Isolation and Characterization of a 94,000-dalton Protein with Thyrotropic Activity from Early Bovine Placenta

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A thyrotropic protein was extracted and purified from the placenta of early bovine gestations. After protein extraction, the 45-60% ammonium sulfate precipitate of maternal and fetal bovine cotyledons was found to compete with thyroid stimulating hormone (TSH) for binding to thyroid cell membranes and to mediate TSH specific biological effects including the stimulation of cyclic AMP production, iodide uptake, and thyroxine secretion. The placental thyrotropin was further purified by gel filtration, ion exchange chromatography, and affinity purification. The effective thyrotropic activity present in placental tissue was found to be a 94,000 dalton protein with a pl of 6.0 and composed of two noncovalently associated chains of 50,000 and 44,000 daltons. The placental 94,000 dalton thyrotropin bound to TSH membrane receptors and induced specific TSH-mediated biological effects, but was structurally and immunologically distinct from TSH and hypophysial or placental gonadotropins.

The placenta may be considered as a remarkable endocrine organ because of the unique quantity and variety of hormones it secretes or metabolizes. In addition to its central role in steroidogenesis as part of the fetal-placental unit, it is also the source of several polypeptide hormones. Gonad-stimulating material of placental origin was first found in human pregnancy urine in 1927 by Ascheim and Zondek (1). Since this early study, the placental peptide hormones chorionic gonadotropin and chorionic somatomammotropin have been extensively characterized in several species (2). Both were found to be closely related to the hormones of the anterior pituitary in their structural and biologic properties. Several authors have suggested that the placenta might also produce growth hormone (3), prolactin-like (4), corticotropin, and thyrotropic (5-7) activities.

In previous reports, we have shown that the thyroid cells of the 40-day-old bovine embryo bear functional membrane receptors for TSH (8), that mediate the response to TSH by an increase in cAMP production (8), iodide uptake and organification (9), and T4 secretion (10). It was further established that TSH is essential for the in vitro aggregation of thyroid epithelial cells and the formation of follicle-like structures in cultured thyroid cells from 75-day-old bovine embryos (8, 11). Moreover, at this early developmental stage, the in situ thyroid colloid formation and folliculogenesis were demonstrated (10). As on one hand no fetal bovine pituitary TSH is detected before 90 days of gestation (10, 12) and on the other hand the placenta is relatively impermeable to maternal TSH (13), we investigated the possibility that the bovine placenta is the source of a thyrotropic activity between 40 and 90 days of gestation.

In the present study, we report the extraction and purification of a protein with thyrotropic activity from bovine placental cotyledons of the first trimester of pregnancy. The isolated chorionic thyrotropin, although chemically and immunologically distinct from bovine TSH, binds to TSH membrane receptors and induces the early and delayed biological effects mediated by TSH.

EXPERIMENTAL PROCEDURES

Materials

Highly purified bovine TSH (30 IU/mg) was a generous gift from Dr. J. G. Pierce (Los Angeles, CA). 7-nitrobenz-2-oxa-1,3-diazole phallacidin (NBD-phallacidin) was a kind gift from Dr. L. Barak (Cornell University, Ithaca, NY). [γ-32P]ATP (16 Ci/mmol) was purchased from the Radiochemical Centre, Amersham. Protein A-bacterial adsorbent and concanavalin A-Sepharose were from Miles-Yeda. The TSH-radiolmmunoassay kit (KHTO 1012) was purchased from Diagnostica Products Corporation and the rabbit antiserum against TSH was a gift from Drs. A. Neimann-Sorensen and V. Kruse (Copenhagen, Denmark). TSH and placental proteins were iodinated by the lactoperoxidase method described by Sato et al. (14) to a specific activity of 5×10^9 cpm/μg of protein.

Binding Assays to Thyroid Cell Membranes

Bovine thyroid glands were collected 20 min after killing and placed immediately on ice. Within 2 h after killing, thyroid cell plasma membranes were prepared as described by Yamashita and Field (15). Membranes were kept frozen at ~70°C in aliquots until use. Under these conditions, the membranes lost neither binding nor cyclic AMP production activity for at least 3 months. Binding of iodinated TSH and placental proteins to the membranes was performed according to

1. The abbreviations used are: TSH, thyroid stimulating hormone; T4, thyroxine; NBD-phallacidin, 7-nitrobenz-2-oxa-1,3-diazole phallacidin; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.
Placental Thyrotropin

Maternal and fetal cotyledons were removed from the chorion and embryonic tissue of pregnant cows at days 40-120 of gestation and placed immediately on ice. The conception-day date was estimated by the crown-rump length of the embryos (17). Cotyledons and intercotyledonic tissue were subjected to sequential extractions at 4°C with 5 (v/w) acetic acid, 0.3 M KCl, and 30% ethanol in the presence of 2 x 10^{-4} M phenylmethylsulfonyl fluoride (7). The extract was then subjected to gradual ammonium sulfate precipitation (10, 20, 30, 45, 60, and 75%, v/v). Solid ammonium sulfate was added to the extract at 4°C with constant stirring for 3 h, followed by centrifugation at 9000 x g for 30 min. Precipitates were extensively redissolved against H2O and lyophilized. The dry ammonium sulfate precipitate (100 mg) was then dissolved in 2 ml of 10 mM phosphate buffer, pH 8.0, and loaded on a Sephadex G100 column (3 x 110 cm). The bioactive protein peak was further fractionated by ion exchange chromatography on a Whatman DE-52 column (1 x 10 cm) equilibrated with 10 mM phosphate buffer, pH 8.0, and eluted with a 0 to 1 M NaCl gradient. Bioactive material was finally absorbed to bovine thyroid membranes by incubation at 37°C for 1 h. Membranes were washed as in the binding assay described above and bound material was eluted by a 5-min treatment with 0.1 M acetic acid before rechromatography on an Ultrogel ACA-34 column. Proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (17) on 5-15% acrylamide gradient slab gels. Gels were stained with Coomassie brilliant blue, vacuum dried, and autoradiography was performed on Kodak Royal X-Omat films. Two-dimensional electrophoresis was performed according to O’Farrell (18). Iodinated proteins were eluted from SDS-PAGE gel slices and tryptide peptide maps on cellular thin layer chromatography plates were carried out (19).

Functional Assays

(a) CAMP Production—Cyclic AMP production in bovine thyroid membranes was measured as described by Salomon (20).
(b) Iodide Uptake—Radiodine uptake by bovine fetal thyroid slices (5-15 mg) was examined after incubation at 37°C for 3 h with 1-2 μCi of Na125I (9). Specific uptake of radiodine is expressed as the radioactivity/g of tissue (T) divided by the radioactivity/ml of medium (M) at the end of the incubation yielding the (T/M) ratio. It is considered to be specifically incorporated when the T/M ratio exceeds 1.
(c) T, Secretion—Stimulation of T, secretion in fetal bovine thyroid slices was measured by a radioimmunoassay technique (10, 21).
(d) Cellular Trophic Effects—The ability of TSH and placental proteins to induce trophic effects was studied on the Fisher rat thyroid cell line FRTL5 (22). The FRTL5 cells were grown in Coon’s modified Ham’s F12 medium supplemented with 5% calf serum and 10 milliunits/ml of TSH, 10 μg/ml of insulin, 5 μg/ml of transferin, 10 ng/ml of somatostatin, 10^{-5} M hydrocortisone, and 20 ng/ml of glycylhistidyl-l-lysine. The FRTL5 cell line and its growth medium were kindly supplied by Drs. Ambesi-Impimbiato and D. Tramontano (University of Naples, Italy). To label F actin, the cells were fixed with 3% paraformaldehyde, permeabilized with 0.1% Triton X-100, and incubated with 2 μg/ml of NBD-phallacidin (11). Cells were observed with a Zeiss inverted fluorescence microscope IM 35 and photographs were taken on Kodak plus X- or Tri X films.

RESULTS

Cotyledons, the patches of placental villi, were removed from the fetal and maternal layers of the placenta of early bovine pregnancies and extracted by the procedure originally described by Reisfeld et al. (23) and also adapted for the purification of human chorionic thyrotropin (7). Maternal and fetal parts of the cotyledons were pooled separately. In addition, the intercotyledonic tissue served as a control. The bovine placental tissue (250-500 g, wet weight) was sequentially extracted with acetone, KCl, and ethanol. The extract, about 10% of the original tissue, wet weight, was then subjected to gradual ammonium sulfate precipitation. After dialysis and lyophilization, each precipitate was tested for its ability to interact with TSH receptors on adult bovine thyroid cell membranes and to stimulate a wide range of TSH-mediated biological effects.

Binding of Placental Ammonium Sulfate Precipitates to Thyroid Cell Membranes

The inhibition of binding of 125I-TSH (7.5 milliunits/ml, 230 ng/ml) to thyroid cell membrane preparation (100 μg of protein/ml) by different ammonium sulfate precipitates from: the placental extract is shown in Fig. 1. The 45-60% precipitates of both maternal and fetal cotyledons from 90-day-old concepts inhibited 125I-TSH binding in a concentration-dependent fashion. Fifty per cent displacement was achieved with 50 μg of protein/ml of both maternal and fetal placental extracts. A slight inhibitory effect was also observed with the maternal and fetal 30-45% and 60-75% ammonium sulfate cuts. Other maternal or fetal fractions and the fractions of the intercotyledonic tissue did not inhibit significantly the binding of 125I-TSH. When 125I-TSH was mixed with the crude placental extract, 78% of the labeled hormone was recovered with the 30-45% ammonium sulfate precipitate. The amount of TSH-like binding activity in the 45-60% ammonium sulfate salt was evaluated in placenta from different gestational ages (Fig. 2). The TSH-like binding activity increased progressively from 17 ± 3 to 157 ± 25 equivalent milliunits of TSH/ng of protein in placenta from 40 to 90 days of gestation. There was, however, no further increase in TSH-like binding activity after this stage. Extracts of the intercotyledonic tissue from the same placenta did not compete for TSH binding to its membrane receptors at any gestational stage studied.

Biological Effects of Placental Ammonium Sulfate Precipitates

(a) Cyclic AMP Production—Maximal cyclic AMP production (4-fold above the basal level) in adult bovine thyroid membranes was achieved with 1.7 μg/ml of TSH. 100 μg of

Fig. 1. Inhibition of 125I-TSH binding to thyroid membranes by either TSH or different ammonium sulfate precipitates of placental tissues. Thyroid membranes (100 μg of protein/ml) were incubated at 37°C for 1 h with 220 ng/ml (7.5 milliunits/ml) of 125I-TSH and increasing concentrations of TSH (△) or fractions of the ammonium sulfate precipitates from maternal or fetal cotyledons: maternal and fetal 20-30% ammonium sulfate precipitate (▲); maternal 45-60% ammonium sulfate precipitate (●); fetal 45-60% ammonium sulfate precipitate (□); and the 45-60% ammonium sulfate precipitate of the intercotyledonic tissue (○). Each data point is the mean of four independent assays performed in triplicates; the standard deviation is less than 5% of the mean values.
protein/ml of the maternal and fetal ammonium sulfate precipitate induced a 3-fold increase in cyclic AMP production (Table 1). None of the other maternal and fetal fractions, nor the fractions of intercotyledonic tissue, induced a significant cyclic AMP production above the basal level concentrations up to 200 µg of protein/ml.

(b) Iodide Uptake—In thyroid slices from 70-day-old bovine embryos, maximal stimulation of 125I trapping, reflected by the T/M ratio (cpm/g of tissue divided by cpm/ml of medium), was reached at a concentration of about 17 ng/ml of TSH. Out of all the placental fractions, only the maternal and fetal precipitate from the 45-60% ammonium sulfate cut enhanced significantly the iodide uptake (Table 1). The stimulation was concentration-dependent; 10 µg of protein/ml induced a stimulation about 2-fold above the basal level and almost equivalent to the effect of TSH.

(c) T₄ Secretion—Maximal secretion of T₄ (7.1 ± 0.8 ng/mg of tissue) by fetal thyroid slices was induced after 6 h exposure to 17 ng/ml of TSH. T₄ secretion was enhanced only by the maternal and fetal 45-60% ammonium sulfate precipitates of the placental extracts at concentrations higher than 10 µg of protein/ml (Table 1). The stimulatory effect of the placental fraction, although significant, was much lower than that observed with TSH; there was a 100-fold discrepancy in stimulatory potency if the effective placental protein concentrations were converted to equivalents of TSH-like binding activity or cyclic AMP production.

### Purification of the Placental Thyrotropic Protein

Maternal and fetal fractions of the 45-60% ammonium sulfate cut were pooled (100 mg of protein) and subjected to gel chromatography on a Sephadex G-100 column in the presence of tracer amounts of 125I-TSH. Each fraction was examined for protein content and for its ability to inhibit the binding of 125I-TSH to thyroid cell membranes. Three poorly resolved protein peaks were eluted close to the void volume of the column, followed by several smaller peaks (Fig. 3). Almost all the TSH-like binding activity eluted with the three major protein peaks, clearly separated from the elution pattern of 125I-TSH (Fig. 3). The fractions with the highest TSH-like binding activity (almost equivalent to 70 µg of TSH/mg of protein) were pooled and analyzed by SDS-PAGE. The major protein components revealed by Coomassie blue staining were of molecular weight 150,000 in nonreducing conditions, 50,000 and 25,000 in reducing conditions, co-migrating with, respectively, intact bovine IgG and its heavy and light chains. This major component was identified as bovine IgG, it could be adsorbed on fixed Staphylococcus aureus bacteria bearing Protein A, specific for the Fc part of IgG molecules (24) and it was immunoprecipitated by goat anti-bovine IgG antibodies. These specific procedures were, however, not sufficient for a quantitative removal of all the bovine IgG present in the fractions that contained the TSH-like binding activity. These fractions (10 mg of protein) were therefore loaded on a Whatmann DE-52 anion exchange cellulose column in a continuous NaCl gradient. The TSH-like binding activity (4

![Fig. 2. TSH-like binding activity of the 45-60% ammonium sulfate precipitate from bovine placenta at different stages of pregnancy. The fractions were prepared from placental cotyledons of 40 to 110 days of gestation, as estimated by the crown-rump length. The TSH-like binding activity was determined by the ability to inhibit the binding of 7.5 milliliters/ml (250 ng/ml) of 125I-TSH to thyroid membranes (100 µg of protein/ml) as in Fig. 1. Each data point is the mean ± S.D. of extracts pooled from 6 to 12 placenta.

![Fig. 3. Gel filtration of the 45-60% ammonium sulfate precipitate of bovine cotyledons. 100 mg of protein of the pooled maternal and fetal 45-60% ammonium sulfate cuts were loaded on a Sephadex G-100 column (3 x 110 cm). A, elution profile; B, TSH-like binding activity of the fractions determined as in Fig. 1. C, elution profile of 125I-TSH tracer.](https://example.com/figure3.png)
mg of protein) eluted exclusively with the second peak and at a different ionic strength than the $^{125}$I-TSH tracer (Fig. 4). The fractions with the highest TSH-like binding activity (equivalent to about 120 $\mu$g of TSH/mg of protein) were iodinated and analyzed by SDS-PAGE. The iodinated placental protein fraction bound specifically to thyroid cell membranes. Saturation was reached at a total concentration of 5 $\mu$g/ml of iodinated placental protein and nonspecific binding, assessed in the presence of 200 $\mu$g/ml of unlabeled protein, did not exceed 20% of the membrane-associated radioactivity. Unlabeled TSH competed for binding of the iodinated placental protein; 2 $\mu$g of TSH/ml displaced about 80% of the membrane-bound label. The SDS-PAGE analysis of the purified placental fraction, both under reduced and nonreduced conditions, revealed the presence of residual bovine IgG, two major protein bands of 50,000 and 44,000 daltons, and several other proteins. A further purification step was thus attempted by binding the placental material to thyroid membranes and eluted by mild acid treatment. Placental protein (5 $\mu$g/ml) was incubated for 1 h at 37 °C with thyroid membranes (80 $\mu$g of protein/ml) in 400 ml of Tris-HCl, pH 7.4. Membranes were washed by ultracentrifugation four times with Tris-HCl buffer and incubated at 4 °C for 5 min with 40 ml of 0.1 M acetic acid. Membranes were pelleted by centrifugation and the supernatant was lyophilized, dissolved in H$_2$O, and relyophilized. The eluted placental material represented between 10 and 20% of the original DE-52 purified proteins and was identified by SDS-PAGE as two bands of 50,000 and 44,000 daltons both under reducing and nonreducing conditions (Fig. 5). The eluted material was chromatographed on an Ultrogel AcA 34 column and recovered as a single peak of estimated molecular weight of 94,000 daltons (Fig. 5). Table II summarizes the yield and enrichment in TSH-like binding activity of the successive isolation steps. About 400 $\mu$g of purified 94,000-dalton protein was recovered, representing 2 × 10$^{-4}$% of the original wet weight of an average placenta from a 90-day-old gestation. Only trace amounts of low molecular weight peptides were eluted from thyroid membranes incubated with inactive placental extracts or acetic acid alone; none of these fractions was found to complete for $^{125}$I-TSH binding to thyroid membranes.

Characterization of the 94,000-dalton Placental Protein

The isolated placental TSH-like protein was readily soluble in aqueous buffers over a range of pH 5 to 9 and could be stored at 4 °C for a week or at −70 °C for over three months without significant loss in binding activity. In contrast to TSH, the placental protein did not bind to a Concanavalin A-Sepharose column. Upon analysis by two-dimensional electrophoresis, the 94,000-dalton protein migrated as three spots with a pI of 6.0 as compared to the pI 6.8 of pituitary TSH (not shown). The purified placental 94,000-dalton protein exhibited a specific affinity to thyroid cell membranes equivalent to 150 $\mu$g (4.5 units) of TSH binding activity/mg of protein. On the basis of the gel chromatography molecular weight estimate and direct binding studies, it was found that thyroid cell membranes bound at saturation 9.3 pmol of placental protein/mg of membrane protein. The purified 94,000-dalton protein was found to be almost as potent as TSH on a molar basis for the stimulation of cyclic AMP.

### Table II

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Recovery</th>
<th>% wet weight of cotyledonic tissue</th>
<th>TSH-like binding activity$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>30% ethanol extract</td>
<td>50 g</td>
<td>5 × 10$^{-4}$</td>
<td>Not measured</td>
</tr>
<tr>
<td>45-60% ammonium sulfate precipitate</td>
<td>100 g</td>
<td>10</td>
<td>5.2 ± 0.1</td>
</tr>
<tr>
<td>Sephadex G-100 peak</td>
<td>10 mg</td>
<td>5 × 10$^{-3}$</td>
<td>38 ± 3</td>
</tr>
<tr>
<td>DE-52 peak</td>
<td>4 mg</td>
<td>2 × 10$^{-3}$</td>
<td>74 ± 5</td>
</tr>
<tr>
<td>Eluate from thyroid membrane-bound material</td>
<td>0.4 mg</td>
<td>2 × 10$^{-4}$</td>
<td>150 ± 7</td>
</tr>
</tbody>
</table>

$^a$ Equivalent of $\mu$g TSH/mg of protein.
production on bovine thyroid membranes and of iodide uptake by fetal thyroid slices. It also significantly enhanced the \( T_4 \) secretion from fetal bovine thyroid slices, although to lower levels than TSH and with the same discrepancy in potency versus binding activity as was noted with the crude placental extracts i.e. 5 \( \mu \)g of placental protein/ml stimulated the secretion of 4.2 \( \pm \) 0.5 ng of \( T_4/ \)mg of tissue as compared to 7.1 \( \pm \) 0.6 ng of \( T_4/ \)mg of tissue with 20 ng of TSH/ml.

The characteristic trophic effects induced by TSH on thyroid cells (11) were also examined with the isolated 94,000-dalton protein. This particular assay could not be performed along the purification steps as the crude placental material appeared to be toxic to the cultured cells. As depicted in Fig. 6A, the Fisher rat thyroid cells FRTL6, deprived of TSH for one week, appeared epithelial-like and well spread on the substrate. Well organized bundles of actin, oriented in different directions, were decorated after labeling of the fixed and permeabilized cells with NBD-phallacidin (Fig. 6B). After a 24-h exposure to either 300 ng of TSH/ml (Fig. 6, C and D) or 2 \( \mu \)g/ml of 94,000-dalton placental protein (Fig. 6, E and F), the cells rounded up and most of the actin appeared diffuse or in dots, most abundant at the cell periphery, at areas of cell to cell contact and around the nucleus. TSH and the placental protein did not induce the appearance of follicle-like chambers in the FRTL6 cell line, in contrast to what could be observed in primary cultures of thyroid cells. This phenomenon appeared to be dependent on the presence of fibroblastic cells in the primary cultures, and will be described elsewhere.

Although the placental thyrotropic protein was clearly chemically distinct from TSH or other hormonal glycoproteins, we found it important to investigate their possible structural or antigenic relatedness. Tryptic peptide maps of the iodinated 94,000-dalton protein eluted from SDS-PAGE gel slices did not show any peptide homology either with TSH (Fig. 7) or with luteinizing hormone, follicle stimulating hormone, or human chorionic gonadotropin (data not shown). Neither antibodies to bovine TSH nor the receptors to human chorionic gonadotropin cross-reacted with the 94,000-dalton placental protein. Finally, the placental protein did not show any gonadotropic activity when examined in a sensitive assay for its stimulatory effect on progesteron secretion from ovary.

**DISCUSSION**

The study of the synthesis and function of placental hormones along the progression of pregnancy is of essential importance to the understanding of the ontogeny of endocrine glands and of the organs effected by these hormones. The differentiation of the pituitary-thyroid axis has been studied in rat, sheep, and man (25, 26) and to a lesser extent in guinea pig (27) and cow (12). The embryonic thyroid gland is necessary for the adequate development and maturation of the respiratory and nervous systems and is critical for the survival of the newborn (28). In previous studies on the bovine embryonal thyroid, we have shown that the fetal thyroid cells

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A. Avivi, manuscript in preparation.

A. Avivi, S. Lavi, and M. Shemesh, unpublished data.
bear membrane receptors for TSH and respond to TSH as reflected by a wide range of biological functions (8-11) before the development of a functional pituitary (10, 12). This finding may simply reflect one aspect of asynchronous differentiation in which the maturation of hormone receptors precedes the presence of their corresponding ligand. Our further evidence that TSH induces the in vitro folliculogenesis of the bovine embryonal thyroid (8) at a developmental stage corresponding to the in situ beginning of colloid formation (10) and before fetal hypophysial TSH secretion (10, 12) constituted the impetus to our search for a placental thyrotropin from early bovine placenta.

Maternal and fetal cotyledons from early bovine gestations were subjected to protein extraction and gradual ammonium sulfate precipitation. The intercotyledonic tissue that functions as connective tissue between mother and fetus was treated similarly to serve as control. To screen for the presence of a truly thyrotropic material we decided to assay specific TSH-mediated biological effects on addition to the inhibition of binding of labeled TSH to thyroid cell membranes. The selected biological assays included the stimulation of cyclic AMP production in adult bovine thyroid cell membranes and the stimulation of iodide uptake and T₄ secretion in fetal thyroid slices in order to ascertain that the TSH-like material would have a relevant interaction with embryonal thyroid tissue. The maternal and fetal 45-60% ammonium sulfate cuts which reacted positively in all these assays were further fractionated by conventional gel and anion exchange chromatography. The resulting thyrotropin preparation represented 2 × 10⁻⁸% of the original wet weight of the placenta and was about 20-fold enriched in TSH-like binding activity compared to the 45-60% ammonium sulfate precipitate. A final isolation step consisted of binding to thyroid cell membranes and elution by mild acid treatment. This procedure was necessary to obtain the purified thyrotropic protein but resulted in either a poor yield or inactivation as only 400 µg of protein with 150 µg of TSH binding activity/mg of protein was recovered from 4 mg of protein with about 75 µg of TSH binding activity/mg of protein.

The isolation of a TSH-like activity from human placenta was reported by Hennen (6) and Hershman and Starnes (7), from which reacted positively in all these assays were further submitted to protein extraction and gradual ammonium sulfate precipitation. The intercotyledonic tissue that functioned as a placental thyrotropic material we decided to assay specific binding of labeled TSH to thyroid cell membranes. The resulting thyrotropic preparation represented 2 × 10⁻⁸% of the original wet weight of the placenta and was about 20-fold enriched in TSH-like binding activity compared to the 45-60% ammonium sulfate precipitate. A final isolation step consisted of binding to thyroid cell membranes and elution by mild acid treatment. This procedure was necessary to obtain the purified thyrotropic protein but resulted in either a poor yield or inactivation as only 400 µg of protein with 150 µg of TSH binding activity/mg of protein was recovered from 4 mg of protein with about 75 µg of TSH binding activity/mg of protein.

The isolation of a TSH-like activity from human placenta was reported by Hennen (6) and Hershman and Starnes (7), but later studies demonstrated that the presumed choricorionic thyrotropin was identical to hypophysial TSH (29) or that the observed effects resulted from an intrinsic thyrotropic activity of human chorionic gonadotropin (30). The bovine placental 94,000-dalton thyrotropin we isolated is an unique protein apparently composed of two noncovalently associated chains of 50,000 and 44,000 daltons and devoid of a carbohydrate moiety reactive with Concanavalin A. It is clearly distinct both structurally and antigenically from hypophysial TSH as revealed by tryptic peptide map analysis and lack of cross-reactivity in a sensitive, specific radioimmunoassay. The placental thyrotropin did not show any affinity to choricorionic gonadotropin receptors.

The placental 94,000-dalton protein and TSH were mutually competitive for binding to thyroid cell membranes. At saturation, the binding capacity of the placental thyrotropin was very close to that of hypophysial TSH. These findings taken together probably indicate that the two proteins bind to the same membrane receptor binding site although allosteric mechanisms or interactions of closely associated receptor entities cannot be excluded. The placental thyrotropin was as potent as TSH for the induction of specific membrane-related effects (cyclic AMP production and iodide uptake) and of trophic effects on cultured thyroid cells. It also significantly stimulated T₄ secretion from fetal thyroid slices although to lower levels and at 100-fold higher concentrations as compared to hypophysial TSH. Apparently, the placental thyrotropin is less effective than TSH in the transmembrane signalling of delayed biological effects that require an intracellular cascade of metabolic processes.

The amount of placental thyrotropic activity increased between 40 and 90 days of gestation. A plateau value was then reached at the end of the first trimester of pregnancy, to be correlated with the time at which thyrotrophic cells first become evident in the bovine fetal pituitary along with TSH secretion. The specific association of the 94,000-dalton thyrotropic protein with the cotyledones of the early bovine placenta before the development of a functional fetal pituitary leads us to suggest that it plays an important role in the differentiation of the embryonal thyroid gland.

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REFERENCES


Metab. 47, 681-685