Inhibin and activin modulate the release of gonadotropin-releasing hormone, human choriionic gonadotropin, and progesterone from cultured human placental cells

(follitostatin/follicle-stimulating hormone-releasing protein/follitropin/follicle-stimulating hormone)

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ABSTRACT Although it is clear that human choriionic gonadotropin (hCG) and progesterone play fundamental roles in pregnancy, the regulation of placental production of these hormones remains to be defined. Recent evidence suggests that the human placenta expresses proteins related to inhibit (αβ subunits) or activin (ββ subunits). Inhibin and activin (follicle-stimulating hormone-releasing protein) possess opposing activities in several biological systems including placental follicle-stimulating hormone (follitropin) secretion, erythroid differentiation, and gonadal sex-steroid production. The actions of purified inhibin and activin on hormonogenesis by primary cultures of human placental cells were studied. The addition of activin increased gonadotropin-releasing hormone (GnRH) and progesterone production and potentiated the GnRH-induced release of hCG. Inhibin by itself did not modify placental immunoreactive GnRH, hCG, and progesterone secretion but reversed the activin-induced changes. Neither inhibin nor activin influenced the release of human placental lactogen. Furthermore, transforming growth factor β, structurally related to inhibin/activin, did not significantly influence hormone release from cultured placental cells. These results support the hypothesis that inhibin and activin may play a role in regulating the release of GnRH, hCG, and progesterone from placenta and implicate inhibin-related proteins in the endocrine physiology of human pregnancy.

Inhibins are heterodimeric proteins, consisting of an α subunit and one of two β subunits (βA or βB), that were originally identified based upon their abilities to selectively suppress follicle-stimulating hormone (FSH) secretion (1-6). FSH-releasing proteins or activins were subsequently isolated and characterized as dimers comprised of inhibit β subunits (βAβA or βBβB) (7, 8). Although inhibin and activin were first isolated from the gonads, various tissues including the placenta contain inhibit subunit mRNAs (9-12). Whereas the gonads contain a large excess of α over β-chain mRNAs, the placenta has an excess of β-subunit mRNAs; such observations raise the possibility that the placenta might have a preponderance of βB dimers (12). However, there is not yet conclusive evidence that intact activin is synthesized by human placenta.

Placental inhibit α-subunit immunoreactivity is localized in the cytrophoblast layer of the vill (11), a region shown earlier to contain other regulatory peptides including gonadotropin-releasing hormone (GnRH; refs. 13-20), somatostatin (21), and corticotropin-releasing factor (22). The evidence that the placenta produces these hypophysiotropic peptides, which modulate the secretion of various hormones related to those in the pituitary (human chorionic gonadotropin (hCG), human placental lactogen (hPL), and corticotropin), supports the hypothesis that placental hormonogenesis may be regulated in part by locally produced peptides (13-22).

A recent report (11) showed that the addition of inhibin antiserum increased hCG production in primary human placental cultures, suggesting that endogenous inhibit might tonically inhibit secretion of the placental gonadotropin. To evaluate the possible roles of inhibin-related proteins in the regulation of placental hormones more directly, we have investigated the effects of inhibit and activin on the secretion of GnRH, hCG, and progesterone by cultured placental cells. Moreover, because inhibit and activin are structurally related to the transforming growth factor β (TGF-β) family of growth factors (23) and because TGF-β and its receptors are present in human placenta (24, 25), we also evaluated the possible action of TGF-β on placental hormone release.

MATERIALS AND METHODS

Preparation of Placental Cell Cultures. Placentae were obtained from pregnant women (n = 7) undergoing elective caesarean section at term. Permission to obtain the tissue was granted by the Human Investigation Committee of University of California, San Diego and The Salk Institute.

Immediately after the collection, a placenta was placed on ice and chunks of tissue were minced (50-70 g). After being rinsed three times in cold Hepes dissociation buffer (HDB; ref. 26), chunks were dissected free of membranes, and connective tissue and the soft tissues were minced. Cells were dissociated with 50 ml of HDB containing collagenase solution [0.1% bovine serum albumin/0.4% collagenase (type II, Coop Biomedical)/300 Kunitz units of DNase II (type IV, Sigma)] in a water-jacketed Spinner suspension flask (Wheaton Scientific). The dissociation mixture was maintained at 37°C and continuously stirred at 200-300 rpm for 1 hr. Trypsin (0.4%; Sigma) was then added, and the solution was stirred for an additional 10 min. The tissue fragments were filtered through a 53-μm mesh filter (Spectrum Medical Industries). Filtered cells were transferred to a sterile plastic tube and centrifuged at 300 X g for 10 min. The resultant supernatant was further centrifuged at 400 X g for 10 min. The pelleted cells from both centrifugations were resuspended in culture medium (β-PJ containing 10% fetal bovine serum; ref. 16). The remaining unfiltered cells and tissue fragments were further digested with trypsin for 10 min and subjected to the above procedure. All cells obtained were

Abbreviations: hCG, human choriionic gonadotropin; GnRH, gonadotropin-releasing hormone; FSH, follicle-stimulating hormone; TGF-β, transforming growth factor β; hPL, human placental lactogen; rGnRH, immunoreactive GnRH.

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resuspended in the culture medium and plated in 35-mm six-well multiwell dishes (Costar) previously coated with poly(d-lysine) (Sigma). This procedure yielded 3 × 10^6 cells per g of tissue, and 5.0–6.5 × 10^6 cells were plated per well.

**Experimental Procedures.** The cultures were maintained at 37°C in a water-saturated atmosphere containing 5% CO₂; the culture medium was changed every 2 days. The secretion experiments were conducted between 5 and 10 days after plating. The incubation medium consisted of B-PJ medium supplemented with 0.1% bovine serum albumin. Treatments were added in a small volume (<50 μl) to triplicate wells containing 1.0 ml of incubation medium. The incubation time was 48 hr.

**Peptide and Protein Hormones.** Purified ovine inhibin (inhibin αβ heterodimer) was isolated from rete testis fluid by immunoaffinity chromatography using an antiserum to the amino-terminal portion of the porcine inhibin α subunit (27). On NaDodSO₄/PAGE, purified nonreduced ovine inhibin shows a single band of Mr 23,000; after reduction, two bands of Mr 21,000 and Mr 14,000 are observed. Porcine activin, a homodimer of inhibin βα, was purified to homogeneity from porcine follicular fluid as described by Vale et al. (7). On NaDodSO₄/PAGE, purified porcine activin shows a single band of Mr 28,000 nonreduced and a single band of Mr 14,000 upon reduction. GnRH was prepared by solid-phase methodology (28). hCG, α-subunit hCG, and the antiserum against hCG were gifts of the National Hormone and Pituitary Program of the National Institute of Diabetes and Digestive and Kidney Diseases. TGF-β was purchased from R & D Systems, (Minneapolis).

**Placental Hormone RIA.** The GnRH RIA used a rabbit anti-[Lys⁸]GnRH as first antibody (1:400,000 final dilution) and a sheep antiserum to rabbit gamma globulins as precipitating antibody (16). Synthetic GnRH was used as the standard and for preparation of tracer (^{125}I-labeled by the chloramine-T method and HPLC-purified). The assay sensitivity was 0.5 pg per tube; the ED₀ was 20 ± 0.5 pg per tube. The inter- and intraassay coefficients of variation were 7.5% and 3.3%, respectively.

hCG was measured by double-antibody RIA (Diagnostic Products, Los Angeles). Tracer bound to antibody was precipitated with sheep anti-rabbit gamma globulins and 10% polyethylene glycol. The assay was standardized against two World Health Organization preparations: the First International Reference Preparation for Immunoassay 75/537 and the Second International Standard for Bioassay 61/6. This assay did not recognize GnRH, hPL, corticotropin, or corticotropin-releasing factor and showed 0.2% cross-reactivity with luteinizing hormone. The assay sensitivity was 1 international milliunit/ml; the inter- and intraassay coefficients of variations were 4% and 2%, respectively.

The hPL concentrations were measured by using a Coat-A-Count solid-phase RIA kit (Diagnostic Products). This assay did not cross-react with hCG, GnRH, growth hormone releasing factor, or growth hormone and only 0.005% with prolactin. The assay was standardized against the World Health Organization First International Reference Preparation for Immunoassay 73/545. The assay sensitivity was 25 ng/ml. The inter- and intraassay coefficients of variations were 5% and 2.5%.

Placental hormone concentrations were measured by using a Coat-A-Count solid-phase RIA kit (Diagnostic Products). This assay did not recognize estradiol, androstenedione, dehydroepiandrosterone, or cortisol. The assay sensitivity was 50 pg/ml. The inter- and intraassay coefficients of variations were 4% and 2%, respectively.

**Statistical Analysis.** Each data point represents the mean ± SEM of three wells assayed in duplicate. Statistical analysis of the results was performed by using analysis of variance, followed by the multiple-range test of Duncan and by Tukey’s test.

**RESULTS**

The addition of purified ovine inhibin to the cultured placental cells did not alter the basal secretion rate of hCG but reduced the GnRH-induced release of hCG (Fig. 1). Activin, which only slightly and not significantly increased the release of hCG when added alone, strongly potentiated the stimulatory action of GnRH on hCG secretion (Fig. 2). This effect was dose-related (Fig. 2) and was reversed by inhibin (Fig. 1).

While inhibin did not modify the immunoreactive GnRH (irGnRH) concentrations in placental cell cultures, the highest doses of activin (105 pM and 350 pM) significantly increased the secretion of irGnRH (from 12.2 ± 2.5 to 42.2 ± 3.1 and 53.3 ± 4.6 pg of irGnRH per ml, respectively). Inhibin (90 pM) completely reversed the activin-induced irGnRH increase. Moreover, activin stimulated progesterone release from placental cultures in a dose-related manner (Fig. 3). Inhibin did not significantly change progesterone concentration but completely reversed (90 pM) the effect of activin on progesterone release (Fig. 3).

The addition of moderate doses of TGF-β (from 4 to 400 pM) neither altered the release of hCG, irGnRH, and progesterone from cultured placental cells nor influenced the action of activin and/or of inhibin on the secretion of these hormones (data not shown). Only the highest concentrations of TGF-β (4 nM) significantly increased secretion of irGnRH (from 11.5 ± 2.6 to 24.7 ± 3.3 pg of irGnRH per ml).

Neither inhibin nor activin nor TGF-β affected the release of hPL from cultured placental cells (data not shown).

**DISCUSSION**

These results show that inhibin and activin influence hormone secretion from cultured human placental cells. Activin

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**Fig. 1.** The effect of GnRH (**○**) on hCG release from cultured human placental cells is increased by 105 pM activin (**△**) and inhibited by 90 pM inhibin (**●**). The activin-induced potentiation of GnRH effects on hCG is also reversed by the addition of inhibin (**●**). Each point represents the mean ± SEM of three wells assayed in duplicate.
increases the release of GnRH and progesterone and augments the release of hCG induced by GnRH. Although inhibin alone does not directly affect the spontaneous secretion of the various placental hormones measured, the effects of activin on GnRH, hCG, and progesterone production were reversed by inhibin. These results agree with previous observations showing functional antagonism between inhibin and activin in various tissues. Indeed, the two dimers exert opposite effects on FSH release from pituitary gland (7), on androgen production by the gonads (29), on the induction of hemoglobin synthesis in a human erythroleukemic cell line, and on cell proliferation in human bone marrow (30). The evidence that neither dimer influenced the secretion of hPL and that moderate doses of the related protein, TGF-β, did not influence the release of the hormones from cultured placental cells supports the specificity of the actions of inhibin and activin in this system.

There is immunologic and chromatographic evidence for the presence of inhibin in placental extracts and cultures (11). Although activin has not yet been demonstrated to be produced in the placenta, the excess of β-subunit relative to α-subunit mRNAs in that tissue (12) would favor the formation of ββ rather than αβ dimers (31). The presence of mRNAs for inhibin subunits in human placenta (9, 12) indicates that inhibin and activin could be locally produced. The effects of inhibin and activin on placental hormone release from cultured cells may reflect autocrine or paracrine effects of the locally produced peptides. Although an endocrine effect of circulating inhibin or activin cannot be excluded, the placenta itself has been shown to be the major source of circulating inhibin during pregnancy (32).

Even though the actions of these dimers on placental functions remain to be explored in vivo, the present studies suggest that activin and inhibin, by modulating the secretion of placental GnRH, hCG, and progesterone, could play a role in the regulation of pregnancy.

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