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Inhibin A-subunit cDNAs from porcine ovary and human placenta

(reproduction/folliculostatin/follicle-stimulating hormone/pituitary)

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ABSTRACT **Inhibin, a gonadal protein that preferentially suppresses the secretion of pituitary follicle-stimulating hormone, has been isolated from porcine follicular fluid and characterized as a 32-kDa protein composed of 18-kDa and 14-kDa subunits. In the present work, oligonucleotide probes predicted from amino-terminal inhibin amino acid sequences have been used to isolate, from a porcine ovarian λgt11 cDNA library, clones encoding the 18-kDa subunit, or A chain, of inhibin. DNA sequence analysis showed that the inhibin A chain is initially synthesized as a larger precursor protein and is predicted to be a glycopeptide. Inhibin A-chain mRNA is present specifically in the gonads, and its synthesis can be induced by treatment of the animal with gonadotropins. The porcine probe was used to isolate a human inhibin A-subunit cDNA from a placental cDNA library. The human precursor is highly homologous to its porcine counterpart and is predicted to generate an 18-kDa glycosylated inhibin A subunit.**

The biological basis for gonadal regulation of pituitary function was formulated in 1923 by Mottram and Cramer (1), who observed hypertrophy of rat pituitary cells following radiation-induced testicular damage. In 1932 McCullaugh (2) demonstrated that the appearance of these hypertrophied cells could be inhibited by the injection of a water-soluble substance derived from bovine testes, and he termed this substance inhibin. Following the discovery of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), the two pituitary hormones known to regulate the development and activity of the gonads, Klinefelter *et al.* (3) postulated that a testicular factor, inhibin, exerted a specific negative feedback action on pituitary FSH secretion. This hypothesis was substantiated when numerous investigators demonstrated a direct suppression of peripheral FSH in animals treated with steroid-free testicular or ovarian preparations (4–6). Inhibin activity has since been observed in testicular or ovarian extracts and in cultured Sertoli or granulosa cells from a variety of species (7–10). The inhibin activity in ovarian extracts has also been commonly termed folliculostatin (6).

Although the existence of inhibin has been realized for more than 50 years, reports of its purification have been widely discrepant with regard to the purity, activity, and physicochemical properties of the respective preparations (11). Recent use of the *in vitro* cultured pituitary cell bioassay (12), which measures suppression of immunoassayable FSH, and HPLC techniques has led to the purification of inhibin from porcine follicular fluid by several groups, including our own (13–15). Porcine inhibin is a 32-kDa protein composed of two disulfide-bound polypeptide chains of 18 kDa (A chain) and 14 kDa (B chain). Amino acid sequences at the amino terminus of both chains have recently been determined; using this information, we have synthesized oligonucleotide probes specific for the porcine inhibin A and B chains and used these to screen a porcine ovarian cDNA library. We report here the isolation of cDNAs encoding a precursor protein that includes the A chain of porcine inhibin, and the subsequent identification of a human inhibin A-chain cDNA.

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                                  5                  10
peptide:  NH2- SerThrAlaProLeuProTrpProTrpSerProAlaAla
mRNA:      5'- TCCACAGCCCCCCTGCCATGGCCATGGTCCCCTGCTGCC
oligo:     3'- AGGTGTCGGGGGGACGGTACCGGTACCAGGGGACGACGG

          15                  20                  25
     LeuArgLeuLeuGlnArgProProGluGluProAlaVal -COOH
     CTGCGGCTGCTGCAGCGGCCCCCTGAGGAGCCTGCTGTG -3'
     GACGCCGACGACGTCGCCGGGGGACTCCTCGGACGACAC -5'
```

FIG. 1. Amino acid sequence of and oligonucleotide probe specific for the porcine inhibin A chain. The first 26 residues determined by direct protein sequencing are shown. The line labeled mRNA indicates the consensus mRNA predicted from the codon usage rules of Lathe (21). The line labeled oligo indicates the 78-mer that was synthesized and used to probe the cDNA library.

MATERIALS AND METHODS

Protein Analysis. Porcine follicular fluid provided by the Contraceptive Development Branch of the National Institute of Child Health and Human Development was used as starting material for the purification of inhibin. Purification procedures have been described in detail (13). The final inhibin preparations showed a single silver-stained band at about 25 kDa when analyzed by $NaDodSO_4$/PAGE under nonreducing conditions and two bands at 18 and 14 kDa, under reducing conditions (13, 16). The individual chains were separated, following carboxymethylation, and subjected to Edman degradation on an Applied Biosystems 470A gas-phase protein sequencer.

cDNA Library Construction. A single female pig was injected i.p. with 50 milliunits of pregnant mare serum gonadotropins (Sigma) per kg of body weight. After 72 hr, she was killed and the ovaries were removed. RNA was isolated by homogenization in guandidinium isothiocynate and centrifugation through CsCl (17) and enriched for poly(A)$^+$ RNA by chromatography on oligo(dT)-cellulose (Collaborative Research, Waltham, MA). cDNA was synthesized with Moloney murine leukemia virus reverse transcriptase using oligo(dT) as primer, made double-stranded with DNA polymerase I, treated with *Eco*RI methylase, ligated to *Eco*RI linkers, and inserted into the phage expression vector λgt11 (18–20). Starting with 10 μg of poly(A)$^+$ RNA, a library of 2 × 10^7 members was constructed.

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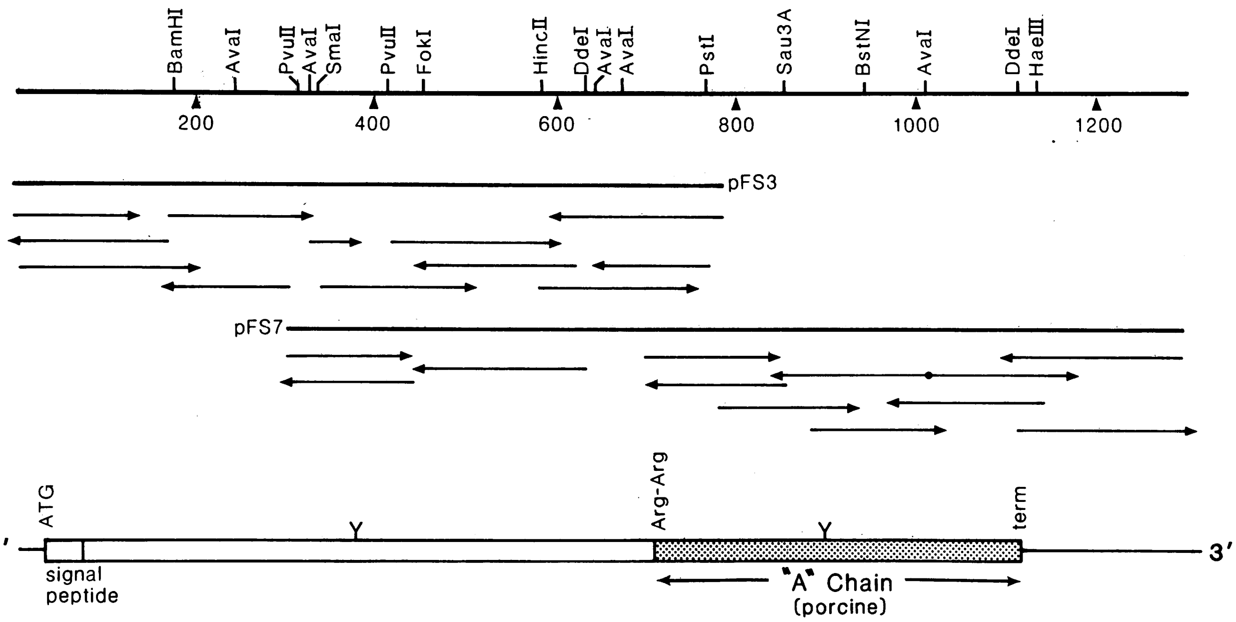
Abbreviations: FSH, follicle-stimulating hormone; LH, luteinizing hormone; bp, base pair(s); kb, kilobase(s).

A



B

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                                                10                                  20
GTTGGCAGGGCCAGGTGAGCTATGTGGCCTCAGCTGCTCCTCTTGCTGTTGGCCCCACGGAGTGGGCATGGCTGCCAGGGCCCGGAGCTG    90
                       MetTrpProGlnLeuLeuLeuLeuLeuLeuAlaProArgSerGlyHisGlyCysGlnGlyProGluLeu

              30                                40                                  50
GACCGGGAGCTTGTCCTGGCCAAGGTGAGGGCTCTGTTCCTGGATGCCTTGGGACCCCCGGCAGTGACTGGGGAAGGTGGGATCCTGGA   180
AspArgGluLeuValLeuAlaLysValArgAlaLeuPheLeuAspAlaLeuGlyProProAlaValThrGlyGluGlyGlyAspProGly

              60                                70                                  80
GTCAGGCGTCTGCCCCGAAGACATGCTGTGGGGGCTTCATGCGCAGGGGCTCCGAGCCCGAGGAGGAGGATGTCTCCCAGGCCATCCTT   270
ValArgArgLeuProArgArgHisAlaValGlyGlyPheMetArgArgGlySerGluProGluGluGluAspValSerGlnAlaIleLeu

              90                                100                                 110
TTCCCGGCTACAGGTGCCCGCTGTGGGGACGAGCCAGCTGCTGGAGAGCTGGCCCGGGAGGCTGAGGAGGGCCTCTTCACATATGTATTC   360
PheProAlaThrGlyAlaArgCysGlyAspGluProAlaAlaGlyGluLeuAlaArgGluAlaGluGluGlyLeuPheThrTyrValPhe

              120                               130                                 140
CGGCCGTCCCAGCACACACGCAGCCGCCAGGTGGCTTCAGCTCAGCTGTGGTTCCACACGGGACTGGACAGACAGGGGATGGCAGCCGCC   450
ArgProSerGlnHisThrArgSerArgGlnValAlaSerAlaGlnLeuTrpPheHisThrGlyLeuAspArgGlnGlyMetAlaAlaAla

              150                               160                                 170
AATAGCTCTGGGCCCCTGCTGGACCTGCTGGCACTATCATCCAGGGGTCCTGTGGCTGTGCCCATGTCACTGGGCCAGGCGCCCCCTCGC   540
AsnSerSerGlyProLeuLeuAspLeuLeuAlaLeuSerSerArgGlyProValAlaValProMetSerLeuGlyGlnAlaProProArg

              180                               190                                 200
TGGGCTGTGCTGCACCTGGCCGCCTCTGCCCTCCCTTTGTTGACCCACCCAGTCCTGGTGCTGCTGCTGCGCTGTCCTCTCTGTTCCTGC   630
TrpAlaValLeuHisLeuAlaAlaSerAlaLeuProLeuLeuThrHisProValLeuValLeuLeuLeuArgCysProLeuCysSerCys

              210                               220                                 230
TCAGCCCGGCCCGAGGCCACCCCCTTCCTGGTGGCCCACACTCGGGCCAGGCCACCCAGCGGAGGGGAGAGGGCCCGACGCTCCACCGCC   720
SerAlaArgProGluAlaThrProPheLeuValAlaHisThrArgAlaArgProProSerGlyGlyGluArgAla[ArgArg]SerThrAla

              240                               250                                 260
CCTCTGCCCTGGCCTTGGTCCCCCGCCGCGCTGCGCCTGCTGCAGAGGCCCCCGGAGGAACCCGCTGTGCACGCCGACTGCCACAGAGCT   810
ProLeuProTrpProTrpSerProAlaAlaLeuArgLeuLeuGlnArgProProGluGluProAlaValHisAlaAspCysHisArgAla

              270                               280                                 290
TCCCTCAACATCTCCTTCCAGGAGCTGGGCTGGGACCGGTGGATCGTGCACCCTCCCAGTTTCATCTTCCACTACTGTCACGGGGGCTGC   900
SerLeuAsnIleSerPheGlnGluLeuGlyTrpAspArgTrpIleValHisProProSerPheIlePheHisTyrCysHisGlyGlyCys

              300                               310                                 320
GGGCTGCCGACCCTGCCCAACCTGCCCCTGTCTGTCCCTGGGGCCCCCCCTACCCCTGTCCAGCCCCTGTTGTTGGTGCCAGGGGCTCAG   990
GlyLeuProThrLeuProAsnLeuProLeuSerValProGlyAlaProProThrProValGlnProLeuLeuLeuValProGlyAlaGln

              330                               340                                 350
CCCTGCTGCGCTGCTCTCCCGGGGACCATGAGGTCCCTACGCGTTCGCACCACCTCGGATGGAGGTTACTCTTTCAAGTACGAGACAGTG  1080
ProCysCysAlaAlaLeuProGlyThrMetArgSerLeuArgValArgThrThrSerAspGlyGlyTyrSerPheLysTyrGluThrVal

              360
CCCAACCTTCTCACCCAGCACTGTGCCTGCATCTAAGGGTGTCCCGCTGGTGGCCAAGCTCCCACAGGCACCAGCCTGGAGGAAGGCAGA  1170
ProAsnLeuLeuThrGlnHisCysAlaCysIle

GTTCCCACCTCCCCTTTCCTTCCGCCTCTCCGCCTGGAGGCTCCCCTCCCTGTCCGCCCCTGTCCCATGGGTAATGTGACAATAAACAGC  1260

ATAGTGCAGATGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
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FIG. 2. (*Legend appears at the bottom of the opposite page.*)

Oligonucleotide Screening. A single oligodeoxynucleotide (78-mer) was predicted from the amino-terminal 26 amino acids of the inhibin A chain (Fig. 1), based upon the codon-usage rules of Lathe (21). Purified oligonucleotide was 5′ end-labeled, using bacteriophage T4 polynucleotide kinase and [γ-^{32}P]ATP, and used as a hybridization probe to screen the porcine ovarian cDNA library. Hybridization was in 0.9 M NaCl/0.05 M sodium phosphate, pH 7.4/5 mM EDTA/10% dextran sulfate/20% formamide/0.1% $NaDodSO_4$/denatured salmon sperm DNA (200 μg/ml) at 45°C at a probe concentration of about 250 ng/ml. Filters were washed in 75 mM NaCl/7.5 mM sodium citrate, pH 7/0.1% $NaDodSO_4$ at 45°C.

Human Library Screening. Nick-translated inserts from porcine clones pFSA-3 (5′ probe) and pFSA-7 (3′ probe) were used to screen in duplicate a human placental λgt11 cDNA library. Analysis of the *Eco*RI inserts from these clones indicated cDNAs of 400 base pairs (bp) and about 1.5 kbp; only the large cDNA was further examined.

DNA Sequencing. cDNA inserts from positive phage were subcloned into the *Eco*RI site of plasmid pBR322, restriction enzyme maps were determined, and purified restriction fragments were 5′ end-labeled with T4 polynucleotide kinase and [γ-^{32}P]ATP and subjected to the chemical-modification sequence reactions of Maxam and Gilbert (22).

RNA Hybridization. Poly(A)$^+$ RNA was electrophoresed in denaturing formaldehyde/agarose gels, transferred to nitrocellulose, and hybridized to cDNA inserts labeled by nick-translation using [α-^{32}P]dCTP (23).

RESULTS

The purification of porcine inhibin and the determination of amino-terminal amino acid sequences were described in a previous report (13). Subsequent sequence runs have identified 26 amino acids at the amino terminus of the inhibin A chain and 25 amino acids at the amino terminus of the B chain. The A-chain sequence was used to predict a long, unique oligonucleotide probe (Fig. 1).

Treatment of rats with gonadotropins leads to a marked rise in serum levels of bioassayable inhibin (24). We therefore treated a pig for several days with pregnant mare serum gonadotropins, isolated ovarian poly(A)$^+$ RNA, and used this RNA as template for cDNA synthesis. A λgt11 cDNA library was constructed, and 400,000 plaques were screened by hybridization with the inhibin A-chain oligonucleotide. Eight plaques showing positive hybridization to the A-chain probe were identified in the primary screen.

Restriction enzyme mapping of the inhibin A-chain cDNAs showed that two of the clones, pFSA-3 (800 bp) and pFSA-7 (1000 bp), contained overlapping inserts that together spanned about 1350 nucleotides. The structural features of these clones are schematically represented in Fig. 2*A*. The DNA sequence was determined (Fig. 2*B*) and found to include an open reading frame of 1092 nucleotides that predicts a 364 amino acid precursor to the inhibin A chain. Following the presumptive initiation codon is a hydrophobic stretch of about 20 amino acids that is most likely the signal sequence for secretion of the inhibin A-chain precursor. The amino-terminal amino acid sequence of the mature inhibin

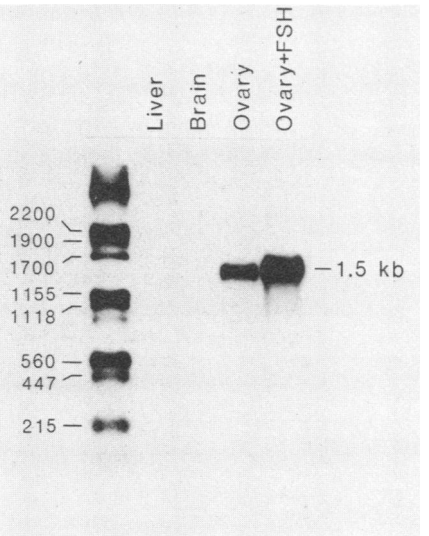


FIG. 3. RNA blot analysis of the porcine inhibin A-chain mRNA. Each lane contained 10 μg of poly(A)$^+$ RNA from the indicated porcine tissue. +FSH indicates prior treatment of the animal with pregnant mare serum gonadotropins. The probe was nick-translated insert from cDNA clone pFSA-7. Markers (left lane) were a mixture of *Hin*dIII-digested simian virus 40 and phage λ DNA; sizes of markers are indicated in nucleotides.

A-chain is found at position 231 in the precursor and immediately follows the sequence Arg-Arg, a site for proteolytic processing of peptides from their precursors (25). The inhibin A chain is predicted to be composed of 134 amino acids leading to a calculated molecular mass of 14.6 kDa. Potential sites for N-linked glycosylation are found at position 144 in the precursor (-Asn-Ser-Ser-) and position 266 within the A-chain peptide (-Asn-Ile-Ser-). We have recently determined by direct protein sequencing that the amino-terminal 25 residues of the inhibin B chain are Gly-Leu-Glu-Cys-Asp-Gly-Arg-Thr-Asn-Leu-Cys-Cys-Arg-Gln-Gln-Phe-Phe-Ile-Asp-Phe-Arg-Leu-Ile-Gly-Trp. A search of the inhibin A-chain precursor indicates that it does not include this sequence, pointing to the existence of an independent mRNA encoding the smaller subunit of inhibin.

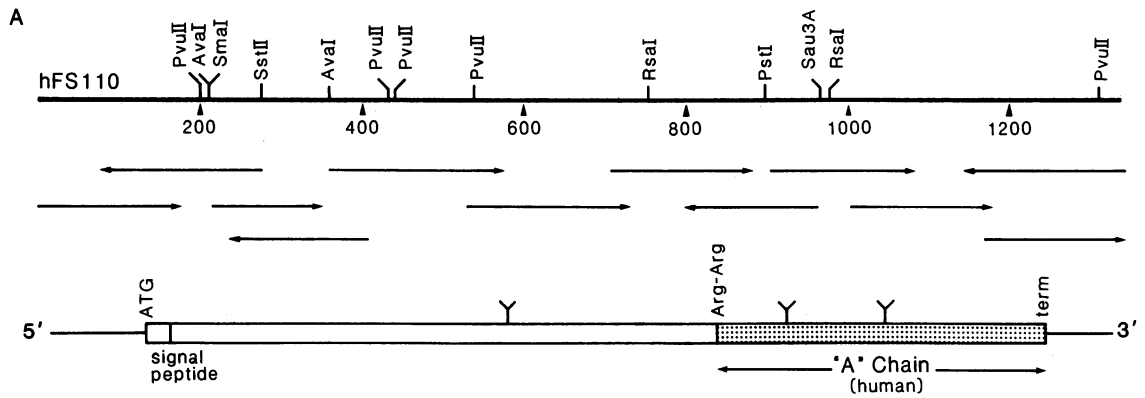
To determine the full-length size of the mRNA encoding the A-chain precursor, we examined RNA from various porcine tissue by RNA blotting using the inhibin cDNA inserts as probe. Fig. 3 shows that the inhibin A-chain cDNA detects a 1.5-kilobase (kb) mRNA that is present in ovary, but not in liver or brain. Furthermore, the abundance of this RNA is increased about 5-fold by treatment of the animal with pregnant mare serum gonadotropins.

Inhibin bioactivity has been reported in the human placenta (26). To test whether this tissue might provide an accessible source of human inhibin, we probed RNA blots containing term human placental poly(A)$^+$ RNA with the porcine inhibin A-chain cDNAs. A specific band of hybridization was observed at 1.5 kb, the same size as the porcine A-subunit mRNA (data not shown). We therefore screened a human placental λgt11 cDNA library previously constructed for other purposes with the porcine inhibin A-subunit probes.

FIG. 2 (*on opposite page*). (*A*) Schematic structure of and strategy for sequencing the cDNA encoding porcine inhibin A-chain precursor. The top line shows a composite restriction enzyme map (numbers indicate length in bp), the central portion of the figure indicates the DNA sequencing strategy for clones pFS3 and pFS7, and the bottom line is a schematic representation of the inhibin A-chain precursor. Initiation and termination codons flank the open reading frame (box), Arg-Arg indicates the site for proteolytic generation of the mature A-chain amino terminus, and Y indicates a potential site for N-linked glycosylation. Arrows indicate the direction and extent of DNA sequence information. (*B*) DNA sequence and predicted amino acid sequence of the porcine inhibin A-chain precursor. The A-chain sequence is underlined, and the site for its proteolytic removal from the precursor is boxed. The consensus poly(A)-addition signal sequence (AATAAA) is also underlined. Arrowheads indicate the extent of the putative signal peptide.

Plaques showing positive hybridization to both pFSA-3 and pFSA-7 probes were identified, and the 1.5-kb insert from one of these (hFSA-110) was characterized.

DNA sequencing revealed that this cDNA encodes a precursor that has substantial homology to the porcine inhibin A subunit and is most likely the human inhibin A subunit. A schematic structure of the human inhibin A-chain precursor is shown in Fig. 4*A*, and the DNA sequence of cDNA clone hFSA-110 is shown in Fig. 4*B*. The 366 amino acid human precursor protein includes the 134 amino acid A subunit at its carboxyl terminus, following two basic residues (Arg-Arg). Potential glycosylation sites are found within both the amino-terminal part of the precursor and within the mature A subunit, as was previously shown for the porcine

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B GAAGGACTGGGGAAGACTGGATGAGAAGGGTAGAAGAGGGTGGGTGTGGGATGGGGAGGGGAGAGTGGAAAGGCCCTGGGCAGACCCTGG      90

                                                                                          10
CAGAAGGGGCACGGGGCAGGGTGTGAGTTCCCCACTAGCAGGGCCAGGTGAGCTATGGTGCTGCACCTACTGCTCTTCTTGCTGCTGACC     180
                                                                MetValLeuHisLeuLeuLeuPheLeuLeuLeuThr

                    20                                  30                                  40
CCACAGGGTGGGCACAGCTGCCAGGGGCTGGAGCTGGCCCGGGAACTTGTTCTGGCCAAGGTGAGGGCCCTGTTCTTGGATGCCTTGGGG     270
ProGlnGlyGlyHisSerCysGlnGlyLeuGluLeuAlaArgGluLeuValLeuAlaLysValArgAlaLeuPheLeuAspAlaLeuGly

                    50                                  60                                  70
CCCCCCGCGGTGACCAGGGAAGGTGGGGACCCTGGAGTCAGGCGGCTGCCCCGAAGACATGCCCTGGGGGGCTTCACACACAGGGGCTCT     360
ProProAlaValThrArgGluGlyGlyAspProGlyValArgArgLeuProArgArgHisAlaLeuGlyGlyPheThrHisArgGlySer

                    80                                  90                                  100
GAGCCCGAGGAAGAGGAGGATGTCTCCCAAGCCATCCTTTTCCCAGCCACAGATGCCAGCTGTGAGGACAAGTCAGCTGCCAGAGGGCTG     450
GluProGluGluGluGluAspValSerGlnAlaIleLeuPheProAlaThrAspAlaSerCysGluAspLysSerAlaAlaArgGlyLeu

                    110                                 120                                 130
GCCCAGGAGGCTGAGGAGGGCCTCTTCAGATACATGTTCCGGCCATCCCAGCATACACGCAGCCGCCAGGTGACTTCAGCCCAGCTGTGG     540
AlaGlnGluAlaGluGluGlyLeuPheArgTyrMetPheArgProSerGlnHisThrArgSerArgGlnValThrSerAlaGlnLeuTrp

                    140                                 150                                 160
TTCCACACCGGGCTGGACAGGCAGGGCACAGCAGCCTCCAATAGCTCTGAGCCCCTGCTAGGCCTGCTGGCACTGTCACCGGGAGGACCC     630
PheHisThrGlyLeuAspArgGlnGlyThrAlaAlaSerAsnSerSerGluProLeuLeuGlyLeuLeuAlaLeuSerProGlyGlyPro

                    170                                 180                                 190
GTGGCTGTGCCCATGTCTTTGGGCCATGCTCCCCCTCACTGGGCCGTGCTGCACCTGGCCACCTCTGCTCTCTCTCTGCTGACCCACCCC     720
ValAlaValProMetSerLeuGlyHisAlaProProHisTrpAlaValLeuHisLeuAlaThrSerAlaLeuSerLeuLeuThrHisPro

                    200                                 210                                 220
GTCCTGGTGCTGCTGCTGCGCTGTCCCCTCTGTACCTGCTCAGCCCGGCCTGAGGCCACGCCCTTCCTGGTGGCCCACACTCGGACCAGA     810
ValLeuValLeuLeuLeuArgCysProLeuCysThrCysSerAlaArgProGluAlaThrProPheLeuValAlaHisThrArgThrArg

                    230                                 240                                 250
CCACCCAGTGGAGGGGAGAGAGCCCGACGCTCAACTCCCCTGATGTCCTGGCCTTGGTCTCCCTCTGCTCTGCGCCTGCTGCAGAGGCCT     900
ProProSerGlyGlyGluArgAlaArgArgSerThrProLeuMetSerTrpProTrpSerProSerAlaLeuArgLeuLeuGlnArgPro

                    260                                 270                                 280
CCGGAGGAACCGGCTGCCCATGCCAACTGCCACAGAGTAGCACTGAACATCTCCTTCCAGGAGCTGGGCTGGGAACGGTGGATCGTGTAC     990
ProGluGluProAlaAlaHisAlaAsnCysHisArgValAlaLeuAsnIleSerPheGlnGluLeuGlyTrpGluArgTrpIleValTyr

                    290                                 300                                 310
CCTCCCAGTTTCATCTTCCACTACTGTCATGGTGGTTGTGGGCTGCACATCCCACCAAACCTGTCCCTTCCAGTCCCTGGGGCTCCCCCT    1080
ProProSerPheIlePheHisTyrCysHisGlyGlyCysGlyLeuHisIleProProAsnLeuSerLeuProValProGlyAlaProPro

                    320                                 330                                 340
ACCCCAGCCCAGCCCTACTCCTTGCTGCCAGGGGCCCAGCCCTGCTGTGCTGCTCTCCCAGGGACCATGAGGCCCCTACATGTCCGCACC    1170
ThrProAlaGlnProTyrSerLeuLeuProGlyAlaGlnProCysCysAlaAlaLeuProGlyThrMetArgProLeuHisValArgThr

                    350                                 360
ACCTCGGATGGAGGTTACTCTTTCAAGTATGAGACAGTGCCCAACCTTCTCACGCAGCACTGTGCTTGTATCTAAGGGTGGGGGGTCTTC    1260
ThrSerAspGlyGlyTyrSerPheLysTyrGluThrValProAsnLeuLeuThrGlnHisCysAlaCysIle

CTTCTTAATCCCATGGCTGGTGGCCACGCCCCCACCATCATCAGCTGGGAGGAAAGGCAGAGTTGGGAAATAGATGGC
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FIG. 4. (*A*) Schematic structure of and sequencing strategy for the human inhibin A-chain precursor. A restriction map of clone hFSA-110 is shown; arrows indicate the direction and extent of DNA sequence information derived from fragments end-labeled at the arrow origins. The schematic structure of the human A-subunit precursor is shown below. Initiation and termination codons flank the open reading frame (box), Arg-Arg indicates the site for proteolytic generation of the mature A-chain amino terminus, and each Y indicates a site for potential glycosylation. (*B*) DNA sequence and predicted amino acid sequence of the human inhibin A subunit. The sequence is derived entirely from clone hFSA-110, which lacks a 3' poly(A) tract. The A-chain sequence as deduced from homology to the pig sequence is underlined, and the site of proteolytic processing is boxed. Arrowheads indicate the extent of the putative signal peptide.

precursor. The porcine and human A-subunit precursors share 84% amino acid identity; this homology is equally spread throughout the molecule.

DISCUSSION

We have isolated from porcine ovarian and human placental cDNA libraries clones encoding the 18-kDa subunit of the reproductive hormone inhibin. Four lines of evidence indicate that these are indeed authentic inhibin A-chain cDNAs. First, the A-chain cDNAs include sequences capable of encoding the amino terminus of the A-chain peptide as determined by the protein chemistry approach. Second, it was previously shown (13) that antibodies raised against a synthetic peptide related to the inhibin A-chain amino terminus can significantly immunoneutralize inhibin bioactivity as measured using the dispersed rat pituitary cell culture assay. Third, the mRNA detected by the A-chain cDNAs is present in ovary, but not in brain or liver, consistent with the role of inhibin as an ovarian hormone. Last, the mRNA detected by the A-chain cDNAs is inducible by the administration of gonadotropins to the animal. As mentioned previously, FSH-inducibility of inhibin bioactivity has previously been postulated, based upon its known physiological role and based upon direct experimental results in the rat (24). Our results confirm that such regulation does occur and suggest that it is a result of increased inhibin synthesis in the presence of elevated FSH. Inhibin cDNA probes will allow us to directly measure the transcriptional activity of the inhibin genes in animals maintained in various hormonal states.

DNA sequence analysis indicates that the inhibin A chain is synthesized as a larger precursor protein and predicts the complete structure of this precursor. In addition to the Arg-Arg sequence that precedes the inhibin A-chain amino terminus, the inhibin A-chain precursor contains several additional paired basic residues that might be potential sites for further or alternative proteolytic processing. Proteins of >32 kDa with inhibin bioactivity have been observed in ovarian extracts from several mammalian species, including pig (11, 13–15). The precursor to the inhibin A chain contains two consensus sequences at which N-linked glycosylation might potentially occur. It has been shown (27) that the inhibin activity present in some preparations can be retained by concanavalin A affinity chromatography. Glycosylation would also explain the difference between the calculated molecular mass of the inhibin A chain (14.6 kDa) and its apparent molecular mass on $NaDodSO_4$/PAGE (18 kDa). The emerging picture of inhibin as a multichain glycoprotein is intriguing, considering that it regulates and in turn is regulated by FSH, also a multichain glycoprotein.

In addition to its synthesis in the ovary, inhibin is also apparently made in the mammalian placenta. The role of inhibin in this tissue during pregnancy remains to be elucidated; however, its presence in placenta has allowed us to isolate and characterize a cDNA clone encoding the human placental inhibin A-subunit precursor protein. The human and pig inhibin A-subunit precursors are highly homologous, and structural features such as the amino-terminal signal sequence, potential glycosylation sites, dibasic proteolytic processing (Arg-Arg) sites, and cysteine residues that might be involved in interaction with the B subunit are all well conserved.

There are numerous reasons for interest in the characterization of inhibin. The function of inhibin in the control of FSH secretion suggests that it is an important component of the many hormones that control the mammalian reproductive cycle. In agreement with this idea, roles suggested for inhibin in the rat include maintenance of an appropriate FSH/LH ratio, triggering of the secondary surge of FSH in late proestrus, and control of folliculogenesis (28). These functions suggest that inhibin, its derivatives, or other agents that regulate its production might find use as contraceptive agents of "natural" origin. Derangements in the production or metabolism of inhibin may explain a number of infertile situations, for example atresia of the dominant follicle or the development of an inadequate luteal phase (29). Abnormal inhibin activity has also been associated with certain reproductive diseases, for example polycystic ovarian disease (30). The characterization of a human inhibin cDNA provides a structural basis for clinical research to further characterize the involvement of inhibin in reproduction and reproductive disorders.

Note. After this work was completed, Mason *et al.* (31) reported the structures of cDNAs encoding the precursors to both porcine inhibin subunits.

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