Gonadotropin Releasing Hormone Does Not Affect Steroidogenesis in JEG-3 Cells*

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ABSTRACT

Gonadotropin releasing hormone (GnRH) affects ovarian and testicular steroidogenesis, is produced by the placenta, and increases chorionic gonadotropin (hCG) production in preterm placenta. It was hypothesized that GnRH might also regulate placental synthesis of progesterone (P) and estradiol (E₂) and the effects were studied of GnRH and the [D-Trp⁶]-LH-RH analog on the production of P, E₂, and hCG by JEG-3 choriocarcinoma cells. Neither GnRH nor [D-Trp⁶]-LH-RH had any effect on the production of P, E₂ or hCG. Our findings are similar to a previous observation which showed GnRH did not increase hCG in term placenta, but differed from preterm placenta in which GnRH increased hCG. This suggests there could be maturational changes in the placental response to GnRH and that JEG-3 cells more closely resemble term placenta. It is our conclusion that although GnRH and the GnRH receptor are found in the placenta, GnRH might have no effect on steroid hormone synthesis near term.

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Introduction

Factors which control the timing of human labor are only minimally understood. Initial theories that a hypothalamic-pituitary-fetal-adrenal axis regulates this process have been challenged by studies documenting the full-term delivery of human infants lacking the pituitary gland in some cases and adrenal glands in others. In humans, a central physiological event occurs at approximately seven weeks gestation and marks the transition from luteal to placental dependence. After this time, progesterone (P), estrogen (E2), oxytocin, and the prostanoids are synthesized by the placenta and play central roles in the maintenance of pregnancy and the onset of labor. In the pregnant ewe, a fall in P accompanied by an increase in E2 and prostaglandin F2α are temporally related to the onset of parturition. In humans, however, a relationship between P or E2 and the timing of labor has not yet been shown.

Current thought suggests that the uterus itself controls human parturition. This alternative theory, proposed by Challis and Mitchell, emphasizes the local synthesis of steroid and peptide hormones that have autocrine, paracrine, and possibly endocrine effects. A change in the local hormone concentration is thought to affect the intrauterine environment without a concomitant change in circulating hormone levels.

This concept has received a great deal of support following the discovery in placental tissues of both pituitary trophic hormone-like proteins [human chorionic gonadotropin (hCG), human chorionic somatomammotropin, and human chorionic corticotropin]; and hypothalamic peptides [gonadotropin releasing hormone (GnRH), somatostatin, corticotropin-releasing hormone, and thyrotropin-releasing hormone].

This discovery has raised the possibility that the feto-placental unit may be largely independent from maternal endocrine factors and subject to regulation by endogenous feedback mechanisms.

Gonadotropin releasing hormone was first identified in the placenta by Gibbons et al in 1975. In addition to GnRH, the GnRH receptor is also expressed in the placenta. Recent studies have shown that GnRH affects steroid hormone production in many steroidogenic tissues including the ovary and the testis, and that GnRH increases the production of hCG by preterm placenta. To our knowledge, however, no study has investigated the possibility that GnRH might also regulate the production of P or E2 by the placenta.

Because GnRH and the GnRH receptor are produced by the placenta, and because GnRH has effects on other steroidogenic tissues, it was hypothesized that GnRH might be important in the regulation of placental steroidogenesis. The present study was designed to examine the effects of GnRH on the production of placental hormones using an in vitro JEG-3 choriocarcinoma cell culture model. This study investigated the in vitro effects of GnRH and the hydrolysis resistant [D-Trp6]-LH-RH analog on the production of P, E2, adenosine 3',5'-cyclic monophosphate (cAMP), and hCG by JEG-3 cell cultures.

Materials and Methods

Approval

This study received prior approval by the Institutional Review Board of the Department of Clinical Investigation, Walter Reed Army Medical Center, Washington, DC.

Materials

JEG-3 choriocarcinoma cells were obtained from American Type Cell Culture* and routinely maintained as previously described. Materials to perform radioimmunoassays for P, E2, and hCG were obtained from ICN Bio-

* Catalog Number ATCC HTB 36, American Type Cell Culture, Rockville, MD 20852.
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medicals.† Gonadotropin releasing hormone (GnRH) and [D-Trp⁶]LH-RH were obtained from Sigma Chemical Co.,‡, and used as supplied. Materials for the determination of cAMP by radioimmunoassay were obtained from Amersham Corp.§ All other chemicals were of reagent grade and obtained from standard vendors.

CELL CULTURES

The JEG-3 cells were maintained in complete media (Media) which consisted of minimum essential medium (MEM) supplemented with 10% fetal bovine serum, penicillin (50 U/ml) and streptomycin (0.05 mg/ml). For hormone studies, cells were plated (1 x 10⁶ cells/cm²) into Costar multiwell (12 well) culture plates. On the day of study, media were replaced with serum free media (SFM) containing peptides as indicated.

BASELINE HORMONE PRODUCTION

To determine the production of P and hCG, JEG-3 cell cultures were divided into groups of 12 each, and incubated in either (a) control serum free media (SFM), (b) SFM containing GnRH (1 to 1000 ng/ml), or (c) SFM containing [D-Trp⁶]-LH-RH (1 to 1000 ng/ml). Cultures were then incubated for 24 hours, after which, the media were collected for determination of P and hCG content. The cells were digested (1 M NaOH) for determination of cell protein.

To determine the production of E₂, testosterone (T, 0.5 µM) was added to all cultures as a substrate for aromatization.²⁷ Cells were incubated in either (a) SFM containing T, (b) SFM containing T plus GnRH (1 to 1000 ng/ml), or (c) SFM containing T plus [D-Trp⁶]-LH-RH (1 to 1000 ng/ml). Media and cells were collected and analyzed in a fashion similar to that outlined above.

CYCLIC NUCLEOTIDE PRODUCTION

To determine the accumulation of cyclic adenosine monophosphate (cAMP), cell cultures (n = 12 for each condition) were incubated 15 min in either SFM or SFM containing GnRH (1000 ng/ml), and then extracted with ethanol (95%, 0°C, 10 min). The extracts were evaporated to dryness and the cAMP content determined by radioimmunoassay. The cell pellets were digested (1 M NaOH) and used for determination of cell protein.

CELL VIABILITY

Cell viability was determined following all incubations by trypan blue exclusion.²⁷

DETERMINATION OF PROTEIN CONTENT

At the end of all incubations, cells were digested in NaOH (0.5 ml, 1M O M) and analyzed for protein content using the method of Lowry.³⁰

STATISTICAL ANALYSIS

Statistical significance for the differences between groups was assessed using one-way analysis of variance (ANOVA), without repeat measure. All experiments were performed in triplicate. Data are expressed as the mean ± standard error of the mean (SEM).

Results

Cell viability, as determined by trypan blue exclusion, was >95% in all cultures. There was no effect of either GnRH or [D-Trp⁶]-LH-RH on cell viability.

In figure 1 are shown the results of P synthesis. The JEG-3 cells constitutively produced significant amounts of P, but there was no effect of either GnRH or [D-Trp⁶]-LH-RH at any of the test concentrations.

† Catalog Numbers 07-170102 (P), 07-138102 (E₂), and 06B-254355 (hCG) ICN Biomedicals, Costa Mesa, CA 92626.
‡ Catalog Numbers L-7134 (GnRH) and L-9761 [D-Trp⁶]-LH-RH Sigma Chemical Co, St Louis, MO 63175.
§ Catalog Number TRK-432, Amersham Corporation, Arlington Heights IL, 60005.
In figure 2 are shown the results of hCG synthesis. Again, there was no effect of either GnRH or [D-Trp⁶]-LH-RH at any of the tested concentrations.

In figure 3 are shown the results of E₂ production. As previously shown,²⁷ with the addition of T, JEG-3 cells produced significant amounts of E₂. There was no effect, however, of either GnRH or [D-Trp⁶]-LH-RH at any of the tested concentrations.

The accumulation of cAMP was also determined in control cells and cells incubated with GnRH (1000 ng/ml). There was no difference between the cAMP content of the control cells (0.65 ± 0.07 pmol/mg cell protein) and the cells incubated with GnRH (0.59 ± 0.08 pmol/mg cell protein).

**Discussion**

It is well known that GnRH is produced in the hypothalamus and released into the hypophyseal portal system from which it binds to specific receptors in the pituitary.³¹ How-
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FIGURE 3. The effect of gonadotropin releasing hormone (GnRH) and [D-Trp6]-LH-RH on estradiol synthesis. Cultures were divided into groups of 12 each, and incubated 24 hr in either (a) serum free media (SFM) + testosterone (T) (0.5 μM, open bars), (b) SFM containing T + GnRH (1 to 1000 ng/ml, cross hatched bars), or (c) SFM containing T + [D-Trp6]-LH-RH (1 to 1000 ng/ml, solid bars). Estradiol was determined by radioimmunoassay, and the data are presented as ng/mg cell protein (mean ± standard error of the mean [SEM]). There is no significant difference for any comparison.

ever, peripheral GnRH levels are normally undetectable. Recent studies have reported that the placenta,20,21,22,23 as well as several tumor cell line, (JEG-3, MDA-MB-231 and ZR-75-1 breast tumor cells)32,33 produce GnRH and the GnRH-receptor. Transcription of the pro-GnRH gene in reproductive tissues is regulated from a unique start site whose location is upstream from the well characterized hypothalamic start site.34 These observations suggest that GnRH is produced locally in the reproductive tract where it may have an autocrine or paracrine action.25

Our laboratory has a long-standing interest in the relationships between chorioamnionitis, activation of the immune system, and the induction of preterm labor. It has been shown that macrophage conditioned media and tumor necrosis factor-α (TNF-α) inhibit P and stimulate E2 synthesis by both placental explants and JEG-3 cell cultures.37,35 This is consistent with the hypothesis that steroid hormone synthesis is critical for maintenance of pregnancy and that immune modulators such as TNF-α could have an effect on labor through changes in steroid hormone production.

The current study was undertaken to examine whether or not GnRH might also effect placental steroid hormone synthesis using this JEG-3 cell culture model. Since GnRH and its receptor are produced in the placenta, it was hypothesized that GnRH is a likely candidate to have paracrine or autocrine effects in this system.

Our results show that GnRH (1 to 1000 ng/ml) appears to have no effect on basal hormone synthesis by JEG-3 cell cultures. This observation is similar to a previous study using term placenta in which GnRH had no effect on hCG production.25 The GnRH, however, can be rapidly hydrolyzed and might therefore have no effect in these in vitro cell culture systems which are known to hydrolyze other peptides.36 For this reason, the same experiments were performed using the [D-Trp6]-LH-RH analog which is resistant to hydrolysis and has a prolonged serum half-life compared to the unsubstituted GnRH molecule.29 As with native GnRH, [D-Trp6]-LH-RH also had no effect on placental steroid hormone synthesis.

These results suggest that although GnRH and the GnRH receptor are produced in the placenta, GnRH appears to have no effect on steroid hormone synthesis. Our observations are limited, however, by several factors. First, they are based on an in vitro system which uses JEG-3 choriocarcinoma cells as a model of placental steroidogenesis. These cells are
thought to be an appropriate model since they produce steroid hormones and respond to some of the factors which affect placental function. However, JEG-3 cells are malignant and have no known trophic hormone regulators.

The GnRH is believed to have a complex mechanism of action. In pituitary gonadotrophs, GnRH activates a specific G-protein (Gq), enhances phosphoinositide turnover, and increases the levels of inositol triphosphate (IP3), diacylglycerol, [Ca2+]i, protein kinase C, arachidonic acid, and leukotriene C4. Cross-talk between all of these regulators then determines the extent of activation of gonadotrophin secretion. In other cell types, however, GnRH signals through alternate mechanisms. In transfected GH3 pituitary cells, GnRH receptors are coupled to the generation of cAMP, and desensitization after tonic GnRH exposure occurs distal to the binding of GnRH, the regulation of GnRH receptors, and the generation of cAMP. The repertoire of intracellular messenger for JEG-3 cells is not fully known. However, one of the principal regulators of JEG-3 cell function is cAMP. For this reason, the accumulation of cAMP following exposure of JEG-3 cells to GnRH was also examined. There was no change in cAMP levels in response to either GnRH or [D-Trp6]-LH-RH. These observations suggest that JEG-3 cells could have an interrupted GnRH signal transduction system which abrogates the response to GnRH. Taken together, these data suggest that GnRH might fail to have an effect on JEG-3 cells but might still have an effect on placental function.

Second, it is possible there could be matu-rational changes in the ability of the placenta to respond to GnRH. Previous studies using preterm placenta have shown that GnRH increases the production of hCG. In contrast, GnRH had no effect on hCG production by term placenta. The number of GnRH receptor binding sites changes during gestation, increasing during the first trimester and then declining throughout the remainder of gestation. Term human placenta binds very little GnRH, and responds only minimally to GnRH exposure. This correlates very closely with the production of hCG, and the hCG response to GnRH stimulation. To our knowledge, no studies have yet investigated the number and affinity of GnRH binding sites on JEG-3 cells as compared to placenta of different gestational ages. The current data, however, suggest that JEG-3 cells more closely resemble term rather than pre-term placenta.

Lastly, previous investigations have shown that GnRH modulates steroidogenesis in other cell systems after the cell culture media has been “primed” with either calcium (0.25 to 1 nM) or gonadotropins. No experiments were performed in which the cell culture media was primed in this manner, because MEM contains calcium, and JEG-3 cells are now known to respond to gonadotropins.

Based on these observations, it is our conclusion that GnRH has no direct effect on basal hormone production by JEG-3 cell cultures. This observation suggests that JEG-3 choriocarcinoma cells may behave in a manner similar to term placenta.

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