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Steroid–Protein Interaction in Human Placenta

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Human placenta produces a large variety of bioactive substances with endocrine and neural competence: pituitary and gonadal hormones, hypothalamic-like releasing or inhibiting hormones, growth factors, cytokines and neuropeptides. The most recent findings indicate that locally produced hormones regulate the secretion of other placental hormones supporting a paracrine/autocrine regulation. In placental endocrinology, a particular relevance is played by steroid hormones. In fact, a specific gonadotropin-releasing hormone (GnRH)–human chorionic gonadotropin (hCG) regulation of placental steroidogenesis has been proposed as a placental internal regulatory system acting on steroids production from human placenta. In addition, activin and inhibin have been proposed as further regulatory substances of the synthesis and secretion of steroids; the addition of activin A to placental culture augments GnRH, hCG and progesterone, and this effect can be significantly reduced by the addition of inhibins. Finally, a steroid–steroid interaction is suggested by the evidence that placental estrogen has a positive role in the regulation of progesterone biosynthesis. Other steroid–protein interactions have been observed in human placenta. In fact, recent data indicate that progesterone inhibits placental corticotropin-releasing factor (CRF) and estrogens act on placental conversion of cortisol to cortisone, activating cortisol secretion by the fetal adrenal and enhancing fetal adrenal function with advancing gestation.

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INTRODUCTION

Human placenta produces a large variety of bioactive substances with endocrine and neuronal competence: pituitary and gonadal hormones, hypothalamic-like releasing or inhibiting hormones, growth factors, cytokines and neuropeptides [1–4]. The most recent findings indicate that in the study of placental endocrinology a new concept has to be introduced: production of placental hormones is no longer seen as autonomous, an increasing number of *in vitro* studies point to paracrine/autocrine regulation. Thus, locally produced hormones regulate the secretion of other placental hormones [3].

A particular relevance in placental function is played by steroid hormones. The role of steroid hormones in the physiology of pregnancy is suggested by several observations. Estrogens and progesterone are involved in the events related to initiation and maintenance of pregnancy, while an action of glucocorticoids is suggested at parturition. The regulation of steroid hormones secretion and their possible effect on other hormonal activities will be reviewed in the present paper.

PARACRINE ACTIVITY OF PROGESTERONE AND ESTROGENS

A series of proteins and peptides have been proposed to modulate progesterone and estrogen production by placental cells, as well as these, steroid hormones may influence the synthesis and release of proteins and peptides from placental cells.

A classical observation is that human chorionic gonadotropin (hCG) stimulates progesterone release from corpus luteum and placental cells. In the last decade, it has been demonstrated that a placental

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gonadotropin-releasing hormone (GnRH) actively stimulates the release of hCG from cultured trophoblasts [5–7], acting on specific GnRH-binding sites [8]. A GnRH–hCG regulation of placental steroidogenesis has been proposed as an internal placental regulatory system (Fig. 1). Although hCG stimulates progesterone production, GnRH inhibits the formation of progesterone and estrogen [9, 10]. The presence of androgens attenuates progesterone production by the inhibitory effect on 3β-hydroxysteroid dehydrogenase Δ^5,Δ^4-isomerase enzyme (3β-HSD), thereby reducing the formation of progesterone from pregnenolone [10, 11].

Vice versa, a positive effect of estrogen and a negative effect of progesterone on basal and GnRH-stimulated hCG release and hCG mRNA levels has been shown [10, 12, 13]. From these data, an interesting hypothesis suggests that the physiological decline of hCG secretion after 10 weeks of gestation may be causally related to the inhibitory effect of progesterone [13].

Inhibin, a glycoprotein hormone synthesized in the trophoblast, regulates hCG and GnRH activity [14]. The addition of inhibin antiserum to placental cell culture increases hCG and GnRH release. Therefore, inhibin plays an inhibitory role in regulating hCG release [15, 16]. The suppressive effect of inhibin on hCG secretion may be expressed in the later part of pregnancy when inhibin levels in maternal serum reach the highest values.

The family of inhibin-related peptides also includes activins. Activins are glycoprotein hormones/growth factors which have a stimulatory effect on hCG secretion. The addition of activin A to placental culture augments GnRH, hCG and progesterone, and this effect can be significantly reduced by the addition of inhibin [14, 17].

The interaction with steroid hormone is reciprocal. In fact, the GnRH release induced by the addition of activin is significantly decreased in cell cultures preincubated in the presence of increasing doses of progesterone and potentiated when estradiol is added to culture medium [18]. The effect of estriol is blocked by tamoxifen or progesterone, while RU-486 reverses the inhibitory influence of progesterone [18]. These results suggest an inhibitory influence of placental progesterone and a stimulatory effect of estrogens on the activin action of the GnRH–hCG axis.

This functional interaction of inhibin, activin and GnRH in placental cells is supported by morphological findings showing a common localization of the mRNAs for the three hormones in placental villi at term [19]. The colocalization of immunoreactive inhibin, activin and GnRH in the cytoplasm of trophoblast cells further supports the hypothesis of a paracrine/autocrine interaction of these three regulatory factors within human trophoblast.

The picture is becoming even more complex following recent findings. Indeed, another FSH-suppressing protein hormone, follistatin, has been found in placental and decidual cells [20]. The addition of recombinant human follistatin does not induce significant changes of hCG or progesterone release from cultured human placental cells [20]. However, the dose-dependent activin A-induced release of hCG or progesterone is completely reversed by the presence of follistatin (Fig. 2). Progesterone has no effect in modulating immunoreactive follistatin release [20]. Follistatin in the human placenta is localized in the external syncytial layer of placental villi, the same cells that contain immunoreactive activin [15, 19] and produce hCG and progesterone [21]. This cellular localization correlates with the action of follistatin as activin A binding protein [20].

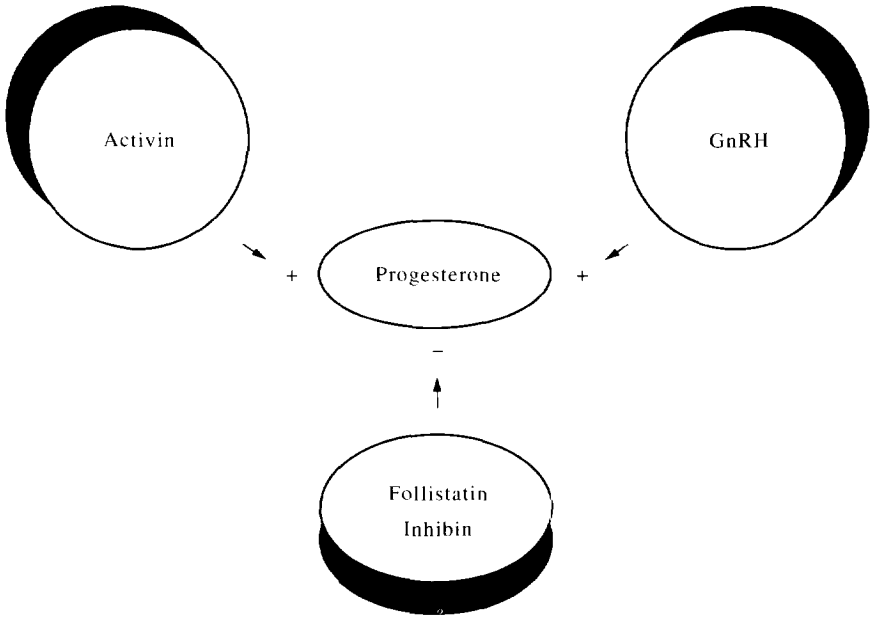

Fig. 1. Progesterone release from cultured human placental cells is modulated by peptides and proteins.

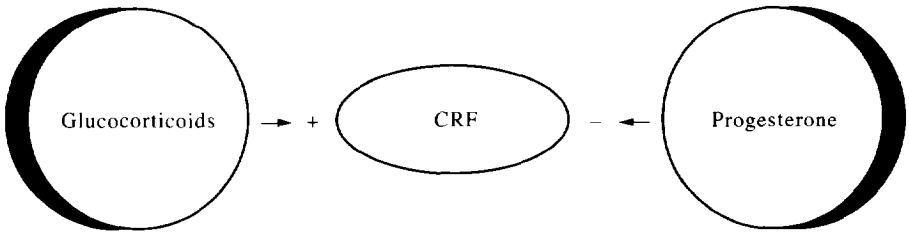

Fig. 2. Glucocorticoids stimulate and progesterone inhibits corticotropin-releasing factor (CRF) from cultured human placental cells, fetal cells, fetal membranes or decidual cells.

Other substances have been demonstrated to modulate progesterone release from placental cells. A stimulatory effect is provided by β_2-adrenergic while an inhibitory action is correlated with high concentrations of dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) [9, 22].

Finally, a steroid–steroid interaction is suggested by the evidence that placental estrogen has a positive role in the regulation of progesterone biosynthesis. This local control via autocrine or paracrine actions is accomplished by two interdependent mechanisms: estrogen (1) induces an increased low-density lipoprotein (LDL) receptor uptake for LDL; and (2) promotes cytochrome *P*450 enzyme-dependent side-chain cleavage ($P450_{scc}$) enzymatic activity. These effects of estrogens may thus accelerate the biosynthesis pathways for progesterone production [11].

EFFECTS OF GLUCOCORTICOIDS ON PLACENTAL CORTICOTROPIN-RELEASING FACTORS

The hypothalamus–pituitary–adrenal (HPA) axis plays an important role in pregnancy. In pregnant women cortisol secretion increases throughout gestation, both at serum and urinary free cortisol levels. Corticotropin-releasing factor (CRF), proopiomelanocortin (POMC), adrenocorticotropic hormone (ACTH), β-endorphin (β-EP), β-lipotropin (β-LP), and α-melanocyte-stimulating hormone (α-MSH) are the components of the HPA axis produced by human placenta [23–27]. The CRF extracted from human placenta stimulates the release of ACTH and βEP from rat pituitary cells from placental cells [28, 29]. This effect of CRF is mediated through CRF receptors in the placenta and can be inhibited by a synthetic antagonist to CRF [29]. Paracrine/autocrine control of placental CRF has been shown. The addition of prostaglandins, neurotransmitters, neuropeptides and cytokines stimulates the release of CRF from cultured placental cells. Both prostaglandins F_2 (PGF_2) and E_2 (PGE_2) increase CRF concentration in the culture medium, with a dose-dependent effect [29]. Norepinephrine (Ne) and acetylcholine (ACh) are active neurotransmitters in increasing CRF release [30]. Human placenta synthesizes ACh and contains higher ACh concentrations than in mammalian brain tissue [31–33]. The close correlation between hypothalamic [34] and placental regulation of CRF release is further supported by the evidence that angiotensin-II, arginine vasopressin or oxytocin increase the release of placental CRF from cultured trophoblasts [30]. Neurotransmitters and neuropeptides are involved in stress-induced neuroendocrine CRF responses [34]. Prostaglandins and glucocorticoid hormones also increase placental CRF release *in vitro* [29, 35], and in view of the important role of prostaglandins and cortisol at parturition, the activation of placental CRF during labor is strongly suggested. In agreement with the regulation of the hypothalamic CRF, interleukin-1 stimulates the release of CRF from cultured placental cells [30]. This regulatory function led us to postulate a neuroendocrine-immune interaction in human placenta.

The interaction between glucocorticoids and CRF deserves particular attention (Fig. 2). Unlike the classical HPA axis, the addition of glucocorticoids does not modify CRF-induced ACTH release [29]. A relevant finding indicates a significant increase of CRF concentration in culture media of placental cells following the addition of dexamethasone, cortisol or corticosterone [35, 36]. Glucocorticoids also stimulate CRF release from amnion, chorion and decidua [36]. The addition of dexamethasone increases CRF mRNA expression in cultured human placental cells at term [35]. These data indicate that glucocorticoids as well as several other stress factors actively stimulate placental CRF release, suggesting an involvement of placenta in the response to the stress of parturition. The marked increase in CRF expression at the end of gestation and the capacity of glucocorticoids to enhance this expression could explain the hypothesis of a biological role for CRF in the fetoplacental unit and in parturition [35]. The rise in placental CRF that precedes parturition could result from the rise in fetal glucocorticoids that occurs at this time, the increase in placental CRF may stimulate, via fetal ACTH, a further rise in fetal glucocorticoids, completing a positive feedback loop that would be terminated by delivery.

Recent data indicate that progesterone inhibits placental CRF release [36]. Increasing progesterone concentration resulted in a significant decrease of CRF concentration which resulted in a culture medium of trophoblast decidua, amnion and chorion at term. This

effect has also been confirmed in tissues connected at an early gestation [37].

CRF stimulates the trophoblast release of ACTH [29], thus suggesting the existence of a CRF–ACTH axis. However, maternal plasma ACTH levels do not increase during pregnancy in parallel with CRF expression and secretion. This disparity can be explained by the presence of a CRF-binding protein (CRF-BP) that binds circulating CRF and renders it unavailable to the corticotrope receptors [38, 39]. Recently, local production of CRF-BP and the expression of CRF-BP mRNA have been demonstrated in human trophoblast and intrauterine tissues [40], possibly representing a mechanism to control CRF activity in target tissues during pregnancy.

From early to mid gestation placental 11β-HSD catalyzes interconversion of cortisol and to its biologically inactive metabolite, cortisone, favouring the formation of cortisol. The passage of cortisol to the fetus inhibits ACTH release by the fetal adrenal. In primates it has been shown that increasing estrogen production in the second half of gestation, enhanced 11β-HSD oxidative activity occurs resulting in increased placental conversion of cortisol to cortisone [41]. As a consequence, the fetal hypothalamic–ACTH axis becomes disinhibited and cortisol secretion by the fetal adrenal is activated [42]. These data strongly suggest that placental estrogen plays a role in enhancing fetal adrenal function by converting cortisol to biologically inactive cortisone with advancing gestation.

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