IMMUNOHISTOCHEMICAL IDENTIFICATION OF PROLACTIN IN HUMAN PLACENTA AND MEMBRANES AT TERM

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SUMMARY

Placentas and membranes of seven healthy women after full-term gestation and spontaneous labor and delivery were studied in order to verify the presence and distribution of prolactin-containing cells. An unlabeled antibody-PAP-method, applied to formalin-fixed, paraffin-embedded tissue sections, showed varying degrees of cytoplasmic positivity in the elements of the amniotic epithelium, decidua and trophoblast.

A comparison between these results and those previously reported in the literature is made.

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The amniotic fluid (AF) of man and other primates contains very large amounts of a peptidic hormone, which is biochemically and biologically similar to pituitary prolactin (PRL) ([1, 5-7, 9, 10, 12, 13, 16-18, 21]).

The concentration of this hormon – which we will designate as amniotic fluid-prolactin (AF-PRL) – increases progressively during early pregnancy and reaches in man the highest levels between 12 and 32 weeks of gestation ([1, 2, 5, 9, 10, 12, 18]).

While its functions are rather well known – influence of water and electrolyte balance in the AF, maintenance of the decidual reaction and inhibition of myometrial contractions ([9-11, 16]) – its source has yet to be determined.

According to Rosemberg *et al.*, "several observations tend to refute the possibility that the major source of AF-PRL is either the maternal or fetal pituitary gland, or both: 1) suppression of both maternal and fetal pituitary PRL production to very low levels with bromocriptine does not influence AF-PRL levels; 2) progressive changes in maternal and fetal serum PRL throughout gestation are discordant with changes in AF-PRL; 3) AF-PRL levels do not change after maternal hypophysectomy or after intrauterine death of the fetus in rhesus monkey; and 4) isotopically labeled PRL injected into the pregnant rhesus monkey or fetus shows as extremely low level of transfer to AF" ([18]).

This has led several Authors to detect an extrapituitary source for AF-PRL. Recent studies have suggested that this hormone might be produced by cells of the placental membranes and/or periplacental tissues. In particular, it has been shown that explants of human decidua or choriondecidua are able to synthesize and secrete AF-PRL *in vitro* ([1, 8, 10, 12, 16-18]).

Furthermore, employing immunofluorescence (I.F.), Healy *et al.* have identified cytoplasmic positivity for AF-PRL in

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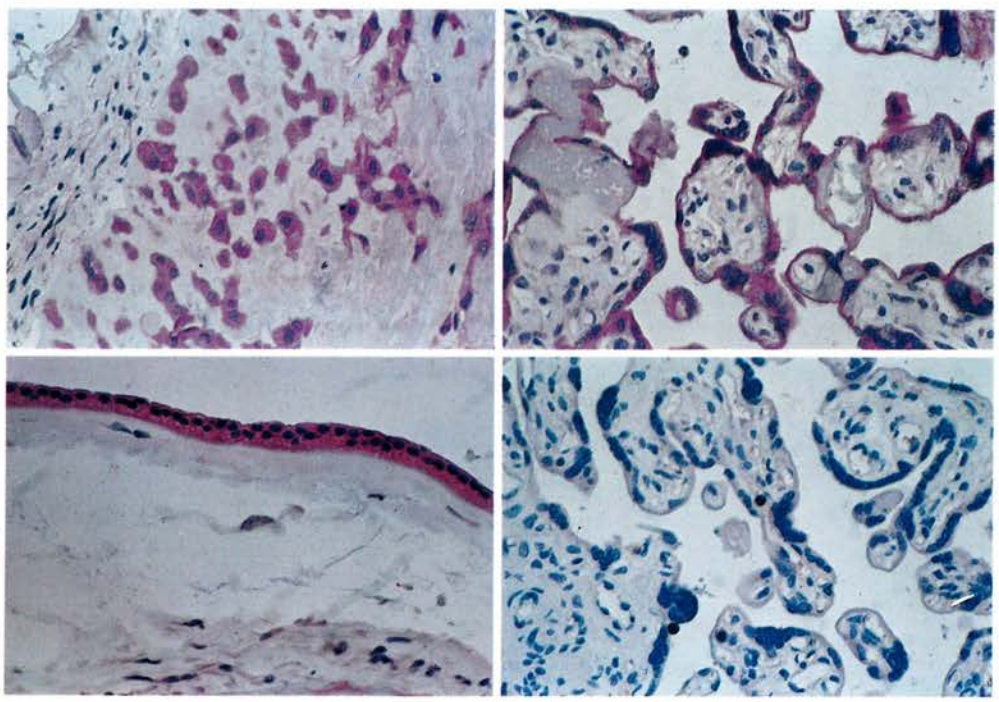

Fig. 1. — Staining of basal decidua cells (unlabeled antibody-PAP-method; counterstained with haemalum; × 264).

Fig. 2. — Positivity of trophoblastic elements of villi (unlabeled antibody-PAP-method; counterstained with heamalum; × 422.4).

Fig. 3. — Staining of amniotic epithelium cells (unlabeled antibody-PAP-method; counterstained with haemalum; × 264).

Fig. 4. — The specific staining is completely abolished by the absorption of the antiserum anti-AF-PRL with the purified antigen (unlabeled antibody-PAP-method; counterstained with haemalum; × 422.4).

amniotic epithelial cells ([9]), while Frame and coworkers observed a definite staining only in the maternal decidua elements that adhere to term fetal membranes and trophoblastic cells of the chorion laeve ([5]).

Recently, Meuris *et al.* have localized by an immunoenzymatic method a prolactin-like immunoreactivity in the decidual cells of the endometrium in cases of early normal pregnancy, molar pregnancy, tubal pregnancy and of a non-pregnant woman, under prolonged lynestrenol treatment ([13]).

The aim of this study is to verify by an unlabeled antibody-PAP-method ([4, 14, 19, 20]) the localization of AF-PRL positive elements in placentas and membranes of seven women after a full-term gestation.

MATERIAL AND METHODS

Placentas and membranes were obtained from seven healthy women after full-term gestation and spontaneous labor and delivery. After rinsing with physiological saline at 37 °C, samples of placentas and membranes were taken, fixed in 10% unbuffered formalin for 48 h and embedded in Paraplast (Oxford/Lancer) at 57 °C. 5 mμ tick-sections were cut with a Leitz rotary-microtome, deparaffinazed in xylol and rehydrated in alcohol. During rehydration, after absolute alcohols, the endogenous peroxidase activity was blocked with 0.3 H_2O_2 in methanol for 30 min. at room temperature ([15]).

After washing in 0.5 M PBS (pH 7.6) for 20 min. with 3 changes at 37 °C, sections were incubated in a 0.05% solution of Protease XIV (Sigma No. P-5147) in 0.5 M PBS (pH 7.6) for 15 min. at 37 °C.

This step was succeeded by washing in 0.5 M PBS (pH 7.6) with 3 changes for 15 min. Then, the sections were incubated in normal swine serum (Dako Code X 901) diluted 1:5 in 0.5 M PBS (pH 7.6) for 30 min. at room temperature. After removing the excess of normal serum, they were incubated in specific antiserum anti-human prolactin (Biodata code 2807), raised in rabbit and diluted 1:100 - 1:200 in 0.5 M PBS (pH 7.6), for 30 min. at room temperature.

After washing in 0.5 M PBS (pH 7.6) for 15 min. with 3 changes, they were treated with swine anti-rabbit IgG (Dako code Z 196), diluted 1:50 in 0.5 M PBS (pH 7.6), for 30 min. at room temperature. After washing as previously described, PAP complexes (Dako code Z 113) raised in rabbit were applied diluted 1:100 for 30 min. at room temperature. After washing with 0.5 M PBS (pH 7.6) for 30 min. with 3 changes, the horseradish peroxidase was demonstrated employing a solution of 0.02% 3 amino-9-ethylcarbazole (Sigma No. A-5754), 0.03% H_2O_2, 5% N,N dimethylformamide (Fluka 40240) in 0.1 M acetate buffer (pH 5.2) ([3]). After washing with 0.5 M PBS (pH 7.6) and distilled water, the sections were counterstained with Mayer's haemalum and mounted in Kaiser's glycerol gelatin (Merck 9242).

Controls of the method and specificity of the antisera consisted of:

a) absorption of the antiserum anti-human prolactin with the specific antigen supplied by Biodata (0.5 mg of pure antigen/1 ml of antiserum diluted 1:100);

b) omission of the primary antiserum;

c) substitution of the primary antiserum with swine, rabbit and goat normal sera;

d) application of PAP complexes only.

RESULTS

At microscopic examination, all the cases showed the same pattern of staining. In particular, the samples taken from the placental disc demonstrated an evident positivity of basal decidua cells (fig. 1) and trophoblastic elements of villi (fig. 2).

The stroma and Hofbauer's cells did not show any degree of immunoenzymatic reaction. Furthermore, the evaluation of membranes revealed a clear-cut positivity in the elements of the amniotic epithelium (fig. 3) trophoblast and parietal decidua. Again no staining was observed in the stroma. It is noteworthy that both in the placental disc and membranes the decidual cells displayed a higher degree of positivity when compared with the elements of the trophoblast and amniotic epithelium.

Regarding the controls, the absorption of the antibody anti-human prolactin with the specific antigen abolished staining completely (fig. 4), while no kind of positivity was observed either with the omission and substitution of the primary antiserum, or with the application of PAP complexes only.

DISCUSSION

The results of the present immunohistochemical study partially confirm the data of Healy *et al.* [9] and Frame and coworkers [5]. In fact, we were able to detect in the examined membranes a definite cytoplasmic positivity for AF-PRL not only in the amniotic epithelium as described by Healy *et al* [9], but also in the trophoblast and decidua as reported by Frame and coworkers [5].

The latter result is also in agreement with the observations of Meuris *et al.* [13], who studied prolactin-like immunoreactivity in the decidual cells of the endometrium of pregnant and non-pregnant women.

Furthermore, we were able to recognize an evident staining in the trophoblastic elements of placental villi, which to our knowledge represents the first example of such a kind of positivity.

We think that the differencies between our series and those previously reported may be due to the diversity of the material and methods employed.

In fact, Haley *et al.* [9] studied not-fixed samples of placentas and membranes collected from pregnancies, which had been terminated for psychiatric reasons in the second trimester by hysterectomy, while we always employed fixed tissue-fragments of placentas and membranes of healthy women after full-term gestation and spontaneous labor and delivery.

Moreover both Healy *et al.* [9] and Frame and coworkers [5] utilized for their investigations indirect I.F., which is less and less sensitive than the unlabeled antibody-PAP-method applied in the present series [4, 19, 20].

From the comparison of our immunohistochemical data with those obtained *in vitro* by others [1, 8, 10, 12, 16-18] it emerges that there is a good correspondence between the strong positivity observed by us in the decidual cells and the ability of these elements to contain and release very large amounts of AF-PRL in culture.

On the other hand, the staining of the amniotic epithelium and trophoblast does not fit with the majority of the observations [1, 8, 10, 16-18], that these structures are not able to produce AF-PRL. As our results are not due to technical artifacts because of the available controls, two possibilities must be taken into consideration: either the amniotic epithelial cells and trophoblastic elements can synthesize small quantities of AF-PRL, not previously detected by experiments *in vitro*, or they may uptake this hormon via specific receptors.

In order to solve this problem, we think that further investigations are required.

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