Release of Human Chorionic Gonadotropin (hCG) and its Alpha-subunit (hCG-α) from Perifused Human Placenta

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Abstract

The release of human chorionic gonadotropin (hCG) and its α-subunit (hCG-α) from the normal human placenta and the effect of some stimulatory agents on their release were studied in vitro using a perifusion system. Each perifusate was assayed for hCG and hCG-α in its own homologous radioimmunoassay systems.

Both hCG and hCG-α were released from the placenta at any stage of gestation in our perifusion system. Much more hCG than hCG-α was released from the placenta in early gestation. By comparison, however, hCG-α increased gradually with the gestational age. The amount of hCG-α released was almost equal to that of hCG in the placenta in the 17th gestational week. After the 22nd gestational week, hCG-α was released in larger quantities than hCG, and about 10 times more hCG-α than hCG was released from the term placenta.

These results were also confirmed by gel filtration of perifusates on a Sephadex G-100 column. hCG-α, compared with hCG, was present in excess in gel filtrated perifusates in the last two trimesters.

By adding 1 mM dibutyryl cyclic AMP to the perifusion medium, the release of both hCG and hCG-α was stimulated significantly. Synthetic luteinizing hormone releasing hormone (LH-RH) at concentrations of 10 ng/ml and 100 ng/ml had no effect, but at a high concentration (1 μg/ml), LH-RH stimulated the release of them. Moreover, mouse epidermal growth factor (EGF) stimulated not only the release of hCG and hCG-α but also their production, because both hCG and hCG-α levels rose progressively with the time course in the presence of EGF.

The present studies demonstrate that the perifusion system of chorionic tissues is a useful method for investigating the release of hCG and its subunits in vitro.

The glycoprotein hormone human chorionic gonadotropin (hCG) is normally secreted in larger quantities by the placenta throughout pregnancy, and it is composed of two non-identical, non-covalently bound subunits, designated α and β.

It is well known that the change in hCG levels in the sera of pregnant women throughout pregnancy shows a very characteristic pattern. Moreover, it has been demonstrated that free hCG-α subunit is also present in the maternal serum, but its secretion pattern is quite different from that of hCG (Ashitaka et al., 1974). Thus there may be some mechanisms regulating the secretion of hCG and its subunits by the normal placenta. However, despite extensive studies so far, these mechanisms are not yet well understood.

The in vitro perifusion system is superior to the static in vitro incubation.
method, because it permits the observation of the releasing dynamics of hormones with a time course and their time-pattern responses to various stimuli. Because it offers these advantages, this system has been initially used to study the release of steroids from rat adrenal glands (Tait et al., 1967). Although recently several investigators have applied this system to other endocrine tissues or hormones, such as luteinizing hormone (LH), follicle stimulating hormone (FSH), growth hormone (GH), estrogen and progesterone (Serra and Midgley, 1970; Carlson et al., 1974; Watson and Leask, 1975; Dowd et al., 1975), applied studies on hCG release have hardly been reported.

The present investigation was undertaken to study the mechanism of the release and the production of hCG and hCG-α in the normal chorionic tissue by the observation of their releasing patterns after the addition of some stimulatory agents using a perfusion system.

**Materials and Methods**

**Chorionic tissue**

Placentas from normal uncomplicated pregnancies at different gestational weeks were obtained after therapeutic abortions and vaginal deliveries. Tissues were washed several times within 30 min with Hanks' Balanced Salt Solution, and clots and membranes were removed as much as possible by means of scissors and pincers.

Chorionic tissue weighing approximately 200 to 300 mg (wet weight) was employed in each single experiment.

**Perfusion procedure**

A schematic diagram of the perfusion apparatus is shown in Fig. 1. This perfusion system consists of medium reservoirs, sample chambers, a peristaltic pump and fraction collectors. Medium reservoirs and sample chambers were placed in the thermoregulated incubation box with water bath, and incubation temperature was maintained at 37°C throughout the experiments. A glass tube (5 × 1 cm) sealed with rubber plugs at each end was used as a sample chamber of which the capacity was about 2 ml. The tubing system was made of Tygon plastic tube with an internal diameter of 3/32 inches. The perfusion medium was Krebs-Ringer-bicarbonate-1.8 mg/ml glucose (KRBG) buffer at pH 7.4 saturated with a mixture of 95% O₂ - 5% CO₂. All test substances were dissolved in KRBG buffer and were kept under the same gas mixture. The medium was pumped from the reservoir through the sample chamber by a multichannel peristaltic pump (Gilson HP-4) at a constant rate of 0.2 ml/min, and effluent buffer (perifusate) was collected at 10 min intervals on the fraction collector (Gilson FC-80 K). Test substances were introduced into the perfusion system at the same rate through a three-way stopcock connected to the inlet of a sample chamber after a preincubation period of 100 to 120 min.

**Radioimmunoassay of hCG and hCG-α**

The concentration of hCG and hCG-α in each perifusate were measured in their homologous radioimmunoassay systems as previously described (Ashitaka et al., 1974 a; Nishimura et al., 1979). Highly purified hCG (CR-119) and hCG-Kobe (Ashitaka et al., 1970) served as reference preparations and hCG-α was prepared according to the method described by Swaminathan and Bahl (1970). Radioiodinations of hCG and hCG-α were performed by an enzymatic method (Ashitaka and Koido, 1974) using Na¹²⁵I.

The results were expressed as nanograms per milligram wet weight of chorionic tissue, amounts of hCG and hCG-α released from 1 mg of chorionic tissue for 10 min respectively.

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Fig. 1. Diagram of the perfusion apparatus.
Column chromatography

Perifusates were gel filtrated on a 90×1.5 cm Sephadex G-100 column. Perifusate of different gestational weeks were lyophylized initially, and later dissolved with 1 ml of 0.05 M phosphate buffer, pH 7.4. This buffer was used for the equilibration of the column and for the subsequent gel filtration studies. Fractions (2.5 ml) were collected at 4°C with a flow rate of approximately 10 ml/hour. The column was standardized with blue dextran, 125I labeled hCG and hCG-α. Elution patterns of hCG and hCG-α in each fraction were determined by their homologous radioimmunoassay systems as described previously.

Test substances

N6,2'-O-dibutyryl-adenosine 3', 5'-cyclic monophosphate (dibutyryl cyclic AMP) was obtained from Sigma Chemical Co. Ltd. and synthetic luteinizing hormone releasing hormone (LH-RH) was obtained from Tanabe Pharmaceutical Co. Ltd.

Epidermal growth factor, isolated from mouse submaxillary gland according to the method described by Cohen and Savage (1974), was supplied by Mr. Satoshi Nishimuro (Hayashibara Co. Ltd., Technical Division, Japan).

Results

Perifusion studies of the chorionic tissue of different gestational weeks

Both hCG and hCG-α were released from the chorionic tissue at any stage of pregnancy in our perifusion system. The release of hCG and hCG-α declined slowly within about 120 min after the initiation of perifusion and reached a relatively constant level which lasted for about 300 min under the present conditions of incubation.

Fig. 2 shows the pattern of hCG and hCG-α release from chorionic tissue in the 7th gestational week. In the present perifusion studies of chorionic tissues of the first trimester, much more hCG was released than hCG-α (Fig. 3). The amounts of hCG and hCG-α released per milligram wet weight of chorionic tissue were greatest in the first trimester, and they decreased thereafter.

Fig. 2. The pattern of hCG and hCG-α release from perifused chorionic tissue in the 7th week of gestation.

Fig. 3. The pattern of hCG and hCG-α release from perifused chorionic tissue in the 12th week of gestation.
Results of second trimesteral tissues, 17th and 22nd gestational week, are shown in Fig. 4 and Fig. 5, respectively. In the 17th gestational week, the amount of released hCG-α was almost equal to that of hCG (Fig. 4). In the 22nd gestational week and thereafter, hCG-α was released in larger quantities than hCG, and about 10 times as much hCG-α as hCG was released from the term chorionic tissue (Fig. 5 and 6).

**Gel filtration studies on Sephadex G-100**

The void volume (Vo) of the column was 45 ml. The elution/void volumes (Ve/Vo) for hCG and hCG-α were 1.22 and 1.72. Fig. 7-a, -b and -c show the elution profiles for hCG and hCG-α of perifusates of the 7th, 22nd and 38th gestational weeks, respectively. The arrows across the top indicate the elution position of blue dextran (Vo), 125I-hCG and 125I-hCG-α.

As shown in Fig. 7, separate peaks for hCG and hCG-α were recognized in all perifusates, and their peaks were found at the positions where 125I-hCG and 125I-hCG-α were eluted.

These studies confirmed the previous results obtained from perifusion studies in which the proportion of the amount of hCG-α to that of hCG increased with gestational age. hCG-α, in comparison to hCG, was present in excess in gel filtrated perifusates of the last two trimesters. There was virtually no hCG but a large peak corresponding to hCG-α in the perifusate of the term.

**Effects of some stimulatory agents on the release of hCG and hCG-α.**

Chorionic tissue obtained from the first trimester of pregnancy were used in these studies.

Fig. 8 shows the stimulatory effect of dbc AMP (1 mM) on the release of hCG and hCG-α. The control perifusion is defined as the perifusion incubated with
Fig. 6. The pattern of hCG and hCG-α release from perifused chorionic tissue in the 38th week of gestation.

Fig. 7. Column chromatography of perifusates of the different gestational weeks, (a) 7th, (b) 22nd and (c) 38th week of gestation. Each fraction was assayed for hCG (●) and hCG-α (○) in their homologous radioimmunoassay systems. The vertical arrows indicate the elution positions of blue dextran (Vo), ¹²⁵I labeled highly purified hCG and hCG-α.
KRBG buffer alone. After a preincubation period, dbc AMP was introduced to the perifusion system for 2 hours. Compared to control levels, a significant transient increase in hCG release was observed after the addition of dbc AMP, whereas the stimulated level of hCG-α was maintained significantly higher than the control in the presence of dbc AMP.

The effects of synthetic LH-RH at various concentrations are shown in Fig. 9. LH-RH at concentrations of 10 ng/ml and 100 ng/ml had no significant effect on the release of hCG and hCG-α (Fig. 9-a, -b). However, when LH-RH at a concentration of 1 μg/ml was infused, significant increases were observed, as shown in Fig. 9-c. The level of hCG release rose rapidly after the addition of LH-RH and returned promptly to the control level after its termination. A similar response was found with repeated equal stimulus, and the maximal increase in hCG release occurred at 20 to 30 min after the stimulation. The pattern of hCG-α release was not always similar to that of hCG, and no increase in its release was observed until the second stimulus.

The addition of mouse epidermal growth factor (EGF) to the perifusion medium at a concentration of 1 μg/ml showed a dif-
Fig. 9. Effect of synthetic luteinizing hormone releasing hormone (LH-RH), at various concentrations on the release of hCG and hCG-α. The concentration of LH-RH was 10 ng/ml (a), 100 ng/ml (b) and 1 μg/ml (c), respectively.
ferent pattern from those observed with dbcAMP and LH-RH (Fig. 10); both hCG and hCG-α levels rose progressively with time after their transient increases in the presence of EGF.

Discussion

Static incubation methods, including short-term tissue culture of placental explants and monolayer culture of placental cells, have normally been used to investigate the release of hCG and its subunits in vitro. However, these methods have provided only a static view of the releasing pattern of hormones. Recently the in vitro perifusion (superfusion; continuous flow incubation) system has been developed in order to eliminate this problem. The greatest advantage of this system over static incubation methods is the observation of changes in minute-to-minute rates of hormone release which may be obscured in the usual static methods and responses to any stimulus with time course.

It is well known that peak hCG concentrations in sera, urine, and chorionic tissues occur between the eighth and the twelfth week of pregnancy and subsequently decline to approximately ten percent of the peak levels in the last two trimesters. Moreover, it has been demonstrated that the free α subunit of hCG is also present independently of hCG in sera of pregnant women and in chorionic tissues. Ashitaka et al. (1974) reported that the secretion pattern of hCG-α was quite different from that of hCG in sera of normal pregnant women and that concentrations of hCG-α increased progressively with low concentrations of hCG-β throughout pregnancy. Vaitukaitis (1974) also showed that hCG and hCG-α, but little or no hCG-β, were present in placental extracts by gel filtration chromatography and found that the proportion of hCG-α relative to hCG increased with gestational age.

The present perifusion and gel filtration studies clearly showed a quantitative changing profile of released hCG and hCG-α from normal chorionic tissues throughout pregnancy. hCG was released in much larger quantities than hCG-α in the first trimester, but the amount of hCG-α released increased gradually with gestational age compared to that of hCG. Larger amounts of hCG-α than hCG were released after the 22nd week of gestation until term, and the ratio of hCG to hCG-α reached about one tenth at term. These results suggest that the relationship between released amounts of hCG and hCG-α is reversed at or around the 20th gestational week, and that about 10 times as much hCG-α as hCG is secreted by the term placenta. In the maternal peripheral circulation at term, however, it was determined by the specific radioimmunoassay that the level of hCG-α was lower than that of hCG (Ashitaka et al., 1974; Hagen and McNeilly, 1975). This discrepancy may be due to the difference between the plasma half life of hCG and that of hCG-α. Because the half life of hCG-α is much shorter than that of hCG (Braunstein et al., 1972), hCG-α should be directly secreted by the placenta in a higher concentration than that detected in the peripheral circulation.

It is reasonable to conclude that the synthesis of hCG may be controlled by the α- and β- subunit syntheses, and the synthesis of hCG-β may be a rate-limiting step in the secretion of hCG in the placenta (Ashitaka et al., 1974 b; Ross, 1977; Daniels-McQueen et al., 1978). According to these hypotheses, possible mechanisms in which such larger amounts of hCG-α than hCG are secreted in the last two trimesters are considered to be: 1) the synthesis and secretion of hCG-α independently increases with gestational age, and 2) with the decrease of the secretion of hCG reflecting the decrease of hCG-β synthesis, hCG-α as
a precursor for hCG synthesis would be excessive, and consequently large amounts of free hCG-α are secreted by the placenta in the last two trimesters.

It has been indicated that the adenylyl cyclase-cyclic AMP system, which had been found in the human placenta (Satoh and Ryan, 1971), mediates the release of hCG and hCG-α in vitro. Many investigators have demonstrated that the release of hCG and hCG-α is stimulated by the addition of dibutyryl cyclic AMP (dbc AMP) which induces an increase in intracellular cyclic AMP with the static incubation methods (Handwerger et al., 1973; Hussa et al., 1974; Chou et al., 1978). As shown in Fig. 8, we confirmed that dbc AMP stimulated the release of hCG and hCG-α from normal chorionic tissues, but the increases observed after the addition of dbc AMP, particularly the transient increase in hCG release, would be difficult to demonstrate in vitro other than by perifusion. Although the mechanism of action of cyclic AMP on the release of hCG and hCG-α is not elucidated, the difference between the patterns of hCG and hCG-α release in response to dbc AMP may suggest that the stimulatory mechanism of cyclic AMP in hCG release is not always similar to that in hCG-α release.

Since Gibbon et al. (1975) initially reported that LH-RH, which was biochemically and biologically indistinguishable from that of hypothalamic origin, was synthesized by the human placenta in vitro, much interest has been focused on the significance of this hormone. Moreover, recently Siler-Khodr and Khodr (1978) reported that large amounts of immunoreactive LH-RH were contained in the human placenta throughout pregnancy. Synthetic LH-RH has been well employed to assess the pituitary gonadotropin responses in vivo, and it has been demonstrated that the serum hCG levels of pregnant women showed no significant change after the administration of LH-RH of 100 or 200 μg (Tamada et al., 1976; Rubinstein et al., 1978). On the other hand, in vitro studies, synthetic LH-RH stimulated the release of hCG from the human placenta (Khodr and Siler-Khodr, 1978). The present results agree with their in vitro observations and 1 μg/ml of synthetic LH-RH caused a significant increase in the release of hCG and hCG-α. The amounts of LH-RH required in vitro appear to be larger than those used for administration in vivo. Although such large amounts, which might not always be in the unphysiologic range for in vitro studies, can not be directly applied in vivo, it is possible that the amounts of LH-RH administered to pregnant women are too small to result in a significant change in serum hCG levels.

The mechanism by which LH-RH stimulates the release of hCG and hCG-α was not investigated in the present study, and it is a subject of much speculation. The cyclic nucleotide may be involved in the mediation of the release of hCG and hCG-α by placental LH-RH as pituitary LH released by hypothalamic LH-RH. There are many reports that cyclic AMP or cyclic GMP acts as the second messenger in the increase in LH release by LH-RH in vitro (Kaneko et al., 1973; Makino, 1973; Naor et al., 1980). They demonstrated that synthetic LH-RH stimulated the production of these cyclic nucleotides in the rat pituitary within 10 min after the stimulation and that an increase in LH release followed. These data are similar to our results in that the increase in hCG and hCG-α release was clearly found at 20 min after the addition of LH-RH. However, definitive proof of this hypothesis can be obtained only by measuring the concentrations of cyclic nucleotides in the chorionic tissue after the addition of LH-RH with time course. The perfusion system is a useful experimental method for studying the hormone release with the
time course, but it is not a suitable one to use in measuring the change in their tissue levels simultaneously. Because of this disadvantage, it should be noted that other in vitro experimental methods are necessary to support this hypothesis.

Epidermal growth factor (EGF) is a polypeptide originally isolated from the submaxillary glands of adult male mice and it has been reported that EGF stimulates the growth of a variety of tissues in many species, including mouse, rat, chick, rabbit and human, both in vivo and in vitro (Cohen and Tayler, 1974). Like other peptide hormones, the binding of EGF to the cell membrane of target organs is thought to be the first step necessary for its biological activity. EGF has been demonstrated to bind specifically to membranes derived from various tissues including the human placenta and rat liver (O'Keefe et al., 1974), and therefore these membrane fractions have been used as the source of receptor in the specific radioreceptor assay for EGF. We confirmed that human placental membrane fractions (100,000 × g fraction) obtained from placentas of both the first trimester and the term bound in a specific manner to 125I labeled EGF. These findings suggest that EGF has some growth-promoting effects on human placenta, but little is known about these effects, especially the effect on the release of placental hormones in vitro (Benveniste et al., 1978).

Our present data indicate that EGF may have a stimulatory effect on the production of hCG and hCG-α as well as on their release, because the amounts released increased progressively with the time course after their transient increases following the addition of EGF. These progressive increases may simply reflect the proliferation of trophoblasts by EGF as a potent mitogen as in the culture of human fibroblasts and HeLa cells (Cohen and Tayler, 1974), or they may be due to some other stimulatory effect of EGF on the synthesis of hCG and hCG-α. For example, EGF may stimulate hCG synthesis by directly stimulating the synthesis of mRNA, possibly acting as a regulator of hCG synthesis.

The present studies demonstrate that the perifusion system for chorionic tissues, as for other endocrine tissues, is a useful method for investigating the release of hCG and its subunits in vitro. Although we also demonstrated that some agents, such as dbc AMP, LH-RH, and EGF, stimulated the release and production of hCG and hCG-α by using this system, further studies are necessary to clarify the correlation among these agents and other agents that have influence on the release and production of hCG and hCG-α. Moreover, the system reported here may be useful for studying the process from biosynthesis to the release of hCG and its subunits in vitro.

Acknowledgements

Highly purified hCG (CR-119) was generously donated by the Center for Population Research of the National Institute of Child Health and Human Development of National Institutes of Health.

This work was supported in part by grants from the Ministry of Education of Japan (No. 00548285, 00567284, and 56770726) and by a grant for Cancer Research from the Ministry of Health and Welfare, Japan (No. 5613).

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