CHANGING RATIO OF HUMAN CHORIONIC GONADOTROPIN SUBUNITS SYNTHESIZED BY EARLY AND FULL-TERM PLACENTAL POLYRIBOSOMES

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SUMMARY

Subunits of hCG[1] were synthesized in vitro by incubating placental polyribosomes in a cell-free system with [^{3}H]leucine. The α and β subunits were precipitated by specific antisera and resolved on SDS-polyacrylamide gels, yielding single radioactive peaks at 10,000 and 16,000 daltons molecular weight. The α and β subunits accounted for 5 and 4%, respectively, of total incorporation into protein by first-trimester ribosomes, but only 1.3 and 0.6%, respectively, by full-term ribosomes. This reflects the declining maternal blood levels of hCG in late pregnancy. The change in α:β ratio from 1.2 to 2.3 suggests uncoordinated synthesis of the subunits and coincides with the appearance in the plasma of free α subunits in the third trimester.

INTRODUCTION

Plasma concentrations of hCG increase during early pregnancy to a maximum at 10 weeks and thereafter decline, whereas hPL[1] concentrations are minimal early in pregnancy and increase progressively up to term. We (1) have shown that hCG accounts for some 11% of peptide chains on polyribosomes prepared from first-trimester placentas, but only 2% of total peptide chains on the ribosomes of term placentas. In contrast, hPL chains could not be detected on placental ribosomes in the first trimester but accounted for 8% of the protein synthesized by ribosomes at full term. Thus, the decreasing plasma levels of hCG and increasing levels of hPL with advancing gestational age reflect the relative rates of synthesis of these hormones by the ribosomes of the placenta in response to the availability of messenger RNAs for each.

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[1]Abbreviations: hCG, human chorionic gonadotropin; hPL, human placental lactogen; SDS, sodium dodecyl sulfate; hCT, chorionic thyrotropin; hCFSH, chorionic follicle-stimulating hormone

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HCG consists of two dissimilar peptide chains, designated α and β. It has been reported that the plasma contains small amounts of free hCG-α subunits, which increase in quantity during pregnancy, whereas the concentration of free β subunits remains low and constant (2,3). This finding suggests that the synthesis of the α and β subunits of hCG may be independently controlled, with the β subunit limiting the appearance of complete hCG chains. We have therefore examined the relative amounts of the two peptides on human placental ribosomes at different stages in pregnancy. We now report that, although the proportion of polysomes synthesizing hCG declines in later pregnancy, the ratio of α subunits to β subunits increases twofold between the first and third trimesters.

MATERIALS AND METHODS

Freshly collected placentas from normal full-term deliveries or prostaglandin-induced first-trimester abortions were trimmed of cord and membranes, washed extensively, and homogenized in a buffer containing 50 mM Tris-HCl (pH 7.6), 25 mM KCl, 100 mM NH_4Cl, 10 mM $MgCl_2$, 0.25 M sucrose, 0.5 mM EDTA, and 15 mg% sodium heparin. Total placental polyribosomes were harvested as previously described (4). It is well known that such cell fractionation procedures result in a selective loss of part of the membrane-bound ribosome population; our previous studies (1) indicate that about equal amounts of membrane-attached and free ribosomes are recovered in the total polyribosome population by our procedure.

Polyribosomes were incubated in a cell-free system for protein synthesis using [^{3}H]leucine as the labeled precursor, as described in detail elsewhere (1). After incubation, the reaction mixture was centrifuged to remove ribosomes with nascent peptide chains; about 20% of the chains were released. Incorporation of radioactivity into total TCA-precipitable protein of the released chains was measured in an aliquot of the post-ribosomal supernatant fluid by the Mans-Novelli method (5).

Incorporation by polyribosomes of radioactive precursor into hCG and its α and β chains was measured after incubation with a specific immunoprecipitation technique. Antisera to pure α and β chains, generated in rabbits by a previously described method (6), were generously provided by Dr. J. Vaitukaitis. An antiserum to intact hCG was purchased from Miles Laboratories, Elkhart, IN. The specificities of the antisera have been previously established as follows: hCG and hCG-β cross-react less than 1% with hCG-α in the homologous hCG-α radioimmunoassay; in the homologous hCG-β assay, hCG and hCG-α cross-react approximately 10% and 1%, respectively, with hCG-β (6). In contrast, antisera to whole hCG are less specific for the intact molecule, cross-reacting with hCG-α and hCG-β chains as well.

These antisera were used in the presence of carrier antigen to precipitate the specific hormone peptides made in vitro. The released chains were isolated from 100 to 200 μl of the post-ribosomal supernatant fluid by addition of 5-10 μg of carrier hCG, hCG-α subunit, or hCG-β subunit, followed by an excess of the appropriate antibody.

After addition of carrier antigen and the appropriate antiserum to the

reaction mixture, formation of a complex was first allowed to occur by incubating at 37° for 2 hr and at 4° overnight, and then precipitating by addition of goat anti-rabbit gammaglobulin (Pacific Biologicals, Biorad). The resulting immunoprecipitates were washed with a detergent solution and then through a discontinuous sucrose gradient to remove non-specifically bound [^{3}H]leucine (1). Studies using [^{125}I]-labeled hPL added to the incubation mixture showed that a consistent 80% was recovered in the washed immunoprecipitate after sucrose gradient purification (1). The immunoprecipitate was then dissolved by heating for 3 min in a solution containing 2% SDS,[1] and was finally resolved on a 12-15% gradient SDS-polyacrylamide slab gel prepared according to Maizel (7), using electrophoresis at 175 v for 4 hr. Under these conditions of resolution, the antigen-antibody complex, the light and heavy chains of the gammaglobulins, and the α and β subunits of hCG are all dissociated and separate on the gel according to molecular size. By staining the gel with 0.2 % Coomassie blue, these proteins can be identified on the gel; in the case of the hCG subunits, the migration is affected by the presence of carbohydrate. The gels were sliced into sections of 3.3 mm and counted for radioactivity. The proportions of the radioactivity in total released peptides accounted for by hCG, by the α subunit or by the β subunit, were computed as counts per min recovered on the gel as a percentage of total precipitable peptide counts.

RESULTS AND DISCUSSION

Fig. 1 shows a typical radioactivity profile of an SDS-polyacrylamide gradient gel used to separate the completed hCG chains synthesized in vitro by polyribosomes harvested from a first-trimester placenta. Following incubation of the placental polysomes with [^{3}H]leucine, labeled peptide chains were precipitated by anti-hCG serum or antisera specific for the α or β subunits. In agreement with our previous report (1), precipitation with anti-hCG serum resulted in a product that resolved partially into two peaks of radioactivity on the SDS gel, one being approximately 10,000 daltons in molecular weight, and the other 16,000. The middle panel in Fig. 1 shows the gel profile after the same incubation mixture had been treated with antiserum to the α subunit, and the lower panel shows the gel profile after precipitation with anti-β subunit serum. Single sharp peaks of radioactivity representing newly synthesized chains and corresponding in molecular size to the peptide portions of the subunits are seen after precipitation with each specific antiserum. This confirms published evidence (8) that the carbohydrate portion is not necessary for antibody recognition. The position of the α chain radioactivity on the middle gel coincided closely with that of the α peak of the intact hCG, but, in all gel runs, the peak of the isolated β subunit was

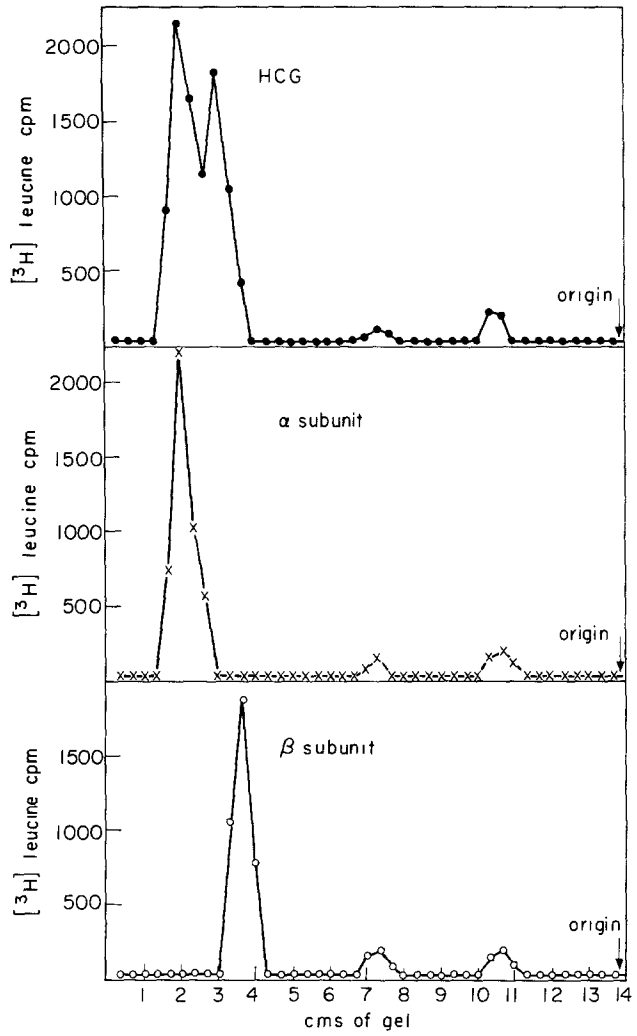

Figure 1. Radioactivity profile on an SDS-polyacrylamide gradient gel of [^{3}H]-labeled peptides synthesized during in vitro incubation of polyribosomes prepared from a first-trimester placenta and immunoprecipitated by anti-hCG serum (top), antiserum to the hCG-α subunit (middle), or antiserum to the hCG-β subunit (bottom). Placental polysomes were incubated in the presence of rat liver pH 5 enzyme and [^{3}H]leucine: the ribosomes were removed, and released [^{3}H]-labeled peptides were precipitated with the antibodies. The immunoprecipitates were resolved on SDS gels, and radioactivity was measured in 3.3 mm slices. The figure is representative of three replicates of the experiment using first-trimester polysomes. The locations of the two major radioactivity peaks correspond in molecular size to the peptide portions of the α and β chains, respectively. Two very small additional peaks (present regardless of the organ from which the polyribosomes were obtained) represent traces of non-hormonal radioactivity co-migrating with the light and heavy chains of the antibody (1). It is important to note that, since the labeled products of incubation were resolved on gels in the presence of SDS, any intact hCG precipitated by antibody would be resolved into its two subunits. Consequently, radioactivity observed at 10,000 and 16,000 daltons includes subunits arising from intact hCG as well as free subunit chains without discriminating between these. The finding that no radioactivity for β chains is precipitated from the reaction mixture by the α-chain antiserum, and vice versa, implies high specificity of these antisera under our conditions.

Table 1. In vitro synthesis of hCG and its α and β subunits by placental polyribosomes.

Placenta examined	Percent of total peptide radioactivity incorporated			Ratio of α to β subunit radioactivity	Percentage recovery of hCG activity in α and β subunits
	hCG	α subunit	β subunit		
First trimester					
1. 12 wk	11.4	6.0	4.6	1.3	93
2. 13 wk	8.1	4.9	4.0	1.2	110
3. 16 wk	6.7	3.9	3.2	1.2	106
Mean	8.7	4.9	3.9	1.2	103
Full term					
4. > 38 wk	-	1.7	0.7	2.3	-
5. > 38 wk	1.7	1.2	0.5	2.5	100
6. > 38 wk	1.9	1.0	0.5	2.0	79
Mean	1.8	1.3	0.6	2.3	89

The polyribosomes were incubated with rat liver pH 5 enzyme fraction and [^{3}H]leucine. Uptake into total released peptides was measured, and [^{3}H] incorporation into the hormone peptides precipitated with anti-hCG, anti-α, or anti-β subunit sera were separately obtained by resolution on SDS gels. The amount of radioactivity in these three hormone fractions is expressed as percent of total peptide incorporation.

slightly retarded compared with the β peak for intact hCG (Fig. 1). This may be due to some interaction between the α and β chains when both are present on the gel or to precipitation of slightly larger chains by the antibody specific for the β subunit.

This technique was applied to polyribosomes harvested from three first-trimester placentas obtained following prostaglandin induction of normal pregnancies and three placentas from normal full-term deliveries. Table 1 shows that some 7 to 11% of peptide chains were accounted for by total hCG in early placental incubations. This is within the range observed previously (1). It will be noted that the lowest value among early placentas occurred for a 16-week pregnancy, when plasma hCG levels are already declining. At full

term, incorporation into hCG had fallen to 1.8% of total peptide synthesis, in agreement with our earlier values (1). This picture is reflected in the radioactivity recovered for both the α and β subunits during the first and third trimesters. The α subunit declines from 4.9 to 1.3 % of total peptide synthesis, while the β subunit falls from 3.9 to 0.6%. Because the hCG antiserum was added in excess, we would expect free hCG-α and hCG-β to be precipitated under our conditions. On the other hand, the subunit antisera were sufficiently specific that, even in the presence of excess of these antisera, they would not cross-precipitate the other subunit or intact hCG. The use of antisera specific for each subunit thus allows quantitation of each more precisely than when both were precipitated by hCG antibody (see Fig. 1). In this connection, the sum of the radioactivity recovered by precipitation with α-chain and β-chain specific antibodies agrees closely with the radioactivity for total hCG precipitated with the anti-hCG serum from the same incubation (Table 1). This validates the overall quantitation of the procedure. It may be noted that, using analysis of tryptic peptides, Landefeld et al. (9) found a fourfold reduction in synthesis of α subunits directed by mRNA from term placentas as compared with first trimester preparations. Their experimental technique did not allow measurement of β subunit synthesis.

The decline in the hCG-specific β subunit confirms the reduced rate of hCG production at term. Furthermore, the ratio of α-chain radioactivity to β-chain radioactivity increases from 1.2 at 12-16 weeks to 2.3 at full term. This coincides with the presence of free α chains in the plasma in increasing amounts as pregnancy proceeds (3). There is no similar progressive accumulation of β chains in the plasma, even though the half-life of the free α chain is shorter than that of the free β chain (10). The presence of the α subunit in the placenta may not be related solely to hCG production. It is claimed that the placenta contains hCT[1] (11) and hCFSH[1] (12), which show increased plasma concentrations towards term. Although the analogous anterior pituitary hormones carry the same α subunit as hCG, the subunit structures of hCT and

hCFSH have not been established and it seems improbable that the excess of α chains made at term is accounted for by these two hormones.

The absolute ratio of α to β chains on the placental ribosomes is not established by our data. First, the number of leucine residues in the α chain is one-third that in the β chain, and most of the α chain leucine residues are located near the N-terminal end, and will thus not be labeled in half-finished nascent chains. Second, the antibodies to the α and β chains and to intact hCG may recognize and precipitate incomplete peptide chains to different extents. For example, the slightly broader α peak (Fig. 1) may represent small differences in peptide size, including variations due to incompletely cleaved precursor peptides, such as those described for many secreted proteins and recently suggested for hCG-α subunit by the data of Landefeld et al. (9).

Our studies demonstrate that there is no fixed ratio in which the chains of hCG are synthesized. There is also evidence from studies of ectopic hCG-producing tumors that the α and β subunit peptides are not synthesized in tandem (13). Unbalanced production of α and β subunits has been recorded for a number of other non-trophoblastic tumors (14). The evidence presented here confirms that a similar situation exists with regard to α and β subunit synthesis and hCG production by normal placentas.

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