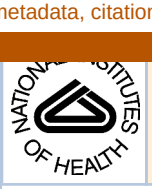

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Purification and Partial Characterization of Relaxin and Relaxin Precursors from the Hamster Placenta[1]

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Abstract

Previous immunological studies have indicated that the molecular structure of hamster relaxin is quite different from that of porcine relaxin. In the present study, hamster relaxin was purified from placentas and characterized in order to investigate its biochemical properties. Placentas from Days 14 and 15 of gestation were homogenized in 0.26 N HCl-62.5% acetone containing protease inhibitors. After centrifugation, soluble proteins were acetone precipitated. Soluble proteins were applied to a carboxymethyl cellulose ion-exchange column and bound proteins were eluted with 0.1 and 0.3 M NaCl. Western blot analysis detected 16.5-, 18.7-, and 36.0-kDa relaxin-immunoreactive (IR) proteins within the 0.1 M NaCl eluant and detected a 5.6-kDa relaxin-IR protein within the 0.3 M NaCl eluant. The 5.6-kDa protein was purified to homogeneity by gel filtration (Sephadex G-50), ion-exchange HPLC, and C_{18} -HPLC. Reduction of the 5.6-kDa protein prior to electrophoresis resulted in a single band of lower molecular mass, suggesting that hamster relaxin consists of two chains of approximately equal molecular mass. Isoelectric point of the 5.6-kDa protein was 7.78. The 16.5- and 18.7-kDa IR proteins were copurified by gel filtration and ion-exchange HPLC. At least five isoelectric point variants were observed for the 16.5- and 18.7-kDa proteins. The N-terminal amino acid for the 5.6 and 18.7 relaxin-IR proteins was arginine, and subsequent cycles indicated an identical partial sequence that was consistent with that for relaxins from other species.

INTRODUCTION

Relaxin is a polypeptide hormone normally associated with pregnancy. A role for this hormone in reproductive tract metabolism, connective tissue remodeling, and myometrial contractility has been demonstrated in several species [1].

The tissue source for relaxin varies among species. Relaxin was first isolated and purified from corpora lutea of the pig ovary [2], and this molecule remains the standard with which others are compared. Porcine relaxin is composed of two polypeptide chains bound by two disulfide bonds and has a molecular mass of approximately 6000 daltons. Relaxin has also been purified and characterized from the ovary of the rat [3], human [4], shark [5,6], whale [7], and skate [8]. Endometrial glands of the pregnant guinea pig contain relaxin immunoreactivity (IR) [9], and preprorelaxin mRNA was recently reported to be present in the endometrium of this species [10]. Relaxin has been purified and characterized from the placenta of the rabbit [11] and human [12]; the amino acid sequences of relaxin purified from horse [13,14] and dog [15] placenta have been reported.

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Extracts of hamster placenta contain relaxin immunoreactivity (IR) and bioactivity [16], and relaxin is localized primarily to giant trophoblast cells of the developing and mature placenta [17]. Serum relaxin IR is first detected on Day 8 of pregnancy [18], which coincides with initial detection of placental relaxin by immunocytochemical methods. While ovarian relaxin is contained within storage granules of the luteal cell in the rat [19,20] and pig [21], the fact that giant trophoblast cells of the hamster placenta do not contain storage granules [22] suggests that synthesized hormone is rapidly secreted from the cell. The effect of this difference in cellular handling on the processing of relaxin preprohormone is not known.

The objectives of the present study were to isolate and biochemically characterize hamster relaxin from placental extracts. In addition to isolation of relaxin hormone, several possible relaxin precursors were identified and characterized.

MATERIALS AND METHODS

Animals and Tissue Recovery

Adult male and female Golden (Syrian) hamsters (Charles River Laboratories, Wilmington, MA) were maintained on a 12L:12D schedule (lights-on: 0700 h). Water and Purina Laboratory Chow (Ralston-Purina, St. Louis, MO) were available ad libitum. Female hamsters (120–150 g) were checked daily for estrus as determined by expression of lordosis in the presence of a male, and females in estrus were housed overnight with a male. The following day was designated as Day 1 of gestation.

On Day 14 or 15 of gestation, hamsters were anesthetized with methoxyflurane (Metofane; Pitman-Moore, Mundelein, IL) and killed by cervical dislocation. The uterus was exposed and removed through a midventral abdominal incision. Placentas were dissected free of the uterus, fetus, and placental membranes and were frozen by immersion of the storage tube into a slurry of dry ice and acetone. Placentas were stored at −80 °C.

Protein Measurement

Protein concentrations were determined via the Bio-Rad Protein Assay (Bio-Rad, Richmond, CA) or, when material was limiting, were estimated by absorbance at 280 nm.

Preparation of Crude Extract

Tissues were processed in batches of approximately 150 g (n ~ 40 hamsters). The aqueous extraction solution (0.26 N HCl-62.5% acetone), similar to that reported by Griss et al. [23], contained phenylmethylsulfonyl fluoride (PMSF; 30 μg/ml), sodium EDTA (10 mM), leupeptin (0.5 μg/ml), pepstatin (0.7 μg/ml), and sodium azide (0.02%) to inhibit proteolysis. All extraction steps were conducted at 4°C. Frozen tissues were homogenized (Polytron; Brinkmann Instruments, Westbury, NY) in extraction solution (5 ml/g tissue), and the homogenate was stirred overnight. After centrifugation (11 000 × *g*) for 30 min, pellets were resuspended in 1 volume of extraction medium and the suspension was centrifuged as before. The supernatants were then combined and centrifuged for 90 min. After passing through cheesecloth to remove lipids, five volumes of ice-cold acetone (final concentration = 93.7%) were added to the supernatant and the resulting precipitate was allowed to settle overnight. Excess acetone was aspirated off, and the pellet was collected by centrifugation for 60 min and air dried.

Ion-Exchange Chromatography

The acetone pellet was resuspended in dH_2O containing protease inhibitors and the solution was dialyzed (Spectrapor 3; Spectrum Medical, Los Angeles, CA) overnight at 4°C against carboxymethyl (CM) buffer (50 mM ammonium acetate, pH 5.5) containing PMSF. After

dialysis, the solution was clarified by centrifugation (16 000 × *g*) at 4°C for 60 min. Protein (~750 mg) was loaded (40 ml/h) onto a column (2.6 × 18.0 cm) of carboxymethyl cellulose (CM-52; Whatman, Clifton, NJ) equilibrated with CM buffer. Protein elution (60 ml/h) was monitored by absorbance at 280 nm, and 5-ml fractions were collected. Unbound protein was removed by washing with CM buffer until absorbance reached zero. A two-step gradient of 0.1 M NaCl and 0.3 M NaCl in CM buffer was used to elute bound protein. Western blot analysis indicated that relaxin IR was associated with high- and low-molecular-mass proteins after elution with 0.1 M NaCl and 0.3 M NaCl, respectively.

Gel Filtration

The relaxin-IR proteins that eluted subsequent to 0.1 and 0.3 M NaCl elution were dialyzed against G-50 buffer (100 mM ammonium acetate, pH 6.8) at 4C for 24 h, centrifuged as before, and loaded onto a column (2.6 × 95 cm) of Sephadex G-50 (Pharmacia; LKB Biotechnology, Piscataway, NJ). Protein elution (30 ml/h) was monitored by absorbance at 280 nm and 3-ml fractions were collected. Fractions containing relaxin IR were pooled for analysis and further purification.

Ion-Exchange HPLC

Following gel filtration, pooled relaxin-IR fractions were concentrated and equilibrated with Buffer A (10 mM ammonium acetate, pH 5.5) via an ultra filtration cell (Amicon, Beverly, MA) equipped with a 1000 molecular mass cutoff filter. Samples were loaded onto a 10 × 100-mm propyl sulfonic ion-exchange column (Protein-PAK SPR, Waters Chromatography Division, Milford, MA). A Waters HPLC system with dual pumps, a gradient programmer, and an absorbance monitor measuring at 258 nm was used. When relaxin-IR fractions of low molecular mass were chromatographed, unbound protein was removed by washing with 60% buffer A and 40% buffer B (10 mM ammonium acetate, 1.0 M NaCl, pH 5.5). Bound protein was eluted by a linear increase in buffer B to 85% over a 70-min period. When high-molecular-mass fractions containing relaxin were loaded, the column was washed with 100% buffer A and bound protein was eluted by a linear increase in buffer B to 100% over an 80-min period.

C_{18}-HPLC

Final purification of low-molecular-mass relaxin-IR protein was accomplished using a 3.9 ×300-mm reverse-phase column (μBondPak C18, Waters Chromatography Division). Solution A contained 0.1% trifluroacetic acid and solution B contained 100% acetonitrile. The column was developed through use of a linear gradient of 20–40% B over a 60-min period.

Radioimmunoassay

Highly purified porcine relaxin (kindly supplied by MJ. Fields, University of Florida, Gainesville, FL) was iodinated with ^{125}I-Na (Amersham Corp., Arlington Heights, IL) by the method of Bolton and Hunter [24] as modified by McMurtry et al. [25]. Specific activity of the iodinated hormone was 45–60 μCi/μg with trichloroacetic acid (TCA) precipitation values of 75–85%.

Rabbit antibody to porcine relaxin (R6, kindly provided by Dr. B. Steinetz) was used for radioimmunoassay and Western blot analysis. This antiserum had been previously used for measurement of relaxin by RIA in serum of pregnant hamsters [18] and for immunolocalization of relaxin in hamster placental tissues [22].

Relaxin IR in chromatography fractions was measured by use of the homologous porcine relaxin radioimmunoassay previously described [22] except that UF-1 porcine relaxin antiserum was replaced by the R6 antiserum (1:30 000 dilution in assay buffer). Binding

of ^{125}I-relaxin was 30–40% and the working range of the assay was from 78.1 to 1250 pg. Intra- and interassay coefficients of variation were 9.0 and 5.1%, respectively. Because binding curves produced by increasing concentrations of porcine relaxin and hamster placental extract were not parallel, serial dilutions of the crude placental extract were also used as standard hormone. This allowed for a better determination of relative differences in hormone content among samples being assayed. To expedite measurement of relaxin in chromatography fractions, assay incubation times following each addition (that of R6 antibody and that of rabbit immunoglobulin-G plus second antibody) were each reduced to 5 h at room temperature. Satisfactory binding was achieved with this method (20%), and porcine relaxin standard curves were not different from those obtained by standard methods.

SDS-PAGE

SDS-PAGE was used to evaluate protein content of crude placental extracts and that of ion-exchange, gel filtration, and HPLC fractions containing relaxin IR. Samples dissolved in SDS sample buffer were loaded onto 1-mm-thick, 16% polyacrylamide minigels (Novex Experimental Technology, San Diego, CA), and electrophoresis was conducted through use of a Tris-tricine buffer system. Gels were run at 125 volts for 2–2.5 h and protein bands were identified by staining with a solution of Coomassie blue R-250.

Western Blot Analysis

After SDS-PAGE, gels were equilibrated in blotting buffer (12 mM Tris, 96 mM glycine, 20% methanol, pH 8.3) and transferred (Western Transfer Apparatus; Novex) to nitrocellulose (0.2-μm pore size; Hoefer Scientific Instruments, San Francisco, CA). Transfer was conducted at 30 volts for 2 h. Completeness of transfer and identification of molecular mass standards were monitored by brief staining of the nitrocellulose in an aqueous solution containing 0.1% (w/v) amido black. After removal of portions of the membrane containing molecular mass standards, the nitrocellulose was washed several times in dH_2O and agitated in blocking solution (TTBS; 100 mM Tris, 0.9% NaCl, 0.1% Tween-20, pH 7.5) for 18–24 h. It was necessary to use this long blocking time to achieve satisfactory immunostaining, perhaps because of protein refolding on the membrane. The blocked nitrocellulose membrane was incubated with R6 antibody (diluted 1:1000 in TTBS) for 12–24 h at room temperature and then washed twice (5 min each) with TTBS. The membrane was next incubated with goat antirabbit-IgG conjugated to alkaline phosphatase (GAR-AP; Bio-Rad) for 1 h at room temperature and washed as before. Bound relaxin antibody was visualized by incubation with alkaline phosphatase substrate (5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium; Bio-Rad).

Isoelectric Focusing (IEF)

Protein isoelectric point was determined using 5% polyacrylamide IEF gels (range, pH 3–10; Novex) and IEF marker peptides (Pharmacia, Piscataway, NJ). Protein preparations recovered from Sephadex G-50 chromatography were subjected to IEF, and completed gels were fixed in a solution containing 10% TCA prior to staining with Coomassie blue. For identification of relaxin-IR proteins, focused proteins were electroblotted to nitrocellulose and immunostained as described above.

Partial Amino Acid Sequence Analysis

For N-terminal amino acid sequence analysis, high- and low-molecular-mass relaxin-IR proteins purified via Sephadex G-50 were separated by SDS-PAGE and blotted to polyvinyldifluoride (PVDF) membrane. Blotted proteins were identified by staining with Coomassie blue and previously identified relaxin-IR bands were cut out of the membrane. Partial N-terminal sequence was determined by the Edman degradation procedure using a gas-phase protein sequencer (Applied Biosystems Model 475A, Foster City, CA).

RESULTS

Purification

Relaxin-IR proteins were eluted, along with other proteins, by 0.1 and 0.3 M NaCl from the CM-52 column (Fig. 1). SDS-PAGE analysis of the 0.3 M NaCl eluant (Fig. 2) detected a protein weakly stained with Coomassie Blue dye that had a molecular mass similar to that of relaxin; Western blot analysis (Fig. 2) indicated that relaxin IR was associated with this protein band. Two relaxin-IR proteins with molecular masses of 16.5 and 18.7 kDa were detected by Western blot analysis (Fig. 2) in the 0.1 M NaCl eluant. Fractions containing these proteins were combined for further purification of what were hypothesized to be relaxin precursors. A third relaxin-IR protein with a molecular mass of 36.8 0.5 kDa (n = 6 determinations) was also observed in the 0.1 M NaCl eluant by Western blot analysis (Fig. 2). Further purification of this protein was not attempted.

The Sephadex G-50 gel filtration chromatography profile of CM cellulose fractions containing relaxin and relaxin precursors is shown in Figure 3. Fractions containing relaxin IR (as determined by RIA) and detectable relaxin or relaxin precursor protein (as determined by SDS-PAGE analysis) were pooled for further purification. SDS-PAGE analysis indicated that relaxin was a major component of that pooled sample (Fig 3A) with significant contamination by three other proteins (not shown). SDS-PAGE analysis also indicated that the relaxin precursors were a major component of that pool (Fig. 3B); however, this fraction contained several other proteins in equal or greater concentration.

Sephadex G-50 fractions containing relaxin or relaxin precursors were subjected to SP-HPLC. Relaxin IR was associated with a single irregular protein peak following SP-HPLC (Fig. 4A) of the relaxin preparation. SDS-PAGE analysis indicated minor contamination of the relaxin preparation by one protein of slightly higher molecular mass (data not shown). Relaxin was subsequently purified to homogeneity by reverse-phase C_{18}-HPLC chromatography (Figs. 5 and6, lane 3). Recovery of hamster placental relaxin at each purification step is shown in Table 1. SDS-PAGE and Western blot analysis indicated that the SP-HPLC relaxin precursor preparation contained two relaxin-IR proteins (Fig. 6, lane 2). Purification of each of these proteins was not attempted.

Physicochemical Properties

The average molecular mass of hamster relaxin was determined by SDS-PAGE analysis to be 5600 ± 200 daltons (n = 7 determinations). The apparent molecular weight of relaxin decreased to approximately 3000 daltons following reduction by β-mercaptoethanol (Fig. 6, lane 4), indicating the presence of two disulfide-bonded chains. The isoelectric point of relaxin was estimated to be 7.78 (Fig. 7, lane 3). The SP-HPLC-purified relaxin precursor preparation contained two proteins with molecular masses of 16 400 ± 200 and 18 700 ± 300 daltons (n = 7 determinations). Five major isoelectric point variants with values from 5.03 to 6.33 were observed (Fig. 7, lane 2).

Partial Sequence Analysis

Amino-terminal sequence analysis was performed through use of gel filtration-purified relaxin and a single relaxin precursor (18.7 kDa) after separation by SDS-PAGE and electroblotting to PVDF membrane. Sequence analysis of the 18.7-kDa precursor continued for 36 cycles, at which time low yields prevented identification of subsequent amino acids. The partial amino acid sequence of the N-terminus corresponded to the B-chain sequences of other relaxins described to date (Fig. 8); rat and hamster relaxins had the most identity (58%) over the region evaluated. Analysis of hamster relaxin yielded an incomplete (23 amino acid) sequence that corresponded to that of the B-chain. The sequence of a minor contaminant was also identified;

however, this sequence did not correspond to that of other relaxins described to date, suggesting that the A-chain of hamster relaxin was blocked.

DISCUSSION

Relaxin has previously been purified and characterized from placenta of a limited number of species. The methods used in the present study for extraction of hamster relaxin were similar to those previously reported; however, subsequent chromatographic procedures were modified to insure isolation of both the high- and low-molecular-mass forms of relaxin. Immunocytochemical localization using antibodies to porcine relaxin indicates that the placenta is the source of relaxin in the hamster [17]. Although values for recovery of relaxin (mg/g tissue) from hamster placental tissues were low compared to those reported for pig [2] and rat 3] ovary, they were similar to values previously reported for horse [13] and rabbit [11] placenta. Secondary giant trophoblast cells are the major placental cell type containing relaxin within the hamster placenta [17], and it is likely that the quantities of relaxin were low because relaxin is not contained within storage granules in placental giant trophoblast cells [22].

High-molecular-mass proteins with relaxin IR and bioactivity have been detected in extracts of ovaries from the pig [26,27] and rat [28]; and multiple forms of relaxin IR have been detected in serum during pregnancy in the rat [29]. This is the first report of high-molecular-mass forms of relaxin produced by placental tissue. Soloff et al. [15] reported that ovarian tissue from pregnant rats contained relaxin-IR proteins of 16.5 and 18 kDa with identical N-terminal sequences corresponding to rat prorelaxin. A hamster prorelaxin cDNA has not been identified. That the 18.7-kDa relaxin-IR protein from hamster placenta is relaxin prohormone was indicated by the similarity of the partial N-terminal amino acid sequence and apparent molecular mass to that of relaxin prohormone reported for other species. Although the identity of the 16.5-kDa protein was not confirmed by sequence analysis, it is likely that this protein represents the same processing intermediate reported for the rat [28]. The function of the 16.5- and 18.7-kDa proteins remains to be determined. Although precise quantification of relaxin and the 16.5- and 18.7-kDa relaxin precursors was not performed, the intensity of stained bands following Western blot analysis indicated that the 16.5- and 18.7-kDa proteins are present in much greater amounts (data not shown). In the rat the 16.5- and 18.0-kDa proteins are also present in excess as compared to relaxin [28]. This similarity with the hamster was surprising, since rat relaxin is stored within secretory granules of luteal cells whereas relaxin-containing giant trophoblast cells of the hamster placenta do not contain storage granules. These facts suggested that relaxin in the hamster may be processed differently than in the rat and other species with an ovarian source of relaxin. However, data from the present study and from investigations in the rat suggest that relaxin preprohormone processing is dictated by molecular structure rather than by factors associated with the secretory pathway utilized by the cell synthesizing the molecule.

Identity of the 36-kDa relaxin-IR protein was not confirmed by sequence analysis; however, Sherwood et al. [29] identified relaxin-IR components with molecular masses greater than 28 kDa in the serum of pregnant rats. The relationship of these proteins to relaxin preprohormone is not known.

Isolation and purification of relaxin and its precursors in sufficient quantities for production of hamster relaxin-specific antibodies has proven to be difficult. However, currently the effort is underway to separate relatively crude preparations of hormone through SDS-PAGE and immunization using relaxin and relaxin precursors recovered from polyacrylamide gels. Generation of hamster-specific antibodies will allow development of a homologous RIA for hamster relaxin. In addition, the N-terminal amino acid sequence of the prohormone has been

utilized to produce a peptide for immunization and to generate oligonucleotides for molecular biology applications.

References

1. Sherwood OD. Relaxin. In: Knobil E, Neill JD (eds.), The Physiology of Reproduction, vol 1. New York: Raven Press; 1988: 585–673.
2. Sherwood OD, O'Byrne EM. Purification and characterization of porcine relaxin. Arch Biochem Biophys 1974;160:185–196. [PubMed: 4828522]
3. Sherwood OD. Purification and characterization of rat relaxin. Endocrinology 1979;104:886–892. [PubMed: 436762]
4. Winslow J, Shih A, Laramee G, Bourell J, Stults J, Johnston P. Purification and structure of human pregnancy relaxin from corpora lutea, serum and plasma. In: Program of the 71st annual meeting of the Endocrine Society; 1989; Seattle, WA. Abstract 889.
5. Reinig JW, Daniel LN, Schwabe C, Gowan LK, Steinetz BG, O'Byrne EM. Isolation and characterization of relaxin from the sand tiger shark (*Odontaspis taurus*). Endocrinology 1981;109:537–543. [PubMed: 7250055]
6. Büllesbach EE, Gowan LK, Schwabe C, Steinetz BG, O'Byrne E, Callard IP. Isolation, purification, and the sequence of relaxin from spiny dogfish (*Squalus acanthias*). Eur J Biochem 1986;161:335–341. [PubMed: 3780747]
7. Schwabe C, Büllesbach EE, Heyn H, Yoshioka M. Cetacean relaxin: isolation and sequence of relaxins from *Balaenoptera acutorostrata* and *Balaenoptera edeni*. J Biol Chem 1989;264:940–943. [PubMed: 2910872]
8. Büllesbach EE, Schwabe C, Callard IP. Relaxin from an oviparous species, the skate (*Raja erinacea*). Biochem Biophys Res Commun 1987;143:273–280. [PubMed: 3827922]
9. Pardo R, Larkin LH, Fields PA. Immunocytochemical localization of relaxin in endometrial glands of the pregnant guinea pig. Endocrinology 1980;107:2110–2112. [PubMed: 6775935]
10. Lee YA, Bryant-Greenwood GD, Mandel M, Greenwood FC. The complementary deoxyribonucleic acid sequence of guinea pig endometrial prorelaxin. Endocrinology 1992;130:1165–1172. [PubMed: 1537282]
11. Eldridge RK, Fields PA. Rabbit placental relaxin: purification and immunohistochemical localization. Endocrinology 1985;117:2512–2519. [PubMed: 3905361]
12. Fields PA, Larkin LH. Purification and immunohistochemical localization of relaxin in the human term placenta. J Clin Endocrinol & Metab 1981;52:79–85. [PubMed: 6778893]
13. Stewart DR, Papkoff H. Purification and characterization of equine relaxin. Endocrinology 1986;119:1093–1099. [PubMed: 3732157]
14. Stewart DR, Nevins B, Hadas E, Vandlen R. Affinity purification and sequence determination of equine relaxin. Endocrinology 1991;129:375–383. [PubMed: 2055195]
15. Stewart DR, Henzel WJ, Vandlen R. Purification and sequence determination of canine relaxin. J Protein Chem 1992;11:247–253. [PubMed: 1388669]
16. Steinetz BG, O'Byrne EM, Goldsmith LT, Anderson MB. The source of relaxin in pregnant syrian hamsters. Endocrinology 1988;122:795–798. [PubMed: 3342753]
17. Renegar RH, Cobb AD, Leavitt WW. Immunocytochemical localization of relaxin in the golden hamster (*Mesocricetus auratus*) during the last half of gestation. Biol Reprod 1987;37:925–934. [PubMed: 3318945]
18. O'Byrne EM, Steinetz BG. Radioimmunoassay (RIA) of relaxin sera of various species using an antiserum to porcine relaxin. Proc Soc Exp Biol Med 1976;152:272–276. [PubMed: 819937]
19. Anderson MB, Sherwood OD. Ultrastructural localization of relaxin immunoreactivity in corpora lutea of pregnant rats. Endocrinology 1984;114:1124–1127. [PubMed: 6368200]
20. Fields PA. Intracellular localization of relaxin in membrane-bound granules in the pregnant rat luteal cell. Biol Reprod 1984;30:753–762. [PubMed: 6372879]
21. Fields PA, Fields MJ. Ultrastructural localization of relaxin in the corpus luteum of the nonpregnant, pseudopregnant, and pregnant pig. Biol Reprod 1985;32:1169–1179. [PubMed: 3893554]

22. Johns TC, Renegar RH. Ultrastructural morphology and relaxin immunolocalization in giant trophoblast cells of the golden hamster placenta. Am J Anat 1990;189:167–178. [PubMed: 2244585]

23. Griss G, Keck J, Engelhorn R, Tuppy H. The isolation and purification of an ovarian polypeptide with uterine-relaxing activity. Biochim Biophys Acta 1967;140:45–54.

24. Bolton AE, Hunter WM. The labeling of proteins to high specific radioactivities by conjugation to a ^{125}I-containing acylating agent. Biochem J 1973;133:529–539. [PubMed: 4733239]

25. McMurtry JP, Kwok SCM, Bryant-Greenwood GD. Target tissues for relaxin identified in vitro with ^{125}I-labeled porcine relaxin. J Reprod Fertil 1978;53:209–216. [PubMed: 211231]

26. Frieden EH, Yeh L-A. Evidence for a “pro-relaxin” in porcine relaxin concentrates. Proc Soc Exp Biol Med 1977;154:407–411. [PubMed: 847002]

27. Kwok SCM, Chamley WA, Bryant-Greenwood GD. High molecular weight forms of relaxin in pregnant sow ovaries. Biochem Biophys Res Commun 1978;82:997–1005. [PubMed: 697780]

28. Soloff MS, Shaw AR, Gentry LE, Marquardt H, Vasilenko P. Demonstration of relaxin precursors in pregnant rat ovaries with antisera against bacterially expressed rat prorelaxin. Endocrinology 1992;130:1844–1851. [PubMed: 1547714]

29. Sherwood OD, Key RH, Tarbell MK, Downing SJ. Dynamic changes of multiple forms of serum immunoreactive relaxin during pregnancy in the rat. Endocrinology 1984;114:806–813. [PubMed: 6697963]

FIG. 1.

Ion-exchange chromatography of the acetone extract (750 mg) on a 2.6 × 18.0-cm column of CM-52. Column buffer was 50 mM ammonium acetate, pH 5.5, and contained PMSF to inhibit proteolysis. A step gradient of 0.1 and 0.3 M NaCl was used to elute bound protein. Flow rate was maintained at 60 ml/h and 5-ml fractions were collected. The black bar indicates fractions containing 5.6-kDa (0.3 M NaCl eluant) and 16.5-, 18.7- and 36.0-kDa (0.1 M NaCl eluant) relaxin-IR proteins.

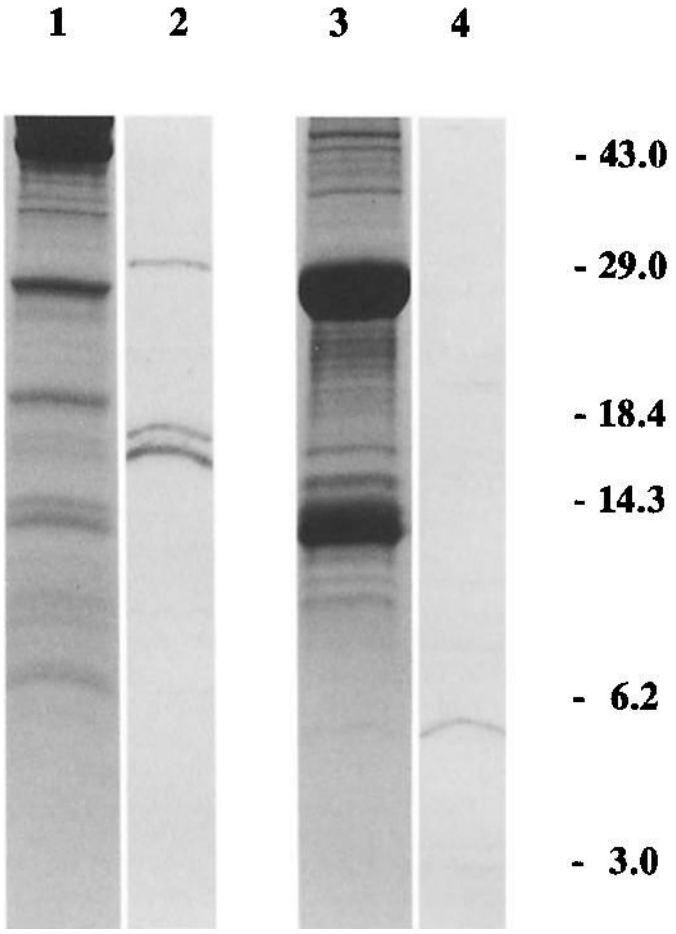

FIG. 2.

SDS-PAGE of relaxin-IR ion-exchange fractions. Lane 1) Coomassie-stained proteins eluted by 0.1 M NaCl. Lane 2) Western blot analysis of proteins eluted by 0.1 M NaCl. Lane 3) Coomassie-stained proteins eluted by 0.3 M NaCl. Lane 4) Western blot analysis of proteins eluted by 0.3 M NaCl.

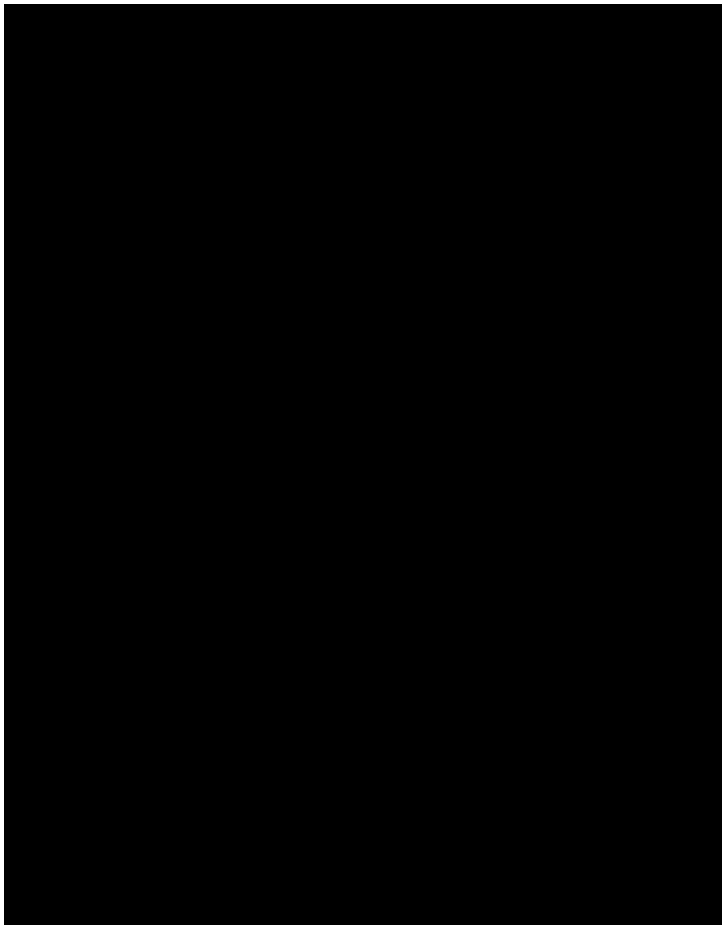

FIG. 3.

Gel filtration of 0.3 M (A) and 0.1 M (B) NaCl proteins on a 2.6 × 95-cm column of Sephadex G-50. Column buffer was 100 mM ammonium acetate, pH 6.8. Flow rate was 30 ml/h; 3-ml fractions were collected. The black bars indicate fractions containing the 5.6-kDa (A) and 16.5- and 18.0-kDa (B) relaxin-IR proteins.

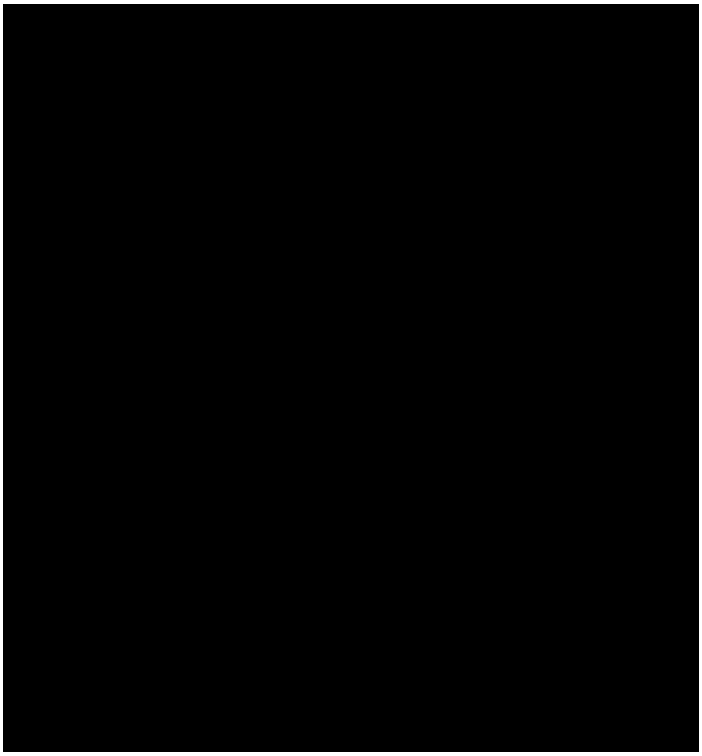

FIG. 4.
Ion-exchange HPLC separation of relaxin-IR gel filtration fractions. Samples were chromatographed on a 10 × 100-mm propyl sulfonic ion-exchange column (buffer A: 10 mM ammonium acetate, pH 5.5; buffer B: 10 mM ammonium acetate, 0.5 M NaCl, pH 5.5). Tubes were changed at 1-min intervals. A) The 5.6-kDa relaxin-IR protein was eluted using a linear gradient of 40–85% buffer B over 70 min. B) The 16.5- and 18.7-kDa relaxin-IR proteins were eluted using a linear gradient of 0–100% buffer B over 90 min. The 16.5- and 18.7-kDa proteins were copurified by this chromatographic step. The black bars indicate fractions containing relaxin-IR proteins.

FIG. 5.
Purification of 5.6-kDa relaxin was accomplished using a 3.9 × 300-mm reverse-phase column (μBondPak C18, Waters Chromatography Division). Solution A contained 0.1% TFA and solution B contained 100% acetonitrile. The column was developed using a linear gradient of 20–40% B over a 60-min period. Purification of the 16.5- and 18.7-kDa proteins was not attempted.

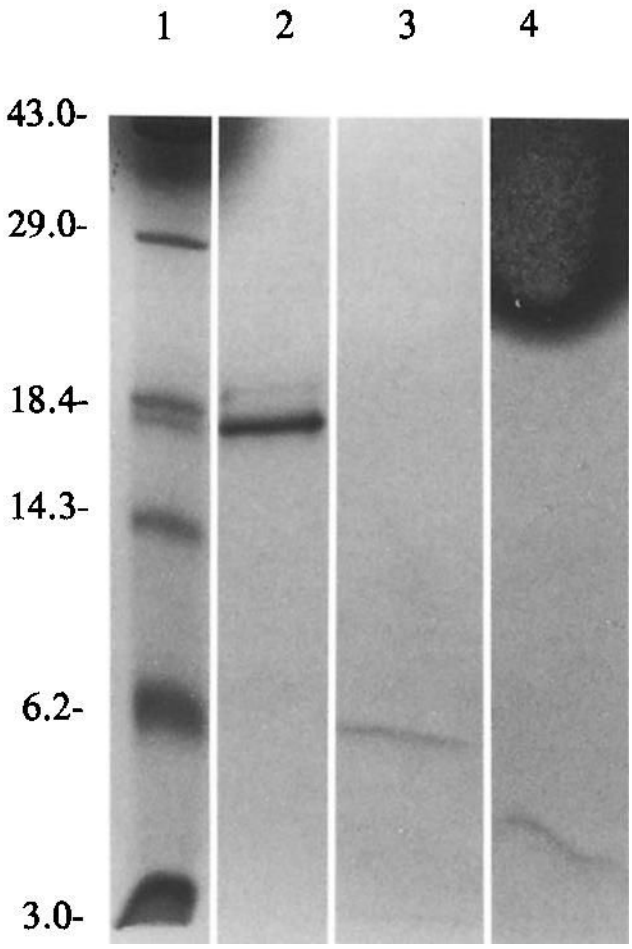

FIG. 6.

Coomassie-stained proteins following SDS-PAGE. Molecular mass standards (lane 1); SP-HPLC-copurified 16.5- and 18.7-kDa (lane 2) and C_{18}-HPLC-purified 5.6-kDa (lane 3) relaxin-IR proteins; 5.6-kDa relaxin-IR protein reduced by addition of β-mercaptoethanol (lane 4). Darkened area at top of gel is an artifact caused by inclusion of β-mercaptoethanol.

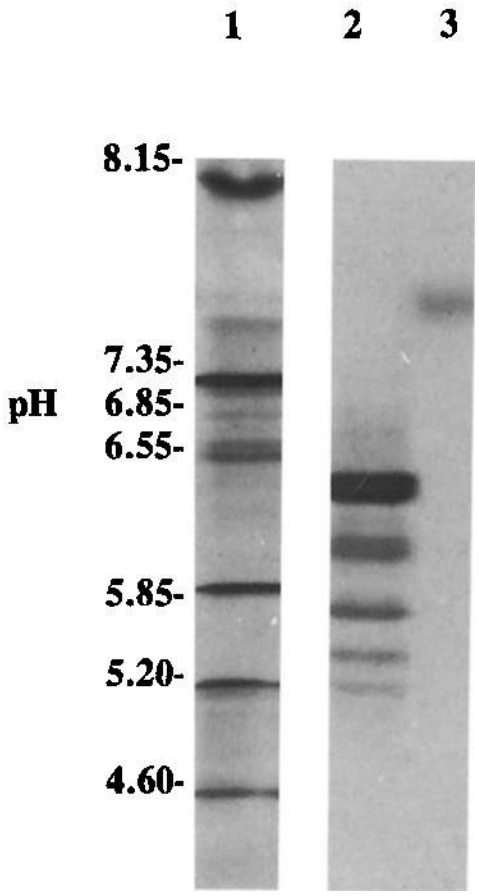

FIG. 7.

The isoelectric point of relaxin-IR proteins was determined by IEF-PAGE and Western blot analysis of Sephadex G-50-purified proteins. Lane 1) Isoelectric point standards. Lane 2) Western blot analysis of the relaxin precursor fraction. Five isoelectric point variants (5.03–6.33) are associated with the 16.5- and 18.7-kDa proteins. Lane 3) Western blot analysis of the relaxin-containing fraction. A single isoelectric point species was identified.

FIG. 8.

Amino acid sequence of hamster 18.7-kDa relaxin-IR protein and relaxin B-chains from several species (reviewed in 1]). [a]Italics indicates amino acids common to rat and hamster. [b]Bold indicates amino acids common to all species listed. [c]Cysteine was identified by the presence of a dehydroalanine adduct peak and the absence of a serine peak.

TABLE 1

Recovery of relaxin and relaxin prohormone from the hamster placenta.

	Fraction	Yield (mg/kg fresh tissue)	Recovery (%) from previous	Total (mg)
Relaxin	(165.53 g tissue)			
	Acetone precipitate	14 800		2457
	Ion exchange	2400	16.2	397
	Sephadex G-50	18	7.0	2.9
	HPLC-ion exchange	4.2	24.0	0.7
	C_{18}-Reverse phase	0.34	8.0	0.03
Precursors (16.5/18.7 kda)				
	Ion exchange	1100	7.2	178
	Sephadex G-50	290	26.6	47
	HPLC-ion Exchange	5	1.7	0.8