RADIOIMMUNOASSAY OF HUMAN PLACENTAL LACTOGEN SYNTHESIZED ON RIBOSOMES ISOLATED FROM FIRST TRIMESTER AND THIRD TRIMESTER PLACENTAE

Irving BOIME and Sophie BOGUSLAWSKI
Departments of Obstetrics and Gynecology and Pharmacology, Washington University Medical School, St. Louis, Missouri 63110, USA

Received 28 May 1974

1. Introduction

The human placenta synthesizes a lactogenic hormone, (HPL), as one of its major proteins. During pregnancy, HPL appears at maximal levels at about 30 weeks [1–3]. It has also been shown that in term placental tissue slices, HPL constitutes one of the primary proteins synthesized [4–6]. Although term serum HPL levels are much higher than at first trimester, it is not clear as to the rate of HPL synthesized during pregnancy [7].

We have previously shown that labeled HPL could be synthesized in a reconstituted cell-free system containing purified placental ribosomes and a cell sap fraction derived from ascites tumor cells [8]. However, it was difficult to quantitate the cell-free synthesis of the labeled hormone as a function of pregnancy due to high backgrounds of radioactivity. Here, we report on the levels of HPL synthesized by ribosomes isolated from human placentae at different periods of gestation using a convenient and reliable commercial radioimmunoassay system. The data are consistent with the hypothesis that the amount of this human hormone synthesized reflects the in vivo differentiation of the placenta during pregnancy.

2. Materials and methods

[^{35}S]Methionine was obtained from Amersham Searle. Human placental lactogen (95% pure) was purchased from Nutritional Biochemicals. The radioimmunoassay kit for HPL, the components of which will be described below, was obtained from New England Nuclear. Rat liver tRNA was generously provided by Dr. Dolph, Hatfield.

Polysomes derived from either first trimester or third trimester placenta and ascites tumor cell sap were prepared as previously described [8]. The cell-free system was composed of 30 mM Tris–HCl (pH 7.5), 3.3 mM magnesium acetate, 70 mM KCl, 7 mM 2-mercaptoethanol, 1 mM ATP, 0.1 mM GTP, 0.6 mM CTP, 10 mM creatine phosphate, 0.16 mg/ml creatine kinase, 40 μM each of 20 unlabeled amino acids, and 60 μg/ml of rat liver tRNA. The volume of the reaction mixtures was 0.6 ml. The reaction mixtures were incubated for 120 min at 33°C. Following incubation, the reactions were terminated with 0.2 mg/ml of pancreatic ribonuclease. This was incubated for an additional 15 min at 33°C. The samples were then dialyzed overnight in the cold against 500 vol of 0.1 M NH_4HCO_3. Following dialysis, the samples were lyophilized and the residue resuspended in a buffer containing 0.1 M Tris acetate (pH 7.5) and 0.25% bovine serum albumin. This buffer, HPL antiserum and [^{125}I]HPL (specific activity 10–20 μCi/μg) are all components of an immunoassay unit (Cat. #NEA-23) supplied by New England Nuclear Co. The resulting suspension was centrifuged at 3000 *g* for 10 min. Aliquots of the supernatant solution were then assayed according to the protocol that accompanies the radioimmunoassay unit except that the total volume of the incubation mixture was reduced by

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1/2. This immunoassay utilizes some of the methodology employed by Saxena et al. [2].

3. Results and discussion

In assays employing precipitating antibody systems, the synthesis of radioactive protein is measured. Here, the immunoassay is based on an isotope dilution which measures the synthesis of unlabeled protein by its competition with a labeled ligand for antibody sites [9].

Immunoreactive material was synthesized on polysomes prepared from term placenta (table 1). Pancreatic ribonuclease, or puromycin addition, or omission of cell sap eliminated the net synthesis. (A background of immunoreactive material in the system may come from partially completed chains or adsorption of HPL to the polysomes during homogenization). The recovery of immunoreactive protein was determined by processing a known quantity of authentic HPL in the complete system treated with ribonuclease. The recovery of immunoassayable HPL was greater than 70%.

The synthesis of this immunoreactive material was specific for the placental cell-free system. No detectable product was observed in a cell-free system containing polysomes prepared from either rat liver or a solid hamster tumor. The synthesis of immunoreactive material, specific for placental polysomes strongly suggests that the material detected in the placental system is HPL whose synthesis is directed by the corresponding mRNA. In further support of this, all the specifically immunoreactive protein co-migrated with authentic HPL in acrylamide gel electrophoresis (data not shown).

In terms of the amount of ribosomes added to the immunoassay system, the level of detectable HPL was linear up to the equivalent of 1.5 A_{260} of ribosomes (fig. 1). It can also be seen that it is possible to detect at least 0.3 ng of HPL.

Table 1
Immunoassay using HPL antibody for protein synthesized in a cell-free system containing term placental ribosomes

Conditions of cell-free system	Immunoreactive protein (ng/A_{260} of ribosomes)
Complete	2.4
Complete, unincubated	0.50
Complete + RNase at zero time	0.60
Complete + 1 mM puromycin	0.55
Minus cell sap	0.40

The reaction mixtures, in addition to the components described in the Materials and methods section, contained 9 A_{260} units of ribosomes and the equivalent of 360 μg of cell-sap protein per 0.60 ml. The reaction mixtures were treated as described in the text. Aliquots of the supernate were added to the immunoassay incubation medium. The amount of immunoreactive material was expressed as ng of HPL/A_{260} of ribosomes initially added to the cell-free system (1 A_{260} = 20 pmoles).

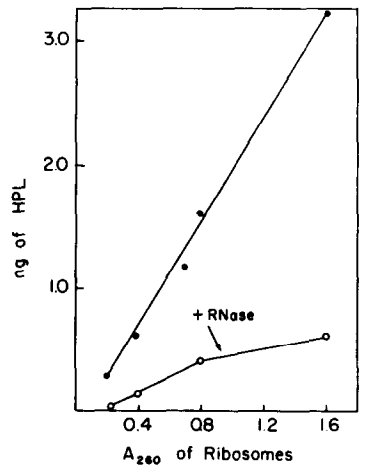

Fig. 1. The effects of increasing quantities of the in vitro synthesized reaction mixture on the radioimmunoassay system. Two reaction mixtures, one containing ribonuclease at zero time, the other treated with ribonuclease after 2 hr of incubation, were processed for immunoassay as described in the text. The data are expressed as ng of HPL detected as a function of the amount of ribosome equivalents added to the immunoassay incubation medium.

The levels of HPL synthesized in the cell-free system containing term polysomes was about 4 times greater than in a system containing first trimester polysomes (table 2). The lower amounts of HPL synthesized in the first trimester system were not the apparent result of lower efficiency of protein synthesis since with equal quantities of ribosomes, the amount of [^{35}S]methionine incorporated in both cell-free

Table 2
The levels of HPL synthesized in cell-free system containing first trimester or term ribosomes

Expt.	Source of ribosomes	[^{35}S]Met. incorp./0.06 ml	Immunoreactive protein (ng/A_{260} of ribosomes)	
			− RNase	+ RNase
A	Term	830 000	2.80	0.53
	First trimester	640 000	0.42	Not detect.
B	Term	660 000	3.00	0.50
	First trimester	690 000	0.62	Not detect.

The 0.6 ml reaction mixture contained 360 μg of protein ascites tumor cell sap, 6 A_{260} units (Expt. A) and 2.6 A_{260} units (Expt. B) of term ribosomes and first trimester ribosomes. Before the addition of unlabeled amino acids, aliquots of the assay system were incubated with 0.5 μM [^{35}S]methionine (specific activity − 195 Ci/mmole), and a mixture of 19 minus methionine cold amino acids. To the remaining mixture, all 20 unlabeled amino acids were added. Both labeled and unlabeled reactions were incubated for 2 hr at 33°C. The amount of [^{35}S]methionine incorporated was quantitated by precipitating the proteins with trichloroacetic acid, filtering, and then counting the filters in a liquid scintillation counter. The first trimester placentae were obtained at 8–12 weeks of gestation.

systems was comparable. The data thus parallel the serum levels of hormone seen during pregnancy [1–3] and the levels of HPL secreted from first trimester and term placental slices incubated in vitro [16].

The trophoblast cellular population of the placenta is composed of 2 types: relatively undifferentiated cytotrophoblasts and a more differentiated syncytium. The cytotrophoblasts are apparently the generative cells for the syncytium [10–13], and the latter is the site of synthesis for HPL [14]. It has been shown morphologically that during gestation there is a decrease in the number of cytotrophoblasts and an increase in the proportion of syncytium of the placental villi [15]. Thus, the increased HPL synthesis seen in the cell-free system containing term ribosomes may be indicative of the continual differentiation of the placenta. More specifically, the higher hormone levels may result from a recruitment of the more primitive cytotrophoblasts to the differentiated syncytium. Thus, it is probable that as a result of an increased fraction of syncytium at term, there is a correspondingly greater proportion of HPL-mRNA.

Acknowledgements

Supported by Population Council Grant #M73.135, and by a departmental grant from the Ford Foundation.

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