Perspectives in Diabetes

Is Islet Amyloid Polypeptide a Significant Factor in Pathogenesis or Pathophysiology of Diabetes?

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Islet amyloid polypeptide (IAPP) or amylin, a recently discovered minor secretory peptide of the β-cell related to calcitonin gene–related peptide (CGRP), is a constituent of amyloid deposits in the islets of many non-insulin-dependent (type II) diabetic individuals and some elderly nondiabetic subjects. IAPP is synthesized as a small precursor at a level of ~1% that of insulin and is processed, amidated, stored in β-granules, and released along with insulin and C-peptide. Analysis of its gene (located on chromosome 12) supports an evolutionary relationship to calcitonin and CGRP, peptides with which it shares some biological actions. Like CGRP, IAPP antagonizes the action of insulin mainly at the level of muscle glycogen synthesis, but the levels required for this effect seem to be considerably higher than reported circulating levels. No evidence for overproduction of IAPP in diabetic subjects has been found thus far, but much more work is necessary to define its normal secretory rates and clearance. Other proposed actions of IAPP include serum calcium–lowering effects and smooth muscle relaxation; the latter effect might promote the uptake of insulin into the circulation within the islets. Deposition of amyloid is species selective due to structural differences within the central part of the molecule and may be initiated intracellularly in type II diabetes by several mechanisms. No differences in the structure of IAPP or its precursor have been found in individuals with maturity-onset diabetes of the young or type II diabetes. The evidence available at this time does not support the view that IAPP plays a significant role in the insulin resistance of type II diabetes or that deposition of amyloid is a primary event in its pathogenesis. However, further studies of the expression and roles of IAPP may provide new insights into islet molecular biology and physiology.

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Although it is usually assumed that the pancreatic β-cell releases only a single hormonal product into the blood—insulin and its related precursor forms—studies over the past decade have revealed that β-cells also secrete smaller amounts of several other peptides and proteins. By far the most intriguing of these is the recently discovered 37–amino acid neuropeptidelike molecule islet amyloid polypeptide (IAPP) or amylin. This peptide is a major component of the amyloid deposits that occur in the islets of elderly diabetic individuals (i.e., those with non-insulin-dependent [type II] diabetes mellitus), in many benign insulinomas of the pancreas, and in the normal pancreases of the aged (1,2). The presence of amyloidlike material in specimens of human pancreas was first noted in 1901 by the pathologist Opie (3), but it was not until 1986 that its constituents were solubilized. When analyzed, this material turned out to consist mainly of the single peptide IAPP (Fig. 1). Determination of its amino acid sequence quite unexpectedly revealed that IAPP is similar in structure to a 37–amino acid neuroendocrine peptide, calcitonin gene–related peptide (CGRP) (1,2). CGRP is a second product of one of the calcitonin genes and is generated through alternative splicing of the calcitonin I gene in neural tissues (4).

HUMAN IAPP PRECURSOR AND ITS GENE

Analyses of cDNAs encoding IAPP precursors from humans and other mammals have shown these to be relatively small proteins of ~90 amino acids (5–7). The structure of the rather typical IAPP precursor is shown in Fig. 1. The presence of a glycine residue just after the COOH-terminal tyrosine of the IAPP sequence followed by the basic dipeptide cleavage signal indicates that IAPP is carboxyamidated, as are CGRP and many other neuroendocrine peptides. Analysis of the human IAPP gene has shown this to be a single-copy gene with an intron-exon pattern similar to the CGRP genes (8).

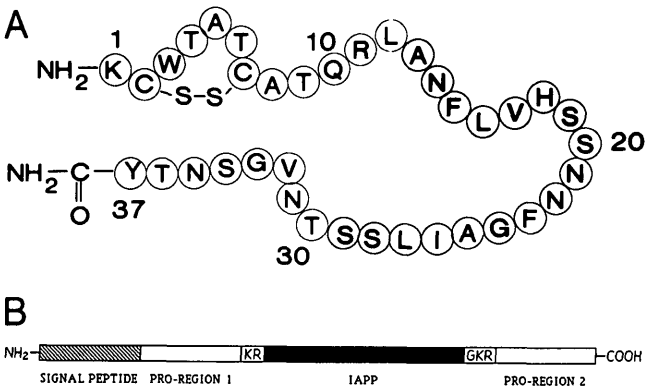

FIG. 1. *A*: structure of human islet amyloid polypeptide (IAPP). Extended region (residues 20–29) is believed to nucleate β-pleated sheets in forming amyloid fibrils (11). *B*: structure of IAPP precursor. G, glycine; K, lysine; R, arginine.

This gene is located on the short arm of chromosome 12 (8,9), whereas the CGRP genes are located on the homologous chromosome 11. These relationships are consistent with the notion that IAPP, CGRP, and calcitonin all arose from the same ancestral gene.

SPECIES VARIATIONS IN IAPP STRUCTURE

It has been known for many years that amyloid deposition occurs in the islets of diabetic animals only in certain species, among them several primates, cats, raccoons, and the degu (*Octodon degus*), a New World rodent related to the guinea pig (1,7,10). The partial amino acid sequence of cat IAPP revealed several interesting differences in the central portion of the IAPP molecule (1). Glenner et al. (11) have shown that this region within IAPP (residues 20–29; Fig. 1) has a high probability of forming insoluble β-pleated sheets. Synthetic peptides spanning this region form fibrils spontaneously in solution, as does intact IAPP in some species (11–13).

We have used the powerful technique of polymerase chain reaction to determine the structures of IAPP precursors from numerous mammals, including the macaque, rat, mouse, cat, dog, guinea pig, hamster, and degu (Fig. 2). Interestingly, in all these species, the central region of IAPP shows the greatest interspecies variations. Westermark et al. (12), Betsholtz et al. (13), and Jordan et al. (14) have concluded that residues 25 and 26 are the most important determinants of amyloid deposition. Replacement of residue 25 with proline seems to be especially critical for preventing deposition of amyloid in all the rodents except the degu (14); the central region in degu IAPP differs only by a single substitution from that of the guinea pig (15), one of the species that lacks amyloid deposits. However, Hellman et al. (16) have recently found that degu amyloid consists of insulin rather than IAPP, and the significance of this observation will be discussed below.

The cDNA sequences have also demonstrated that IAPP is highly conserved, consistent with its probable role as a hormone (7). Proregions 1 and 2 of the precursor are much more variable, reminiscent of the proinsulin C-peptide, and thus probably have no biological activity (Fig. 1). Comparison of the sequences of IAPP and CGRP reveals canonical similarities and differences between these peptides that suggest they may bind to structurally related but not identical receptors (10).

BIOSYNTHESIS AND LEVELS OF IAPP IN ISLETS

Studies with antibodies specific for IAPP have demonstrated that it is present in normal islets in significant amounts, as judged by immunocytochemical staining (17), and it has been localized by electron microscopy to the secretory granules of the β-cells (18,19). Very low levels of IAPP mRNA have been detected in the stomach and other regions of the gastrointestinal tract, in the lung, and also in the dorsal root ganglia of the spinal cord (20). The significance of the extraislet expression of IAPP is unknown. Recent biosynthetic studies in our laboratory indicate that prepro-IAPP is handled very similarly to preproinsulin (S.N., D.F.S., unpublished observations). In normal rat islets, pro-IAPP is efficiently processed into the mature amidated IAPP, stored, and subsequently cosecreted with insulin. We have not observed any significant nongranule secretion of either pro-IAPP or IAPP except in insulinoma lines, such as the mouse βTC3 line, which usually exhibit more prominent constitutive or unregulated hormone secretion.

One rather surprising result has been the finding that IAPP is a very small fraction of the level of insulin in the β-cell. Leffert et al. (6) reported that the content of IAPP mRNA in isolated rat islets is ~10% that of insulin mRNA. However, high-performance liquid chromatography analysis of freshly isolated rat islets in our laboratory showed that IAPP amounted to only ~1–2% of the level of insulin on a molar basis (9). Other studies with islets or whole-pancreas extracts also indicate that IAPP-related peptides amount to only ~1% of the level of insulin (i.e., ~0.1–0.2 nmol IAPP/g; 21–23). Biosynthetic labeling experiments confirm that IAPP synthesis in rat islets occurs at a rate that is ~100-fold lower than that of insulin and suggest that IAPP mRNA is less efficiently translated than insulin mRNA (S.N., D.F.S., unpublished observations). Obviously, a higher synthetic rate would be expected to lead to a higher level of stored IAPP than has actually been observed unless it is selectively degraded before secretion. Numerous studies in animals and humans have shown that IAPP secretion is stimulated by glucose and usually amounts to 2–5% of the amount of insulin released (23–27). However, in the basal state, IAPP

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            .         .         .         .         .
Human       KCNTATCATQRLANFLVHSSNNFGAILSSTNVGSNTY NH2
Monkey      ----------------R------T---------D-- NH2
Cat         ----------------IR----L-----P-------- NH2
Dog         ----------------RT---L-----P-------- NH2
Rat         ----------------R----L-PV-PP-------- NH2
Mouse       ----------------R----L-PV-PP-------- NH2
Hamster     ------------------N--L-PV--P-------- NH2
Guinea pig  ------------T----R--H-L--A-LP-D------ NH2
Degu        ------------T----R--H-L--A-PP-K------ NH2
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FIG. 2. Comparison of known islet amyloid polypeptide amino acid sequences. Note that differences are more frequent in central amyloidogenic portion of molecule and may partially explain species differences in amyloid occurrence (see text). Sources of sequences: human (1,2,5); rat (6,7); cat (7,13); mouse, guinea pig (7); degu (14); hamster (54); dog and monkey (15; S.O., M.N., G.I.B., D.F.S., unpublished observations). A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine.

amounts to only ~30% of the total immunoreactive material due to the presence of smaller circulating fragments (24). Basal values for IAPP in humans are typically reported to be 1.5–2.5 pM rising to 7–10 pM after glucose ingestion. Higher levels are found in obesity (27), but no study shows any increase in IAPP levels in individuals with type II diabetes relative to suitable controls (25,26). As expected, IAPP levels are markedly reduced in individuals with insulin-dependent diabetes receiving insulin (26).

BIOLOGICAL ACTIONS OF IAPP

Great controversy surrounds the possible biological actions of IAPP in modifying the secretion or responses to insulin in the organism. Synthetic rat IAPP-amide inhibits insulin secretion from rat islets of Langerhans, but the doses required for this effect (10^{-5} M) are extremely high (28). At lower doses (10^{-7}–10^{-9} M), no significant effects of IAPP (amidated or nonamidated) on either insulin secretion or biosynthesis in isolated rat islets have been seen. On the other hand, Leighton and Foot (29) and Cooper et al. (30) have shown that 10 nM IAPP significantly inhibits glycogen synthesis in muscle exposed to the peptide in vitro, an effect it shares with CGRP. Moreover, euglycemic glucose-clamp studies with dogs (31) and rats (32) have demonstrated that IAPP-amide inhibits insulin-stimulated glucose disposal over short infusion periods. Again, similar effects were noted with CGRP (32). In these experiments, the rates of IAPP infusion were 3- to 6-fold (31) or 385-fold (32) higher on a molar basis than the rates of insulin infusion. Such high ratios of secretion of IAPP relative to insulin cannot conceivably occur under normal physiological conditions in vivo, where IAPP is, at most, 10% of the level of insulin. Binding of IAPP to liver membranes has been reported (33), but no effects on insulin sensitivity were found in perfused liver by Roden et al. (34). Thus, the observed induction of an insulin-resistant state in vivo must be viewed as a pharmacological rather than physiological effect.

A more plausible possibility might be that IAPP plays a local role in the islets, although it does not appear to affect the secretion of insulin (28) or the other islet hormones (35). However, it may affect the surrounding acinar tissue or alter the rate of blood flow through the islets when insulin release is stimulated. The potent vasodilating effects of CGRP are shared by IAPP (36), lending credence to the latter possibility. An additional interesting action of IAPP is its serum calcium–lowering effects in animals in vivo and cell-culture systems (37). A direct effect on uptake of calcium by bone tissue has been demonstrated, but it is not clear whether this effect is mediated via calcitonin or IAPP receptors. MacIntyre (37) proposed that IAPP may be secreted along with insulin to promote the utilization of ingested calcium; however, the physiological relevance of such an action remains to be demonstrated.

Because IAPP seems to share some actions of CGRP, a family of neuropeptides that are expressed in the nervous system and at nerve endings in many organs throughout the body (4,10,38,39), it is plausible that they both act through similar receptors. The main function of CGRP in peripheral tissues appears to be mediated via cAMP and involves smooth muscle relaxation leading to bronchial dilation, lowering of blood pressure, and decreases in intestinal motility (39). CGRP may also play a role in regulating growth hormone secretion (40) or as a growth factor, regulating the development of certain neurons during embryogenesis (41). Although CGRP binding sites have been identified in some tissues, the nature of its receptor has not been well characterized. In view of its modulating effects on adenylate cyclase, it is conceivable that the CGRP (and IAPP) receptors are members of the G-protein–coupled receptor family (42).

MECHANISM OF AMYLOID DEPOSITION IN DIABETIC ISLETS

Amyloid fibrils occur in various disease syndromes, but all of them are characterized by the deposition of β-pleated sheets arranged in insoluble fibrillar arrays, often caused by mutations or cleavages in proteins that render them susceptible to fibril formation (43). The presence of IAPP in islet amyloid has been demonstrated by both light and electron microscopic immunocytochemistry (17,18). Although, in normal β-cells, IAPP is found only within the insulin secretory granules (19,20), in some patients with type II diabetes, fibrillar immunoreactive amyloid deposits have also been found within the cytoplasm of β-cells (17). High concentrations of IAPP immunoreactivity have been noted in lysosomes and lipofuscin bodies within the β-cells of the islets of both nondiabetic and diabetic individuals (44,45). These findings suggest that, in some individuals, amyloid forms during the intracellular degradation of secretory granules, as occurs in the normal turnover of unused secretory stores, a process known as *crinophagy*. It is possible that, during crinophagy, acidic conditions within the lysosomes may bring about the precipitation of protease-resistant aggregates of IAPP. This material may then remain behind, along with other undegradable by-products, e.g., lipofuscin, and be extruded from the β-cell. Alternatively, it may be retained, possibly leading to altered cell function, degeneration, and necrosis (10,44,45).

What is it about the diabetic state that leads to amyloid deposition? One possibility, often discussed, is that insulin is overproduced in type II diabetes due to acquired or inherent resistance to its action (46). This may also result in increased secretion of IAPP (46–48). On the other hand, there is also a large body of evidence that glucose responsiveness is impaired in the islets of individuals with type II diabetes (49). This could result in the intracellular accumulation of increased numbers of secretory granules, formed in response to hyperglycemia but not released normally. These granules will eventually age and then undergo crinophagy, as discussed above, generating fibrillar material in the lysosomes and multivesicular bodies that later will be extruded from the cells. Both of the foregoing hypotheses envision overproduction of IAPP (and insulin) as a primary factor, but the latter theory posits an associated block in granule release, resulting in increased intracellular turnover of both IAPP and insulin in type II diabetes. Selective overexpression of IAPP (relative to insulin) occurs in some islet cell tumors and could account for the selective deposition of amyloid in insulinomas in the dog (50; S.N., D.F.S., unpublished obervations). The dog is curious, because although its IAPP has essentially the same amyloidogenic sequence as the cat (Fig. 2), islet amyloid deposition is never seen (14). Dexamethasone administration and streptozocin-

induced diabetes have both been reported to lead to the relative enhancement of IAPP gene expression in rats (51). Although the latter finding seems paradoxical in view of the loss of β-cells, this kind of mechanism could conceivably play a role in the increased deposition of amyloid in some diabetic individuals and not others.

Antiserums against the NH_2-terminal propeptide of the IAPP precursor have demonstrated immunoreactivity in amyloid deposits (Fig. 1), suggesting a possible role for altered processing of IAPP in the generation of the deposits (52). This immunoreactivity could either represent intact pro-IAPP or its NH_2-terminal prosegment (proregion 1), which, after proteolytic cleavage, would probably be cosecreted with IAPP. However, note that pro-IAPP–related peptide sequences have not been described as components of solubilized amyloid. We recently sequenced the IAPP genes of 25 selected individuals with type II diabetes without finding any abnormalities in the structure of either IAPP or its precursor (53). The fact that amyloid indistinguishable from that occurring in diabetic individuals is formed to a lesser extent in normal islets during aging argues strongly that amyloid deposition is simply a secondary manifestation of disordered islet function. The fact that islet amyloid consists of insulin in the diabetic degu (16) also greatly strengthens the conclusion that IAPP is not per se a diabetogenic molecule but rather is deposited as a result of disordered β-cell function associated with diabetes and aging. Whether the amyloid deposits intensify islet dysfunction in type II diabetes remains an unresolved issue.

Much remains to be learned about the intracellular processing and secretion of IAPP and the genetic mechanisms that may modulate its expression as well as its receptors and mechanism of action. Its role in the normal physiology of the islets of Langerhans represents a challenging and unresolved issue and, in the final analysis, may prove to be more illuminating than its proposed role in the causation of type II diabetes.

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