Islet amyloid polypeptide: actions and role in the pathogenesis of diabetes

A. Clark*, S. B. P. Charge*, M. K. Badmant, D. A. MacArthurt and E. J. P. de Koning†


Introduction

Islet amyloid polypeptide (IAPP), also known as 'amylin', is the component peptide of amyloid deposits found in islets of 90% of Type-2 diabetic patients. Since its identification in 1987 in extracts of diabetic pancreas and insulinomas [1,2], IAPP has become better known as a putative hormone involved in glucose metabolism. Establishment of a physiological function for IAPP has been driven by the potential of IAPP analogues as therapeutic agents for treatment of diabetes; putative physiological actions of IAPP include regulation of insulin action and secretion, inhibition of gastric emptying, vasodilatation and modulation of Ca2+ metabolism [3,4]. If all these actions could be confirmed, IAPP would be a major controlling agent for nutrient metabolism.

Expression, secretion and degradation of IAPP

IAPP is a 37-amino acid peptide with close similarity to calcitonin-gene-related peptide (CGRP) [1,2] and is expressed in the β-cells of both normal and diabetic humans and in all animal species examined so far (Figure 1) [5]. IAPP is also expressed in cells of the gastric mucosa of humans and rodents and in somatostatin-containing δ-cells of rats and mice [6,7]. It is co-localized with insulin in the β-cell granules [8] and co-secreted with insulin in response to β-cell secretagogues [9]. Factors controlling IAPP production appear to be closely linked to insulin production, and therefore to nutrient intake, in normal, obese and diabetic conditions [10–12]. However, expression of IAPP can be dissociated from insulin production by dexamethasone: a disproportional increase in IAPP mRNA compared with that of insulin is induced by dexamethasone in rats [13]. The role of steroids on β-cell gene expression is unclear since dexamethasone also increases the proportion of secreted unprocessed proinsulin in humans [14]. This suggests that steroid-induced changes in expression of processing enzymes in the pancreas can affect secretion.

Like insulin, IAPP is derived from a larger propeptide by proteolytic processing in the β-cell (Figure 1). Since IAPP is present in insulin granules, the prohormone convertases PC2 and PC1/3, which are responsible for production of

Abbreviations used: IAPP, islet amyloid polypeptide; CGRP, calcitonin-gene-related peptide.

Figure 1

Amino acid sequence of prolAPP in 12 mammals

The sequence of IAPP-(1–37) is well conserved. Residues 20–29 determine the amyloidogenic potential of the peptide: compared with primate and feline IAPP, substitution of proline in the region IAPP-(24–28) prevents rat, mouse and hamster IAPP forming β-sheets and amyloid fibrils. IAPP is derived from prolAPP by proteolytic cleavage at the C- and N-termini and the processing sequences are well conserved.
mature insulin from proinsulin [15], are candidate enzymes for proIAPP processing. Studies in an in vitro translation/translocation system have shown that human proIAPP is cleaved by PC2 but not by PC3 or by furin [16]. There is an increase in the proportion of proinsulin secreted in Type-2 diabetes [17] and it is unclear whether abnormal processing is a factor in islet amyloid fibril formation in diabetes.

IAPP is co-secreted with insulin in response to β-cell secretagogues in a ratio of 10–50 to 1 (insulin to IAPP) [9–12]. The amount of circulating IAPP is between 5 and 15 pmol/l in fasting conditions and is elevated after β-cell stimulation. Plasma IAPP concentrations increase in parallel with insulin in insulin-resistant states in humans and animals [10]. IAPP is absent or reduced in Type-1 diabetes and in C-peptide-negative Type-2 diabetic subjects [18] but is unchanged in Type-2 diabetes. It is therefore unlikely that elevated IAPP levels promote pathological features of Type-2 diabetes, such as insulin resistance and amyloid deposition.

Degradation and excretion of IAPP may be important factors in amyloid fibril formation. The circulating concentrations of IAPP are elevated in patients with renal failure on dialysis treatment suggesting that it is largely excreted via the kidney [19]. These patients also have an increased prevalence of islet amyloid, suggesting that impaired clearance from the circulation could be a factor in fibril formation [20].

IAPP is degraded at intracellular sites. Its highest intracellular concentration can be found in β-cell lysosomes in normal and diabetic humans and monkeys [8]. Soluble human IAPP enters lysosomes largely by crinophagy (fusion of unsecreted insulin granules with lysosomes) but, unlike insulin, IAPP is inefficiently degraded by lysosomal enzymes in primate cells. This lack of degradation by lysosomal enzymes is a feature only of primate (and possibly feline) IMP. Rodent IAPP is more efficiently degraded in β-cell lysosomes since IAPP immunoreactivity is only apparent in mouse islets when proteolytic enzymes are inhibited [22].

Extracellular IAPP is taken up by tissue macrophages. IAPP immunoreactivity is found in macrophage lysosomes in amyloid-containing pancreatic tissue in diabetic monkeys and human insulinomas [23]. This may represent uptake of proteolysis-resistant fibrillar material since synthetic human IAPP in fibrillar form is taken up by macrophages in vitro and retained in lysosomes for periods of days [24].

Potential roles for IAPP in nutrient metabolism

IAPP has been proposed as a circulating hormone with several physiological roles (Figure 2) many of which it shares with CGRP [3]. IAPP, like CGRP, has been shown to decrease insulin-induced glycogenesis in skeletal muscle in vitro and has therefore been proposed as a regulator of glucose disposal with an important role in insulin resistance in diabetes [25]. However, this hypothesis could not be substantiated in vivo in humans and other animal experiments: glucose disposal after an infusion was not reduced by very high circulating concentrations of synthetic IAPP in humans and experimental animals [26,27]. In addition, it has been suggested that IAPP, by promoting lactate formation in muscle, potentiates hepatic glycogenesis from lactate [28,29]: this potential effect of IAPP is the basis of a putative therapy for Type-1 diabetes to maintain hepatic glycogen stores and reduce insulin-induced hypoglycaemia. However, transgenic mice expressing elevated levels of rat IAPP have neither insulin resistance nor elevated plasma lactate [30].

IAPP has been shown to have an effect on β-cell secretion and to be able to act as a paracrine regulator of islet function [31]. It decreases insulin secretion in perfused pancreas in vivo and in isolated β-cells [32,33]. Its mode of action is not entirely clear but an action on stimulus-secretion coupling has been proposed [34]. It has been suggested from observations made with the so-called IAPP blocker that IAPP exerts a tonal inhibition of β-cell secretion. CGRP (8–37) not only prevents the inhibitory effects of exogenous IAPP on β-cell secretion but increases insulin output [33]. IAPP could therefore join somatostatin and glucagon as paracrine regulators of insulin secretion.

A hormone mediating nutrient metabolism requires specific receptors on target organs. Specific binding sites for IAPP on muscle, islet cells and in the liver have yet to be identified. IAPP interacts with binding sites for CGRP and calcitonin in many other tissues, and high-affinity IAPP-binding sites have been identified in the lung [35] and brain [36]. However, IAPP is not expressed in these organs.
IAPP, like CGRP and calcitonin, causes vasodilatation and modulates Ca²⁺ metabolism but is less effective than the two latter hormones. Recently synthetic IAPP and its homologue triproamylin (a substituted form of rat IAPP) has been shown to reduce the rate of gastric emptying in rats and humans [37]. This effect of IAPP may prove to have a therapeutic role in diabetic subjects to prolong the period of glucose absorption and reduce rapid postprandial elevations of blood glucose. It is unclear if this is a normal physiological effect of IAPP: a small number of cells containing IAPP are present in the gastric mucosa [6] but IAPP receptors have not been found on gastric smooth muscle. It is possible that effects of IAPP on gastrointestinal motility are mediated via the central nervous system and vagus nerve since high concentrations of exogenous IAPP cause nausea and vomiting [38].

Despite the many and various potential physiological roles of IAPP on nutrient metabolism, there is no clear indication that circulating IAPP precipitates the onset of hyperglycaemia or contributes to insulin resistance in Type-2 diabetes.

The role of IAPP in the pathogenesis of diabetes

At post mortem, islet amyloid formed from IAPP is present in up to 90% of diagnosed Type-2 diabetic patients and has been described in Caucasian, Japanese, Asian and Pima Indian diabetic subjects. Amyloid can be found in 0.5–90% of islets and may occupy up to 80% of the islet mass [39]. The relationship between the development of islet amyloid and the onset and progression of diabetes is unclear: amyloid is present before the onset of hyperglycaemia in spontaneously diabetic cats and monkeys and progressively increases during the course of the diabetic syndrome in these species [40,41]. Amyloid deposition is associated with a reduction in islet cell population in both humans and animals: the β-cell mass is decreased by 90% in diabetic monkeys and associated with a massive deposition of amyloid in every islet. It is unlikely that amyloid deposition precipitates the onset of hyperglycaemia in humans since some diabetic subjects have less than 2% of islets affected even after 30 years of diabetes [39]. At the early stages of amyloid formation, amyloid deposits are in the
form of relatively thin rings surrounding islet capillaries. However, the extent of islet amyloidosis (more amyloid in a larger number of islets) is increased in subjects with severely decreased islet function as shown by the need for insulin therapy [42]. This suggests that, in susceptible diabetic subjects, amyloid deposition is more rapid leading to β-cell destruction.

The factors causing amyloidosis-associated islet cell loss are unknown. Extensive perivascular amyloid deposits could reduce nutrient transfer to the islet cells resulting in necrosis. Recent reports indicate that synthetic IAPP fibrils are toxic to islet cells in vitro and that the cell death occurs within a period of 24 h by a process of apoptosis [43]. Such a rapid response to deposition of fibrils is not characteristic of any form of amyloidosis in vivo. Deposition of amyloid at many sites in the body is a pathological process which is clinically silent until the size of the deposit affects the function of the host tissue. Amyloid fibrils are formed in islets isolated from transgenic mice expressing the gene for human IAPP. However, these islets containing biosynthetic IAPP fibrils showed no signs of cell death during culture for 2–3 weeks [44], suggesting that either cells are protected when in the islet environment or that biosynthetic fibrils are not toxic to cells. We have shown that, although high concentrations of fibrillar synthetic human IAPP cause necrosis of isolated β-cells in vitro, no effects of the fibrils on the viability of intact mouse or human islets could be identified [45]. The mechanism of interaction of fibrils with cultured cells remains to be determined.

Fibrils are formed initially adjacent to the β-cells in the perivascular space so that even small deposits may contribute to decreased islet function by reducing the efficiency of nutrient transfer to the β-cell and subsequent insulin release. Furthermore the plasma membrane of β-cells adjacent to the amyloid deposits have deep invaginations filled with amyloid fibrils (Figure 3). These changes in the membrane could impair normal function of nutrient signalling and insulin release at this region of the cell.

The causal factors for amyloid deposition are known but could be related to nutrient-induced hypersecretion of β-cells. Amyloid is formed in hypersecreting human insulinomas and massive amyloidosis has been described in a patient with severe insulin resistance and hyperinsulinaemia [46].

Fibrillogenesis of synthetic human IAPP is concentration- and pH-dependent: fibrils form more readily at neutral or alkaline pH and aggregation is promoted by the presence of insulin [47]. Fibrillar amyloid is not present in islets of transgenic mice hyperexpressing the gene for human IAPP despite circulating concentrations of IAPP up to 30 times higher than in control mice [21]. However, fibrillar amyloid develops in islets isolated from these transgenic mice within 24 h of culture in elevated glucose concentrations (11–28 mM) [44]. Fibrils form between and within the cells and the amount of amyloid is dependent upon the glucose concentrations. However, fibril formation is not due to extracellular interaction of glucose with secreted IAPP (e.g. non-enzymic glycosylation) since fibrils are also formed in the presence of the non-glucose secretagogues, tolbutamide, α-ketoisocaproic acid and leucine [48]. The amount of amyloid deposited is related to the degree of β-cell stimulation and the amount of insulin and IAPP produced. Nutrient stimulation of β-cells in diabetic humans will therefore promote fibril formation.

Summary
IAPP has been postulated to have a role as a modulating factor in glucose homoeostasis and to be involved in the pathophysiology of diabetes. However, the normal physiological functions of the peptide remain obscure: exogenous IAPP
Amyloid deposits formed from polymerized IAPP in Type-2 diabetes, but progressive accumulation of evidence to suggest that circulating IAPP has an acts on many experimental systems to modulate diabetic patients. These insoluble deposits do not precipitate the onset of hyperglycaemia in Type-2 diabetes, but progressive accumulation of amyloid is associated with islet cell destruction and decreased islet function in the later stages of the disease. Although the causative factors of formation of the first IAPP fibril are unknown, continued high levels of insulin and IAPP secretion as a result of nutrient stimulation or insulin resistance will promote binding to preformed fibrils and extension of the deposits. It is important that methods to identify patients susceptible to amyloid deposition are developed and therapeutic agents are produced that can reduce or prevent polymerization of IAPP to form amyloid and minimize severe deterioration of islet function in Type-2 diabetes.

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