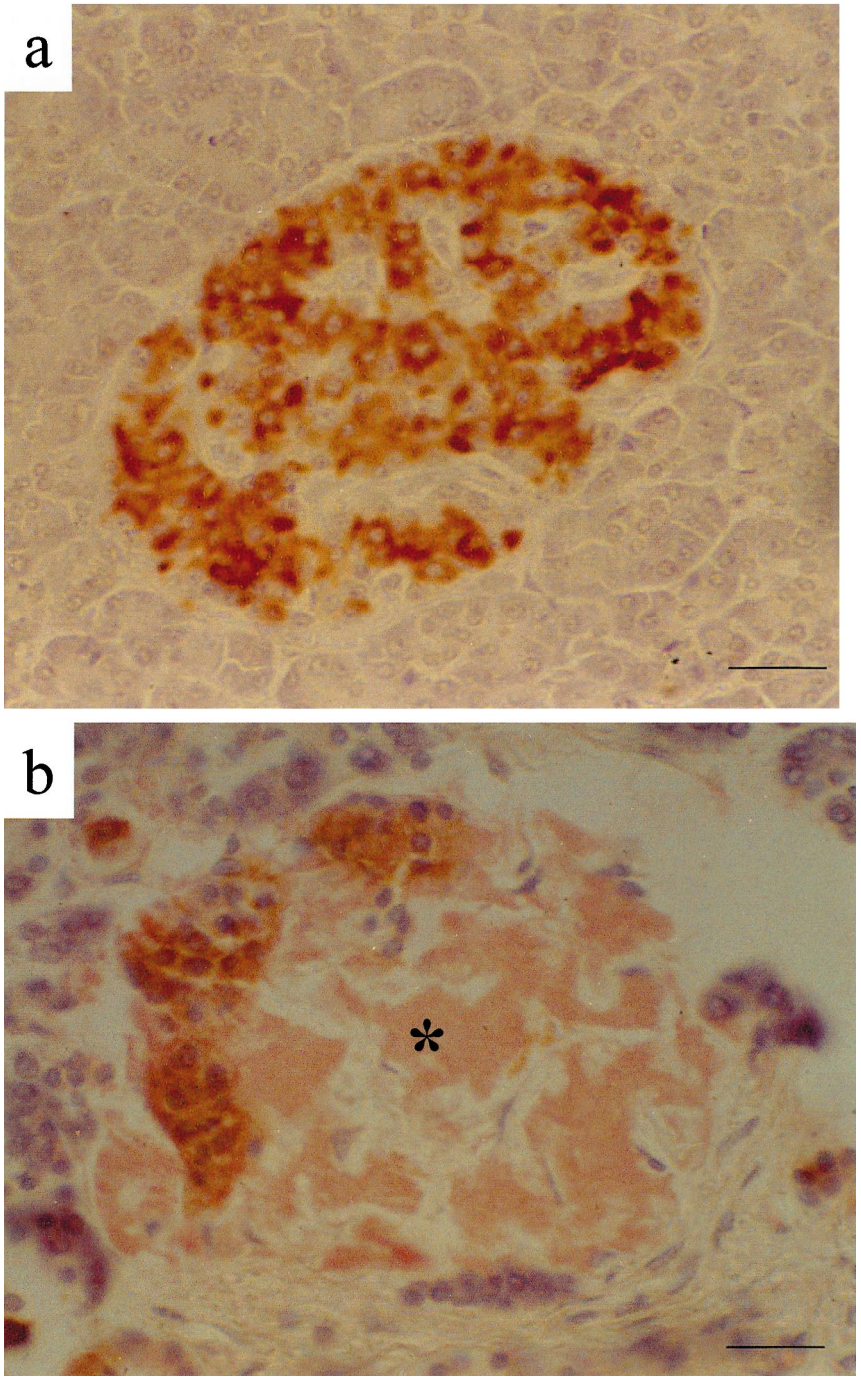


Fig. 1. Pancreatic islet structure in non-diabetic and type 2 diabetic subjects. (a) Islet of a non-diabetic subject immunolabelled for insulin (brown); insulin-containing β-cells occupy more than 80% of the islet space. (b) Islet of a diabetic subject labelled for insulin (brown) and stained with congo red for amyloid (pink); more than 50% of the islet space is filled with congo red stained, amorphous amyloid deposit (asterisk). The remaining β-cells are localised at the edges of the deposits. (a,b) Scale bar = 20 μm.

proaches to the problem of how amyloid forms and how it affects islet function.

Islet amyloid fibrils are formed from islet amyloid polypeptide (IAPP), also known as 'amylin', which is a 37 amino acid peptide co-secreted with insulin from the β-cells of pancreatic islets in both diabetic and non-affected subjects [3–6]. Under normal circumstances, IAPP is released into the circulation and is

excreted via the kidney [7,8]. The molecular mechanisms that result in refolding of the peptide to convert it from a normally soluble monomer into insoluble fibrils have not been identified. Amyloid fibrils are formed from misfolded IAPP and are deposited in pancreatic islets adjacent to the islet cells (Fig. 1); islet amyloidosis can affect less than 1% or up to 80% of islets in a diabetic individual [9–12]. This localised pancreatic amyloid deposition differs from systemic amyloidoses where the fibrils are formed from other peptides including serum amyloid A and IgGs, and are deposited in many organs. The occurrence of islet amyloid in non-diabetic subjects is low, less than 15% in elderly, apparently non-diabetic individuals, but is high in more than 90% of diabetic subjects at post-mortem [4,10,13]. Although small changes in the amino acid sequence of IAPP play a role in the species specificity of diabetes-associated amyloid formation, there is no evidence for production of a mutated form of IAPP in the majority of diabetic patients [14,15]. This suggests that disease-associated changes in the islet and in production of IAPP play a role in fibrillogenesis in diabetes.

The relationship between the complex pathophysiology of the diabetic syndrome in man and islet amyloid formation remains unclear; severe islet amyloidosis is correlated with the need for insulin replacement therapy and loss of islet β-cells [10,12,13]. Experiments in vitro have demonstrated that synthetic amyloid fibrils are cytotoxic [16–19]. Similar cytotoxic effects have been seen with fibrils formed from synthetic Aβ (the Alzheimer's disease amyloid peptide) [19–21]. The mechanism of fibril-induced toxicity in Alzheimer's disease (AD) and diabetes involves interaction between the cell membrane and misfolded proteins but the toxic form of the protein has yet to be determined.

This review discusses the process of amyloid formation from the misfolding of the IAPP molecule to the effects of fibril formation on organ function in diabetes in animal models and man. Following a brief description of some of the relevant aspects of the pathophysiology of type 2 diabetes, the structure of the IAPP molecule is discussed in relation to molecular events that could occur in the refolding of IAPP and its conversion to fibrils. The potential changes in the biochemistry of islet function in diabetes are related to misfolding of IAPP. The feedback of amyloid on biochemical processes and islet secretion is examined. Animal models of IAPP amyloidosis including transgenic mice expressing the human IAPP gene are discussed in relation to the course of the diabetic syndrome in man. A diagrammatic plan of how these processes interact is presented to highlight the relationship between islet function and misfolding of human IAPP (hIAPP) in type 2 diabetes.

2. Type 2 (non-insulin-dependent) diabetes and amyloid

There are two major types of diabetes in man, both of which result in a disturbance of the normally tight control of glucose homeostasis effected by insulin secreted from pancreatic islets. Type 1 (insulin-dependent) diabetes develops in individuals mostly under the age of 30 years and is an autoimmune disease resulting in destruction of insulin-secreting cells and the requirement for insulin replacement therapy. Type 2 diabetes develops in individuals mostly over the age of 40 years [22] and is a multifactorial disease with genetic and environmental components; it is characterised by a progressive deterioration in the regulation of blood glucose concentrations usually associated with obesity, decreased physical activity and increased calorie intake. Recently the incidence of type 2 diabetes has increased in both younger individuals as well as in the older age group; the increase of affected individuals under the age of 20 is attributed to similar factors [23]. Amyloid is formed only in type 2 diabetic subjects since destruction of the islet β-cells in type 1 diabetes removes the source of IAPP. Therefore, this review is restricted to features of type 2 diabetes.

Type 2 diabetes is characterised by a disruption of the secretion of insulin from islet β-cells and decreased insulin sensitivity of peripheral tissues (increased insulin resistance) [24–26]. These two pathophysiological features are present to varying degrees in different patients. Increased circulating insulin concentrations are a marker for the decreased insulin sensitivity occurring in obesity whereas β-cell dysfunction results in lack of appropriate insulin secretion in relation to the ambient glucose concentrations. At diagnosis, many patients can be treated

with dietary advice. If this is ineffective, insulin secretion can be augmented by sulphonylurea drugs, which stimulate β-cell secretion. When this fails during the progression of the disease, insulin replacement therapy in the form of injections is required. This usually marks the final stages of the syndrome when endogenous insulin secretion is severely compromised. Although amyloid deposition is a recognised feature of the islets in type 2 diabetic subjects at post-mortem, it is unclear if the deposits are a causal factor for any aspect of the disease, or develop as a result of the disease, and whether they affect the progressive deterioration of insulin secretion and by what mechanism.

The highest prevalence of type 2 diabetes is found in indigenous populations that have become 'Westernised'. These include the Pima Indians of Arizona [27] where the prevalence is greater than 50% in age groups over 35 years and amyloid is present in diabetic subjects [28], the Australian aboriginal peoples [29] and the Nauruans of the Pacific Islands [30]. Type 2 diabetes has a familial association but although many candidate genes have been proposed, clear associations with mutant genes have been identified in only a small percentage of individuals affected by the disease [31].

3. What molecular features of IAPP are involved in transition from monomer to fibril?

3.1. Amyloidogenic regions of the IAPP molecule

Observations of the process of folding of human IAPP and amyloid fibril formation in vitro have been complicated by the fact that most synthetic IAPP preparations contain fibrils before investigations commence, making it very difficult to define a soluble 'native state' of this peptide. Furthermore, in vitro observations are confounded by differences in the method of peptide preparation, pH of the experiments, the use of concentrated stock solutions which may contain fibrils and the vast range of concentrations and solvents used in different laboratories. In the presence of helix-inducing organic solvents, such as 2,2,2-trifluoroethanol (TFE) or 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), the circular dichroic (CD) spectrum of hIAPP suggests an α-helical structure [32,33]. These solvents, together with DMSO, have been used extensively to examine the conformation of IAPP and the amyloidogenic Aβ peptide [34,35] but do not permit extrapolation to in vivo conditions where the three-dimensional structure will be different. Recent careful studies of purified peptide have shown that a 'native state' of human IAPP can be predicted; in the absence of organic solvents and with the elimination of potential 'seeds' by filtration, the CD spectra of hIAPP solubilised in water remained stable for many days. The peptide exhibited a random coil structure suggesting an unfolded state in aqueous medium [36,37]. However, the state in which hIAPP is found in the cell and circulation is still unknown.

IAPP is expressed in pancreatic islets of all mammalian species so far examined but the potential to form amyloid is related to species-specific differences in the amino acid sequence (Fig. 2). Diabetes-associated amyloid is found in primates [38,39] and cats [40,41] but not in rodents. This has been attributed to alterations in the amino acid sequence in the region IAPP 20–29 [42,43]: proline substitutions in this region in rodents and some other species are thought to be the major factors preventing refolding of the molecule into β-conformation and assembly into typical amyloid fibrils [43,44].

Until recently, the single amyloidogenic region, spanning residues 20–29, had been proposed to be involved in fibril formation of hIAPP, [43,45–47]. This β-strand peptide fragment has been used to model the formation of intermolecular interactions and β-sheet formation of oligomers of hIAPP and was considered to be the only β-sheet region of the peptide. However, recent studies have identified a second amyloidogenic region, hIAPP 30–37, which forms amyloid-like fibrils in aqueous medium [48]. In addition, a peptide fragment of IAPP consisting of residues 8–20 has been shown to form fibrils [49]. These two additional regions of potential β-conformation have important consequences for folding not only of human but also of rodent IAPP and of IAPP in all other species where the sequence is largely conserved. The region IAPP 8–20 of rodent IAPP contains a single amino acid substitution, His18Arg (Fig. 2), which has been shown to have no affect on formation of fibrils from rat IAPP 8–20. This lack of effect of histidine as a major determinant of fibril

formation is in contrast to the important role of His in fibril formation from the amyloidogenic peptide Aβ [50]. At the C-terminus of the molecule, rat IAPP 30–37 and hIAPP 30–37 are completely homologous and form fibrils. Thus, in the rodent molecule, two potentially amyloidogenic domains exist [49] but are separated by the proline rich domain, 20–29, which prevents β-sheet formation. These recently acquired data indicate that there are three molecular domains of the human IAPP sequence which could have a role in the assembly of β-sheets, namely amino acid regions 8–20, 20–29, and 30–37. With the presence of more than one potential β-strand region, interactions *within a monomer* as well as *between molecules* could occur during β-sheet formation. Therefore, the folding processes involved in fibril formation via the creation of unfolded intermediates, as has been proposed for other amyloidogenic proteins [51,52], are likely to be more complex than those envisaged for molecular association via a single strand involving only the region IAPP 20–29 [47].

3.2. Modelling hIAPP folding Intermediates

The intermediate molecular forms of hIAPP created during fibrillogenesis can be modelled in various conformations dependent upon the number of β-sheet components present in a native monomer or folding intermediate. A β-turn has been predicted at asparagine 31 [36], which would result in two adjacent β-strands, hIAPP 32–37 and 24–29, creating an antiparallel β-sheet. The third β-strand contained in the fragment 8–20 could extend this sheet with a turn in the region 18–23 (predicted at serine 20) [36]. A computer model incorporating three strands, 8–20, 20–29 and 30–37, with two turns predicts a structure which could form an intramolecular β-sheet (Fig. 3a). The first turn between amino acids 17 and 19 is depicted as a loop rather than a tight turn. Residues Asp22, Gly24, Ile26, Leu27 and Ser28 have been shown to be important for fibril formation from proline substitution experiments [43,44]. These residues would lie in the second strand region and be localised on the outer edge of the β-sheet where they would be involved in interactions between molecules or protofilaments necessary for assembly of two or more protofilaments into fibrils. The β-sheet would be disrupted in rat IAPP by the proline substitutions in the region 24–27, which would prevent both intra- and intermolecular H-bonding. Residue 28 is in the second turn and a proline at 28 affects the folding and conversion to fibrils as has been demonstrated by substitution experiments [43,44]. The mutation Ser20Gly, found in small numbers of Japanese type 2 diabetic subjects [53,54], is thought to accelerate formation of fibrils from hIAPP in vitro [55]; in the proposed model, the S20G substitution would result in a tighter angle on the loop due to the smaller size of glycine. However, the mutation is relatively uncommon in type 2 diabetes [56] and not all individuals with this mutation develop severe insulin-requiring diabetes which would be associated with extensive amyloid deposition [54]. It is, therefore, difficult to reconcile the biophysical data with diabetic pathophysiology.

It is unlikely that the disulphide bridge between residues 2 and 7 would allow the N-terminal portion of hIAPP to adopt a β-sheet conformation: in the

```
                          1       8          18          29      37
Human   TPIES:::HQVEKR  KCNTATCATQRLANFLVHSSNNFGAILSSTNVGSNTY  GKRNAVEVLKREPLNYLPL
Monkey  -----:::------  ----------------R------T---------D--  ------------------
Cat     -----:::N-----  ----------------IR----L-----P--------  ---ST-DI-N-------F
Dog     ---K-:::N-----  ----------------RT---L-----P--------  ----TI-I-N-G------
Rat     --VG-GTNP--D--  ----------------R----L-PV-PP--------  ----VA-DPN- S-DF-L-
Mouse   --VR-GSNP-MD--  ----------------R----L-PV-PP--------  -----AGDPN- S-DF-KV
Hamster--VR-GTN--MD--  ------------------N- L-PV-P----------  ---S-A-IPDGDS-DLFL-
G-pig   -S-A-DTG---G--  ----------T-----R--H-L- A-LP-D-------  -----PQISD- LCH----
Degu    ---A-DTD-R-D--  ----------T-----R- -H-L-A-PP-K-------  -R---:Q-VDV-L-H----
Rabbit                          --------I---------F-PPS
Hare                            --------I---------F-PP-
Cougar                  -*----*---------IRSS*------*
Pig                       -M------H-----DR-R-L:-T-F-P-K---
```

Fig. 2. ProIAPP amino acid sequences in different species indicated by single letter code. Three residues are missing (:) in human, monkey, cat and dog when aligned with other sequences. Porcine IAPP is missing one amino acid. Amino acids identical to those in human proIAPP are indicated by a dash (–). Asterisks (*) represent amino acids not yet determined. Proline residues are present in the region IAPP 20–29 in rat and mouse IAPP which prevent folding to form fibrils. G-pig, guinea pig.

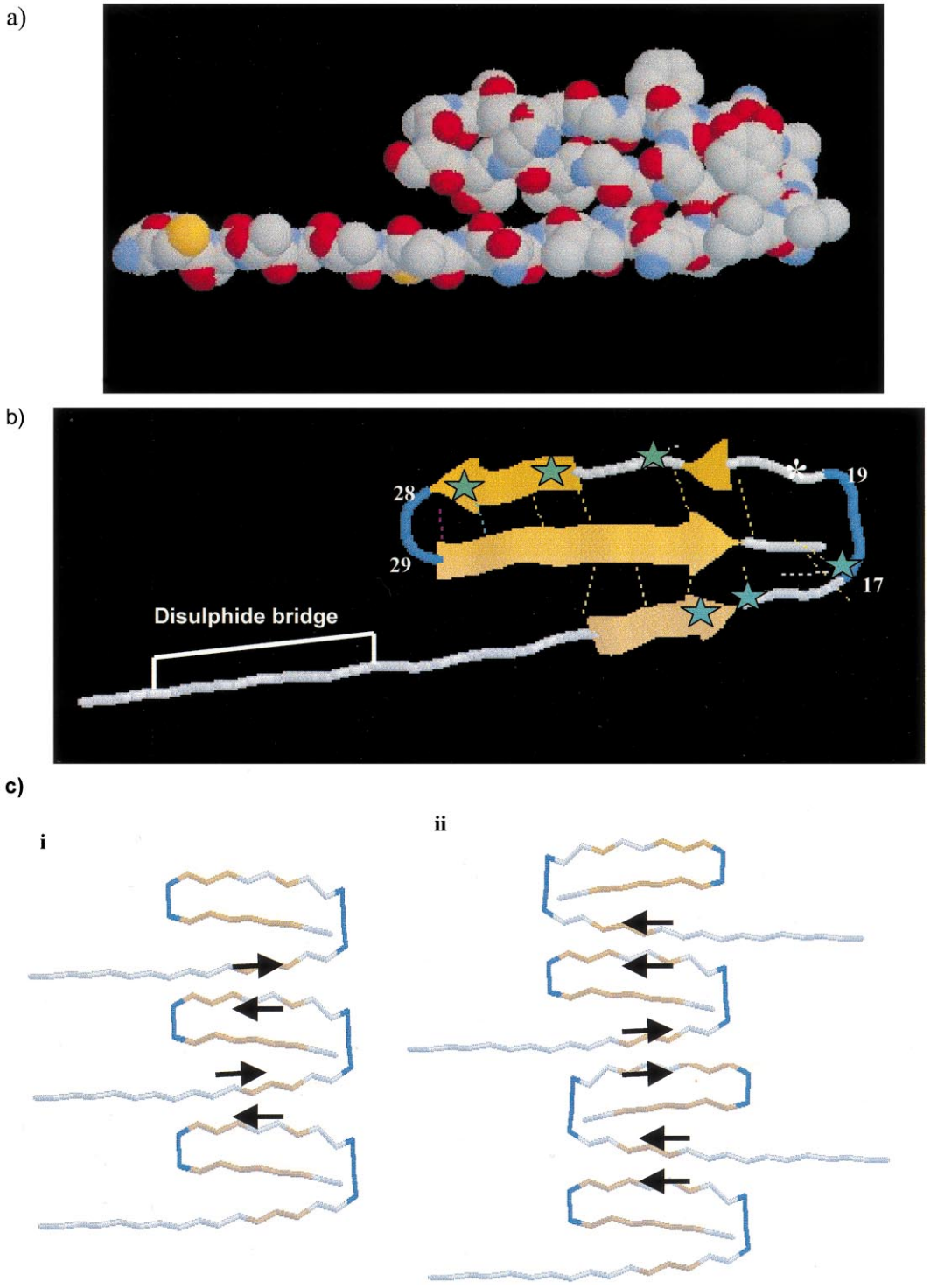


Fig. 3. Models of human IAPP folding intermediates and assemblies. (a) A hypothetical model of a folded β-sheet monomer of hIAPP represented in a space filling diagram. (b) Linear diagram of the model. In (b) regions of β-strand are shown in yellow, turns are shown in blue. Hydrogen bonds are shown with dotted lines. Hydrophobic residues (green stars) are involved in hydrogen bonding between the backbones of the β-strands. Serine 20, which is substituted with a glycine in a small subset of type 2 diabetic subjects, is labelled with an asterisk. The model was generated using Insight II. (c) Possible conformation of stacked hIAPP molecules within a protofilament. (i) Parallel stacking of molecules with parallel and antiparallel β-strands. (ii) Antiparallel stacking of peptide molecules with parallel interaction between molecules. β-Strands are shown in yellow and turns in blue. Direction of β-strands is marked with arrows.

model, the N-terminal region, including the intact cysteine bridge, would most likely lie outside the boundaries of the β-sheet in a similar way to that predicted for non-β-sheet regions of larger amyloidogenic proteins [51,52]. The disulphide bridge is conserved in all species and is believed to be necessary for effective biological activity of IAPP [57]. However, it is unclear if this bridge is intact in the molecular species that forms amyloid deposits since synthetic IAPP without the bridge forms fibrils in vitro.

IAPP fibril formation was found to be independent of pH in the physiological range (5.5–7.4) [49] and therefore it is unlikely that the charge on histidine 18 is a major determining factor for fibril assembly. During fibril growth, and as the protofilament extends in length, intermolecular interactions would occur due to the addition of monomers, protofibrils or small oligomers. Parallel and antiparallel orientations of peptide monomers are possible in this model (Fig. 3b). A parallel arrangement of molecules would result in antiparallel intermolecular interactions. An antiparallel arrangement of monomers results in parallel intermolecular β-strands. Determining the orientation of molecules by identification of residues or charges exposed on the surface of the native and synthetic protofilaments will indicate how the protofilament is assembled and provide clues for the hIAPP misfolding process.

3.3. Fibril assembly from protofilaments

The folding characteristics of the proposed model would determine the width of the protofibril and protofilament. The width of the IAPP molecule (the strand between residues 17 and 28 in the model) (Fig. 3a) would be predicted to be ∼3 nm. A single β-sheet monomer aligned perpendicular to the protofilament axis would result in a width of protofibril/protofilament of 3–4 nm which is consistent with the 5 nm measured diameter of synthetic IAPP protofibrils and protofilaments observed by atomic force microscopy (AFM) and electron microscopy (EM) (Fig. 4a). Protofilaments formed from the similarly sized Aβ 1–40 and from IAPP 25–35 have been shown to have a width of ∼3 nm [58]. Fibril formation incorporating between three and six protofilaments of IAPP would therefore result in a fibril diameter of 9–18 nm which agrees with measured diameters of fibrils formed from synthetic hIAPP in vitro and from biosynthetic hIAPP in islets in vivo and in vitro (Fig. 4b) [59]. Fibrils formed from hIAPP 8–37 have been shown to differ from fibrils formed from full length IAPP 1–37 in that they appear to be composed of two rather than three protofilaments [59]. Therefore, rather than participating in the actual fibril the N-terminal region may be a modulating influence on the interactions between protofilaments.

Assembly of the protofilaments into fibrils is likely to be dependent upon interaction of side groups exposed on the surface of the protofilament. As an example, histidine residues are involved in Aβ amyloid fibril assembly affecting lateral association of protofilaments [50,60] possibly via the protonation state of Aβ His13 which is a binding site for zinc [61–64]. Although a role of zinc in IAPP fibril formation is attractive since zinc concentration is high in the β-cell secretory granule [65], the absence of His18 in rat IAPP does not affect fibril formation from rat 8–20. It is more likely that assembly of protofilaments into fibrils is dependent upon many different features of the environment (salt and ionic concentrations), extracellular proteins, the concentration of IAPP and interactions with other factors such as heparan sulphate proteoglycans (HSPG). Observations on ex vivo IAPP fibrils extracted from a pancreas of a diabetic patient (Fig. 4c) suggest that fibrils formed in vivo in diabetes in man are relatively uniform in morphology, approx. 10 nm wide, short, straight and without the twisted and ribbon-like assemblies seen with synthetic IAPP fibrils (Fig. 4a). Experimental conditions that simulate the in vivo situation would be more appropriate to determine how fibrils are assembled.

4. What features of the diabetic islet environment could affect IAPP peptide misfolding?

4.1. ProIAPP and aberrant proteolytic processing in diabetes

Very little is understood about the cause of defective insulin secretion in type 2 diabetes despite considerable laboratory and clinical investigations into the effects of elevated glucose (glucose toxicity), lip-

ids (lipotoxicity) and potential genetic defects in the machinery of stimulus-secretion coupling in the islet β-cell (reviewed in [25]). However, some specific biochemical abnormalities have been identified and are used as markers of abnormal islet activity in diabetic patients. Increased secretion of the precursor of insulin, proinsulin, together with intermediates of proinsulin proteolysis indicate β-cell dysfunction [66,67]. Proinsulin and proIAPP are processed by the same prohormone convertase enzymes, PC1 and PC2, and by carboxypeptidase E during their passage through the Golgi and are packaged into maturing secretory granules [68–70]. Therefore, proIAPP processing could be affected by the same unknown β-cell pathology which affects proinsulin proteolysis; there would therefore be concurrent hypersecretion of precursors of both insulin and IAPP in diabetes. In support of this hypothesis, mice lacking the gene for PC2 secrete incompletely processed proIAPP [71] and proinsulin and proIAPP processing intermediates have been identified in extracts of human islets in culture [72].

ProIAPP has been shown to have amyloidogenic characteristics when examined both as a recombinant and synthetic peptide [73,74]. The process of fibril formation from this peptide appears to be less aggressive than that observed for IAPP 1–37 [73]. However, if proIAPP were a causal factor for islet amyloid deposition, proIAPP or processing intermediates would be expected to be present as components of the deposits. Extraction studies indicated that IAPP amyloid in both insulinomas and pancreatic material of diabetic subjects and cats was formed from IAPP 1–37 and not a larger peptide [3,4]. However, if the process of fibril assembly is initiated by a 'seeding' process [75], secretion and aggregation of incompletely processed proIAPP fragments could act as the 'seed' or 'nidus' for subsequent deposition of IAPP. In diabetes, the most frequently secreted processing intermediate of proinsulin is des 31,32 proinsulin, resulting from incomplete cleavage by PC2 at the C-peptide/A chain junction of proinsulin [66,70]. Mice lacking the gene for PC2 have elevated levels of the N-terminal intact proIAPP intermediate [71] and the N-terminal processing intermediate has been identified in extracts of human islets cultured in elevated glucose concentrations [72]. It would be expected, therefore, that this fragment with the N-terminus intact would be the processing fragment, which, together with intact proIAPP, might be associated with hyperproinsulinaemia of diabetes. This fragment might therefore be localised in some amyloid deposits. Immunoreactivity for the N-terminal region of proIAPP has been identified in some amyloid deposits [76,77] supporting this hypothesis.

Recent studies on proIAPP fragments have shown that a binding site for heparan sulphate spans the N-terminal cleavage site of proIAPP [78]. If proIAPP is incompletely cleaved at the N-terminal site, it would be secreted from the cell in this form. In the extracellular islet space this intermediate could bind to HSPG on the basement membrane. HSPG, together with other proteoglycans, has been proposed as a co-factor for fibril assembly [79–81]. These observations taken together suggest that increased secretion of proIAPP processing intermediates in diabetes could accumulate adjacent to the islet capillary and form the 'nucleus' or 'seed' for misfolding and assembly of secreted hIAPP. This would result in initiation of fibrillogenesis close to the capillary border, which is where many very small deposits are located in vivo. This hypothesis requires development of assay methods to determine if proIAPP and processing intermediates are co-secreted with proinsulin in diabetes and to determine if HSPG is a co-factor for accumulation. Binding studies with synthetic peptides and model building to determine if HSPG will interfere with subsequent protofilament assembly of IAPP would be required to confirm this suggestion.

4.2. Glycation and islet amyloid in diabetes

Increased glycation of proteins is characteristic of the hyperglycaemia of diabetes resulting in increased fragility of red cell membranes, increased glycated haemoglobin, and tissue damage as a result of formation of advanced glycation end products (AGE) [82]. IAPP glycation has been proposed as an important factor in generation of fibrils in diabetes [83]. However, IAPP amyloid is deposited also in human, feline and canine insulinomas when glucose concentrations are low and glycation is minimal; this indicates that formation of AGE products are not essential for IAPP fibrillogenesis. AGE conversion is associated with proteins with a long half-life such

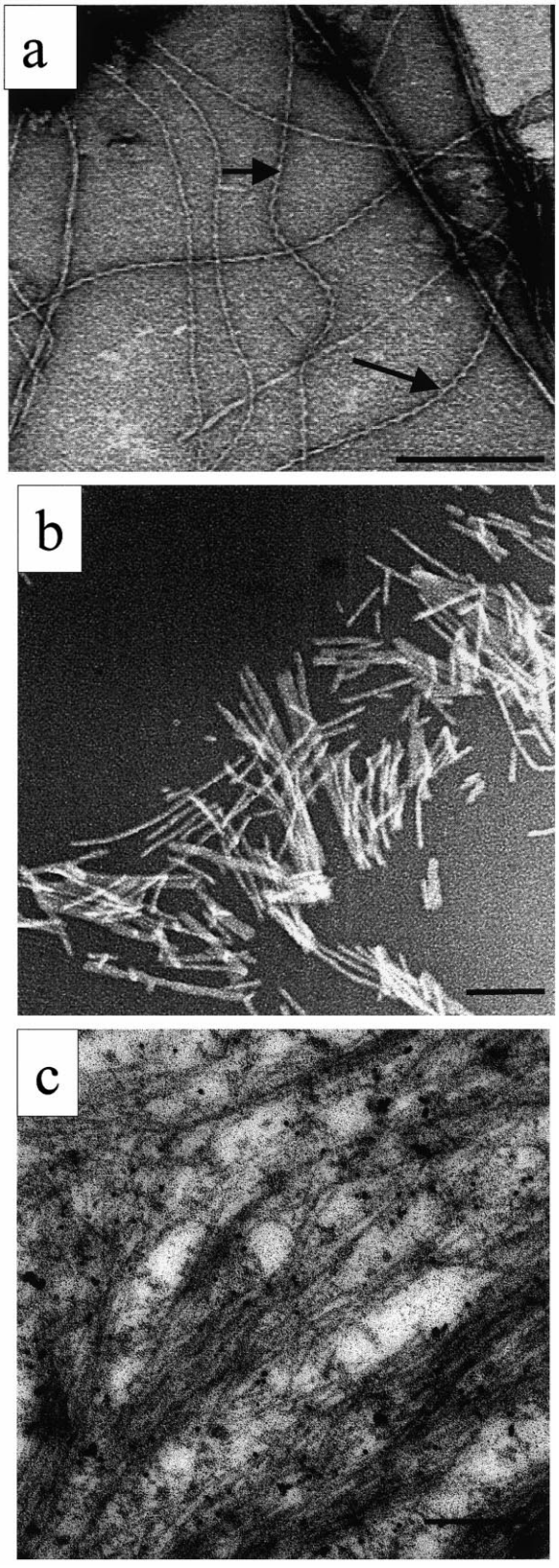


Fig. 4. Fibrils formed from synthetic and biosynthetic human IAPP. (a) Synthetic hIAPP visualised with EM following negative staining with uranyl acetate. Fibrils in the form of twisted protofilaments (arrow) and ribbons were formed when synthetic human IAPP was solubilised in sodium acetate. Scale bar = 200 nm. (b) Fibrils formed in vivo in the pancreas of a diabetic patient. The fibril-containing extract was visualised with platinum carbon shadowing and EM. The fibrils were relatively short and uniform in width (approx. 6 nm) and morphology; there was no evidence of complex twisting or sheet formation. Scale bar = 200 nm. (c) Electron micrograph of amyloid fibrils in an islet section of a diabetic monkey examined with EM; fibrils which were relatively straight and not in the form of sheets were immunolabelled for IAPP with 10 nm gold. Scale bar = 200 nm.
←

as collagen and haemoglobin. Monomeric secreted IAPP has a half-life comparable with C-peptide of 30–40 min [7,84]. Therefore, glycation of the monomeric peptide is unlikely. However, once IAPP is deposited as amyloid, the fibrils would be targets for glycation and sites for AGE conversion in hyperglycaemia of diabetes.

4.3. *Additional components of islet amyloid and diabetes*

Amyloid deposits contain many components in addition to the protein fibrils. Serum amyloid P component (SAP) is found in all amyloid deposits including islet amyloid [85] and has been proposed to protect amyloid from degradation rather than promote fibrillogenesis [86]. Acute induction of splenic AA amyloidosis in SAP deficient mice has been shown to be retarded and the amyloid cleared more effectively after the induction compared to that seen in normal mice; this suggests that SAP has a role in shielding amyloid from degradation [86]. SAP binding to amyloid deposits is calcium dependent and the pancreas is high in calcium which accumulates as insoluble aggregates both in pancreatic ducts in pancreatitis and in amyloid deposits [87]. Thus, SAP could have an important role in protecting pancreatic islet deposits from removal and degradation.

Apolipoprotein (Apo) E is also a component of the deposits [88] and has been identified in islet cells [89]. The commonly occurring isoform, ApoE ε4 has been implicated in plaque formation in AD [90]. ApoE has an important role in disorders of lipid

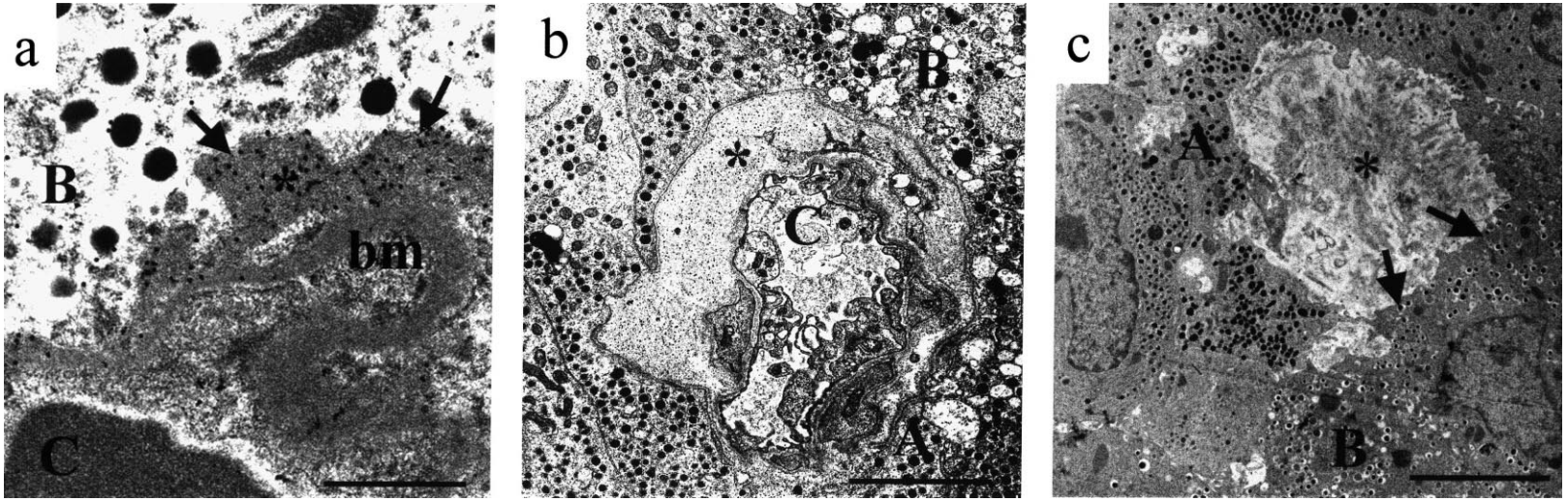


Fig. 5. Perivascular and extracellular amyloid in islets of (a) human, (b) primate and (c) hIAPP transgenic mouse. (a) Small amyloid accumulation (arrows) adjacent to basement membrane (bm) in islet from a diabetic subject. This accumulation, which may not be fibrillar, was immunogold labelled for IAPP (arrows) and would be too small to be visualised by light microscopy. (b) Perivascular amyloid deposits were immunogold labelled for IAPP in an islet from a non-diabetic monkey; the amyloid fibrils (asterisk) were located between the islet cells and the capillaries. (c) Extracellular IAPP fibrils deposited between cells of an islet isolated from a mouse expressing human IAPP and cultured in 11.1 mM glucose for 6 days. Fibrils (asterisk) form between the cells and adjacent to the plasma membrane of the β-cells (arrows). B, β-cell; A, alpha cell; C, capillary. Scale bars: (a) 1 μm; (b) 5 μm; (c) 5 μm.

metabolism [91] which are frequently associated with type 2 diabetes. However, no association has been found between ApoE genotype and the degree of islet amyloidosis in post-mortem specimens or with severity of diabetes [92]. Furthermore, islet amyloid is deposited in ApoE deficient hIAPP transgenic mice (S. Kahn, personal communication).

5. Where is the process of fibrillogenesis initiated?

5.1. Fibril formation at extracellular sites

Spontaneously developing islet amyloid deposits associated with diabetes in man, monkeys and cats are almost exclusively at extracellular sites and, in diabetic animals, are clearly visible with the light microscope within the islet envelope [9,11,39,93–95]. However, much smaller deposits adjacent to islet capillaries can be identified only with electron microscopy (Fig. 5a), suggesting that sub-pathological deposits may be present early in the disease adjacent to capillaries; as these deposits expand there could be an impact on islet function. Such pericapillary deposits are visible in islets of non-diabetic monkeys (Fig. 5b); this amorphous mass could affect nutrient signalling and transfer of secretion between the islet space and capillary. As the deposit expands due to progressive IAPP deposition, the fibril mass extends into the islet space, occupying areas that were previ-

Table 1
Concentrations of IAPP in different species in relation to islet amyloid formation

Species	ND/D m/f	Age (years)	Rx	IAPP (pmol/l)	Insulin (pmol/l)	Amyloid	Ref.
Man	ND	>40	none	7 (5.5–17)	65 (30–72)	n/a	[136]
	D	>40	diet	12.5 (3–16.5)	85 (52–132)	n/a	
	D	>40	sulph	6 (1.5–35)	107 (38–226)	n/a	
	D	>40	insulin	3 (0.5–5.5)	–	n/a	
Cat	ND	>2	–	3.1	50	0	[105]
	D Dex+GH	>2	sulph	9.8	–	Y 4/4	
	D Dex+GH	>2	insulin	2.9	–	Y 1/4	
Monkey	ND	<10	none	–	187 (201–431)	0	[39]
	ND	>10	none	–	250 (101–618)	Y 2/9	
	ND obese	>10	none	–	1206 (847–2230)	Y 4/6	
	D	>10	none	–	160 (65–345)	Y 8/8	
		Age (weeks)					
hIAPP T mice	ND	82	–	157	38	N	[101]
	ND	32	–	(63–38)	(36–183)	N	[126]
	ND	24	–	15	36	N	[127]
	ND	–	–	333	205	N	[133]
	D m/f	4 wk Rx	Dex+GH	1135	595	Y 8/30	
	D m	32	fat diet	n/a	360	Y 10/32	[130]
	ND f	32	fat diet	n/a	150	N	
	D m	16–20	homozy	85	70	Y	[96]
	ND f	16–20	homozy	130	220	N	
	D m	52	hIAPP×Y	51	213	Y 8/8	[97]
	D m	70	hIAPP×ob	600	7255	Y 5/6	[99]

Data published from non-diabetic (ND) and diabetic (D) human and animal studies are related to the treatment (Rx) by sulphonylurea (sulph), diet or insulin. Diabetes/amyloid deposition was induced in animal models by administration of dexamethasone (Dex) and growth hormone (GH), by a high fat diet, by cross-breeding to produce mice expressing hIAPP in a 'homozygous' condition (homozyg), or breeding against a background of obesity with the Agouti viable yellow mutation (Y) or the *ob*/*ob* strain (ob). Fasting concentrations of plasma IAPP and insulin are expressed in pmol/l and the occurrence of amyloid as detected by light microscopy (yes (Y) or no (N)) in different sized cohorts of animals is presented where assessed (not assessed, n/a). There is no clear association of the occurrence of amyloid with the circulating concentration of IAPP. The occurrence of amyloid in genetically determined obese hIAPP transgenic mice (8/8, 5/6) is similar to that found in man (>90%) but other mouse models show a much lower incidence of amyloid deposition (8/30, 10/32).

ously filled with endocrine cells. Progressive amyloid deposition is therefore associated with destruction/replacement of islet cells in both man and animal models [39,96,97].

Islet amyloid formation in experimental animal and cellular models is largely associated with increased production of hIAPP [96,98–100] (Table 1). If increased secretion/decreased clearance of IAPP is a causal factor for fibril formation, accumulation would be expected to occur at extracellular sites within the islet domain, on the diffusion path between the cells and the capillaries (as shown in Fig. 5b). β-Cell secretagogues have been shown to increase extracellular fibril formation in isolated cultured hIAPP transgenic mouse islet preparations [100,101]; in this experimental model where the capillaries are no longer functional, fibrils formed between the islet cells (Fig. 5c) and the extent of extracellular fibril accumulation was proportional to the secretion of insulin and IAPP and independent of whether the stimulus was glucose or an amino acid. This indicates that increased secretion/decreased clearance from the islet were important factors for fibril formation. The extent of *intracellular* fibril accumulation in these transgenic mouse islets was minimal and little cell death was observed concluding that biosynthetic hIAPP forms extracellular fibrils which are not acutely (over a period of 24–48 h) toxic to islet cells.

Conversion of synthetic IAPP is accelerated both in the presence of pre-formed fibrils [37] or a 'nucleus' [102]. In addition, IAPP fibril formation can be stimulated by the presence of other amyloidogenic peptides in fibril form [103]. This suggests that, in vivo, once an extracellular 'nidus' is formed within the islet space, IAPP monomers released during the process of β-cell secretion could be converted rapidly to β-conformation at the nucleation site and assembled into fibrils. The nature of the 'nucleus' or 'nidus' in diabetes is unclear but could consist of insoluble aggregates (not necessarily in β-conformation) of proIAPP intermediates or IAPP fragments; such fragments of IAPP have been identified in extracts of human pancreas and plasma [104]. Thus, once a nucleation point had been established in the islet, even small increases in β-cell activity as occurs due to increased glucose concentrations in impaired fasting glucose (IFG), in insulin resistant individuals and in sulphonylurea therapy in diabetes, would promote fibril deposition. This has been demonstrated in a feline experimental model of diabetes (a species in which islet amyloid forms). Cats were made diabetic by removal of part of the pancreas and insulin resistance was increased by hormone treatment. At this time no amyloid was formed but these animals were then treated with either sulphonylurea to increase β-cell secretion of insulin and IAPP or insulin to reduce the requirement for endogenous secretion of IAPP (and insulin) from β-cells [105]. Following this treatment regime, islet amyloidosis was more extensive in the sulphonylurea-treated animals than those treated with insulin (Table 1): these data suggest that prolonged stimulation of β-cells by sulphonylurea therapy or sustained high glucose concentrations in diabetes could lead to more extensive islet amyloid formation and β-cell failure. This appears to be in conflict with the findings of the United Kingdom Prospective Diabetes Study, UKPDS, where treatment with diet, sulphonylurea or insulin was compared in 5000 newly diagnosed patients [106]. There was no apparent difference in the rate of decline of islet function from the time of diagnosis in patient groups randomised to insulin or sulphonylurea treatment. However, at present, there is little evidence that islet amyloidosis is a major causative factor for the onset and initial progression of the disease in man. It would be of interest to compare, in these well characterised patients, the extent of pancreatic amyloid at post-mortem with the longitudinal progression of the disease.

5.2. *Intracellular fibril formation*

Fibril-like material has been described in β-cell granules of hIAPP transgenic mice suggesting that fibrils form before secretion of the peptide [107]. However, it remains to be confirmed if these very small accumulations, which are visible only by electron microscopy, have the characteristic feature of amyloid (congo red birefringence). Intracellular IAPP fibrils are found in unidentified organelles in the cytoplasm in some human cells, notably human insulinoma cells [108,109].

Such intracellular fibrils, oriented in parallel arrays, are frequently not enclosed by a visible membrane (Fig. 6a) and are congo red positive and bi-

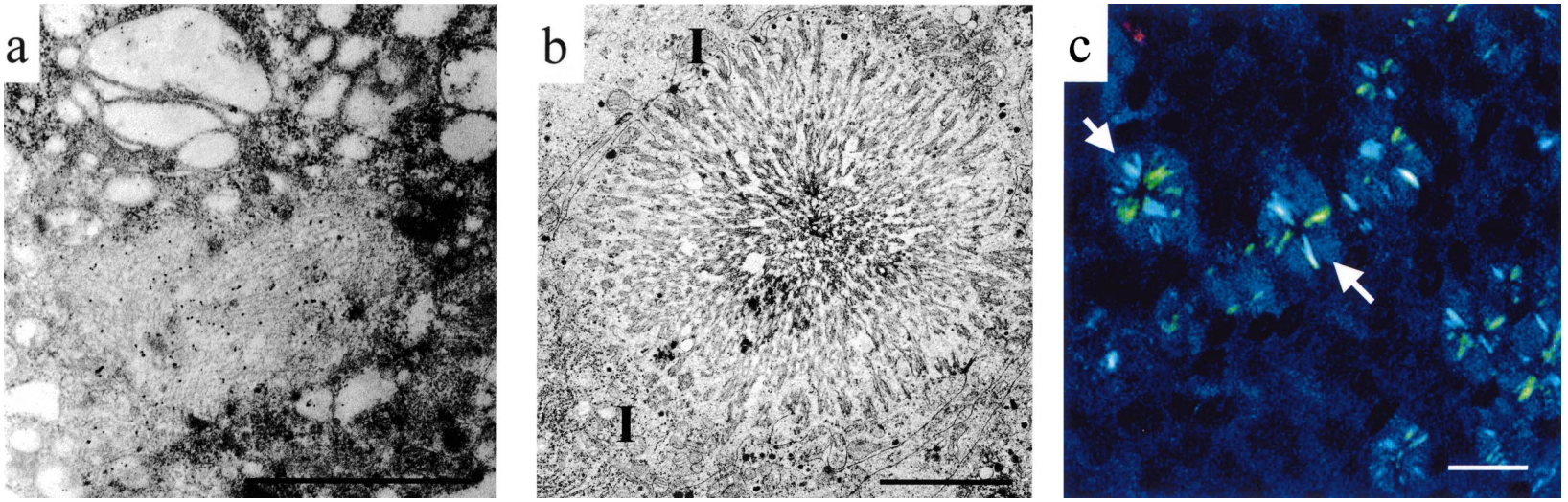


Fig. 6. Intracellular amyloid fibrils. (a) Fibrils in the cytoplasmic domain of a human insulinoma cell. There was no apparent membrane enclosing the bundle of fibrils which were largely in parallel orientation and immunoreactive for the N-terminus of proIAPP (10 nm gold) suggesting origin from the Golgi or ER organelles. (b) Starburst-like orientation of intracellular fibrils in an insulinoma cell. Fibrils were in close proximity to insulin granules and other cytoplasmic organelles. (c) Light micrograph of specimen shown in panel b. When stained with congo red, the starburst fibrils exhibited star-like green birefringence in polarised light (arrows). There was no morphological evidence suggesting apoptosis in cells containing IAPP fibrils. I, insulin granules. Scale bars: (a) 1 μm; (b) 5 μm; (c) 20 μm.

refringent in polarised light (Fig. 6b). Intracellular fibrils have been reported in hIAPP transgenic mouse islets [101], human transplanted islets [110] and in massive amyloidosis associated with diabetes in hIAPP transgenic mice treated with steroids [96]. Some intracellular fibrils show immunoreactivity for the N-terminus of proIAPP (Fig. 6c) and have been shown to be ubiquitinated [109]. These data suggest that the component peptide of intracellular fibrils is proIAPP or an intermediate and that the fibrils are located in a pre-Golgi part of the secretory pathway, possibly the endoplasmic reticulum (ER). Ubiquitin targets misfolded intracellular proteins in the ER and cytoplasm and directs them to the proteosomal degradation pathway [111]. This could be a normal pathway for disposal of misfolded IAPP intermediates before or after fibrils are formed in the ER or other cytoplasmic organelles.

Intracellular accumulation of hIAPP has been noted in COS-1 cells transfected with the gene for hIAPP [53,112]; DNA constructs were used to transcribe either rat, human wild type IAPP or the S20G mutant IAPP form. IAPP was shown to accumulate in the ER and Golgi in these transfected cells and cells expressing hIAPP showed a high degree of apoptosis. However, similar studies in other laboratories with hIAPP cDNA transfected into COS-7 cells [113] or AtT20 cells (C.E. Higham, personal communication) were unable to detect fibrils. Accumulation of transfected gene products in Golgi and ER suggests that the peptide is not being transferred efficiently through the constitutive secretory pathway present in COS cells. This may be related to aggregation of proIAPP in these organelles either in the form of fibrils or aggregates as COS cells do not possess PC1 or PC2 required to process proIAPP [68]. In a similar way, misfolded protein in the form of insoluble aggregates accumulates in the ER in human and rodent pituitary cells expressing mutant vasopressin/oxytocin [114]. These types of intracellular accumulations of misfolded/aggregated protein are likely to result in cellular dysfunction and increased cell death even if fibrils are not formed. Intracellular accumulations of IAPP-immunoreactive material (but not necessarily in the form of fibrils) have been identified in β-cell lysosomes of both diabetic and non-diabetic human subjects [115] and in hIAPP transgenic mice [101]. Lysosomal IAPP accumulations are derived from the process of crinophagy whereby secretory granule components, including insulin and IAPP are recycled within the cell via a lysosomal degradation pathway [116,117]. Degradation of human IAPP by lysosomal enzymes appears to be less efficient than that for rodent IAPP or for insulin. This high concentration of hIAPP in lysosomes is potentially a source for intracellular fibril formation during cellular disintegration resulting from apoptosis. Thus cell death in the islet, whatever the cause, will result in release of hIAPP from intracellular compartments, notably granules and lysosomes, and under appropriate conditions fibrils, could form.

Fibril formation from hIAPP is therefore possible at more than one site and more than one aberrant biochemical or physiological process can result in IAPP fibrillogenesis. Experimental modulation of IAPP production in cellular systems in vitro or in vivo will initiate one or more of these mechanisms. More than one site of production of aberrantly folded hIAPP has been demonstrated in a single cellular system of hIAPP transgenic mouse islets cultured under different conditions; IAPP was deposited as extracelluar fibrils but a small percentage of IAPP accumulated as fibrils at intracellular sites or in non-fibrillar form outside the cells [100,101]. However, the major site in the islet relevant for diabetes in man is extracellular and adjacent to islet capillaries.

6. Is islet amyloid responsible for β-cell destruction and/or dysfunction in diabetes?

Synthetic human IAPP is acutely toxic to cultured cells and islets in vitro [16,118,119]; the mechanism of this cytotoxicity is mediated by apoptosis. These effects of synthetic peptide fibrils has been attributed to interaction of the fibrillar form with cell membranes to create pores and affect normal transmembrane ionic currents [120]. Similar cytotoxic effects have been described for fibrillar synthetic Aβ peptide [18,121] but only when fibrils are formed with ageing since Aβ is not toxic when freshly prepared [19,21,122]. It has been suggested that the toxic species of the amyloidogenic peptides is not the mature fibril but an aberrantly folded form of the fibril pre-

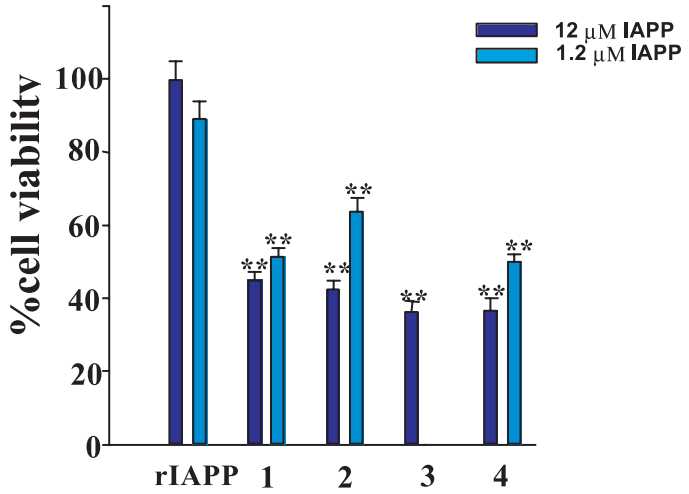


Fig. 7. Cytotoxicity of different preparations of human synthetic IAPP in vitro. The cytotoxic effects of hIAPP were examined on mouse islet βTC cells in culture and quantified by MTT reduction as a colorimetric test. Cell viability was expressed as a percentage of MTT reduction in the wells containing medium plus rat IAPP (rIAPP) which was non-toxic. Conditions: 1, hIAPP diluted into culture medium from a 1 mg/ml stock solution in dH_2O; 2, hIAPP reconstituted directly into medium from a freeze dried aliquot that had previously been in 100% HFIP and filtered (0.2 μm) to ensure all 'seeds' were removed; 3, freeze dried aliquot as in 2, made up at 1 mg/ml in dH_2O and incubated for 1 week at 24°C to 'age the fibrils' before further dilution into culture medium; 4, freeze dried aliquot as in 2, made up at 1 mg/ml in dH_2O and incubated for 1 week at 37°C to accelerate 'ageing' process. Electron microscopy confirmed the presence of fibrils in all hIAPP preparations. No differences were seen in the toxic properties of these preparations.

cursor, possibly a protofibril or small oligomer [123]. In the case of IAPP, this hypothesis was derived partly from in vitro experiments demonstrating that 'ageing' of synthetic hIAPP preparations for days or weeks decreased the degree of cytotoxicity [123]. However, these data have not been universally confirmed in all laboratories. Ageing of hIAPP under different conditions did not affect the degree of toxicity [18]; different preparations aged for more than 1 week inhibited similar degrees of cytotoxicity (Fig. 7). One explanation of these conflicting data is that with time, synthetic IAPP fibrils aggregate into larger insoluble precipitates which are not equally dispersed in the culture medium and, when added to the medium will come in contact with less cells than if the fibrils were dispersed. The nature of the toxic form of hIAPP both in vitro and in vivo and the method of induction of cell damage remains to be determined. However, mechanisms operative in vitro may not reflect the events occurring in vivo in man where the time scale for islet amyloid deposition and associated cellular destruction could be much longer – in the order of months or years – during the course of the disease.

Biosynthetic IAPP fibrils appear to be less toxic than fibrils formed from synthetic peptides since fibrils formed in cultured transgenic islets do not result in acute onset of cell death of adjacent cells during the culture period [100,101]. However, the margins of β-cells adjacent to extracellular IAPP deposits show deep invaginations filled with fibrils when IAPP is deposited in islets in vivo or in cellular systems in vitro [4,40,109,124,125] (Fig. 8a,b). Clathrin coated plasma membrane invaginations are more frequently found adjacent to the fibrils than in the absence of fibrils or other regions of the cell, suggesting that recovery of membrane by endocytosis is partly arrested by the presence of the fibrils (Fig. 8a). The interaction of extracellular fibrils with the adjacent β-cell membrane in amyloid deposition could play an important role in decreasing membrane fluidity resulting in interruption of transmembrane signalling. Thus a very small accumulation of fibrils adjacent to β-cells could modulate glucose signalling via membrane GLUT2 transporters and affect potassium and calcium channels, insulin granule exocytosis and membrane recovery. If this hypothesis is correct, β-cell function could be compromised with a small accumulation of IAPP fibrils without any substantial changes in

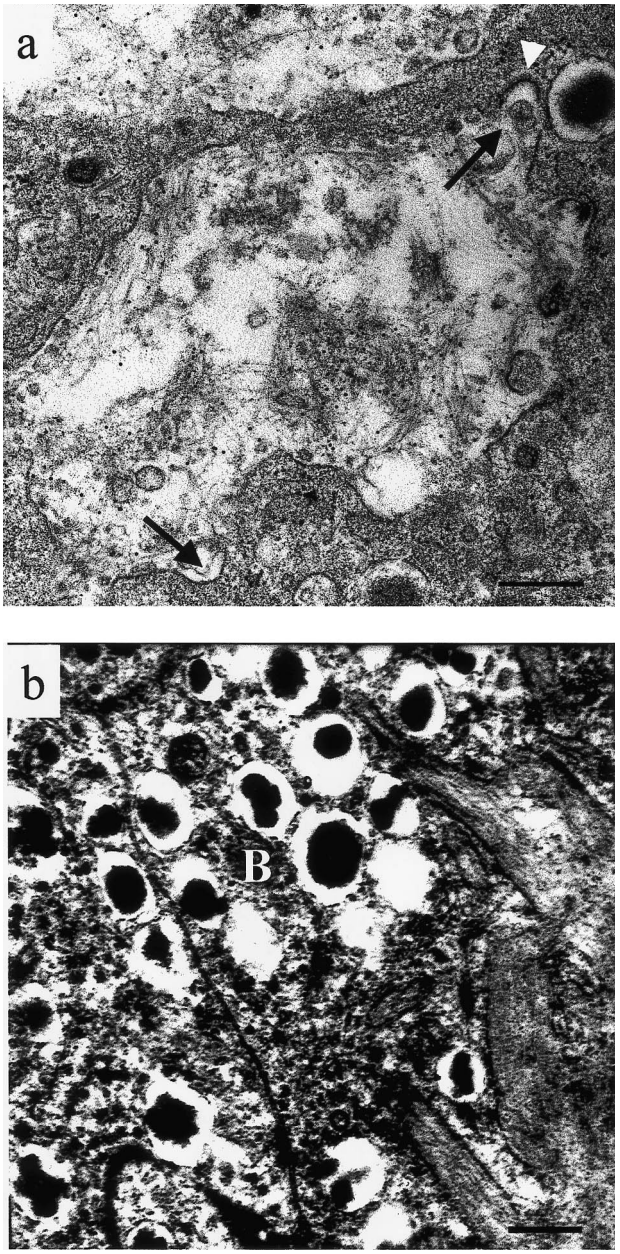


Fig. 8. Fibrils and morphology of the β-cell plasma membrane. (a) Islet isolated from a hIAPP transgenic mouse and cultured in vitro in 16.7 mM glucose. Fibrils (immunogold labelled for IAPP, 10 nm gold) formed in the extracellular space close to the margins of the cells. The plasma membrane was irregular and some invaginations (arrows) have a thickened appearance consistent with clathrin coating of an endocytotic figure (arrowhead). Interaction of fibrils with the plasma membrane could interrupt the fluidity of the lipid bilayer and affect insulin granule release and membrane recapture and mobility of membrane proteins such as GLUT2 glucose transporters. (b) Islet from a human diabetic subject showing similar irregularity of the plasma membrane adjacent to amyloid deposit with invaginations into the β-cell. B, β-cell. (a,b) Scale bar = 200 nm.

β-cell population arising as a result of fibril-induced cell death.

7. Are laboratory and animal models accurate representations of the amyloid deposition and disease processes in diabetes?

The major problem in determination of the relationship between type 2 diabetes and islet amyloidosis is the difficulty in making observations in vivo in man as pancreatic biopsies are ethically unacceptable. Animal models of spontaneously developing, diabetes-associated islet amyloidosis are restricted to older cats and monkeys kept in captivity [38,39,41,93]. The monkey and feline models of diabetes have a similar diabetic syndrome to that seen in man; the pathophysiology includes older age, obesity, impaired glucose tolerance and finally insulin dependence. To address this research problem, at least three different colonies of transgenic mice expressing the gene for human IAPP (hTM) have been created [96,126–128] (Table 1). Animals in these colonies do not develop diabetes spontaneously and islet amyloid deposition appears rarely even in very old mice [128,129]. Amyloid deposition and diabetes have been induced in these animals by creating a background of obesity and increased insulin resistance by various strategies including increased dietary fat [130], treatment with steroids or hormones [96,131] or by co-expression of obesity genes [97,99]. However, it must be remembered that although obesity is a risk factor for diabetes in man this is not a complete model of human type 2 diabetes and progression of the syndromes is not identical.

It is not possible to determine longitudinal progression of amyloidosis in man and the degree of islet amyloidosis in diabetic patients is very variable at post-mortem examination; the number of affected islets visible by light microscopy can be less than 1% or up to 80% in patients with long-established diabetes (Fig. 9a,b). The distribution of islets affected by amyloid in the pancreas is not uniform [132] and the degree of amyloidosis within an islet can be less than 0.5% or more than 90% of the islet volume [9,11]. These features indicate that islet amyloid is variable and is not a major causative factor for onset

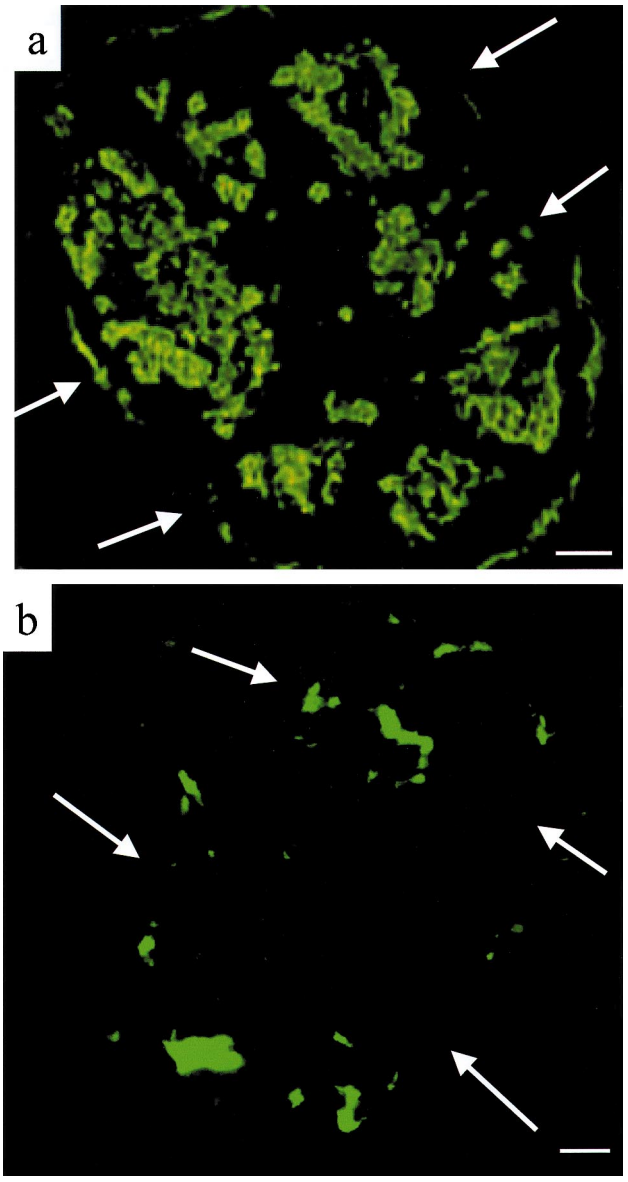
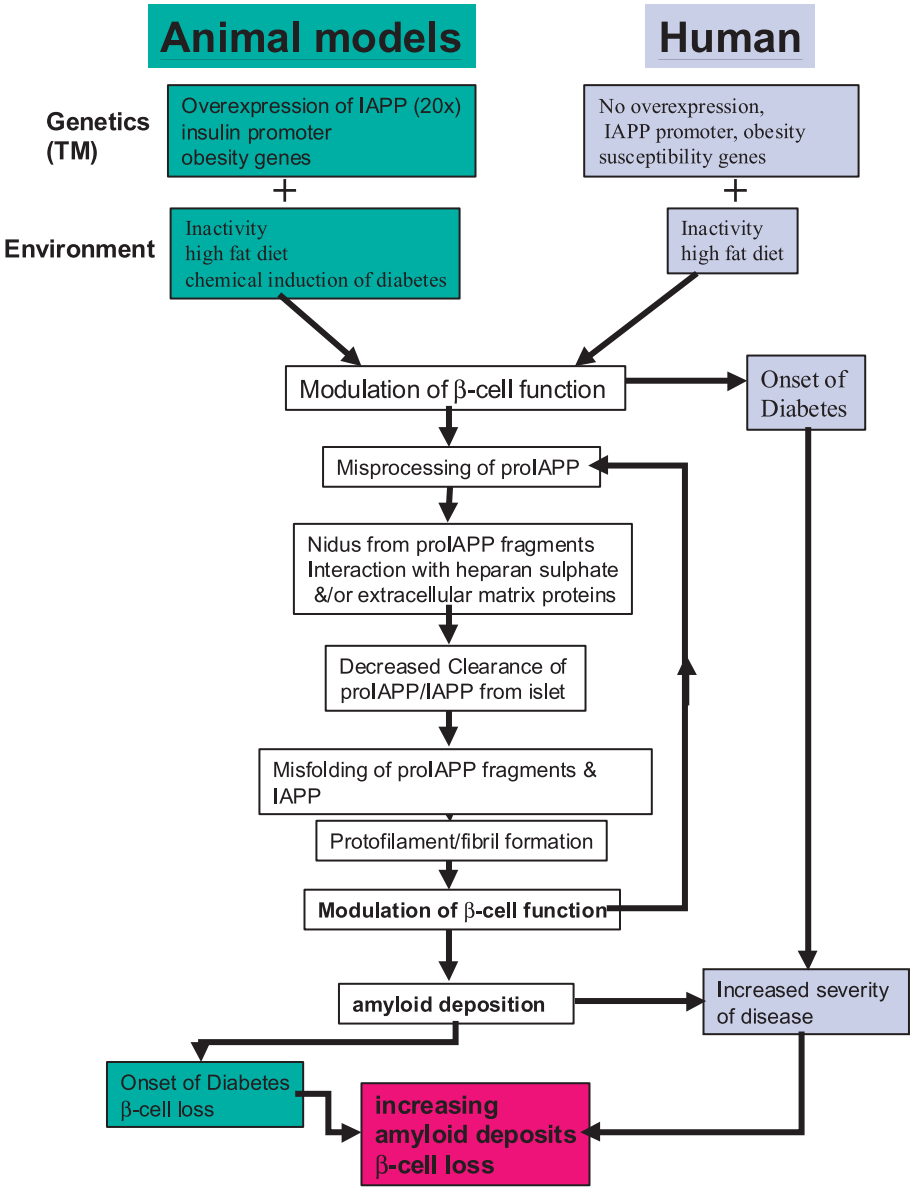


Fig. 9. Different degrees of islet amyloidosis in two patients with similar duration and clinical features of type 2 diabetes. Amyloid was labelled with fluorescent thioflavin S in islets from two diabetic subjects. (a) An islet from a subject (aged 84 years) who died after 12 years of type 2 diabetes treated with sulphonylurea; quantitation demonstrated that 80% of islets were affected with amyloid and more than 85% islet area was replaced with amyloid. (b) Subject aged 65 years, died after 10 years of diabetes treated with sulphonylurea; $<1\%$ islets contained amyloid deposits and $<1\%$ islet area replaced with amyloid. Amyloid was present as thin perivascular deposits adjacent to the capillary surrounding the islet and in larger accumulations within the islet domain; margins of islets are indicated with arrows. (a,b) Scale bar = 20 μm.

of diabetes in man. There is, however, a positive relationship between more extensive islet amyloidosis at post-mortem in man and more severe islet dysfunction as shown by the requirement for insulin therapy [10,12,13]. In comparison, in most animal models, there is progressively increasing islet amyloid formation before the onset of hyperglycaemia [39,93] and the degree of amyloidosis (numbers of islets affected and size of amyloid deposits) appears to relate to the onset of hyperglycaemia [39,97,99]. This implicates islet amyloid (and the associated destruction of islet cells) directly with development of hyperglycaemia in these animal models (Fig. 10). In addition, amyloid deposition in the relatively young hTM (less than 1 year old) is more pronounced in males and is increased by oophorectomy in females [130,133,134]. No such clear gender differences in diabetes susceptibility are seen in humans. The question of the identity of the relationship of islet amyloidosis to the syndrome of diabetes in man and animal models remains unanswered at present. It is likely that the animal models are a better representation of the later stages of the syndrome in man when islet dysfunction is severe and β-cell mass is diminishing [135] (Fig. 10).

Development of amyloid in transgenic animal models is thought to be promoted by increased production of IAPP but that cannot be the only factor since islet amyloid is not formed in hTM overexpressing IAPP unless against a background of obesity (Table 1). Many studies in intact non-transgenic animals and man have shown that IAPP and insulin production change in a parallel fashion in response to different β-cell stimulating agents and at different stages of the diabetic syndrome; secretion of both peptides is elevated in increased insulin resistance and decreased with islet failure [136–139]. Although it has been suggested that amyloid formation is initiated by specific overproduction of IAPP, no convincing evidence has been produced to demonstrate disassociation of insulin and IAPP production at any stage of diabetes (Table 1). Expression of the IAPP transgene in hTM is under the control of the insulin promoter and therefore is driven and controlled in parallel with insulin by hyperglycaemia and transcription factors. The IAPP and insulin gene promoters have some degree of similarity but are not identical [140–144]. In particular, cellular IAPP



gene expression is increased whilst that of insulin is reduced by dexamethasone [145]. Studies on human isolated islets and β-cells have demonstrated that the IAPP and insulin genes are not always co-ordinately regulated in response to secretagogues and agents that modulate β-cell gene expression [72,141,146,147]. This would suggest that in non-transgenic animals and man, different regulatory systems could control expression of these two β-cell genes. To begin to answer the question of regulation, it will be necessary to examine human IAPP expression and fibril formation in a model system where the IAPP gene is regulated by its own promoter such as in human islets or cells.

It has been assumed that islet amyloid deposition in diabetes is an irreversible and progressively increasing process. However, the absence of amyloid in hIAPP transgenic mice could reflect an efficient removal of fibrils or nucleating aggregates rather than a lack of fibril formation. It has been proposed that amyloidosis is reversible and that there is continual turnover of fibrils in deposits [86]. Systemic

Fig. 10. Hypothetical model indicating potential pathways linking IAPP fibril formation with diabetes in animal models and man. In man, genetic factors, susceptibility genes, coupled with adverse environmental conditions (inactivity and high fat diet) lead to changes in function of insulin-secreting β-cells. No phase of inappropriate overexpression of IAPP has been identified in diabetes, although the IAPP and insulin gene promoters are not identical. Abnormal proteolytic processing of proIAPP (and proinsulin) in susceptible individuals results in accumulation of misprocessed proIAPP either in the cell (which will form fibrils under some conditions or be cleared via the ubiquitin pathway) or in the extracellular space. Interaction with basement membrane components (HSPG) will delay transfer to the circulation and generate a 'nidus' or 'seed' in the islet. The onset of obesity-linked increased insulin resistance and hyperglycaemia of type 2 diabetes will further modulate β-cell function although there is no evidence for disproportionate increased IAPP secretion. This will result initially in an increase in β-cell secretion/decreased clearance and exacerbate the accumulation of proIAPP/IAPP in the islet. Increased concentration of hydrophobic hIAPP in the accumulations will create the environment that promotes molecular rearrangement of the peptide and misfolding to form β-sheets, then protofibrils, protofilaments and finally fibrils. Interaction of the oligomers, protofilaments or fibrils with the β-cell plasma membrane will further compromise β-cell function. However, severe deterioration of β-cell dysfunction (and requirement for insulin therapy) will result only from extensive accumulation of amyloid and associated loss of β-cells by fibril-induced toxicity. In animal models, islet amyloidosis is artificially/experimentally created either by increased consumption of food (animals in captivity or domestic environment) or by genetic manoeuvres (transgenic mice TM expressing the hIAPP gene regulated by an insulin promoter, genetic background of obesity) or environment or drugs (high fat diet, steroids). These factors which promote β-cell secretion could increase the production of misprocessed proIAPP but this has yet to be demonstrated. ProIAPP/IAPP production is increased by more than 20 times in most transgenic mouse models but additional factors are required to generate amyloid. In most animal models, islet amyloid is present before the onset of hyperglycaemia and the degree of amyloidosis (and/or the associated loss of β-cells) appear to determine the onset and severity of hyperglycaemia.
←

amyloid deposits have been shown to regress following reduction of the source of the protein component of the fibrils [148]. However, islet amyloid deposits are most extensive in patients and hTM where few β-cells remain to continue to provide IAPP for fibril formation. Since IAPP fibrils are insoluble in aqueous medium, islet amyloidosis has all the hallmarks of a progressive and irreversible disease process.

8. Conclusions

In spite of the increased information from transgenic mouse and cellular models, the process of amyloid deposition and its relationship to diabetes remain an enigma. It appears that there are specific factors/processes associated with human diabetes (which are also ongoing in insulinomas) which cause changes in the molecular conformation of monomeric IAPP resulting in accumulation and formation of fibrils. These aberrant processes occur in normal physiological conditions in vivo and have little in common with the extreme denaturing biochemical conditions required to convert intrinsically non-amyloidogenic proteins into β-sheet [149]. It is likely that β-cell dysfunction in diabetes creates an environment which promotes or permits misfolding of IAPP. Research over the past 13 years, since the identification of IAPP, has increased our understanding of the biophysics of IAPP folding and the process of amyloid deposition in islets. However, more questions still remain to be answered:

- What are the primary factors for β-cell dysfunction in diabetes?
- Are they genetically determined and associated with IAPP?
- How do these factors affect production, folding and misfolding of IAPP?
- How does amyloid deposition affect β-cell function in diabetes?

Islet amyloid and hyperglycaemia are present in more than 90% of patients. Type 2 diabetes has very heterogeneous clinical and pathophysiological features. If amyloid contributes to the final stages of the syndrome when insulin therapy is required, it is important that strategies be developed to identify patients at risk and to devise therapies which reduce fibril deposition and the final demise of the β-cell. Over the past decade, the increased interest in protein/peptide folding and amyloid structure has

changed the focus from the putative physiology and function of 'amylin', IAPP, to the process of misfolding in diabetes. A century of scientific development has transformed Opie's landmark observations [1] from hand drawn pictures of a microscope field to computer modelling of complex molecular processes. Will advances in proteomics during the next century solve the molecular puzzle of protein folding and the biochemistry of diabetes and allow development of appropriate therapies?

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