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Biochemical Evidence That Human Placental Lactogen and Human Chorionic Gonadotropin Are Not Stored in Cytoplasmic Secretion Granules[1]

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ABSTRACT

The intracellular storage sites for the human placental hormones placental lactogen (hPL) and chorionic gonadotropin (hCG) are unknown. To determine whether hPL and hCG are stored in cytoplasmic secretion granules, we have compared the localization of hPL and hCG in placental homogenates following differential and density-gradient centrifugations to those of prolactin (PRL) and luteinizing hormone (LH) in human and rat pituitary homogenates. In the differential centrifugation studies, 93.1 ± 4.1% (mean ± SE) of the hPL and 79.4 ± 6.0% of the hCG were detected in the postmicrosomal supernatant of placental homogenates. In contrast, 95–98% of the hPRL and hLH in the pituitary homogenates were detected in particulate fractions. Following centrifugation on sucrose-density gradients, particulate hPL and hCG were distributed diffusely throughout the gradients, while greater than 90% of the pituitary hormones sedimented as single peaks with densities of 1.22 g/cm^3. When human placental and rat pituitary tissues were homogenized together prior to differential and density-gradient centrifugations, similar marked differences were observed between the distribution of the placental and pituitary hormones. These results strongly suggest that the placental hormones hPL and hCG, unlike pituitary PRL and LH, are not stored in large secretory granules. Differences in the intracellular storage sites of the hormones may explain, in part, differences in the regulation of peptide hormone secretion by placental and pituitary tissues.

INTRODUCTION

The subcellular storage sites for the human placental hormones placental lactogen (hPL) and chorionic gonadotropin (hCG) are unknown. While the protein hormones of the anterior pituitary gland and most other endocrine tissues are stored in large dense secretory granules (O'Connor et al., 1983), ultrastructural studies of the placenta have not identified large secretory granules comparable to those present in the pituitary and other endocrine tissues. Rather, ultrastructural studies of the placenta have identified a relatively sparse number of large pleomorphic granules and small round or elongated granules (Wynn, 1975; Thliveris and Speroff, 1977; Sheppard and Bonnar, 1982; Tedde, 1982). The large granules do not have the appearance of secretion granules. The small granules, on the other hand, have the appearance of dense, membrane-bound, proteinaceous secretory granules that maintain their membranes until incorporated into a promontory of syncytium prior to discharge into the intervillous space.

Although it has been suggested that the small secretory granules may contain protein hormones (Wynn, 1975), the presence of hormones in these granules has not been demonstrated. In fact, the demonstration in a single ultracentrifugation study (Weinstein et al., 1982) that hPL is localized in the postmicrosomal supernatant of a placental homogenate suggests that hPL may not be stored in granules. An investigation of the ultrastructural localization of hCG in normal term placenta utilizing the horseradish-anti-horseradish peroxidase immunocytochemical technique demonstrated immunostaining for hCG on

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the maternal surface of the apical plasma membrane and in the rough endoplasmic reticulum of the syncytiotrophoblast, but not in the lamellae and vesicles of the Golgi complex or the large granules (Dreskin et al., 1970). Examination of the small dense granules gave inconclusive results, since some staining of the dense granules was observed in both immunostained and control tissue sections.

Since earlier studies of the subcellular distribution of hPL and hCG have yielded inconclusive results about the localization of these hormones in secretion granules, we have examined the subcellular localizations of hPL and hCG following both differential ultracentrifugation and density-gradient centrifugation of five placental homogenates.

MATERIALS AND METHODS

Materials

Placental tissue was obtained from five women with normal pregnancies of 38–40 wk gestation within 5 min of delivery, as previously described (Handwerger et al., 1981). Three of the women delivered vaginally and two were delivered by repeat caesarian section. Human pituitary tissue was obtained at autopsy from a 58-yr-old male within 2 h of death from a myocardial infarction. Both kinds of tissue were transported to the laboratory on ice and processed immediately. Permission to use the human tissues for research purposes was granted by the Human Investigation Committee of Duke University Medical Center, and informed consent was obtained in each instance. Female adult Sprague-Dawley rats were purchased from Zivic Miller Company (Allison Park, PA). The animals were killed by decapitation and the pituitary glands were quickly removed and placed in Hanks' Balanced Salt Solution.

Subcellular Fractionation

All subcellular fractionation procedures were performed at 0–4°C using techniques similar to those characterized by us in previous studies of human decidual, human pituitary, and rat pituitary tissues (Handwerger et al., 1984). Centrifugal forces given are those occurring at the maximum radius of rotation for each rotor.

Differential centrifugation. Tissues were blotted, weighed (human placental tissue, 5.0–15.0 gm; human pituitary tissue, approximately 0.1 gm), and homogenized in 9 ml of 0.32 M sucrose per gram wet weight with 5 strokes of a glass-to-glass, motor-driven conical Duall tissue grinder (100- to 150-μm clearance). The resulting homogenate was centrifuged at 800 × g for 10 min. (Sorvall SS-34 rotor), and the supernatant fluid was decanted and saved. The sedimented material was homogenized again in one-half the initial volume of 0.32 M sucrose by 5 strokes of the Duall homogenizer plus 5 strokes of a Dounce homogenizer with a tight-fitting pestle (25- to 75-μm clearance). This resuspended material contained no intact cells larger than erythrocytes as monitored by exclusion of trypan blue. This homogenate was centrifuged at 800 × g for 10 min to produce a crude nuclear fraction (P1). The supernatant fluids from both centrifugations were combined and centrifuged at 26,000 × g for 10 min to yield a crude mitochondrial fraction (P2). The supernatant fluid (S2) was further centrifuged at 111,000 × g for 60 min (Beckman 40 rotor) to produce the microsomal fraction (P3) and the postmicrosomal supernatant (S3). The P1, P2, and P3 fractions were each resuspended in 0.32 M sucrose and, in some cases, were washed by a second centrifugation, under similar conditions to the first, before final resuspension in 0.32 M sucrose. In two experiments, human placental and rat pituitary tissues were homogenized together with the Duall homogenizer prior to the initial centrifugation. The total weight of the rat pituitary tissues was 30–40 mg.

Density-gradient centrifugation. Linear sucrose gradients were formed from equal volumes of 0.5 M and 2.0 M sucrose (16.5 ml/gradient), overlayed with 0.55 ml–1.0 ml of resuspended sample in 0.32 M sucrose, and centrifuged in a Beckman SW27.1 rotor at 25,000 rpm (116,000 × g) for 15–19 h. Individual tubes were unloaded from the bottom using a peristaltic pump, and 20 or 21 fractions were collected from each. Identically prepared gradients overlayed with only 0.32 M sucrose were fractionated similarly, and the sucrose concentration of each fraction was determined by refraction using known standards. In two instances, gradients were performed on the homogenates prepared by homogenizing human placental and rat pituitary hormone tissues together.

Hormone and Protein Determinations

Human prolactin (hPRL), rat PRL (rPRL) and rat luteinizing hormone (rLH) were measured by homologous radioimmunoassays (RIAs) using kits

supplied by the National Hormone and Pituitary Program, NIADDK. hPL and hCG were measured by specific homologous radioimmunoassays (Handwerger and Sherwood, 1974; Tyrey et al., 1974). The anti-hCG serum was generated against intact hCG. hPRL and rPRL did not cross-react in the PRL radioimmunoassay (<0.001%), and hCG and rLH did not cross-react in the hCG and rLH assays (<0.001%). Before assay, the subcellular fractions were sonicated and then frozen and thawed five times. The fractions were subsequently centrifuged at 12,000 × *g* for 0.5 h, and analyses were performed on the supernatants. The amounts of hPL and hCG extracted by sonication and repeated freezing and thawing were shown to be identical to those extracted by alkali or Triton X-100.

RESULTS

Differential centrifugation studies of five human placental homogenates indicated that approximately 95% of the hPL and 80% of the hCG were present in the postmicrosomal supernatant (Table 1). Homogenization and differential centrifugation of human pituitary tissue by the same methods indicated that 98% of the hPRL and 95% of the hLH were present in particulate fractions, primarily in the mitochondrial fraction (Table 1). When human placental and rat pituitary tissues were homogenized together and subjected to differential centrifugation, the subcellular distribution patterns for the placental and pituitary hormones were similar to those of the individual tissue homogenates (Table 2). Approximately 86% of the hPL and 70% of the hCG were detected in the postmicrosomal supernatant, while 94% of the rPRL and 90% of the rLH were present in the particulate fractions.

Following density-gradient centrifugation through a gradient of 0.5–2.0 M sucrose, the small amounts of particulate hPL and hCG from four different placental homogenates were broadly distributed in the gradient; no peaks of hPL or hCG were observed. In contrast, nearly all of the hPRL and hLH sedimented as single peaks with mean densities of 1.22 g/cm^3 (Fig. 1). Sucrose-gradient centrifugation of the two homogenates prepared by homogenizing human placental and rat pituitary tissues together yielded similar results (Fig. 2). Again, hPL and HCG were distributed over most of the gradient, while rPRL and rLH sedimented as sharp peaks with densities of approximately 1.22 g/cm^3.

DISCUSSION

The results of this study suggest that the placental hormones hPL and hCG are not stored in secretory granules, but are localized in the postmicrosomal supernatant of placental homogenates. While, as anticipated, almost all of the pituitary peptide hormones were detected in mitochondrial and microsomal particulate fractions and sedimented in sucrose gradients with densities of 1.22 g/cm^3 (Hymer, 1975), approximately 95% of the hPL and 80% of the hCG were present in the postmicrosomal supernatant. Unlike PRL and LH, the small amounts of hPL and hCG in the particulate fractions were relatively evenly

TABLE 1. Differential centrifugation of human placental and pituitary tissues.[a]

Item	Placental homogenate		Pituitary homogenate	
	hPL n=5	hCG n=4	hPRL	hLH
	mean ± SEM			
Mitochondrial fraction, % total recovery[b]	4.5 ± 2.0	14.7 ± 2.5	74.1	58.8
Microsomal fraction, % total recovery	2.4 ± 2.0	5.7 ± 2.8	23.7	36.2
Postmicrosomal supernatant % total recovery	93.1 ± 4.1	79.4 ± 6.0	2.2	5.0
Total hormone centrifuged	4.1 ± 0.5 mg	512 ± 63 IU	4.1 μg	262.0 mIU
% total recovery	96.2 ± 2.4	96 ± 2.2	95.7	91.6

[a]Homogenates of five human placental tissues and a homogenate of one human pituitary tissue were centrifuged as outlined in *Materials and Methods*. hPL = human placental lactogen; hCG = human chorionic gonadotropin; hPRL = human prolactin; hLH = human luteinizing hormone.

[b]The percentage of each hormone of the homogenate fractions is expressed as a percentage of the total hormone recovered in the nonnuclear fractions. The amount of each hormone in the nuclear fraction (not shown) was less than 9.5% of the total hormone.

TABLE 2. Differential centrifugation of combined homogenates of human placental and rat pituitary tissues.

Item	hPL[a]		hCG		rPRL		rLH
	Exp I	Exp II	Exp I	Exp II	Exp I	Exp II	Exp I
Mitochondrial fraction, % total recovery[b]	3.6	2.8	10.5	11.4	83.6	85.8	60.8
Microsomal fraction, % total recovery	11.2	10.0	20.3	16.8	10.3	9.0	28.7
Postmicrosomal supernatant % total recovery	85.1	87.2	69.2	71.8	6.1	5.2	10.4
Total hormone centrifuged	0.7 mg	1.1	93.0 IU	136.4	2.0 μg	2.1	11.5 mIU
% recovery	94.9	93.1	100.1	96.0	104.2	100.2	89.2

[a]The distribution of human placental lactogen (hPL), human chorionic gonadotropin (hCG), and rat prolactin (rPRL) was determined in both homogenates, whereas the distribution of rat luteinizing hormone (rLH) was determined only in Experiment I.

[b]In two separate experiments, human placental tissue and rat pituitary tissue were homogenized together and subjected to differential centrifugation, as outlined in *Materials and Methods*. The percentage of each hormone in the homogenate fractions is expressed as a percent of the total hormone recovered in the nonnuclear fractions. In each instance, the amount of hormone in the nuclear fraction (not shown) was less than 9.0% of the total hormone.

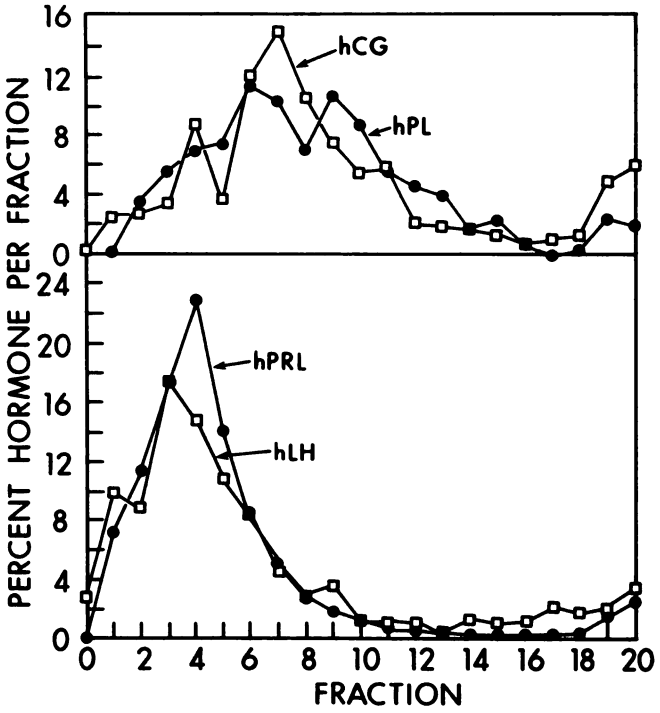

FIG. 1. Density-gradient centrifugation of the mitochondrial fractions of human placental (*top*) and human pituitary (*bottom*) tissues through 0.5–2.0 M sucrose. The amounts of hormone in each of the fractions are expressed as the percentage of the total hormone (human placental lactogen [*bPL*] 180.0 μg; human chorionic gonadotropin [*bCG*] 75.1 U; human prolactin [*bPRL*] 3.0 μg; human luteinizing hormone [*bLH*] 154.0 mU) in the gradient tubes. Density-gradient centrifugation of the microsomal fraction of human placental and pituitary tissues gave nearly identical results. The mitochondrial and microsomal fractions from three other placentas gave similar distributions of hPL and hCG.

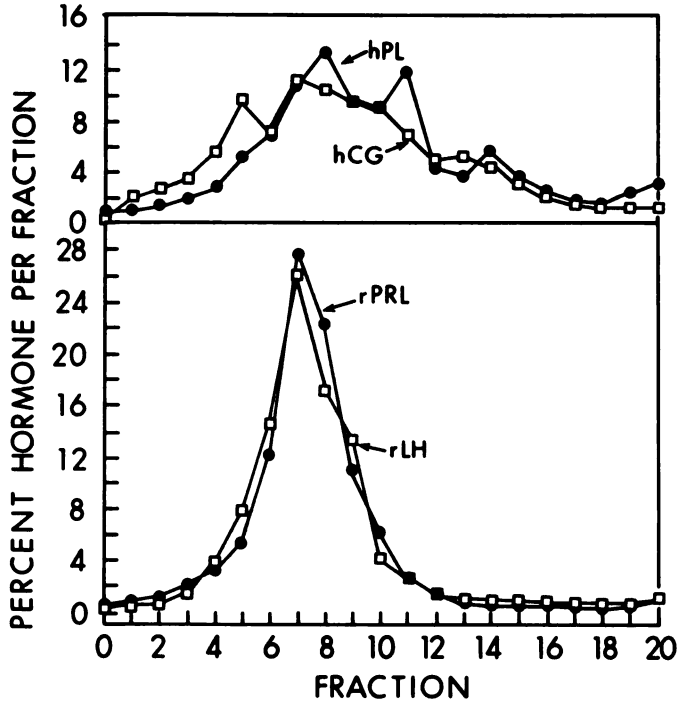

FIG. 2. Density-gradient centrifugation of the mitochondrial fractions of a combined homogenate of human placental (*top*) and rat pituitary (*bottom*) tissues through 0.5–2.0 M sucrose. Human placental and rat pituitary tissues were homogenized together and then subjected to density gradient centrifugation as outlined in *Materials and Methods*. The amounts of the hormones in each fraction are expressed as a percentage of the total amounts of each hormone (human placental lactogen [*bPL*] 28.0 μg; human chorionic gonadotropin [*bCG*] 9.7 U; rat prolactin [*rPRL*] 17.0 μg; rat luteinizing hormone [*rLH*] 6.9 mU) in the gradient tubes. Density-gradient centrifugation of the microsomal fraction of the same combined homogenate gave nearly identical results. The mitochondrial and microsomal fractions from one other combined homogenate gave similar distributions of hPL and hCG.

distributed in sucrose gradients, suggesting that the hormones were not localized in granules but were nonspecifically bound to cellular components. The percentage of hPL in the postmicrosomal supernatant in the five placental homogenates was somewhat higher than that reported by Weinstein et al. (1982) in a single homogenate.

The possibility that the large percentage of hPL and hCG in the postmicrosomal supernatant was due to rupture of secretory granules seems unlikely. In the ultracentrifugation and density-gradient centrifugation studies of the homogenates prepared by homogenizing human placental and rat pituitary tissues together, almost all of the hPL and hCG were detected in the postmicrosomal supernatant, whereas nearly all of the rPRL and rLH were in the particulate fractions. To account for the large percentage of hPL and hCG in the postmicrosomal supernatant, all or nearly all of the placental secretion granules would have had to have been ruptured under homogenization conditions that ruptured few or none of the pituitary secretion granules. Unfortunately, it is not possible to determine by biochemical methods whether the placental secretion granules were ruptured during the homogenizing procedure since biochemical markers for the placental granules are unknown.

Although the present study strongly suggests that hPL and hCG are not stored in secretory granules, our biochemical approach does not indicate the precise intracellular localization of these hormones. Since only relatively small percentages of the hormones were present in the microsomal and mitochondrial fractions, no attempt was made to characterize those fractions further. Although hPL and hCG were not detected in secretion granules, some of the hPL and hCG may have been present in small vesicles involved in the transport of the hormones to the plasma membrane.

The fact that the placental hormones hPL and hCG do not appear to be stored in secretory granules clearly distinguishes these hormones from most protein and polypeptide hormones, including those of the anterior pituitary, parathyroid, pancreas, and thymus (O'Connor et al., 1983). hPL and hCG, however, are not the only protein hormones that are not stored in secretory granules. Recent biochemical and immunohistochemical studies from our laboratories also have demonstrated that decidual prolactin is not stored in granules (Handwerger and Capel, 1985). Like hPL and hCG, decidual prolactin is localized primarily in the postmicrosomal supernatant of tissue homogenates. Furthermore, like placental tissue, decidual tissue does not react to a specific monoclonal antibody to chromogranin. Since hPL and hCG are not stored in secretory granules, differences in the intracellular storage sites of the placental and pituitary protein hormones may explain, at least in part, the differences in the regulation of hormone secretion by these tissues.

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REFERENCES

Dreskin RB, Spicer SS, Greene WB, 1970. Ultrastructural localization of chorionic gonadotropin in human term placenta. J Histochem Cytochem 18:862–74

Handwerger S, Capel D, 1985. Differences in the cell surface antigens of prolactin-producing cells of human decidual and pituitary tissues. J Clin Endocrinol Metab 61:830–33

Handwerger S, Conn PM, Barrett J, Barry S, Golander A, 1981. Human placental lactogen release *in vitro*: paradoxical effects of calcium. Am J Physiol 240:550–55

Handwerger S, Sherwood LM, 1974. Human placental lactogen. In: Jaffe J, Behrman H (eds.), Methods of Hormone Radioimmunoassay. New York: Academic Press, pp. 417–26

Handwerger S, Wilson S, Conn PM, 1984. Different subcellular storage sites for decidual- and pituitary-derived prolactin: possible explanation for differences in regulation. Mol Cell Endoc 37:83–87

Hymer WC, 1975. Separation of organelles and cells from the mammalian adenohypophysis. In: Tixier-Vidal A, Farquhar MG (eds.), The Anterior Pituitary. New York: Academic Press, pp. 137–80

O'Connor DT, Burton D, Deftos LJ, 1983. Immunoreactive human chromogranin A in diverse polypeptide hormone producing human tumors and normal endocrine tissues. J Clin Endocrinol Metab 57:1084–86

Sheppard BL, Bonnar J, 1982. Ultrastructure of the maternal placenta in human pregnancy. Bibl Anat 18:74–79

Tedde G, 1982. Morphometric ultrastructure study of the trophoblast during gestation. Bull Assoc Anat 66:407–18

Thliveris JA, Speroff L, 1977. Ultrastructure of the placental villi, chorion laeve, and decidua perietalis in normal and hypertensive pregnant women. Am J Obstet Gynecol 129:492–98

Tyrey L, Handwerger S, Sherwood LM, 1974. Human chorionic gonadotropin (hCG). In: Jaffe J, Behrman H (eds.), Methods of Hormone Radioimmunoassay. New York: Academic Press, pp. 427–36

Weinstein D, Galski H, Schenker J, Lorberboum H, de Groot N, Ilan J, Folman R, Hochberg AA, 1982. The synthesis and secretion of human placental lactogen (hPL) in cultured term placenta. Mol Cell Endocr 26:189–99

Wynn RM, 1975. Development and ultrastructural adaptations of the human placenta. In: Brosens IA, Dixon G, Robertson WB (eds.), Human Placentation. Amsterdam: Excerpta Medica, pp. 3–21