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Review

Islet amyloid polypeptide – a novel controversy in diabetes research

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The discovery of a previously unknown polypeptide in the islet Beta cells was unexpected. This putative hormone, named islet amyloid polypeptide (IAPP) or amylin, has been implicated in the normal regulation of glucose metabolism and has been proposed to have a role in the pathogenesis of Type 2 (non-insulin-dependent) diabetes mellitus. IAPP is therefore of great interest in the field of diabetes research at present. Its potential role(s) in diabetes has made several pharmaceutical companies interested in IAPP, and a new company has been created solely to exploit its commercial potential.

Historical background

It has often been stated that the islets of Langerhans are morphologically normal in Type 2 diabetes in spite of the fact that amyloid strictly limited to the pancreatic islets occurs in more than 90% of patients with this disease [1, 2]. This morphologic alteration in islets was demonstrated early this century, and was named hyalin [3, 4]. In some patients with Type 2 diabetes the amyloid deposits are minimal, but in more than 50% of patients with such deposits the amyloid is widely spread and affects many islets in the body and the tail of the pancreas. The fact that islet amyloidosis has often been overlooked in the discussion of Type 2 diabetes is surprising today. This might be partly explained by the fact that histological demonstration of amyloid requires both appropriate staining and some experience in its evaluation. Furthermore, most experimental animal species used in diabetes research do not develop islet amyloid. Over the years, the subject of islet amyloid has therefore been of interest to mainly a small group of pathologists.

Amyloid in general consists of aggregated small proteins forming fibrils with a high degree of β-pleated sheet structures [5, 6]. The amyloid proteins often are generated by cleavage of larger precursors which, in the case of local amyloid deposits, are synthesized in cells close to the deposition. A close topographical relationship between islet amyloid fibrils and islet Beta cells was demonstrated nearly 20 years ago [7], and it was logical to presume that islet amyloid should consist of aggregated insulin or proinsulin fragments. It was therefore a surprise when purification and amino acid sequence analysis of the major amyloid protein, first from an insulinoma [8] and then also from islets of human Type 2 diabetic patients and from diabetic cats, revealed a previously unknown polypeptide that did not resemble insulin. This polypeptide had 43–46% identity with the neuropeptide calcitonin gene-related peptide (CGRP) [9–11]. The novel polypeptide was designated islet amyloid polypeptide (IAPP) by us [9] and was later called amylin by others [12]. Like CGRP, IAPP is 37 amino acid residues long, has an N-terminal intramolecular disulphide bridge, and is C-terminally amidated [9–11, 13–15]. The N- and C-terminal parts of IAPP are strinkingly homologous to CGRP while there is a 10 residue long segment (positions 20–29) which is completely different.

The IAPP gene and its expression

Human IAPP is expressed as an 89-amino-acid prepropolypeptide with a 22 amino acid N-terminal signal peptide for transport through the endoplasmic reticulum [13–15]. The propeptide has two short flanking peptides which are removed by proteolytic cleavage at pairs of basic amino acid residues (as is also the case with proinsulin and many other polypeptide hormones) (Fig. 1). Comparative analysis of the cDNA sequences of IAPP from several mammalian species have revealed that the N- and C-terminal regions are almost completely conserved [16–19] (Fig. 2). The propeptides, on the other hand, vary greatly between species and probably have no biological function [17]. It is now well established that IAPP in humans and other mammalian species is co-stored with insulin in the Beta-cell secretory granules and is co-released with insulin [20–22].

The human IAPP gene contains three exons of which the first is non-coding and the third codes for almost the complete propeptide [23–25]. The gene is present on chro-

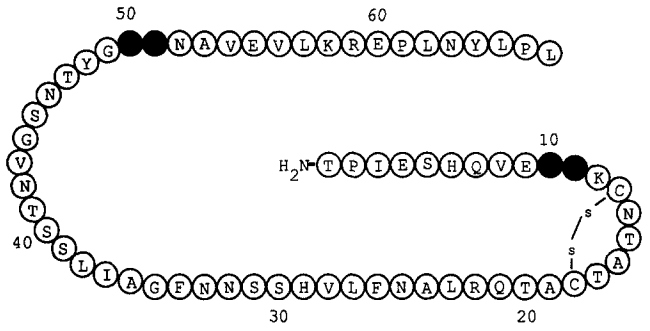

Fig. 1. Human islet amyloid polypeptide (IAPP) precursor after the signal peptide has been removed. IAPP is formed by cleavage at double basic amino acid residues N- and C-terminally. The glycine residue in position 49 gives rise to the amidation of the C-terminal residue of the mature IAPP molecule

mosome 12, probably on the short arm (although the data are slightly conflicting) [23, 24, 26, 27]. A functional promoter region has been identified in the 5′ flanking DNA sequence [24] and, interestingly, no significant homology to the insulin promoter was identified [23–25]. This is in accordance with some data on the mRNA and protein levels which indicate that the two hormones are not co-regulated [28].

In normal human and rat islets, IAPP seems to be expressed exclusively by the Beta cells. However, in transformed rat islet cell cultures, IAPP has been seen co-localized with glucagon and somatostatin and in the absence of insulin [28]. There are also some reports indicating extrapancreatic expression of IAPP. IAPP mRNA has been found in the stomach, duodenum, jejunum, ileum, caecum and colon as well as in dorsal root ganglia of the rat [29]. The levels of IAPP mRNA in the stomach were about 5 % of that in the pancreas. Small amounts of immunoreactive IAPP have been found in extracts of stomach and intestines of rat and human [30, 31]. Furthermore, in a recent study, using immunohistochemical methods, Toshimori et al. reported IAPP immunoreactive cells in the gastric and duodenal mucosa [32]. The possible existence of an islet hormone in the gastrointestinal mucosa should not be surprising, but this finding is at variance with our results and those of others [33]. We have studied the gastrointestinal tract from man, cat, mouse and rat using several antisera to IAPP without finding any IAPP-positive cells [34 and unpublished results]. The reason for this discrepancy is not clear and further studies are needed.

IAPP release and plasma concentration

Since IAPP co-exists with insulin in the Beta-cell secretory granules, the substances should be co-released into the extracellular space. Several in vitro and in vivo studies in rats, and in vivo studies in humans, have shown that insulin and IAPP are released together and that the relative amounts of the substances generally increase in a parallel manner after Beta-cell stimulation with glucose or other secretagogues [35–43]. However, it has been shown that the relative amounts of IAPP and insulin produced and secreted by the Beta cells may vary under certain metabolic conditions. For example, dexamethasone has been shown to increase the relative proportion of IAPP to insulin mRNA in Beta cells [44], and to increase the relative amount of IAPP to insulin secreted from rat islets [45].

Although there are difficulties in measuring the plasma levels of IAPP, there are now several studies which indicate fasting plasma concentrations between 2–10 pmol/l in normal humans, i.e. about 10 % of the insulin levels [30, 37, 40, 42, 46] although higher levels have been reported [47]. Information regarding the plasma levels of IAPP in Type 2 diabetes is of considerable interest in that it has been considered to play a role in the pathogenesis of this disease [27, 48]. Studies published so far indicate that the IAPP concentrations in non-obese persons with impaired glucose tolerance or Type 2 diabetes are normal, as they are with a glucose load [37, 40]. Higher than normal IAPP levels were reported in obese patients with impaired glucose tolerance or Type 2 diabetes but the insulin levels were even higher resulting in a decreased IAPP to insulin ratio [37]. Thus, no data have been published to date indicating that a considerable increase of IAPP expression occurs in the development of Type 2 diabetes. It can also be noted that Beta cells in patients with Type 2 diabetes [49] and in diabetic cats [50] possess lower than normal IAPP immunoreactivity. However, there must be an explanation for the occurrence of IAPP-derived islet amyloid and it is still possible that there is an overproduction of IAPP during some period of the development of Type 2 diabetes [50]. There also is the possibility of a non-continuous oversecretion of IAPP, which is difficult to demonstrate. Interestingly, Beta cells of cats with glucose impairment but without overt diabetes show a very high level of IAPP immunoreactivity as compared to normal control animals [50]. It is also possible that the accuracy of current radioimmunoassay procedures is not adequate to detect small but physiologically relevant alterations in IAPP levels. This uncertainty regarding measurement of plasma IAPP levels is also of significance as it pertains to the evaluation of the biological effects found experimentally with IAPP. However, it is questionable whether the nanomolar concentrations of IAPP used to induce in vivo effects are relevant to the pathogenesis of Type 2 diabetes.

Ratio between IAPP and insulin

Several studies have shown that the ratio between IAPP and insulin in basal and stimulated conditions is usually constant, i.e. there is a parallel relationship between the concentrations of these two polypeptides. However, a relative decrease of IAPP immunoreactivity in plasma has

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                  10        20        30
Human    KCNTATCATQRLANFLVHSSNNFGAILSSTNVGSNTY-NH2
Cat      ----------------IR----L-----P---------NH2
Rat      ----------------R----L-PV-PP---------NH2
Hamster  ----------------N--L-PV--P---------NH2
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Fig. 2. The primary structure of islet amyloid polypeptide (IAPP) of four mammalian species. IAPP is N- and C-terminally conserved but with considerable variation in a central segment

been seen in Type 2 diabetes [37]. An altered plasma IAPP/insulin ratio has also been seen in obese-diabetic viable yellow mice [51] and in rats after administration of a borderline diabetogenic dose of streptozotocin [52, 53] or dexamethasone [45]. All of these findings are consistent with the idea that production and secretion of IAPP and insulin are regulated differently and that disturbances in the IAPP/insulin ratio may be of importance in the development of Type 2 diabetes.

Normal function of IAPP

The structure of IAPP suggests that it is a regulatory polypeptide and its co-production with insulin is consistent with it having an important relationship to glucose metabolism. It is therefore not surprising that most studies concerning the normal function of IAPP have focused on this possibility. Other studies have concentrated on effects known for CGRP.

1. IAPP as an autocrine or a paracrine substance

The reported effects of IAPP on insulin secretion are contradictory. Inhibition of glucose-stimulated insulin release from Beta cells has been seen with IAPP in some studies [54, 55] while in others no effect on insulin release has been found [56–58]. Also, increased insulin release has been reported after IAPP administration [59]. Interestingly, CGRP has a dual effect on insulin secretion causing inhibition at low concentrations and stimulation at high concentrations [60]. In those in vitro or in vivo experiments in which IAPP was reported to have an effect, the concentration of IAPP was high or very high (nano- to micromolar) and it has therefore been claimed that IAPP is not likely to be a physiologically relevant modulator of Beta-cell insulin secretion in vivo [57]. However, the concentration of IAPP at the surface of Beta cells is probably very high compared to that found in plasma and it is therefore our opinion that it is too early to exclude the possibility that IAPP has functions as a modulator of insulin release.

2. IAPP causes insulin insensitivity in skeletal muscle and liver

Several studies have shown that IAPP is capable of inducing insulin resistance in vivo, although at higher concentrations than so far found in plasma [61–64] and that both IAPP and CGRP decrease the basal and insulin-stimulated glycogen synthesis in vitro [65–66]. IAPP has therefore been proposed as an important modulator of basal and insulin-mediated glucose uptake in skeletal muscle [27]. In hyperinsulinaemic glucose clamp studies in rats and dogs, IAPP and CGRP significantly inhibited glucose uptake [62, 64] and decreased the effect of insulin in suppressing hepatic glucose output [64]. The level at which IAPP exerts its effect on skeletal muscle is not completely understood. Studies on both rat and human muscle with the use of corresponding IAPPs indicate that IAPP inhibits insulin-stimulated glucose transport [67, 68] at a point distal to the insulin receptor [68], decreases glycogen synthesis [63] by inhibition of glycogen synthase [69] and activates glycogen phosphorylase [69, 70]. As in almost all studies on the physiology of IAPP, pharmacological (nano- to micromolar) rather than physiological concentrations of IAPP have been used. Therefore, it is difficult to judge the relevance of these data concerning the normal function of IAPP and its potential relationship to the development of Type 2 diabetes.

An effect of IAPP on metabolism of glucose and glycogen in the liver is not yet fully established. Receptors binding CGRP and IAPP and linked to cAMP production have been identified on liver cell membrane preparations [71, 72]. In a recent study by Stephens et al. [73], IAPP/CGRP receptors were found only on non-parenchymal liver cells. These authors, and others, have failed to demonstrate an effect of IAPP on the glucose production or glycogen synthesis while the opposite has also been reported [63, 64]. Obviously, these discrepancies must be clarified before a possible effect of IAPP on the liver can be accepted.

3. Other effects of IAPP

As with CGRP, IAPP lowers plasma calcium levels [74]. This effect seems to be due to an inhibition of osteoclastic degradation of bone. The effect of IAPP is stronger than that of CGRP but much weaker than that of calcitonin. It has been proposed that an important normal function of IAPP is to promote calcium absorption from the intestines after a meal [74]. CGRP is a strong vasodilator, an effect also exerted by IAPP although not as potently and only at high concentrations [75 and unpublished results]. There are also several reports indicating that IAPP, as with many other polypeptide hormones, is present in the nervous system. IAPP immunoreactivity has thus been found in extracts of the hypothalamus of rats [76].

Why does IAPP form amyloid deposits in Type 2 diabetes?

The mechanism by which certain small proteins aggregate as amyloid fibrils is not completely understood in any type of amyloidosis. X-ray crystallographic and infrared spectrographic studies have indicated that proteins in amyloid fibrils are arranged in cross β-pleated sheet conformation [5, 6] and proteins which normally contain a high degree of β-structure are more prone to form amyloid fibrils [5, 6]. Thus, intrinsic properties of involved polypeptides seem to determine their proneness to form amyloid. In familial amyloidoses single amino acid substitutions make certain proteins more amyloidogenic. However, the predicted sequences of the IAPP precursor determined from the IAPP coding regions of genomic DNA of 25 patients with Type 2 diabetes was competely normal [77], thus indicating that an abnormality in the primary structure of IAPP or its precursor is not of importance in the amyloidoge-

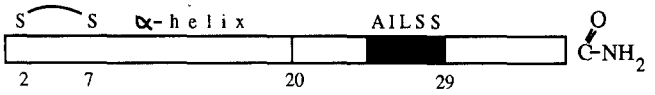

Fig. 3. Schematic outline of human islet amyloid polypeptide (IAPP). The 37 amino acid residue IAPP molecule is C-terminally amidated and contains a disulphide bridge between Cys 2 and Cys 7. The 20–29 segment is completely different from calcitonin gene related peptide and shows the most pronounced interspecies variations. The 25–29 Ala-Ile-Leu-Ser-Ser segment constitutes the amyloidogenic part of human IAPP while the 8–18 segment probably has a α-helix conformation in the normal IAPP molecule

nesis of IAPP. It has previously been shown that fibrils with amyloid properties can easily be made in vitro from some small proteins [5, 6]. We recently demonstrated that synthetic human IAPP also spontaneously forms amyloid-like fibrils in vitro [78]. The secondary structure of IAPP is not known but it is reasonable to assume that IAPP and CGRP have closely related secondary structures. A recent study of CGRP in solution showed an N-terminal loop followed by an α-helix (residues 8–18) and a short loop while the rest of the molecule is disordered [79]. It is therefore unlikely that residues 1–21 determine the amyloidogenicity of IAPP. This also is consistent with a secondary prediction of IAPP structure that indicated a short β-strand in the 25–29 segment (-Ala-Ile-Leu-Ser-Ser-) of human IAPP [78]. Interestingly, this segment falls within a 10 amino acid residue part of IAPP (positions 20–29) which differs completely from CGRP, which is a molecule not known to form amyloid fibrils. Like the full-length human IAPP, the synthetic IAPP decapeptide ($IAPP_{20-29}$) easily forms amyloid-like fibrils in vitro [14, 80] while the corresponding segment of CGRP or $IAPP_{7-17}$ do not [14 and unpublished result]. These findings focus the amyloidogenicity of IAPP to the Ala-Ile-Leu-Ser-Ser sequences (Fig. 3). As a matter of fact, the hexapeptide Gly-Ala-Ile-Leu-Ser-Ser forms amyloid-like fibrils in vitro [81].

One part of the explanation for the lack of islet amyloid formation in most mammalian species is likely to be related to the structure of the 20–29 segment of the IAPP molecule. A first indication for this was the finding that an antiserum to human $IAPP_{20-29}$ reacted immunohistochemically with islet Beta cells only in species where islet amyloid occurs [14, 82]. It was subsequently shown that this segment of IAPP in contrast to the conserved N- and C-terminal parts, shows considerable interspecies variations. Specifically, proline residues were found in IAPP positions 25, 28 and 29 of several rodents [16, 17]. Of these, $proline_{28}$ seems to be of special importance for hindering amyloid fibril formation [78]. The Ala-Ile-Leu-Ser sequence was identified not only in IAPP of human, cat, and cougar [14, 17, 18, 83], species where islet amyloid occurs, but also in the dog [84]. This latter species never develops islet amyloid but, interestingly, does develop IAPP-derived amyloid in insulinomas [85]. Therefore, not only an amyloidogenic $IAPP_{20-29}$ sequence but also other as yet unidentified factors (e. g. production or concentration factors) are responsible for the amyloid formation. Whether or not the N-terminal proIAPP segment, present in small amounts in the islet amyloid [86], is of any significance in the formation of amyloid is unclear.

Is IAPP pathogenetically involved in Type 2 diabetes?

Is there an IAPP gene abnormality?

The abnormal behaviour of IAPP with the resultant amyloid fibril formation in Type 2 diabetes indicates that the polypeptide is in some way linked to the development of the disease. There are at present no indications that hereditary factors are associated with altered expression of the IAPP gene (see above) and RFLP analysis has failed to indicate a mutation in or close to the IAPP gene [87]. Also, there seems to be no abnormal splitting of the precursor since IAPP purified from normal pancreata is identical to that found in amyloid deposits [88].

Importance of islet amyloid in Type 2 diabetes

It has been argued that islet amyloid per se cannot be of more than marginal importance in the development of diabetes, since it does not occur universally in Type 2 diabetes and also is present in many non-diabetic patients. Furthermore, some individuals with Type 2 diabetes and islet amyloid have only very small amyloid deposits [2, 89]. However, several facts contradict such statements. Firstly, careful autopsy examinations have shown that islet amyloid is present in the vast majority of patients with Type 2 diabetes [1, 2, 89]. Secondly, even if the amyloid deposits are small, they tend to be widely spread among and within the islets [89]. Thirdly, Beta cells close to even very small deposits show quite extensive cell membrane disruption [7]. It is not known when islet amyloid first occurs in the development of Type 2 diabetes but animal studies indicate that they occur early and are often present even in the pre-diabetic state [50, 90]. Formation of islet amyloid might be a continuous process but the severity of islet amyloidosis does not correlate significantly with the duration of diabetes (P Westermark, unpublished data). Although islet amyloidosis is probably not a primary cause of diabetes, it could potentiate other diabetogenic factors and also sometimes be responsible for the late Beta-cell failure seen in Type 2 diabetes. This is consistent with the finding of most severe islet amyloidosis in Type 1 (insulin-dependent) patients in one study [91], although this was not confirmed in our larger material (P Westermark, unpublished data).

Another possible mechanism through which IAPP could contribute to the diabetic state (as reviewed earlier in this paper) would be by an abnormal autocrine action on islet Beta cells e. g. by over-expression. However, to-date, no data convincingly support this possibility.

Induction of insulin resistance by IAPP

Much interest has been focused on the possibility that an overproduction of IAPP is responsible for insulin resistance in Type 2 diabetes [27]. Such a hypothesis is very attractive since, (1) IAPP is produced by the Beta cells and hyperinsulinaemia is often present in Type 2 diabetes, and (2) the insulin resistance in Type 2 diabetes greatly resem-

bles the effect of IAPP [65]. However, as has been discussed previously, comparably low plasma levels of IAPP have been demonstrated in Type 2 diabetes and, hence, there are valid concerns regarding the importance of IAPP in the development of the insulin resistance in this condition. However, the methods determination of IAPP are possibly not fully reliable, and it is also possible that small changes in the ratio between insulin and IAPP release have so far escaped detection. If IAPP normally acts as a long-term modulater of insulin action on peripheral tissues, as some results might indicate [61], minor shifts of the action of insulin may occur even with small changes in the levels of IAPP or of the ratio between IAPP and insulin. Such changes could perhaps cause small but important shifts in the metabolism of glucose, glycogen and fat but would probably not give rise to insulin resistance. It is tempting to speculate whether such changes contribute in the long run to the development of late complications, especially in Type 1 diabetes where the change in ratio between plasma levels of IAPP and insulin are most pronounced. The lack of insulin in Type 1 diabetes can be therapeutically substituted but the lack of IAPP has obviously not been therapeutically substituted [48].

In conclusion, IAPP is a novel putative Beta-cell hormone, stored and released together with insulin. IAPP is responsible for the islet amyloid deposits (observed in some species only) by an intrinsic amyloidogenic sequence in positions 25–29 of IAPP, and possibly also facilitated by an overproduction of the polypeptide. The variation in effects of IAPP found by different research groups is disturbing and difficult to explain. One possibility is that different synthetic IAPPs have different activities. Furthermore, we have found that human (but not rat) IAPP has a strong tendency to convert to biologically inactive fibrils in vitro. Many effects of IAPP have been demonstrated but without exceptions only at concentrations above those found in plasma. Therefore, the normal functions of IAPP or its importance in the development of Type 2 diabetes are still not known.

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