Sequences of islet amyloid polypeptide precursors of an old world monkey, the pig-tailed macaque (*Macaca nemestrina*), and the dog (*Canis familiaris*)

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Summary. The 37-amino acid islet amyloid polypeptide represents the major protein component present in islet amyloid deposits. Although the presence of islet amyloid is a characteristic pathological feature of the islets of humans, monkeys and cats with Type 2 (non-insulin-dependent) diabetes mellitus, it is not found in the islets of diabetic rats, mice or dogs. To further explore the molecular basis for these species differences in amyloid deposition we have used a polymerase chain reaction based method to clone cDNAs encoding the monkey (*Macaca nemestrina*) and dog (*Canis familiaris*) islet amyloid polypeptide precursors. The predicted amino acid sequence of the monkey precursor is 96% identical to that of the human protein; differences include one replacement in the signal peptide and three in the islet amyloid polypeptide domain. The sequence of the dog precursor is most closely re-

lated to that of the cat protein (85% identity); the sequences of dog and cat islet amyloid polypeptide differ only at two positions and are identical in the region of amino acids 20–29, the region thought to be primarily responsible for amyloidogenesis. Thus, amino acid residues in addition to those at positions 20–29 may facilitate the aggregation of islet amyloid polypeptide. The presence of amyloid deposits in some dog pancreatic endocrine tumours suggests that the dog protein can be amyloidogenic, perhaps due to elevated expression of islet amyloid polypeptide by the tumours relative to normal islets.

Key words: Islet amyloid polypeptide precursors, monkey (*Macaca nemestrina*), dog (*Canis familiaris*), amyloidogenic properties.

Islet amyloid polypeptide (IAPP) is a normal secretory product of the pancreatic Beta cell and is a major constituent of amyloid deposits in patients with Type 2 (non-insulin-dependent) diabetes mellitus [1–4]. The levels of IAPP in the islet and in the peripheral circulation are about 1% those of insulin [5]. The presence of amyloid deposits is the most common pathological lesion seen in the islets of human subjects with Type 2 diabetes. These deposits are also present in the islets of aged individuals although the degree of amyloidosis is much less than that seen in diabetic subjects. Although IAPP is predicted to have hormone-like properties, its normal physiological function(s) are unknown. However, its pathological deposition in the islets disrupts the normal architecture of the islet and may account at least in part for the impaired Beta-cell function seen in patients with Type 2 diabetes. Amyloid deposits have also been noted in the islets of diabetic monkeys [6, 7] and cats [8]; the protein component of cat islet amyloid has been isolated and partially sequenced and shown to be IAPP [3]. Amyloid deposits also occur in the islets of the diabetic degu, a South American rodent related to the guinea pig; however, the protein

component of degu islet amyloid is insulin rather than IAPP [9].

Although IAPP-derived amyloid deposits are a feature of diabetes mellitus in humans, monkeys and cats, amyloidosis is not present in the islets of diabetic mice, rats or dogs. The molecular basis for this difference in islet pathology may be related to the structure of the IAPP molecule, especially amino acid sequence differences in the putative fibril-forming region (residues 20–29) [10]. As part of our ongoing studies of the biosynthesis and functional properties of IAPP as well as its role in the impairment of islet function, we have determined the sequences of the IAPP precursors in two useful animal models of diabetes, one of which is characterized by the presence of islet amyloid deposits, the old world monkey, *Macaca nemestrina*, and the other which is not, the dog, *Canis familiaris*.

Materials and methods

Genomic DNA was prepared from peripheral blood lymphocytes obtained from a single monkey and from two dogs. RNA was isolated from the pancreases of a fasted monkey and dog using the gua-

GAGGGGGTAAATATTCCAGTGGATACAAGCTTGGGCTCTTTTCTTGAAGCTTTCTTCTGTCTCAGAGGCATTTGCTGATATTGCTGACGTTGAAACAGTAAAAGAAAAATTTGAGAAGCA

-22	-20	Signal peptide										-10	1 NH ₂ -terminal propeptide																		
Met	Cys	Ile	Leu	Lys	Leu	Gln	Val	Phe	Leu	Ile	Val	Leu	Ser	Val	Ala	Leu	Asn	His	Leu	Lys	Ala	Thr	Pro	Ile	Glu	Ser	His	Gln	Val		
ATG	TGC	ATT	CTG	AAG	CTG	CAA	GTA	TTT	CTC	ATC	GTG	CTC	TCT	GTT	GCA	TTA	AAC	CAT	CTG	AAA	GCT	ACA	CCC	ATT	GAA	AGT	CAT	CAG	GTG		
10				IAPP				20				30																			
Glu	Lys	Arg	Lys	Cys	Asn	Thr	Ala	Thr	Cys	Ala	Thr	Gln	Arg	Leu	Ala	Asn	Phe	Leu	Val	Arg	Ser	Ser	Asn	Asn	Phe	Gly	Thr	Ile	Leu		
GAA	AAG	CGG	AAA	TGC	AAC	ACT	GCC	ACG	TGT	GCA	ACG	CAG	CGC	CTG	GCA	AAT	TTT	TTA	GTT	CGT	TCC	AGC	AAC	AAC	TTT	GGT	ACC	ATT	CTC		
40				50				COOH-terminal propeptide												60				67							
Ser	Ser	Thr	Asn	Val	Gly	Ser	Asp	Thr	Tyr	Gly	Lys	Arg	Asn	Ala	Val	Glu	Val	Leu	Lys	Arg	Glu	Pro	Leu	Asn	Tyr	Leu	Pro	Leu	AM		
TCA	TCT	ACC	AAC	GTG	GGG	TCC	GAT	ACA	TAT	GGC	AAG	AGG	AAT	GCA	GTA	GAG	GTT	TTA	AAG	AGA	GAG	GAG	CCA	CTG	AAT	TAC	TTG	CCC	CTT	TAG	
AGGACGATGTAACCTCTATAGTTATTGTTTTATGTTCTAGAGATTTCTCTGTATAATTTAACTGCCTTTTTCATTTCCAGTGTGAATATATGGTCTGTGTATCTGATGTTTGTGCCAGG																															
ACATATACATTGTCAAAGATTGTTTTATAT																															

	-22	-20	Signal peptide								-10																	1 NH ₂ -terminal			
TTGAGAAACA	Met	Cys	Leu	Leu	Lys	Leu	Pro	Val	Val	Leu	Ile	Ile	Leu	Ser	Val	Ala	Leu	Asn	His	Leu	Lys	Ala	Thr	Pro	Ile	Lys	Ser				
ATG	TGT	CTC	CTG	AAG	CTG	CCA	GTA	GTT	CTC	ATC	ATA	CTC	TCG	GTT	GCA	TTA	AAC	CAT	CTG	AAG	GCT	ACT	CCC	ATT	AAA	AGT					
propeptide			10		IAPP							20		30																	
His	Gln	Met	Glu	Lys	Arg	Lys	Cys	Asn	Thr	Ala	Thr	Cys	Ala	Thr	Gln	Arg	Leu	Ala	Asn	Phe	Leu	Val	Arg	Thr	Ser	Asn	Asn	Leu	Gly		
CAC	CAG	ATG	GAA	AAG	CGG	AAA	TGC	AAC	ACT	GCC	ACG	TGT	GCG	ACT	CAA	CGC	CTG	GCA	AAT	TTC	TTG	GTT	CGT	ACT	AGC	AAT	AAT	CTT	GGT		
			40		COOH-terminal propeptide										60																
Ala	Ile	Leu	Ser	Pro	Thr	Asn	Val	Gly	Ser	Asn	Thr	Tyr	Gly	Lys	Arg	Asn	Thr	Ile	Glu	Ile	Leu	Asn	Arg	Gly	Pro	Leu	Asn	Tyr	Leu		
GCC	ATT	CTC	TCG	CCT	ACC	AAT	GTG	GGA	TCC	AAT	ACA	TAT	GGC	AAG	AGA	AAC	ACA	ATT	GAG	ATT	TTA	AAC	AGG	GGA	CCA	TTG	AAT	TAC	TTA		
67																															
Pro	Leu	AM																													
CCA	CTT	TAG	AAGACAACGCACACCCCTATCATTTCTTACTTTTCATGTATAAAAAATATTCTAGTGATTTCCTGTGTAAGTTAACATTAGCATGAACATAAGTTTTTATGTACTATGTTT																												
GTTACGACTACATATAAATTGGTTATGGTAAGAATTG																															

Fig.1A,B. Nucleotide and deduced amino acid sequences of monkey (**A**) and dog (**B**) islet amyloid polypeptide (IAPP) precursor cDNAs. The IAPP domain of the precursor is indicated in bold type

nidinium thiocyanate/CsCl procedure. Monkey (*Macaque nemestrina*) tissues were kindly provided by the Regional Primate Research Center at the University of Washington (supported by NIH Grant RR 00166).

Amplification of homologous DNA fragments and rapid amplification of cDNA ends

Amplification of homologous DNA fragments (AHF) was carried out as described previously [11]. Briefly, a 119-base pair DNA fragment coding for mature IAPP was amplified from genomic DNA by the polymerase chain reaction (PCR) using the following two oligonucleotide primers: 5'-AAGTGCAACACTGCCACATGTGC-3' and 5'-CGCTTGCCATATGTATTGGA-3'. PCR was carried out for 30 cycles as follows: denaturation for 1 min at 94°C, annealing for 2 min at 50°C, and extension for 3 min at 72°C. Amplified fragments of the expected size, 119 base pairs, were isolated, subcloned into the HincII site of M13 mp18, and sequenced by the dideoxy nucleotide chain-termination procedure. The remainder of the sequence of the IAPP precursor nucleotide sequence was obtained using the 5'- and 3'-RACE protocol as described previously [11]. The rapid amplification of cDNA ends (RACE) reaction products were subcloned into the HincII site of pGEM4Z, identified by colony hybridization and sequenced. Sequences of six individual clones were usually determined to rule out any errors due to the amplification procedure.

RNA blotting

RNA was separated in a 1.5% agarose-0.66 mol/l formaldehyde gel, blotted onto nitrocellulose filters, and hybridized with nick-translated cDNA. Filters were washed under high stringency conditions (15 mmol/l sodium chloride/1.5 mmol/l sodium citrate/0.1% sodium dodecyl sulphate, 60°C) and autoradiographed with intensifying screens.

Results

The sequences of monkey and dog IAPP precursors were deduced from nucleotide sequences generated by a combination of AHF and RACE. The cDNA and predicted amino acid sequences of monkey and dog IAPP are shown

Fig. 2a,b. RNA blot of monkey and dog islet amyloid polypeptide mRNAs. Five micrograms of poly(A)⁺ RNA isolated from monkey (lane a) and dog (lane b) pancreas were electrophoresed, blotted, and hybridized with the corresponding nick-translated cDNA and autoradiographed for 24 h. Positions of 18S and 28S rRNA are shown

	Signal peptide	NH ₂ -terminal propeptide	Islet amyloid polypeptide	COOH-terminal propeptide	
Human	MGILKLQVFLIVLSVALNHLKA	TPIES:::HQVEKR	KCNTATCATQRLANFLVHSSNNFGAILSSSTNVGSNTY	GKRNAVEVLKREPLNYLPL	(89)
Monkey	-C-----	-----	-----R-----T-----D-----	-----	(89)
Cat	-CL---P-V---L---H---	-----:N-----	-----IR---L---P-----	---ST-DI-N-----F	(89)
Dog	-CL---P-V---I---	---K---:M-----	-----RT---L---P-----	---TI-I-N-G-----	(89)
Rat	MRC-SR-PAV-LI---G-R-	--VG-GTNP-D---	-----R---L-PV-PP-----	---VA-DPN--S-DF-L-	(93)
Mouse	M-C-S-PAV-LI---S-R-	--VR-GSNP-MD---	-----R---L-PV-PP-----	---AGDPN--S-DF-KV	(93)
Hamster	-H-S-PAA-LIF---	--VR-GTN--MD---	-----N---L-PV-P-----	---S-A-IPDGDS-DLFL-	(92)
Guinea pig	-CL-R-P-T-L-C---E---	-S-A-DTG--G---	-----T---R-H-L-A-LP-D-----	---PQISD--LCH---	(92)
Degu	-CL-Q-P-V-LL-FA---T---	---A-DTG-R-D---	-----T---R-H-L-A-PP-K-----	-R---:Q-VDV-L-H---	(91)

Fig. 3. Comparison of deduced amino acid sequences of nine mammalian islet amyloid polypeptide (IAPP) precursors. Signal peptide, IAPP motif and NH₂- and COOH-terminal propeptide segments are indicated. Dashes indicate amino acid residues identical to the

human sequence. Colons indicate gaps inserted to generate this alignment. The length of each precursor is shown. The sequences of the IAPP precursors are from references 11, 15–17

in Figure 1. The sizes of monkey and dog pancreatic IAPP mRNA are 2.2 and 2.1 kilobases, respectively, (Fig. 2) indicating that the nucleotide sequences presented in Figure 1 do not correspond to all of the mRNA.

The amino acid sequences of IAPP precursors of nine mammals are shown in Figure 3. The percent identity and similarity between these sequences are summarized in Table 1. The identity varies from 59–96%. The sequence of the IAPP portion of the precursor is more highly conserved than the other domains which is consistent with IAPP being the only biologically active peptide derived from this protein.

Discussion

Islet amyloid deposits in association with diabetes have been reported in several species of monkeys, including *Macaca nemestrina* [6, 7]. In longitudinal studies of *Macaca nigra*, it was noted that islet amyloidosis preceded development of overt diabetes and the extent of amyloid de-

position correlated well with the degree of metabolic deterioration [12]. Furthermore, in view of the relatively close relationship of monkeys to humans, the macaque provides a highly suitable animal model for human Type 2 diabetes. The sequence of the monkey IAPP precursor is very similar to that of the human (96% identity and similarity). Remarkably, no differences were found in the NH₂- and COOH-terminal propeptide regions between the monkey and human precursors. In the critical fibril-forming region, i.e. residues 20–29, monkey IAPP differs by only one amino acid from that of human: residue 25, which is alanine in human and cat IAPP, is changed to threonine. Since the substitution of proline for alanine at position 25 in human IAPP had only minor effects on its in vitro fibril-forming ability [10], the threonine substitution at this position in monkey IAPP is unlikely to cause a significant reduction in its amyloidogenic properties.

Although dogs have IAPP immunoreactivity in the Beta cells of their pancreases, diabetic animals do not develop islet amyloidosis. Moreover, a recent report of the partial sequence of dog IAPP indicated that it is very simi-

Table 1. Identity and similarity between mammalian islet amyloid polypeptide (IAPP) peptides and precursors

	Percent similarity								
	Human	Monkey	Cat	Dog	Rat	Mouse	Hamster	Guinea pig	Degu
A. IAPP									
Percent identity									
Human		92	92	92	86	86	89	78	78
Monkey	92		89	89	86	86	84	76	76
Cat	89	86		100	95	95	92	84	86
Dog	89	86	95		95	95	92	86	86
Rat	84	84	89	89		100	92	84	86
Mouse	84	84	89	89	100		92	84	86
Hamster	86	81	86	86	92	92		78	78
Guinea pig	78	76	81	84	84	84	78		95
Degu	78	76	84	84	86	86	78	95	
B. IAPP precursor									
Percent identity									
Human		96	85	89	73	73	74	72	71
Monkey	96		85	89	74	74	72	72	71
Cat	81	81		92	77	76	71	72	73
Dog	82	82	85		80	80	75	75	75
Rat	65	66	66	69		92	82	69	71
Mouse	63	65	65	69	88		82	70	71
Hamster	65	63	61	69	77	77		66	66
Guinea pig	67	67	69	70	63	61	60		84
Degu	65	65	65	67	63	62	59	80	

For calculations of percent similarity the following amino acid residues were considered to be equivalent: D, E; F, Y, W; K, R; I, L, M, V; N, Q; and S, T

lar to that of the cat, a species known to have such deposits [13]. Our results confirm that the sequences of dog IAPP and its precursor are closely similar to those of the cat (95% and 85% identity, respectively [Fig. 3, Table 1]). In the fibril-forming region of IAPP, the amino acid sequence of dog IAPP is identical to that of the cat, as recently reported [13]. However, our sequence differs from that reported by Jordan et al. [13]; there are three nucleotide differences, one of which results in an amino acid difference. Residue 34 of dog IAPP, which is serine in our sequence and in eight other mammals, is threonine in the report by Jordan et al., perhaps indicating an allelic difference in this species. However, both of the dog samples studied in this work gave identical prepro IAPP sequences. From the results of our present study, the sequence of dog IAPP differs by only two amino acids from that of the cat, at residues 18 and 20. It is possible that one or both of these changes may account for the absence of islet amyloid deposits in diabetic dogs. The mRNA levels in Northern analysis appear to be comparable in dog and monkey pancreases (Fig. 2), but there could be differences in the translational efficiency of these mRNAs in these two species. A recent study demonstrated the presence of IAPP-immunoreactivity in amyloid deposits associated with pancreatic endocrine tumours in dogs [14]. This finding suggests that increased expression of IAPP or its precursor, as may occur in such tumours (18; ref. 19, unpublished results) plays a role in the generation of these amyloid deposits. The reasons for the absence of such deposits in the islets of diabetic dogs remain unclear but, if not the result of reduced levels of expression of IAPP in dogs relative to other species, may indicate that the threshold concentration of IAPP required to initiate the aggregation of dog IAPP into fibrils is higher than that for the cat or human proteins and these levels are not normally exceeded in diabetic dogs whereas they may be in pancreatic endocrine tumours.

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