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REGULATION OF PROGESTERONE BIOSYNTHESIS IN THE HUMAN PLACENTA BY ESTRADIOL 17ß AND PROGESTERONE

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SUMMARY

Ex vivo addition of estradiol 17ß to first trimester or term human placental minces caused a significant increase in the quantity of progesterone produced. Addition of an aromatase inhibitor, CGS 16949 A, or the estrogen receptor antagonist, ICI 182780, significantly inhibited progesterone production confirming the role of estradiol 17ß in the regulation of progesterone synthesis in human placenta. RU 486 and ZK 98299, which are antagonists of progesterone receptor, significantly modulated progesterone synthesis in the human placenta but exhibited paradoxical effects on the first trimester and term placenta. We conclude that progesterone synthesis in the human placenta is regulated by estradiol 17ß and progesterone. This is the first report providing evidence for autoregulation of progesterone synthesis in the human placenta.

Key Words: Progesterone, synthesis, human placenta, regulation, autoregulation, estradiol 17β.

INTRODUCTION

The human placenta, besides serving the function of transport of nutrients from the maternal circulation to the fetus and providing protection from the attack of maternal immune system against the fetal semi-allograft, also serves as a very efficient endocrine gland by its ability to synthesize and secrete a variety of protein, peptide and steroid hormones.

One of the most important steroid hormones produced by the human placenta is progesterone (P4), which is absolutely indispensable for the maintenance of pregnancy (1). The levels of P4 continuously increase throughout the course of pregnancy and the quantity of P4 produced by the

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Abbreviations : P4- Progesterone, E2- Estradiol 17ß, FTHP- First Trimester Human Placenta, LDL- low density lipoprotein, EBSS- Earle's Balanced Salt Solution, RIA- Radioimmunoassay.

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human placenta can be as much as 250 - 600 mg per day during late gestation (2). The intraplacental concentration of P4 is estimated to be approximately as high as 7μM (3). However, it has little capacity to synthesize cholesterol, the precursor for the synthesis of P4, *de novo* from acetate (4). The human placenta obtains cholesterol from the maternal system in the form of low density lipoprotein {LDL} (5). Since the human placenta is completely dependent on the supply of cholesterol in the form of LDL from the maternal system, uptake of LDL by specific LDL receptors is a rate limiting step for the synthesis of P4. However, recent studies have established that the levels of LDL-receptor decline with the progress of pregnancy and the α2-macroglobulin receptor takes over the function of uptake of LDL during the course of pregnancy (6).

There is very little information on the regulation of P4 synthesis and the factors controlling it in the human placenta are largely unknown. Chorionic Gonadotropin, which is known to rescue the *corpus luteum* and maintain its P4 production until the placenta acquires steroidogenic capacity, does not have any direct role in the regulation of P4 synthesis by human placenta (7). Feinberg *et al* (8) have reported that P4 secretion by JEG-3 choriocarcinoma cells is stimulated by cytokines like IL-1 and TNF-α. However, these may not be the major regulatory factors as these cytokines are produced by monocytes upon immune-activation of these cells. Hence, this observation may not be of much significance *in vivo*. Kato *et al* (9) have demonstrated that phorbol esters stimulate P4 production by human term placental explants implicating a role for Protein Kinase C and Ca^{2+} in this process. However, the extracellular signals which might initiate these intracellular pathways are largely unknown.

The placental steroids, estradiol 17ß (E2) and P4 are good candidates for regulating P4 synthesis in the human placenta. In fact, E2 is known to facilitate the uptake of LDL and thereby stimulate P4 synthesis in the baboon placenta (10). In fact, Wunsch *et al* (11) demonstrated that the antiestrogen, MER-25, inhibited P4 production by human placental cells cultured *in vitro*. However, subsequently it was demonstrated that MER-25 can exhibit agonistic activities (12) and hence act like estrogen itself. Consequently, it is not yet clear whether estrogen stimulates or inhibits human placental P4 production. Our recent studies have provided unequivocal proof for the presence of progesterone receptors in the human placenta (13) and thus, human placenta is very likely a target tissue for the action of P4. Also, P4 synthesis has been suggested to be under autoregulation (14). In the present study, we have investigated the role of P4 and E2 in the regulation of P4 production by the first trimester and term human placenta. Our findings reveal that both these hormones have an important role in regulating placental P4 production.

MATERIALS AND METHODS

CGS 16949 A was a gift from Dr. A.S. Bhatriagar, Ciba-Giegy, Basel, Switzerland. ICI 182780 was obtained from Zeneca Pharmaceuticals Chesire, England. RU 486 was obtained from Roussel-Uclaf, Paris, France. ZK 98299 was obtained from Schering AG, Berlin, Germany. [^{3}H]-Progesterone was obtained from Amersham, UK. Aminoglutethimide was obtained from Sigma Chemical Co., St. Louis, USA. Estradiol 17 ß was obtained from Steraloids Inc. USA. All other chemicals used were of analytical grade.

Preparation of human placental minces for ex vivo incubations

First trimester human placental minces were prepared as described earlier (15). In brief, first trimester human placenta (FTHP) [7-12 weeks gestation] from cases of medical termination of pregnancy or term placenta from cases of Caesarean section, were collected in cold Earle's balanced salt solution (EBSS), pH 7.2, on ice. The tissue was extensively washed with normal saline to remove blood contamination. The villi were separated from membranes and other tissues by visual examination and the villi were minced using a fine scissors. The minced villi were pelleted by centrifugation at 500 x g, at 4°C for 2-3 minutes and after washing, the pellet was resuspended in a known volume of EBSS and about 50-100 mg wet weight of tissue from this preparation was aliquoted into the tubes and used for *ex vivo* studies. In order to study the effect of various modulators such as CGS 16949 A and RU 486, placental minces were incubated for 30 minutes in EBSS in an atmosphere of 95% O_2 and 5% CO_2 at 37°C in order to allow the endogenous steroids to be secreted out. In the human placenta, due to the presence of high endogenous level of steroid hormones, it is not possible to study the effect of added steroids. In order to overcome this problem, we have employed inhibitors of hormone synthesis or action to minimize the effects of endogenous hormones and study the effect of deprival of hormones. Following the pre-incubation, the minces were collected by centrifugation, washed with EBSS, and incubated with the desired concentration of modulators for 2 hours under identical conditions. The tissue was homogenized in EBSS; steroids were extracted with diethyl ether and used for the measurement of P4 by a radioimmunoassay (RIA).

RIA for Progesterone

Progesterone was estimated by a specific RIA which was standardized in our laboratory. The antiserum was used at a final dilution of 1: 5000 and the range of the assay was 2.5 pg to 1000pg. The minimum detectable quantity of P4 was 15 pg. The inter-assay variation was 6.5% while the intra-assay variation was 2%. The extraction efficiency varied from 85-90% and the values reported are uncorrected for recovery.

RESULTS

Fresh synthesis of progesterone occurs during the *ex vivo* incubation

One of the primary requirements for the present study is to ascertain the fact that the placental villi are capable of synthesizing P4 during the period of incubation. In order to establish this, placental villi were incubated with 0.43 mM aminoglutethimide, which inhibits side-chain cleavage enzyme, which converts cholesterol to pregnenolone. Aminoglutethimide was able to decrease P4 levels (data not shown) to a very significant extent in both FTHP (90% inhibition) and term placental

(75% inhibition) minces, confirming that fresh synthesis of P4 takes place during the incubation. In addition, externally added substrate in the form of LDL or serum also stimulated P4 production by the placental minces (data not shown), providing additional support for the above conclusion. Further, a time course study of P4 production by placental minces revealed that P4 production is maximal at 2 hours of incubation. Hence, the effects of all the modulators described in this study were examined at 2 hours of incubation.

Effect of addition of Estradiol 17 ß

In order to investigate the role of E2, the effect of E2 was studied after inhibiting the endogenous biosynthesis of E2 using CGS 16949 A, an aromatase inhibitor, and then adding E2. Results presented in Fig. 1 (a & b) demonstrate that E2 can increase the P4 synthesis by both FTHP as well as term placenta in a dose-dependent manner and at near physiological concentrations.

Effect of addition of CGS 16949 A and ICI 182780

In order to confirm that E2 stimulates P4 synthesis, we examined the effect of CGS 16949 A, which is an aromatase inhibitor, and ICI 182780, which is an estrogen receptor antagonist. Results presented in Fig. 2 (a & b) clearly indicate that CGS 16949 A was able to inhibit P4 production by FTHP as well as term placenta in a dose dependent manner. ICI 182780 (Fig.2c&d)

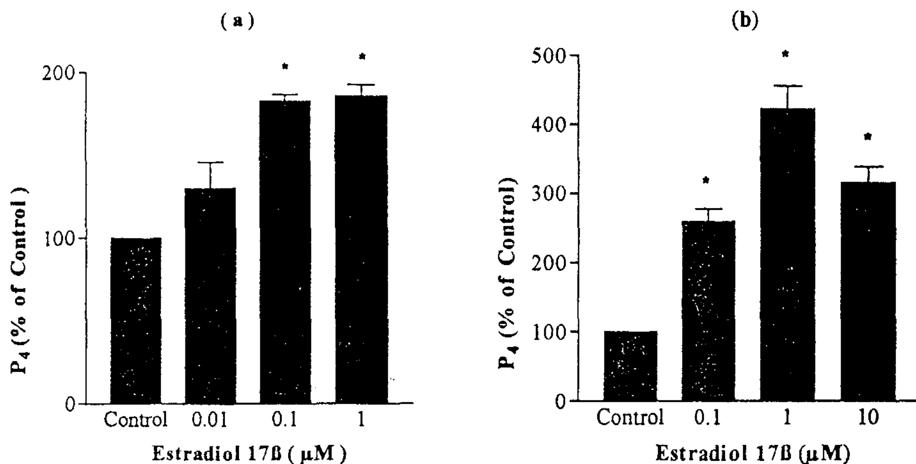

Figure 1 : Effect of addition of estradiol 17β on P4 production by human placental minces. Placental minces were incubated for 2 hours with the specified concentration of E2 in EBSS at 37°C in an atmosphere of 95% O_2 and 5% CO_2. Tissue was extracted with diethyl ether and the extract was used for the quantification of P4. (a) FTHP (b) term placenta. The values presented are mean ± SEM of triplicates. The level of statistical significance is indicated over the respective bars. * denotes P< 0.01.

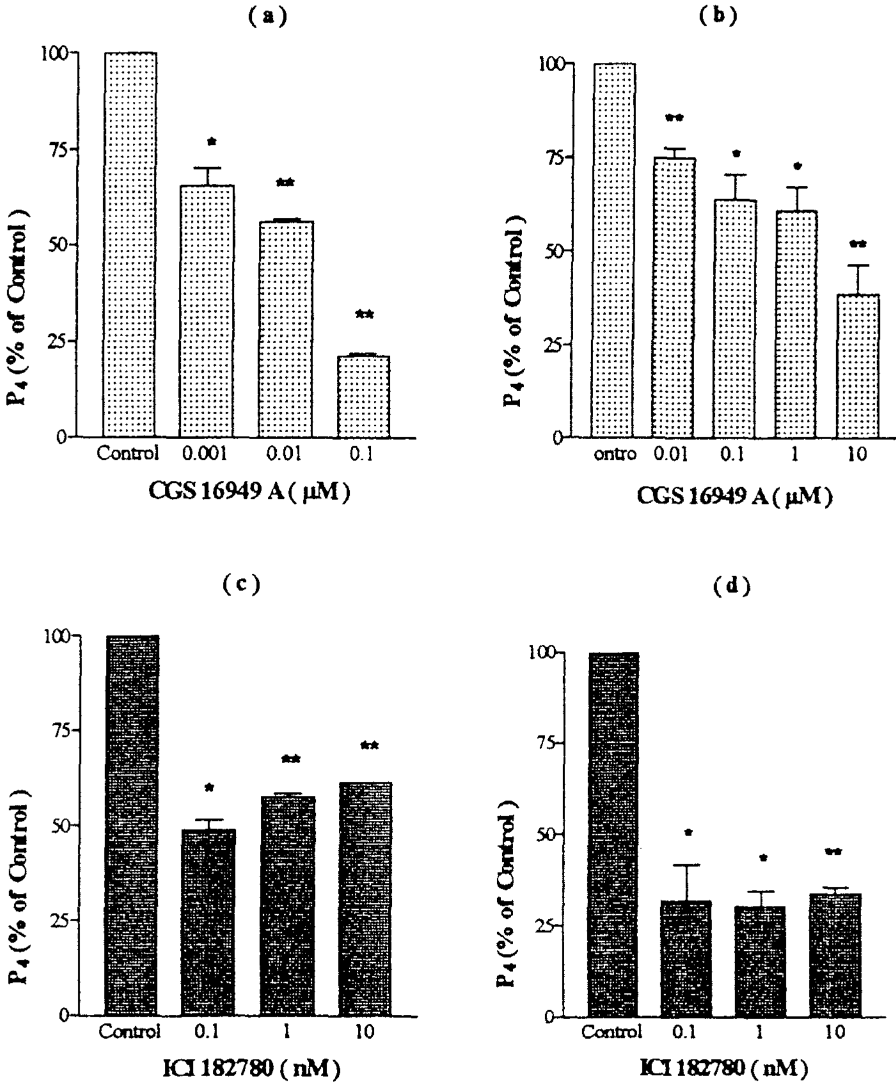

Figure 2 : Effect of addition of CGS 16949 A and ICI 182780 on P4 production by human placental minces. Experimental conditions are as described in legend to fig.1. (a) Effect of CGS 16949 A on P4 production by FTHP; (b) Effect of CGS 16949 A on P4 production by term placenta; (c) Effect of ICI 182780 on P4 production by FTHP; and (d) Effect of ICI 182780 on P4 production by term placenta. * denotes P< 0.01, ** denotes P< 0.001.

was also able to inhibit P4 production to a very significant extent. However, while the inhibition observed with CGS 16949 A was dose dependent, the inhibition observed with ICI 182780 did not conform to such a pattern. Also, the concentration of CGS 16949 A required to bring about an inhibition was much higher than ICI 182780, which could be due to the high endogenous level of E2 synthesis in the placental minces..

Effect of addition of RU 486 and ZK 98299 on progesterone production by FTHP

Although our results provide additional evidence for the important role E2 has in regulating steroid synthesis, it was of importance and interest to examine the role of P4, as it is produced in large quantities and as it has been suggested that P4 synthesis is under autoregulation. However, unlike in the case of E2, it is not possible to study the effects of added P4 as it will interfere in the RIA. In view of this, we have employed antagonists of progesterone receptor, viz. RU 486 and ZK 98299. Both these antagonists did not cross-react with the antiserum and hence did not interfere in the RIA (data not shown). It was observed that addition of RU 486 or ZK 98299 resulted in an increase in P4 levels in FTHP. 1 μM RU 486 increased P4 levels by 220% (Fig.3a) and 22.5 μM ZK 98299 caused a 288% increase in P4 levels (Fig.3b).

Effect of addition of RU 486 and ZK 98299 on progesterone production by term placenta

In contrast to the stimulatory effects of antiprogestins in FTHP, they inhibited P4 production in term placenta, although as expected, RU 486 (Fig.3c) was more effective (88% inhibition at 1μM) as compared to ZK 98299 (50% inhibition at 22.5 μM, Fig. 3d). Interestingly, the inhibitory effects of RU 486 and ZK 98299 were observed at the same concentrations of these compounds which increased P4 levels in FTHP. It is pertinent to note that while the inhibition observed in term placenta was concentration dependent over a wider range, the stimulation observed in FTHP was only over a narrow range of concentrations.

DISCUSSION

The synthesis of large quantities of P4 by the human placenta despite its inability to synthesize cholesterol and its dependence on the maternal supply of LDL has continued to attract the attention of several investigators to identify the possible factors in the regulation of P4 synthesis in human placenta. The factors identified to date include cytokines like IGF-I, TNF- α and IL-1 (8) as well as intracellular molecules like Ca^{2+} and protein kinase C (9). The ability of placenta to produce steroid hormones like E2 and P4, which have a very crucial role to play in the maintenance of pregnancy, makes these hormones good candidates for regulating placental steroidogenesis.

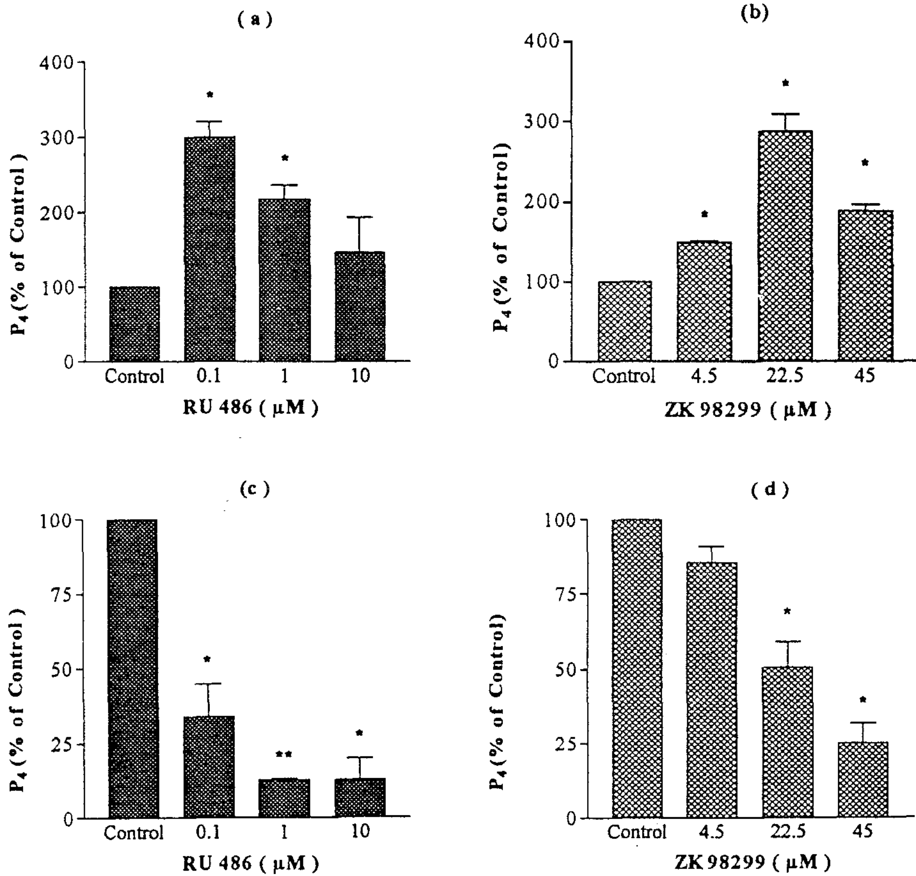

Figure 3 : Effect of RU 486 and ZK 98299 on P4 production by human placental minces. Experimental conditions are described in legend to fig.1. (a) Effect of RU 486 on P4 production by FTHP; (b) Effect of ZK 98299 on P4 production by FTHP; (c) Effect of RU 486 on P4 production by term placenta; and (d) Effect of ZK 98299 on P4 production by term placenta. * denotes $P < 0.01$; ** denotes $P < 0.001$.

The role of estrogen in the regulation of P4 production has been well studied in the case of baboon placenta (16). It has been demonstrated in the baboon placenta that E2 upregulates P4 production by increasing the uptake of LDL and thereby increasing the availability of cholesterol for P4 synthesis (10). However, such detailed studies have not been carried out with the human placenta. There has been only one report on the role of E2 in P4 production by the human placenta. Wunsch *et al* (11) reported that the antiestrogen, MER-25 was able to inhibit P4 production by human placental syncytiotrophoblasts in culture. However, Cantabrana and Hidalgo (12) demonstrated that MER-25 can exhibit agonistic activities. Hence, the role of E2 in the regulation of P4 production by the human

placenta is not yet clear. We have demonstrated here that E2 can increase, in a dose dependent fashion, P4 synthesis in placental minces obtained from early (first trimester) or late (term) gestation during the *ex vivo* incubation. Further, CGS 16949 A, a potent aromatase inhibitor, and ICI 182780, a novel, pure antiestrogen were able to inhibit P4 synthesis to a very significant extent in a dose dependent manner. All these observations are in agreement with Wunsch *et al*'s conclusion (11) and establish that E2 upregulates P4 synthesis in the human placenta. Recent unpublished observations from our laboratory suggest that this increase is mediated by an increase in the LDL-R mRNA levels under the influence of E2 (17), which in turn might result in an increased uptake of LDL.

In contrast to the studies pertaining to the role of E2, very few studies deal with the role of P4 in the regulation of its biosynthesis. Runnebaum and Rabe (14) have hypothesized, as early as 1983, that P4 synthesis in the human placenta is subject to autoregulation. The results of the present study suggest that P4 does indeed regulate its own synthesis. Since our approach involved quantification of P4 levels, it is not possible to study the effect of added P4. In order to overcome this problem we have employed the antiprogestins RU 486 and ZK 98299, which are progesterone receptor antagonists. We observed that RU 486 and ZK 98299 exhibited paradoxical effects on P4 synthesis, i.e. they increased the levels of P4 in FTHP but decreased its levels in term placenta.

It is well documented that both, RU 486 and ZK 98299, can exhibit agonistic effects (in addition to the antagonistic effects) in a cell-, stage- and gene- specific manner (18,19). Thus, it is possible that RU 486 and ZK 98299 are exhibiting agonistic effects in FTHP or term placenta on P4 production. Such paradoxical effects of antiprogestins on P4 production by *corpus luteum* have been reported during rat pregnancy. Telleria and Deis (20) have reported that RU 486 both raised and lowered P4 secretion by the *corpus luteum* in pregnant rats. RU 486 raised P4 secretion on Day 4 or 6 of pregnancy, lowered it between Days 8 and 15 and again raised it between days 16 and 21. In a recent review, Rothchild (21) has hypothesized that P4 always stimulates its own synthesis and that the paradoxical effects of RU 486 are artifacts of RU 486 treatment depending on the condition of the *corpus luteum*. We have found similar paradoxical effects of RU 486 as well as ZK 98299 on P4 synthesis by human placenta. Considering the fact that P4 is indispensable for the maintenance of pregnancy, it is difficult to conceive that P4 can inhibit its own production. It is possible that, as hypothesized in the case of *corpus luteum*, P4 may stimulate its own synthesis in the human placenta also. Such a hypothesis would explain the continuous increase in the levels of P4 throughout the course of gestation. Thus, the inhibitory effects of RU 486 and ZK 98299 in term placenta could be due to their antagonistic effects while the stimulatory effect observed in FTHP could be due to their agonistic effects.

While our results demonstrate that P4 does indeed regulate its own synthesis, the question as to whether this regulation is positive or negative is yet to be answered. Further studies aimed at delineating the mechanism of autoregulation of P4 synthesis should answer this question.

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