Metabolism of ^{14}C -pregnenolone in the Placenta Throughout Pregnancy in Organ Culture

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Abstract

The present study was undertaken to assess the metabolism of radiolabeled pregnenolone in the placenta throughout pregnancy in organ culture. The product generated from pregnenolone was only progesterone regardless of the weeks of gestation. The amount of progesterone formed by the placenta was $5.60 \pm 0.15 \times 10^{-11}$ moles/mg protein/hour in the 1st trimester ($n=4$), $6.47 \pm 0.09 \times 10^{-11}$ moles/mg protein/hour in the 2nd trimester ($n=3$) and $7.72 \pm 0.25 \times 10^{-11}$ moles/mg protein/hour in the 3rd trimester ($n=6$). The increase in the formation of progesterone as gestation advanced was statistically significant. The increase in the placental weight throughout pregnancy was in proportion to the rise in maternal plasma levels of progesterone. These data indicate that the manifold increase in progesterone in the maternal circulation as gestation advances mainly reflects the increase in the functional mass of the placenta rather than the increased rate of production of progesterone in the placenta.

During pregnancy, the placenta synthesizes a large amount of progesterone and estrogen and secretes them into both maternal and fetal circulation (Tulchinsky *et al.*, 1975). Maternal plasma levels of progesterone rise from 30 to 180 ng/ml from the 1st to the 3rd trimester of pregnancy (Tulchinsky *et al.*, 1972), of which the physiological mechanism has been attributed partly to the increase in 3β -HSD (Δ^5 - 3β -hydroxysteroid dehydrogenase; Δ^5 , 4 -isomerase) activity in the placenta catalyzing the formation of progesterone as demonstrated by the histochemical studies (Bertha *et al.*, 1962; Baillier *et al.*, 1965).

However, the metabolism of pregnenolone throughout pregnancy has not been shown

in the placenta in organ culture. Therefore, the elaboration of the organ culture method for the human placenta in our laboratory prompted us to investigate the metabolism of pregnenolone in the placenta at 7 to 38 weeks of pregnancy to assess the mechanism of the rise in maternal plasma levels of progesterone during pregnancy.

Materials and Methods

Chemicals and culture medium

A nutrient mixture of F-10 (Ham) and fetal bovine serum were purchased from GIBCO. Kanamycin sulfate was obtained from Takeda Pharmaceutical Co. ^{14}C -pregnenolone (S.A. 57.2 mCi/mmol) was purchased from New England Nuclear. The radiochemical purity of ^{14}C -pregnenolone was in excess of 98%. Unlabeled steroids were purchased

from Sigma Chemical Co. (St. Louis, Missouri).

Tissue preparation and culture method

Specimens of normal placenta were obtained at the time of vaginal delivery and induced abortion. The gestational ages were 7, 15, 16, 18, 24, 35, 37 and 38 weeks. The specimens were prepared under sterile conditions for organ culture as soon as possible after expulsion of the placenta. The placental villi were isolated and minced into pieces approximately 1 to 2 mm³ in size under a microscope equipped with a 1/100 cm objective lens scale. The outer well of the culture dishes (Falcon 3037, Bacton, Dickinson and Co.) was filled with physiological saline for humidification. A stainless steel organ culture grid (Falcon 3014, Becton, Dickinson and Co.) was placed over the center well containing 450 μ l of nutrient mixture of F-10 supplemented with 20 % fetal bovine serum and kanamycin sulfate at a concentration of 80 μ g/ml. As a supporting matrix, a millipore filter with 1.2 μ m pores (Gelman Instrument Co., USA) was selected and draped over the metal organ culture grid. After tissue preparation, two pieces of the placental tissue were transferred onto the supporting matrix (Fig. 1). The placental tissue was pre-incubated for 24 hours at 37°C in a static chamber with a gas mixture of 95 % air and 5 % CO₂ prior to the incubation to measure the amount of metabolites generated from pregnenolone.

Formation of metabolites from pregnenolone

The placental tissues pre-incubated for 24 hours were thoroughly washed and transferred to new culture dishes containing ¹⁴C-pregnenolone (1.75 n moles, 0.1 μ Ci) dissolved in 5 μ l of ethanol as the substrate. The final concentration of ethanol in the culture medium was less than one per cent. No cofactors were added to the culture medium, since the placental tissues in this organ culture have been demonstrated to keep the cell function intact. The incubation was terminated at 20 hours, which was determined from the results of the time course study. Extraction of the steroids was carried out

three times with two volumes of dichloromethane from the medium. Fifty μ g of pregnenolone, ten μ g of progesterone and 20 α -dihydroprogesterone were added to the extracts as carriers. The extracts were chromatographed on a silica gel thin layer plate in a system of isopropyl ether: petroleum ether: acetic acid (70: 30: 2, vol/vol). Radioactive regions on the thin layer chromatogram were detected with a β -chromatogram camera (Aloka, BCC-101B) and scraped off the plate. Each radioactive substance was once eluted from silica gel with 0.2 ml of the mixture of chloroform and methanol (2: 1, vol/vol). The amount of radioactivity recovered during the whole procedure ranged between 50 % and 70 %. An aliquot of each eluate was quantitated with a liquid scintillation counter (Aloka, LSC-700). The amount of the product was calculated by dividing the radioactivity found in the product by the initial specific activity of the substrate added to the culture medium. Identification of the product was carried out by repeated crystallization of the radioactive product with the corresponding authentic reference. The placental tissues obtained at the end of incubation were homogenized by sonication in PBS. An aliquot of the homogenates was used for protein determination by the Lowry method with bovine serum albumin as a standard. The data are the mean values obtained with duplicate incubations.

Expression of the production rate

The production rate of the metabolites from pregnenolone was tentatively expressed as the mean value of the amount of products in $\times 10^{-11}$ moles/mg protein/hour.

Results

Metabolism of ¹⁴C-pregnenolone in the placental tissues in organ culture

The placental tissues cultivated for 20 hours converted ¹⁴C-pregnenolone only to radioactive progesterone. No significant radioactivity was detected in the region corresponding to the authentic 20 α -dihydroprogesterone (Fig. 2).

Time course study of ¹⁴C-pregnenolone metabolism

The placental tissues obtained at 37 weeks of pregnancy were incubated with ¹⁴C-pregnenolone (1.75 n moles, 0.1 μ Ci) for 2, 4, 8, 12 and 24 hours respectively.

Fig. 1. Schema of the organ culture dishes. The details of the procedure are described in the text.

Fig. 2. Radiochromatogram of the thin layer chromatography detected by β -chromatogram camera. Solvent system is composed of isopropyl ether: petroleum ether: acetic acid (70:30:2, vol/vol).

Radioactive progesterone was quantitated at the end of each incubation time. Results of the time course study of the formation of progesterone are shown in Fig. 3. Formation slowly increased with time up to 8 hours, followed by a linear increase from 8 to 24 hours.

The amount of progesterone formed in the placental tissues obtained at different periods of gestation

The amount of progesterone generated from pregnenolone in the placental tissues was $5.60 \pm 0.15 \times 10^{-11}$ moles/mg protein/hour in the 1st trimester ($n=4$), $6.47 \pm 0.09 \times 10^{-11}$ moles/mg protein/hour in the 2nd trimester ($n=3$) and $7.72 \pm 0.25 \times 10^{-11}$ moles/mg protein/hour in the 3rd trimester ($n=6$). Thus, the rate of production of progesterone in the placental tissues increased linearly and significantly as gestation advanced. In the placenta of the 3rd trimester, it was 137.5 % of that in the 1st trimester of pregnancy as a corresponding control ($p<0.01$).

The correlation among maternal plasma levels of progesterone, weight of the placenta and production rate of progesterone in the placenta throughout pregnancy is illustrated in Fig. 4. This shows that the rate of the increase in maternal plasma levels of progesterone was in proportion to that of the

Fig. 3. Time dependent conversion of pregnenolone to progesterone by the placental tissues in organ culture. Incubations were carried out in duplicate. Values are nano moles/mg protein.

placental weight throughout pregnancy, while the rate of production of progesterone rose only 37.5 % between the 1st and 3rd trimesters of pregnancy. Therefore, it is very likely that the main reason for the increase in maternal plasma levels of progesterone as gestation advances is due to the increase in

Fig. 4. Correlation among maternal plasma levels of progesterone (P_4 ;), weight of the placenta (PW; —•—) and production rate of progesterone (Δ — Δ) throughout pregnancy.

the functional mass of the placenta rather than to an increase in the rate of production of progesterone.

Discussion

Steroid metabolism in the human placenta has so far been investigated intensively by using homogenates (Ryan *et al.*, 1966), subcellular fractions (Pearlman *et al.*, 1954), minced tissues (Nissim *et al.*, 1952), perfusion (Jaffe *et al.*, 1966) and monolayer cell culture (Hall *et al.*, 1977; Paul *et al.*, 1980) of the placenta.

These experimental methods to assess the steroid metabolism in the placenta have some drawbacks in that the tissue prepared lacks physiological integrity in terms of structure and function of the placenta. Therefore, the organ culture method for the human placenta was elaborated in our laboratory to assess the metabolism of pregnenolone in the placenta under as natural conditions as possible.

Prior to the incubation of the placental tissues in the culture medium containing ^{14}C -pregnenolone to investigate the metabolism of pregnenolone, the placental tissues were pre-incubated for 24 hours to adapt the tissues to the culture environment and

to deplete the endogenous substrate for progesterone. In fact, the concentration of pregnenolone in the tissue homogenates after 24 hours of pre-incubation was less than 0.316 pM in the term placenta. It follows that the amount of endogenous substrate remaining in the tissues was less than 1/10,000 of the added radioactive substrate. Therefore, the concentration of the endogenous substrate was not taken into consideration when a sufficient amount of radiolabeled exogenous substrate was added.

The metabolite from ^{14}C -pregnenolone under these conditions was only progesterone regardless of the number of weeks of gestation, although hydroxylation and a reduction in progesterone were demonstrated in the other tissue preparations (Tabei *et al.*, 1975; Diaz-zagoya *et al.*, 1977; Laatikainen *et al.*, 1980). Considering the fact that the placental 20α -hydroxysteroid oxidoreductase activity was approximately one tenth of that of 3β -HSD in the term placenta (Diaz-zagoya *et al.*, 1977), it might be possible that the specific activity of ^{14}C -pregnenolone used in this study was too low to detect the 20α -dihydroprogesterone formed.

The amount of progesterone formed did not vary linearly with time after 2 to 8 hours. The initial delay in the formation of progesterone, which was also reported by Ferre

et al. (1980), might be due to the slow incorporation of the substrate into the cells or to insufficient interaction between substrate and enzyme.

A significant increase in the formation of progesterone in the placental tissues as gestation advanced was clearly demonstrated in our study. However, the manifold increase in the amount of progesterone in the maternal circulation towards term cannot be explained by the 37.5% rise in the production rate of progesterone in the placental tissues from the 1st to the 3rd trimester of pregnancy. On the other hand, the weight of the placenta increases as gestation advances in proportion to the rise in maternal plasma levels of progesterone. Since the weight of the placenta reflects the volume of the trophoblast cells, its rise from the 1st to the 3rd trimesters implies an increase in the volume of the syncytiotrophoblast (Teasdale, 1980) as well as an increase in DNA content in the placental tissues (Winick *et al.*, 1967).

In conclusion, therefore, the manifold rise of progesterone levels in the maternal circulation is due mainly to the increase in the functional mass of the placenta as gestation advances.

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