

# Newly Identified Pancreatic Protein Islet Amyloid Polypeptide

## What Is Its Relationship to Diabetes?

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Islet amyloid polypeptide (IAPP) or amylin is a newly identified 37-amino acid COOH-terminal-amidated polypeptide that is the major protein constituent of amyloid deposits in insulinomas and amyloid deposits in pancreatic islets of non-insulin-dependent (type II) diabetic humans and adult diabetic cats. IAPP is stored with insulin in  $\beta$ -cell secretory vesicles and is cosecreted with insulin in response to glucose and several secretagogues. IAPP has been demonstrated in normal pancreatic islets of many species, but IAPP-derived amyloid develops commonly in the islets of only a few species (e.g., humans and cats), especially in association with age-related diabetes. IAPP from the human and cat inherently contains a short amyloidogenic sequence that is not present in species that do not form islet amyloid. Studies in animals indicate that an aberration in the synthesis or processing of IAPP, leading to a local increase in concentration of IAPP in the islet, is also required to facilitate the conversion of IAPP to amyloid. The formation of islet amyloid may contribute to the development of type II diabetes by causing disruption of islet cells and by replacement of islets. It has also been proposed that an abnormality of IAPP homeostasis underlies the pathogenesis of type II diabetes. A significant causal relationship between IAPP and type II diabetes is based on reports that IAPP inhibits glucose-stimulated insulin release by  $\beta$ -cells and that IAPP inhibits insulin-stimulated rates of glycogen synthesis and glucose uptake by skeletal muscle cells. These findings clearly have potentially great relevance to type II diabetes in that impaired insulin secretion and peripheral resistance to insulin are the clinical hallmarks of this form of diabetes. However, studies generally have not supported a role

for IAPP as a physiologically relevant modulator of insulin secretion, and it is yet to be demonstrated whether IAPP in physiological concentrations can induce the peripheral insulin resistance that is characteristic of type II diabetes. The potential role of this newly identified pancreatic polypeptide in the genesis of type II diabetes thus needs further investigation and confirmation in model systems utilizing physiological concentrations of IAPP. The significance of the strikingly greater responsiveness of IAPP secretion relative to insulin in severely hyperglycemic states also is not clear. However, this observation may provide an important clue to the normal function of IAPP and points to an important area of future exploration. *Diabetes* 40:310-14, 1991

**T**he recent discovery of a previously unknown pancreatic islet protein, known as islet amyloid polypeptide (IAPP) or amylin, has provided a new focus of interest and investigation in the field of diabetes. These investigations have led to the consideration of IAPP as a significant pathogenetic factor in the development of non-insulin-dependent (type II) diabetes mellitus, a condition in which IAPP is known to aggregate and form islet amyloid deposits in the vast majority of cases.

A considerable amount of information has been obtained about IAPP since the identification of its partial sequence in 1986, but significant aspects of the relationship between IAPP and diabetes are yet to be clearly elucidated. The implications of these various findings with respect to the development of type II diabetes are discussed here.

### IS IAPP A NEW ISLET HORMONE?

"Hyalinosis" of the pancreatic islets (1), now known to represent localized amyloid deposits, was detected as a common morphological feature of type II diabetic humans as early as 1900. These islet amyloid deposits, also known to occur in diabetic nonhuman primates (2) and adult diabetic cats (3,4), are composed of characteristic 8- to 10-nm pro-

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tein fibrils that bind Congo red dye and subsequently elicit green birefringence when viewed with polarized light (5).

The significance of islet amyloid and its role in the pathogenesis of type II diabetes have always been a matter of controversy. Its potential significance was especially obscured (and often dismissed) as a simple consequence of aging due to its occasional presence in the pancreatic islets of apparently nondiabetic adults (5–8). Also, its significance was questioned because it is not a feature of insulin-dependent (type I) diabetes and does not occur in commonly studied animal models of diabetes.

Our early investigations clearly showed that islet amyloid in humans and cats was histochemically and physiochemically distinct from the so-called *primary* or *secondary* (i.e., reactive) systemic forms of amyloid that commonly affect the liver, kidneys, and spleen (9–11). These observations led us to postulate that islet amyloid was derived from a local secretory product and that determination of the chemical nature of this amyloid protein might provide an important new clue or insight into the pathogenesis of type II diabetes. This rationale was significantly linked to the knowledge that islet amyloid deposits are present in the islets of >90% of type II diabetic humans (12) and ~80% of adult diabetic cats (13), which is a much greater incidence than in nondiabetic age-matched control subjects.

Delineation of the chemical nature of the islet amyloid protein was a significant challenge because of the extreme insolubility and low concentration of islet amyloid in the pancreas. Using amyloid-containing human insulinoma (14) and pancreases from type II diabetic humans and aged diabetic cats (15,16), we purified the major protein component of islet-derived amyloid and showed that it was composed of a previously unknown 37-amino acid polypeptide. This newly identified protein, which we named IAPP, has substantial NH<sub>2</sub>- and COOH-terminal homology to the 37-amino acid neuropeptides identified as calcitonin gene-related peptides (CGRP) 1 and 2 (17,18). CGRP 1 and 2 are widely distributed in the nervous system and have been reported to have several biological effects, including vasodilation, inhibition of gastric acid secretion, and inhibition of insulin secretion. A group of investigators at Oxford University subsequently isolated IAPP from pancreases of patients with type II diabetes (19) and later called the polypeptide amylin (20).

Several reviews of IAPP have already been published (21–23); refer to these for more complete details of studies related to this putative hormone. Briefly, investigations have shown that IAPP is normally stored with insulin in  $\beta$ -cell secretory vesicles (24–26) and is cosecreted with insulin in response to glucose and several secretagogues (27–33). Studies employing in situ hybridization with cDNA probes for IAPP have also shown that the  $\beta$ -cells are the predominant site for IAPP expression (34). These findings explain why islet amyloidosis is not known to occur in type I diabetes, in which  $\beta$ -cells have been selectively destroyed. Genomic and cDNA studies indicate that human and rat IAPP are derived from 89- and 93-amino acid prepro-IAPP molecules, respectively, and that IAPP is normally carboxamidated (22,34–36). The NH<sub>2</sub>- and COOH-terminal regions are highly conserved between species (and also with CGRP), whereas considerable heterogeneity is present between species within the 20–29

region. Therefore, the NH<sub>2</sub>- and COOH-terminal regions are probably important for the biological activity of IAPP. Biological activity of IAPP also appears to be importantly linked to amidation of tyrosine at position 37 and the presence of an intramolecular disulfide bond between cysteine residues at positions 2 and 7 (37). The gene coding IAPP in humans is located on the short arm of chromosome 12, which may be an evolutionary homologue of chromosome 11, where the CGRP genes are located (38,39).

It is interesting that IAPP, which is cosecreted with insulin by islet  $\beta$ -cells, was not discovered until nearly 65 yr after the discovery of insulin by Banting, Best, and colleagues. It is also ironic that the islet amyloid deposits, which were observed for >85 yr but considered by many to be relatively insignificant bystanders in the development of type II diabetes, provided the abnormally high concentration of hormone that importantly facilitated the discovery of IAPP.

#### AMYLOIDOGENICITY OF IAPP

Only certain species (e.g., humans, nonhuman primates, and cats) develop islet amyloid, usually in conjunction with diabetic syndromes that are associated with aging (21). Several avenues of in vitro and in vivo investigations have provided important clues to factors contributing to the polymerization of IAPP to form islet amyloid in these species. It is not likely that an abnormal form of IAPP is involved in the formation of islet amyloid, because the structure of IAPP derived from islet amyloid is identical to the sequence predicted via cDNA. However, the normal inherent differences in the primary structure within the 20–29 region of IAPP between different species appear to be importantly linked to the ability of IAPP to aggregate and form amyloid fibrils. For example, synthetic IAPP peptides corresponding to the human (36,40) and cat (41) sequence in this region aggregate to form Congoophilic amyloidlike fibrils in vitro, whereas synthetic IAPP 20–29 of the hamster, rat, and mouse (which do not develop islet amyloid in vivo) lacks the ability to form fibrils (42,43). The 25–28 region of human and cat IAPP is identical in structure (i.e., Ala-Ile-Leu-Ser [AILS]) and appears to be the most important amyloidogenic sequence common to human and cat IAPP. Single-amino acid substitutions in the 25–28 region of synthetic human IAPP 20–29 significantly reduce or eliminate the amyloidogenicity of the peptides in vitro (43). Also, IAPPs from species that do not form islet amyloid in vivo diverge significantly from the human and cat in the 25–28 region.

We have recently shown that dog IAPP, like human and cat IAPP, incorporates the putative amyloidogenic AILS sequence at positions 25–28 (44). However, dogs do not develop IAPP-derived islet amyloid but do form IAPP-derived amyloid deposits in insulinomas (45). The studies in dogs show that a species-specific IAPP structural motif alone is clearly not adequate for the conversion of IAPP to amyloid fibrils in vivo. It appears likely that aberrations in  $\beta$ -cell synthesis (or processing) of IAPP, leading to increased concentration of IAPP in the local milieu, provides a second prerequisite for the conversion of IAPP to amyloid. We have also demonstrated increased IAPP immunoreactivity in  $\beta$ -cells of normoglycemic cats with impaired glucose tolerance (13). These cats have an increased incidence of IAPP-derived islet amyloid, which provides additional evidence for

an aberration in IAPP production and/or processing that facilitates amyloidogenesis. However, we do not know whether the increased IAPP immunoreactivity in the  $\beta$ -cells of cats reflects a primary or secondary  $\beta$ -cell alteration.

### BIOLOGICAL FUNCTION OF IAPP AND ITS RELATIONSHIP TO DIABETES

The biological functions and roles of IAPP are yet to be clearly elucidated. However, IAPP is a highly conserved  $\beta$ -cell hormone that has been shown by numerous investigations in humans, animals, and in vitro systems to be cosecreted in a biphasic pattern (in parallel with insulin) in response to glucose administration (27–33). Interestingly, IAPP output in hyperglycemic states (or supraphysiological ranges of glucose) far exceeds that of insulin output, i.e., the effects of glucose on IAPP and insulin release are strikingly dissociated when high glucose levels are achieved. It has been shown in perfused rat pancreases, for example, that although glucose or arginine stimulated the secretion of insulin and IAPP in a parallel fashion and in similar relative potencies, the amount of IAPP relative to insulin increased with the more potent combined glucose-arginine stimulus (28). Utilizing perfused pancreases from rats made severely hyperglycemic, we also observed extremely high IAPP secretion with high IAPP-to-insulin ratios (unpublished observations). Similarly, in adult rats injected with 12 daily injections of dexamethasone, a 16-fold elevation in pancreatic IAPP mRNA was observed in contrast to only a 4-fold increase in insulin mRNA levels (46). The relatively greater responsiveness of IAPP secretion relative to insulin in hyperglycemic states appears paradoxical if it is presumed that the physiological role of IAPP is to oppose insulin release or activity, i.e., the demands of glucose homeostasis would seem to require suppression of IAPP synthesis in this situation (46). The explanation for this apparent paradox is not clear on the basis of our knowledge of IAPP.

A significant pathogenetic relationship between IAPP and type II diabetes has been considered probable on the basis of both early and recent observations. This hormone, which is cosecreted with insulin by islet  $\beta$ -cells in response to hyperglycemia, is known to be conserved and expressed in humans and many animal species. Islet amyloid, which is the most consistent and conspicuous morphological feature of type II diabetes, is a polymerized product of IAPP known to occur in the islets of only a relatively few species (e.g., humans, cats, nonhuman primates) that also develop age-related diabetes. Also, IAPP is structurally similar to CGRP, and CGRP has earlier been shown to inhibit insulin secretion in several animal species (47).

The potential roles of IAPP in the pathogenesis of type II diabetes may be classified into at least three general categories: 1) formation of islet amyloid with resultant damage to and replacement of  $\beta$ -cells, 2) local (or paracrine) effects on the secretion of insulin or other islet hormones, and 3) hormonal effects on peripheral tissues. It is possible that more than one of these mechanisms or other as yet undefined roles may be operative.

Potentially significant causal relationships between IAPP and type II diabetes have recently been proposed by reports indicating that IAPP can inhibit glucose-stimulated insulin

release by  $\beta$ -cells and that IAPP is a potent inhibitor of insulin-stimulated rates of glycogen synthesis and glucose uptake by skeletal muscle cells. These observations clearly have potentially great relevance to type II diabetes in that impaired insulin secretion and peripheral resistance to insulin are the clinical hallmarks of this form of diabetes.

The relationship of IAPP to insulin secretion has been evaluated in several laboratories. An initial in vitro study indicated that insulin secretion by rat pancreatic islets was significantly inhibited by synthetic IAPP but only at the very high dose of  $10^{-5}$  M (48). Subsequent in vitro and in vivo studies clearly have not supported a role for IAPP as a physiologically relevant modulator of insulin secretion (49–53). The finding that IAPP does not inhibit insulin secretion at dose levels used in previous CGRP-induced insulin suppression models (53) is especially interesting and somewhat surprising in that there is a high degree of  $\text{NH}_2$ - and  $\text{COOH}$ -terminal homology between IAPP and CGRP. The recent studies by Nagamatsu et al. (54), showing no effect of  $10^{-6}$  M IAPP on insulin mRNA levels or of  $10^{-4}$ – $10^{-9}$  M IAPP on proinsulin biosynthesis by isolated rat islets, are also consistent with the likelihood that IAPP is not a physiologically relevant modulator of insulin secretion.

Cooper et al. (22) proposed that IAPP is a glucoregulatory hormone that can contribute to the pathogenesis of type II diabetes when it is overproduced by islet  $\beta$ -cells. They proposed that IAPP is normally a glucoregulator that can act in concert with insulin as a signal to switch the site of carbohydrate disposal from glycogen in skeletal muscle to longer-term stores of triacylglycerol in adipose tissue. It was suggested that this could be achieved by making skeletal muscle relatively insulin resistant while leaving rates of insulin-stimulated carbohydrate metabolism in adipose tissue unaltered (22). These investigators thus proposed a specific role for IAPP in the development of peripheral insulin resistance in type II diabetes based on the premise that increased secretion of this polypeptide can downregulate insulin-stimulated rates of glycogen synthesis and glucose disposal in skeletal muscle cells (20,55–57). The postulation that IAPP is responsible for the insulin resistance observed in type II diabetes is based on in vitro and in vivo studies utilizing  $10^{-9}$  M concentrations of IAPP and CGRP (20,55–57). Therefore, although the rates of insulin-stimulated glycogen synthesis and glucose disposal were inhibited by >50% with both CGRP and IAPP, the concentrations used to produce these effects were ~1000-fold greater than the circulating levels (i.e., 2–20 pM) of IAPP that have been documented in nondiabetic humans. Therefore, it is questionable whether IAPP in physiological concentrations can be responsible for the insulin resistance observed in type II diabetes.

Hothersall et al. (58) recently reported that CGRP and IAPP at 1- $\mu$ M concentrations inhibited insulin-stimulated 2-deoxy- $^3\text{H}$ glucose transport in isolated rat diaphragm by 30 and 60%, respectively. Their study thus implicated decreased glucose transport rather than reduced glycogen synthesis (as reported by Leighton and Cooper [55] and Leighton and Foot [57]) as the specific mechanism for insulin antagonism by IAPP. The significance of this study, implying a potential role for IAPP in causing increased peripheral insulin resistance by impairment of insulin-stimulated glucose transport,

is obviously also questionable on the basis of the extremely high concentrations of IAPP and CGRP used in the study.

Although impaired glucose tolerance and decreased glucose clearance have been reported in cats (59) and dogs (60), other *in vivo* efforts to modify glucose tolerance with IAPP have provided negative results. Bretherton-Watt et al. (49) were unable to demonstrate any effect of IAPP-amide on glucose clearance in seven nondiabetic humans that were infused to circulating IAPP plasma levels  $>1$  nM. Likewise, Ghatei et al. (51), after administration of a single intravenous bolus of IAPP-amide (500 pmol) to rats or a continuous infusion of IAPP-amide in rabbits, failed to demonstrate either suppressed plasma insulin levels or elevated blood glucose levels.

In summary, the role of IAPP in the pathogenesis of type II diabetes has not as yet been clearly established. In particular, studies have not supported a role for IAPP as a physiologically relevant modulator of insulin secretion. However, based on evidence that an increased local concentration of IAPP is a necessary factor for amyloidogenesis within the islet, a possible paracrine effect of IAPP that is in fact associated with unusually high hormone levels within the islets cannot be ruled out. The implicated role for IAPP in the induction of the peripheral resistance in type II diabetes is of substantial potential significance, but confirmation for such a role is needed in model systems utilizing physiological concentrations of IAPP.

#### SUMMARY AND FUTURE DIRECTIONS

Evidence most clearly indicates that IAPP is cosecreted with insulin by islet  $\beta$ -cells in response to hyperglycemic states. A relatively greater responsiveness of IAPP secretion relative to insulin secretion is observed in marked hyperglycemia. The significance of this profound responsiveness to marked hyperglycemia is not clear at this time. We have considered a possible role for IAPP in the prevention of glucose-induced modifications of structural proteins, which are known to occur in prolonged hyperglycemic states. This appears to be an important area of future exploration in that evidence for such a role for IAPP would obviously have important ramifications in the treatment of diabetes.

Prolonged hyperglycemia may provide the essential stimulus for increased production of IAPP leading to a concentration in the local milieu that promotes the aggregation of IAPP to form islet amyloid deposits. However, aggregation of IAPP to form amyloid occurs only in those few species (e.g., humans and cats) where IAPP inherently contains the short amyloidogenic sequence (i.e., AILS) at positions 25–28. Therefore, islet amyloid is not a morphological marker for chronic hyperglycemia (i.e., diabetes) in other species. Islet amyloid deposition in humans, cats, and monkeys may contribute to the further development of the diabetic condition through its progressive deposition and local disruption of islet morphology and function. Islet amyloid is not a feature of the prolonged hyperglycemic condition induced in type I diabetes because of the selective destruction and subsequent absence of islet  $\beta$ -cells. A primary defect in the synthesis of IAPP by  $\beta$ -cells, resulting from genetically determined aberrations in expression of the IAPP gene, cannot be excluded on the basis of available information.

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