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Islet amyloid polypeptide: identification and chromosomal localization of the human gene

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Islet or insulinoma amyloid polypeptide (IAPP) is a 37 amino acid polypeptide isolated from pancreatic amyloid. Here, we describe the isolation and partial characterization of the human gene encoding IAPP. The DNA sequence predicts that IAPP is excised from a larger precursor protein and that its carboxy-terminus is probably amidated. The predicted normally occurring IAPP is identical to the reported polypeptides isolated from pancreatic amyloid, except for the amidated carboxy-terminus. IAPP specific polyadenylated RNAs of 1.6 kb and 2.1 kb are present in human insulinoma RNA. The human IAPP gene is located on chromosome 12.

Islet amyloid polypeptide gene; Amyloid; Diabetes type 2; Insulinoma; Calcitonin gene family

1. INTRODUCTION

Amyloid is a pathological deposit of peptide subunits arranged in β-pleated sheet fibrils. It is commonly found in pancreatic islets of patients with diabetes mellitus type 2 (non-insulin-dependent) and in insulinomas. Recently, islet or insulinoma amyloid polypeptide (IAPP) was isolated as a major constituent of amyloid present in a human insulinoma [1,2]. IAPP was also isolated from islet amyloid in a human type 2 diabetic patient and in the diabetic cat [2,3]. A similar polypeptide, termed diabetes associated peptide (DAP), was also isolated from islet amyloid [4,5]. Most likely IAPP and DAP represent the same polypeptide.

Human IAPP (hIAPP) is 37 amino acids long with cysteine residues in positions 2 and 7. These features are also found in all known calcitonin gene-related peptides (CGRPs) [6–8]. Furthermore, hIAPP shows 46% amino acid sequence homology with hCGRP-II [2,5,9]. IAPP has been demonstrated immunochemically in normal β-cells of several mammals [2,4,10]. These data suggest that IAPP has important hormonal significance with respect to pancreatic islet function and is expressed from a gene distantly related to the calcitonin gene family.

The isolation and chromosomal localization of the human IAPP gene are presented in this report. Furthermore, expression of this gene in a human insulinoma was confirmed by the detection of IAPP specific polyadenylated RNAs on a Northern blot. Investigations of the structure and expression of the IAPP gene are of importance with respect to pancreatic amyloid formation and with respect to the evolution of the calcitonin/CGRP (CALC) gene family.

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2. MATERIALS AND METHODS

2.1. *Phage λ library of human genomic DNA*

An amplified human genomic library in λEMBL3 [11], prepared from chromosomal DNA from chronic myeloid leukemia cells, was kindly provided by Dr G. Grosveld (Erasmus University, Rotterdam, The Netherlands).

2.2. *Oligonucleotides (see fig.1)*

Oligonucleotide IAPP 1–18 corresponds to amino acids 1–18 of hIAPP [5]. Oligonucleotide IAPP 30–39 corresponds to amino acids 30–37 [5], whereas a glycine residue was predicted in position 38 and an arginine residue in position 39. These residues are highly conserved in all known CGRP precursors [6–8]. The nucleotide sequences of the oligonucleotides were selected using the criteria of Lathe [12]. Oligonucleotide CGRP 12–21 is 100% homologous to the regions encoding amino acids 12–21 of hCGRP-I and -II [6].

2.3. *Oligonucleotide labeling*

Labeled oligonucleotide probes were synthesized on template oligonucleotides by elongation of hybridized primers (see fig.1), using the Klenow fragment of DNA polymerase I [13]. [α-^{32}P]dGTP (IAPP 30–39) or [α-^{32}P]dCTP (IAPP 1–18 and CGRP 12–21) was incorporated during the reaction. A mixture of 8 pmol primer and 6 pmol template oligonucleotide in 10 mM Tris-HCl, pH 8.5/10 mM $MgCl_2$ and 0.1 mM of each of the unlabeled dNTPs was heated to 95°C for 3 min and allowed to cool to 20°C. The mixture was added to 250 μCi [α-^{32}P]dGTP or [α-^{32}P]dCTP, which had been dried in advance. After the addition of Klenow enzyme (5 U, Pharmacia) the mixture was incubated for 45 min at 16°C, 45 min at 20°C and 30 min at 37°C. A chase reaction was performed by the addition of dGTP or dCTP to 0.1 mM and incubation for 15 min at 37°C. Due to the difference in length, the labeled strand could be separated from the unlabeled template strand on a 10% polyacrylamide/7 M urea gel. After autoradiography, the labeled strand was excised from the gel and eluted for 2 h in 10 mM Tris-HCl, pH 7.5/1 mM EDTA. The specific activity of the oligonucleotide probes was $6–7 \times 10^9$ dpm/μg.

2.4. *Plaquelifts, Northern blotting, Southern blotting and hybridizations*

One million plaques were screened on Hybond-N membranes (Amersham) at a density of 35000/135 mm filter. Plaquelifts were made as described by the supplier. RNA was isolated from a human insulinoma [14] and used for a Northern blot on a Hybond-N membrane [15]. Southern blotting of agarose gels was performed on Hybond-N membranes overnight, using 20 × SSC, as described in [16]. Double-stranded DNA probes were labeled by random priming [17] using [α-^{32}P]dCTP. Hybridization of membranes with probes was performed in the presence of 50% formamide and 6 × SSC overnight at 30°C (probes IAPP 30–39 and CGRP 12–21), at 35°C (IAPP 1–18) or at 42°C (ds-DNA probes). Filters were washed in 2 × SSC, 2 × 30 min at hybridization temperature, except for filters hybridized with ds-DNA probes (2 × SSC/0.5% SDS at 65°C, 2 × 30 min). Filters were exposed to Fuji RX films using intensifying screens. As an IAPP-specific probe we used a *Pst*I-*Bam*HI fragment, which contains 4 nucleotides of the M13 polylinker and nucleotides 8–171 (see fig.2).

2.5. *Nucleotide sequence analysis and chromosomal assignment*

The 170 bp *Sau*3A and the 570 bp *Alu*I fragments subcloned in M13 mp9 were sequenced using the dideoxy-nucleotide chain-termination method [18]. ds-DNA fragments of these clones were labeled 5′-terminally using [γ-^{32}P]ATP and T_4 kinase and sequenced according to the chemical modification method [19]. The method used for the chromosomal assignment of a human gene has been described [20]. A Southern blot prepared from *Taq*I-digested DNA from 14 human-Chinese hamster and 2 human-mouse somatic cell hybrids was hybridized to the *Pst*I-*Bam*HI probe.

3. RESULTS

Plaquelift filters were successively hybridized with the oligonucleotide probes listed in fig.1. Under low stringency hybridization conditions, these oligonucleotides each detect many genomic clones (100–200). One clone (λh201) hybridized to both oligonucleotides IAPP 1–18 and IAPP 30–39, but not with oligonucleotide CGRP 12–21. 33 clones were identified as CALC-I or CALC-II clones because of their strong hybridization with the CGRP 12–21 oligonucleotide probe. Nucleotide sequence analysis of a 570 bp *Alu*I and a 170 bp *Sau*3A fragment, obtained by subcloning h201 in M13 mp9, reveals the DNA sequence shown in fig.2.

The deduced amino acid sequence of part of the genomic *Alu*I fragment of λh201 (nucleotides 75–185 in fig.2) predicts a 37 amino acid polypeptide identical to the polypeptides isolated from pancreatic amyloid [2,5]. Apparently, hIAPP is cleaved from a larger precursor polypeptide at the proteolytic processing sites Lys-Arg (nucleotides 69–74) and Gly-Lys-Arg (nucleotides 186–194), the latter being a combined site for proteolytic cleavage and subsequent amidation of the preceding amino acid. The carboxy-terminal polypeptide of the hIAPP precursor is 16 amino acids long, which might be cleaved at the Lys-Arg proteolytic site corresponding to nucleotides 213–218. These data clearly indicate that λh201 contains (part of) the human IAPP gene. The oligonucleotide probes used to identify this gene are 86% (IAPP 30–39) and 76% (IAPP 1–18) homologous to the corresponding parts of the IAPP gene.

The *Pst*I-*Bam*HI probe hybridizes with an 11 kb *Eco*RI, a 9 kb *Pst*I and a 4.0 kb *Taq*I fragment on a Southern blot of human genomic DNA (fig.3).

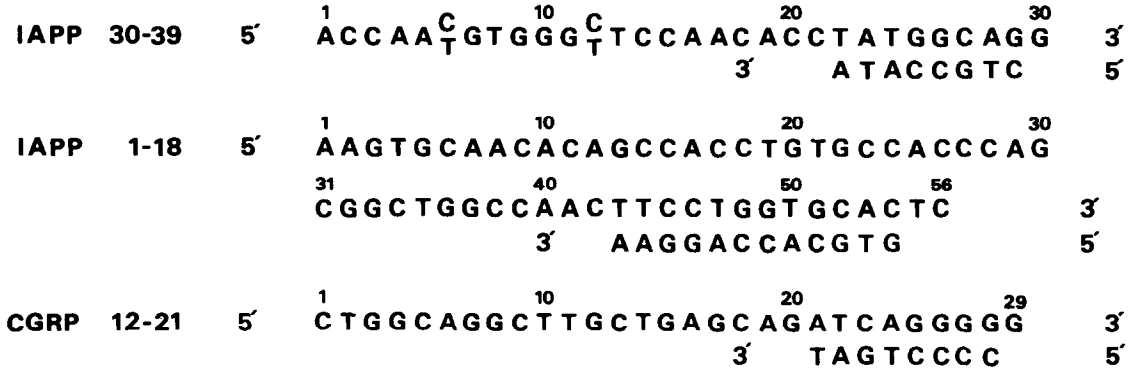

Fig.1. Sequences of oligonucleotides IAPP 30–39, IAPP 1–18, CGRP 12–21 and their respective primers. Primers are elongated using Klenow polymerase. Incorporation of labeled dNTPs results in a labeled strand, which can be separated from the template oligonucleotide, due to the difference in length.

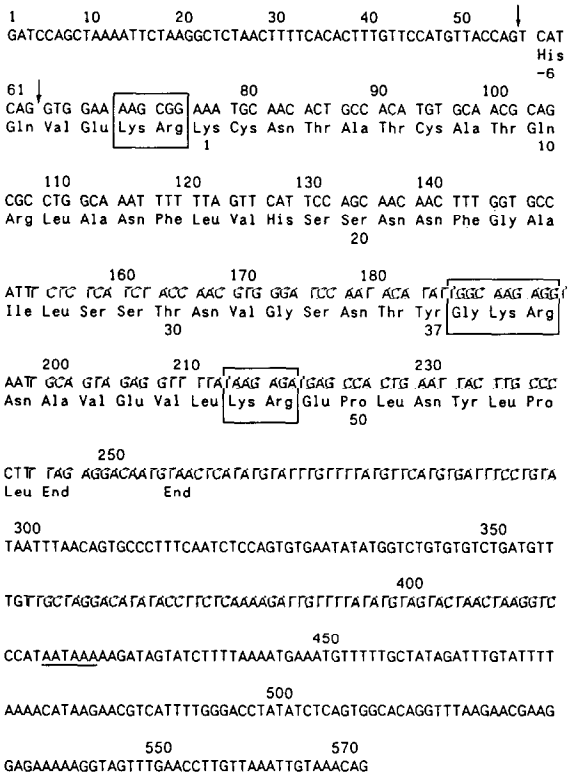

Fig.2. Nucleotide sequence of part of the human IAPP gene. Putative 3′-splice sites are indicated by arrows. Translation from nucleotide 56 downstream reveals part of the IAPP precursor. Potential proteolytic processing sites are boxed. The stop codon (243–245; end) is followed by a second stop codon in the same reading-frame (255–258; end). A putative polyadenylation signal (419–424) is underlined. The 170 bp *Sau* fragment corresponds to nucleotides 1–171; the 570 bp *Alu*I fragment to nucleotides 8–571.

On a Northern blot containing RNA isolated from a human insulinoma, the same probe detected two polyadenylated RNA species of 1.6 and 2.1 kb, respectively (fig.4). Hybridization of *Taq*I-digested DNA of human-rodent somatic cell hybrids with the hIAPP probe reveals the presence of the human IAPP gene on chromosome 12 (table 1). Two hybrids were available containing chromosomes derived from the reciprocal translocation t(12;21)(q14;q21) [21]. A1WBF-2

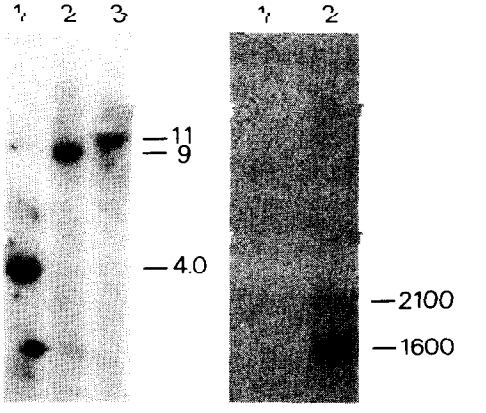

Fig.3. (Left) Southern blot analysis of human chromosomal DNA digested with *Taq*I (lane 1), *Pst*I (lane 2) and *Eco*RI (lane 3), hybridized with the *Pst*I-*Bam*HI probe (see section 2). Sizes of hybridizing fragments are indicated in kilobases (kb).

Fig.4. (Right) Northern blot analysis of RNA isolated from a human insulinoma. Total cellular (3.3 μg, lane 1) and poly(A)-enriched (3 μg, lane 2) RNA preparations were hybridized with the *Pst*I-*Bam*HI probe (see section 2).

Table 1

Segregation of the human IAPP locus with human chromosomes in 16 human-rodent somatic cell hybrid clones

Chromosome	Chromosome/IAPP locus				% discordance
	+/+	+/−	−/+	−/−	
1	7	4	4	1	50
2	2	1	9	4	63
3	7	2	4	3	38
4	5	2	6	3	50
5	7	2	4	3	38
6	3	2	8	3	63
7	6	1	5	4	38
8	7	4	4	1	50
9	6	2	5	3	44
10	5	1	6	4	44
11	6	5	5	0	63
12	10	0	1	5	6
13	6	1	5	4	38
14	8	1	3	4	25
15	9	2	2	3	25
16	8	4	3	1	44
17	10	3	1	2	25
18	3	2	8	3	63
19	6	4	5	1	56
20	9	2	2	3	25
21	7	4	4	1	50
22	7	3	4	2	44
X	8	3	3	2	38

Numbers in columns indicate the number of hybrid clones which contain both the numbered chromosome and the IAPP gene (+/+), the numbered chromosome but not the IAPP gene (+/−), the IAPP gene but not the numbered chromosome (−/+), or neither the numbered chromosome nor the IAPP gene (−/−)

containing the q14-pter part of chromosome 12 was positive for IAPP. A1WCB containing the q14-qter part of chromosome 12 was negative for IAPP (not shown).

4. DISCUSSION

We have isolated from a human genomic library the gene encoding hIAPP by (i) usage of two oligonucleotide probes corresponding to different parts of the amino acid sequence of hIAPP and (ii) labeling of oligonucleotide probes to a high specific activity using the primer extension method.

IAPP is a major constituent of pancreatic amyloid and since amyloid is found in the majority of type 2 diabetic patients as well as in insulinomas, an important role is suggested for IAPP in the pathogenesis of pancreatic islet dysfunction. The deduced amino acid sequence of the IAPP encoding part of the gene reveals that the normally occurring polypeptide is identical to the polypeptides isolated from pancreatic amyloid [2,5]. This observation implies that amyloid is not formed due to the presence of a mutated form of IAPP, as has been hypothesized [5]. The glycine residue following Tyr-37 indicates that, like all known CGRPs and calcitonins, hIAPP is probably carboxy-terminally amidated. This has not been reported by the amino acid sequence analysis, but it is in agreement with the reported problems concerning the carboxy-terminal identification with carboxypeptidase Y [5]. Amidation of IAPP is probably important for studies on the physiological role of IAPP.

Like many other polypeptide hormones mature IAPP is processed from a larger precursor protein at Lys-Arg proteolytic cleavage sites. When we compare the deduced amino acid sequence of the IAPP precursor (fig.2) with the reported deduced sequences of CGRP precursors [6–8], it appears that IAPP (amino-terminally cleaved) and the CGRPs are cleaved at a Lys-Arg proteolytic site. The highly conserved Gly-Arg-Arg-Arg-Arg sequence at the carboxy-terminus of the CGRPs is replaced by Gly-Lys-Arg in the IAPP precursor. The deduced carboxy-terminal peptides of the known CGRP precursors consist of four amino acids, whereas the IAPP carboxy-terminal peptide is 16 amino acids long, and is possibly cleaved to form two small peptides of 6 and 8 amino acids. Whether these have physiological functions, remains to be determined.

The IAPP gene probably is a single copy gene in the human genome, because only one genomic *Eco*RI, *Pst*I or *Taq*I fragment is detected with the IAPP-specific probe. Since the polypeptide is present in pancreatic islets and in insulinomas, this gene is supposed to be expressed in pancreatic β-cells. In a human insulinoma two IAPP-specific RNA species of approx. 1.6 and 2.1 kb have now been detected. The presence of two different polyadenylated IAPP RNAs in this tissue remains to be investigated. The IAPP RNAs are rather large in comparison to the human CGRP mRNAs (1.1–1.2 kb) [22,23].

We assume that hIAPP is encoded by an exon starting at position 56 or position 63 (in fig.2). Both potential 3′-splice sites resemble the consensus sequence $(Py)_nNCAG$ [24]. Splicing between nucleotides 55 and 56 would result in an exon starting at exactly the same distance (19 nucleotides) upstream of the hIAPP- and hCGRP-encoding sequences, respectively. Two potential branch point sequences are present (positions 14–20 and 22–28), which would result in branching at the A-residues at positions 19 or 27 (−37 and −29, respectively). It is unclear whether this putative exon is spliced at the 3′-end or whether the potential poly(A) addition signal at position 419–424 is used. The sequence information of an IAPP cDNA will be important in this respect.

The hIAPP gene was assigned to chromosome 12 using the human-rodent somatic cell hybrid technique. Limited regional localization, using hybrids containing translocation-derived chromosomes, placed the IAPP locus within the q14-pter region. The gene is thus not located in close proximity to the related CALC-I, CALC-II and CALC-III genes, which were assigned to chromosome 11 [20,25,26]. This observation implies that the exon 2- and 3-like sequences of CALC-III [26] are not directly related to the IAPP gene. Probably the IAPP gene arose by duplication and translocation to chromosome 12 of a progenitor CALC-gene and subsequent sequence divergence. An evolutionary relationship between chromosomes 11 and 12 has been suggested [27].

The availability of DNA probes may be important in determining the role of IAPP in normal pancreatic islet function and its possible role in the pathogenesis of type 2 diabetes mellitus. The resemblance of part of the IAPP gene to the CALC genes makes it tempting to consider the possibility of the IAPP gene revealing alternative RNA processing, since this phenomenon occurs in the CALC-I gene.

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