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Conservation of the sequence of islet amyloid polypeptide in five mammals is consistent with its putative role as an islet hormone

(pancreatic beta cell/insulin/RIN-m5F insulinoma cells/calcitonin-gene-related peptide/polymerase chain reaction)

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ABSTRACT **Islet amyloid polypeptide (IAPP) is a 37-amino acid peptide found in the pancreatic amyloid deposits of type II (non-insulin-dependent) diabetic patients and insulinomas. We previously reported the nucleotide sequence of a human cDNA, which indicated that IAPP is a C-terminally amidated peptide derived by proteolytic processing of an 89-amino acid precursor. We now report the isolation of cDNA clones coding for cat, rat, mouse, and guinea pig IAPP precursors, obtained using the combination of "amplification of homologous DNA fragments" (AHF) and "rapid amplification of cDNA ends" (RACE). The predicted structure of IAPP precursors from these four mammals revealed that the IAPP moiety of each is derived from an 89- to 93-amino acid precursor by proteolytic processing and is likely to be amidated at the C terminus. The predicted amino acid sequence identities between the IAPP domains of these four mammals and human IAPP were 89% (cat), 84% (rat and mouse), and 78% (guinea pig). Within the IAPP domains, the N-terminal and C-terminal amino acid sequences are very highly conserved among the mammals, as is also the case with a structurally related neuropeptide, calcitonin-gene-related peptide (CGRP), suggesting that IAPP and CGRP interact with similar though not identical receptors. By contrast, the N- and C-terminal propeptides of the IAPP precursor show very little sequence conservation, which suggests that these regions do not represent additional biologically active molecules. Interspecies variations in the amino acid sequence of residues 20–29 of IAPP may account for the presence of amyloid deposits in the islets of humans and cats and their absence in rats and mice.**

A peptide structurally related to calcitonin-gene-related peptide (CGRP) has been identified as a major protein component of amyloid deposits in the islets of Langerhans of diabetic humans and cats and in human insulinomas (1–5). Immunohistocytochemical studies (2, 6, 7) have identified the beta cells of the islets as the source of this islet amyloid polypeptide [IAPP; designated diabetes-associated peptide (DAP) or amylin by some investigators]. Amyloid deposits also occur in the islets of aging normal individuals and in some insulinomas (8); however, they are more extensive in islets of type II diabetics, suggesting a possible role in the pathogenesis of this disorder (4). Recent studies on the effects of IAPP on the metabolism of glucose suggest that it selectively inhibits insulin-stimulated glucose utilization and glycogen deposition in muscle, while not affecting adipocyte glucose metabolism (9, 10). These observations have led to the hypothesis that IAPP may be a normal islet hormone that modulates the action of insulin on target tissues. If IAPP is a hormone of consequence, certain features of its structure should be well conserved among various species. To examine this question, we have sequenced cDNAs encoding the IAPP precursor from cats, rats, mice, and guinea pigs and have compared their deduced amino acid sequences to that of the human IAPP precursor (11). The results are consistent with the view that IAPP (or its C-terminally amidated form) is a peptide hormone of the islets of Langerhans.‡

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MATERIALS AND METHODS

Materials. Oligonucleotides were synthesized on an Applied Biosystems model 380B DNA synthesizer. Genomic DNA was prepared from human peripheral blood lymphocytes, rat liver (Sprague–Dawley), mouse kidney (BALB/c), guinea pig (Hartley strain), and cat spleen as described (12). RNA was isolated from a rat insulinoma cell line (RIN-m5F; ref. 13) and mouse, guinea pig, and cat pancreas by the guanidinium thiocyanate/CsCl procedure (14).

Amplification of Homologous DNA Fragments (AHF) Partially Encoding IAPP from Genomic DNA. A 119-base-pair (bp) DNA fragment coding for mature IAPP was amplified from genomic DNA by the polymerase chain reaction (PCR) using oligonucleotide primers A and B (Table 1). Each reaction mixture (100 μl) contained 1 μg of DNA, 100 pmol of each primer, and 2.5 units of *Thermus aquaticus* (*Taq*) DNA polymerase in 50 mM KCl/10 mM Tris Cl, pH 8.3/1.5 mM $MgCl_2$/0.01% (wt/vol) gelatin/200 μM each deoxyribonucleoside triphosphate (15). The PCR was carried out in a Perkin–Elmer Cetus DNA thermal cycler through 30 cycles: denaturation for 1 min at 94°C; annealing for 2 min at 50°C; and extension for 3 min at 65°C. After extraction with chloroform, 30 μl of the reaction mixture was electrophoresed in 7% polyacrylamide or 4% NuSieve GTG (FMC BioProducts) agarose gel. Amplified fragments of the expected size were eluted from the gel, subcloned into the *Hin*cII site of M13mp18, and sequenced by the dideoxynucleotide chain-termination procedure (16).

Rapid Amplification of cDNA Ends (RACE). The RACE protocol for obtaining cDNA clones extended at the 3′ or 5′ ends was carried out essentially as described by Frohman *et al.* (17) with minor modifications. cDNAs were prepared from 10 μg of total RNA (RIN-m5F cells, mouse and cat pancreas) or 0.5 μg of poly(A)$^+$ RNA (guinea pig pancreas) by using 200 fmol of oligo(dT)-adaptor as primer and avian myeloblastosis virus reverse transcriptase. The reaction mixtures were extracted with phenol/chloroform/isoamyl alcohol (25:24:1, vol/vol), precipitated with ethanol, and dissolved in 20 μl of H_2O. The PCR for 3′ RACE was then carried out on 1–4 μl of the cDNA for 30 cycles (94°C, 1 min; 50°C, 2 min; 65°C, 3 min) with specific sense-strand primers (Table 2, 3′Amp).

Abbreviations: IAPP, islet amyloid polypeptide; CGRP, calcitonin-gene-related peptide; PCR, polymerase chain reaction; AHF, amplification of homologous DNA fragments; RACE, rapid amplification of cDNA ends.

‡The sequences reported in this paper have been deposited in the GenBank data base (accession nos. M25387–M25390).

Table 1. Primers used for AHF

Name	Sequence
	^{1}Lys ^{2}Cys ^{3}Asn ^{4}Thr ^{5}Ala ^{6}Thr ^{7}Cys ^{8}Ala
Primer A	5′ AAG TGC AAC ACT GCC ACA TGY GC
	^{34}Ser ^{35}Asn ^{36}Thr ^{37}Tyr ^{38}Gly ^{39}Lys ^{40}Arg
Primer B	3′ AGG TTA TGT ATA CCG TTC GC
Oligo(dT)-adaptor	5′ GAC TCG AGT CGA CAT CGA T_{17}
Adaptor	5′ GAC TCG AGT CGA CAT CG

Amino acid numbering is relative to the N terminus of mature IAPP.

cDNAs for 5′ RACE were prepared by substituting specific antisense primers for the oligo(dT)-adaptor in the reverse transcription reaction (Table 2, 5′RT). The cDNA was precipitated twice in 2.5 M ammonium acetate with 3 vol of ethanol and then tailed with oligo(dA) by using dATP and a kit from Boehringer Mannheim. Double-stranded cDNA was synthesized by the Klenow fragment of DNA polymerase I with oligo(dT)-adaptor (25 pmol) as primer at 15°C for 60 min. The PCR was carried out with specific (internal) antisense primers (Table 2, 5′Amp). The 3′- and 5′-RACE reaction mixtures were extracted with chloroform and the amplified cDNAs were subcloned into the *Hin*cII site of pGEM-4Z. Plasmids with IAPP cDNA inserts were identified by colony hybridization (18) with ^{32}P-labeled primer A or B and sequenced.

RNA Blotting. RNA was separated in a 1.0% agarose/0.66 M formaldehyde gel, blotted onto nitrocellulose filters, and hybridized with nick-translated cDNA (18). Filters were washed under high-stringency conditions (15 mM sodium chloride/1.5 mM sodium citrate/0.1% SDS, 65°C) and autoradiographed with intensifying screens.

RESULTS AND DISCUSSION

Isolation of IAPP cDNAs. To obtain cDNAs coding for IAPP from different species, we have taken advantage of recent developments that have enhanced the utility of PCR for cDNA cloning. Frohman *et al.* (17) recently reported a protocol, which they termed RACE, by which the entire cDNA sequence can be generated if one has a partial sequence. Moreover, in experiments using PCR for the amplification of insulin-like sequences from diverse species, we have observed efficient amplification of target sequences, even when there were significant mismatches between the primer and template, when the annealing temperature during PCR was reduced to 50°C. Although the resulting reaction mixtures also contain artifactual DNA fragments, since the exact size of the target DNA is known, it can be readily purified from the contaminating fragments by electrophoresis in polyacrylamide or agarose gels (S.J.C., unpublished results). This observation suggested to us that oligonucleotide primers corresponding to a conserved region in the human IAPP protein could be used to obtain partial IAPP coding sequences from other species.

Table 2. Primers for 5′ and 3′ RACE

Species*	Sequence (5′ to 3′)	Positions†
5′RT		
Ct	AAT GGC ACC AAG ATT GTT GCT GGA	154–177
Rt	GAC TGG ACC AAG GTT GTT GCT GGA	166–189
Ms	GAC TGG ACC AAG GTT GTT GCT GGA	166–189
Gp	GCA GGA GAG CAG CGC CAA GG	174–193
5′Amp		
Ct-1	GAA ATT TGC CAG GCG TTG GGT C	123–144
Ct-2	CCG CTT TTC CAC TTG GTT AC	80–99
Rt	GAA GTT TGC CAG ACG TTG TGT G	135–156
Ms	AAA GTT TGC CAG GCG TTG TGT G	135–156
Gp	TTG TGG CTG GAA CGA ACC AA	154–173
3′Amp		
Ct	TCT TTC TCC TAC CAA TGT GGG A	177–198
Rt	ACC TTG GTC CAG TCC TCC CAC CAA	176–199
Ms	ACC TTG GTC CAG TCC TCC CAC CAA	176–199
Gp	TCT CCT GCC TAC TGA TGT GG	186–205

*Species abbreviations: Ct, cat; Rt, rat; Ms, mouse; Gp, guinea pig. See text for explanation of 5′RT, 5′Amp, and 3′Amp.
†From Fig. 3.

Exon 3 of the human IAPP gene encodes amino acids 28–89 of the IAPP precursor and includes the IAPP moiety (T. Sanke, M.N., and D.F.S., unpublished results). Since the exon/intron organization of other polypeptide hormone genes has been generally well conserved during evolution, we tested primers A and B (Table 1) to determine whether they could be used to amplify the region coding for IAPP in DNA from other animals. As shown in Fig. 1, a 119-bp DNA fragment representing amino acids 1–37 of IAPP and the C-terminal Gly-Lys as well as two nucleotides of the following arginine codon was the major product in the PCR using human, cat, rat, mouse, or guinea pig DNA. The sequence of the human fragment was identical to that of the corresponding region of the human gene. Similarly, we sequenced the 119-bp fragments from the other species and these were clearly homologous to the human sequence and represented a segment of the IAPP gene of each of these animals.

Using these partial sequences to generate specific primers (Table 2), we obtained full-length cDNAs coding for the cat, rat, mouse, and guinea pig IAPP precursors (Fig. 2). The sequence of each DNA was confirmed by analysis of an independently isolated clone from a separate PCR.

Sequence of IAPP Precursors. The sequence and structural organization of the IAPP precursors of cat, rat, mouse, and guinea pig are similar to that of the human (Figs. 3 and 4). Each has four domains: a signal peptide, N-terminal propeptide, IAPP, and C-terminal propeptide. The IAPP domain of each is flanked by Lys-Arg and Gly-Lys-Arg at the N and C termini, respectively, suggesting that, like the human pep-

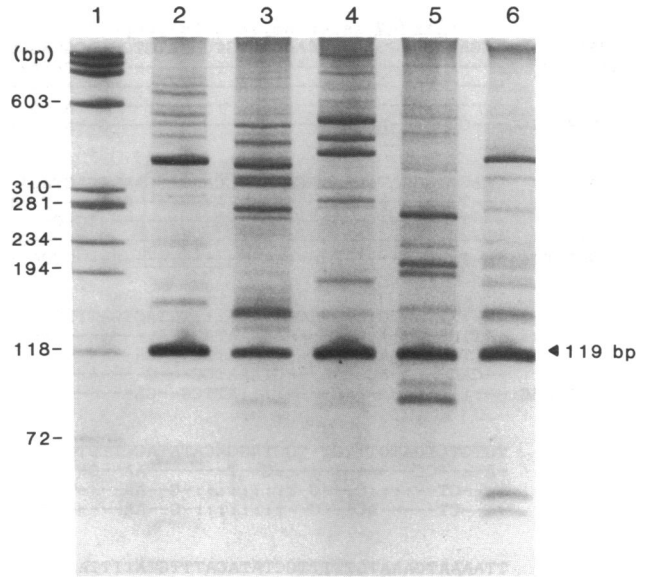

FIG. 1. Amplification of genomic DNA segments containing partial IAPP coding sequences. PCRs were performed using oligonucleotide primers A and B (Table 1) and 1.0 μg of human (lane 2), rat (lane 3), mouse (lane 4), guinea pig (lane 5), or cat (lane 6) DNA. Aliquots (30 μl) of the reaction mixtures were analyzed by electrophoresis in a 7% polyacrylamide gel and the DNA fragments were visualized by ethidium bromide staining. The position of 119-bp fragments containing the IAPP sequence is indicated. The size markers (lane 1) are *Hae* III digestion products of φX174 DNA.

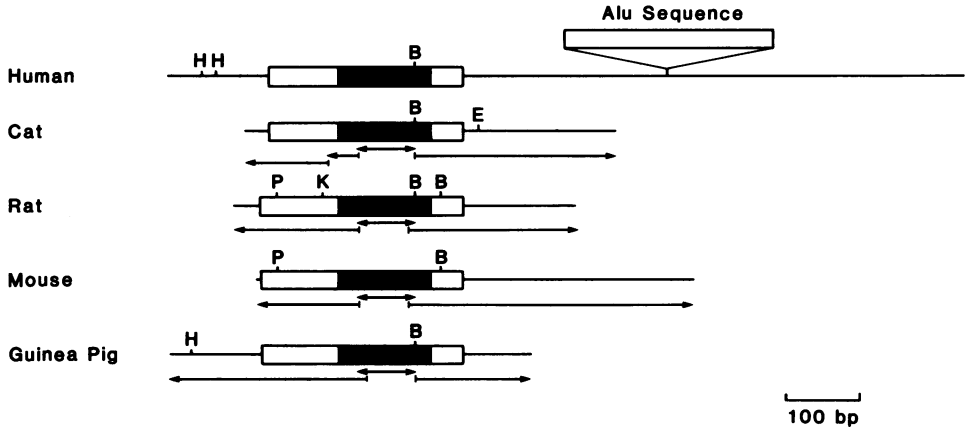

FIG. 2. Schematic map of IAPP cDNAs obtained from PCR amplification of genomic DNAs and from RACE. The regions coding for the IAPP precursor are boxed; the IAPP moiety is denoted by the filled box. Double-headed arrows show the DNA segments from AHF. Leftward and rightward pointing arrows indicate cDNA clones obtained from the 5′-RACE and 3′-RACE reactions, respectively, using specific oligonucleotide primers (Table 2). The human IAPP cDNA was reported by Sanke *et al.* (11). Restriction sites for *Eco*RI (E), *Hin*dIII (H), *Kpn* I (K), and *Pvu* II (P) are shown.

tide, the IAPPs of these mammals are also generated by proteolytic processing and C-terminal amidation. The sizes of the IAPP precursors differ slightly, being 89 amino acid residues in human and cat, 93 residues in rat and mouse, and 92 residues in guinea pig. These differences are due to an increase in size of the putative signal peptide of rats and mice (23 residues) compared to humans, cats, or guinea pigs (22 residues) as well as to the presence of an additional 3-amino

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                                                                HUMAN (-118) GAGGGGGTAAATATTCCAGTGGATACAAGCTTGGACTC -81
                                                      GUINEA PIG (-124) -----A-C-----C-----G-A--G------------T -87

HUMAN      TTTTCTTGAAGCTTTCTTTCTATCAGAAGCATTTGCTGATATTGCTGACATTGAAACATTAAAAG:::::::::::::::::::::AAAATTTGAGAAGCA  -1
CAT                                                    (-30) C-------C------:::::::::::::::::::::-------------  -1
RAT                                          (-36) -------C--C--CC:--C-G----:::::::::::::::::::::GG-TC------C:::  -1
MOUSE                                                                                          (-5) -----:::  -1
GUINEA PIG -C---:::::::::::::::-----T-A-G--G---C---G----CT-C-----------------GACTGATTTATTCTTCTCAG-----C---------  -1

           Met
HUMAN      ATG:::GGCATCCTGAAGCTGCAAGTATTTCTCATTGTGCTCTCTGTTGCATTGAACCATCTGAAAGCTACACCCATTGAAAG:::::::::TCATCAGG  88
CAT        ---:::T--C-------A--T-C----G--------------TG------C-AC----------------C-----------:::::::::-A-C--A-  88
RAT        ---AGGT-----TCC-G-----C--CTG-----C-CA-C-----G--G---C-CGG---CT---G---------TG-C-G---TGGTACCAAC-C-----  100
MOUSE      ---ATGT-----TCC--A----C--CTG-C---C-CA-C---------G---C-------CT---G---------TG-CAG---TGGTAGCAAC-C----A  100
GUINEA PIG ---:::T--C----T-G-----C----ACG---C---------G-------C-A---G-A-----------T------C---TGACACCGGC-------  97

                Lys Arg Islet Amyloid Polypeptide
HUMAN      TGGAAAAAGCGGAAATGCAACACTGCCACATGTGCAACGCAGCGCCTGGCAAATTTTTTAGTTCATTCCAGCAACAACTTTGGTGCCATTCTCTCATCTAC 188
CAT        ----------------------------------G--C--A--------------C---A---G------------TC-------------T--TC---- 188
RAT        ----C--A-----G--------A-----------C--A--A--T--------C--C--G----GC------------C-----C-AG-C---C--C-A-- 200
MOUSE      ----C--A-----G--------G-----G-----C--A--A----------C-----G----G-------------C-----C-AG-C---C--C-A-- 200
GUINEA PIG ---GC--A--A--G-------------G-----G--A--A------A---------G----G-------C-----C----C--TGC----CTGC---- 197

                          Gly Lys Arg                                                                  End
HUMAN      CAACGTGGGATCCAATACATATGGCAAGAGGAATGCAGTAGAGGTTTTAAAGAGAGAGCCACTGAATTACTTGCCCCTTTAGAGGACAATGTAACTCTAT 288
CAT        ---T----------------------A------GCA----T--TA-------C--G--A---T----------A---T-------T-------C-----G 288
RAT        ---T----------------------G--------TG-C------A-CC---T--G--AT-C---G---T---A-T---G--A--:T--------CTC-CG- 299
MOUSE      ------------G--------------------G-C--G--A-CC---T--G--AT-CT--G---T---A-T-G---A--:T-------CT---GC 299
GUINEA PIG TG-T----------------------------C---CCTC--A---C-G-C-----A-TC--AC----TC-A-----A---T-C-CA-CC--T--- 297

HUMAN      AG:::TTATTGTTTTATGT:::::::TCTAGTGATTTCCTGTATAATTTAACAGTGCCCTTTTCATCTCC:::::AGTGTGAATATATGG:::TCTG:: 367
CAT        -ATTC--T------C----ACAAATAT----------------G----------T-:::::::::::::::::::--CA----CC-GCA::::----:: 362
RAT        -TCTC-----AC--CC---GTAAATGC---GA----------A-----G----------T--GC---T---GCCCA----A----G--CAACAACG--CC 399
MOUSE      --CAC---A-AC-------GTAAATGC---G----------A---T-:------A--T-------TC--CCCTC----A----GC-CAA:::-G--CT 394
GUINEA PIG --:::G-C-CA---C----::::ATTCG--GA----------C-CA-C---------TC------T-GT:::::------ 367

HUMAN      TGTGTCTGATGTTTGT:TGCTAGGACATATACCTTCTC:::AAAAGA:TTGTTTTATATGTAGTACTAACTAAGGTCCCATAATAAAAAGATAGTATCTT 462
CAT        -A----CC--------:---C--T-------AA--G-TTTG------A--------A--T--AC-----T---------G---------TC-------- 461
RAT        ----CT--:::::C---G-T:::::::::-G--AA----TATTGT 430
MOUSE      ----CT-----AC---G--:::::::::-G--AA-----ATGCT----A---C----A-CTG----T-G-TC---T--AG-GTGA-GTC-ATGTC-C-AA- 486

HUMAN      TTAAAATGAAATGTTTTTGCTATAGATTTGTATTTTAAAACATAAGAACGTCATTTTGGGACCTA:TATCTCAGTGGCACAGGTTTAAGAACGAAGGAGA 561
CAT        -A---G-- 469
MOUSE      CAC-C---TTC-TGC-A-A-AT-TATA--T--GGGAC-CTT-A--TTT-:-GT----:::----TG--C---TA--A-T--A------C--TA---A--- 582

HUMAN      AAAAGGTAGTTTG 574
MOUSE      CC-T--G-TGA-- 595
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FIG. 3. Nucleotide sequences of human, cat, rat, mouse, and guinea pig IAPP cDNAs. Composite nucleotide sequences were obtained from PCR-amplified cDNAs and genomic DNAs as indicated in Fig. 2. Codons for the initiator methionine, processing sites flanking IAPP, and translation termination are boxed; the hexanucleotide polyadenylylation signal is underlined. Human IAPP cDNA sequence is from Sanke *et al.* (11).

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                    Signal Peptide    N-Terminal Propeptide   Islet Amyloid Polypeptide              C-Terminal Propeptide
Human   MGILKLQVPLIVLSVALNHLKA TPIES:::HQVEKR KCNTATCATQRLANFLVHSSNNFGAILSSTNVGSNTY GKRNAVEVLKREPLNYLPL (89)
Cat     -CL---P-V----L---H---- -----:::N----- ---------------IR----L-----P-------- ---ST-DI-N--------F (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)
Guinea Pig -CL-R-P-T-L--C----E--- -S-A-DTGH--G-- ----------T----R--H-L--A-LP-D------ -----PQISD--LCH---- (92)
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FIG. 4. Comparison of predicted amino acid sequences for human, cat, rat, mouse, and guinea pig IAPP precursors. The IAPP moiety is boxed. The length of each precursor (number of amino acids) is given in parentheses. The predicted boundaries of the signal peptide and N- and C-terminal propeptides are indicated. Dashes indicate amino acid residues that are identical to the human sequence. Colons indicate gaps that were inserted to generate this alignment.

acid segment in the N-terminal propeptide of rodents (Fig. 4). The coding sequence of the additional amino acids corresponded to an exon/intron junction in the human gene (T. Sanke, M.N., and D.F.S., unpublished results), suggesting that this splice acceptor site has moved during evolution, thereby generating precursors having N-terminal propeptides of different sizes. The nucleotide sequence of the rodent cDNAs in this region (e.g., TGGTACCAA in rat) is homologous to that of the splice acceptor site of intron 2 of the human gene (TGTTACCAG), an observation that supports this notion. Similarly, a 20-nucleotide insertion in the guinea pig cDNA sequence (nucleotides −35 to −16) shows significant sequence identity with that of the splice acceptor site of intron 1 of the human gene (TGCTGGATTATTCTTTGACAG), suggesting that this difference is also due to intron sliding. The sequence of the guinea pig IAPP gene in this region indicated that this was indeed the molecular basis for the presence of the insertion in the 5′ untranslated region of the guinea pig cDNA (data not shown).

The nucleotide and predicted amino acid sequences of the IAPP domains of these mammals exhibit 82–93% and 78–100% identity, respectively. In IAPP, 27 residues out of 37 (74%) are conserved in all five sequences (Fig. 5). The sequences of the mammalian IAPPs are also homologous with those of CGRPs from both mammalian and avian species (Fig. 5). The sequences of both peptides are most highly conserved in their N- and C-terminal regions, including the putative disulfide loop (residues 2–7), suggesting that these regions may be involved in receptor binding, possibly to structurally related receptors. However, it is also clear that a number of differences exist between the IAPP and CGRP families, as only 38% of positions are conserved between the two.

RNA Blotting. Analysis of the sizes of the IAPP transcripts in poly(A)$^+$ pancreatic RNA revealed three transcripts of 1.9, 1.2, and 0.75 kilobases (kb) in cat pancreas, two transcripts of 1.2 and 0.9 kb and 1.8 and 1.1 kb in rat insulinoma cells and guinea pig pancreas, respectively, and a single transcript of 1.0 kb in mouse pancreas (Fig. 6). Since the coding sequence is only about 0.3 kb, these findings indicate that the IAPP mRNAs contain extended 5′ and/or 3′ untranslated regions in most species. The presence of multiple bands in cat, guinea pig, and rat may result from alternative RNA splicing or multiple transcription initiation or termination sites. Interestingly, IAPP transcripts of 2.4 and 2.1 kb were present in RNA from a human insulinoma (data not shown), but a 1.5- to 1.6-kb band, as reported previously in different insulinomas (11, 19), was not seen, suggesting that altered mRNA processing occurred in this tumor.

Conclusion. The data presented here extend our previous studies on the precursor of human IAPP (11). All five mammalian IAPP precursors are similar in overall structure but differ slightly in length due to minor differences at their translation start sites and, in the case of the rodent precursors, due to the presence of three additional residues in the N-terminal propeptide. The large number of replacements in the signal peptides (67% of positions) is generally conservative and would not be expected to alter its function or cleavage. However, the very high rate of mutation acceptance in the propeptides (only 25% identity) suggests that these regions are unlikely to have any physiological functions, if secreted with IAPP (Fig. 4).

In addition to the conservation in the sequence of IAPP, the processing signals flanking the IAPP moiety are also conserved, including the glycine residue preceding the C-terminal Lys-Arg, suggesting that all are C-terminally amidated. The sequence conservation of both IAPP and its processing signals is consistent with a putative role for IAPP as a hormone. Evidence that IAPP is colocalized with insulin in beta-cell secretory granules strengthens this conclusion further (20), as does recent evidence that IAPP may selectively inhibit glucose utilization and glycogen deposition in muscle in response to insulin, although in those experiments a nonamidated form of IAPP was used (9, 10).

The mechanism by which IAPP contributes to the formation of amyloid deposits in the islets of type II diabetes remains unclear. A recent report (21) has suggested that residues 20–29 in IAPP, the region that diverges most markedly from CGRP, may contain the nucleation site for amyloid fibril formation. A synthetic oligopeptide corresponding to this region of human IAPP has been shown to form a twisted β-pleated sheet conformation characteristic of amyloid fibrils (21). That the species differences we have observed in this segment of the rodent IAPP peptides may account for their lack of amyloid fibril formation is plausible, but further studies will be necessary to determine whether differences in the level of expression and/or extent of posttranslational processing of pro-IAPP may also contribute to the seemingly selective deposition of amyloid in humans, cats, and a few other species (2, 22–24).

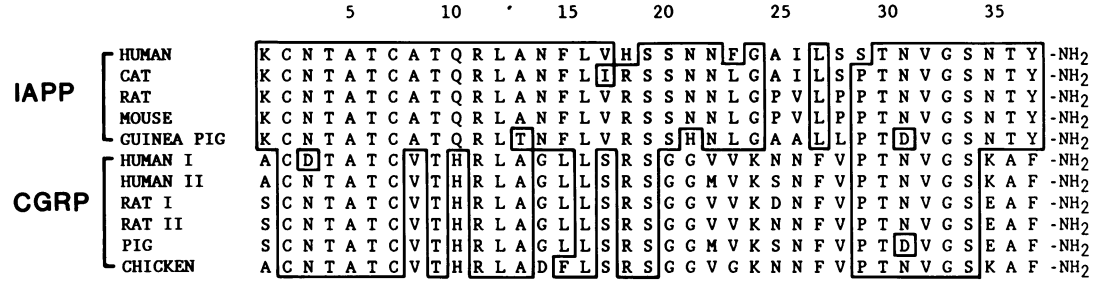

FIG. 5. Comparison of known IAPP and CGRP sequences.

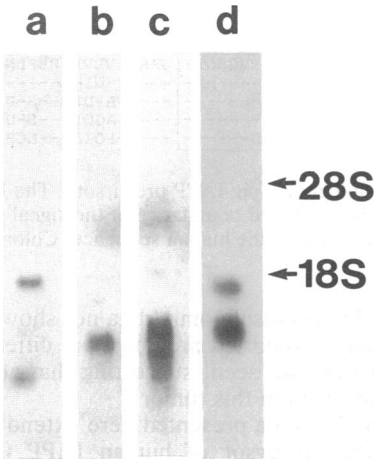

FIG. 6. RNA blot analysis of cat, mouse, rat, and guinea pig IAPP mRNAs. Five micrograms of poly(A)$^+$ RNA isolated from cat pancreas (lane a), mouse pancreas (lane b), or guinea pig pancreas (lane d) and 10 μg of total RNA from rat insulinoma cells (lane c) were electrophoresed in 1.0% agarose/0.66 M formaldehyde gels and blotted onto nitrocellulose filters; each lane was hybridized with the corresponding nick-translated cDNA and autoradiographed. Positions of 18S and 28S rRNA are shown.

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