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W G McGregor, … , R W Kuhn, R B Jaffe

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Research Article

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Fetal Tissue Can Synthesize a Placental Hormone

EVIDENCE FOR CHORIONIC GONADOTROPIN β-SUBUNIT SYNTHESIS BY HUMAN FETAL KIDNEY

W. GLENN MCGREGOR, WILLIAM J. RAYMOURE, ROBERT W. KUHN, and ROBERT B. JAFFE, *Reproductive Endocrinology Center, University of California, San Francisco, California 94143*

ABSTRACT Metabolically active tissues from second trimester human fetuses were examined for their ability to synthesize the placental hormones chorionic gonadotropin and chorionic somatomammotropin. During short-term incubation studies both placenta and fetal kidney were found to synthesize and secrete the β-subunit of chorionic gonadotropin, whereas its synthesis was not observed in fetal liver, lung, or muscle. In addition, chorionic somatomammotropin synthesis and secretion was demonstrated with placental tissue but could not be detected in any of the fetal tissues examined. These observations constitute the first evidence that the genome of a fetal tissue directs the synthesis of what is considered a placental hormone.

INTRODUCTION

Human chorionic gonadotropin (hCG),[1] as well as other proteins primarily of placental origin, has been found in various neoplastic tissues and in the circulation of certain patients with nongestational cancer (1). Although the mechanism of neoplastic elaboration of these proteins is unknown, some workers have postulated that this represents a reversion to fetal gene expression (2). In studying the physiologic role of hCG in the human fetus, we found that the human fetal testes (3) and adrenal (4) responded to hCG administration with increased steroidogenesis in vitro. To determine the relative hCG content in the fetus, the hCG concentration in homogenates of various fetal tissues was measured using an hCG β-subunit (β-hCG) radioimmunoassay (5). Contents of immunoreactive β-hCG greater than could be accounted for by contamination from circulating hormone were found in homogenates of gonad, thymus, and kidney derived from second trimester human fetuses. Because this could result from an accumulation of circulating hCG, its *de novo* synthesis, or both, we sought to determine whether fetal tissues were synthesizing this hormone. We now report that a metabolically active fetal tissue, the kidney, synthesizes a peptide that is indistinguishable from β-hCG. Although the physiologic significance of this observation is unknown, this is the first demonstration that the fetal genome actively expresses what has been considered a placental hormone.

METHODS

Human fetuses were obtained immediately after dilatation-evacuation abortion performed under general anesthesia. Gestation ranged from 16–20 wk menstrual age, as judged by history and physical examination. Intact organs were identified by gross inspection, then placed into Krebs-Ringer bicarbonate buffer containing 1% glucose (KRB-G) at 4°C. The tissues were minced into 2-mm slices and placed in 10 ml of KRB-G at 37°C. After a 30-min preincubation, the medium was replaced by 10 ml of fresh KRB-G buffer and the incubation started by the addition of 30 μCi of [^{35}S]methionine (1,000 Ci/mmol; New England Nuclear, Boston, Mass.). Incubations were performed for 12 h at 37°C with constant agitation in a Dubnoff metabolic incubator (Precision Scientific, Chicago, Ill.) under a 95% O_2/5% CO_2 atmosphere. Preliminary experiments demonstrated linear incorporation of labeled amino acid into total protein for at least 12 h with rates of incorporation being identical whether the media was KRB-G, Eagle's minimal essential medium lacking unlabeled methionine, or Eagle's minimal essential medium with 10% fetal calf serum.

Incubations were stopped by separating the tissue minces from their incubation media. The tissues were placed in 10 ml of NET buffer (0.1 M Tris, 0.1 M NaCl, 5 mM EDTA, 1% Triton X-100) and homogenized in a tight fitting glass homogenizer. Each sample was centrifuged at 100,000 g for 60 min and, to decrease nonspecific binding during subsequent immunoprecipitation, 30-μl *Staphylococcus aureus* capsule (SAC; Sigma Chemical Co., St. Louis, Mo.; final concentration

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[1] *Abbreviations used in this paper*: β-hCG, hCG β-subunit; hCG, human chorionic gonadotropin; KRB-G, Krebs-Ringer bicarbonate buffer containing 1% glucose; NET, buffer solution containing 0.1 M Tris, 0.1 M NaCl, 5 mM EDTA, 1% Triton X-100.

10% in NET buffer) was added to the supernate. After incubation for 30 min at 4°C, the samples were centrifuged at 60,000 *g* for 1 h. The supernate was removed and concentrated 20-fold before analysis for the synthesis of hCG. Concentration was achieved by using powered sugar and dialysis tubing with a molecular weight exclusion limit of 3,500.

Rabbit antiserum to β-hCG was diluted 1:50 in NET buffer. The immunoprecipitation of β-hCG was carried out by adding 100 μl of the antiserum solution to 100-μl samples of homogenate or media. Normal rabbit serum, diluted as above, was added to control samples in lieu of antisera. After incubation overnight at 4°C, 15 μl of *S. aureus* capsule was added. After incubation for 20 min at 4°C and subsequent centrifugation for 1 min at 5,000 *g*, the pellets were washed three times with NET buffer containing 1% Triton X-100. The *S. aureus* capsule-antigen-antibody complexes were disrupted by incubation for 2 h with 0.1 M KOH for incorporation studies or with 2% sodium dodecyl sulfate (SDS) for gel studies. After centrifugation the supernates were analyzed on SDS-polyacrylamide gels or were counted in a liquid scintillation counter.

SDS disc gel electrophoresis was performed according to a modification of the method of Weber and Osborn (6) as previously described (7). After electrophoresis, the gels were cut into 2-mm slices and each slice was placed into a scintillation vial containing 3.5 ml of 3% Protosol in Econofluor (New England Nuclear). After incubation for 16 h at 60°C, the radioactivity was determined at 60% efficiency in a PRIAS liquid scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.). All samples were corrected for radioactive decay. Protein concentrations were determined by use of the micro-Bradford assay (8).

RESULTS

Significant incorporation of [^{35}S]methionine into immunoprecipitable β-hCG was observed in both tissue homogenates and incubation media from placenta and fetal kidney samples (Fig. 1). A very low but consistent incorporation of [^{35}S]methionine also occurred in liver, whereas none could be detected in either lung or muscle tissues. During incubations of both placenta and kidney, the incorporation of radioactive methionine into immunoprecipitable β-hCG per milligram of protein was greater for the media samples than for the corresponding tissue homogenates. This suggests that these tissues not only synthesize but also actively secrete the β subunit of hCG. This conclusion is more strongly supported when the data are normalized to the amount of newly synthesized protein rather than total protein present (Table I). A marked enrichment for β-hCG is observed in incubation media as compared with homogenates of placenta and kidney but not liver.

To determine whether the observed synthesis of β-hCG by the fetal kidney was specific or attributable to a general elaboration of hormones as seen in the placenta, we examined various tissues for their ability to synthesize and secrete human chorionic somatomammotropin (hCS). Specific incorporation of [^{35}S]-methionine was determined with an antiserum to human chorionic somatomammotropin kindly provided by Dr. Henry Friesen, University of Manitoba, Winnipeg, Manitoba, Canada. Table I shows that although

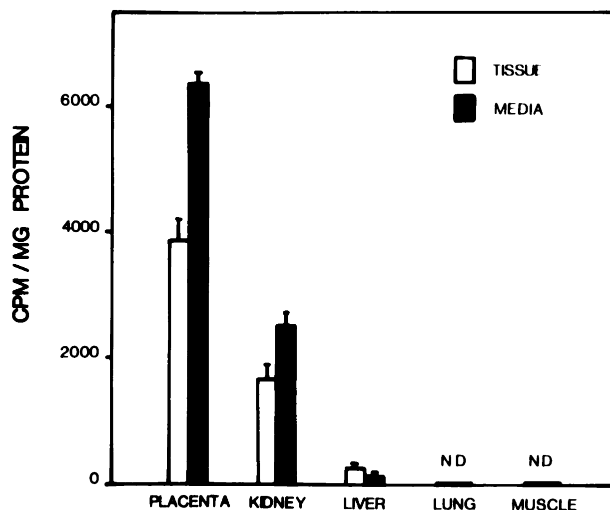

FIGURE 1 Incorporation of [^{35}S]methionine into immunoprecipitable β-hCG. Placenta and fetal tissue samples were incubated for 12 h at 37°C in KRB-G containing 30 μCi of [^{35}S]-methionine. Tissues were homogenized, and both homogenates and media were analyzed for immunoreactive β-hCG as described in Methods. Protein concentrations were determined according to the method of Bradford (8) and all data were expressed as cpm per mg of total protein.

placenta synthesized and secreted human chorionic somatomammotropin, none of the fetal tissues examined did. Thus fetal kidney production of hCG is attributable neither to the generalized elaboration of all placental hormones nor to contamination with trophoblastic tissue.

Because the antibody used in these studies (designated SMR-1) showed ~25% cross-reactivity with human thyroid-stimulating hormone, 10% cross-reactivity with human luteinizing hormone, but virtually no affinity for human follicle-stimulating hormone, further characterization of the nature of the immunoprecipitable material was required. To ensure that the antibody was recognizing β-hCG, immunoprecipitation using an antiserum prepared against the carboxyl terminal fragment from β-hCG (residues 123–145) was performed. This antiserum (H-93), kindly provided by Dr. H. Chen (National Institutes of Health), is highly specific for β-hCG with no affinity for thyroid-stimulating hormone, luteinizing hormone, or follicle-stimulating hormone.[2] Both H-93 and SMR-1 recognized virtually identical amounts of labeled protein from placenta (tissue: 8,260±740 vs. 7,930±860; media: 19,230±2,230 vs. 18,640±1,960) and kidney (tissue: 3,380±420 vs. 3,230±260; media: 38,420±3,180 vs. 36,840±4,250). These data represent counts immunoprecipitated by H-93 or SMR-1 respectively per 10^5 trichloroacetic acid–precipitable counts±SEM. In addition, immunoprecipitable counts were completely

[2] Dr. H. Chen. Personal communication.

TABLE I
Synthesis of Placental Hormones by Placenta and Fetal Tissues

	β-hCG		hCS	
	Specific cpm*	Percent‡	Specific cpm	Percent‡
Placenta				
Tissue	7,930±860	7.9	8,820±960	8.8
Medium	18,640±1,960	18.6	26,400±3,860	26.4
Kidney				
Tissue	3,230±180	3.2	ND	—
Medium	36,840±4,250	36.8	ND	—
Liver				
Tissue	150±120	<1	ND	—
Medium	120±160	<1	ND	—
Lung				
Tissue	ND§	—	ND	—
Medium	ND	—	ND	—
Muscle				
Tissue	ND	—	ND	—
Medium	ND	—	ND	—

* *Specific cpm* is the number of immunoprecipitable counts per 10^5 trichloroacetic acid–precipitable counts.
‡ Represents the percent of total protein synthesis that is immunoprecipitable.
§ ND, not detectable.

displaced by the addition of purified β-hCG; 5 ng caused 50% displacement with 20 ng resulted in virtually complete displacement of newly synthesized protein from both the SMR-1 and H-93 antisera.

SDS gel electrophoresis provided further evidence that the protein precipitated with our antibody was β-hCG. Fig. 2 (dashed line) shows the electrophoretic patterns obtained with ^{125}I-labeled β-hCG standard, and placental and kidney homogenates. After immunoprecipitation had been performed, the supernate was similarly analyzed (dotted line, Fig. 2). With the standard β-hCG, the peak of radioactivity centered at slice 16 was completely removed by this treatment. The samples from both kidney and placenta showed a marked reduction in counts in this region of the gel. The material present in the immunoprecipitate was solubilized and run on SDS gels. In each case, a single band of radioactivity centered at slice 16 and migrating identically to highly purified β-hCG (kindly supplied by Dr. Harold Papkoff) was observed. These results strongly suggest that only β subunit of hCG was being detected by our immunoprecipitation procedure.

DISCUSSION

These data demonstrate that the fetal kidney of 16–20 wk gestation can synthesize a protein that is immunologically identical to and, on the basis of SDS-gel electrophoresis, the same size as the β subunit of hCG. It should be noted that detergent was utilized during assay procedures to decrease nonspecific precipitation of counts. Because this treatment results in the dissociation of α and β subunits, it is not possible to conclude from the data whether the fetal kidney synthesizes α as well as β-subunits resulting in the production of biologically active molecules. However, we have shown

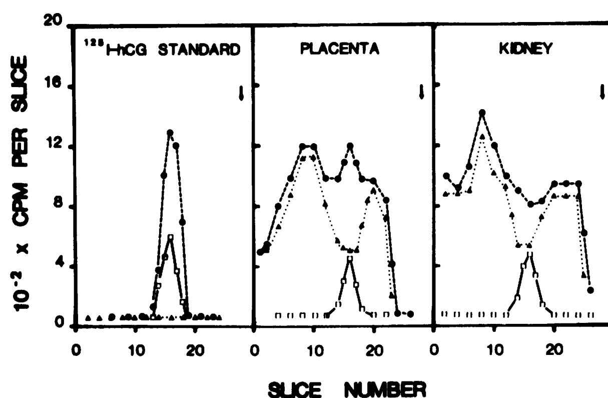

FIGURE 2 SDS gel electrophoretic analysis of the immunoprecipitation reactions. Electrophoretic analysis was performed as described in Methods. In each panel the dashed line (closed circles) represents samples of the homogenate or standard before incubation with antibody. The dotted lines (closed triangles) represent samples of the supernates after removal by centrifugation of the antigen-antibody-SAC complexes. The solid lines (open squares) represent the material eluted from the antigen-antibody-SAC complexes. In each case electrophoresis was from left to right (cathode to anode). The arrow represents the position of the reference tracking dye, bromphenol blue.

that the fetal kidney, in a manner analogous to placental hormone production, not only synthesizes but actively secretes into the media newly synthesized β-hCG. If intact hCG is synthesized and secreted or if the β subunit of fetal origin associates with an α subunit (either of fetal or maternal origin), it could then have a metabolic role in the fetus. Indeed it has been shown that the fetal zone of the fetal adrenal gland responds to hCG administration with an increase in dehydroepiandrosterone sulfate production (4). In addition, Benirschke (9) and Gray and Abramovich (10) have demonstrated that the fetal adrenal gland is normal until midgestation in anencephalic and apituitary fetuses. These observations led to the speculation that hCG and ACTH have a dual role in fetal adrenal gland regulation until midgestation, when ACTH may assume the primary regulatory function. Whether hCG of at least partial fetal origin plays an important role in this regulation remains a matter of speculation and requires further investigation.

The role of hCG in the regulation of human fetal testes is of extreme interest in light of the hCG-stimulated testosterone production and the high-affinity binding of the hormone found in this tissue (3). Unfortunately the minute size of the fetal gonad at this stage of gestation has precluded tissue explant study to date.[3] The origin and role of the high concentration of hCG in ovary and thymus also remain obscure and await further investigation.

The finding that the fetal genome can elaborate a trophoblastic hormone has further interest when one considers that hCG has purportedly been found in the serum of normal adults (11) and in normal adult liver, gastrointestinal tract, kidney, and testes (12, 13). In addition, it has been suggested that sera from patients with proliferating lesions (both neoplastic and nonneoplastic) of the gastrointestinal tract and with nontrophoblastic neoplasms of kidney and gonad contain immunoreactive hCG (14). Whether these phenomena represent atavistic reversion to fetal gene expression or the proliferation of a clone of nonendocrine epithelial cells with hormone production capability (as suggested by Baylin and Mendelsohn [15]) is a central question of tumor biology.

[3] While this manuscript was in preparation, we were successful in growing human fetal testis cells in primary cultures. Preliminary experiments suggest these cells synthesize β-hCG. In addition, using immunocytochemical techniques we have shown that placenta and fetal kidney and testis cells positively stain for β-hCG whereas lung, liver, and muscle do not (manuscript in preparation).

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