ISLET AMYLOID POLYPEPTIDE, ISLET AMYLOID, AND DIABETES MELLITUS

Per Westermark, Arne Andersson, and Gunilla T. Westermark

Departments of Medical Cell Biology and Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden

Westermark P, Andersson A, Westermark GT. Islet Amyloid Polypeptide, Islet Amyloid, and Diabetes Mellitus. Physiol Rev 91: 795–826, 2011; doi:10.1152/physrev.00042.2009.—Islet amyloid polypeptide (IAPP, or amylin) is one of the major secretory products of β-cells of the pancreatic islets of Langerhans. It is a regulatory peptide with putative function both locally in the islets, where it inhibits insulin and glucagon secretion, and at distant targets. It has binding sites in the brain, possibly contributing also to satiety regulation and inhibits gastric emptying. Effects on several other organs have also been described. IAPP was discovered through its ability to aggregate into pancreatic islet amyloid deposits, which are seen particularly in association with type 2 diabetes in humans and with diabetes in a few other mammalian species, especially monkeys and cats. Aggregated IAPP has cytotoxic properties and is believed to be of critical importance for the loss of β-cells in type 2 diabetes and also in pancreatic islets transplanted into individuals with type 1 diabetes. This review deals both with physiological aspects of IAPP and with the pathophysiological role of aggregated forms of IAPP, including mechanisms whereby human IAPP forms toxic aggregates and amyloid fibrils.

I. INTRODUCTION

Islet amyloid polypeptide (IAPP), or amylin, was named for its tendency to aggregate into insoluble amyloid fibrils, features typical of islets of most individuals with type 2 diabetes. This pathological characteristic is most probably of great importance for the development of the β-cell failure in this disease (146, 171), but its possible pathogenetic importance was often denied, since it was not found in all individuals with diabetes (21, 93, 394). Moreover, islet hyalinosis is not unique for diabetes but is also observed in nondiabetic individuals, although it then affects fewer islets and to a lesser extent (22, 214, 394). The similarity of the hyaline substance to amyloid was noted at an early date, and some researchers reported staining reactions typical of amyloid (6, 12, 114). Only later, however, was more general agreement reached about the amyloid nature of the islet deposits (93, 324, 393). At that time this was not of great help since the nature of amyloid in general was not understood. It had been shown in 1959 that amyloid of several types has a characteristic ultrastructure (63), and islet deposits were found to share this appearance (196). When biochemical analyses of amyloid fibrils from systemic primary and secondary amyloidoses showed that these consisted of distinctive proteins (23, 123), it was suspected that the islet deposits might also be a polymerized protein.

The chemical composition of islet amyloid did not attract much attention even after the characteristics of other amyloid fibrils had been elucidated. The finding that the amyloid in C cell-derived medullary thyroid carcinoma is of polypeptide hormonal origin was an important indication that amyloid in other endocrine tissues also comes from the local secretory products (334), and it was believed that insulin, or proinsulin, or split products thereof constitute the islet amyloid fibrils (294). Immunological trials to char-
characterize the amyloid yielded equivocal results, and purification of a major fibril protein was hampered by the much greater insolubility of islet amyloid deposits compared with most other amyloid forms (388), except the amyloid core in Alzheimer’s plaques (236). Only when concentrated formic acid was used on amyloid, extracted from an amyloid-rich insulinoma, was it possible to purify the major fibril protein and characterize it by NH$_2$-terminal amino acid sequence analysis, which very unexpectedly revealed a novel peptide, not resembling any part of proinsulin but with partial identity to the neuropeptide calcitonin gene-related peptide (CGRP) (405). Further characterization of the peptide purified from an insulinoma and from islet amyloid of human and feline origin proved it to be a 37-amino acid (aa) residue peptide. The peptide was initially named “insulina amyloid peptide” (405), later diabetes-associated peptide (DAP) (64), and finally islet amyloid polypeptide (IAPP) (404), or “amylin” (66). The designation IAPP will be used in this review.

III. EXPRESSION OF ISLET AMYLOID POLYPEPTIDE

IAPP is a 37-aa residue long peptide, but by the application of molecular biological methods it was quickly shown that IAPP is expressed initially as part of an 89-aa residue preproprotein containing a 22-aa signal peptide and two short flanking peptides, the latter cleaved off at double basic aa residues (28, 254, 268, 319) similar to proinsulin (272). IAPP is expressed by one single-copy gene on the short arm of chromosome 12, in contrast to insulin and the other members of the calcitonin family, including CGRP, adrenomedullin, and calcitonin, all of which are encoded by genes on the evolutionary related chromosome 11 (see Ref. 414 for review). The preproIAPP gene contains three exons, of which the last two encode the full prepropeptide (55, 254, 256). The signal peptide is cleaved off in the endoplasmic reticulum (ER), and conversion of proIAPP to IAPP takes place in the secretory vesicles. ProIAPP and proinsulin are both processed by the two endoproteases prohormone convertase 2 (PC2) and prohormone convertase 1/3 (PC1/3) and by carboxypeptidase E (CPE) (FIGURE 1). This pH-dependent processing takes place in the late Golgi and secretory granules. PC1/3 cleaves human proinsulin almost exclusively on the carboxyl side of Arg31 Arg32 at the B/C junction of proinsulin, whereas PC2 favors the Lys64 Arg65 site at the A/C junction (335, 336). The processing is sequential and first occurs at the B/C junction. CPE removes the COOH-terminal dibasic amino acids (74). PC2 processes proIAPP at position Lys10 Arg11, and PC1/3 at position Lys50 Arg51 (234). It has been shown that in the absence of PC1/3, PC2 can process proIAPP at the COOH-terminal processing site (371). As for many other hormonal peptides, the COOH-terminal glycine residue is used for

\[ \text{FIGURE 1} \]

A: the amino acid sequence of human pro-islet amyloid polypeptide (proIAPP) with the cleavage site for PC2 at the NH$_2$ terminus and the cleavage site for PC1/3 at the COOH terminus, indicated by arrows. The KR residues (blue) that remain at the COOH terminus after PC1/3 processing are removed by carboxypeptidase E. This event exposes the glycine residue that is used for COOH-terminal amidation. Below is a cartoon of IAPP in blue with the intramolecular S-S bond between residues 2–7 and the amidated COOH terminus. B: the amino acid sequence of human proinsulin with the basic residues at the B-chain/C-peptide junction and the A-chain/C-peptide/junction indicated in blue and the processing sites indicated by arrows. PC1/3 does almost exclusively process proinsulin at the B-chain/C-peptide junction while PC2 preferentially processes proinsulin at the A-chain/C-peptide junction. The basic residues (RR) (position 31, 32) that remain at the COOH terminus of the B-chain is removed by the carboxypeptidase CPE. Below is a cartoon of insulin A-chain and B-chain in red with intermolecular S-S bonds between cystein residues 7 in the A and B chains, between cystein residues at position 19 in the B-chain and 20 in the A-chain and the intermolecular S-S bond between cystein residues at position 6 and 11 of the A-chain.
amidation, which, in addition to a disulfide bridge between residues 2 and 7, is a prerequisite for full biological activity. The removed C peptide from proinsulin and the two flanking peptides from proIAPP remain in the secretory granule, resulting at exocytosis in release of equimolar concentrations of the removed peptides and their respective final hormonal product.

IAPP and insulin genes contain similar promoter elements (117), and the transcription factor PDX1 regulates the effects of glucose on both genes (116, 280, 375). Glucose stimulated β-cells respond with a parallel expression pattern of IAPP and insulin in the rat (173, 257). However, this parallel secretion of IAPP and insulin is altered in experimental diabetes models in rodents. Perforated rat pancreas secreted relatively more IAPP than insulin when exposed to dexamethasone (275), whereas high doses of streptozotocin or alloxan reduced insulin secretion more than that of IAPP (256). Oleate and palmitate increased the expression of IAPP but not of insulin in MIN6 cells (304). In mice fed a diet high in fat for 6 mo, plasma IAPP increased 4.5 times more than insulin compared with mice fed standard food containing 4% fat (383). In human recipients who had become insulin-independent by intrahepatically transplanted islets, there was disproportionately more IAPP than normal secreted during hyperglycemia (308). These examples show that the strictly parallel expression of IAPP and insulin may be disturbed under certain conditions.

In addition to islet β-cells, IAPP is expressed in the δ-cells in rat and mouse; in the gastrointestinal tract of the rat, mouse, cat, and human (248, 258, 260, 357); and in sensory neurons in rats (259). IAPP is also expressed in sensory neurons in mice, and IAPP null mice exhibit a reduced pain response in the Formalin paw test (113). In the gastrointestinal tract of rats, IAPP has been identified from the pyloric content is evenly distributed among the islet microscopical analyses have revealed that whereas the insulin content of islets of two human nondiabetic individuals, there was a population of β-cells containing much more IAPP (292). For instance, in the islets of two human nondiabetic individuals, there was a population of β-cells containing much more IAPP. This may indicate that the relative proportions of IAPP and insulin vary considerably between and perhaps within the cells. Subtle changes in single cells may constitute the starting point for fibril formation.

### IV. ORGANIZATION OF ISLET AMYLOID POLYPEPTIDE IN SECRETORY VESICLES

The crystalline structure of insulin in granules is well characterized (88). Hexameric insulin, together with zinc, constitutes the core of the mature granules, while IAPP, together with a large number of additional components, including the C peptide, is found in the halo region (402). The highly fibrillogenic human IAPP (1) has to be protected in some way from aggregation, which otherwise would take place spontaneously. The fact that very fibril-prone proteins can be kept in solution at high concentrations is known from studies of arthropod silk (29). The composition of the β-cell granule is extremely complex, and it has many components in addition to insulin and C peptide (150), in micromolar concentrations (148). The intragranular pH has been estimated to be 5–6, close to the isoelectric point of insulin (149), but this is favorable for the solution of the basic molecule IAPP. It has recently been proposed that polypeptide hormones are packed in granules with an amyloid-like conformation (“functional amyloid”) (225). However, this is very unlikely, at least with human IAPP, which forms unusually insoluble material when assembled into amyloid fibrils (388).

It is more probable that IAPP is protected from aggregation by interaction with other components. Plausible candidates are proinsulin, insulin, or their processing intermediates. Insulin has been found to be a strong inhibitor of IAPP fibril formation (402). This finding has been verified in a number of subsequent studies, which have also shown the potency of the inhibition (157, 158, 195, 199, 326). The inhibition seems to depend solely on the B-chain, which binds specifically to a short segment of IAPP (119). An insulin-to-IAPP ratio of between 1:5 and 1:100 had a strong inhibitory effect (199). The molar ratio between IAPP and insulin in the granule as a whole is 1–2:50, but the concentrations in the halo region are not known. The possibility cannot be ruled out, therefore, that even minor changes in the relative proportions of IAPP and other halo components can initiate aggregation and start fibril formation. The finding of amyloid-like fibrils in β-cell granules early in amyloidogenesis (see below) may support this assumption (290).

Additionally, careful semiquantitative light and electron microscopical analyses have revealed that whereas the insulin content is evenly distributed among the islet β-cells, there is a much more pronounced heterogeneity concerning the content of IAPP (292) (FIGURE 2). For instance, in the islets of two human nondiabetic individuals, there was a population of β-cells containing much more IAPP. This may indicate that the relative proportions of IAPP and insulin vary considerably between and perhaps within the cells. Subtle changes in single cells may constitute the starting point for fibril formation.

### V. ISLET AMYLOID POLYPEPTIDE IN PLASMA AND PANCREAS

IAPP purified from human pancreas and plasma has been shown to have a composition identical to that of the amyloid protein (264). In addition, a peptide consisting of po-
sitions 17–37 of IAPP was identified at both locations. There seems to be no report on the possible function of this fragment, but it should be noted that IAPP8–37 is a IAPP antagonist (373). The human plasma IAPP concentration is only 1–2% of that of insulin. Since IAPP is costored with insulin in the secretory vesicles (164, 216), a regulated co-secretion should occur and has in fact been demonstrated (172). On glucose stimulation, the IAPP concentration changes in parallel with that of insulin (51, 177, 257). Like the C peptide, IAPP is eliminated in the urine (202), but there are alternative degradation systems. Insulin-degrading enzyme (IDE, also known as Insulysin and insulinase; Ref. 16) is a Zn\(^{2+}\)/H\(^+\)-metalloprotease involved in the clearance of insulin (92). IDE is present in the cytosol (16, 322), peroxisomes (253), mitochondria (207), endosomes (89), and on the cell membrane (124) of a wide variety of cells. IDE lacks a signal peptide but is known to be secreted from different cells, and its release has been shown to be unaffected by known stimulators of protein secretion. Therefore, the secretion is believed to be through an unconventional pathway that still needs to be elucidated (82). IDE has also been shown to degrade A\(\beta\)-peptide, and a missense mutation in the IDE gene in the Goto-Kakizaki (GK) rat, a well-characterized model of type 2 diabetes, reduces the catalytic efficiency of the enzyme and affects the degradation of both insulin and A\(\beta\) (106). Overexpression of IDE in A\(\beta\) precursor transgenic mice markedly reduces amyloid plaque formation (206). IDE has also been shown to be important for IAPP degradation (24), and incubation with the IDE inhibitor bacitracin impairs degradation of IAPP and increases IAPP cytotoxicity and the deposited amyloid load (25).

A second protease capable of degrading amyloid is neprolysin (210), and this was also first shown to degrade A\(\beta\)1–42 in vivo (426). Neprolysin is a type II zinc-containing metalloprotease with a short 23-residue NH\(_2\)-terminally located intracellular domain, a 24-residue transmembrane spanning domain, and a 699-residue catalytic extracellular domain (229). Viral overexpression of neprolysin in presynaptic sites leads to a pronounced deceleration of A\(\beta\) amyloid accumulation in mutant A\(\beta\) precursor expressing mice (251). Neprolysin is also present in islet cells, including \(\beta\)-cells, and its expression is normally reduced during aging. Human IAPP-expressing mice, which are prone to develop islet amyloid during ageing, display sustained elevation of neprolysin mRNA with preserved activity. This indicates that the islet neprolysin has a compensatory mechanism aimed to prevent amyloid accumulation (447). In a recent paper it was stated that neprolysin does not degrade IAPP, but it is suggested that the protease directly interferes with the fibril propagation (445).

VI. ISLET AMYLOID POLYPEPTIDE RECEPTORS

The calcitonin peptide family members act through seven transmembrane domain G-coupled receptors. As a hormone, IAPP should have specific receptors, but efforts to find such receptors were long in vain, although specific binding sites were identified, particularly in the brain (293, 328) and renal cortex (418). The failure to find receptors was explained by the discovery of a family of receptor activity-modifying proteins (RAMPs), which are single-domain proteins (241). Three different RAMPs were identified (241). They are not receptors in themselves, but when they dimerize with the calcitonin receptors, they interact and alter the affinity for ligands (435). When the calcitonin receptor forms complexes with one of the RAMPs, the receptor affinity may change, and certain combinations create high-affinity IAPP receptors (255, 302, 354). In this way, several IAPP receptors may form (57, 134, 252). This novel principle has so far been recognized only within the calcitonin family. Expression of RAMPs and calcitonin receptors has been identified in areas of the mouse and rat brain that can be reached by circulating IAPP (19, 363). Both
calcitonin receptors and RAMPs have been demonstrated in a β-cell line (231). The RAMPs seem to act by several mechanisms. For example, they participate in transport of receptor protein to the cell surface, influence glycosylation (241), and modulate signaling (252).

VII. PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL EFFECTS OF ISLET AMYLOID POLYPEPTIDE

A. General Considerations

The phylogenetically conserved nature of IAPP (FIGURE 3) indicates an important function. On the other hand, the two flanking peptides show a much higher degree of amino acid substitution (FIGURE 3). This may be taken to indicate that they do not have regulatory functions, and no such functions seem to have been described. On the other hand, the same has been claimed for the proinsulin C peptide, which today has been shown to have putatively important physiological effects (98, 130, 345, 368). The possible effects of the two IAPP flanking peptides have not been studied.

The function of IAPP is not fully understood. One major problem is the difficulty in differentiating between its physiological or pathophysiological role and its pharmacological effects achieved experimentally. This cannot always be done. IAPP is cosecreted with insulin in the secretory vesicles, and it is reasonable to believe that it is involved in the regulation of glucose metabolism. Very early after the discovery of IAPP, it was found that the peptide inhibits insulin-stimulated glucose uptake and glycogen synthesis in isolated incubated rat skeletal muscle (66). This inhibition may be mediated by effects on several enzymes (79). It was also shown that IAPP inhibits insulin-stimulated glucose transport in vitro by a post-insulin-receptor effect (444). It was initially hoped, therefore, that the mechanism underlying insulin resistance in type 2 diabetes had been found with the discovery of IAPP, and it was shown that insulin resistance could be induced by infusion of IAPP in vivo (166). However, these effects were achieved at concentrations much higher than those seen physiologically and were therefore to be regarded as pharmacological rather than physiological. Increased plasma IAPP concentration in association with renal insufficiency had no effect on insulin secretion (215). However, the possible role of IAPP in the modification of insulin effects on peripheral tissues is still controversial, and it cannot be excluded that IAPP has an important function in the fine tuning, that is difficult to demonstrate experimentally. These effects, and possible effects, direct or indirect, on the liver and adipose tissues have been reviewed several times (59, 65, 401, 414) and will not be repeated here in detail.

Nowadays, there are two plausible physiological roles of IAPP that are of particular interest. One is its function as an auto- or paracrine molecule in the islets of Langerhans, and the other is its role as a hormone with effects on the central nervous system.

B. IAPP Effects on Cells in the Islets of Langerhans

In vivo, in humans, only a very high plasma concentration (2,240 but not 1,420 pM) of IAPP, much above what is found under physiological conditions, has been reported to affect the insulin response to a glucose load. At this very high plasma concentration both the first and second phase of the insulin response was depressed (34). Studies on in vitro effects of IAPP on insulin secretion have yielded contradictory results. Several investigators, for example, have reported an inhibition of insulin secretion (81, 191, 318, 442), even at as low a concentration as 75 pM in the perfused rat pancreas (331). On the other hand, others have reported no inhibitory effect of IAPP on insulin release (35, 279, 296). For further reading on this matter, see Reference 432. So far, there is no definite explanation for these varying results, but at early dates the strong tendency for human IAPP to aggregate into amyloid-like fibrils was not taken

![FIGURE 3](http://physrev.physiology.org/) The amino acid sequences of proIAPP of some mammalian species with numbering according to the human sequence. IAPP is strongly conserved but with notable variation in the 20–29 region of IAPP. This corresponds to residues 31–40 of proIAPP. This important area is highlighted with a red box. Species without occurrence of islet amyloid dog, rat, mouse, guinea pig, degu, and cow have one or more proline residues at this region. Proline residues 36 and 39 are believed to be essential for inhibition of aggregation.
into consideration (432) and the reported effects of early IAPP preparations may be questioned. Today there are effective methods of synthesizing pure and fully active IAPP (4, 140). Interestingly, in addition, Åkesson et al. (8) found that IAPP may have dual effects on insulin release, with stimulation of basal insulin secretion and suppression under conditions of enhanced insulin secretion. It is of interest that male IAPP knock-out mice showed increased insulin responses paralleled with more rapid blood glucose elimination compared with wild-type controls (112). These findings indicate that endogenous IAPP has a physiological role for and suggest that IAPP limits the degree of glucose-induced insulin secretion and the rate of blood glucose elimination (62). In accordance with this notion, Åhrén et al. (5) observed the opposite effect in human IAPP transgenic mice.

Very different results have been obtained concerning the effect of IAPP on glucagon secretion. Silvestre et al. found no direct IAPP effect on glucagon cells, but their results indicated possibly centrally mediated inhibition of glucagon release by arginine (332). Likewise, Young (434) reported that IAPP had no effect on hypoglycemic stimulation of glucagon secretion. On the other hand, other studies, conducted on isolated mouse islets, have shown an inhibitory effect of IAPP on glucagon secretion (8, 285). The addition of IAPP 8–37, which is an antagonist of IAPP, or immunoneutralization of the endogenous IAPP secretion in cultured rat islets, resulted in a dose-dependent increase in stimulated insulin and glucagon secretion. There were no effects, however, on basal insulin secretion (370).

In summary, there are many studies which indicate a paracrine or autocrine function of IAPP, but the mechanisms are still far from clear.

C. Anorectic Effects and Influence on Gastric Emptying

The inhibitory effect of IAPP (including pharmacological analogs) on eating has been well established in experimental animals and in humans (13, 18, 217, 218). In a study on healthy men, it was found that the drug “pramlintide” (essentially human IAPP with amino acid substitutions altering its solubility) reduced the caloric intake as well as the meal duration (48). Binding sites for IAPP have been found at several locations in the brain, including the nucleus accumbens and the area postrema (56), of which at least the latter is outside the blood-brain barrier, making it a possible target for IAPP produced by the islets of Langerhans. However, although it was initially stated that there is no cerebral production of IAPP, several later studies have demonstrated IAPP immunoreactivity (69, 70, 333) in the hypothalamus and basal ganglia. IAPP mRNA was also identified in the preoptic area of the lactating rat (85). Furthermore, IAPP and IAPP mRNA have been found in the human brain (G. T. Westermark and M.E. Oskarsson, unpublished results). It is still not known whether or not IAPP expressed intracerebrally is of importance for food intake or gastric emptying.

Like cholecystokinin, IAPP also inhibits gastric emptying (307), but while the former seems to act through afferent vagus nerve fibers, IAPP does not (408) and elicits its effects by binding to the brain (13). Gastric emptying is pathologically rapid in type 1 diabetes, and this is considered to be one contributing cause of the postprandial hyperglycemia in this disease (415). It is believed that the almost total lack of islet IAPP production in type 1 diabetes may be pathogenically important in the gastric behavior (415). Inhibition of gastric emptying and the underlying mechanisms of this, particularly with emphasis on the IAPP analog pramlintide, have recently been extensively reviewed (433).

D. Additional Effects of IAPP

The structural similarity of IAPP to calcitonin immediately created interest in the possibility that IAPP may be involved in the regulation of calcified tissues (222), and experimentally it was shown that the peptide inhibits osteoclastic activity (437). Many effects have been ascribed to IAPP, and today there is evidence to indicate that the hormone plays a physiological role in inhibition of bone resorption (for review, see Ref. 266).

IAPP has an effect as vasodilator but is two orders of magnitude less effective than its relative CGRP (32, 111). This vasodilative property of IAPP probably depends on binding to CGRP receptors.

Early binding studies revealed strong binding of radiolabeled IAPP to the renal cortex (346). This was regarded as unspecific uptake of radioactivity due to reabsorption of labeled IAPP in the proximal tubules, but later studies have indicated specific binding (418). A physiological effect on the renin-angiotensin system has been suggested (417).

VIII. ISLET AMYLOID POLYPEPTIDE AND AMYLOID

A. What Is Amyloid?

Amyloid is a generic term for a specific protein aggregation state in which molecules in β-sheet conformation are bound to each other predominantly by hydrogen bonds but also by other bonds (87, 121, 122, 310). This state of aggregation creates thin (~10 nm), stable fibrils in which the β-strands are oriented perpendicular to the fibril axis. In humans, more than 25 proteins are known to create amyloid fibrils, and more will be described (390, 396).
Most of them are not related, and at present it is not known why only certain proteins aggregate in this form. Polypeptide hormones are overrepresented (379), possibly because of their small size, their low degree of native secondary structure, and their local occurrence at high concentration. Many small peptides and proteins are able to create fibrils in vitro with amyloid-like properties (86, 310). However, the designation amyloid should be reserved for the material deposited in vivo which, in addition to its fibril core protein, has components including proteoglycans and usually the glycoprotein serum amyloid P component (396). Thus fibrils made in vitro are not amyloid but “amyloid-like” (39). The aggregation state of the fibrils creates properties by which amyloid is recognized, including typical reactions with certain dyes such as Congo red, a fine fibrillar ultrastructure, and a characteristic X-ray diffraction pattern (122).

Not only IAPP but also several other polypeptide hormones can be deposited as amyloid, and this is a common finding in endocrine tumors (400). Interestingly, insulin is also an islet amyloid fibril protein; this does not occur spontaneously in humans, but has been demonstrated in the hystricomorph rodent degu (137). The association of insulin-derived islet amyloid with diabetes in the degu strongly supports the importance of islet amyloid in human type 2 diabetes. In humans, insulin can aggregate into an iatrogenic form of amyloidosis at the sites of injections (84, 347, 436).

B. Islet Amyloid Formation and Animal Species

As expected for a polypeptide hormone, IAPP is conserved through evolution, and the molecule has been characterized in mammals, birds, and teleostean fishes (168, 230, 247, 268, 381). In particular, the NH₂- and COOH-terminal parts of IAPP are conserved (FIGURE 3). The pathological deposition of IAPP-derived amyloid in the islets of Langerhans occurs not only in humans but also in some other mammalian species. One important reason for the earlier relative lack of interest in islet amyloid may be the fact that it does not occur in the animals commonly used in diabetes research, such as the rat and mouse (401). Rat and mouse IAPP (which are identical) lack fibrillogenicity in vivo and in vitro. The reason for this is to be found in differences in the primary structure. Although IAPP is a conserved molecule, there is an interspecies variation at some critical amino acid residues. Most obvious are the variations detected in the IAPP20–29 region, where five out of six differences between rat and human IAPP are found (FIGURE 3). Notably, rat/mouse IAPP has three proline residues, known as β-sheet breakers, in this region. Whereas synthetic human IAPP20–29 is extremely fibrillogenic, the corresponding rat peptide is not (398). The rat shares this sequence characteristic with several rodents, while species with amyloidogenic IAPP include humans, non-human primates, and cats (26, 27). These differences only explain why amyloid can develop, not why it does.

Later studies have indicated that although the IAPP20–29 region is essential to amyloid formation, other parts of the molecule are also important in fibrillogenesis (2, 125, 155, 192, 267). The IAPP14–20 domain, which is within the amphipathic helical region of the molecule (265), may be of particular importance (118) (see below). It has been suggested that the structure of IAPP in the amyloid fibril form may be a double β-hairpin (β-serpentine) with three β-strands consisting of residues 12–37 (175) (FIGURE 4, A AND B). These are then stacked and held together by H-bonds with the NH₂-terminal part sticking out from the core (FIGURE 4C) (175). In another model, partially based on X-ray crystallography of short IAPP segments, the basic unit is a hairpin, allowing interaction between Phe23 and Tyr37 (412), which is in agreement with data from fluorescence resonance energy transfer (FRET) (284). A solid-state NMR study resulted in a similar although not identical model (213) (FIGURE 5). Based on results with additional methods, Dupuis et al. (91) concluded that the β-hairpin structure is the direct amyloid fibril precursor. The pathological aggregation may be initiated in the helix region with conversion into β-sheet structure and may be catalyzed by membranes (3, 190, 265). Results of a recent study indicated that human IAPP dimerizes when in a high degree of α-helical state (413). This dimer is an on-pathway intermediate in the fibril formation.

C. The Three-Dimensional Structure of IAPP and Amyloid Formation

The 37-amino acid residue peptide IAPP belongs to the calcitonin family, which also contains calcitonin, CGRP, adrenomedullin, and intermedin (312, 414). Human IAPP has 43–46% identity with the two CGRPs (404). IAPP shares with the other peptides in the family a disulfide bridge between amino acid residues 2 and 7 and an amidated COOH terminus, which are posttranslational modifications important for biological function. IAPP has more limited sequence identity with other members of the CGRP family (414). IAPP has been mentioned as having a random coil structure (179). However, both CD (190) and NMR studies (265, 288, 411) have shown that the peptide forms an at least transient amphipathic helix in the NH₂-terminal region (3), except for the very NH₂-terminal part, which forms a rigid ring structure, resulting from the disulfide bridge between residues 2 and 7. In solution, the helix spans residues 5–23 (190). The COOH-terminal part of the molecule is unstructured (265). The helical part is believed to be important in receptor binding and may also be deeply involved in the pathological transformation to amyloid fibrils (see below). The quaternary structure of human and rat IAPP is
essentially the same, but with some important differences depending on the presence of three proline residues in the rat sequence. These differences make human but not rat IAPP amyloidogenic.

D. Impact of Islet Amyloid

The development of islet amyloid is difficult to study in humans. Islet amyloidosis associated with diabetes much
resembling the human type 2 form occurs in the domestic cat and in several monkey species (75, 143, 167). Careful studies by Johnson et al. in domestic cats (163, 277, 278), by Howard in black Celebes monkeys (143, 144), and recently by Guardado-Mendoza et al. in baboons (127) have revealed evidence of the importance of islet amyloid for the diabetic state, indicating that the deposits are not only the result of the disease. The latter authors conclude: “Whether islet amyloid (IA) is a cause or consequence of β-cell dysfunction/hyperglycemia is controversial, but we believe that IA plays a causative role in the β-cell dysfunction/apoptosis that is observed in T2DM in a manner that is similar to other human diseases that are associated with amyloid deposits, causing dysfunction of the affected tissue and organs” (127).

Important information on the impact of IAPP aggregation for the development of diabetes has come from studies with animals, transgenic for human IAPP. A number of transgenic mouse strains have been developed, usually with human IAPP cDNA placed behind the rat insulin I or II promoter (68, 110, 151, 240, 420). Also, a transgenic rat expressing human IAPP behind the rat insulin II promoter (HIP rat) has been created (38). The phenotypes have varied (240), but in several strains diabetes has developed in association with the development of islet amyloid and loss of β-cell mass, comparable to events in human type 2 diabetes (160, 239, 339). In some mouse strains, a diet high in fat or induction of insulin insensitivity has been necessary for the creation of the diabetic phenotype (152, 367). Hemizygous HIP rats became diabetic with pronounced islet amyloidosis. In addition to the support for an important role of IAPP aggregation in the pathogenesis of the β-cell lesion coming from studies of transgenic animals, inhibition of proIAPP expression by small interfering RNA (233) as well as treatment with a peptide inhibitor of IAPP aggregation (301) enhanced β-cell survival in cultured human islets. Taken together, all these studies firmly corroborate the impact of aggregated IAPP in the pathogenesis of type 2 diabetes.

Islet amyloidosis is commonly found in ageing animals of the hystricomorph rodent degu (Octodon degus), living in South America (343). Interestingly, in this species the amyloid fibril is made up of insulin (137), which in this group of animals has certain peculiar characteristics (137, 270). The degu IAPP is not, however, amyloidogenic (270) (FIGURE 3).

**IX. PATHOGENESIS OF TYPE 2 DIABETES**

**A. Insulin Resistance and β-Cell Function**

The pathogenesis of type 2 diabetes is complex and multifactorial and a subject of continuous intense investigation (80, 107, 351). It seems clear today that two main factors, each based on a number of different mechanisms, are involved (170, 174). One is a decreased effectiveness of insulin (“insulin resistance”), leading to an increased demand for the hormone, and the second main factor is the pancreatic β-cell failure. This includes a reduction both in the β-cell mass (61, 188, 314, 399) and in the β-cell function (169). Similar findings have been described in a cat model of type 2 diabetes (277). Also the baboon (Papio hamadryas) is a very promising model of type 2 diabetes, including development of islet amyloid (49, 127).

**B. Function of Islets From Type 2 Diabetic Individuals**

Direct studies of the function of islets from individuals with type 2 diabetes are sparse. In an interesting study by Deng et al. (83), islets isolated from patients with type 2 diabetes showed inferior insulin secretion compared with matched controls and failed to make diabetic recipient animals normoglycemic after transplantation. Notably, the glucagon response was normal in perifusion experiments (83). A concern in this study, however, is the finding that islets purified from diabetic donors were smaller than those from the controls, which is not a general observation in histological studies (61, 223). The authors also state that there was very little amyloid in the diabetic specimens and none in the controls. Knowing the difficulty in finding the small, but widely spread amyloid deposits in almost all diabetic pancreata, it seems very likely that amyloid was indeed present and played a role in the failure of the islets.

**C. Association of Islet Amyloid With Diabetes**

Islet amyloid is clearly linked to type 2 diabetes (21, 61, 93, 394) but also occurs in nondiabetic individuals, although it then usually affects fewer islets and to a less severe degree (22, 394). The facts that islet amyloid could be found in some individuals without diabetes, that the depositions were not pronounced in all diabetic pancreata, and that well-granulated β-cells were seen in islets with a large amount of amyloid led to the somewhat premature conclusion some decades ago that islet amyloid is of no major importance. Careful analyses showed, however, that amyloid deposition is associated with a reduced islet volume due to a reduction of the β-cell mass (61, 399, 406). However, the volume reduction in itself should hardly be an explanation for the insufficient insulin response in type 2 diabetes. It was pointed out at an early date that β-cells in the proximity of amyloid were penetrated by bundles of fibrils, ending deep in the cells (391). It is reasonable to believe that the function of such cells is disturbed. Interestingly, new knowledge indicates that interactions between amyloid fibrils and the cell membrane can lead to unregulated Ca2+ influx, which would seriously affect the function of the cells (178).

It is claimed that in type 1 diabetes clinical symptoms show up when the β-cell mass is reduced to 10% of the normal. In
a recent study it was shown that in this disease, also, individual islets may still contain a considerable number of insulin-containing cells (410). Likewise, islets with amyloid in type 2 diabetes contain β-cells filled with insulin granules (391). This does not mean, however, that the islets function normally (406).

D. IAPP Gene Mutations, Type 2 Diabetes, and Islet Amyloid

In 1996, Sakagashira et al. (316) described a mutation in the IAPP gene found in 12 of 294 individuals with type 2 diabetes. The mutation was not found in type 1 diabetic patients or healthy controls. The missense mutation at amino acid position 20 created a glycine-for-serine substitution of mature IAPP. In another study of 308 Japanese individuals with late-onset type 2 diabetes, the mutation was found in three patients as well as in 1 of 149 controls (135).

In a larger Japanese study, the IAPPS20G frequency was 40 of 1,538 among diabetic subjects and 9 of 1,108 among nondiabetic controls (325). Furthermore, Lee et al. (204) identified 7 of 462 Chinese type 2 diabetic patients with the mutation but no individuals with the mutation in a control population of 126 persons. In the same way as in the first study (316), the majority had developed type 2 diabetes at a comparatively young age. On the other hand, in three other reports, one from Japan (422), one from China (58), and one from Korea (53), the mutation showed no association with diabetes. The studied series were relatively small, however. IAPPS20G has only been identified in Asian populations and not in Caucasians or Mexicans (273).

There is one more reported mutation in the IAPP gene leading to an amino acid substitution (IAPPQ10R); this was found in a single type 2 diabetic Mauri patient (298). The mutation was not seen in any of the 258 controls. In addition, there are at least two other mutations associated with type 2 diabetes, which have been identified in the promoter region of the IAPP gene (274, 298). The functional significance for the development of diabetes is, however, unclear (103).

It is obvious that the IAPPS20G mutation plays no major role in the pathogenesis of type 2 diabetes. It is reasonable to believe that IAPPS20G constitutes a risk factor for the disease, the nature of which is not known at present. Position 20 lies within the most amyloidogenic part of IAPP (398), but other parts of IAPP may also contribute to fibrillogenesis (2, 118). IAPPS20G exhibited increased fibrillogenicity in vitro compared with the wild-type protein (221, 315). As a result of the mutation there is a decreased entropy cost in the assembly process, which may explain the increased amyloidogenicity (419). Insulin has been reported to inhibit fibril formation of both IAPP variants, but there was a tendency towards less effective inhibition with the mutant protein (221). Hypothetically other common risk factors leading to β-cell dysregulation may cause enhanced amyloid formation in individuals with the IAPPS20G mutation.

E. Islet Amyloid Formation: Intra- or Extracellular?

Typical of the deposits seen in the human type 2 diabetic islet is their extracellular location, even when small (FIGURE 6A). When the amount of amyloid increases, the number of endocrine cells, particularly β-cells, is reduced. In spite of this, there is evidence that IAPP aggregation starts intracellularly. The problem is certainly not only an academic one but may have fundamental consequences both for the mecha-

![FIGURE 6](http://physrev.physiology.org/)

A: human pancreatic islet with extracellular amyloid deposits, a typical finding in type 2 diabetes. The section is stained for amyloid with Congo red. B: electron micrograph of a part of a β-cell with intracellular amyloid. Note the thin amyloid fibrils within the membrane-encircled compartments (black arrow). Amyloid between two cells is indicated by red arrows. C: electron micrograph of β-cell granules from human IAPP transgenic mouse fed a diet high in fat. Intragranular fibrils present in the halo region are immunolabeled with proIAPP specific antibodies (red arrows).
nisms by which aggregated IAPP affects the β-cells and for the way in which therapeutic interventions can act on the aggregation process.

It was noted early after the discovery of IAPP that β-cells in islets containing amyloid had completely or partly lost their IAPP immunoreactivity (311, 407). This change was found with several different polyclonal antisera and also in islets in cats with and without islet amyloid (165). The reduction is not due to lost expression of the polypeptide gene, since the presence of mRNA was demonstrated (380). Surprisingly, a monoclonal antibody against IAPP reacted with equal strength to β-cells in islets with and without amyloid deposits (220), suggesting an aberrant structure or binding of IAPP in amyloid-containing islets. On the other hand, in a cat model of type 2 diabetes, animals with impaired glucose tolerance but without diabetes displayed increased IAPP immunoreactivity versus normal in β-cells, indicating that a transient overproduction of the peptide may precede the diabetic state (165, 219).

With the transgenic technique, different strains of human IAPP-expressing mice were developed in the early 1990s (68, 110, 420). Even though islet amyloid did not occur spontaneously after establishment of these strains, reports on islet amyloid appeared not long after (160, 367). Early histological studies on islet amyloid in autopsy specimens indicated that all deposited material was extracellular, and the same was also true for other types of amyloid (391). However, when islet amyloid was studied in systems in which amyloid develops more rapidly such as isolated human islets transplanted into mice, or transgenic mice expressing human IAPP, initial intracellular deposition became evident (156, 160, 290, 382, 387, 397, 421). Similarly, in other fast-forming amyloid systems, β-cell tumors (insulinomas), evidence of intracellular fibrillogenesis was obtained (276). Also in type 2 diabetic baboons, amyloid was found both extracellularly and within β-cells (120). The exact location of the intracellular amyloid has been difficult to determine. O’Brien et al. (276) noted fibrillar material free in the cytoplasm, without any surrounding membrane, as well as some membrane-bound aggregates. Both extracellular and intracellular amyloid was found in type 2 diabetic baboon islets (127). In human islets transplanted into nude mice or cultured in vitro, early amyloid forms an intracellular network, indicating its presence within the ER (FIGURE 6B) (290, 397). In addition, however, tiny intragranular IAPP-immunoreactive fibrils were observed at an early phase of amyloidogenesis (290, 421) (FIGURE 6C). The most likely sites for their development are in the ER, Golgi, and secretory granules.

In amyloidogenesis in general, the molecules deposited in the fibrils have undergone some degree of proteolysis, and only rarely are unprocessed proteins found (77, 102, 243). In the early phase of type 2 diabetes, disproportionately elevated levels of unprocessed proinsulin and of the des-31, 32 processing intermediates of proinsulin are present (374). Since proIAPP processing takes place at the same location, an increase in proIAPP and partially processed proIAPP is expected to occur early in development of type 2 diabetes as well, and this has recently been partly verified (441). When IAPP was purified from an insulinoma and from human and feline islet amyloid, only full-length molecules were identified (64, 403–405). However, immune electron microscopic (EM) studies with antibodies specific for proIAPP have shown that the intracellular amyloid, in addition to mature IAPP contains proIAPP. ProIAPP as well as its two possible processing intermediates are in themselves highly amyloidogenic (see below), and an intracellular abnormality leading to aggregation of one of them may be the initial event in amyloid formation (139, 290, 291). Also, prolonged hyperglycemia per se may lead to a higher proportion of proIAPP and intermediates in islets (142).

We have put forward the following hypothesis for the initiation of amyloid formation in an islet (FIGURE 7). The very first amyloid forms within single β-cells, perhaps from insufficiently processed proIAPP (290). This leads to the death of the cell, now leaving a small extracellular amyloid particle. This acts as a seed for secreted mature IAPP, which adds progressively and forms the extracellular amyloid masses that are typically seen in type 2 diabetes. A primary defect in the prohormone processing machinery has been suggested as part of the pathogenesis of type 2 diabetes (154).

F. Amyloid Fibrils in the Secretory Granules

In transmission EM analysis of β-cells from hIAPP transgenic mice, amyloid deposits appeared to a varying degree, and some cells contained only minute amounts of fibrillar material. This was then present as fibrils in the halo region of the secretory granules (FIGURE 6D). The fibrils were recognized by antibodies raised against the NH₂-terminal and COOH-terminal processing sites of proIAPP (290). Hence, the secretory granule is a putative compartment for initial fibrillogenesis, and at this location proIAPP may participate in the process. There can be multiple reasons for the increase in secretion of proinsulin and/or proinsulin processing intermediates during peripheral insulin resistance. Possible examples are an increased demand for secretion due to a rapid turnover of secretory granules, aberrant processing resulting from altered activity of the prohormone convertases PC1/3 or PC2 or of their activators such as 7B2 (33, 443) and SAAS (205).

There is immunological evidence for the presence of proIAPP in amyloid deposits (384), even though the detected amount constitutes only a minute proportion of the total amyloid mass. In an in vitro study with only synthetic materials, proIAPP was less amyloidogenic in the presence
of artificial lipid membranes (185). We have studied the significance of proIAPP processing for amyloid formation by expressing proproIAPP in cells with different patterns of prohormone convertase expression: in beta-TC-6 cells that express PC2 and PC1/3, in AtT-20 and GH3 that express PC1/3 and PC2, respectively, and in GH4C1 and Cos-7 cells that lack convertase expression (291). Intracellular amyloid accumulated in AtT-20, GH4C1, and Cos-7 cells, lines with aberrant prohormone convertase processing. The number of transfected cells decreased more rapidly over time in cells where amyloid developed, a sign of amyloid toxicity. In cells with a larger intracellular amyloid mass, Congo red staining was shown to colocalize with the apoptosis marker M30. If ER stress-induced apoptosis is linked to the formation of toxic oligomers (see below), amyloid formation might for a limited time serve as rescue pathway. Proteomic analysis of the secretory granule content has revealed the presence of multiple chaperone proteins (138). Their possible role in preventing IAPP aggregation still needs to be elucidated.

G. IAPP and β-Cell Death

During development of type 2 diabetes, peripheral insulin resistance is compensated for by increased insulin production. When the exhausted β-cells fail to produce insulin in sufficient amounts, type 2 diabetes develops (115, 170, 438). Islet amyloid is the cardinal finding in the islets of patients with type 2 diabetes, but the reported percentage of patients with amyloid has varied from almost 100% down to 40% or less (21, 226, 392, 440).

The general characteristics of amyloid include the presence of unbranched fibrils of an indefinite length and with a diameter of 7−10 nm (63). The built-in monomers are assembled into a β-sheet structure, arranged perpendicularly to the fibril axis (see above). Amyloid formation is a nucleation-dependent process that can be divided into three different phases. The first is the lag phase, the rate-limiting step during which nucleation of monomeric peptides occurs. The duration of this period varies from some minutes to a lifetime, depending on the protein and other variables such as concentration and temperature. The second phase is the elongation phase, in which amyloid fibrils are propagated, and the third phase, at least in in vitro systems, is the plateau phase when the fibrillation has reached steady state and the fibrillar mass is constant. Amyloid formation is a self-driven process that after initiation continues as long as the precursor is present at a sufficient concentration.

There is little morphological difference between amyloid fibrils made up of each of the hitherto almost 30 described amyloid proteins. Nevertheless, it is known that the morphology of the fibrils may vary, even within a specific biochemical amyloid form, including that derived from IAPP (389). Regarding systemic amyloidosis, where large amounts, often kilogramms, are deposited in different tissues, such as the liver, kidney, or heart, it is obvious that the amyloid masses themselves are sufficient to cause severe disease (244, 295).

The mature amyloid fibril is presumed to be relatively inert and to have no significant cell toxicity. Rather, smaller oligomeric intermediates formed during fibrillogenesis are thought to be cytotoxic. The amyloid fibril protein in Alzheimer’s diseases is Aβ, which is a 40–42 amino acid fragment of the much larger Aβ protein precursor (AβPP) (for review, see Ref. 77). The whole field of toxicity in the pathogenesis of amyloid-associated diseases started with studies by Yankner and co-workers (424, 425), in which they showed that a COOH-terminal fragment of the Aβ-precursor and Aβ are neurotoxic in vitro. These findings created a severe controversy in Alzheimer’s research, since a number of researchers were not able to reproduce the results (232).
The issue of protein toxicity entered the diabetes field in 1994, again through a study by the Yankner group (211), in which they showed that fibrils of human IAPP are toxic to adult human and rat islet cells in vitro. They also reported evidence that the toxic mechanism leads to apoptosis. These initial studies indicated that amyloid fibrils constitute the toxic forms of both Aβ and IAPP (212). Subsequent reports have underlined that it is small, oligomeric IAPP aggregates and not fibrils that constitute the toxic species (159). The role of amyloid protein toxicity is presently an important open question in the research of Alzheimer’s disease, and is also a central subject in the discussions concerning the cause of the β-cell lesion in type 2 diabetes.

A major problem in this area is that the oligomers are ill-defined. These aggregation intermediates, often referred to as prefibrillar oligomers, oligomers, protofibrils, intermediate-sized toxic amyloid particles, or amyloid oligomers (120, 181), have been studied extensively in vitro. It was suggested at an early date that they are inserted into the cell membrane, wherein they form functioning ion channel-like structures (10, 200, 246, 299). This pore forming capacity has been proposed to be a universal cytotoxic mechanism for all amyloid proteins, and analyses of the composition of pores recovered from artificial lipid bilayers have revealed oligomeric complexes of trimers up to octamers, depending on the amyloid protein. For IAPP the major inserted complexes were of the trimeric and hexameric type (305). Suggested mechanisms by which IAPP may permeabilize membranes are either through formation of toroidal (doughnut-like) pores or by nonspecific membrane disruption due to excessive negative curvature strain (337) (FIGURE 8).

In preparations of oligomers produced in vitro, a variety of prefibrillar structures can be detected, some of which are annular in shape. They resemble the membrane-inserted structures, but they lack the ability to permeabilize membranes (181). Instead, smaller prefibrillar oligomers seem to be inserted into cell membranes. At these locations they form annular structures that allow ions to leak. Oligomer-specific antibodies that are said to recognize common structures independently of the amyloid protein have been produced (180). This finding strengthens the view that amyloid fibril formation is a specific process involving mechanisms that are independent of the actual nature of the amyloid protein (86, 122). However, weaknesses in all conclusions drawn so far are that studies on oligomers are mainly performed in vitro and that the existence of toxic oligomers in
vivo has to be proven more than just indirectly. However, evidence of soluble oligomers has been obtained from extracts of the brain of Alzheimer-model mice (208) and recently also from human brain tissue (355). Some evidence for formation of toxic human IAPP oligomers in vivo has also been obtained from studies in transgenic mice (209) and recently in human pancreatic tissue (128). However, the role of IAPP oligomers is still somewhat controversial, and it is too early to rule out the importance of mature IAPP amyloid fibrils in the pathogenesis of the β-cell failure in type 2 diabetes (448).

In a proposed model on IAPP cytotoxicity, based on findings when IAPP was absorbed onto or inserted into a lipid membrane, the 19 NH2-terminal amino acid residues are inserted in the membrane (100, 184). Insertion in this way leaves the amyloidogenic segment of residues 20–29 free to aggregate, and fibril growth will force the membrane to rupture (100, 101). An important difference from other models is that it is monomeric IAPP that initially interacts with the membrane rather than oligomers or fibrils. Likewise, Soong et al. (342) have suggested that human IAPP monomers associate with the cell membrane. In addition, multiple factors such as protein concentration, other proteins including chaperones, temperature, and pH play a crucial role for aggregation of a fibrillogenic protein into amyloid (42). It is also possible that enrichment of amyloidogenic peptides in the vicinity of the membrane can create microenvironments where the peptide concentration is high enough to induce aggregation.

Cholesterol and other lipids have been suggested to be involved in human IAPP amyloidogenesis and the resulting cell death (162). Exogenous human IAPP aggregated into cytotoxic fibrils in cholesterol- and ganglioside-rich rafts in plasma membranes (369). On the other hand, cholesterol inhibited human IAPP aggregation on synthetic membranes (52). Results of one study indicated that human IAPP may induce apoptosis by activation of acid sphingomyelinase, which would lead to production of ceramide (439).

An interesting recent finding is that aggregated IAPP can activate the inflammasome to produce processed interleukin-1β, which may cause β-cell death (237). IAPP also induced release of interleukin-1α that might be of importance for inflammation in pancreatic islets (237).

**H. Can Amyloid Be Harmless or Even Protective?**

As mentioned above, the mature fibril in all forms of amyloid has been considered to be nontoxic, and therefore small amyloid deposits such as those often seen widely spread in islets in type 2 diabetes have been thought to have no significant impact on islet function. The correctness of this assumption should certainly be questioned, since the presence of amyloid, even in limited amounts, affects the cytoarchitecture of the islets, and insulin secretion is dependent on cell-to-cell contacts (416). When human islets were transplanted to a site under the kidney capsule of nude mice, amyloid developed within 2 wk in 75% of the implants (397). At high resolution, thin streaks of amyloid could be seen to protrude between β-cells, which then became separated.

The pancreatic islet is a highly vascularized tissue with β-, α-, ε-, δ-, and PP-cells, and at least the β-cells make up an electrically synchronized unit (126, 262), with pulsatory release of insulin (129, 197, 341). The β-cell coupling seems to be essential for sustaining optimal insulin gene expression, insulin synthesis (20), and proper oscillatory behavior (9, 353). Gap junction channels made up of connexin-36 (327) form the cell-cell connection of β-cells, and in connexin-36-deficient mice, the synchronized glucose-induced calcium and insulin oscillations are altered (306). Incubation of human islets with 40 μM human IAPP resulted in a 90% reduction of insulin secretion stimulated with 16 mM glucose, and in an increase in islet diameter, a morphological change that was interpreted as resulting from disruption of cell-cell couplings and not from hypertrophy of individual β-cells (309). The synchronization of secretion was found to be markedly disordered from islets incubated with human IAPP and stimulated with 16 mM glucose. The question of whether IAPP oligomers exist in the islets of Langhans in vivo remains to be answered, and if they do, for how long will they remain as cell toxic species? It has been suggested that formation of amyloid fibrils is a protective mechanism of taking care of dangerous protein aggregates and transforming them into inert deposits. It might be appropriate to ask whether amyloid formation is initially a rescue pathway that acts to prolong cell survival (86).

**X. ENDOPLASMIC RETICULUM STRESS AND UNFOLDED PROTEIN RESPONSE**

A common term for the dysfunction of β-cells in type 2 diabetes is “pancreatic β-cell exhaustion,” which in reality may be equivalent to β-cell stress (11). For reviews on the importance of ER stress in diabetes, reference should be made to Eizirik et al. (95) and Fonseca et al. (109).

One important component of type 2 diabetes is peripheral insulin resistance that for some time may be compensated for by an enhanced insulin biosynthesis. The increased demand on the secretory machinery in the β-cells results in the development of ER stress. An increase in insulin biosynthesis in the stressed β-cell is paralleled by an increase in synthesis of IAPP (257). Proteins destined for exocytosis are transported through the ER and trans-Golgi complex and stored in secretory granules prior to secretion. ER is a tubular and saclike system where proteins fold into their native structure and the posttranslational modifications are
started. There is a rigorous quality control system to ensure that only correctly folded proteins are transported to the Golgi compartment. A large variety of physiological and pathological factors can perturb ER homeostasis and trigger ER stress as defined by the UPR. An increase in protein synthesis (15, 131, 321) or obstruction of the ER-Golgi transport (303) can lead to an accumulation of unfolded proteins and trigger UPR. The UPR includes upregulation of ER-located chaperones to assist folding of aggregation-prone proteins; a selective inhibition of overall protein synthesis to reduce the ER work load, while selectively favoring synthesis of proteins that augment the UPR; and transport of misfolded proteins to the ubiquitin-proteasome system (UPS) for degradation; and if these measures fail to reestablish homeostasis, induction of apoptosis (73, 228, 283, 323). The different pathways are regulated by the three sensors ATF6 (activating transcription factor 6 alpha), IRE1 (inositol requiring 1), and PERK (PKR like ER kinase). These pathways seem to function in concert (423, 430). In response to ER stress, the chaperone Bip/GRP78, a negative regulator of ATF6, detaches from ATF6 inserted in the ER-membrane, which leads to a translocation of ATF6 from ER to Golgi complexes (50). The cytosolic part of ATF6 is proteolytically cleaved off from the membrane and transferred to the cell nucleus, and activates transcription of genes encoding for chaperones and folding enzymes and proteins, active in the ER-associated degradation (ERAD). Nuclear ATF6 initiates induction of the X-box binding protein (XBP-1). This second transcription factor becomes activated after mRNA splicing by ER-stress activated IRE1 (41, 141). XBP-1 enhances transcription of ER chaperone genes, and the spliced form of XBP-1 can autoregulate its transcription in the presence of activated IRE1 (203). Incubation of human pancreatic islets with freshly solubilized human IAPP increased expression of hsp90 and splicing of XBP-1 mRNA were analyzed in islets isolated from human IAPP transgenic mice. Islet amyloid de-

Expression and activation of XBP-1 increases ER capacity by upregulation of UPR genes such as the chaperone Bip (203) and protein disulfide isomerase (PDI) and by expansion of rough ER (344). PDI is an enzyme that in addition to catalyzing the native formation of disulfide bonds in peptides entering ER, also assists in folding of nascent peptides and is classified as a foldase (36, 340). Activation of PERK in response to ER stress inhibits overall new protein synthesis (54, 133) in favor of translation of selected mRNAs, e.g., chaperones that are important for persistent proper protein folding.

Persistent ER stress activates apoptosis and IRE1 (372), PERK (132), and ATF6 (431), all of which activate transcription of CHOP (C/EBP homologous protein/GADD153). Under normal conditions, CHOP is present at very low concentrations in the cytoplasm, but upon activation, synthesis increases and CHOP translocates to the cell nucleus and induces cell cycle arrest and DNA fragmentation (17, 94). IRE1 signaling can also activate cell death through activation of the c-jun NH2-terminal kinase (JNK) pathway (364). Pro-caspase-12 in mice, equivalent to caspase-4 in humans (133), is present in ER, and its activation during ER stress is linked to an increase in apoptosis (263). Treatment of human cells with caspase-4 siRNA lowers ER stress-induced apoptosis (133). The mechanism of the signaling cascade downstream of caspase-12 is not fully resolved but might relate to caspase-9-linked apoptosis. ER stress has been reported to occur in β-cells in patients with type 2 diabetes and to result in apoptosis (201). Taken together and with the knowledge that IAPP in vitro is one of the most aggregation-prone amyloid peptides and that islet amyloid is present in pancreata of a great majority of patients with type 2 diabetes, it is obvious that induction of IAPP aggregation as a cause of ER stress and of apoptosis, as proposed by Peter Butler’s team, is highly relevant. This research group showed that overexpression of human IAPP in the rat triggers apoptosis and reduces the β-cell mass (38, 239). A sixfold increase in CHOP-positive cell nuclei was detected in human pancreatic sections of type 2 diabetic subjects, but was not present in nonobese or obese nondiabetic subjects (145). An increased production of the ER-stress markers HSPA5, DDIT3, DNAJC3, and BCL2-associated X protein was detected in human pancreas recovered from diabetic subjects (201). However, in this immunological study, CHOP reactivity was restricted to the cytosol without translocation to the cell nucleus where it could mediate ER-stress related apoptosis (238).

The ER density volume (calculated from the relative area taken up by ER in electron micrographs) and apoptosis rate were increased in β-cells from patients with type 2 diabetes compared with those in islets from nondiabetic individuals (227). Moreover, Bip and XBP-1 expression was upregulated in islets from type 2 diabetic individuals but not in islets from nondiabetic subjects after culture in 11.1 mM glucose for 24 h. This difference shows that islets from patients with type 2 diabetes might be more vulnerable to ER stress (227).

Consensus regarding the association between human IAPP expression and induction of ER stress has not yet been reached. Hull et al. (147), for instance, failed to detect ER-stress induction in human islets and mouse islets expressing human IAPP after culture at high and low glucose concentrations (147). The expression of Bip, ATF4, and CHOP and splicing of XBP-1 mRNA were analyzed in islets isolated from human IAPP transgenic mice. Islet amyloid de-
developed and was associated with a reduced β-cell area in a glucose- and time-dependent manner. However, amyloid formation was not associated with significant increases in expression of ER stress markers (147). Furthermore, β-cell apoptosis induced by IAPP amyloid was in short term independent of oxidative stress and antioxidant treatment inhibited rise in ROS, but did not prevent accumulation of amyloid (446) (FIGURE 9).

**XI. AUTOPHAGY**

Autophagy is a well-preserved catabolic process that is active in degradation and recycling of misfolded proteins and excess of, or defective, organelles. The targeted material is encircled by a double membrane structure (phagophore), which together with the engulfed material is referred to as an autophagosome. The autophagosome
fuses with a lysosome and forms an autolysosome, and degradation begins (14, 90, 104). This type of autophagy is named macroautophagy (78). A panel of autophagy-related genes (Atgs) participate in the autophagy process. Atg1 is involved in the early steps of the formation of the phagophore (356). Microtubule-associated protein 1 light chain 3 (LC3 or Atg8) and Atg12-Atg5 participate in the formation of the autophagosome (281, 348). LC3 is cleaved at the COOH terminus by the protease Atg4 (186) to form cytosolic LC3-I. Atg7 and Atg3 convert it into LC3-II by COOH-terminal conjugation of phosphatidylethanolamine (PE) (153, 350), and LC3-II is often used as a biological marker for autophagy induction (249). Atg4 cleavage of LC3 is the first step for its conversion into LC3-II, but Atg4 is also responsible for the deconjugation of LC3-II that releases LC3-I from the mature autophagosome membrane (250). This deconjugation prevents fusion of the autophagosome with the lysosome and further degradation, but it allows LC3-I to be recycled.

Autophagy can be induced in response to ER stress. The ER stress stimulates both phagophore and autophagosome formation. Atg1 kinase activity, which reflects initiation of autophagy, is upregulated during ER stress-induced autophagy (427–429).

Autophagy is connected to amyloid formation in situations where the maturation of autophagolysosomes is hampered. This leads to an increased accumulation of material in autophagic vacuoles, which is a sign of disturbance in the lysosomal system (271). Autophagy-related gene becn1 (Beclin 1) is the mammalian ortholog of yeast Atg6. Beclin 1 is localized in the trans-Golgi network and participates in the formation of the autophagosome after forming a complex with the class III phosphatidylinositol 3-kinase human vacuolar protein sorting factor protein (hVps34). The proautophagic interaction between hVps34 and Beclin 1 is inhibited by Beclin 1 interaction with antiapoptotic Bcl-2 localized in the ER (224). Binding between Beclin 1 and Bcl-2 requires binding of the redox-regulated NAF-1 (nutrient-deprivation autophagy factor-1) to Bcl-2 (47). Binding decreases under starving condition but may increase, e.g., under growth in the presence of nutrients. Therefore, binding of Beclin 1 to Bcl-2 can either promote or prevent macroautophagy (289). Beclin 1 expression is decreased in degenerating neurons in Alzheimer’s disease, and Beclin 1 heterozygous deficiency in mice causes Alzheimer’s-like pathology (297) (FIGURE 9). Beclin 1 deficiency might interfere with the formation of autophagosomes and may well lead to an increase in intracellular accumulation of aggregated proteins.

Aggrephagy is a special form of macroautophagy used for degradation of protein aggregates present in the cytosol (363, 376). The autophagy receptors p62 [also known as sequestosome1 (SQSTM1)] and NBR1 (neighbor of BRCA gene 1) bind to the ubiquitine present on protein aggregates destined for degradation and to LC3-II present on the autophagosome membrane, and thereby direct and promote degradation of the aggregate (187, 286, 376).

At present, the knowledge of the role (if any) of autophagy in the formation of IAPP amyloid and the development of the β-cell apoptosis that occurs in type 2 diabetes is limited, but Masini et al. (235) detected accumulation of autophagic vacuoles and autophagosomes in islets isolated from diabetic organ donors compared with nondiabetic donors (235). Moreover, they found that the number of β-cells was reduced. Quantitative RT-PCR analysis revealed unchanged expression of Beclin 1 and Atg1, while transcription of LAMP2 and cathepsins B and D was reduced. This indicates a reduction of lysosomal activity (235).

It is reasonable to believe that a variety of mechanisms are involved in amyloid-induced apoptosis. ER-stress, UPR, and ERAD are activated by protein aggregation in the ER compartment. Prefibrillar amyloid aggregates can disrupt mitochondrial membrane (128, 287) and cause metabolic dysfunction with induction of oxidative stress and ROS production, consequently damaging proteins (320). Oxidative stress stimulates the ubiquitin-activating enzymes leading to a selective removal of damaged proteins (329). If the ubiquitin pool is fixed, a change in UCH L-1 activity might deplete ubiquitin available for ubiquitination, or the proteasome may be obstructed by polyubiquitinated proteins (46). A reduction of UCH-L1 protein levels has been detected in islets from type 2 diabetic individuals (67). Atg4 is redox-regulated, and low levels of H2O2 can cause a decrease in Atg4 deconjugating capacity of LC3-II, with an increased accumulation of autophagosomes. An intracellular overload of autophagosomes induces type II cell death (37). More advanced ROS production generates general protein damage with a decrease in protein function that affects different cellular pathways. Protein aggregates cannot be degraded by the proteosome pathway. Instead, intracellular amyloid can be ubiquitinated and degraded by aggrephagy. Some general mechanisms have been summarized in FIGURE 9.

**XII. AMYLOID FORMATION IN TRANSPLANTED PANCREATIC ISLETS**

Almost 15 years ago we conducted a study with the ultimate aim of finding an appropriate experimental in vivo model to investigate the pathophysiological relevance of islet amyloid formation for islet function. For that purpose we grafted isolated human pancreatic islets from nondiabetic subjects to a site beneath the renal capsule of nude mice (397). Human islets survive for a long time at an ectopic site as evidenced by studies regarding, for instance, susceptibility of human β-cells to agents known to be toxic to rodent
\(\beta\)-cells (97). In that first study, we found that antisera against insulin and IAPP stained the same cells in islet grafts. More challenging was our finding of IAPP-positive amyloid deposits in two-thirds of the islet grafts. This section describes how these findings have been extended to include experiments with islets isolated from mice transgenic for human IAPP and to take into account evidence to suggest that amyloid formation is indeed a likely candidate as a cause of the long-term failures of clinically grafted human islets.

A. Experimental Islet Transplantation

Ever since the early 1970s, experimental islet transplantation has been successfully carried out with inbred mouse and rat strains, using collagenase isolated islets as grafts. The islets have been either freshly isolated or cultured for a number of days. Although intraportal injections were mostly used as the infusion route of choice in initial studies (182), the subcapsular site of the kidneys has since become more frequently preferred, mainly because of the fairly simple surgical procedure but also because of the possibility of easily recovering the graft for histological and biochemical investigations (96, 242, 359) or of performing perfusion studies of the graft-bearing kidney (193). In the context of islet xenografting, nude mice have been frequently used as recipients of different preparations of endocrine pancreatic material, such as, for instance, human fetal pancreas (317) and fetal (194) or adult porcine islets (99). When human islets, isolated by fairly reproducible techniques, became available, such recipients, normoglycemic or made diabetic by injections of alloxan or streptozotocin, were used in studies of effects of hyperglycemia on human \(\beta\)-cell function (96, 161), replication (359, 361), or susceptibility to \(\beta\)-cell toxic agents (97, 338, 360). Keeping in mind the difference in the IAPP amino acid sequence that prevents amyloid formation in rodent islets, the availability of human islet specimens in an in vivo setting of this kind has paved the way for experimental studies of the presence of fibrillogenic IAPP and accompanying amyloid formation in human islets.

Comparisons of adjacent sections of human islet grafts that had resided in nude mice for 2 wk and were then subsequently stained for insulin and for IAPP indicated that the antisera stained the same cells (397). EM investigations showed explicitly that IAPP immunoreactivity normally was confined to the secretory granules of the \(\beta\)-cells, while \(\alpha\)- and \(\delta\)-cells were negative. Our finding of a lower percentage of IAPP-positive cells in the grafts of hyperglycemic mice was interpreted as indicating that the storage of the substance was decreased after hyperglycemia. Interestingly, we found amyloid deposits in human islet transplants by means of Congo red stainings in six of eight normoglycemic and two of four hyperglycemic recipients. The deposits seemed to be extracellular, but small and very faintly stained deposits were obviously also present in the cytoplasm. There was, however, no clear difference in occurrence of amyloid between nondiabetic and alloxan-diabetic recipients. As in the light microscopical study, our ultrastructural examinations showed accumulation of amyloid material, strongly labeled with antisera to IAPP, in many grafts. Large amounts of amyloid fibrils were easily recognized, but sometimes the amyloid material also had a granular appearance. The amyloidogenic process was obviously quite rapid and took place in the islet grafts, since no amyloid was found in sections of the donor pancreata collected before they were processed for islet isolation.

Our ultrastructural findings have recently been confirmed in a study published by Davalli et al. (72). They grafted human islets to a site under the renal capsule of streptozotocin diabetic nude mice. A great majority of them remained hyperglycemic, and only recipients of islets from one donor (“fully effective prep”) became normoglycemic. While the number and ultrastructural appearance of the \(\alpha\)-cells remained fairly intact, the \(\beta\)-cells decreased in number and were often only sparsely granulated. The same group also demonstrated densely packed IAPP-positive fibrillar deposits in the reticulum cisternae and in the extracellular spaces of the \(\beta\)-cells (108). Exact information on how long the grafts had resided under the renal capsule was not given in their paper.

Essentially all clinical islet transplantation has so far been carried out by intraportal infusion of the islet graft to the diabetic patient. We were therefore interested in investigating the possible deposition of amyloid in intraportally grafted islets. Nude mice were used as recipients of human islets, and indeed, amyloid exhibiting affinity for Congo red was found in a great majority of the islets of intraportally grafted animals (387). We also found that intrasplenically grafted islets contained amyloid with the same appearance as in the intraportally implanted human islets. The same study also showed that islets that had resided as long as 6 mo under the renal capsule most often contained fairly large amyloid deposits, all of which were located extracellularly.

Since human islets are only irregularly offered for experimental use and their quality and biological origin vary considerably, it would be of great help if the islet availability could be more predictable and quality assured. Obviously transgenic mice overexpressing human IAPP would be of benefit in this context. Most of the mouse strains expressing human IAPP do not form islet amyloid in vivo unless hyperglycemia or hyperlipidemia is imposed on the islets (76, 367, 378). Normally, many months of exposure are necessary for amyloid to develop in these animals. We compared the formation of amyloid in human and hIAPP transgenic mouse islets grafted into nude mice, with the same mouse serving as recipient of both human and mouse islets (386). Again extensive deposition of amyloid occurred after only
14 days in the human islets, whereas amyloid deposition in the transgenic mouse islets was sparse and only detected by electron microscopy. It is worthy of note that the amyloid deposition in islets of transgenic mice expressing hIAPP and in human islets differed considerably. In the human islets amyloid was mainly formed intracellularly, whilst in islets from transgenic mice the amyloid was exclusively deposited extracellularly. These animals carried eight copies of the human IAPP gene but also expressed murine IAPP. Their plasma IAPP levels were elevated fivefold (110). Later studies have shown, however, that in hIAPP transgenic animals not expressing the mouse molecule, the first amyloid occurs inside the β-cells (290). Furthermore, recent studies of human IAPP transgenic islets or control islets grafted into streptozotocin-diabetic mice showed that hyperglycemia occurred only in mice that had received transgenic islets. Amyloid deposition occurred prior to the recurrence of hyperglycemia, and was accompanied by increased rates of β-cell apoptosis and decreased β-cell replication (362). The accumulation of amyloid correlated with the loss of β-cells.

It is quite obvious from the data gathered so far on amyloid formation in transplanted islets from nondiabetic subjects that such a process never develops to the degree of amyloid deposition seen in the islets of type 2 diabetes patients. The reasons for this are still unknown, but clearly the present experimental model should offer unique opportunities for such studies. While awaiting further mechanistic studies, it is tempting to speculate that the first amyloid is formed intracellularly and that amyloid at a later stage acts as a nidus for further, extracellular deposition. One circumstance that might explain the rapid deposition of amyloid in the grafted islets is their fairly low vascular density compared with the endogenous islets in the pancreas (44). Such a relative lack of blood vessels, counteracting an effective export of secretory products from the islets, might facilitate the accumulation of IAPP and, as a result, formation of amyloid.

To test this hypothesis, we looked for the presence of amyloid deposits in microencapsulated islets. Such islets survive for many weeks despite their existence as a totally nonvascularized islet graft (31). For that purpose we encapsulated both human islets and hIAPP transgenic mouse islets in a high-guluronic alginate solution (30). These capsules were subsequently transplanted into the renal subcapsular space of normoglycemic nude mice and retrieved 4 wk later (S. Bohman, A. Andersson, and G.T. Westermark, unpublished data).

The preliminary results of that study suggest that considerable amounts of amyloid had been formed in the β-cells of the encapsulated islets, probably much more than in non-encapsulated islets. This may be taken to indicate that a distorted or at least malfunctioning blood perfusion of the islets enhances amyloid formation. To analyze this further, the encapsulation technology might offer unique opportunities for detailed molecular studies of the amyloid formation process in pancreatic islets.

B. Clinical Islet Transplantation

Trials with clinical islet transplantation were carried out in the 1980s and 1990s, but the results were mainly disappointing (349). Thus overall only ~10% of islet recipients achieved normoglycemia without insulin therapy. Almost 10 years ago the Edmonton group spread more enthusiasm to this field of research when reporting that a handful of diabetes patients had become normoglycemic and free from insulin therapy after two or three intraportal implantations of human islets (330). Most of these patients remained off insulin therapy for at least 1 year when given transplants with a higher number of islets and immunosuppression lacking steroids. A detailed follow-up of subjects with type 1 diabetes having undergone this type of treatment showed that ~80% of the patients had C-peptide production after 5 years, but that only ~10% maintained insulin independence (313). It was concluded that there was a progressive loss of islet function in most subjects, all of whom had become insulin-independent initially. We suggested that aggregation of IAPP may be an important cause of the loss of β-cell function in transplanted islets (395). Reports on the pathology of clinically grafted islets are very sparse, but last year two publications were added to the report by Davalli et al. almost 10 years ago (71). Besides our own report specifically focusing on the presence of amyloid in the grafted islets (385), there was one from the Boston group (338) challenging immunological reasons for the loss of islet function. In our study we demonstrated widespread amyloid deposition in the islets implanted in a patient with type 1 diabetes for more than 35 years. He died of myocardial infarction 5 years after the first of three intraportal islet infusions. In almost every second islet of a total of 89 islets found in the liver tissue blocks, amyloid deposits were identified (FIGURE 10). Our light microscopic findings were corroborated by immuno-electron microscopic investigations. Despite considerable technical difficulties, we were able to demonstrate amyloid fibrils in the grafted β-cells that were positive for antibodies against IAPP. In the Boston study, no attempts were made to identify amyloid in the light microscopic study. Electron microscopy was performed on islets derived from paraffin-embedded and Formalin-fixed tissue. No amyloid fibrils were identified in such specimens. There is a possibility that the amount of tissue scrutinized was too small, keeping in mind our observation that only every other islet contained amyloid. Evidently, the amount of tissue available for studies has been limited, since no light microscopic examinations were carried out in attempts to identify amyloid deposits. In the Italian study (71) amyloid deposits were not looked for.

Long-term results of clinical islet transplantation are fairly discouraging, although the reports on this matter are con-
flicting (183, 245, 366). There is strong evidence, however, to suggest that there is a progressive loss of the grafted \( \beta \)-cells that is not compensated for by regeneration of new \( \beta \)-cells. Moreover, it is obvious from the three published autopsy studies that a non-immune-mediated \( \beta \)-cell loss is the cause of graft functional deterioration. But knowledge on the nature of that process is meager, and the importance of performing autopsies under the guidance of pathologists experienced in different aspects of islet pathology, including islet amyloidosis, cannot be sufficiently strongly underlined. By such means further insights into the nature of the destructive process(es) should be gained. Taken together, our combined experimental and clinical data and the recent confirming publications (72, 362) make it very plausible to suppose that amyloid formation in the grafted human islets is a cause of their long-term failure. This idea was recently supported by the finding that porcine islet grafts, which did not develop amyloid, had much better long-term viability than human islet grafts (300).

**XIII. FUTURE RESEARCH**

It is important to understand what aggregation states of IAPP are devastating and how such aggregates exert their toxicity. Almost all studies of IAPP fibrillogenesis and of the toxic effects of IAPP oligomers have been performed in vitro. There is an obvious lack of in vivo studies in animal models and in humans (448). The latter are difficult to carry out, but it should be possible to analyze different forms of human IAPP aggregates, extracted from pancreatic specimens, when such are available, and very recently Gurlo et al. (128) reported evidence for toxic IAPP oligomers in the ER of human \( \beta \)-cells. Given this localization, these oligom-
ers most likely consisted of proIAPP molecules. Additionally, methods to visualize IAPP amyloid deposits in situ in living experimental animals and finally in humans may become available, and positron emission tomography is of particular interest in this context. It has already been successfully used for the demonstration of cerebral amyloid in Alzheimer’s disease (189).

Inhibition of islet amyloid formation in vivo will become a matter of considerable interest. Already a number of agents have been suggested as having this inhibitory effect including small peptides (43, 176, 261, 352) and other small molecules (7, 40, 136). It will be most interesting to test such substances in animal models of diabetes, e.g., in monkeys or cats, characterized by their deposits of amyloid in the islets. The use of different islet transplantation protocols will open up hitherto unknown possibilities of conducting such studies. Hopefully, future research of this kind will finally show whether IAPP aggregation is important for the loss of β-cell function ultimately leading to type 2 diabetes.

**XIV. CONCLUSION**

Islet amyloid was a puzzling islet phenomenon for more than 80 years until its polypeptide nature was unraveled in 1986–1987. Since then, IAPP has been associated with a double controversy. First, its importance in health and disease as a hormone has been vigorously discussed. Second, the role of aggregated IAPP in the development of the β-cell lesion in type 2 diabetes has not yet become generally accepted (60, 401). However, recent results from transplantation of human and transgenic animal islets firmly indicate that IAPP fibrils or oligomers have a crucial role in the progressive failure of β-cells in transplants and thereby also indirectly support a similar mechanism in type 2 diabetes. These results point to the possibility of development of new treatments in type 2 diabetes (176) and the use of islets that do not develop IAPP-amyloid, e.g., from the pig (300), in clinical transplantation.

**ACKNOWLEDGMENTS**

We thank Maud Marsden for kind linguistic revision. Address for reprint requests and other correspondence: G. T. Westermark, Dept. of Medical Cell Biology, Biomedical Center, Box 571, SE-751 23 Uppsala, Sweden (e-mail: Gunilla.Westermark@mcb.uu.se).

**GRANTS**

Our own research, referred to in this review, was supported by the Swedish Research Council, the European Framework 6 Program “EURAMY,” the Swedish Diabetes Association, the Swedish Juvenile Diabetes Fund, and the Family Ernfors Fund.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**REFERENCES**


124. Higham CE, Hull RL, Lawrie L, Shennan KJ, Morris JF, Birch NP, Docherty K, Clark A. Processing of synthetic pro-islet amyloid polypeptide (proAPP) “amylin” by recom-
IAPP, ISLET AMYLOID, AND DIABETES MELLITUS


IAPP, ISLET AMYLOID, AND DIABETES MELLITUS

physical interaction between Bcl-X(L) and a Bcl-2-like domain in Beclin-1. EMBO J 26: 2527–2539, 2007.


822 Physiol Rev  VOL 91  JULY 2011  www.prv.org

287. Parks JK, Smith TS, Trimmer PA, Bennett JPJ, Parker WDJ. Neurotoxic Abeta pep-


284. Padrick SB, Miranker AD. Islet amyloid polypeptide: identification of long-range con-


280. Ohlsson H, Karlsson K, Edlund T. IPF1, a homeodomain-containing transactivator of

281. Ohsumi Y. Molecular dissection of autophagy: two ubiquitin-like systems.


270. Nishi M, Steiner DF. Cloning of complementary DNAs encoding islet amyloid poly-

277. O'Brien TD, Hayden DW, Johnson KH, Fletcher TF. Immunohistochemical mor-

267. Nilsson MR, Raleigh DP. Analysis of amylin cleavage products provides new insights

266. Naot D, Cornish J. The role of peptides and receptors of the calcitonin family in the

265. Nishi M, Chan SJ, Nagamatsu S, Bell GI, Steiner DF. Conservation of the sequence of

264. Nolan C, Margoliash E, Peterson JD, Steiner DF. Cloning of complementary DNAs encoding islet amyloid poly-

peptide, insulin, and glucagon precursors from a new world rodent, the degu, Octodon degus.


262. Nolan C, Margoliash E, Peterson JD, Steiner DF. The structure of bovine proinsulin. J

261. Ohlsson H, Karlsson K, Edlund T. IPF1, a homeodomain-containing transactivator of

260. Ohsumi Y. Molecular dissection of autophagy: two ubiquitin-like systems.

259. O'Brien TD, Butler AE, Roche PC, Johnson KH, Butler PC. Islet amyloid polypeptide

258. O'Brien TD, Hayden DW, Johnson KH, Fletcher TF. Immunohistochemical mor-

257. O'Brien TD, Hayden DW, Johnson KH, Stevens JB. High dose intravenous glucose

256. O'Brien TD, Westerman P, Johnson KH. Islet amyloid polypeptide and insulin secre-

255. O'Brien T, Westerman P, Johnson KH. Islet amyloid polypeptide and insulin secre-

254. O'Brien T, Butler AE, Roche PC, Johnson KH, Butler PC. Islet amyloid polypeptide

253. Ohlsson H, Karlsson K, Edlund T. IPF1, a homeodomain-containing transactivator of


250. O'Brien TD, Hayden DW, Johnson KH, Fletcher TF. Immunohistochemical mor-

249. O'Brien TD, Hayden DW, Johnson KH, Stevens JB. High dose intravenous glucose

248. O'Brien TD, Hayden DW, Johnson KH, Stevens JB. High dose intravenous glucose

247. O'Brien TD, Butler AE, Roche PC, Johnson KH, Butler PC. Islet amyloid polypeptide

246. O'Brien TD, Hayden DW, Johnson KH, Fletcher TF. Immunohistochemical mor-

245. O'Brien TD, Hayden DW, Johnson KH, Fletcher TF. Immunohistochemical mor-

244. O'Brien TD, Hayden DW, Johnson KH, Stevens JB. High dose intravenous glucose


242. Ohlsson H, Karlsson K, Edlund T. IPF1, a homeodomain-containing transactivator of


239. Ohsumi Y. Molecular dissection of autophagy: two ubiquitin-like systems. Nat Rev Mol

238. Ohsumi Y. Molecular dissection of autophagy: two ubiquitin-like systems. Nat Rev Mol


236. Ohsumi Y. Molecular dissection of autophagy: two ubiquitin-like systems. Nat Rev Mol


231. Ohsumi Y. Molecular dissection of autophagy: two ubiquitin-like systems. Nat Rev Mol


228. Ohsumi Y. Molecular dissection of autophagy: two ubiquitin-like systems. Nat Rev Mol


Schröder M. Endoplasmic reticulum stress responses.

Scheuner D, Kaufman RJ. The unfolded protein response: a pathway that links insulin response, lipid biosynthesis, and biogenesis of the endoplasmic reticulum.


IAPP, ISLET AMYLOID, AND DIABETES MELLITUS


