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DYNAMICS OF VARIOUS PEPTIDE HORMONE RECEPTORS IN HUMAN PLACENTAL CELL MEMBRANES

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ABSTRACT

The function of peptide hormone receptors on the trophoblastic cell membrane was analyzed, and the results obtained were as follows:

1) Receptors for hCG/LH, hPL/hGH, ACTH, insulin, and transferrin were recognized in trophoblastic cells.

2) Cyclic AMP levels in the tissue increased after administration of these hormones.

3) Binding capacity for these receptors was present in hydatidiform mole tissue, and its activity was stronger than that in normal placenta tissue.

These findings strongly suggest that trophoblastic cells may have an autoregulatory mechanism through the membrane receptors, such as hCG/hLH and hPL/hGH, and this mechanism is working mainly through the cyclic AMP system.

INTRODUCTION

The placenta is considered to act as an endocrine organ throughout pregnancy. The placenta secretes two main hormones, i. e., steroid and peptide hormones. They are synthesized by the syncytium cells and are thought to play an important role in maintaining pregnancy and fetal growth[15].

Although receptors for insulin[4], estrogen[8], androgen[9], and transferrin[10] have been found in human trophoblasts, there are no reports on other hormone receptors or their functions.

In addition to analyzing these known receptors, we tried to clarify whether there are also receptors for hCG and hPL and whether these hormones are secreted by the trophoblasts themselves. In addition, we studied other hormone receptors, such as ACTH and, which, though not yet confirmed, are thought to be secreted by the trophoblasts.

First, to investigate whether any differences exist among normal, molar, and choriocarcinoma cells, we attempted to analyze whether trophoblasts themselves may have the ability to autoregulate receptors for the peptide hormones secreted by the placenta.

Then, to clarify the changes in the post-receptor system, we studied the dynamics of cyclic AMP levels in these tissues.

Materials and Methods

1. Materials: One hundred and five specimens of normal human placental tissues, 41 specimens of molar trophoblasts, and a choriocarcinoma cell line (GCH-1) were used. The specimens were rinsed immediately after removal to eliminate any blood by using physiological saline at 0℃ and then storing them at −80℃.

Venous blood samples for hormone assay were drawn immediately after the placenta had been removed from the patients. Sera were kept at −20℃ until use. The activity of each hormone was assayed by using RIA kits.

2. Preparation of Membrane Receptors: Tissues were homogenized with a Polytron PT-10 homogenizer in 5 volumes (V/W) of 0.3 M sucrose solution. After filtlation through several layers of gauze, the filtrate was centrifuged at 1,000 xg for 10 min. The supernatant solution was again centrifuged at 15,000 xg for 30 min. The precipitate was suspended in 1/40 M Tris-HCl buffer (pH 7.6; 10 mM $CaCl_2$; 1% BSA) and used for receptor assays.

3. Radio Receptor Assay Method: I^{125}-labelled and cold hormones were incubated with the prepared cell membrane in the 1/40 M Tris-HCl buffer at 37℃ for 60 min. After incubation, the reaction mixtures were centrifuged for 30 min. The precipitate was washed once with the buffer, and its radioactivity I^{125} incorporated into the cell membrane was counted with a gamma-conunter.

HCG, LH, hGH, ACTH, FSH, and insulin standards were obtained from Sigma, U. S.A. ^{125}I-labelled hormones were prepared by the lactoperoxidase method.

4. Cyclic-AMP Assay: For studying cyclic-AMP activity, tissue which had been kept at −80℃ was minced and assayed with varying amounts of hormone by using various incubation times, i. e., 15, 30, 60, and 120 min.

About 0.1 g of the minced tissue was preincubated in 500 μl of 1/40 M Tris-HCl buffer at 37℃ for 30 min followed by centrifugation. The precipitate was incubated in a 5% CO_2 atmosphere at 37℃ for cyclic AMP assay under the various conditions mentioned above. The assay was carried out with a cyclic-AMP assay kit purchased from Yamasa, Japan.

RESULTS

The lower part of Fig. 1 shows the serum levels of β-hCG and the corresponding maximum binding capacities (B max) for hCG-LH receptors at each stage of normal pregnancy. At weeks 10-12 of pregnancy, the β-hCG levels in the blood reached a peak value, while the number of the hCG-LH receptor sites showed a tendency to decrease. The upper part of Fig. 1 shows the data for molar trophoblasts. The β-hCG value and the hCG-LH receptors in cases of mole exhibited rather high values compared to those of normal trophoblasts, and there was no dissociation between β-hCG and hCG-LH receptors. Slightly higher values were observed with hCG-LH receptors of GCH-1 cells.

Figure 2 shows the results of a similar experiment revealing the changes in hPL

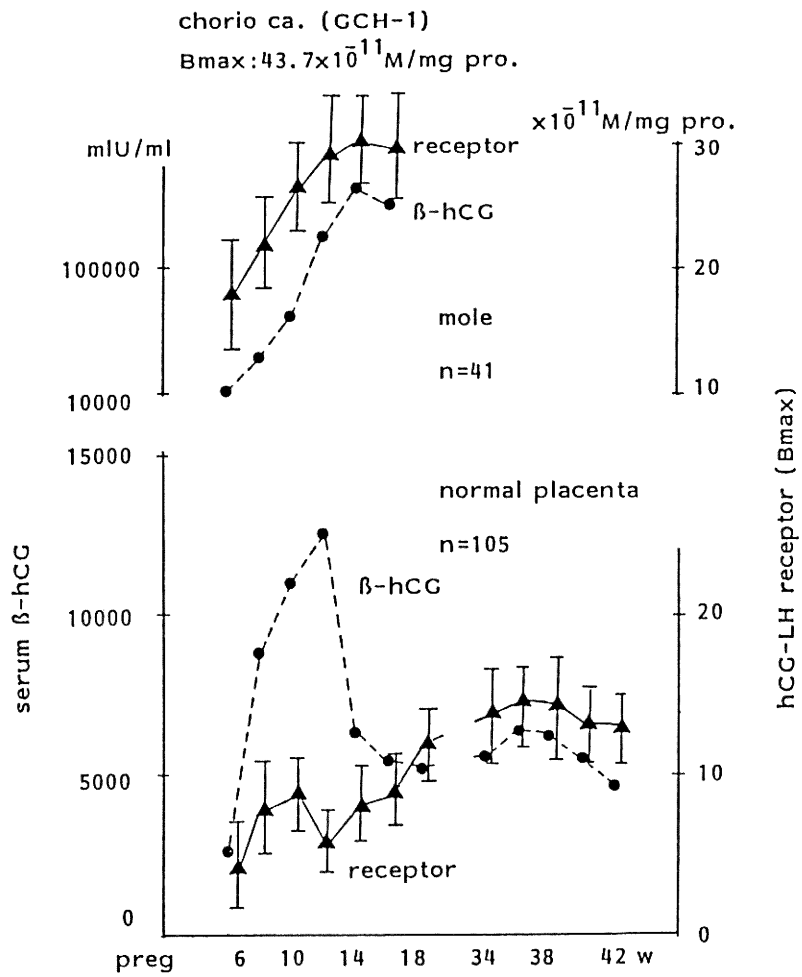

Fig. 1. The changes of hCG-LH receptors in human placental cell membranes

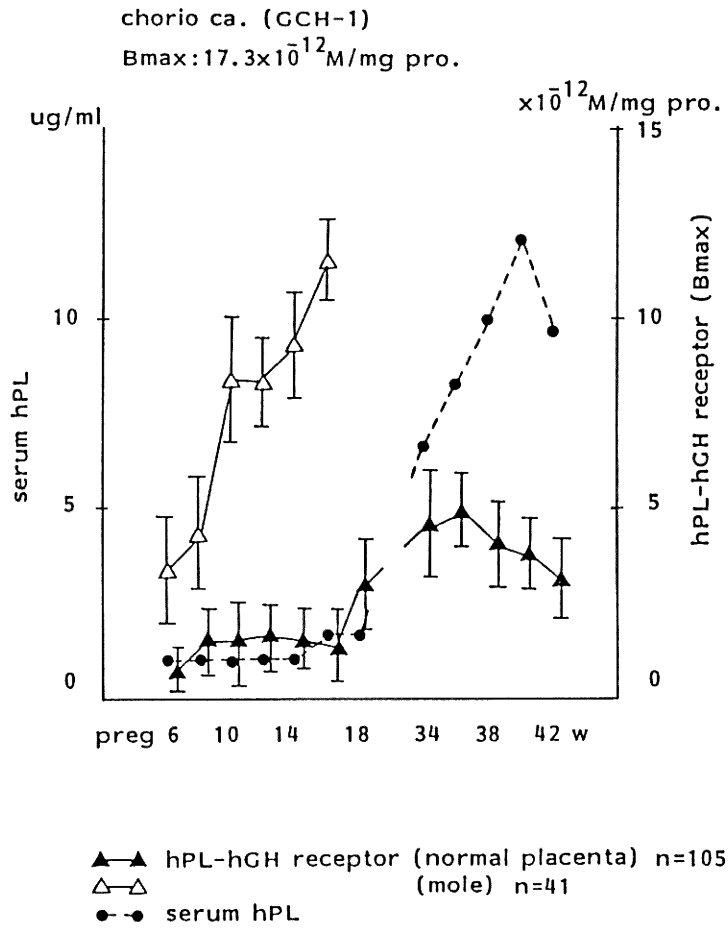

Fig. 2. The changes of hPL-hGH receptors in human placental cell membranes

blood levels and hPL-GH receptors. Similar to the case of hCG-LH receptors, the hPL-GH receptors in molar trophoblasts and choriocarcinoma also showed very high values compared to those of normal trophoblasts.

Figure 3 shows the changes in ACTH receptors. As seen with hPL/GH receptors, a peak was attained in the late stage of pregnancy.

Figure 4 shows that insulin receptors reached a peak at weeks 10-12 and weeks 38-40 of pregnancy.

Figure 5 shows the changes in transferrin receptors. At the early stage of pregnancy, the transferrin concentration in the serum showed a possible tendency to increase with gestational age. A similar tendency was also observed for the transferrin receptors on normal trophoblast cells. However, the tansferrin receptors on molar trophoblasts were approximately twice as numerous as those on normal trophoblasts.

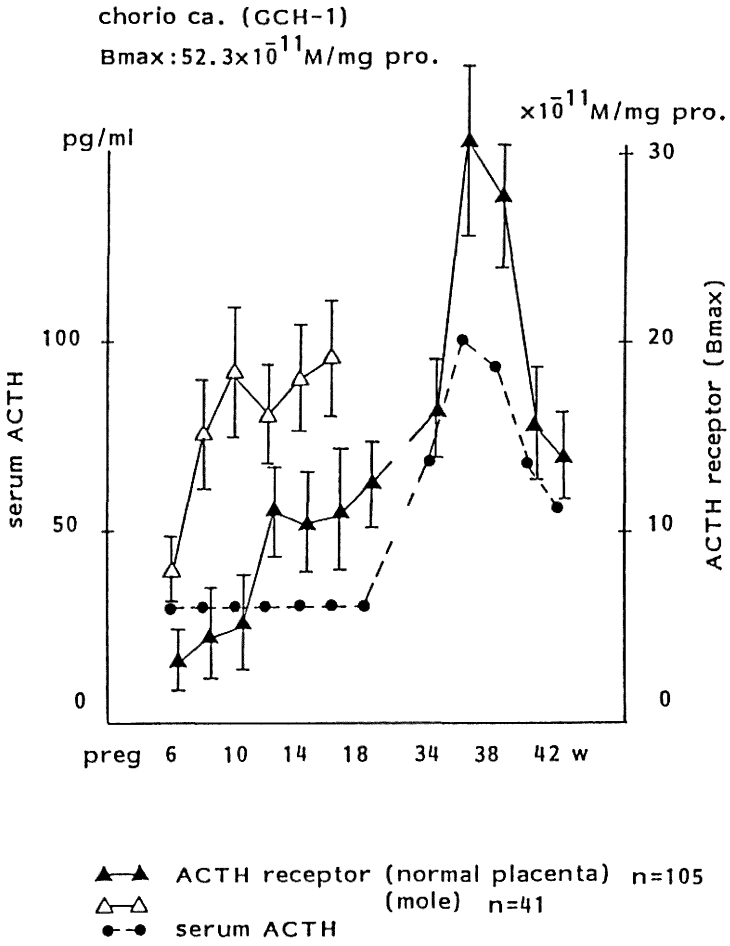

Fig. 3. The changes of ACTH receptors in human placental cell membranes

Furthermore, the transferrin receptors on GCH-1 (choriocarcinoma) cells were ten times more numerous than those on normal trophoblasts. As shown in Fig. 5, the transferrin receptors had a tendency to increase with gestational age.

The results of cyclic AMP assay after addition of 50 m iu hCG to chorionic tissue at week 40 of pregnancy are shown in Fig. 6. The peak value was observed at approximately 60 min after hCG administration. When hPL and ACTH were added in a similar manner, a significant cyclic AMP increase was also seen.

Figure 7 shows the changes in cyclic AMP levels at various concentrations of hCG after preincubation with hCG. These results show that the tissue preincubated with various hormones except hCG showed a dose-dependent effect. Figure 8 shows the results of the same experiment though using hPL instead of hCG as that presented in Fig. 7.

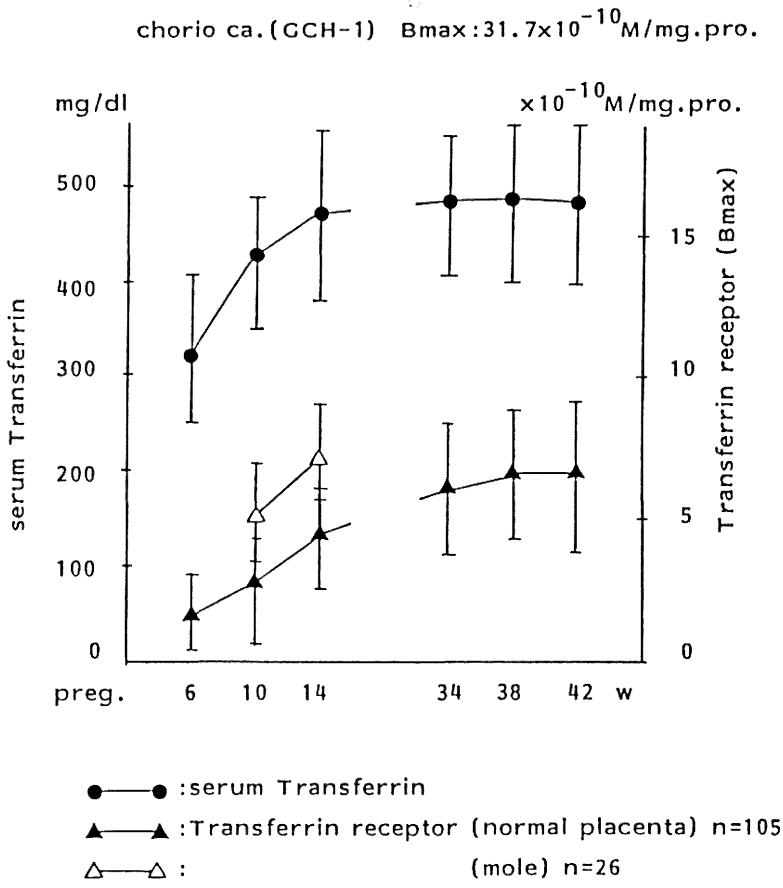

Fig. 4. The changes of insulin receptors in human placental cell membranes

Discussion

In recent years it has become clear that a hormone binds a particular cell site called a "receptor" consisting of high-molecular-weight proteins and induces reactions in the cell according to the information carried. Several action mechanisms for the hormone receptors that are involved in the adenylcyclase system have been proposed[1,4,10].

As is well known, Robinson[13] et al. have postulated the two component models based on a two-messenger theory. When a hormone reaches a target cell, it binds with a regulatory unit of the adenylcyclase system, and then this unit recognizes the molecular structure of the hormone. Finally, a catalytic unit, which is located on the inner surface of the cell membrane, is activated. This unit stimulates conversion of ATP to cyclic AMP. This model is thought to explain most hormonal action mechanisms.

On the other hand, Rodell et al.[14] have proposed a three-component model based on

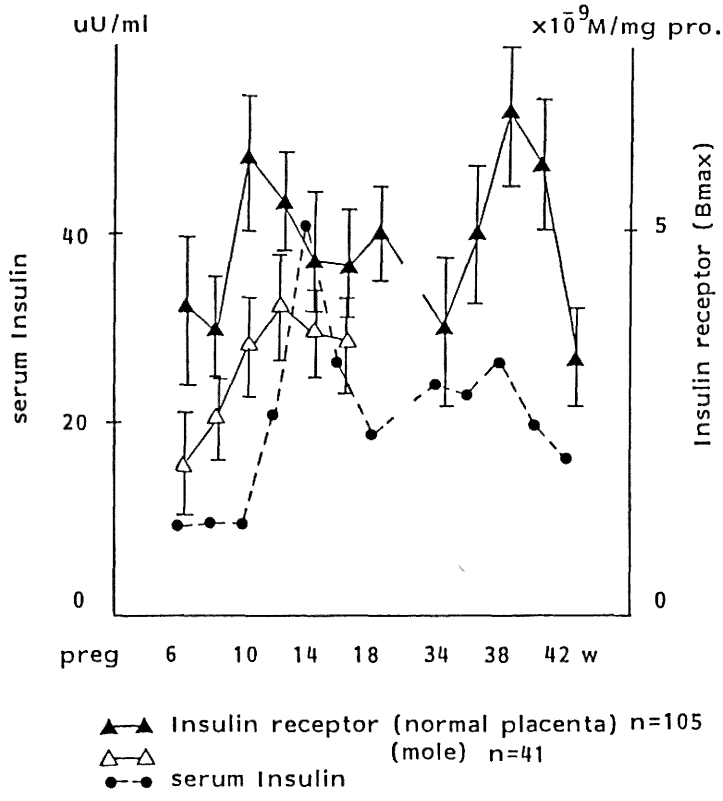

Fig. 5. The changes of transferrin receptors in human placental cell membranes

their observations of the mode of action of glucagon in the liver. In any case the initiation of hormonal action must start at the point where the hormone binds to a specific hormone receptor[21,22].

In the present study, we used human placental cells and molar trophoblasts to identify the receptors. We found that an increase of hCG/hLH receptors between the 8th and 10th weeks of gestation may have some correlation with the increase in serum hCG concentration.

In cases of normal pregnancy, some decreases were apparent in receptor binding sites from the 10th to the 12th weeks. We speculate that a down-regulation of these receptors caused by the large quantity of hCG may occur[2,3].

However, molar trophoblastic tissue did not show any such down-regulation[11]. As to the reason for such a difference between normal and molar trophoblast cells, the possibility could not be ruled out that some changes in the sensitivity and number of these receptors took place in the cells. Since, in the case of molar pregnancy, no fetus would be present in the uterus and a higher hCG concentration would be present in comparison with a normal pregnancy.

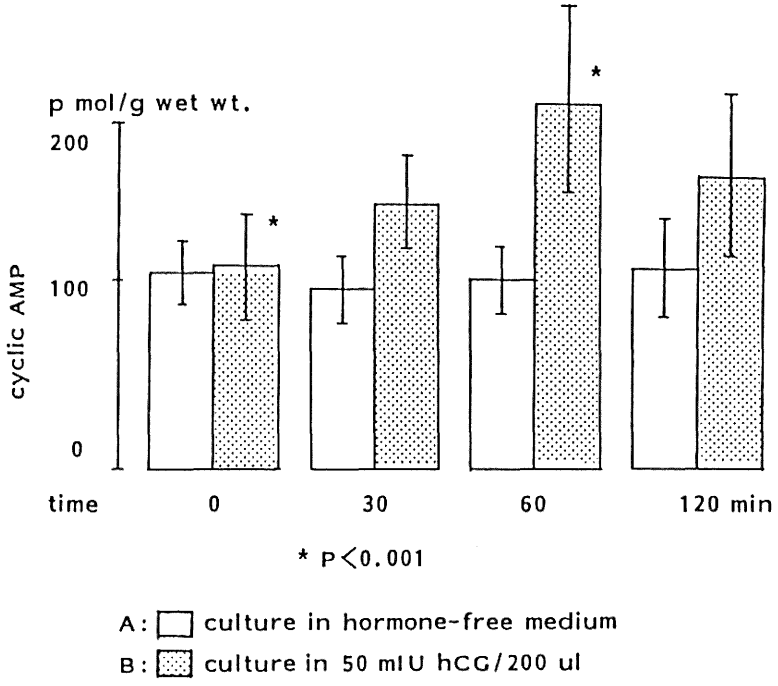

Fig. 6. Two groups are compared: culture in hormone-free medium (A), culture in 50 mIU hCG/200 ul (B). The experiments were performed for three different time periods of culture (30, 60, and 120 min). Norman placenta (40 w) n=14

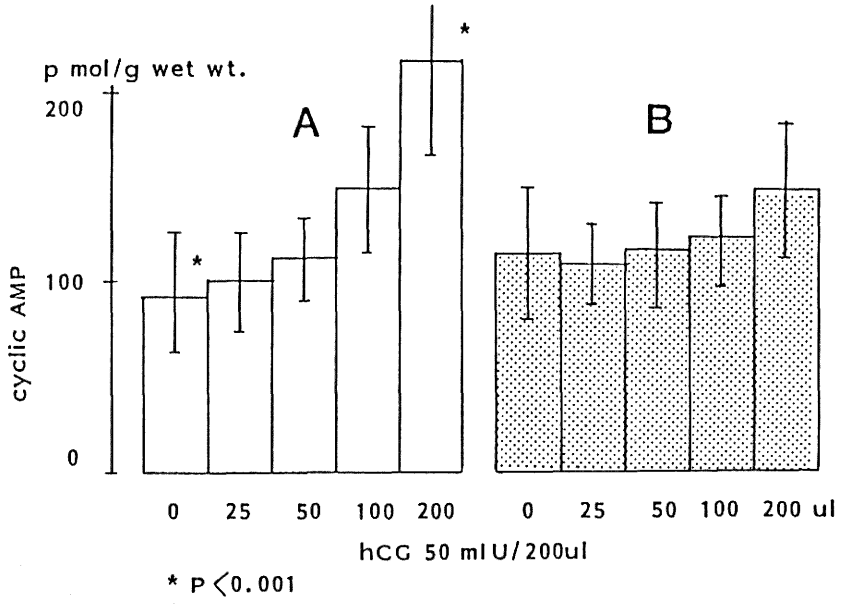

Fig. 7. Two groups are compared: culture in hormone-free medium (A), culture in 50 mIU hCG/200 ul for 6 hr at 4℃ (B). Statistical differences were evaluated by analysis of variance: only in group A was significant stimulation of cAMP concentration obtained. Normal placenta (40 w) n=14

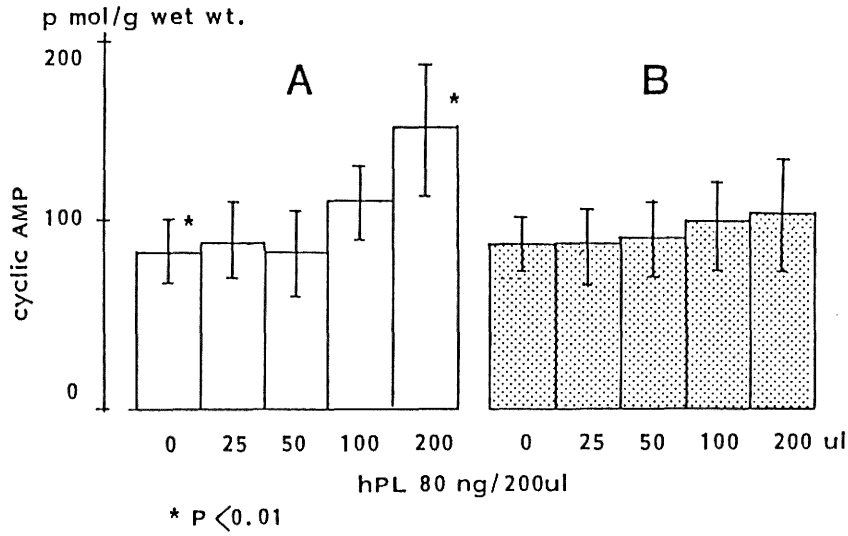

Fig. 8. Two groups are compared: culture in hormone-free medium (A), culture in 80 ng hPL/200 ul for 6 hr at 4℃ (B). Statistical differences were evaluated by analysis of variance: only in group A was significant stimulation of cAMP concentrathon obtained. Normal placenta (40 w) n=14

As to the hPL receptor, the binding capacity for hPL reached its peak between the 34th and 38th weeks. hPL plays a role in glucose fatty acid metabolism and is primarily a hormone for fetal growth. It is thus reasonable as shown in Fig. 2 that the requirement on hPL receptor would increase with fetal growth.

We also investigated receptors for ACTH and insulin, which are not produced in the trophoblasts themselves[16,17,18,19,20], whereas the trophblastic membranes are the target of these hormones. The number of ACTH receptors showed peak growth between the 34th and 38th weeks of pregnancy.

Insulin receptors had two peaks, the first from the 10th to the 12th, the second from the 38th to the 40th weeks of pregnancy. The first peak is easily accounted for because the trophoblasts are undergoing vigorous mitotic proliferation during this stage. As for the second peak, insulin is considered necessary during this period for carbohydrate metabolism in the fetus[4,5,7].

After exposure to a high concentration of hormone, the receptors lose their sensivity for the hormone. This phenomenon is called desensitization and can be explained by "down-regulation," although the mechanism of cell desensitization is not yet well understood.

Internalization of polypeptide hormones after binding with their receptors may be involved in the down-regulation process. The results shown in Fig. 6 indicate that hCG is one of the polypeptide hormones for which cyclic AMP plays a main role as a second messenger. The results also indicate that, as shown in Fig. 7 B, hCG receptors are

controlled by down-regulation when an excess quantity of hCG is added.

Almost all receptors showed a much stronger affinity for each hormone in the choriocarcinoma cell line than in normal trophoblasts. This observation that the down-regulation observed in normal trophoblasts was not detected at all in cancerous cells is of considerable interest, this suggests that cancerous transformation might have caused changes in the characteristics of receptors with regard to their quantity, quality and sensitivity.

Ney et al.[22] reported that membrane receptors of tumors from functional endocrine glands lost their original characteristics in many cases. This observation would support our findings that receptors of target organs change their nature on an equal basis with canceration.

From these results and other observations, we postulate that hCG may stimulate the production of cyclic-AMP, thus activating the synthesis of steroid hormones.

In the early stage of gestation, the desensitization of hormones may take place during periods of cyclic AMP production after hCG and hPL have become to their own receptors[22,23,24]. Futhermore, membrane receptors for hCG and hPL seem to decrease in number, followed by an apparent drop in the enzyme activity of steroid hormone synthesis. Overall, the target cells may become rapidly less sensitive to newly synthesized hCG and hPL.

The mechanism responsible for the post-receptors system in the placental cell membrane is not yet clear. Through this study we have shown some aspects of the role of the placenta as an endocrine organ and the mechanisms of the very complex cooperative hormonal system operating during pregnancy.

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