Synthesis and Secretion of Human Chorionic Gonadotropin Subunits by Cultured Human Malignant Cells*

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The synthesis and secretion of human chorionic gonadotropin (hCG) subunits have been studied by pulse-chase techniques in JAR choriocarcinoma cells, which produce both the α and β subunits of hCG "eutopically," that is, as part of their expected repertoire of gene products, and in three cell lines that produce either α or β subunit "ectopically." JAR cells contain two intracellular forms of the α subunit (Mr = 15,000 and 18,000) and of the β subunit (Mr = 18,000 and 24,000) but do not accumulate fully processed, "mature" α (Mr = 22,000) or β (Mr = 34,000) subunits during chase of pulse-labeled cells. Mature subunits, however, are secreted into the culture medium during this time. Thus, secretion of mature subunits appears to occur rapidly after processing of the intracellular forms. Two cell lines that ectopically secrete α but not β subunit, ChaGo bronchogenic carcinoma cells and HeLa S1 cervical carcinoma cells, also contain the Mr = 15,000 and 18,000 intracellular forms of α subunit and appear to accumulate mature α subunit intracellularly. The CBT cell line, derived from a glioblastoma multiforme, produces the Mr = 18,000 and 24,000 intracellular forms of β subunit, with no evidence for α subunit synthesis or secretion. These four cell lines should provide "biologic reagents" for the further study of α and β subunit processing and secretion.

The production of the α and β subunits of the glycoprotein hormone human chorionic gonadotropin (hCG) by human malignant cells is often unbalanced in vivo (1–3) and in vitro (4–6), with a subunit production frequently predominating. During normal pregnancy, also, there is an excess production of α over β by the placenta, and the synthesis of β appears to be rate-limiting in the production of complete hCG (1). It is now known that, in the placenta, the mRNAs for the α and β subunits are separate and that translation of the α and β mRNAs in cell-free systems is differentially sensitive to Mg2+ ion concentration (7). Thus, from both in vitro and in vivo studies, there is considerable evidence suggesting that there is unbalanced synthesis and secretion of α and β subunits of hCG in both normal and malignant cells. There have also been reports of biochemical differences between hCG subunits produced by normal trophoblastic cells and those secreted by malignant cells (8–10). Moreover, the effects of a number of drugs on the eutopic secretion of hCG subunits by trophoblastic cells are different from the effects on ectopic production by non-trophoblastic cells (5, 6, 11–13). Very little is known, however, about the control of hCG secretion by trophoblastic and non-trophoblastic cells or about the processing of intermediate glycosylated forms prior to secretion. The studies reported here were undertaken to characterize and to compare the synthesis and secretion of hCG subunits in eutopic and ectopic hCG subunit-producing cell lines.

EXPERIMENTAL PROCEDURES

Cell Culture Lines—JAR choriocarcinoma cells (obtained from Dr. Roland Pattillo, Medical College of Wisconsin (14)) were grown in Dulbecco's modified Eagle's medium with 10% fetal bovine serum. ChaGo bronchogenic carcinoma cells and CBT glioblastoma cells (from Dr. Alan Rabson, National Cancer Institute) were grown in RPMI 1640 medium with 20% fetal bovine serum. HeLa S1 cultures (American Type Culture Collection) were grown in Eagle's minimal essential medium with 10% fetal bovine serum.

Radioactive Labeling and Preparation of Cell Lysates—Late log or early confluent cultures (grown in 100-mm diameter Petri dishes) were incubated at 37°C for various times with 100 μCi/ml of [35S]methionine (500 to 700 Ci/mmol, New England Nuclear Corp.) in methionine-free medium. In some experiments, the radioactivity was "chased" by removing the labeling medium, washing the cells three times with complete medium, and incubating for various times in the presence of complete medium. Tunicamycin at various doses was added to some culture plates 16 h prior to a radioactive pulse. After incubation, the medium was removed, and the cells were washed with phosphate-buffered saline solution (0.01 M, pH 7.2) and lysed by the addition of 2 to 5 ml of phosphate-buffered saline containing 1.6% Triton X-100, 0.5% sodium deoxycholate, and 0.1% sodium dodecyl sulfate (lysis buffer), followed by sonication. Culture medium was brought to the same concentration of lysis buffer by the addition of a concentrated solution. Cell lysates were clarified at 100,000 × g for 1 h at 0°C. The 100,000 × g pellet contained no detectable hCG subunits, as determined by SDS-PAGE followed by fluorography (see below), and was discarded. Cell lysates and media were stored at −70°C until analyzed.

Immunoprecipitation and SDS-PAGE—Detection of hCG-specific polypeptides in cell lysates and media was performed with rabbit antiserum directed against complete hCG, hCG-α, or hCG-β (see miniprint supplement)5. In some experiments, immunoprecipitations were carried out with an antiserum raised in rabbits to a synthetic polypeptide representing the 15 COOH-terminal amino acids of the hCG-β subunit (see miniprint supplement). Serum from immununized...
rabbits was used as a control. Cell lysates or media were incubated with immune or nonimmune rabbit serum (1:5000 final dilution) at 4°C for 16 h. Immune precipitates were precipitated by the addition of Protein A Sepharose CL-4B (Pharmacia) followed by mixing for 2 h at 4°C and centrifugation at 1000 g for 15 min. The immunoprecipitates were washed three times in lysis buffer, dissolved in electrophoresis buffer containing 0.062 M Tris HCl (pH 6.7), 1% SDS, 10% glycerol, and 2.5% β-mercaptoethanol, heated at 100°C for 5 min, and layered on 5 to 20% linear gradient gels (unless otherwise indicated) prepared by the method of Laemmli (15). Electrophoresis was carried out for 16 h at 35 V in a Bio-Rad model 220 slab gel apparatus. Radioactivity was visualized by the fluorographic method of Bonner and Laskey (16). The following ¹³C-labeled molecular weight standards (New England Nuclear Corp.) were employed: phosphorylase B (92,500), bovine serum albumin (69,000), ovalbumin (46,000), carbonic anhydrase (30,000), and cytochrome c (12,300). Molecular weights of the hCG-specific polypeptides were determined from logarithmic plots based on the migration of the molecular weight standards.

RESULTS

Ectopic Production of hCG Subunits by JAR Choriocarcinoma Cells—We have previously reported (17) that JAR choriocarcinoma cells contain glycosylated "precursors" of hCG-α and hCG-β with intracellular half-lives of ≥1 h. The precursor of the α subunit had an apparent molecular weight of 18,000 (by SDS-PAGE) and contained high mannose core, asparagine-linked carbohydrate units. The β precursor had an apparent molecular weight of 24,000 and also appeared to contain the asparagine-linked carbohydrate units. Mature α and β subunits secreted by JAR cells were M₉ = 22,000 and 34,000, respectively. We also had noted that, after incubation of JAR cells with tunicamycin for 16 h, a M₉ = 12,000 α polypeptide and a M₉ = 15,000 partially glycosylated α polypeptide were present intracellularly (17).

In order to further differentiate the α- and β-specific polypeptides synthesized in JAR cells, we prepared and characterized a rabbit antiserum directed against the 15 COOH-terminal amino acids of hCG-β. This antiserum has a high degree of specificity for hCG-β with minimal cross-reactivity with the α subunit (see miniprint supplement, Fig. 9). Fig. 1 illustrates the results of an experiment in which cell lysates were prepared from JAR cells pulsed for 1 h with [³⁵S]methionine and then immunoprecipitated with anti-hCG, anti-α, and anti-COOH-terminal β. Anti-hCG precipitated polypeptides at M₉ = 24,000, 18,000, and 15,000 (Lane 1). Anti-α precipitated the M₉ = 18,000 and 15,000 α-specific polypeptides noted above (Lane 2). Polypeptides of M₉ = 24,000 and 18,000 were also immunoprecipitated by the antiserum directed against the COOH-terminal of β (Lane 3). Immunoprecipitation of the M₉ = 18,000 and 15,000 bands by anti-α was inhibited by excess α (Lane 5) but not by excess β subunit (Lane 8), whereas immunoprecipitation of the M₉ = 24,000 and 18,000 polypeptides by the antiserum to COOH-terminal β was blocked by excess unlabeled β subunit (Lane 9) but not by excess α subunit (Lane 6). β-specific polypeptides of M₉ = 18,000 and 15,000 were previously noted after treatment of JAR cells with 1 µg/ml of tunicamycin for 16 h (a concentration that partially blocked glycosylation), whereas only the M₉ = 15,000 polypeptide was seen after treatment of JAR cells with 5 µg/ml of tunicamycin (a concentration that completely inhibited glycosylation) (17). Taken together, these data suggested that the M₉ = 18,000 band immunoprecipitated by various anti-β sera was a partially glycosylated β precursor, whereas M₉ = 15,000 band was the β apoprotein. This apparent molecular weight is consistent with that reported for the β apoprotein by SDS-PAGE (7).

Although intracellular forms of a subunit could be identified in JAR cells, very little accumulation of fully processed, mature α was detected in these cells during a 4-h chase of cells pulsed for 15 min with [³⁵S]methionine (Fig. 2); however, a subunit secreted into the medium (Lanes 7 to 9) migrated identically to that of mature placental hCG-α, and immunoprecipitation of the secreted form of α was blocked by addition of excess placental hCG-α subunit (data not shown).

Ectopic Production of hCG Subunits by ChaGo, HeLa S₉, and CBT Cells—The ChaGo cell line was established from a bronchogenic carcinoma and shown by radioimmunoassay to secrete high amounts of a subunit in culture (18). Some cloned sublines of ChaGo produce and secrete only free α subunit and no hCG-β or complete hCG (4, 19), whereas others produce more β than α subunit (4). The ChaGo cells used in our experiments synthesized and secreted only a subunit; no β subunit was detectable either intra- or extracellularly (6). Different strains of HeLa cells have been shown to secrete a subunit (6, 20, 21) and can be induced by the addition of sodium butyrate to secrete 50 to 100 times more a subunit than in the basal state (6, 21, 22). Little or no free β subunit has been detected in these cells under basal or induced con-
ditions (6, 20, 22). The CBT cell line was established from a glioblastoma multiforme (23) and has been shown to produce hCG or hCG-β, or both, but no free α subunit (6).

All of the data cited above have been derived by radioimmunoassay. It was of interest to examine these cells by pulse-chase techniques to determine the nature of the subunits synthesized and secreted by these cells and to compare these events between cells that produce hCG subunits ectopically and trophoblastic cell lines that produce hCG subunits eutopically.

Fig. 3 shows the results of a pulse-chase experiment in which ChaGo cells were incubated for 1 h with [35S]methionine and then chased for 4 h in the presence of excess unlabeled amino acids. In the lysates from ChaGo cells pulsed for 1 h, the anti-α serum precipitated bands at M = 18,000 and 15,000 (Lane 6) as well as a diffuse band that migrated identically to that of the secreted α subunit (Lanes 1 and 2). The secreted α migrated at the same molecular weight as standard placental hCG-α. No β-specific polypeptides were present intracellularly or extracellularly (Lanes 3, 7, and 11).

In the ChaGo cells chased for 4 h (Lane 10), there was no evidence of the M = 15,000 band and relatively less of the M = 18,000 band compared to the amount of material that migrated like the secreted form of α, suggesting a precursor-product relationship between the low molecular weight and mature forms of the α subunit. The immunoprecipitation of both the intracellular and secreted forms of α subunit from the ChaGo cells was blocked by addition of excess unlabeled placental hCG-α subunit (Fig. 4).

In order to ascertain whether the glycosylated intermediates in the processing pathway for α subunit were similar between JAR and ChaGo cells, cultures of ChaGo were incubated with 1 or 5 μg/ml of tunicamycin (the latter concentration had been previously shown to inhibit glycosylation but not synthesis of α subunit in JAR cells (17)) for 16 h prior to pulsing for 1 h with [35S]methionine. Cells then were lysed and immunoprecipitated with anti-α serum (Fig. 5). It can be

![Fig. 3. Pulse-chase labeling of ChaGo cells: comparison to JAR cells. ChaGo and JAR cells were pulsed for 1 h with [35S]-methionine and chased for 4 h in complete medium. Both media and cell lysates were immunoprecipitated with various antisera. Arrows indicate migration of secreted α subunit and the M, = 18,000 and 15,000 precursors. Lanes 1 to 4 (ChaGo, 4-h chase, medium): 1, anti-hCG; 2, anti-α; 3, anti-β (R279-P1); 4, nonimmune serum. Lanes 5 to 8 (ChaGo, 1-h pulse, cells): same sequence of antisera. Lanes 9 to 12 (ChaGo, 4-h chase, cells): same sequence of antisera; Lane 13 (JAR, 1-h pulse, cells): anti-α. Lane 14 (JAR, 4-h chase, medium): anti-α. Lane 15: molecular weight standards.](http://www.jbc.org/)

![Fig. 4. Identification of putative hCG-α precursors in ChaGo cells by competition with excess unlabeled placental hCG-α. ChaGo cells were pulsed for 1 h with [35S]methionine and chased for 4 h. Immediately before immunoprecipitation with anti-α, a 100-fold excess of placental hCG-α was added to the clarified cell lysate and medium obtained after the 4-h chase. Lane 1, cells, without excess hCG-α; Lane 2, cells, with excess hCG-α; Lane 3, medium, without excess hCG-α; Lane 4, medium, with excess hCG-α; Lane 5, molecular weight standards. Note: the gels used in this experiment were non-gradient 12% polyacrylamide gels rather than 5 to 20% linear gradient gels used in the other experiments depicted here; hence, the bands appear more diffuse.](http://www.jbc.org/)
seen that treatment with 1 or 5 µg/ml of tunicamycin increased the amount of the \( M_r = 15,000 \) band relative to the \( M_r = 18,000 \) band and produced a distinct band at \( M_r = 12,000 \), which was not seen in the untreated cells. This was in agreement with what was observed in JAR cells (17), suggesting that the glycosylated intermediates of \( \alpha \) were similar in JAR and ChaGo.

The secretion of a subunit in HeLa cells treated with sodium butyrate for 72 h prior to pulsing for 1 h with \(^{35} \text{S} \) methionine appeared to be similar to that of ChaGo cells (Fig. 6). Untreated cells pulsed for 1 h contained primarily the \( M_r = 18,000 \) and 15,000 bands (Lane 2); no \( \beta \)-specific polypeptides were evident (Lane 3). Cells pretreated for 72 h with butyrate contained more of the \( M_r = 18,000 \) and 15,000 polypeptides as well as mature \( \alpha \) subunit (Lane 6). The medium from control and butyrate-treated HeLa cells chased for 4 h contained only free \( \alpha \) subunit, and much more \( \alpha \) was secreted by butyrate-induced than by noninduced cells (Lanes 10 and 14). Thus, both synthesis and secretion of \( \alpha \) subunit appeared to be stimulated by treatment with butyrate.

The pattern of hCG subunit synthesis and secretion by CBT glioblastoma cells was the opposite of ChaGo and HeLa in that only the \( \beta \) subunit appeared to be synthesized and secreted. From the lysates of CBT cells pulsed for 1 h with \(^{35} \text{S} \) methionine, anti-hCG and anti-COOH-terminal \( \beta \) precipitated the \( M_r = 18,000 \) and 24,000 bands that were also seen with anti-\( \beta \) in JAR cells (Fig. 7, Lanes 1 and 2). Immunoprecipitation of both the \( M_r = 18,000 \) and 24,000 forms was blocked by addition of excess placental hCG-\( \beta \) subunit (data not shown). Neither free \( \alpha \) subunit nor complete hCG appeared to be synthesized in CBT cells since the only specifically immunoprecipitated band that appeared in the 4-h chase reaction was a \( M_r = 34,000 \) band that migrated identically to that of placental hCG-\( \beta \) subunit (Lanes 7 and 8). If complete hCG were being secreted by these cells in significant amounts, the anti-hCG serum should have immunoprecipitated it, and both the \( \alpha (M_r = 22,000) \) and \( \beta \) bands should have been apparent on the SDS gels under the reducing conditions used in these experiments. Furthermore, anti-\( \alpha \) did not immunoprecipitate \( \alpha \) precursors in pulse-labeled CBT cells (data not shown).

**DISCUSSION**

The data obtained in this study indicate that although the "kinetics" of secretion of hCG-\( \alpha \) subunit is somewhat different between cells that produce \( \alpha \) ectopically and those that produce it eutopically, the intermediates in the processing pathway for a subunit appear to be similar. Intracellular \( M_r = 18,000 \) and 15,000 forms of a subunit occur in JAR, ChaGo, and HeLa cells (Figs. 1, 3, and 6). In JAR cells the intracellular forms are "chased" from the cells without significant accumulation of fully mature \( \alpha \), but mature \( \alpha \) does appear in the chase medium (Fig. 2). In ChaGo and butyrate-induced HeLa cells, on the other hand, there is evidence for the accumulation of mature \( \alpha \) subunit intracellularly prior to secretion into the medium (Figs. 3 and 6).

The reasons for the apparent differences between eutopic and ectopic \( \alpha \)-producing cells are not yet evident, and they may represent quantitative rather than qualitative differences. We have also observed that another ectopic \( \alpha \)-producing cell line, BeWo choriocarcinoma, follows the pattern exemplified by JAR cells. Thus, the secretion of \( \alpha \) subunit by malignant trophoblastic cells may not follow the same route reported for other secretory glycoproteins, i.e. incorporation into secretory granules and secretion by exocytosis. This conclusion is consistent with the observations that there is a scarcity of secretory granules in BeWo cells (24) and that agents, such as colchicine, which inhibit hormone secretion by cells in which exocytosis is involved in the secretory process, stimulate secretion of hCG by BeWo cells (25). These authors (24, 25) postulate that secretory granules and microtubules do not play a significant role in hCG secretion by malignant trophoblastic cells.

The differences in \( \alpha \) secretion between the various cell lines observed in these experiments are not due to differences in the culture growth phase or the growth rate of the cells because in our hands the doubling time of JAR cells and HeLa cells is similar ( 24 h) and that of ChaGo and BeWo is similar ( 48 h). Furthermore, cells taken at any point in the growth phase from midlog to early confluency give identical results. It does not appear that differences in intracellular proteases can explain the discrepancies because lysis of cells in the presence of the protease inhibitor phenylmethanesulfonyl fluoride or incubation of clarified cell lysates for 24 h at 37°C before immunoprecipitation do not alter the banding patterns of the immunoprecipitated polypeptides.

Our results suggest the following hypothesis, which can now be tested experimentally, for the processing of the \( \alpha \) subunit in cultured malignant cells: the \( \alpha \) apoprotein (from which the signal peptide has already been cleaved (17)) is glycosylated during synthesis in a stepwise fashion leading to a \( M_r = 15,000 \) form containing one asparagine-linked high mannose oligosaccharide; this \( M_r = 15,000 \) form is then rapidly glycosylated by addition of the second high mannose oligosaccharide to pre-

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Footnote:

3 R. W. Ruddon and C. Anderson, unpublished observations.
duce an $M_1 = 18,000$ form that accumulates intracellularly because one of the next processing steps is a relatively slow one; the $M_1 = 18,000$ form is further processed by glycosidases and terminal complex carbohydrate synthesis to yield a mature $M_1 = 22,000$ glycoprotein subunit that is secreted. Although the data for the $\beta$ subunit are less clear, the following hypothesis is suggested from the data available: a $M_1 = 15,000$ apoprotein is glycosylated during synthesis to an $M_1 = 18,000$ glycoprotein and subsequently to a $M_1 = 24,000$ glycoprotein containing both high mannose core oligosaccharides and at least one of the serine-linked carbohydrate chains; this moiety is then processed and complex-type and serine-linked carbohydrate chains are added to form a $M_1 = 34,000$ glycoprotein that is secreted. The processing of the $\beta$ subunit is complicated by the fact that it contains four serine-linked carbohydrate units in addition to the two asparagine-linked chains (26) and, hence, the shifts in molecular weight are not analogous to those of $\alpha$. Of course, the molecular weights of all of these putative intermediates are based on SDS-PAGE, a system in which glycoproteins do not migrate strictly according to their molecular weights; thus, the true molecular weights of these intermediates must await more definitive biochemical analysis.

The apparent stepwise addition of high mannose-containing oligosaccharides to asparagine residues of hCG subunits, followed by processing via glycosidases and subsequent addition of complex-type carbohydrate chains, is consistent with what is known about vesicular stomatitis virus G protein (27-30), immunoglobulin G heavy chain (30), and the glycosylated env gene precursor (Pr 80)" of murine leukemia virus (31). The conversion of lower molecular intracellular forms of hCG-\alpha subunit to a higher molecular weight form that is secreted is similar to the observation by Weintraub and Stannard (32) that the $\alpha$ subunit of thyroid-stimulating hormone in mouse thyroptic tumor cells is lower in molecular weight than the secreted form. Bielinska and Boime (33) have shown that mRNA isolated from first trimester placenta and incubated in a cell-free system containing microsomal membranes directs the synthesis of hCG-\alpha subunit containing high mannose oligosaccharides and migrating at approximately $M_1 = 18,000$ by SDS-PAGE. The use of microsomal membranes from tunicamycin-treated cells or incubation of the $M_1 = 18,000$ form with endoglycosidase H produced lower molecular weight forms of $\alpha$ (33). These observations are consistent with our data obtained from intact tunicamycin-treated cells (Fig. 5) and from experiments using endoglycosidase H digestion (17).

It is evident that various human cell lines can produce both $\alpha$ and $\beta$ subunits (JAR), only $\alpha$ (ChaGo), or only $\beta$ (CBT) as well as that some cell lines can be induced to synthesize and secrete much greater amounts of one of the subunits (HeLa). Our studies indicate that these cell lines can be exploited as “biologic reagents” to study further the synthesis, processing, and secretion of hCG subunits by malignant human cells.

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Additional references are found on p. 1007.
Synthesis and Secretion of Human Chorionic Gonadotropin Subunits by Cultured Human Trophoblast Cells: Purification of hCG Subunits and Preparation of Antibodies

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EXPERIMENTAL PROCEDURES

Isolation of hCG

Crude hCG was purchased from Organon, Inc., Boxt, The Netherlands. Purification was performed by a modification of published procedures (1, 9). All steps were performed in the cold room at 4°C and particular attention was given to the proper preparation and timing of column material. The following were performed:

- Crude hCG (200 mg) was dissolved in 0.25 M ammonium acetate, pH 8.0, and dialyzed for 24 h against this solution.
- The solution from step 2 was clarified by centrifugation and applied to a 2.5 x 40 cm column of SP-Sephadex C-50 previously equilibrated with starting buffer. The column was washed sequentially with starting buffer, 1.0 M ammonium acetate, pH 8.0, and then with a linear gradient of ammonium acetate of 0.04 M to 0.4 M in 250 ml of 1.0 M ammonium acetate, pH 8.0, over 2 h at 4°C. The hCG eluted between 0.3 M and 0.5 M.
- Partially purified hCG was dialyzed briefly against water and then overnight against 0.04 M Tris, pH 8.0. It was then applied to a 2.5 x 40 cm column of DEAE-Sephadex A-50 previously equilibrated with the same buffer. The column was washed and then eluted using a salt gradient as described in the legend to Fig. 15.
- Partially purified hCG obtained by DEAE-Sephadex chromatography was dialyzed briefly against water, lyophilized to dryness, resuspended in 15 mM ammonium bicarbonate, and chromatographed on Bio-Rad A430-A agarose as described in the legend to Fig. 15.

Isolation of hCG Subunits

Subunits were isolated by a modification of published procedures (15). Purified hCG was incubated at room temperature for 16 h in 1 M propionic acid, following lyophilization, the precipitate was resuspended in 15 mM ammonium bicarbonate, pH 8.0, clarified by centrifugation, and dialyzed against starting buffer. The insoluble precipitate was removed by centrifugation, and the supernatant fraction was analyzed by polyacrylamide gel electrophoresis to ascertain that the electrophoretic pattern of the starting material was evident from this gel that the anion-exchange chromatography and gel filtration removed contaminating material which included proteins. The yield from this step (based on hCG) was 28%, and 85% of the starting material was recovered. Since a major goal of this work was to obtain the subunits, it was decided to eliminate the step following cation-exchange chromatography in favor of gel filtration. However, absorption data shown in the insets of Figs. 16 and 20 indicate that gel filtration chromatography did not remove contaminating material which could interfere with isolation of the subunits. An overall yield of about 90% of the starting material was obtained by this purification procedure. Major advantages of the propionic acid dissociation procedure to obtain hCG subunits are that 1) the two subunits are easily isolated from each other and 2) this procedure is performed at pH 8.0 without any salt. Purification by dialysis (15) is an alternative method of this dissociation step which was used in the experiments reported in this manuscript. The anti-hCG-a serum (R301-22) detected 7.0 ng/ml hCG at 500 mg/ml and had the following crossreactivities: at 500 mg/ml with related glycoprotein hormones, N.M. 79%, rHCG 1%, LH, 1.2%, FSH, 0.8%, and ACTH, 0.5%. Crossreactivity of the individual subunits of the related hormones was even less for the complete hormones.

The anti-hCG-a serum (R301-22) used in these experiments detected primarily pure alpha subunit, but it did not detect the subunits of the related hormones at concentrations of 100 times or more. The sensitivity and specificity of the anti-hCG-a serum (R301-22) are shown in Fig. 26. There was minimal crossreactivity with hCG subunits and the beta subunits of the related hormones. The sensitivity (anti-hCG-a serum applied) of these experiments (to 0.2 mg/ml hCG at 500 mg/ml and 200 mg/ml) was 7.0 ng/ml. The sensitivity and specificity of the anti-hCG-a serum (R301-22) is shown in Fig. 26.
Synthesis of hCG Subunits

Fig. 5A. Gel dextran B-100 chromatography of hCG disulfide-bonded to pig proenkephalin A. hCG immunized with 10 pig proenkephalin A and purified by phenyl-pheolysis was dissolved in 1% dodecanol, trichloroacetic acid, and applied to a column (2.8 x 30 cm) of Gel dextran B-100. The column was developed with 15% dodecanol trichloroacetic acid, followed by the buffers used for the electrophoreograms shown in Fig. 5A.

Fig. 5B. SDS-polyacrylamide gel electrophorograms of aliquots from the different steps of hCG purification. Electrophorograms were performed with a 25 blastic cells and a SDS-polyacrylamide gel according to Laemmli's procedure (17). The gels were stained on a 15% polyacrylamide gel containing 0.1% Coomassie Brilliant Blue R-250 and 5% SDS, respectively, of hCG after dialysis steps. Lanes 3 and 4: 50 and 75 µg of hCG after PEG precipitation; lanes 5 and 6: 50 and 75 µg of hCG after DEAE-Dextran; lanes 7, 8, and 9: 42, 62, and 77 µg of hCG after Bio-Gel A-0.5m. Amounts of hCG were estimated from published A280 values (17) and can only be rough approximations for the crude samples.

Fig. 5C. SDS-polyacrylamide gel electrophorograms of purified NCG and its subunits. Electrophorograms and gel staining were performed as described in the legend of Fig. 5A. Lane 1 contained 20 µg of hCG (fraction 5) from Fig. 3C; lanes 2 and 3 contained 20 and 40 µg of beta subunit (fraction 4) from Fig. 5C; lanes 4, 5, and 6 contained 25, 40, and 60 µg of alpha subunit (fraction 7) from Fig. 5C.
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Fig. 5B. Dose response curves for anti-hCG-8 (K359-22). The reagents used were: 2nd International Standard hCG (0-0), hCG-o (0-0), hCG-8 (A-A), prepared as described under Experimental Procedures; hCG-o (0-0), hCG-8 (A-A), prepared as described under Experimental Procedures; and 1st International Reference Preparation hLH (1st IRP hLH1, A-A), obtained from the National Pituitary Agency. The double antibody radioimmunoassay was based on a 1:15,000 final dilution (500 µl total tube volume) of anti-hCG-8 F279-P1 and 1-hCG-8 tracer. The percentage crossreactivity of the various competing antigens when compared to hCG-8 at 40% binding were: 2nd International Standard hCG, 3.2%; hCG-o, 0.6%; hLH (LER960), 3.0%; hLH-m, 0.4%; hFSH, 0.21; hFSH-a, 0.0%; hFSH-8, 0.04. Bound/Total = 37.0%; nonspecific binding = 3.0% of total counts.

Fig. 6. Dose response curves for anti-hCG-8 (K359-22) conjugate. The reagents used were: hCG (v-VI, hCG-o (0-0), hCG-8 (A-A), prepared as described under Experimental Procedures; and 1st International Reference Preparation hLH (1st IRP hLH1, A-A). The double antibody radioimmunoassay was based on a 1:30,000 final dilution (500 µl total tube volume) of anti-carboxy-terminal peptide-hemocyanin conjugate (R359-22) and 1st IRP hLH tracer. The percentage crossreactivity of hCG when compared to hCG-8 at 50% binding was: hCG-o, 12.2%; hCG-m, 0.0%; hFSH, 0.05; hFSH-a, 0.05; hFSH, 0.05; hFSH-a, 0.05. Bound/Total = 37.0%; nonspecific binding = 2.0% of total counts.
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