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ISLET AMYLOID POLYPEPTIDE (IAPP)

A short review

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Abstract

The islet amyloid polypeptide (IAPP) was originally identified by chemical analysis of the amyloid component in a human pancreatic islet cell tumor. It consists of 37 amino acids and displays about 50% homology with the neuropeptide calcitonin gene-related peptide (CGRP). In the pancreatic islets the IAPP is confined to the β-cells, co-stored with insulin in the secretory granules and apparently co-secreted with insulin on glucose stimulation. In β-cell depletion states such as streptozotocin diabetes in animals and in human type I diabetes mellitus both the IAPP and the insulin levels display reduction or are even absent. Within the mature IAPP molecule the amino acid sequence 23–29 shows considerable amino acid heterogenicity among various mammalian species. The amino acid composition of human IAPP in this specific region promotes the development of pancreatic islet amyloidosis, a phenomenon related to the ability to develop type II diabetes in that particular species. However, as type II diabetes is an inherited disease affecting a subpopulation of humans, not only the gene coding mature IAPP, but also one or several other hereditary factors of unknown origin are needed for the disease to develop. We have established a radioimmunoassay for plasma measurements of IAPP. During screening investigations of a large material of endocrine tumors we found a patient with extremely elevated plasma levels of IAPP, about 20 000 pmol/l. Immunohistochemical investigations confirmed the IAPP content and also revealed amyloid deposits. While performing an oral glucose tolerance test insulin levels remained unchanged whereas there was an increase in the glucose and IAPP levels. It is thus concluded that IAPP can be used as a tumor marker in pancreatic islet cell tumors and that high plasma levels of IAPP can inhibit glucose stimulated insulin secretion.

Key words: Human pancreatic islets, diabetes mellitus, amyloid, islet amyloid polypeptide, pathogenesis, islet cell tumors.

The isolation, purification and amino acid sequence analysis of the amyloid component in a pancreatic islet cell tumor in 1986 revealed the existence of a previously unidentified native peptide. It consisted of 37 amino acids and was designated as islet (or insulinoma) amyloid polypetide (IAPP) (1, 2). It was followed by the detection of an identical peptide in amyloid material obtained from pancreatic tissues of diabetic individuals (3). Comparative studies showed that IAPP had about 50% homology with the previously identified neuropeptide calcitonin gene-related peptide (CGRP), which is present in the central and peripheral nervous systems and in the calcitonin-producing thyroid c-cells (2). It was also known at that time that the amyloid in the c-cell derived medullary carcinoma was related to calcitonin or procalcitonin (4). These facts pointed towards an endocrine or other regulatory functions of IAPP.

Recent interest for IAPP has mainly been directed towards its possible endocrine role and its significance for a better understanding of the pathogenesis of type II (non-insulin dependent) diabetes mellitus (NIDDM). IAPP may also have some bearing on the discussion of long standing type I (insulin-dependent) diabetes mellitus (IDDM) and its microangiographic complications. In the present review the current knowledge of IAPP is summarized. Furthermore, the IAPP and its interrelationship with types I and II diabetes mellitus is specially emphasized. Some data from a patient with a pancreatic islet cell tumor producing IAPP is also reviewed.

Chemistry and molecular biology

Both IAPP and CGRP I and II are built up of 37 amino acids. The amino acid homology between IAPP and the

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two CGRP molecules is 43–46% (2). There are species differences in the amino acid composition. For instance, the divergence between human and rat IAPP is of six amino acids of which five are found in the 23–29 region of the mature IAPP molecule (5–6). Isolation and characterization of cDNA clones coding for IAPP precursors in humans and various animals has been reported (6–14). The predicted amino acid sequence identities are 89% for cat, 84% for rat and mouse and 78% for guinea pig (6). The nucleotide sequence of a human cDNA indicates that IAPP is a C-terminally amidated peptide derived by proteolytic processing of an 89 amino acid precursor molecule. Within the IAPP molecule the N-terminal and the C-terminal amino acid secquences are highly conserved among different species. This is also the case with the structurally related neuropeptide CGRP. Thus, it is possible that IAPP and CGRP interact with similar or maybe identical receptors (6). The N-terminal and the C-terminal regions of the IAPP precursor molecule show little sequence conservation, indicating that these regions do not represent additional biologically active peptides. The prepro-IAPP begins with a sequence of 22 amino acids, of which most are hydrophobic, which is a characteristic feature of a signal peptide molecule for transport across the membrane of the endoplasmatic reticulum. The final IAPP molecule is represented by amino acids 34 to 70 of the precursor molecule. The human IAPP gene appears to be a single-copy gene and it has been localized to the p12.3 region of chromosome 12 (11). The CGRP gene, like the insulin gene, is present on chromosome 11. However, the IAPP and the CGRP genes may be evolutionarily related (11).

Localization

Antibodies raised against amino acid sequences 7–17 and 20–29 of IAPP have been used for immunohistochemical localization of IAPP in histological material. A specific immunoreaction is observed in the pancreatic islets. Staining of serial sections and application of a sequential staining method, i.e. immunohistochemical staining followed by silver staining of one section from human pancreas, has revealed that the IAPP immunoreactivity is confined to the insulin producing β-cells in the islets of Langerhans, leaving the argyrophil glucagon (α)-cells unreactive. The islet somatostatin (D)-cells and pancreatic polypeptide (PP)-cells have not been separately examined with regard to IAPP immunoreactivity at the light microscopy level. IAPP immunoreactivity can also be seen in amyloid in pancreatic islets and in endocrine pancreatic tumors producing amyloid (15). An antiserum to the N-terminal flanking peptide of pro-IAPP has been utilized to demonstrate that not only mature IAPP but also the N-terminal flanking peptide occurs in islet amyloid deposits (16). Using the immuno-gold technique it has been shown that IAPP is exclusively present in the central electron dense core of the β-cell secretory granules (17). Furthermore, by using a double immunocytochemical staining it was observed that IAPP is co-stored with insulin in these granules. However, in cat islets accumulations of IAPP immunoreactivity have been reported in the electron lucent space between the central core and the surrounding membrane of the β-cell granules (18). Examination of human pancreas from non-diabetic subjects showed that the greatest density of immunoreactivity for IAPP was found in the electron dense regions of some lysosomal or lipofuscin bodies while to a lesser degree in secretory granules and in lamellar bodies (19).

Tissue distribution has been studied using highly sensitive and specific radioimmunoassays (RIA) for IAPP. The content of IAPP in the normal human pancreas was found to be 604-1 448 pg/mg wet weight. A small amount of IAPP immunoreactivity was detected in the stomach, duodenum and jejunum (20). A corresponding investigation in rats also revealed large amounts of IAPP in the pancreas, 304–354 pmol/g wet weight, but only 0.1–0.8% of this amount was detected in the antrum of the stomach, duodenum, jejunum, ileum and colon. IAPP was not detected in the central nervous system (21). Northern blot technique was also used to reveal expression of IAPP mRNA in various rat tissues. IAPP mRNA was found in greatest concentrations in the pancreas giving a hybridization signal intensity approximately 10% that of insulin mRNA. IAPP mRNA was also found in the stomach and lung but not in the thyroid gland. The only nervous tissue in which IAPP could be detected was the dorsal root ganglion (22). Light microscopic immunohistochemistry has not revealed IAPP immunoreactivity in endocrine or other non-endocrine tissues outside the pancreatic islets. At present the production of IAPP in the islet β-cells appears to be quite specific as also in the case of insulin.

Physiology

Several studies have been made in order to evaluate a possible physiological role in IAPP. These studies fall into four main categories namely: 1) Effects of IAPP on insulin secretion, 2) mechanisms behind IAPP release from the β-cells, 3) peripheral actions of IAPP in relation to glucose metabolism and 4) other peripheral actions of IAPP.

1) IAPP shows about 50% homology with CGRP, a peptide that inhibits insulin secretion (3). Several reports have tested if IAPP too can inhibit insulin secretion. Almost all studies conclude that basal secretion of insulin is unaffected by IAPP while in about half of the studies it was shown that IAPP can reduce the glucose-stimulated insulin secretion and in the other half IAPP has no effect on the insulin secretion (23–27).

2) Studies on IAPP secretion after glucose stimulation appear to be more coherent. Several studies indicate that IAPP is co-released with insulin (28–31). IAPP in plasma

has been determined in non-diabetic subjects after oral and intravenous glucose administration (31). These results suggest that IAPP is co-secreted with insulin in reponse to a glucose load. Furthermore, it was found that IAPP secretion was inhibited by hypoglycemia and somatostatin. Similar observations have also been made in experiments on isolated rat islets and neonatal rat pancreas (28–29).

3) It has been reported that human IAPP and rat CGRP I are potent inhibitors of both basal and insulin-stimulated rates of glucogen synthesis in stripped soleus muscle in vitro (32–34). In accordance it was also shown that synthetic amidated IAPP induced impaired glucose tolerance in cats in vivo (35). However, a study on non-obese human volunteers suggested that circulating IAPP may not have an important influence on intravenous glucose tolerance in man (36). During clamp studies in rat it was shown that IAPP inhibited the ability of insulin to stimulate glucose disposal by 65% and to suppress hepatic glucose output by 64% (34). These observations are confirmed by another study which showed that IAPP can bind to and introduce adenylate cyclase activation via CGRP receptors on rat liver plasma membranes (37).

4) Two observations of metabolic effects of IAPP outside the glucose regulatory system have been reported. IAPP has been shown to be a potent osteoclast-inhibiting peptide that induces profound hypocalcaemia in rats and rabbits and inhibits bone resorption by osteoclasts (38–39). IAPP has also been shown to have vasodilatory effects (40). Intradermal injections caused vasodilation and intravenous injections caused a drop in systemic blood pressure in rabbits.

Type I diabetes

The development of diabetes has been assessed in identical, monozygotic twins. When diabetes occurs in one index twin before the age of 40 half of the pairs are concordant (41–43). This observation indicates that in type I diabetes, there is an inherited trait but otherwise the disease is caused by environmental factors. The genetic factors have been demonstrated to be related to the immune system and to result in an increased tendency to autoimmune responses. The environmental causes of diabetes are not completely understood. However, it is not infrequent that diabetes in children arises after endemic viral infections. Light microscopy of pancreatic islets reveals insulitis in early cases. In long standing diabetes, the islet tissue displays pronounced atrophia and the relative frequency of the different islet cells is altered with a decrease in the insulin producing β-cells. The total amount of β-cells has been calculated to represent only 10–20% of the β-cell mass of normal subjects. Consequently, since β-cells are the sole source of insulin synthesis, storage and release, insulin deficiency will develop.

Primarily, type I diabetes is not an insulin depleted but rather a β-cell depleted disease. Thus, it would be of interest to know which additional peptide products are synthesized and stored in the secretory granules and discharged for the β-cells after stimulation. The insulin secretory granule is a complex intracellular organelle comprised of many proteins with different catalytic activities and messenger functions. After extensive analysis of β-cell granular fractions obtained from a rat insulinoma cell line it was concluded that upwards of 100 different polypeptides may be present in the granules (44). Besides insulin, c-peptide and IAPP, several additional proteins have hitherto been identified in the granules. Some of these are chromogranin A and its fragments, pancreastatin, β-granin and synaptophysin. The latter has the chemical composition of an ion channel protein and is localized within the granular membrane. The β-cell granules upon stimulation apparently release a cascade of peptide and protein molecules. If some of these molecules are exclusively transcribed and translated into proteins within the β-cells, as it is in the case of insulin, they subside into type I diabetes. This may result in insufficiency symptoms and should therefore be substituted. Against this background it is of interest to note that when experimental diabetes is induced in rats by injection of streptozotocin the β-cell destruction results in both IAPP and insulin mRNA reduction (22, 45, 46). A decrease of IAPP in plasma in type I diabetes has also been observed (47). It seems that IAPP is reduced or even absent in plasma before the pronounced or near complete damage of the pancreatic islet β-cell occurs. Thus, it is feasible to suggest that the microangiographic complications occurring in some patients with long-standing diabetes is not only dependent on an incomplete glucose regulation but is also caused by an insufficient substitution therapy.

Type II diabetes

Hereditary studies indicate that type II diabetes mellitus is genetically determined. In identical, monozygotic twins above the age of 40 the concordance with respect to diabetes is almost 100% (41–43). Thus, external factors such as body weight, food intake, and physical activity seem to influence symptoms more than the basic pathogenic mechanisms.

Morphologically there is no main alteration of the pancreatic islets with regard to size and total volume. However, amyloid deposits are frequently accumulated in the islet stroma in close contact with the β-cells (48). These amyloid deposits are pronounced in type II diabetes that is of severe and of long duration but the relation between islet amyloid and type II diabetes is not complete. Amyloid deposits may be found without concomitant diabetes and vice versa. On the other hand, in various mammalian species there is an accordance between the ability to produce islet amyloid and to develop type II diabetes. IAPP is the main constituent of islet amyloid and the IAPP

molecule displays variation in its amino acid composition between species. The most pronounced heterogenicity is found from amino acid 23 to 29 of the mature IAPP molecule. For instance, human and rat IAPP differ with respect to five amino acids within this region (5–6). The human sequence can form amyloid in vitro, while the corresponding sequence in the rat cannot. In agreement, rats do not develop islet amyloidosis or type II diabetes. The genomic background for IAPP production can thus explain why type II diabetes occurs only in a limited number of mammalian species (human, cat, racoon) and not in others (mouse, rat, guinea pig, dog) (12, 49, 50). However, the human hereditary traits that select individuals for type II diabetes mellitus are still unknown. The normal islet β-cells of dogs do not form amyloid despite the fact that dog IAPP contains the amyloidogenic amino acid sequence. The corresponding endocrine tumors, though, can form amyloid (14, 51). In human islet cell tumors the amyloid production sometimes starts within the β-cell cytoplasm. These observations may indicate that the amyloid formation is caused by β-cell intrinsic events (unpublished results).

It has been shown by in vitro studies that IAPP inhibits insulin-stimulated glucose uptake by skeletal muscle cells (32–33). IAPP injection in vivo induces impaired glucose tolerance in cats (35, 52). Since an increased peripheral insulin resistance has been recorded in the early phase of type II diabetes, IAPP may play an important role in the development of diabetic hyperglycemia. However, corresponding experiments with IAPP in human volunteers do not support this proposal (36).

Endocrine tumors

Radioimmunoassay measurements of the IAPP content in endocrine tumors have revealed high levels in insulinoma (53). We have developed a sensitive and specific RIA for plasma measurements of IAPP using standard IAPP and polyclonal rabbit antibodies purchased from Peninsula (Peninsula Lab., USA). During investigations of a large material of endocrine tumors we found a patient with extremely elevated plasma levels of IAPP, about 20 000 pmol/l (Stridsberg et al.: unpublished study). This patient had a pancreatic islet cell tumor and multiple, large liver metastasis. Immunohistochemical investigations of biopsy specimens from the tumor confirmed the content of IAPP and the presence of amyloid deposits.

The patient showed no pronounced symptoms except for a newly developed diabetes. Plasma levels of glucagone, CGRP and somatostatin were all within the reference ranges. An oral glucose tolerance test was performed. Blood glucose levels increased in a clearly diabetic way while plasma insulin was still at base line levels. Plasma levels of IAPP increased significantly during the test. Thus, we can conclude that IAPP can be used as a tumor marker for pancreatic islet cell tumors and that these high plasma levels of IAPP can inhibit glucose stimulated insulin secretion.

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