IN VITRO EFFECT OF HUMAN PLACENTAL LACTOGEN ON STEROIDOGENESIS IN HUMAN PLACENTAL TISSUE FROM EARLY PREGNANCY*

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(Received on October 30, 1979)

Summary : Studies were carried out with human placental lactogen (hPL) to elucidate its role in regulation of steroidogenesis (progesterone and estrogens) during early pregnancy in humans. Our *in vitro* studies with early pregnancy placenta under different doses of hPL demonstrated that this hormone could stimulate the synthesis of progesterone as well as estrogens (estrone and estradiol) from their respective precursors.

Key words: steroidogenesis human placental lactogen progesterone estrogens

INTRODUCTION

The placenta occupies a unique position among the endocrine glands. It is a temporary organ and during pregnancy it plays a central role in the endocrinology of pregnancy, producing peptide hormones, human chorionic gonadotropins (hCG) and hPL and steroidal hormones, estrogen and progesterone, which are essential for the maintenance of pregnancy. But knowledge regarding the mechanism of hormone regulation of placental function is not yet clearly understood.

During the last two decades, sufficient knowledge has accumulated regarding the functions of hCG, estrogen and progestins and their interdependence during pregnancy. During early pregnancy, the corpus luteum is sustained by hCG and secretes progesterone till the placenta is fully developed and takes over the functions of the corpus luteum (10).

Another protein hormone, human, placental lactogen (hPL), which is secreted and synthesized by the placenta from 35th day onward of pregnancy (10) is considered to be an important factor in maintaining pregnancy upto term. As pregnancy advances, the level of hPL goes on increasing but falls at once after delivery. Studies of Tomi *et al.* (5,6) on rabbits and rats showed that immunization with hPL caused interruption of placental function during the post-implantation period, and resulted in foetal death and foetal resorption. These authors suggested that circulating antibodies in treated animals might have interfered with placental steroidogenesis. On the other hand, *in vivo* studies indicated that steroidogenesis in human corpus luteum was not affected by large doses of hPL (13).

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*Part of Ph.D. Thesis of first author submitted to the All India Institute of Medical Sciences, New Delhi.

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The present studies also indicated that passive immunization of pregnant rats w antisera against early pregnancy placenta, which possessed antibodies against a place antigen immunobiologically identical to hPL, resulted in foetal death and foetal resorp (8). These observations prompted us to carry out studies, in depth, to explore the rol hPL in the regulation of steroidogenesis in the human placenta. As the corpus lut maintains pregnancy by secretion of progesterone till 10 to 12 weeks of pregnancy and that stage the placenta takes over the function of corpus luteum, so it was deemed fi explore the role of hPL in the regulation of steroidogenesis in the human placenta th early pregnancy (i.e. 10 to 12 weeks).

MATERIAL AND METHODS

Radioactive steroids and chemicals: Pregnenolone-α-^{3}H (specific activity 18 c mole), oestradiol-4-^{14}C (specific activity 56 mci/m mole) Androst-4-ene, 3, 17, dione 7- -^{3}H (specific activity 8.3 ci/m mole), progesterone 4-^{14}C specific activity 50-60 mC mole were obtained from Radiochemical Centre, Amersham. The purity of the labe steroids was checked by thin layer chromatography and scanning the chromatograms on radiochromatogram scanner (Packard Model-7200).

Radioinert hormones: Progesterone, estradiol-17β, estrone, 5-pregnenolone. Androst- one-3, 17 dione were obtained from Sigma Chemical Co., U.SA. hPL was purchas from Nutritional Biochemicals Corporation, U.S.A.

Other chemicals: Laboratory chemicals and solvents were of analytical grade, obtain from B.D.H. (India). The solvents were further purified and distilled before use. Adenosi triphosphate (ATP), Glucose-6-phosphate, glucose-6-phosphate-dehydrogenase, triphos phophyridine nucleotide, (Sigma Chemical Co., U.S.A.) and Sucrose (Schwartz-Mann).

Collection and preparation of placental tissue: Early pregnancy placentae (10 to 12 week were collected from women who underwent medical termination of pregnancy a. All-Indi Institute of Medical Sciences Hospital, New Delhi. The placental tissue was stored unde cold conditions soon after hysterotomy. The tissue was washed and minced repeatedl with chilled buffer to remove clots. This minced tissue was used for *in vitro* biosynthesis o progesterone from its precursor.

Subcellular fractionation: The minced placental tissue was homogenized with a polytron (PT-10) homogeniser in sodium phosphate buffer (pH 7.0, 0.05 M). The homogenate was filtered through cheese cloth and fractionated in a Beckman preparative ultra-centrifuge model-L, using rotor H-40. The nuclear fraction was obtained by centrifugation at 800 x g for 10 min. The supernatant from above was centrifuged at 17.500 x g for 30 min for separation of mitochondrial pellet and this supernatant was further centrifuged at 105,000 x g for 60 min and microsomal pellet was obtained.

In vitro biosynthesis of progesterone and estrogens :

To study the biosynthesis of progesterone, 500 *mg* of minced placental tissue was incubated in Kreb Ringer's phosphate buffer (0.15 M; pH 7.2) with pregnenolone-^{3}H (1.5 to 2.0 uCi) in the incubating flask at 37°C for 2 hr with air as a gas phase in a Dubnoff metabolic shaker. Similarly for the study of biosynthesis of estrogens the microsomal fraction obtained from 2.5 to 5 g placental tissue was incubated with ^{3}H-androstenedione (1-2 uCi) suspended in 5 *ml* of sodium phosphate buffer (0.05 M; pH 7.0) containing sucrose (0.2 M) and nicotinamide (0.04 M) and TPNH generating system (10 uM TPN, 60 uM glucose-6-PO_4 and 1.5 K unit glucose-6-PO_4-dehydrogenase) for 1 hr at 37°C in metabolic shaker with air as a gas phase (1). Four sets of incubations for progesterone biosynthesis and four sets of incubations for estrogens biosynthesis in duplicate were prepared, one out of which served as control. For progesterone biosynthesis to the other three sets 100 *µgs*, 200 *µgs* and 500 *µgs* of hPL was added respectively and for estrogen biosynthesis to the other three sets 100 *µg*, 200 *µg* and 500 *µg* of hPL was added to the incubation flasks respectively.

Extraction of total radioactive steroids from tissue :

Four volumes of acetone were added to centrifuge tubes containing samples. The tubes were centrifuged in a refrigerated centrifuge at 2,500 rpm for 15 min. The supernatant was preserved and to the sediment, acetone was added again, stirred and centrifuged. The acetone extracts were pooled. The steroid from the sediment was again extracted with methanol and pooled with the acetone extract. The total extract was dried completely under nitrogen at 40-45°C. The dried extract was dissolved in 5 *ml* of alkaline water, pH 10, which facilitated the extraction of neutral steroids. For the extraction of estrogens, the dried extract was dissolved in 5 *ml* of acidic water (pH 4.5). To the aqueous extract, 15 *ml* of diethyl ether was added. The free steroid was extracted in ether. The ether extraction of the steroids from the aqueous phase was carried out thrice. The labelled steroids thus extracted were dried under nitrogen and the sample was then ready for further analysis by thin layer chromatography.

Chromatograph of the radioactive steroids :

Thin layer chromatography (TLC) was carried out on silica gel G and HF (0.25 mm) coated plates. The plates were activated at 110°C for 1 hr prior to use. The samples along with standard progesterone and estrogens (estrone and estradiol) were spotted on TLC plates, and ascending chromatography was performed in the following solvent systems for progesterone and estrogens.

(1) Hexane : Ethylacetate (5:2), (2) Dichloromethane : Ethylacetate (5:2) (Progesterone), Benzene : Methanol (9:1) (estrogens). The reference progesterone was located on the plates under ultraviolet light and in iodine chamber the estrogens were located. The

standards were marked and the whole plate was divided into one cm - fractions and el with chloroform methanol (1:1), three times and dried under nitrogen.

Radioactivity counting :

Radioactivity was counted in a Tricarb liquid scintillation spectrometer (Pack Model, 3380), using simple scintillation fluid, containing 2, 5-diphenyloxazole (PP 4.00 *gm* and 1,4-bis-2 (4 methyl-5 phenyl-oxazolyl) benzene (POPOP) 100 *mg*, disso in 1000 *ml* of toluene.

RESULTS

Influence of different doses of hPL on estrogens and progesterone biosynthesis :

The *in vitro* conversion of androstenedione to estrone (E_1) and estradiol (E_2) expressed as dpm x 10^6/*gm* tissue microsomes of placenta from early pregnancy h been presented in Fig. 1. It was observed that hPL stimulated the conversion of p precursor to E_1, showing an increase of 12.3% over the control at the 100 μg dose le followed by the maximum stimulation of 25.7% at the 200 μg dose. hPL also stimulate the synthesis of E_2 which followed a pattern similar to that of E_1. Furthermore, th conversion of androstenedione was markedly in favour of E_1 being 4 times greater tha that of E_2. At the 500 μg dose the conversion was decreased to the same level as th 100 μg dose i.e. 12.3% (Fig. 1).

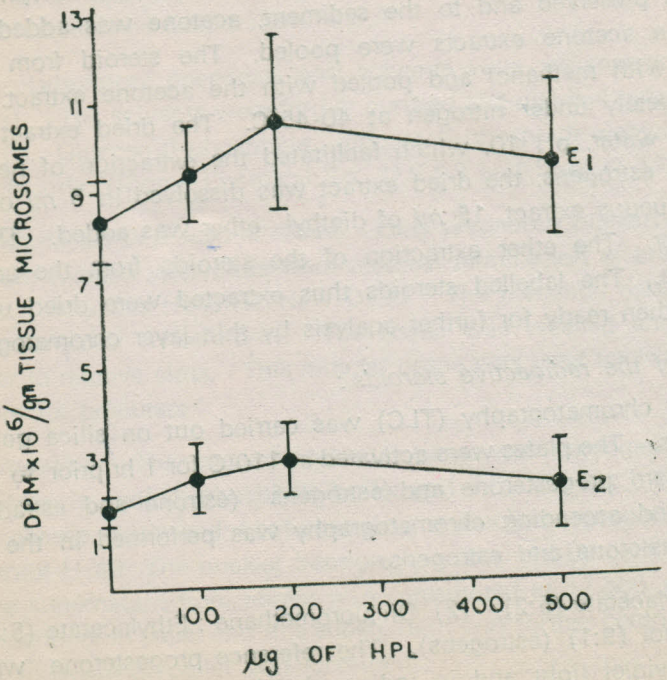

Fig. 1 : Dose response of hPL action on the synthesis of estrone (E_1) and estradiol-17 B (E_2). (Mean of 5 experiments ± SEM)

Fig. 2 shows the progesterone synthesis in placental tissue expressed in dpm x 10^{-6}/*gm* tissue in the presence of different concentration of hPL. As is evident from the dose response curve, hPL at a concentration of 100, 200 and 500 μg per incubation stimulated the synthesis of progesterone by 29.6%, 34.1% and 35.1% respectively over the control.

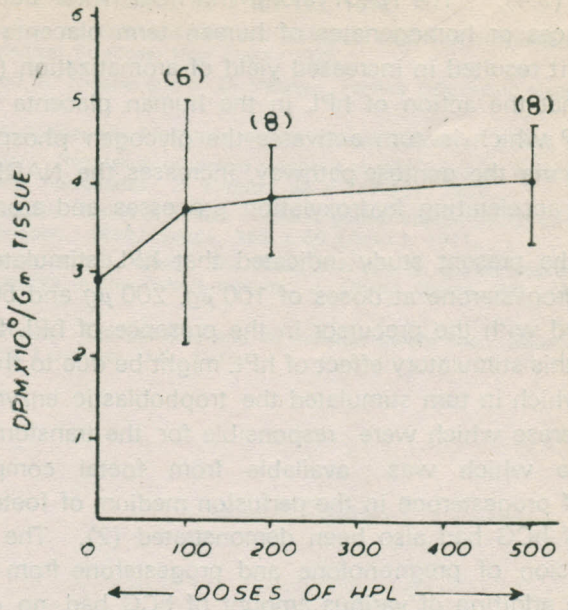

Fig. 2 : Dose response of hPL action on the synthesis of progesterone. Figures in parenthesis denote the number of experiment. (Mean ± SEM).

DISCUSSION

Effect of hPL on placental steroidogenesis :

Our studies showed that hPL binding components existed in 800 x g pellet from early pregnancy placenta of humans (8). In these *in vitro* studies, a positive effect of hPL on the biosynthesis of progesterone and estrogens from their precursors in the human placenta from early pregnancy was demonstrated. As far as the *in vitro* biosynthesis of estrogens from its precursor Δ^4-Androstenedione - ^3H in the early pregnancy placental microsomes is concerned, the results depicted that in the absence of exogenously added hPL the precursor was transformed into both estrone and estradiol. Conversion from androstenedione to estrone was 4 fold that of estradiol. The addition of hPL to the incubates resulted in a stimulation of biosynthesis of E_1 and E_2 at all the three dose levels i.e. 100, 200 and 500 μg, the maximum increase being at the 200 μg. dose (Fig. 1). This stimulatory effect of hPL may be due to the stimulation of syncytiotrophoblastic

cells indirectly stimulating the NADPH generating system which might, in turn, fa the hydroxylation of androstenedione on 19th position under the influence of hydroxy and demelase enzyme (1,3,7,9). The stimulatory effect of gonadrophins and cA on the aromatization of neutral steroids by human full term placenta when perfused *in v* had also been shown (3,4). The NADP alongwith hCG when added to the incubat media containing minces or homogenates of human term placenta and ^{14}C-testoster and androstenedione it resulted in increased yield of aromatization (9,12). It is, therefo possible to assume that the action of hPL in the human placenta from early pregna is mediated by cAMP which in turn activates the glycogen phosphorylase, the glyco breakdown, produced via the pentose pathway, increases the NADPH levels, and the c factors necessary for accelerating hydroxylation processes and aromatization.

The data of the present study indicated that hPL stimulated the conversion pregnenolone-^{3}H to progesterone at doses of 100 μg, 200 μg and 500 μg when the tiss minces were incubated with the precursor in the presence of hPL for two hours (Fig. 2 It is conceivable that this stimulatory effect of hPL might be due to the stimultion of hum trophoblast by hPL, which in turn stimulated the trophoblastic enzymes $\triangle^{5}$-3β-ol-dehdr genase and $\triangle^{5}$-isomerase which were responsible for the transformation of progesteron from $\triangle^{6}$-pregnenolone which was available from foetal compartment (14). T increased secretion of progesterone in the perfusion medium of foeto-placental unit *in v* under the infuence of hCG had also been demonstrated (2). The addition of co-fact increased the conversion of pregnenolone and progesterone from cholesterol by placen homogenates, but the addition of various amount of hCG had no effect on conversion cholesterol to pregnenolone and progesterone (9). The stimulatory effect of hPL on t transformation of pregnenolone to progesterone, demonstrated in the early pregann placenta in this study might be due to the activation of adenyl cyclase which was bou to the inner side of cell membranes and activated the enzymes for energy production a specific hydroxylases. The role of cyclic AMP which could mimic the function of LH a stimulate progesterone biosynthesis in rabbit ovarian homogenates had been indica (9,11).

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