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Primary structure of bovine pituitary secretory protein I (chromogranin A) deduced from the cDNA sequence

(parathyroid/adrenal/S1 nuclease mapping)

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ABSTRACT **Secretory protein I (SP-I), also referred to as chromogranin A, is an acidic glycoprotein that has been found in every tissue of endocrine and neuroendocrine origin examined but never in exocrine or epithelial cells. Its co-storage and co-secretion with peptide hormones and neurotransmitters suggest that it has an important endocrine or secretory function. We have isolated cDNA clones from a bovine pituitary λgt11 expression library using an antiserum to parathyroid SP-I. The largest clone (SP4B) (≈1.6 kilobases) hybridized to a transcript of 2.1 kilobases in RNA from parathyroid, pituitary, and adrenal medulla. Immunoblots of bacterial lysates derived from SP4B lysogens demonstrated specific antibody binding to an SP4B/β-galactosidase fusion protein (160 kDa) with a cDNA-derived component of 46 kDa. Radioimmunoassay of the bacterial lysates with SP-I antiserum yielded parallel displacement curves of ^{125}I-labeled SP-I by the SP4B lysate and authentic SP-I. SP4B contains a cDNA of 1614 nucleotides that encodes a 449-amino acid protein (calculated mass, 50 kDa). The nucleotide sequences of the pituitary SP-I cDNA and adrenal medullary SP-I cDNAs are nearly identical. Analysis of genomic DNA suggests that pituitary, adrenal, and parathyroid SP-I are products of the same gene.**

Chromogranin A is an acidic glycoprotein (48 kDa) of the adrenal medulla chromaffin granule that represents 40% of its soluble protein (1-7). Chromogranin A is co-secreted with epinephrine and other granule contents. Chromogranin A was believed to be unique to the adrenal, but this view changed when it was reported that parathyroid gland secretory protein I (SP-I), a protein that earlier had been found to be co-stored and co-secreted with parathyroid hormone (8), was biochemically similar to chromogranin A (6). A reported difference in 2 of the first 20 amino-terminal residues (compare refs. 6, 9, and 10) suggested that SP-I and chromogranin A might be generated from different genes, but subsequent studies by Kruggel *et al.* (11) revealed that the amino-terminal sequences were, in fact, identical. Furthermore, the recent cloning and sequencing of the cDNA for bovine adrenal medulla SP-I¶ (4, 5) has yielded a deduced amino-terminal polypeptide sequence that agrees exactly with that for parathyroid SP-I (9). SP-I immunoreactivity has been shown to be present in every endocrine or neuroendocrine cell type examined (12–17). In marked contrast, no exocrine cell has yet been found to contain this protein. There is much speculation on the as-yet-unknown physiological function of SP-I (6, 17–19), with emphasis on it playing a role in endocrine secretory processing.

To gain insight into the structure of SP-I and/or SP-I-like proteins expressed in different endocrine tissues, we isolated and characterized a cDNA encoding SP-I from bovine pituitary. Comparison of the nucleotide sequence of this cDNA with those of recently cloned adrenal medullary SP-I cDNAs (4, 5) reveals that the coding regions, and hence the primary amino acid sequences, are nearly identical. These data confirm the immunologic localization of SP-I in the adrenal medulla, pituitary, and parathyroid (10–15), and together with the results of blot hybridization of genomic DNA, indicate that this protein is the product of a single gene.

MATERIALS AND METHODS

Purification of Antibodies to Parathyroid SP-I. SP-I antiserum R-2, a polyclonal rabbit antiserum raised against purified bovine parathyroid SP-I (9), was used as an antibody probe. Prior to screening the bovine pituitary λgt11 library, antibodies present in the SP-I antiserum that were directed against *Escherichia coli* or phage antigens were removed by adsorption to a lysate prepared from a nonrecombinant λgt11 lysogen (*E. coli* BNN 97) that was covalently linked to cyanogen bromide-activated Sepharose (20).

Immunoscreening of the Bovine Pituitary cDNA Library. A λgt11 expression library in which 75% of the phage are recombinant and contain DNA sequences complementary to bovine pituitary mRNA (a gift of R. Maurer, University of Iowa) was screened with SP-I antiserum by described methods (20). Positive plaques were purified to homogeneity by repeated antibody screening. Phage DNA was isolated (21) and analyzed by digestion with *Eco*RI.

Analysis of Lysogens for Fusion Proteins Containing SP-I Antigens. Lysogenization of *E. coli* Y1089 with λSP4B phage and induction of the fusion protein with isopropyl β-D-thiogalactoside (IPTG), a gratuitous activator of *lacZ* transcription, was performed as described (20, 22). The lysates of IPTG-induced and noninduced lysogens were subjected to electrophoresis through $NaDodSO_4$/7.5% polyacrylamide gels (23), and the proteins were either stained with Coomassie blue or electrophoretically transferred to nitrocellulose filters for immunostaining.

Radioimmunoassay of Pituitary SP-I. Radioimmunoassay was performed with R-9 antiserum raised in rabbits against purified parathyroid SP-I (9). The bacterial lysates (100 μl) from λSP4B lysogen or λgt11 lysogen (BNN97) at the indicated dilutions (see Fig. 2) were incubated overnight at 4°C with 9000 cpm of ^{125}I-labeled SP-I and antiserum (final dilution, 1:3200) in 0.1 M sodium phosphate buffer (pH 7.4) containing 0.15 M NaCl, 0.05% ovalbumin, 0.05% sodium

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Abbreviations: SP-I, secretory protein I; IPTG, isopropyl β-D-thiogalactoside.

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¶We use the term secretory protein I (SP-I) when referring to this protein in the adrenal and in other tissue.

azide, and 0.063 M EDTA. One hundred microliters each of 2% normal rabbit serum and a 1:40 dilution of sheep anti-rabbit IgG (both from Antibodies, Davis, CA) were added. After an additional 24 hr, the antibody–antigen complex was separated from unbound ^{125}I-labeled SP-I by centrifugation, and radioactivity was counted.

RNA Transfer and Blot Hybridization Analysis. RNA was extracted from bovine tissue by the guanidinium isothiocyanate method and isolated by centrifugation through a 5.7 M cesium chloride cushion (21). Ten micrograms of total RNA was denatured, electrophoresed in 1% agarose gels containing 2.2 M formaldehyde, and transferred to nylon membranes (GeneScreen *Plus*, New England Nuclear–DuPont) (24). Membranes were prehybridized with 37% deionized formamide, 5× SSPE (1× SSPE = 0.15 M NaCl/10 mM $NaPO_4$/1 mM EDTA, pH 7.4), 1% $NaDodSO_4$, 10% dextran sulfate, 500 μg of heparin per ml, at 42°C for 4–6 hr and hybridized overnight in the same solution with 1.6 kilobase pairs (kbp) of SP4B cDNA probe (1–2 × 10^6 cpm/ml) that was labeled with [^{32}P]dCTP by the random-primer method (specific activity, 10^9 cpm/μg) (25). Filters were washed twice at 25°C for 30 min in 2× SSC (1× SSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7.0)/1% $NaDodSO_4$, and twice at 65°C for 30 min in 0.1× SSC/1% $NaDodSO_4$.

DNA Isolation and Analysis. High molecular weight DNA was prepared from leukocytes as described (26) and analyzed by the technique of Southern (24). Hybridization and high-stringency washing conditions were as described for RNA hybridization analysis.

Nucleotide Sequence Analysis. The 1.6-kbp SP4B cDNA insert was subcloned into the *Eco*RI site of phages M13mp18 and M13mp19. The DNA sequence was determined by sequencing both strands via the dideoxy chain-termination method of Sanger *et al.* (27–29) using Klenow fragment or reverse transcriptase isolated from avian myeloblastosis virus (New England Nuclear–DuPont). The M13 universal primers and synthetic primers derived from bovine pituitary SP-I sequences (kindly provided by E. Doran and R. Kutny, Oligonucleotide Synthesis Facility, DuPont Central Research and Development, Wilmington, DE) were used.

S1 Nuclease Mapping. S1 nuclease mapping of mRNA was performed by described methods (21, 30). The 324-bp *Eco*RI/*Bam*HI fragment of SP4B, which includes the 33 nonhomologous nucleotides from the 5′ noncoding region was end-labeled with [^{32}P]dCTP and T4 polynucleotide kinase (21). Approximately 50 ng of the [^{32}P]cDNA probe was hybridized with 5 μg of parathyroid and adrenal medulla total RNA and 50 μg of pituitary total RNA for 16 hr at 60°C. After S1 nuclease digestion, protected fragments were analyzed by electrophoresis through an 8% polyacrylamide/8.3 M urea gel (16 V for 3 hr).

Other Reagents. Purified restriction endonucleases, T4 DNA ligase, T4 kinase, and S1 nuclease were purchased from Bethesda Research Laboratories, chemicals were from Sigma or Accurate Chemicals, and radioactive compounds were from New England Nuclear.

RESULTS

Isolation and Identification of Bovine Pituitary SP-I cDNA Clones. Approximately 225,000 recombinant phage were screened with the SP-I antibody and 12 positive clones were identified. The largest recombinant phage (λSP4B) contained a 1.6-kbp cDNA insert and was used in the remainder of the studies.

To confirm the immunologic identity of the fusion protein produced by λSP4B, we analyzed bacterial lysates of nonrecombinant λgt11 lysogens and a λSP4b lysogen by $NaDodSO_4$/polyacrylamide gel electrophoresis (Fig. 1*A*) and immunoblotting (Fig. 1*B*). In the presence of IPTG, the nonrecombinant lysogen produced a protein corresponding in molecular mass (Fig. 1*A*, lane 4) and antigenicity (Fig. 1*B*, lane 5) to β-galactosidase. The SP-I antiserum did not react with this or other proteins present in this bacterial lysate (Fig. 1*B*, lane 3). In contrast, in the presence of IPTG the recombinant λSP4B lysogen produced a fusion protein with an estimated mass of 160 kDa (Fig. 1*A*, lane 2). This protein showed both β-galactosidase (Fig. 1*B*, lane 4) and SP-I (lane 2) immunoreactivity. Because 114 kDa of this fusion protein can be accounted for by β-galactosidase, the cDNA-encoded peptide would have a calculated molecular mass of ≈46 kDa.

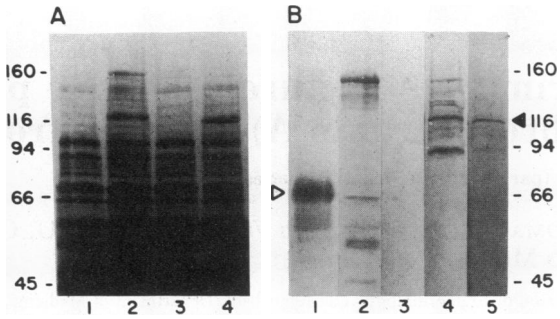

FIG. 1. (*A*) $NaDodSO_4$/PAGE analysis of the SP4B/β-galactosidase fusion protein. Bacterial lysates (20 μl) were electrophoresed on $NaDodSO_4$/7.5% polyacrylamide gels and stained with Coomassie brilliant blue. Positions in kDa are noted. Proteins from noninduced or IPTG-induced lysates of SP4B (lanes 1 and 2) and wild-type λgt11 lysogens (lanes 3 and 4) were analyzed. The noninduced lysates (lanes 1 and 3) yield identical protein patterns, whereas the IPTG-induced lysates contain additional proteins corresponding to β-galactosidase (lane 4, 116 kDa) and the SP4B/β-galactosidase fusion protein (lane 2, 160 kDa). Several other stained bands in lane 2 most likely represent proteolytic breakdown products of the fusion protein. (*B*) Immunoblots of bacterial lysates: lane 1, authentic SP-I is denoted by open arrowhead; lanes 2 and 4, IPTG-induced SP4B lysogen; lanes 3 and 5, IPTG-induced wild-type λgt11 lysogen. Proteins were detected by sequential hybridization with antibodies to SP-I (lanes 1–3), monoclonal antibodies to β-galactosidase (lanes 4 and 5), and alkaline phosphatase-linked second antibodies. The SP4B fusion protein has an apparent mass of ≈160 kDa. The position of β-galactosidase is denoted by solid arrowhead. Several apparent proteolytic breakdown products are again noted using the monoclonal anti-β-galactosidase antibody.

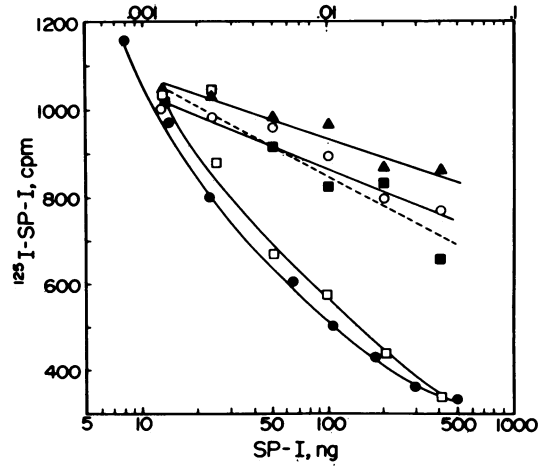

FIG. 2. Results of radioimmunoassay of the bacterial lysates using antibodies to SP-I and ^{125}I-labeled SP-I tracer. A standard RIA curve using increasing amounts of authentic SP-I (●) is compared to the displacement of SP-I tracer by lysates of induced SP4B lysogen (□), or noninduced SP4B lysogen (■), and induced (○) or noninduced (▲) wild-type λgt11 lysogen.

This agrees well with the size calculated from the amino acid sequence predicted by the cDNA.

Radioimmunoassay of bacterial lysates from IPTG-induced and noninduced λSP4B and nonrecombinant λgt11 were performed using SP-I antiserum (Fig. 2). The induced λSP4B lysate and authentic SP-I revealed parallel displacement of SP-I tracer.

Nucleotide and Deduced Amino Acid Sequence. The complete nucleotide sequence of the bovine pituitary SP-I cDNA insert in the λSP4B clone is shown in Fig. 3. An open reading frame extends from the first ATG codon at nucleotide 130 to the in-phase termination signal, the TAG codon at nucleotides 1477–1479. The encoded polypeptide of 449 amino acids has a molecular mass of ≈50 kDa.

The cDNA sequence shown in Fig. 3 includes 129 bases in the 5′ untranslated region and 138 bases in the 3′ untranslated region. Because a polyadenylylation signal is not found, we conclude this cDNA does not extend to the 3′ terminus of the mRNA. The deduced SP-I protein sequence (Fig. 3) contains an 18-amino acid hydrophobic "signal" sequence with leucine residues at positions 7, 9, 10, and 11, consistent with the structure predicted by analysis of the translation products of parathyroid SP-I mRNA in a cell-free system (31). Moreover, the amino-terminal amino acid sequences of bovine adrenal medulla SP-I (11) and bovine parathyroid SP-I (9) are found to be precisely encoded within the pituitary SP-I cDNA sequence.

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                                                                CCGACTCGTCCTCGGCGCCGACAGTACCGCTCT 33

GCAGCTTGCCTGGAGCGAGCAGTCCAGCCGCCCCTCGCCCGAGCGCGCGCCGTGGCCCGCCCCAGACCACCAGCT 108

                          1                                   10
                          MetArgSerAlaAlaValLeuAlaLeuLeuLeuCysAlaGlyGlnValIleAla
GCTCGGCGCCCCGGCTTCGCCATGCGCTCCGCCGCGGTCCTGGCGCTTCTGCTCTGCGCGGGGCAAGTCATTGCC 183

    20                                  30                                  40
LeuProValAsnSerProMetAsnLysGlyAspThrGluValMetLysCysIleValGluValIleSerAspThr
CTGCCTGTGAACAGCCCCATGAATAAAGGGGACACTGAGGTGATGAAGTGTATCGTCGAGGTCATCTCTGACACA 258

                          50                                  60
LeuSerLysProSerProMetProValSerLysGluCysPheGluThrLeuArgGlyAspGluArgIleLeuSer
CTCTCCAAGCCCAGCCCCATGCCAGTCAGCAAGGAGTGTTTTGAGACACTCCGAGGAGATGAACGGATCCTCTCA 333

    70                                  80                                  90
IleLeuArgHisGlnAsnLeuLeuLysGluLeuGlnAspLeuAlaLeuGlnGlyAlaLysGluArgThrHisGln
ATCCTGCGACATCAGAATTTGCTGAAAGAGCTCCAAGACCTCGCTCTCCAAGGAGCCAAGGAGCGGACACATCAG 408

                         100                                 110
GlnLysLysHisSerSerTyrGluAspGluLeuSerGluValLeuGluLysProThrAspGlnAlaGluProLys
CAGAAGAAGCACAGCAGTTACGAGGATGAACTCTCAGAGGTGCTTGAGAAGCCGACCGACCAGGCCGAGCCGAAA 483

    120                                 130                                 140
GluValThrGluGluValSerSerLysAspAlaAlaGluLysArgAspAspPheLysGluValGluLysSerAsp
GAGGTGACAGAAGAGGTGTCCTCCAAGGATGCTGCAGAAAAAAGAGACGATTTTAAAGAGGTGGAGAAGAGTGAT 558

                         150                                 160
GluAspSerAspGlyAspArgProGlnAlaSerProGlyLeuGlyProGlyProLysValGluGluAspAsnGln
GAAGACTCGGACGGAGACAGGCCTCAGGCCTCCCCAGGGCTTGGGCCGGGGCCCAAGGTTGAGGAGGACAACCAG 633

    170                                 180                                 190
AlaProGlyGluGluGluGluAlaProSerAsnAlaHisProLeuAlaSerLeuProSerProLysTyrProGly
GCCCCTGGGGAGGAGGAGGAGGCCCCCTCCAACGCCCACCCCCTAGCCAGCCTCCCCAGCCCGAAATACCCAGGC 708

                         200                                 210
ProGlnAlaLysGluAspSerGluGlyProSerGlnGlyProAlaSerArgGluLysGlyLeuSerAlaGluGln
CCACAGGCCAAGGAGGACAGCGAGGGTCCCTCCCAGGGTCCAGCCAGCAGGGAGAAGGGCCTGAGTGCAGAGCAA 783

    220                                 230                                 240
GlyArgGlnThrGluArgGluGluGluGluLysTrpGluGluAlaGluAlaArgGluLysAlaValProGlu
GGGAGGCAGACAGAGAGAGAAGAGGAGGAGGAGAAGTGGGAGGAGGCGGAGGCTAGAGAGAAGGCCGTCCCGGAG 858

                         250                                 260
GluGluSerProProThrAlaAlaPheLysProProProSerLeuGlyAsnLysGluThrGlnArgAlaAlaPro
GAAGAAAGCCCCGCCCACCGCAGCGTTTAAACCCCCACCGAGCCTCGGCAACAAGGAGACGCAGAGGGCTGCTCCA 933

    270                                 280                                 290
GlyTrpProGluAspGlyAlaGlyLysMetGlyAlaGluGluAlaLysProProGluGlyLysGlyGluTrpAla
GGTTGGCCCGAGGATGGAGCCGGGAAGATGGGGGCTGAGGAGGCCAAGCCCCCTGAGGGGAAGGGGGAGTGGGCA 1008

                         300                                 310
HisSerArgGlnGluGluGluGluMetAlaArgAlaProGlnValLeuPheArgGlyGlyLysSerGlyGluPro
CACTCCCGGCAGGAGGAAGAGGAGATGGCAAGGGCCCCTCAAGTCCTCTTCCGTGGTGGGAAGAGCGGGGAGCCC 1083

    320                                 330                                 340
GluGlnGluGluGlnLeuSerLysGluTrpGluAspAlaLysArgTrpSerLysMetAspGlnLeuAlaLysGlu
GAGCAGGAGGAGCAGCTCTCCAAGGAGTGGGAGGACGCCAAGCGATGGAGCAAGATGGACCAGCTGGCCAAGGAG 1158

                         350                                 360
LeuThrAlaGluLysArgLeuGluGlyGluGluGluGluGluGluAspProAspArgSerMetArgLeuSerPhe
CTGACGGCCGAGAAGCGGCTGGAGGGGGAGGAGGAGGAAGAGGAGGACCCCGACCGCTCCATGAGGCTCTCCTTC 1233

    370                                 380                                 390
ArgAlaArgGlyTyrGlyPheArgGlyProArgLeuGlnLeuArgArgGlyTrpArgProAsnSerGlnGluAsp
CGGGCCCGGGGCTACGGCTTCAGGGGTCCCAGGCTGCAGCTGCGGCGAGGCTGGAGGCCGAACTCCCGGGAGGAC 1308

                         400                                 410
SerValGluAlaGlyLeuProLeuGlnValArgGlyTyrProGluGluLysLysGluGluGluGlySerAlaAsn
AGCGTGGAGGCCGGCCTGCCCCTCCAGGTGCGCGGCTACCCGGAAGAGAAGAAGGAGGAGGAGGGCAGCGCCAAC 1383

    420                                 430                                 440
ArgArgProGluAspGlnGluLeuGluSerLeuSerAlaIleGluAlaGluLeuGluLysValAlaHisGlnLeu
CGCAGACCAGAGGACCAGGAGCTGGAGAGCTTGTCGGCCATCGAGGCAGAGCTGGAGAAGGTGGCCCACCAGCTG 1458

GluGluLeuArgArgGly
GAGGAGCTTCGGCGGGGCTGAGGCACTGACTGGCCCCACCAGCCAGGGCCCCGAGACACCTCGTGGTCCCGGCTC 1533

TGTTGCCCCCTCTGCAGGTCCTGGCCAGACGGCCCCAGGCACTGCTTCCGGGAGGGAGGCCGCCCCCAGCCTGCC 1608

CAAGCC
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FIG. 3. Nucleotide sequence of the bovine pituitary SP-I cDNA (SP4B). The nucleotides are numbered in the 5′ to 3′ direction with the first number beginning at the ATG triplet encoding the initiating methionine. The deduced amino acids are shown above the nucleotides. Solid lines denote nucleotides that differ from the adrenal medullary cDNAs.

The coding sequences of the bovine pituitary SP-I cDNA differ from the bovine adrenal SP-I cDNA sequences reported by Benedum *et al.* (4) by eight noncontiguous nucleotides, and by Iacangelo *et al.* (5) by five noncontiguous nucleotides. In both comparisons, the nucleotide discrepancies result in differences in five amino acids. The cDNA reported by Iacangelo *et al.* predicts three amino acids within the amino acid sequence 153–160 that differ from those predicted by the cDNAs reported here and by Benedum *et al.* This appears to result from differences in two nucleotides—one within the codon for amino acid 153 and the other within the codon for amino acid 160. The pituitary sequence agrees with one or the other adrenal medullary SP-I cDNA sequences in all but two nucleotides of the coding region, resulting in two amino acid differences (threonine for asparagine at position 112; arginine for glycine at position 379).

Although the nucleotide sequence of the coding and 3′ noncoding regions of pituitary and adrenal SP-I cDNAs are nearly identical, there is no homology within the first 33 nucleotides in the 5′ noncoding region. To determine whether this dissimilarity represented true differences in adrenal and pituitary mRNA or resulted from cloning artifacts, S1 nuclease mapping studies were performed. S1 nuclease digestion of total RNA from parathyroid, adrenal medulla, and pituitary tissues after hybridization with the 324-bp *Eco*RI/*Bam*HI cDNA fragment of pituitary SP-I (Fig. 4) demonstrated a single protected fragment of 291 nucleotides. This indicates that the dissimilar 5′ region of the cloned pituitary cDNA is not present in native RNA and is likely an artifact of cloning λSP4B. No evidence of a hybridizing mRNA was detected in control RNA from bovine liver.

Tissue Distribution of SP-I mRNA. Hybridization of the radiolabeled 1.6-kbp SP4B cDNA insert to total RNA from bovine parathyroid, adrenal medulla, pituitary, adrenal cortex, and liver is shown in Fig. 5. In tissues known to express SP-I immunoreactivity (pituitary, adrenal medulla, and parathyroid), a single 2.1-kb signal was found (lanes 1–3). No transcript was detected in RNA from bovine adrenal cortex (lane 4) or liver (lane 5).

Southern Blot Analysis of Genomic DNA. Southern blot analysis of bovine genomic DNA digested with *Eco*RI, *Hin*dIII, and *Sst* I is shown in Fig. 6. Hybridization using the 1.6-kbp SP4B cDNA produced a single prominent band with each of the three restriction endonucleases, consistent with the presence of a single SP-I gene within the haploid genome.

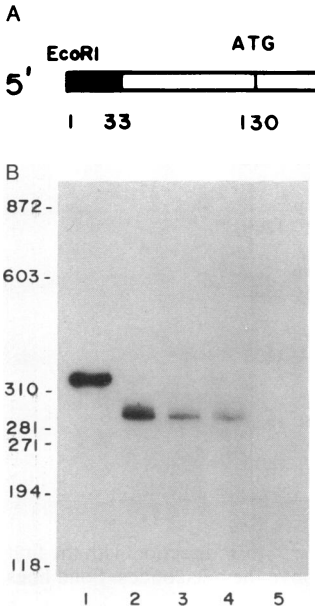

FIG. 4. (*A*) Diagram of the 324-bp *Eco*RI/*Bam*HI fragment of the pituitary SP-I cDNA used as a probe for S1 nuclease mapping studies. Nucleotide sequences that differ from the adrenal SP-I cDNA are shaded. (*B*) Autoradiograph of protected mRNA fragments after polyacrylamide gel electrophoresis. Lanes: 1, ^{32}P-end-labeled 324-bp SP4B *Eco*RI/*Bam*HI fragment; 2, 5 μg of parathyroid RNA; 3, 5 μg of adrenal medulla RNA; 4, 50 μg of pituitary RNA; 5, 10 μg of liver RNA. Numbers on left indicate marker sizes in bases.

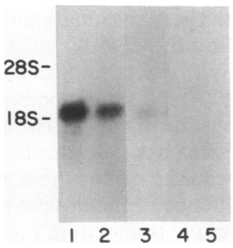

FIG. 5. RNA blot analysis of total RNA (10 μg) from bovine parathyroid (lane 1), adrenal medulla (lane 2), pituitary (lane 3), adrenal cortex (lane 4), and liver (lane 5). Hybridizations were performed using the ^{32}P-labeled 1.6-kb pituitary SP-I insert of SP4B. Positions of the 28S and 18S rRNAs are indicated.

DISCUSSION

To determine whether SP-I is similar in diverse endocrine tissues, we isolated a bovine pituitary SP-I cDNA from an expression library. The identity of the clone was initially achieved by a displacement radioimmunoassay in which the expressed fusion protein was recognized as SP-I. Immunoblot analysis of the fusion protein indicated a molecular mass of 45–50 kDa for the SP-I protein, confirming the notion that previous estimates of the molecular mass of SP-I (70–80 kDa) may have been incorrect because of the anomalous migration of the protein upon $NaDodSO_4$/polyacrylamide gel electrophoresis (4, 5).

The nucleotide sequence of the bovine pituitary SP-I cDNA is nearly identical to that of bovine adrenal SP-I described by Benedum *et al.* (4) and Iacangelo *et al.* (5). When the pituitary cDNA sequence is compared to either of the adrenal SP-I cDNA sequences, only two nucleotide discrepancies are found within the coding region and result in the assignment of different amino acids at two sites. The differences in amino acids 153–160 reported by Iacangelo *et al.* (compared to those reported here and by Benedum *et al.*) are likely the result of sequencing errors that cause a frameshift for seven codons. All other differences are single nucleotide substitutions that may be the result of sequencing errors or DNA polymorphisms. Whereas the coding se-

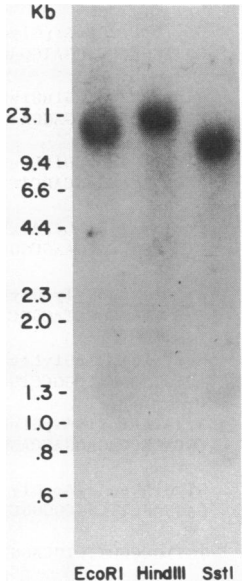

FIG. 6. Southern blot analysis of bovine genomic DNA (5 μg) digested with *Eco*RI, *Hin*dIII, and *Sst* I restriction endonucleases. Molecular size markers (kb) are indicated on the left. Hybridizations were performed using the radiolabeled 1.6-kb SP4B insert.

quences of the adrenal and pituitary cDNAs are nearly identical, the first 33 nucleotides of the 5′ noncoding regions were found to be completely dissimilar. Although discrepancies in the 5′ noncoding sequences of similar cDNAs may result from tissue-specific differences in transcriptional initiation or mRNA processing, our S1 nuclease data indicate that this was due to a cloning artifact [occasionally encountered in the 5′ region of cloned cDNAs (32–34)].

Earlier studies have described the synthesis of multiple immunoreactive SP-I-like proteins after cell-free translation of mRNA and have suggested the possibility that several SP-I genes exist. Majzoub *et al.* (31) noted that translation of parathyroid pre-SP-I mRNA in cell-free systems gave rise to four closely related pre-SP-I products. Partial amino acid sequencing of these proteins revealed microheterogeneity in the lengths of the amino-terminal leader sequences. These workers could not conclude, however, whether these differences in amino acid sequence were due to differences in the site of initiation of translation of a single mRNA or to translation of separate but closely related mRNAs. Subsequently, Serck-Hanssen and O'Connor (35) noted that cell-free translation of mRNAs from bovine adrenal medulla, anterior and neurointermediate pituitary gland, and human pheochromocytoma results in the synthesis of a series of "chromogranin-like" proteins that exhibit apparent molecular mass between 25 and 120 kDa. They hypothesized that the different-sized proteins did not derive from a common high molecular weight precursor, but rather were each encoded by a distinctive mRNA.

Although the precise relationship of SP-I mRNA and SP-I-like proteins of various molecular masses remains to be clarified, our studies suggest that there is a single gene that encodes pituitary, parathyroid, and adrenal SP-I. First, Southern blot analysis of bovine DNA digested with three separate restriction endonucleases reveals a simple restriction pattern consistent with the presence of a single SP-I gene (Fig. 6). Second, the nucleotide sequences of pituitary and adrenal SP-I cDNAs are nearly identical, and a similar 2.1-kb message is detected in RNA from pituitary, adrenal, and parathyroid tissues. Third, blot hybridization analysis of RNA isolated from human pheochromocytoma, medullary thyroid carcinoma, and lung carcinoma using a human medullary thyroid carcinoma (36) or a human pheochromocytoma SP-I cDNA probe (L. J. Helman, T.G.A., A. Allison, M.A.L., and M. A. Israel, unpublished data) similarly reveals the presence of a single hybridizing band of 2.1 kb in each tissue. Finally, S1 nuclease analysis of RNA isolated from bovine pituitary, adrenal medulla, and parathyroid with a DNA probe from the 5′ region of the pituitary SP-I cDNA demonstrates a single identical fragment. This latter finding suggests that the microheterogeneity in the lengths of the leader sequences of four pre-SP-I proteins described by Majzoub *et al.* (31) did not result from the translation of multiple mRNAs.

Attempts at understanding the function of SP-I must take into consideration its widespread distribution in endocrine and neuroendocrine secretory tissues as well as its absence in other polypeptide-secreting cells. The availability of these cDNAs should allow further investigation of the function of these secretory proteins.

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