Perspectives in Diabetes

Islet Amyloid

A Long-Recognized but Underappreciated Pathological Feature of Type 2 Diabetes

Steven E. Kahn, Sofianos Andrikopoulos, and C. Bruce Verchere

Islet amyloid has been recognized as a pathological entity in type 2 diabetes since the turn of the century. It has as its unique component the islet β-cell peptide islet amyloid polypeptide (IAPP), or amylin, which is cosecreted with insulin. In addition to this unique component, islet amyloid contains other proteins, such as apolipoprotein E and the heparan sulfate proteoglycan perlecan, which are typically observed in other forms of generalized and localized amyloid. Islet amyloid is observed at pathological examination in the vast majority of individuals with type 2 diabetes but is rarely observed in humans without disturbances of glucose metabolism. In contrast to IAPP from rodents, human IAPP has been shown to form amyloid fibrils in vitro. Because all human subjects produce and secrete the amyloidogenic form of IAPP, yet not all develop islet amyloid, some other factor(s) must be involved in islet amyloid formation. One hypothesis is that an alteration in β-cell function resulting in a change in the production, processing, and/or secretion of IAPP is critical to the initial formation of islet amyloid fibrils in human diabetes. This nidus of amyloid fibrils then allows the progressive accumulation of IAPP-containing fibrils and the eventual replacement of β-cell mass by amyloid and contributes to the development of hyperglycemia. One factor that may be involved in producing the changes in the β-cell that result in the initiation of amyloid formation is the consumption of increased dietary fat. Dietary fat is known to alter islet β-cell peptide production, processing, and secretion, and studies in transgenic mice expressing human IAPP support the operation of this mechanism. Further investigation using this and other models should provide insight into the mechanism(s) involved in islet amyloidogenesis and allow the development of therapeutic agents that inhibit or reverse amyloid fibril formation, with the goal being to preserve β-cell function and improve glucose control in type 2 diabetes. ***Diabetes*** **48:241–253, 1999**

The presence of deposits that replace the endocrine cells within the pancreatic islet has been recognized since the turn of the century as a feature of the pancreatic pathology in patients with hyperglycemia (1). These deposits were originally described as "hyalinosis" by Eugene Opie (1), were subsequently demonstrated to be composed of amyloid, and are a characteristic feature of type 2 diabetes (2–4). Although amyloid as a pathological entity is well recognized in a number of disease states, such as multiple myeloma, rheumatoid arthritis, and Alzheimer's disease (5–7), its potential importance in the pathogenesis of type 2 diabetes is only now starting to gain attention. In this perspective, we review what is known about amyloid and, specifically, islet amyloid deposits; highlight the potentially important role of islet amyloid in the pathogenesis of the islet dysfunction of type 2 diabetes; consider possible mechanisms responsible for its formation; and discuss approaches we believe will be important in unraveling the contribution of the role of this pathological entity in type 2 diabetes.

AMYLOIDOSES

Amyloid deposits consist of small proteins that ultrastructurally form fibrils with a β-pleated sheet structure (5). These fibrils have characteristic features, including green birefringence when viewed by polarized microscopy after Congo red staining and a staining affinity for thioflavin S. Although the classic description of amyloid has always included visualization by light microscopy using one of these staining methods, the development of amyloid commences with the formation of fibrils, which are, on average, 7–10 nm in diameter and of varying length and which are observed by electron microscopy before evidence of amyloid is observed by light microscopy. These characteristic microscopic features occur whether the amyloid is formed as part of a systemic disease or as a more localized process. The classification of these deposits is based essentially on the nature of the precursor protein that forms the backbone of these fibril deposits (8).

Systemic amyloid occurs in a number of forms, some of which are familial. The three major forms are primary or AL

From the Division of Metabolism, Endocrinology and Nutrition (S.E.K., S.A.), Department of Medicine, University of Washington, Seattle; the Veterans Affairs Puget Sound Health Care System (S.E.K., S.A.), Seattle, Washington; and the British Columbia Research Institute for Children's and Women's Health (C.B.V.), University of British Columbia, Vancouver, British Columbia, Canada.

Address correspondence and reprint requests to Steven E. Kahn, MB, ChB, VA Puget Sound Health Care System (151), 1660 S. Columbian Way, Seattle, WA 98108. E-mail: skahn@u.washington.edu.

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Aβ, β-amyloid protein; apo, apolipoprotein; CGRP, calcitonin gene–related peptide; GAG, glycosaminoglycan; GAILS, Gly-Ala-Iso-Leu-Ser; IAPP, islet amyloid polypeptide.

amyloidosis, familial transthyretin-associated amyloidosis (ATTR), and secondary or AA amyloidosis. These forms of amyloidosis are invariably associated with deposits of a circulating product such as monoclonal immunoglobulin light chains, transthyretin, or amyloid A protein, respectively (6). AL and AA amyloidoses include those observed in multiple myeloma and rheumatoid arthritis, respectively, with deposits found in organs such as the kidney, bone marrow, and liver. On the other hand, amyloid deposits occurring as part of a localized process are characteristically associated with a specific organ and result from the deposition of a unique product invariably produced at that site, with other organs being spared (8,9). Conditions associated with localized amyloid formation include Alzheimer's disease (7), medullary thyroid carcinoma (10), and type 2 diabetes (2,3,11). This perspective deals primarily with type 2 diabetes, but because all of these amyloid disease states have features in common, observations made in other localized amyloid-associated disease states are highlighted where appropriate.

Localized amyloid deposition results from the production of a unique polypeptide, which contains an amyloidogenic sequence and is capable of forming a β-pleated sheet structure. In the case of Alzheimer's disease, the polypeptide is the β-amyloid protein (Aβ) (7); in medullary thyroid carcinoma, it is calcitonin (12); and in type 2 diabetes, it is islet amyloid polypeptide (IAPP), which is also known as amylin (13–15) (Fig. 1). However, in each case, these peptides are not the sole components of the amyloid deposit. As with systemic amyloid deposits, these localized deposits can contain a variety of other products, which may include serum amyloid P component (16), complement components C1q and C3 (17), apolipoprotein (apo) E (18), and perlecan, a matrix proteoglycan (19,20). The fact that many of these molecules may be common to the different forms of amyloid suggests that they may have a role in the pathogenesis of these deposits. However, it is also a prerequisite that the unique polypeptide be present in order for amyloid fibril formation to occur. These fibrils form the basis of what ultimately become the larger amyloid deposits that are visible on light microscopy.

PREVALENCE OF ISLET AMYLOID IN TYPE 2 DIABETES

Islet amyloid deposits are observed in the vast majority of individuals with well-established clinical type 2 diabetes and would appear to be a characteristic feature of the disease process. At autopsy, islet amyloid has been demonstrated in up to 90% of individuals with type 2 diabetes (3,4). In some individuals, only a minimal number of islets are affected, but in many patients, the deposits are widespread and affect many islets. The degree of islet (predominantly β-cell) mass that has been replaced by amyloid may be a determinant of the severity of the diabetic disease process, with those individuals requiring insulin treatment having the greatest islet mass reduction and amyloid formation (21). Because islet amyloid has been observed in autopsy samples obtained from different populations, it appears to be a phenomenon common to the disease rather than to a subset of individuals with the syndrome (3,4). In older individuals who are not known to have type 2 diabetes, islet amyloid can be observed but with markedly reduced frequency and severity (4). The prevalence of islet amyloid deposits increases with age (2), which is not surprising because normal aging is associated with a deterioration in glucose tolerance and an increased prevalence of type 2 diabetes (22). On the other hand, in maturity-onset diabetes of the young (MODY) due to a glucokinase mutation, in which glucose intolerance appears at an earlier age and is often mild, islet amyloid was not observed in the single subject whose pancreas was available for examination (23).

It is important to remember that although islet amyloid formation is a characteristic feature of type 2 diabetes, it does not occur solely with this disease process. Islet amyloid has also been observed in humans with insulinomas, which are β-cell tumors characterized by abnormalities in peptide processing and secretion (24). Because type 2 diabetes is also characterized by alterations in peptide processing and secretion, the possibility that these changes may be partly responsible for the initiation of amyloid fibril formation in both disease states has been suggested (24). Data supporting this possibility are discussed later in this perspective.

ISLET AMYLOID POLYPEPTIDE: THE NOVEL COMPONENT OF ISLET AMYLOID IN TYPE 2 DIABETES

The major and unique component of islet amyloid is IAPP, or amylin, a 37–amino acid peptide identified in 1987 (13–15). It is ~45% homologous with calcitonin gene–related peptide (CGRP) but differs substantially from CGRP in its midportion (amino acid positions 20–29); this midregion appears to be critical to the pathogenesis of islet amyloid (25). Human IAPP and the forms in nonhuman primates and cats are potentially amyloid-forming, whereas the sequences of IAPP observed in other species, such as rodents, do not appear to be capable of forming amyloid in vitro (25). This difference is due to amino acid sequence variations at positions 20–29 and, specifically, the critical Gly-Ala-Iso-Leu-Ser (GAILS) sequence at amino acids 24–28. When certain amino acids in this GAILS sequence are replaced by prolines, as occurs in rodents, amyloid fibrils do not form, and those species do not develop islet amyloid when they have diabetes. However, the fact that islet amyloid is not typically observed in healthy normoglycemic human subjects (2,4) makes it apparent that this critical amyloidogenic sequence alone is not sufficient for amyloid formation and that some other factor(s) must be operative.

The gene for human IAPP is located on the short arm of chromosome 12, contains three exons and two introns, and transcribes an 89–amino acid precursor peptide (26). This precursor molecule contains a signal peptide, which is compatible with its being a secretory product, and proregions, which flank the final product and have dibasic amino acid sites that provide the substrate for cleavage by proprotein convertases. The intact IAPP peptide includes a glycine residue following the COOH-terminal tyrosine of IAPP, which indicates that the peptide is carboxyamidated, and several threonines at the NH_2-terminal region, which appear to provide the substrate for O-linked glycosylation in the Golgi apparatus. Furthermore, the precursor peptide contains a consensus sequence for binding the heparan sulfate moiety of proteoglycans (9).

A number of studies followed the isolation and sequencing of IAPP to determine the normal physiology of IAPP production and/or release and to learn whether derangements in these processes could be related to the pathophysiology of type 2 diabetes. Along with others, we were able to demonstrate that the peptide is a normal secretory product of the pancreatic β-cell and is released in response to glucose and nonglucose secretagogues both in vitro and in vivo (27–31).

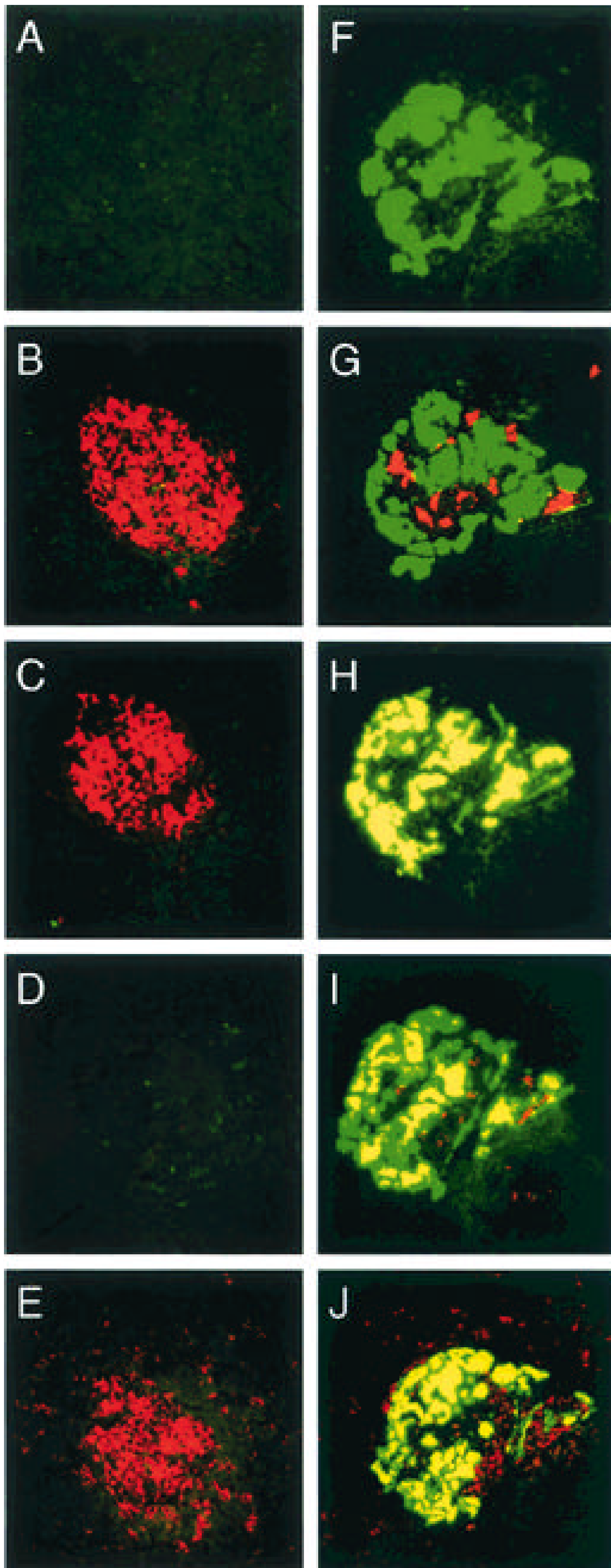

FIG. 1. Human pancreas from a healthy subject (*A–E*) and from an individual with type 2 diabetes (*F–J*) stained for amyloid with thioflavin S (*A* and *F*) and immunostained for insulin (*B* and *G*), IAPP (*C* and *H*), apoE (*D* and *I*) and the heparan sulfate proteoglycan perlecan (*E* and *J*). The immunostained sections (*B–E* and *G–J*) were also stained with thioflavin S. The β-cells in the healthy subject contain insulin (*B*), IAPP (*C*), and perlecan (*E*), demonstrated by the red fluorescence. However, these β-cells do not appear to contain apoE (*D*). Perlecan, which is a basement membrane protein, is also present in smaller amounts in the exocrine tissue (*E*). The subject with type 2 diabetes has marked islet amyloid demonstrated as green fluorescence with thioflavin S (*F*). These amyloid deposits contain IAPP (*H*), apoE (*I*), and perlecan (*J*), all of which appear yellow when the thioflavin S and antibody immunostaining images are merged. However, these deposits do not contain insulin, although the remaining β-cells do (*G*), as indicated by red fluorescence.

Among the initial hypotheses for the impact of the peptide were that the peptide could reduce insulin-mediated glucose uptake (32) and inhibit insulin secretion (33) and that it was therefore critical in the pathogenesis of the hyperglycemia of type 2 diabetes. However, substantial evidence to support either of these hypothesized effects in type 2 diabetes depended on high doses and was not convincing at more physiological levels (34). By virtue of the fact that IAPP slows gastric emptying (35,36), it has been postulated that the peptide has a physiological role in decreasing postprandial glucose excursions and thus may be useful in improving glycemic control in people with diabetes. In addition, by virtue of its ability to form amyloid fibrils and thus initiate the process of amyloidogenesis, IAPP has been hypothesized to have an important role in the pathogenesis of type 2 diabetes through its impairment of β-cell function and reduction of β-cell mass (37). Besides being able to form islet amyloid deposits that replace β-cell mass, amyloid fibrils appear to be able to damage islets directly. Studies in which islets were incubated for 24 h in the presence of human or rat IAPP demonstrated that in a concentration-dependent manner, the human peptide formed amyloid fibrils, which were associated with the death of islet cells, but that cell death did not occur in the presence of rat IAPP, which does not form fibrils (38). Interestingly, a recent study suggested that the toxicity of IAPP may occur only when freshly formed amorphic prefibrils exist and that the effect wanes as mature fibrils form over time (39).

To determine the mechanism(s) underlying islet amyloid formation, we and several other groups have developed transgenic mouse models expressing the human form of IAPP in their β-cells (40–44). These animal models have provided answers to a number of questions. First, they have confirmed that the human amino acid sequence is required for amyloid formation. Second, they have largely confirmed the human observation that simple overproduction of IAPP cannot be responsible for islet amyloid formation, because most lines, even when bred to homozygosity, do not develop classic amyloid deposits. However, in our transgenic mouse model, we have observed the development of IAPP-derived islet amyloid when these mice were fed a high-fat diet (45). We have used tissue from this animal model along with that from humans with type 2 diabetes to learn more about the pathogenesis of human disease.

ISLET AMYLOID IN TYPE 2 DIABETES: OTHER COMPONENTS OF POTENTIAL IMPORTANCE

Although a large body of work on IAPP and its role in islet amyloidosis has been performed, it must be remembered that IAPP is just one component of these deposits. Examination of autopsy samples from individuals with type 2 diabetes has demonstrated the presence of at least two additional components in islet amyloid: apoE and the heparan sulfate proteoglycan perlecan (Fig. 1). Both of these proteins have also been identified as components of other forms of amyloid (9).

ApoE binds triglyceride-rich lipoproteins and is important in reverse cholesterol transport (46,47). The human apoE gene is polymorphic, resulting in the expression of three different isoforms in humans. Homozygosity for one of these isoforms, apoE2, is associated with an increase in plasma concentrations of the intermediate-density lipoproteins and the development of premature atherosclerosis (48). Recently presented studies provide evidence that the presence of the apoE4 allele is strongly associated with an earlier age of onset of Alzheimer's disease in susceptible individuals (49,50), and apoE has also been shown to be a component of the fibrillar tangles of Alzheimer's disease (51). We and others have found evidence for apoE in islet amyloid (52,53). On immunocytochemistry, islet amyloid deposits associated with type 2 diabetes fluoresce intensely for apoE immunoreactivity, whereas islets from subjects with normal glucose tolerance do not, suggesting that human islets normally do not contain this apolipoprotein (Fig. 1). It would also appear that apoE is the sole apolipoprotein present in islet amyloid deposits, because our immunostaining studies have failed to demonstrate the presence of either apoA and apoB in islets or amyloid deposits from either subjects with normal glucose tolerance or those with hyperglycemia (S.E.K. et al., unpublished observations). Although we were able to show that apoE is present in islet amyloid of humans, monkeys, and our transgenic mice that develop islet amyloid, we have been unable to demonstrate the presence of apoE mRNA in normal human and mouse islets (53; S.E.K. et al., unpublished observations), suggesting that its presence in islet amyloid deposits results from its transport from a distant site via the circulation rather than from its being a product of the β-cell, as is IAPP. Distant biosynthesis contrasts with the scenario in Alzheimer's disease, in which an abundance of apoE mRNA has been demonstrated in astrocytes shown to synthesize and secrete the protein (54), which is compatible with apoE production near the site of amyloid deposition. Because it appears that the source of the apoE in islet amyloid is extrapancreatic, it seems most likely that it is produced in the liver, which is a major site of production of this apolipoprotein or macrophages circulating through the islet (55).

The potential role of apoE in islet amyloidogenesis is not fully understood. Studies of amyloid formation in Alzheimer's disease suggest that apoE can promote fibrillogenesis and appears to stabilize amyloid fibrils formed from Aβ (56–58), the novel peptide found in the amyloid deposits of Alzheimer's disease. Whatever role this apolipoprotein has in the pathogenesis of Alzheimer's disease, there is experimental evidence to suggest its importance—namely, that when bred with mice lacking apoE, transgenic mice expressing the human form of Aβ develop amyloid in the hippocampal region much more slowly than transgenic mice containing a full complement of the apoE gene (59). Based on these findings in Alzheimer's disease and our finding of apoE in islet amyloid, it is very possible that apoE participates in islet amyloidogenesis.

Another component of islet amyloid is perlecan (60). This molecule is a heparan sulfate proteoglycan that is one of a family of proteoglycans, including dermatan sulfate, chondroitin sulfate, and keratan sulfate (61). Perlecan is a major component of the endothelial cell basement membrane (62) and is thus ubiquitous. Amyloidogenic proteins, including IAPP, contain a consensus sequence that appears to bind the glycosaminoglycan (GAG) chains of this protein (9). Perlecan has been shown to be a component of Alzheimer's amyloid, in which it is hypothesized to enhance amyloid formation from Aβ, stabilize amyloid fibrils, and prevent amyloid degradation (20,63,64). Support for the concept that the GAG chains of perlecan may also be important in the pathogenesis of islet amyloid was recently obtained in an in vitro study in which perlecan was shown to accelerate fibril formation by IAPP (65). Thus, it appears that perlecan may facilitate

amyloid fibril formation, thereby providing the nidus on which continued deposition occurs, leading ultimately to the amyloid deposits visible by light microscopy.

We have found perlecan to be present in the islet amyloid deposits in humans with type 2 diabetes (Fig. 1), as well as in islet deposits in monkeys (S.E.K. et al., unpublished observations) and in our transgenic mice that bear the human IAPP gene (53). To determine the likely site of biosynthesis of this molecule, we have performed Northern and Western blot analyses on isolated mouse islets and found perlecan mRNA and protein, in contrast to our findings with apoE (53). Given that islets contain endothelial cells, it is possible that perlecan synthesized in these vascular cells may be involved in islet amyloidogenesis. However, the presence of perlecan mRNA in βTC3 cells (S.E.K. et al., unpublished observations) indicates that perlecan may be arising from the same cells as IAPP.

IS ISLET AMYLOID AN EARLY OR LATE EVENT IN THE PATHOGENESIS OF TYPE 2 DIABETES?

While islet amyloid is a feature of the islet pathology in the vast majority of individuals with type 2 diabetes, it is unclear how early in the natural history of the disease it develops. Thus, there has been debate as to whether islet amyloid is a critical and early component of the pathogenesis of hyperglycemia or simply an epiphenomenon. Part of the difficulty in providing an answer to this question has been the inability to study islet amyloid formation in a large number of humans longitudinally and relate these deposits to changes in glucose metabolism. Furthermore, until the recent resurgence of interest in islet amyloid as a distinct pathological entity, all studies of islet amyloid had relied on light microscopy, in which the presence of visible abnormalities is compatible with a long-standing process and does not represent the early stages of amyloidogenesis, which can only be observed on electron microscopy. Thus, those debating whether islet amyloid is an early or late phenomenon have had to rely on the somewhat limited work performed in animal models of type 2 diabetes, much of which was performed with light microscopy.

In studies of the nonhuman primate *Macaca nigra*, which develops hyperglycemia and islet amyloid, Howard (66,67) was the first to precisely examine islet pathology and glucose metabolism. This formidable body of work demonstrated that at the time of an initial pancreatic biopsy, monkeys with islet amyloid had reduced insulin secretion that was associated with mild impairments of glucose tolerance but no change in the fasting plasma glucose concentration (67). Longitudinal follow-up of these animals, which included serial pancreatic biopsies, highlighted the fact that continued amyloid deposition was associated with a further reduction in insulin secretion and deterioration in intravenous glucose tolerance. The development of fasting hyperglycemia was a late phenomenon and occurred in these animals only in association with substantial islet amyloid deposits. These studies were performed before IAPP was identified, and the presence of IAPP in these deposits, although likely, has yet to be shown.

More recently, we have taken advantage of the transgenic approach to examine islet amyloidogenesis in a transgenic mouse line expressing human IAPP (45). These studies have allowed us to obtain data that not only support Howard's observations of monkeys (66,67) but also extend them, suggesting that development of IAPP-derived islet amyloid does not depend on hyperglycemia and is progressive. In these studies, we fed transgenic mice a diet containing an increased quantity of fat for up to 16 months and monitored plasma glucose levels during this period. We observed the development of hyperglycemia in 10 (31%) of our 32 male transgenic mice but in 5 (14%) of the 35 male nontransgenic animals. When pancreatic sections from these mice were examined, we found islet amyloid in every transgenic mouse with diabetes. To our surprise, however, we found that two-thirds of our male transgenic animals that had been consistently normoglycemic also developed islet amyloid deposits, indicating that hyperglycemia is not a prerequisite for islet amyloid formation. In all mice, the quantity of amyloid varied from islet to islet. In islets in which less amyloid was deposited, amyloid was present around blood vessels and in the islet periphery, with electron microscopic examination demonstrating marked separation of the basement membrane and the accumulation of fibrillar material containing human IAPP immunoreactivity between β-cells and between β-cells and endothelial cells in islet capillaries (Fig. 2). The fibrils demonstrated by electron microscopy were not visible between endothelial cells nor between islet α- or δ-cells, which is a finding consistent with the production of IAPP in islet β-cells. Of further interest in these mice was the fact that we never observed evidence of intracellular amyloid fibril formation, which would suggest that initiation of amyloid formation may not occur within the β-cell. Although this observation regard-

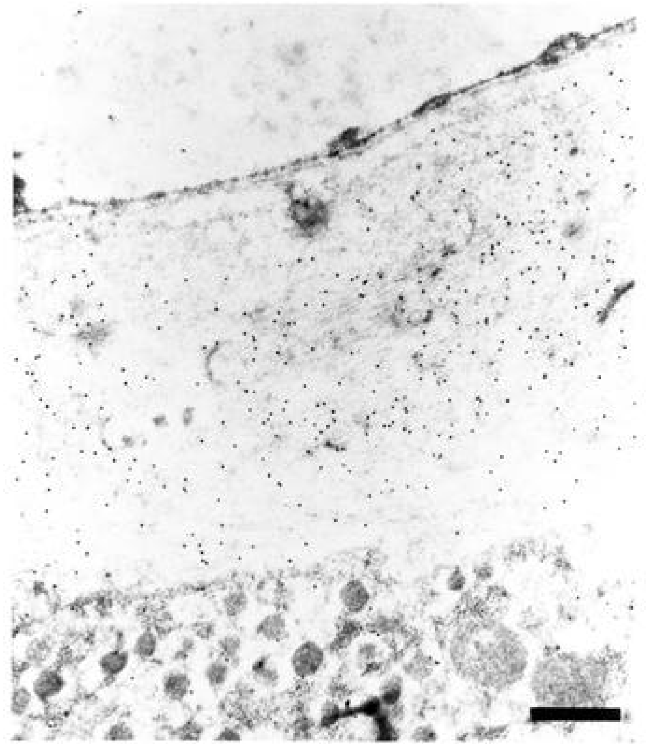

FIG. 2. Electron micrograph of an islet from a human IAPP transgenic mouse, demonstrating the presence of fibrils between the basement membranes of a β-cell and an islet capillary. The fibrils contain IAPP immunoreactivity as demonstrated by the immunogold positive labeling. Fibrils were observed only extracellularly between β-cells and between β-cells and endothelial cells, never adjacent to α- or δ-cells or intracellularly. Bar = 0.5 μm. Reprinted with permission from Verchere et al. (45).

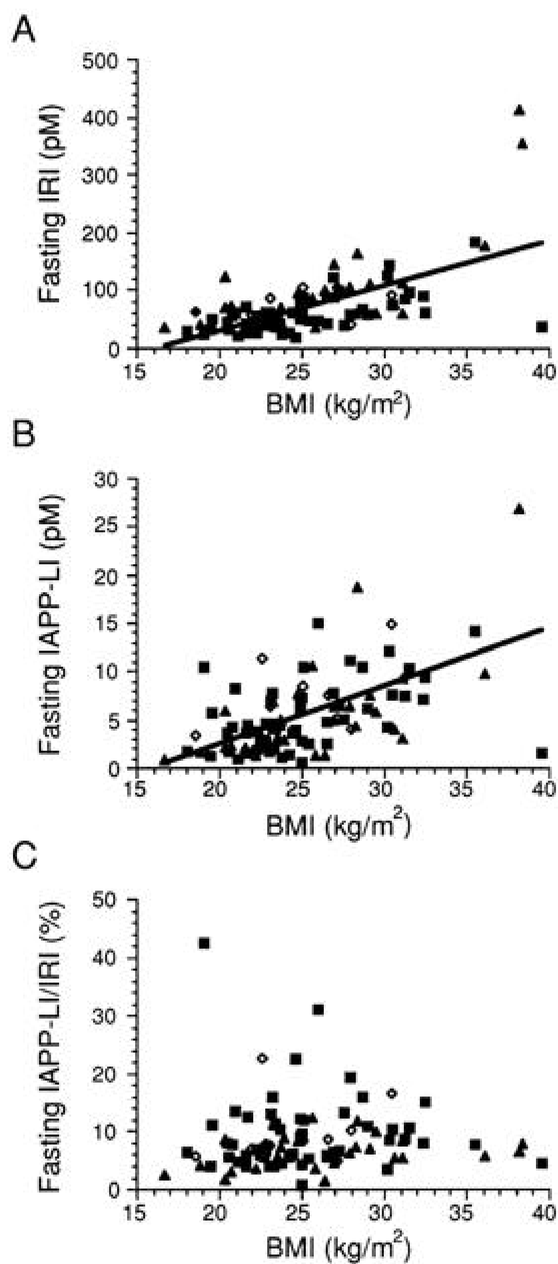

FIG. 3. Relationship between body adiposity, determined by BMI, and fasting plasma immunoreactive insulin (IRI) (*A*), fasting plasma IAPP-like immunoreactivity (LI) (*B*), and fasting IAPP-LI/IRI (*C*) in 94 Japanese Americans with normal glucose tolerance (■), impaired glucose tolerance (◇), and type 2 diabetes (▲). Both fasting IRI (r = 0.63; P < 0.0001) and fasting IAPP-LI (r = 0.58; P < 0.0001) increase with increasing BMI, whereas the fasting IAPP-LI/IRI does not (r = 0.004, NS). Reproduced from Kahn et al. (75).

ing the site of initiation of islet amyloidogenesis is consistent with findings in human samples, it contrasts with observations made by others who have used mostly in vitro preparations, as discussed below.

Collectively, the data from the studies in monkeys and in our transgenic mice provide a number of important clues pertaining to the pathogenesis and importance of islet amyloid in type 2 diabetes. First, it would appear that amyloid fibrils are a precursor to the islet amyloid deposits observed on light microscopy and that these human IAPP fibrils may be cytotoxic to β-cells and thus could produce early alterations in islet function (38,39). Second, islet amyloid deposition is an early feature of the islet lesion of type 2 diabetes, and progressive accumulation of islet amyloid is associated with further β-cell mass reduction (11,68). Thus, we believe a progressive reduction in islet mass by increased amyloid deposition is associated with a progressive impairment in insulin secretion, reduction in glucose tolerance, and eventually the development of fasting hyperglycemia. Indirect support for the importance of the relationship between the quantity of islet mass replaced by amyloid and the degree of impairment in insulin release, and thus the severity of the hyperglycemia, comes from studies in monkeys (67,69) and from Westermark's observation of autopsy samples obtained from human subjects with type 2 diabetes (21). In Westermark's study (21), a greater degree of islet replacement by amyloid was found in patients using insulin than in those taking oral agents, which is compatible with the concept that the need for insulin therapy is the result of a more severe reduction of insulin secretory capacity due to amyloid deposition (70). Third, our studies in human IAPP transgenic mice reviewed above suggest not just that hyperglycemia is associated with the development of islet amyloid but that amyloid contributes to the development of hyperglycemia by replacing β-cells.

MECHANISMS THAT MAY UNDERLIE ISLET AMYLOID FORMATION

In a number of studies, investigators have attempted to determine why IAPP forms islet amyloid in type 2 diabetes and insulinomas. Although many of these studies have provided important information, collectively they do not provide a complete answer. What they highlight is that the presence of the amyloidogenic sequence within the midregion of the IAPP molecule is absolutely necessary, but not sufficient, for islet amyloid fibril formation and accumulation of amyloid deposits.

Two early hypotheses on the mechanism for islet amyloid formation were common to much of our thinking regarding the pathogenesis of type 2 diabetes. The first was that mutations of the IAPP gene would increase the propensity for IAPP to aggregate. This possibility was largely ruled out when Cook et al. (71) found no evidence of linkage of the IAPP gene with type 2 diabetes and Nishi et al. (72) failed to demonstrate any abnormality of the IAPP gene, including the coding region for the precursor molecule, in 25 subjects with type 2 diabetes. However, a Ser Gly substitution at position 20 in the IAPP molecule has been reported (73). This finding was observed in 4.1% of 294 Japanese subjects with type 2 diabetes who appeared to have an earlier onset and more severe form of the disease, but it is unclear whether this alteration produced a peptide that was inherently more amyloidogenic. This mutation has not been demonstrated in other populations with type 2 diabetes (74), suggesting that if it is a pathogenic factor, it is an unusual one.

The second hypothesis was that simple overproduction of IAPP may result in its accumulation and thus aggregation. Several lines of evidence suggest that this is not the case. Perhaps one of the strongest arguments against this possibility comes from the observation that in most normoglycemic human subjects that are obese and/or insulin resistant, and therefore predicted to be producing and secreting more IAPP, islet amyloid is rarely observed (4). In fact, evaluation of IAPP secretion in obese subjects suggests a parallel increase of

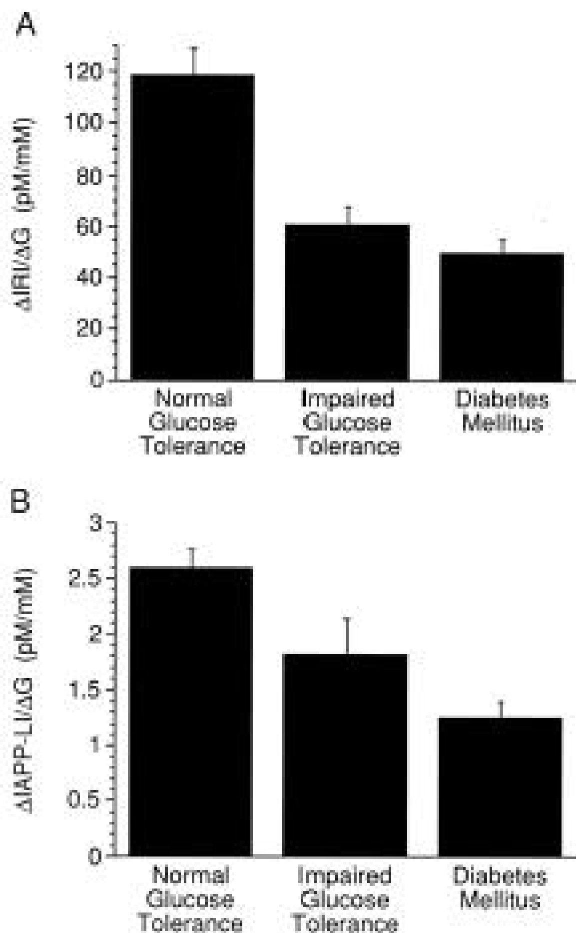

FIG. 4. Ratio of the incremental responses of immunoreactive insulin (IRI) (*A*) and IAPP-like immunoreactivity (LI) (*B*) to the incremental glucose response (ΔG) over the first 30 min after oral glucose ingestion in 94 Japanese-American subjects with varying glucose tolerance. Of the 94 subjects, 56 had normal glucose tolerance, 10 had impaired glucose tolerance, and 28 had type 2 diabetes. Significant decreases in both the ΔIRI/ΔG ($P < 0.0001$) and the ΔIAPP-LI/ΔG ($P < 0.0001$) occurred with decreasing glucose tolerance. Reproduced from Kahn et al. (75).

IAPP with insulin (30,75) (Fig. 3). By contrast, the β-cell dysfunction present in individuals with impaired glucose tolerance includes relative reductions in both insulin and IAPP release when compared with individuals with normal glucose tolerance who are matched for body adiposity (30,75) (Fig. 4). Furthermore, IAPP secretion decreases in parallel with the progressive loss of insulin secretion observed in type 2 diabetes (30,75) (Fig. 4). Thus, it appears that the increased production of IAPP associated with obesity is likely to be moderate and that marked overproduction of IAPP is not present at a very early stage in the disease process of type 2 diabetes. Whether amyloid fibrils are present in islets of human subjects with impaired glucose tolerance and could thus be contributing to β-cell dysfunction and reduced IAPP output is not known.

Because islet amyloidogenesis cannot readily be studied in humans, a significant portion of the work addressing questions related to this issue has been performed using whole animal and cell culture studies. Confirming the observation that simple overproduction of IAPP in humans does not result in accelerated islet amyloid formation is the fact that islet amyloid was not observed in transgenic mice expressing the human IAPP gene, even when IAPP production was increased by crossbreeding lines of these mice with the genetically obese and diabetic *ob/ob* and *db/db* mice (76), breeding mice to homozygosity (77), or producing experimental insulin resistance in mice by administration of corticosteroids (76) or nicotinic acid (77), and following them for periods of up to 1 year. However, a line of mice with greater overexpression of the human IAPP transgene has subsequently been shown to develop islet abnormalities spontaneously when bred to homozygosity (44) or when treated with steroids and growth hormone (78). This greater degree of transgene expression resulted in marked overproduction of IAPP, widespread β-cell death with a resultant marked reduction in islet size shown on morphologic studies, limited amyloid deposits evident on electron microscopy, and no evidence of amyloid on light microscopy (44). This observation is not the typical histopathologic finding in humans, nonhuman primates, or cats with hyperglycemia. More recently, heterozygous mice from the same transgenic line have been crossed with the obese agouti viable yellow (A^{vy}/a) mouse (79), a model of obesity and diabetes. The resultant line developed marked insulin resistance and the early onset of hyperglycemia, which was associated with the development of islet amyloid at 10 months of age. Thus, it would appear that under certain conditions associated with high expression of the transgene, these mice may produce sufficient quantities of IAPP for spontaneous amyloid formation. However, in most transgenic mice, the level of production appears to be insufficient for spontaneous amyloid formation, and under most circumstances, some other alteration is required. One possible explanation is the change in lipid metabolism that occurs with a high-fat diet, insulin resistance, and hyperglycemia. We interpret these studies to suggest that islet amyloid usually does not develop after simple overproduction of IAPP, even in human subjects who have obesity-associated insulin resistance (4) and who secrete increased amounts of IAPP (30,75). However, it is possible that in rare cases of chronic massive secretory demand on the β-cell, amyloid may form in humans (80).

In vitro work has also examined the issue of amyloid fibril formation during increased IAPP production. Transfection of a human IAPP cDNA into COS cells resulted in expression of the human peptide and the development of intracellular amyloid fibrils (81). Whether the peptide was processed from the larger precursor to the intact 37–amino acid product was not determined. However, this conversion may not have occurred, because the proprotein convertases (PC1/3 and PC2) found in β-cells are lacking in COS cells. In addition, unlike β-cells, COS cells do not sort proproteins to secretory granules. Thus, typical sorting, processing, and release of (pro)IAPP are unlikely. In another series of experiments, isolated islets obtained from human IAPP transgenic mice demonstrated the presence of both intra- and extracellular amyloid fibrils, but only when the islets were isolated and cultured at 28 mmol/l glucose for 7 days and not when a lower glucose concentration was used (82). In another report, intracellular amyloid fibrils were observed in human islets that had been transplanted into nondiabetic and diabetic nude mice (83). In this experimental scenario, establishment of vascular access, and thus of IAPP drainage away from the islet, is not immediate.

The finding of intracellular fibrils in these above-mentioned studies raises the question of whether fibril formation commences intracellularly in the pathogenesis of islet amyloid in type 2 diabetes or whether the process is initiated only once IAPP has been secreted from the cell. If apoE, perlecan, and other factors are vital in the pathogenesis of islet amyloid fibrils, intracellular formation of fibrils would likely require that these products either be produced by the β-cell or be taken up from the circulation. On the other hand, if the site of initiation of amyloid fibrillogenesis is extracellular, then uptake of these molecules into the cell would not be necessary. In the studies discussed above, intracellular compartments including the secretory granules and lysosomes have been shown to contain IAPP immunoreactivity (84,85). Despite the presence of this immunoreactivity within the β-cell, electron microscopic studies of islets from human subjects with type 2 diabetes and our human IAPP transgenic mice have demonstrated the presence of extracellular fibrils but have never shown accumulation of intracellular fibrillar material (21,23,45). However, it must be remembered that examination of these human samples is complicated by the state of the autopsy material and by the fact that in many instances, the islets are likely to contain substantial amyloid deposits. Thus, further work in this area is necessary.

The promoters of both the insulin and IAPP genes are known to contain elements that may be regulated by glucose. Thus, glucose can stimulate IAPP biosynthesis (86), and it is conceivable that it may play a role in islet amyloid deposition as an accelerator rather than as a primary factor. The initial event could be the formation of amyloid fibrils followed by alterations in β-cell function and the development of increased glucose levels. However, it is also possible that the increase in glucose levels enhances IAPP synthesis and amyloid formation. Eventually, however, the continued accumulation of islet amyloid and its replacement of β-cells result in progressive β-cell dysfunction and the marked reductions in insulin and IAPP release observed clinically (30,75). What, then, is the evidence suggesting that hyperglycemia is a secondary rather than a primary event in the pathogenesis of islet amyloid? First, amyloid develops in human islets transplanted into nude mice in the presence of euglycemia (83). Second, some of the islet amyloid we observed in our transgenic mice (45) and that seen by Howard (67) in his monkeys and by Johnson et al. (87) in their studies of cats was often present when glucose levels were still within normal limits. Thus, it would appear that if glucose does enhance islet amyloid formation (82), it does so only after the beginning of fibril formation that is secondary to some other event. This effect of glucose would lead to a vicious cycle of accelerated reduction in islet function and progressive hyperglycemia as amyloid forms and islet mass is lost. Similarly, one may postulate that once a nidus of amyloid fibrils has formed for some reason, obesity and sulfonylurea therapy, both of which likely increase IAPP biosynthesis and/or release as they do insulin (30,75,88–91), could possibly also feed forward to accelerate amyloid formation and contribute to islet β-cell destruction.

If amyloid fibril formation is an important participant in the pathogenesis of β-cell loss in type 2 diabetes, it is logical to ask what the primary event is in islet amyloidogenesis. Shortly after the identification of IAPP, we proposed that an abnormality in β-cell peptide processing and/or release may underlie the development of islet amyloid (24). Today, we know that although the presence of the critical amyloidogenic sequence as exists in the human form of the peptide is necessary, it alone is insufficient for amyloid formation and thus must require some other coexisting event or abnormality. Our thesis that this abnormality involves a fundamental alteration in β-cell function still appears viable. In examining what evidence adds credence to this proposal, it is important to describe the normal biosynthetic and secretory mechanisms operative in the β-cell.

After translation of mRNA in the endoplasmic reticulum, the large precursor forms of the major peptides (proinsulin and pro-IAPP) traverse the Golgi apparatus and at the trans-Golgi apparatus are either sorted into secretory granules of the regulated secretory pathway or are released via constitutive secretory vesicles (92). Vesicles in the constitutive pathway traverse the cell interior rapidly and are released ~20 min after their formation. Because the transit time of these vesicles is short and they lack the endopeptidases (PC1/3 and PC2) responsible for peptide processing in the β-cell, propeptides are predominantly released. The quantity of peptide released via this pathway depends on the rate of peptide synthesis and thus is accelerated by nutrients such as glucose, which are capable of enhancing proinsulin and pro-IAPP biosynthesis (86,93,94). On the other hand, granules destined for release from the regulated secretory pathway contain not only proinsulin and pro-IAPP but also the endopeptidases PC1/3 and PC2, which are responsible for converting proinsulin, and presumably pro-IAPP (95), to their fully processed products before the release of the granule content by calcium-dependent exocytosis. This conversion process is highly dependent on the pH and calcium concentration within the granule and occurs over the course of ~120 min as the granule matures (96,97). At the completion of this conversion process, nearly all the propeptide has been processed to the mature product, but a small quantity of incompletely processed proinsulin still exists within the granule (98,99), and the same is likely true for pro-IAPP. Release from the regulated secretory pathway, as implied by the name of the pathway, depends on the nature and magnitude of the stimulus and, unlike the constitutive secretory pathway, only indirectly depends on synthesis (92).

Determination of exactly where the abnormality in β-cell function resides has been hampered in large part by the difficulty in obtaining viable islets from human subjects with type 2 diabetes. Thus, observations in humans have been made on the basis of plasma-based studies and examination of autopsy material. From the regulated pathway, release of IAPP and of insulin-like immunoreactivity is frequently coordinated at a fixed molar proportion based on in vitro and in vivo studies, some of the latter having been performed in humans with varying degrees of glucose tolerance (30,75,100). Examination of the insulin-like immunoreactivity released from the regulated pathway in subjects with type 2 diabetes reveals a disproportionate increase in the quantity of proinsulin relative to insulin released (98,99), which is compatible with reduced proinsulin-to-insulin conversion within granules in this pathway. Because both proinsulin and pro-IAPP are likely to be cleaved by the same endopeptidases present in secretory granules in the regulated pathway, it seems that the increased regulated release of proinsulin observed in type 2 diabetes should be associated with similar increases in pro-IAPP secretion. While the regulated path-

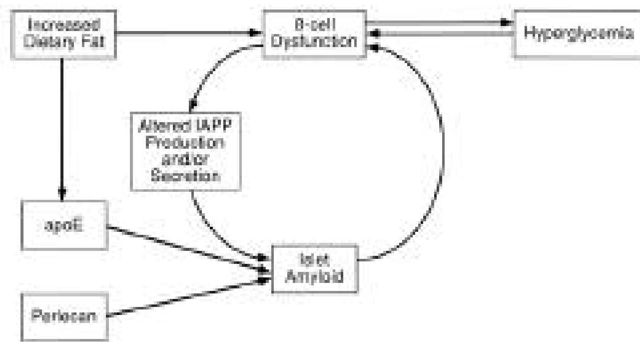

FIG. 5. Model to explain the development of islet amyloid in type 2 diabetes and its contribution to the hyperglycemia of the disease. β-Cell dysfunction is associated with altered biosynthesis, sorting, processing, and/or secretion of IAPP, leading to the development of a nidus of islet amyloid fibrils that is not necessarily associated with hyperglycemia. The fibrils themselves may be toxic to β-cells and produce β-cell dysfunction. Continued accumulation of fibrils results in the development of the classical islet amyloid deposits visible by light microscopy, which progressively replace islet mass. These amyloid deposits contain not only IAPP but also apoE and the heparan sulfate proteoglycan perlecan. As the quantity of amyloid increases, islet (and β-cell) mass decrease, resulting in progressive β-cell dysfunction and progressive hyperglycemia. Hyperglycemia per se alters β-cell function and stimulates IAPP production, resulting in further accumulation of islet amyloid and islet mass reduction. β-Cell dysfunction could be genetically determined but is also affected by the environment, specifically a diet containing increased quantities of fat. Fat may also contribute to amyloid fibril formation by altering the production of other components of islet amyloid, including apoE.

way is active in insulinomas (101), the constitutive secretory pathway is also a major route for the release of proinsulin in these tumors (92,102). Because both proinsulin and pro-IAPP appear to enter the constitutive pathway (94,103) and this pathway lacks the endopeptidases responsible for cleaving proinsulin (92), it seems likely that in human insulinomas, pro-IAPP will also be a major secretory product released via this pathway. It is not known whether this alternative secretory pathway is a contributor to the increased proinsulin levels observed in the basal state in subjects with type 2 diabetes.

It should be recognized that although the patterns of release of insulin and IAPP immunoreactivity are usually similar and processing of proinsulin to insulin is incomplete in subjects with type 2 diabetes and insulinomas, the release of increased quantities of pro-IAPP in human disease has not yet been conclusively demonstrated, because assay systems capable of measuring pro-IAPP have not been developed. However, although an association of type 2 diabetes and/or insulinomas with increased pro-IAPP release has not been definitively demonstrated, other lines of evidence do support this possibility. First, the presence in plasma of a high-molecular-weight form of IAPP immunoreactivity in a patient with an IAPP-secreting islet tumor has been reported (104), suggesting that incomplete processing of pro-IAPP to IAPP may occur in islet tumors. Second, immunohistochemical studies have demonstrated the presence of the NH_2-terminal sequence of pro-IAPP in the islet amyloid deposits in subjects with type 2 diabetes (105), supporting the possibility that an alteration in pro-IAPP processing may play a role in islet amyloid formation in subjects with type 2 diabetes. Finally, results of work performed in a murine insulinoma–derived cell line demonstrated that proinsulin release is increased in association with an increase in pro-IAPP secretion (103), which is compatible with the concept that both unprocessed products are secreted together. Thus, although it has not been established, it appears likely that in humans pro-IAPP is released in parallel with proinsulin from the regulated secretory pathway. Therefore, we would expect that both pro-IAPP and proinsulin secretion may be disproportionately elevated in type 2 diabetes.

Whereas over the last decade we have learned a great deal about the role of IAPP in islet amyloidogenesis, we still do not understand the mechanism by which this amyloidogenic peptide forms amyloid fibrils. Work to elucidate this mechanism will rely in large part on human studies and on experiments using animal models of islet amyloid. Our recent observation of islet amyloid formation in human IAPP transgenic mice fed a higher-fat diet (45) provides a plausible unifying hypothesis that would also appear to apply to the development of human type 2 diabetes (106).

DIETARY FAT: A UNIFYING HYPOTHESIS FOR THE ISLET AMYLOID OF TYPE 2 DIABETES?

Increased intake of dietary fat has long been associated with westernization and has been suggested to be an important risk factor in the development of type 2 diabetes, especially in migrant populations (106). Increased dietary fat has also been shown to be capable of inducing hyperglycemia in mice, and this approach has been used to produce rodent models of diabetes (107). The question is whether this increased fat intake could possibly impair islet β-cell function in addition to producing obesity and insulin resistance? In mice studied while their dietary fat consumption was increased, β-cell function was reduced, as measured by decreased insulin release from perifused isolated islets (108). Similarly, our group has recently demonstrated that dogs gain weight and become insulin resistant when they are fed a diet containing lard, but rather than observing a reciprocal change in β-cell function, we observed lower or unchanged insulin responses; this observation is compatible with β-cell dysfunction and a failure to increase insulin output to the increased secretory demand of insulin resistance that results in the development of glucose intolerance (S.E.K. et al., unpublished observations). The mechanism by which a high-fat diet produces β-cell dysfunction is not entirely clear. It may be related to exposure to increased circulating levels of free fatty acids, to triglyceride accumulation in the β-cell, and ultimately to apoptosis of the cell (109,110). When isolated islets are used, 24 h of exposure to fatty acids reduces both insulin biosynthesis and release; removal of the fatty acids from the medium results in the reversal of this suppression (111). Recently, it has also been reported that fatty acids can reduce posttranslational processing of the endoproteases PC1/3 and PC2, which are responsible for processing of proinsulin to insulin (and of pro-IAPP to IAPP) (112). Whether reversal of some or all of these effects can occur after more prolonged exposure is unclear.

Based on the association between dietary fat intake and the development of type 2 diabetes in humans (106), on the association between the effect of increased dietary fat and a lack of β-cell adaptation in dogs and mice, and on our observation that an increase in dietary fat induces islet amyloid in our human IAPP transgenic mice, we propose the following hypothesis to explain the development of amyloid in type 2

diabetes (Fig. 5). β-Cell dysfunction is a necessity that may be genetically determined and/or influenced by the environment. We propose that any factor that impairs (pro)IAPP processing, sorting, storage, secretion, or degradation may result in the initiation of islet amyloid formation. The propensity for this amyloidogenic product to form fibrils in subjects with type 2 diabetes and not in healthy subjects, rather than depending solely on the absolute amount of IAPP being produced or released, may be due to the release of incompletely processed pro-IAPP or to an alteration in intragranular constituents resulting in reduced IAPP solubility. The initiation of amyloid fibril formation would need only a minor alteration in β-cell function that would not necessarily be associated with hyperglycemia. Once a nidus of amyloid fibrils has formed, the process is progressive, and amyloid can eventually be visualized using electron microscopy and then with the typical staining techniques using light microscopy. These amyloid fibrils result in the loss of islet cell mass either through a direct toxic effect of extracellular fibrils, as demonstrated in vitro (38,39), or by their progressive space occupancy, which may reduce the ability of nutrients to reach the cell, or may destroy cells, simply by their occupying islet space (11,68). The toxic effect of fibrils may be mediated through different mechanisms, such as reactive oxygen species (113,114) and apoptosis (115,116), as observed with other types of amyloid, such as that from Aβ in Alzheimer's disease. As islet mass is reduced, insulin release declines and progressive hyperglycemia ensues. Hyperglycemia per se, by virtue of its ability to stimulate IAPP biosynthesis (86,94), also feeds forward in a vicious cycle that results in further islet amyloid development and β-cell destruction. It has also been suggested that glycation of IAPP by circulating glucose may enhance amyloid fibril formation (117). The presence of apoE and perlecan in amyloid fibrils, and their potential ability to stabilize these fibrils, suggest that they also have a role in islet amyloidogenesis. Given that dietary fat increases apoE biosynthesis (118,119), it seems plausible that this production of enhanced apoE (and perlecan) may stabilize and could thus reduce degradation of existing amyloid fibrils, thereby providing a mechanism for continued amyloidogenesis and further promoting the ultimate development of the clinical syndrome.

The natural history of established type 2 diabetes is one of progressive hyperglycemia. This increasing severity of hyperglycemia, we suggest, may be largely a function of increasing amyloid formation, resulting in progressive β-cell mass loss and deficient insulin release. The increase in the degree of hyperglycemia is associated with a greater need for medications because ongoing destruction of β-cells limits the cell's responsiveness to secretagogues, including sulfonylureas. Progressive β-cell destruction by amyloid may well explain the secondary failure so frequently observed with the use of these agents. The end result is a feed-forward scenario that results in marked islet destruction, hyperglycemia, and later, an absolute need for insulin replacement therapy in subjects with type 2 diabetes. However, although this scenario may apply to the vast majority of subjects with type 2 diabetes, it may not apply to all clinical situations associated with hyperglycemia.

CONCLUDING REMARKS

The recent resurgence of interest in the role of islet amyloid in the pathophysiology of β-cell failure in type 2 diabetes coincides with the identification of IAPP and an increased understanding of the pathogenesis of the localized amyloids. We believe that islet amyloid formation plays a central role in the development of the β-cell failure of type 2 diabetes. With the use of current technology, it should be possible to test the theory that an abnormality in the processing of the amyloidogenic peptide pro-IAPP underlies the development of islet amyloid in individuals with type 2 diabetes. It is hoped that studies of the mechanisms underlying the development of islet amyloid deposits in experimental animal models will provide opportunities to test compounds capable of interrupting this process, thus potentially reducing the burden of type 2 diabetes some 100 years after Opie's original observations.

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