Immunohistochemical analysis of corticotropin-releasing hormone (CRH) in human trophoblast of normal pregnancy and hydatidiform mole

Eisaku Okamoto, Nagatoshi Sugita
Department of Obstetrics and Gynecology, Sakai Municipal Hospital
Tetsu Takagi, Itsuko Iwata, Eriko Nishino
Nobuaki Mitsuda, Osamu Tanizawa
Department of Obstetrics and Gynecology, Osaka University Medical School
(Accepted: January 23, 1990)

Immunoreactive corticotropin-releasing hormone (IR-CRH) in maternal plasma increases progressively during pregnancy and decreases rapidly after delivery, suggesting that IR-CRH is produced in the placenta. We studied immunohistochemical localization of CRH in developing human placenta, decidua, the amniotic membrane and a fresh surgical specimen of hydatidiform mole by Avidin-Biotin Complex staining. Our results were as follows:

1. IR-CRH was localized in cytotrophoblasts of the placenta, and decidua in the first trimester, and in the amniotic membrane at term.

2. Immunohistochemical localization of CRH was not detected in trophoblasts of a hydatidiform mole.

These results suggest that the sources of the increased level of IR-CRH in human plasma and amniotic fluid during pregnancy are the placenta, decidua and amniotic membrane. The production of IR-CRH in trophoblast of hydatidiform mole may be suppressed because of the characteristic change of trophoblast.

Key words: corticotropin-releasing hormone (CRH), Avidin-Biotin Complex staining, immunohistochemical localization.

Introduction

Corticotropin-releasing hormone (CRH) was originally supposed to be a product of the hypothalamus (Saffran 1955, Guillemann 1955), and Vale et al. purified ovine-CRH for the first time from ovine hypothalamus in 1981 (Vale 1981). Soon afterwards the amino acid sequence and primary structure of the biosynthetic precursor of human CRH were determined (Shibahara 1983). Subsequently, Sasaki et al. reported that the concentration of immunoreactive (IR)-CRH in human plasma increases progressively during pregnancy and decreases rapidly after delivery (Sasaki 1987), and supposed that the IR-CRH was produced by the placenta.

In this paper, we studied the localization of IR-CRH in human placenta, decidua, amniotic membrane and hydatidiform mole by the use of immunohistochemical method.

Materials and methods

Placenta and decidua at 10 weeks of pregnancy was obtained from women who underwent therapeutic abortion. Placenta and amniotic membrane at 40 weeks of pregnancy
were obtained from woman who underwent repeated, elective cesarean section. A hydatidiform mole, obtained after 10 weeks of pregnancy, was used as a specimen of trophoblastic disease. All patients gave their informed consent to participate in this study. For immunohistochemical staining, samples of tissues obtained at 10 and 40 weeks of pregnancy, and of the hydatidiform mole were fixed in 10% formalin/PBS for 48 hours.

Formalin-fixed tissues were dehydrated and embedded in paraffin. Sections were cut at 4 μm, deparaffinized and rehydrated. Immunostaining, using anti-hCRH antibody as first antiserum, was carried out by the avidin-biotin complex method (Hsu 1981), using a vectastin ABC kit (Vector Laboratories, Inc., Burlingame, CA). CRH antiserum was raised in rabbits as described in detail elsewhere (Okamoto 1989). Diamino-benzidine tetrahydrochloride was used as peroxidase substrate. The sections were treated with 0.3% H2O2 in methanol and 3% normal rabbit serum to reduce nonspecific background staining and block endogenous peroxidase activity. Immune serum diluted 1:100, and normal rabbit serum were used as controls.

Results

In placenta at 10 weeks of pregnancy, the cytotrophoblast layer beneath the syncytiotrophoblast layer was evenly stained, as shown in Fig. 1. In the decidua, the glandular epithelium was stained, and stromal cells also showed diffuse positive staining (Fig. 2). No staining reaction was detected in placenta at term (Fig. 3), but the amniotic membrane was heavily stained (Fig. 4). No immunostaining was seen in tissue of the hydatidiform mole (Fig. 5).

Discussion

IR-CRH was shown to be localized in cytotrophoblasts of the placenta and decidua in early pregnancy, and in the amniotic membrane at term of pregnancy. These observations support the notion that the production sites of IR-CRH during pregnancy are not only placenta, but also decidua and amniotic membrane. The explanation for negative immunohistochemical finding in term placenta may be due to the small proportion of cytotrophoblast. Our finding that CRH is not present in the placenta at term is in accordance with previous reports (Petralgia 1987, Soijonmaa 1988).

Laatikainen et al. reported that IR-CRH is...

![Fig. 1](image_url)  
**Fig. 1** Immunohistochemical analysis of normal placenta at 10 weeks of pregnancy. (A) Control staining, (B) staining with anti-CRH immune serum. In (B), note that a positive reaction is confined to cytotrophoblasts. Original magnification : x 200.
Fig. 2 Immunohistochemical analysis of normal decidua at 10 weeks of gestation. Staining was as for Fig. 1. In (B), note the positive reactions in both the glandular epithelium and many stromal cells. Original magnification: x 200.

Fig. 3 Immunohistochemical analysis of normal placenta at 40 weeks of pregnancy. Staining was as for Fig. 1. Note that there is no staining reaction in frame (B), for which immune serum was used. Original magnification: x 200.

Fig. 4 Immunohistochemical analysis of normal amniotic membrane at 40 weeks of gestation. Staining was as for Fig. 1. Note the strong CRH reaction in the epithelium in frame (B). Original magnification: x 200.
Immunohistochemical analysis of corticotropin-releasing hormone (CRH) in human trophoblast of normal pregnancy and hydatidiform mole.

ADVANCES IN OBSTETRICS AND GYNECOLOGY
Vol.42 No.4

present in the amniotic fluid and it increases greatly during the latter half of pregnancy (Laatikainen 1988). Their findings, together with the present results, indicate that the IR-CRH in the amniotic fluid is produced and secreted from the amniotic membrane.

The physiological role of CRH during pregnancy is unknown. There is a CRH binding protein (CRH-BP) in human plasma and most plasma CRH is bound to CRH-BP and inactivated (Orth 1987, Suda 1988). Thus although a large amount of IR-CRH, most of which is produced in the placenta, may be preferentially secreted into the maternal circulation, its influence on the maternal pituitary-adrenal axis may be within a physiological range because most of it is bound to CRH-BP and inactivated. There is a report that CRH may be an initiator of labor, because the level of plasma IR-CRH of pregnant women who went into premature labor was raised several weeks before the onset of labor (Campbell 1987). Increased IR-CRH and cortisol levels in the amniotic fluid are associated with a raised lecithin/sphingomyelin ratio and increase in phosphatidylglycerol (Laatikainen 1988). Similar observations have been reported on prolactin (PRL): IR-PRL in maternal plasma and in the amniotic fluid increases during pregnancy, and human decidua contains a high level of IR-PRL (Ziegler 1982). PRL produced by the decidua has also been suggested to be secreted through the fetal membranes into the amniotic cavity and to accelerate fetal lung maturation. Judging from these findings, the CRH produced by the amniotic membrane is probably secreted into the amniotic fluid and accelerates fetal maturation in obstetric stress by stimulating the fetal-adrenal cortex to produce corticosteroids. The maternal plasma IR-CRH level is reported to be in the normal, non-pregnant range in hydatidiform mole (Wolfe 1988), but there has been no report of immunohistochemical studies on CRH in molar tissue. In this study we could not detect IR-CRH in trophoblastic tissue of a hydatidiform mole. Hydatidiform mole, which is the most common trophoblastic tumor, has the potential for DNA synthesis and rapid growth and proliferation. In this tumor, production of CRH is probably suppressed due to the characteristic change of the trophoblasts.

In summary, we showed by immunohistochemical technique that CRH is produced in not only placenta but also decidua and amniotic membrane in normal pregnancy, and that its production is suppressed in trophoblastic disease.

Fig. 5 Immunohistochemical analysis of hydatidiform molar tissue at 10 weeks of gestation. Note that there is no staining reaction in frame (B), in which anti-CRH serum was used.
References


正常妊娠と胞状奇胎におけるcorticotropic-releasing hormone（CRH）の免疫組織学的局在について

岡本 栄作，杉田 長敏
市立堺病院産婦人科
高木 哲，岩田以津子，西野栄里子
光田 信明，谷澤 修
大阪大学産婦人科学教室

妊娠と共に母体血漿中のimmunoreactive (IR)-corticotropic-releasing hormone（CRH）が急増し，分娩直後に著減することにより，胎盤での産生が示唆されている。今回我々は妊娠各期の胎盤，脱落膜，羊膜，及び胞状奇胎組織におけるIR-CRHの局在を，Avidin–Biotin 染色法により検討し以下の結果を得た。

（1）妊娠初期において，IR-CRHの局在が胎盤のcytotrophoblast層に認められた。

（2）妊娠末期において，IR-CRHの局在が羊膜上皮にみとめられた。

（3）胞状奇胎組織においては，IR-CRHの局在は明かでなかった。

以上のことより，妊娠中に増加する母体血漿中及び羊水中のIR-CRHは，胎盤，脱落膜，及び羊膜であることが示唆された。また，胞状奇胎組織では絨毛の組織分化の変化によりIR-CRHの産生が抑制されていることが示唆された。