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Chronic overproduction of islet amyloid polypeptide/amylin in transgenic mice: lysosomal localization of human islet amyloid polypeptide and lack of marked hyperglycaemia or hyperinsulinaemia

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Summary. Type 2 (non-insulin-dependent) diabetes mellitus is characterised by hyperglycaemia, peripheral insulin resistance, impaired insulin secretion and pancreatic islet amyloid formation. The major constituent of islet amyloid is islet amyloid polypeptide (amylin). Islet amyloid polypeptide is synthesized by islet beta cells and co-secreted with insulin. The ability of islet amyloid polypeptide to form amyloid fibrils is related to its species-specific amino acid sequence. Islet amyloid associated with diabetes is only found in man, monkeys, cats and racoons. Pharmacological doses of islet amyloid polypeptide have been shown to inhibit insulin secretion as well as insulin action on peripheral tissues (insulin resistance). To examine the role of islet amyloid polypeptide in the pathogenesis of Type 2 diabetes, we have generated transgenic mice with the gene encoding either human islet amyloid polypeptide (which can form amyloid) or rat islet amyloid polypeptide, under control of an insulin promoter. Transgenic islet amyloid polypeptide mRNA was detected in the pancreas in all transgenic mice. Plasma islet amyloid polypeptide levels were significantly elevated (up to 15-fold) in three out of five transgenic lines, but elevated glucose levels, hyperinsulinaemia and obesity were not observed. This suggests that insulin resistance is not induced by chronic hypersecretion of islet amyloid polypeptide. Islet amyloid polypeptide immunoreactivity was localized to beta-cell secretory granules in all mice. Islet amyloid polypeptide immunoreactivity in beta-cell lysosomes was seen only in mice with the human islet amyloid polypeptide gene, as in human beta cells, and might represent an initial step in intracellular formation of amyloid fibrils. These transgenic mice provide a unique model with which to examine the physiological function of islet amyloid polypeptide and to study intracellular and extracellular handling of human islet amyloid polypeptide in pancreatic islets.

Key words: Islet amyloid polypeptide, amylin, transgenic mouse, islet beta cell, islet amyloid, glucose metabolism, insulin resistance, Type 2 (non-insulin-dependent) diabetes mellitus.

Diabetes associated islet amyloid occurs only in man, monkeys, cats and racoons but not in rats or mice. The major protein component of islet amyloid is islet amyloid polypeptide (IAPP) (amylin) [1–3]. IAPP is produced in islet beta cells and co-secreted with insulin in response to beta-cell secretagogues, although the amounts are several times lower [4–6]. It was shown that the amino acid sequence of a particular region of IAPP (amino acids 25–29) is important for amyloid fibril formation [7]. Non-amyloidogenic rat and mouse IAPP (which are identical) diverge considerably in this particular region from amyloidogenic IAPP molecules of e. g. man and cat. In the dog [8], rabbit and hare [9], the amino acid sequence of IAPP predicts amyloidogenicity, but islet amyloid has not been detected. In the dog IAPP-immunoreactive amyloid is associated with insulinoma [8], and in the rabbit and hare low levels of IAPP production may contribute to the lack of islet amyloid in these species [9]. The causative factors for amyloidogenesis of IAPP are unknown, but are not related to a genetically determined change in the amino acid sequence of IAPP or of its precursor (preproIAPP) [1, 10–12]. Overproduction, decreased breakdown or abnormal cellular handling of the peptide could be involved. The potential importance of IAPP overproduction is indicated by the occurrence of IAPP-immunoreactive amyloid in IAPP-overproducing insulinomas in man [1] and in the dog [8].

Until now, biological actions of human and rat IAPP have been described almost exclusively in short-term experiments using synthetic IAPP preparations at high concentrations. It was shown that IAPP can inhibit insulin secretion [13–16] and antagonize insulin action on glucose metabolism, in particular by inhibiting glycogen synthesis in skeletal muscle and stimulating glucose production from lactate by the liver [3, 17–21]. However, in some studies such effects of IAPP could not be demonstrated

[22–28]. In Type 2 diabetes peripheral insulin resistance is often associated with obesity. Elevated plasma IAPP levels have been demonstrated in some obese glucose-intolerant patients [29], in glucose-intolerant first-degree relatives of Type 2 diabetic patients [30] and in animal models of diabetes [31–33]. Thus, an increase in production of IAPP might be important in the development of islet amyloid and of insulin resistance in Type 2 diabetes.

To test this hypothesis we generated transgenic mice which overproduce human (h) IAPP or rat (r) IAPP in their pancreatic beta cells. This represents a more natural and powerful model with which to study biological actions of native IAPP, produced in vivo in (supra)physiological amounts from pancreatic islet beta cells. This model allows the study of effects of chronically elevated plasma IAPP concentrations on glucose metabolism and on islet beta-cell function. In addition, transgenic mice producing hIAPP may represent a model for examination of factors involved in islet amyloid formation in vivo.

Materials and methods

Generation of the transgenic constructs

Plasmid RIP-DIP contains sequences from positions − 695 (*BamHI* site) to + 8 (*XbaI* site) relative to the transcription start site of the rat insulin 2 gene [34, 35]. RIP-1910 was derived from RIP-DIP and contains *SalI* linkers in the *BamHI* site at position −695 and in a *BamHI* site at a position approximately 50 base pairs (bp) downstream of the *XbaI* site. Plasmids containing the human (RIP-hIAPP) or the rat (RIP-rIAPP) IAPP gene linked to the rat insulin 2 gene promoter fragment, were generated by ligation of suitable IAPP gene fragments [36, 37] (Fig. 1) in the *XbaI* site of plasmid RIP-1910. The correct orientation of the IAPP gene fragments relative to the insulin gene promoter was confirmed by nucleotide sequence analysis.

Generation of the transgenic mice

Plasmids RIP-hIAPP and RIP-rIAPP were digested with *SalI* and the ± 8.7 kilobase (kb) and ± 8.3 kb fragments, containing the insulin promoter fragment linked to the IAPP gene fragments, were isolated and purified. These *SalI* fragments were used for microinjection into pronuclei of mouse zygotes, obtained from matings of (C57Bl/6J × CBA/J) F1 males and females [38].

Mice which developed from injected zygotes were tested for transgene integration by Southern blot hybridization of chromosomal DNA isolated from tail biopsies [39]. The human transgene was detected with the 588 bp *EcoRI* cDNA insert of clone λhIAPP-c1 [36], which does not hybridize to the endogenous mouse IAPP gene. The rat transgene was detected as a 2 kb *TaqI* fragment when hybridized to the 120 bp *AxyI/BamHI* fragment of the rat IAPP cDNA clone RPC-1 (nucleotides 11–130 in Fig. 1 b in [37]). This probe detects the mouse IAPP gene (nucleotide sequence homology is 93 %) as a 1 kb *TaqI* fragment. Southern blot positive mice (founders) and their transgenic offspring (F1) were back-crossed to C57Bl/6J mice and the F1 and F2 mice were used for the experiments described.

RNA isolation/Northern blot analysis

Specimens of mouse tissues and of human and rat pancreas were snap frozen in liquid nitrogen and stored at − 80 °C. Total cellular RNA was isolated and analysed by Northern blotting as previously described [36]. The probes for detecting human and rat/mouse IAPP RNAs were as described for the Southern blot hybridizations. The human insulin probe, showing cross-hybridization to mouse insulin RNA, was a *SmaI* fragment with nucleotides 499 to 1250 of the human insulin gene [40]. Quantification of mRNAs was performed by densitometric scanning of autoradiographs, using an Ultroscan XL (LKB, Bromma, Sweden).

Light microscopy

Tissue from pancreas and kidney was fixed in 4 % paraformaldehyde (weight/volume, w/v) in phosphate buffer (pH 7.2), dehydrated and embedded in wax. Sections were stained with a human-specific rab-

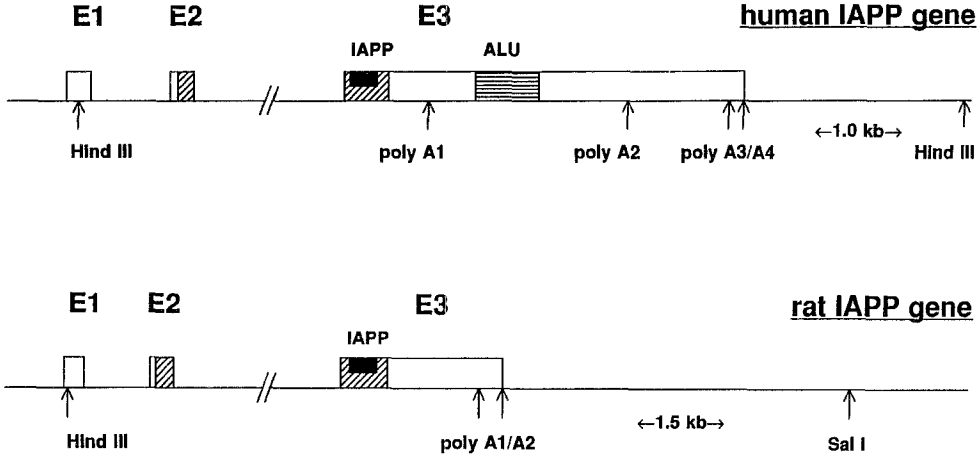


Fig. 1. Schematic representation of the *human* and *rat IAPP* genes. Exons are represented by *numbered boxes, hatched parts* indicate protein-encoding regions, *black boxes* indicate parts encoding the 37 amino acids of IAPP. Human IAPP and rat (= mouse) IAPP differ at 6 amino acid positions [10], 4 of which are in the amyloidogenic region 25–29 [7]. An *Alu* repetitive sequence in human IAPP exon 3 and identified polyadenylation signals are also indicated. The size of intron 1 is approximately 5 kilobases (kb) in both genes. The ± 8.0 kb *HindIII* fragment of the human IAPP gene and the ± 7.6 kb *HindIII/SalI* fragment of the rat IAPP gene were cloned downstream of a 703 base pair rat insulin 2 gene promoter fragment. The resulting plasmids RIP-hIAPP and RIP-rIAPP were digested with *SalI* and the ± 8.7 kb and ± 8.3 kb *SalI* fragments containing the insulin promoter/IAPP gene construct were used for generating transgenic mice. *E* exon; *polyA* polyadenylation signal

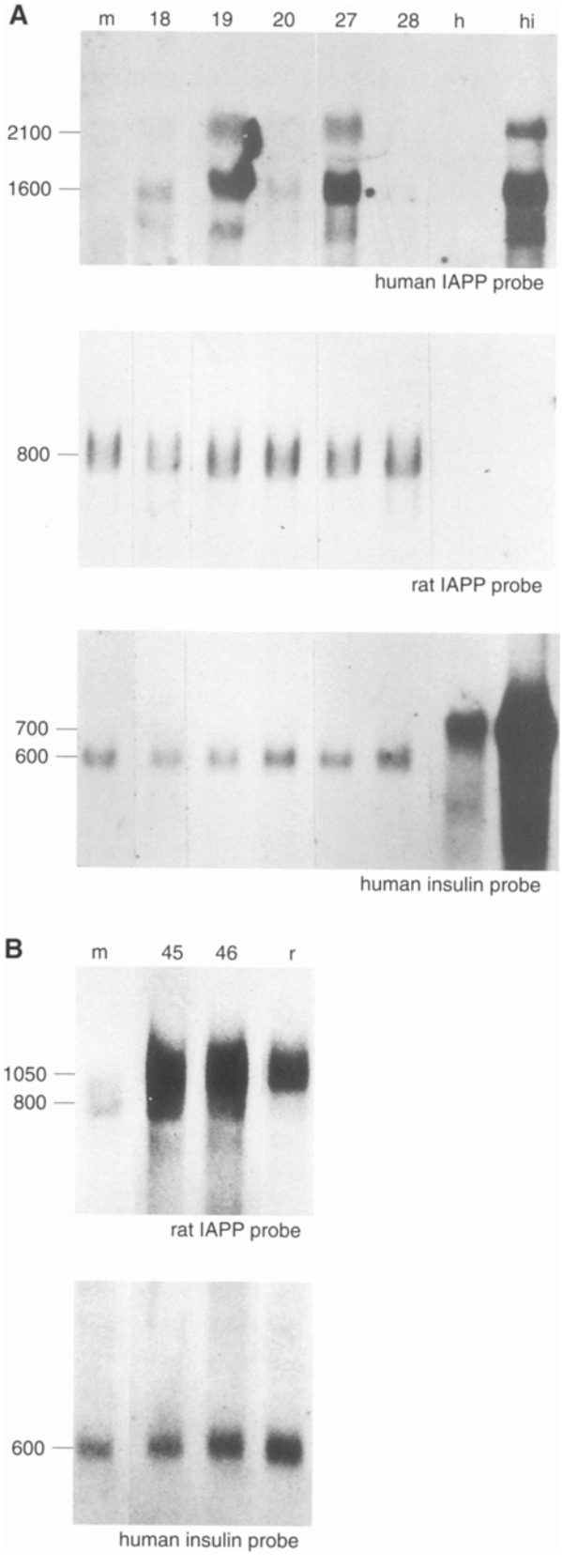
bit antiserum (dilution 1:1200), biotin-conjugated swine anti-rabbit IgG (Dako, Glostrup, Denmark), peroxidase-conjugated streptavidin-biotin complex (Dako) and 3,3′-diaminobenzidine as substrate. The human-specific polyclonal rabbit antiserum was raised against the 16 amino acid peptide of proIAPP, which flanks IAPP at its carboxy terminus (K. L. v. H., unpublished data). This carboxy-terminal flanking peptide is only 38% homologous between man and mouse [10]. IAPP immunostaining was performed with a polyclonal rabbit antiserum to hIAPP (1–37) (1:80) (Peninsula, Belmont, Calif., USA), which also detects rat and mouse IAPP. Specificity of the antisera was determined by lack of immunoreactivity following preabsorption with the carboxy-terminal flanking peptide and with hIAPP, respectively. For the detection of islet amyloid tissue sections were stained with sodium sulphate alcian blue (SAB), congo red or thioflavin S.

Electron microscopy

Fresh tissue samples of pancreas were cut into small pieces (approximately 1 mm^3) and fixed immediately in 2.5% glutaraldehyde (v/v) in phosphate buffer (pH 7.2), post-fixed in 1% osmium tetroxide, dehydrated and embedded in Spurr's resin (Taab Laboratories, Reading, UK). IAPP was detected with a polyclonal rabbit antiserum to rat IAPP (1–37) (1:1000) (Peninsula) and protein A gold (Biocell, Cardiff, UK). The antiserum reacts with IAPP in beta cells of mouse, rat and human pancreatic islets. Specificity of the antiserum was determined by lack of immunoreactivity following preabsorption with human or rat IAPP (1–37). Contrast was enhanced with lead citrate and uranyl acetate. Specimens were examined for IAPP immunoreactivity with the investigators unaware of the genetic status of the animals.

Blood analyses/radioimmunoassays

Blood samples were taken from the retroorbital plexus of ether anaesthetised animals and collected in EDTA-tubes, which were kept on ice until centrifugation at 3500 rev/min for 5 min at 4 °C. Plasma was taken and stored at −80 °C until determination of the levels of plasma IAPP, insulin and glucose. A radioimmunoassay (RIA) for determining IAPP concentrations in 50–100 µl plasma samples was developed using acid-acetone extraction and a polyclonal rabbit antiserum against hIAPP (1–37) (K. L. v. H., unpublished data). Equal volumes of plasma and acid-acetone were mixed and after centrifugation the IAPP-containing supernatant was freeze-dried and resuspended in 250 µl assay buffer. A 200 µl sample was incubated with the primary antiserum for 90 h at 4 °C. Then 5000 cpm ^{125}I-



Figs. 2 A, B. Northern blot analysis of total cellular RNA (30 µg) isolated from pancreas tissue and (3 µg) from an insulinoma. **A** RNA from the pancreas of a control mouse *(m)*, of transgenic mice expressing the human IAPP transgene (derived from five different founders), of a non-diabetic human *(h)* and from a human insulinoma *(hi)*. The 1600 and 2100 nucleotides human IAPP mRNAs are generated by usage of polyadenylation signals 2 and 3/4 in the human IAPP gene respectively ([56], see Fig. 1). These RNAs were also detected in the mouse derived from founder # 28 and in normal human pancreas, but only after a longer exposure time (not shown). **B** RNA from the pancreas of a control mouse *(m)*, of transgenic mice expressing the rat IAPP transgene (derived from two different founders) and of a rat *(r)*. In the rat IAPP gene both polyadenylation signals (Fig. 1) are used in generation of mRNAs of approximately 1050 nucleotides [37]. Rat IAPP mRNAs were present in mice from both 'rat IAPP lines'. The *blots of panel* **A** and **B** were hybridized consecutively, by stripping and reprobing, to different probes (see Materials and methods). In mice of the 'human IAPP lines' transgenic IAPP mRNA was specifically detected by the *human IAPP probe*. Endogenous mouse IAPP mRNA (800 nucleotides) was detected by cross-hybridization to the *rat IAPP probe*. Endogenous mouse insulin mRNA was detected by cross-hybridization to the *human insulin probe*

IAPP (Peninsula) was added and the incubation continued for another 48 h at 4 °C. A second (goat-anti-rabbit) antiserum was added and incubated for 4 h at 4 °C. Immune-complexes were precipitated and the ratio bound/not bound ^{125}I-IAPP was calculated. The recovery of synthetic IAPP in this procedure (extraction plus RIA) was 82 ± 6% (range 73–94%, n = 16) and the minimal detection limit was 2.3 ± 0.1 femtomol (fmol) per sample. Intra-assay variation was 16.3% (4.3 pmol/l) to 6.2% (32.0 pmol/l) (n = 10) and inter-assay variation was 12.2% (4.1 pmol/l) to 7.4% (29.7 pmol/l) (n = 8). The cross-reactivity in this RIA of rat (= mouse) IAPP is 100%. Thus, the sum of endogenous mouse IAPP and transgenic IAPP was measured. Plasma insulin concentrations were measured by RIA in duplicate using 50 µl samples and a polyclonal guinea pig antiserum against swine insulin [41]. The recovery in this RIA was approximately 95% and the sensitivity was 1 fmol per sample. Because the actual degree of cross-reactivity with mouse insulin was unknown, we have expressed plasma insulin levels as pmol equivalents of swine insulin per litre (pmolE/l). Plasma glucose levels were determined in 50 µl samples using the hexokinase method [42].

Statistical analyses

Since plasma IAPP levels were not normally distributed within the transgenic lines, statistical calculations for investigating differences in plasma IAPP levels, plasma insulin or glucose levels between the different groups of mice (transgenic and control) were performed using the non-parametric Kruskal-Wallis Test and Multiple Comparison Z-Values ($z > 1.96$).

To investigate relations between plasma levels of IAPP and plasma levels of insulin or glucose of individual animals within each of these groups of mice, multiple regression analysis was performed. Multiple regression analysis was also used to compare the body weights between the different groups of mice. The limit of significance was set at $p = 0.05$.

Results

Seven mice which were Southern blot positive for the human construct and two for the rat construct were obtained (founders). Tissue specific expression of the transgenes was examined by Northern blot analysis of RNA isolated from different tissues. Human IAPP mRNAs of 2100 and 1600 nucleotides were detected in the pancreas of all mice with the human construct, whereas rIAPP mRNA of 1050 nucleotides was detected in the pancreas of all mice with the rat construct (Fig. 2). As compared to normal human pancreas, hIAPP mRNAs were more abundant (4–22 fold) in the pancreas of hIAPP transgenic mice. The level of expression was highly variable between mice from different founders (Fig. 2A), but was not related to the transgene copynumber (5–25, data not shown). As compared to normal rat pancreas, rIAPP mRNA was two- to three-fold more abundant in the pancreas of rIAPP transgenic mice (Fig. 2B). The levels of endogenous mouse IAPP mRNA and insulin mRNA in the pancreas were apparently not influenced by expression of the human or rat IAPP transgene (Figs. 2A and 2B). Three hIAPP lines (# 18, # 19 and # 20) and two rIAPP lines (# 45 and # 46) were established and mice from 21–63 weeks of age were used for further characterization. Transgenic RNA was also detected in the kidney, except for mice of line # 45. No IAPP RNA was detected in brain, liver, spleen, stomach, heart, lung, salivary gland or urogenital tract.

Table 1. Median of plasma IAPP, insulin and glucose levels of non-fasted transgenic and control mice

Line	n	IAPP (pmol/l)	Insulin (pmolE/l)	Glucose (mmol/l)
# 18	2f/2m	258 (146–380)[a]	80 (29–102)	9.3 (7.9–10.2)
# 19	4f/2m	122 (69–218)[a]	80 (65–145)	8.2 (7.2–10.6)
# 20	2f/2m	53 (37–105)	87 (44–123)	10.9 (8.5–13.7)
# 45	2f/3m	63 (24–170)	102 (22–109)	9.5 (8.8–13.1)
# 46	2f/2m	463 (351–665)[a]	65 (29–73)	9.4 (8.5–9.7)
Con	5f/5m	32 (14–42)	73 (36–102)	10.1 (7.9–12.7)

[a] $p < 0.05$ vs control mice. Con, Non-transgenic controls (littermates); n, number of animals investigated; f, female mice; m, male mice. Range is indicated in parentheses. pmolE/l, pmol equivalents of swine insulin per litre

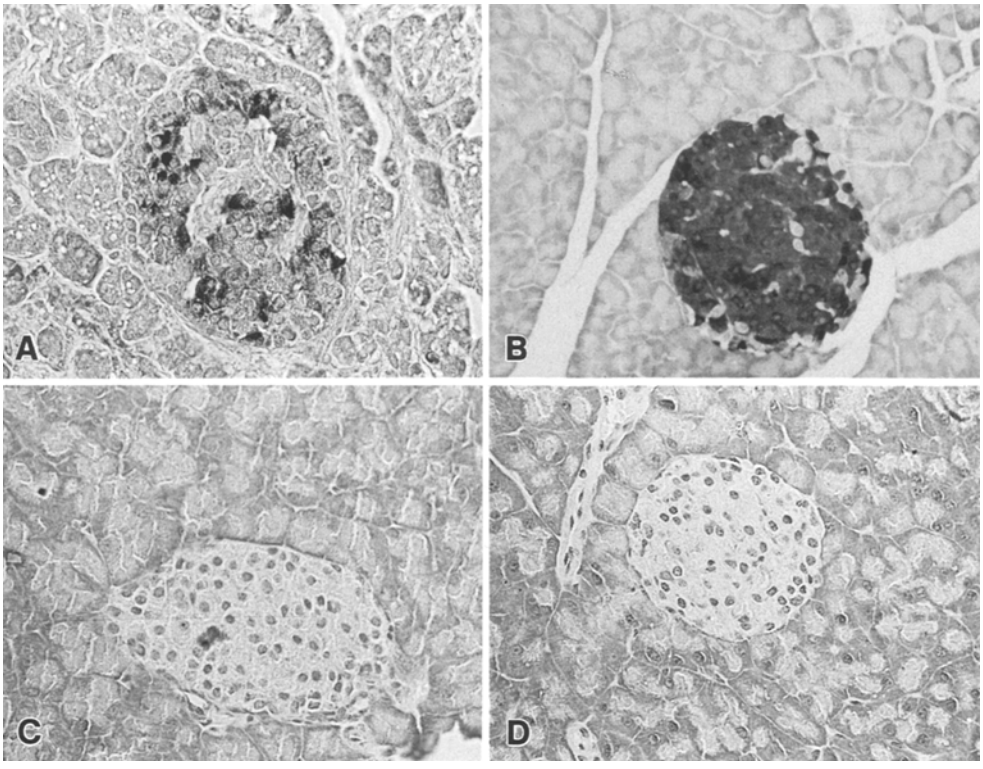
Translation of transgenic hIAPP mRNA was investigated by immunohistochemistry, using an antibody raised against the carboxy-terminal flanking peptide to hIAPP. This antibody immunostained beta cells in human pancreas and in hIAPP transgenic mouse pancreas, but not in rIAPP transgenic or control mouse pancreas, thus confirming the production of transgenic human protein (Figs. 3A–D). Stronger IAPP immunostaining of beta cells in rIAPP transgenic mice as compared to non-transgenic control mice, indicated translation of transgenic rIAPP mRNA in the pancreas. In the kidney specific IAPP immunoreactivity could not be detected, except for rIAPP transgenic mice of line # 46 where IAPP was localized to proximal tubular cells (data not shown).

Plasma levels of IAPP were significantly elevated in mice from three transgenic lines (# 18, # 19 and # 46), as compared to non-transgenic mice of the same genetic background (Table 1). In some animals a more than 15-fold elevated plasma IAPP concentration was measured. As indicated by the large range-values in Table 1, the plasma IAPP levels varied greatly between mice within the same line. Due to this variation the elevation of plasma IAPP levels in lines # 20 and # 45 was not statistically significant compared to control mice.

Plasma insulin or glucose levels were not significantly different between the transgenic mice and controls (Table 1). Also, within each of the transgenic lines there was no significant correlation between plasma levels of IAPP and insulin or glucose from individual mice.

Body weights of animals between 20 and 60 weeks of age were not significantly different between transgenic mice ($n \geq 12$ for each of the lines) and controls ($n = 39$).

Amyloid deposits were not detected in hIAPP transgenic mice (n = 6–8 for each of the three lines) or rIAPP transgenic mice (n = 6 for both lines) or in non-transgenic control mice (n = 16), up to 63 weeks of age. As a positive control, islet amyloid was detected in the pancreas of a patient with Type 2 diabetes. At the subcellular level, IAPP immunoreactivity was localized to beta-cell secretory granules in all mice examined, both transgenic for hIAPP (n = 12) or rIAPP (n = 8) and non-transgenic (n = 10). However, the highest level of immunoreactivity was observed in beta-cell lysosomal bodies in all hIAPP transgenic mice (n = 4 for each line) (Fig. 4). IAPP immunoreactivity in lysosomes was not seen in similarly aged non-transgenic mice nor in mice expressing the rIAPP transgene (n = 4 for each of the two lines).



Figs. 3 A–D. Detection of human IAPP gene encoded protein in pancreas tissue by immunohistochemical staining with an antiserum raised against the carboxy-terminal flanking peptide to human IAPP. Immunoreactivity was limited to islet beta cells of (**A**) human and (**B**) human IAPP transgenic mouse pancreas (from line # 19), but was not detected in (**C**) control mouse or (**D**) rat IAPP transgenic mouse pancreas (from line # 45). Magnification: × 200

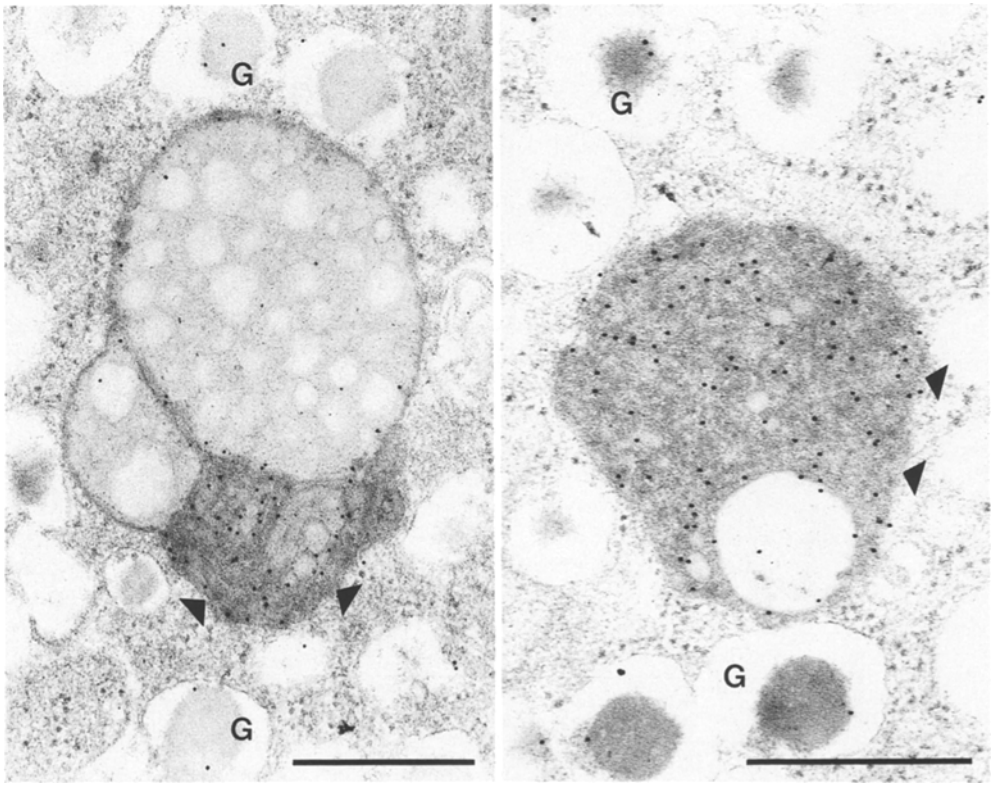


Fig. 4. Subcellular localization of IAPP in islets of human IAPP transgenic mice, using electron microscopy and immunogold labelling. Secretory granules *(G)* in beta cells of all transgenic and control mice showed immunoreactivity. In addition a much stronger immunoreactivity was observed in beta-cell lysosomal bodies *(arrows)* only in animals producing human IAPP. Scale bar = 0.5 μm

Discussion

The rat insulin 2 gene promoter/IAPP gene constructs directed the production of high levels of transgenic IAPP mRNA in the pancreas of most transgenic mice. Exact quantitative comparisons between transgenic and endogenous mouse IAPP mRNA levels per beta cell are difficult, due to differences in nature and specific activity of the probes used. Furthermore, differences in islet density between human, rat and mouse pancreas confound these comparisons. Downregulation of the endogenous mouse IAPP gene was not observed in any of the transgenic lines. Downregulation of the endogenous mouse insulin gene was also not seen despite the presence of multiple copies of the transgenic constructs containing the rat insulin 2 gene promoter fragment, which might compete for common transcription factors. Using immunohistochemistry, translation of transgenic IAPP mRNA in the pancreas was confirmed.

Transgenic RNA was also detected in the kidney, as previously described for other rat insulin 2 gene promoter/transgene constructs [43, 44]. This 'ectopic' expression is probably due to the nature of the rat insulin 2 gene upstream region in the constructs used. Thus, upstream of position − 695 relative to the transcription start site, sequences might be located which prevent activity of the rat insulin 2 gene promoter in tissues other than the pancreas. Translation of transgenic IAPP RNA in the kidney could be demonstrated only in mice of line # 46, which express the rIAPP transgene.

Reported circulating IAPP levels are 5–15 pmol/l in man [45] or 55–90 pmol/l in mouse [46]. However, for hyperinsulinaemic ob/ob and db/db mice, plasma IAPP levels up to 290 pmol/l have been reported [46]. In other animal models of obesity and diabetes high circulating levels of both IAPP and insulin have also been reported [47, 48]. In man both plasma IAPP and insulin levels are higher in some obese, glucose-intolerant subjects than in controls [29, 30]. Apparently, IAPP and insulin secretion are co-ordinately regulated in fasting [49], as well as in obesity and diabetes. In our mouse model the level of transgene expression is independent of endogenous mouse insulin gene expression. Thus, effects of elevated plasma IAPP levels on insulin secretion could be examined, as well as other effects which might be due to elevated plasma IAPP levels irrespective of high insulin levels.

Our data show that IAPP exerts no obvious long-term effect on insulin gene expression or insulin secretion by beta cells at (supra)physiological concentrations of the native peptide (median 53–463 pmol/l). This is in accordance with several in vitro and in vivo studies, which failed to demonstrate an effect of IAPP on insulin biosynthesis or secretion, even at concentrations of 100 nmol/l [22–25]. In some short-term studies IAPP-induced inhibition of insulin secretion has been demonstrated, mostly at pharmacological concentrations of 0.1–10 μmol/l [13–15] but, more recently, also at a concentration of 75 pmol/l [16]. However, changes of insulin secretion in a situation of chronically elevated IAPP concentrations have not yet been explored.

Hyperglycaemic effects of IAPP described in the literature have been established at very high concentrations (mostly at least 1 nmol/l) of synthetic human or rat IAPP [3, 17–21]. We did not observe elevated plasma glucose levels in any of our transgenic lines, at plasma IAPP levels ranging from 53 to 463 pmol/l (median). This does not exclude a local effect of IAPP on glucose metabolism in skeletal muscle or liver, which might not result in long-term elevated plasma glucose levels due to counterregulation. In theory, both an inhibitory effect on insulin secretion (direct effect of IAPP on beta cells) and a stimulatory influence on insulin secretion (due to IAPP-induced insulin resistance in peripheral tissues) might counterbalance each other without an obvious effect on plasma insulin levels. However, the resulting insulin level would probably be insufficient to prevent elevated glucose levels as a consequence of the insulin resistance.

Type 2 diabetes is an age-related disease which is usually not diagnosed before the age of 40 years and which may have very diverse pathogenic origins [50]. As demonstrated in diabetic man [51] and diabetic monkeys [52], the extent of islet amyloid formation increases with disease severity. Formation of insoluble amyloid deposits around capillaries is likely to be involved in the progressive deterioration of islet function. Although islet amyloid in diabetic man and diabetic animals is found predominantly extracellularly, there are several indications suggesting that amyloid fibrils might result from accumulation of IAPP at intracellular sites [53]. The highest density of IAPP immunoreactivity was localized to beta-cell lysosomal bodies in human IAPP transgenic mice, as in man [54]. In human insulinoma cells intracellular IAPP-immunoreactive amyloid fibrils have been demonstrated [55]. If amyloid fibril formation in pancreatic islets occurs as a consequence of accumulation of IAPP, lysosomal bodies would represent a potential intracellular site for the initiation of amyloidogenesis, in particular since both lysosomal accumulation and amyloid fibril formation are related to the amino acid sequence of IAPP. Deposits of islet amyloid were not found in hIAPP transgenic mice up to the age of 63 weeks, but might develop later in life. Alternatively, mice simply do not grow old enough to develop islet amyloid deposits as we know them in man or perhaps additional factors, such as those present in diabetes, are required for islet amyloidogenesis (e.g. hyperglycaemia or hyperinsulinaemia or both).

In conclusion, our present data indicate that chronically elevated IAPP levels per se do not inhibit insulin biosynthesis and do not cause elevated glucose levels, hyperinsulinaemia or obesity, suggesting no insulin resistance, in mice up to 63 weeks old. Human IAPP is handled by transgenic mouse beta cells in a similar manner as by human beta cells, causing accumulation of IAPP within lysosomal bodies. Further studies are needed to investigate whether this lysosomal pathway can lead to fibrillogenesis and amyloidogenesis. In the pathogenesis of Type 2 diabetes, increased production of IAPP may induce and/or contribute to progressive amyloid formation, which in the long term may lead to beta-cell dysfunction, insufficient insulin secretion and hyperglycaemia. The overproduction of human IAPP in this transgenic mice

model offers a unique opportunity to study the biosynthesis, intracellular handling, secretion and extracellular handling of human IAPP in vivo.

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