Changes in the Concentrations of Plasma Steroid Hormone and Plasma 13, 14-Dihydro-15-Oxo-Prostaglandin F₂ α in Late Pregnancy Rabbits Treated with an Inhibitor of 3 β -Hydroxysteroid Dehydrogenase.

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Abstract

Changes in the concentrations of progesterone, 17 β -estradiol and 13, 14-dihydro-15-oxo-prostaglandin F₂ α (PGFM) were evaluated in the peripheral plasma of rabbits during late pregnancy by treating trilostane, an inhibitor of 3 β -hydroxysteroid dehydrogenase, in an attempt to obtain further insight into the involvement of progesterone and prostaglandin (PG) in the initiation of parturition. The concentrations of progesterone were 18.8 ± 2.2 ng/ml (mean $\pm$ SE, n=6) before administration of the inhibitor, significantly ($p < 0.05$) fell to 7.6 ± 1.0 ng/ml (n=6) at 30 min, and remained low until 10 h after the drug administration. The concentrations of progesterone were still low (5.4 ± 0.5 ng/ml, n=6) at 20-24 h after administration of the inhibitor, and were also low (4.9 ± 2.2 ng/ml, n=6) at delivery. Premature deliveries occurred at 28.8 ± 2.0 h after injection of trilostane (on days 29 of gestation). Increased concentrations of PGFM were observed at delivery. However, administration of trilostane induced no discernible changes in the concentration of estradiol. These findings suggest that delivery is induced by progesterone withdrawal and that synthesis of prostaglandin F₂ α is remarkably increased at delivery in the rabbit.

It is evident that concentrations of progesterone in the peripheral circulation decrease both in spontaneous (Challis *et al.*, 1973, 1974, Petter, 1970) and drug-induced (Challis *et al.*, 1975, Mitchell *et al.*, 1978) deliveries. Csapo (1956) has advanced the theory that progesterone "blocks" myometrial activity throughout pregnancy and

implied that progesterone withdrawal is essential to parturition. However, the precise mechanism accounting for the effects of the decrease in the progesterone concentration on the initiation of delivery is not yet clear. Administration of progesterone was reported to block the response of uterine muscle to exogenous oxytocin and PGF₂ α in sheep, and progestin was considered to have a direct inhibitory effect on the myometrium rather than an inhibitory effect on myometrial PGF₂ α synthesis (Lye *et al.*, 1978). However, Taylor *et al.* (1982)

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has suggested that progesterone withdrawal is one mechanism that initiates increased PGF 2α secretion in pregnant sheep based on their findings of low progesterone and increased PGFM concentrations in the sheep during late pregnancy treated with trilostane. The purpose of the present study was to evaluate the interaction between progesterone and PG in the initiation of delivery in rabbits, a species in which a study on "progesterone block" was performed by Csapo (1956). We will describe in this communication the effects of trilostane on the endocrine milieu and on the occurrence of premature delivery in rabbits of late pregnancy.

Materials and Methods

General

(2, 3, 5, 17)-4, 5-Epoxy-17-hydroxy-3-oxo-androstane-2-carbonitrile (trilostane) was kindly supplied by Mochida Pharmaceutical Co., Japan. Trilostane was dissolved in dimethyl sulphoxide (DMSO, 10 mg/0.1 ml) and the volume made up to 5 ml by adding heparinized saline (200 units heparine/ml, 0.9% (w/v) NaCl). Eight Japanese white rabbits were used. The day of mating to a fertile buck was designated 0 of pregnancy. Two rabbits were given vehicle (DMSO) alone and samples were collected as described for the trilostane treated animals. A lateral ear vein catheter was placed on the 28th day of gestation. It was filled with heparinized saline. In 6 rabbits, trilostane (2-3 mg/kg) was administered as an intravenous bolus on the 28th day of gestation. Blood samples were taken with heparinized syringes, transferred at once to chilled polystyrene tubes on ice, centrifuged within 30 min at 1,500 g for 5 min at 4°C, and stored at -20°C until analysis. Blood samples were obtained immediately before injection of trilostane, at 30 min, at hourly intervals for the first 6 h, at 10 h after injection of trilostane, and at delivery. Plasma progesterone and 17 β -estradiol concentrations were measured by radioimmunoassay with the radioimmunoassay kits obtained from Commissariat A l'Energie Atomique. Progesterone and estradiol were extracted from samples using special grade diethylether (Wako Pure Chemical Industries,

Ltd., Japan). Average recovery of progesterone was 98.6%, and that of estradiol 92.5%. The inter-assay coefficient of variation averaged 9.2% (progesterone), and 13.8% (estradiol).

Radioimmunoassay for PGFM

Extraction of PGFM from plasma: The plasma sample was adjusted to pH 8.2, and a plasma sample (1.0 ml) was extracted twice with petroleum ether (4.0 ml, Special Grade) to remove neutral lipids. The aqueous layer was then exposed to 3.0 ml of 3:3:1 ethyl acetate: isopropanol: 0.1 N HCL, apparent pH 5.8, and vortexed. After 30 min, a mixture of 2.0 ml of ethyl acetate and 3.0 ml of distilled water was added. After further mixing, the upper (ethyl acetate) phase was taken, dried, and dissolved in tricine buffer (pH 8.2) containing 0.025% gelatin (Dojin Chemical Laboratory, Japan).

Radioimmunoassay: 13, 14-dihydro-keto PG-F 2α was obtained from P-L Biochemicals, Inc., Milwaukee, USA. Antiserum to PGFM was a gift from Dr. Kirton, Upjohn Ltd., Kalamazoo, USA. 5, 6, 8, 9, 11, 12, 14(n)- 3 H-PGFM was purchased from Amersham Japan Co., Japan. Sample, 3 H-PGFM and antiserum to PGFM were diluted with tricine buffer. Sample (0.1 ml), 3 H-PGFM (5,000 cpm, 0.1 ml) and antiserum to PGFM (1:5,000, 0.1 ml) were added to the tube, mixed and incubated for 3 h at 4°C. Separation of bound and free 3 H-PGFM was performed by the addition of a suspension containing 1.0% (w/v) charcoal, 0.01% (w/v) dextran T-40 in tricine buffer. Dextran-coated charcoal was removed by centrifugation at 4°C. The supernatant (0.25 ml) was added to 10 ml of scintillation medium (Atomlight, New England Nuclear Co., Boston, USA), and counted in a scintillation counter (Aloka LSC-903 SP). The intra-assay coefficient of variation averaged 2.7%, and inter-assay coefficient of variation 11.4%. The water blank was 28 ± 7.5 pg/ml (mean $\pm$ SE). Recovery averaged 74.0%. Statistical analyses were performed using Wilcoxon's signed ranks test.

Results

Experimental details for the rabbits treated with trilostane are shown in Table 1. Premature delivery of fetuses occurred

Table 1. Experimental details for six rabbits injected with trilostane

No.	At start of experiment (days)	Dose of trilostane (mg/kg)	Induction -delivery time (h)	State of fetuses at birth
1	28	2	36	asphyxia
2	28	2	26	alive
3	28	2	23	alive
4	28	3	32	alive
5	28	3	28	asphyxia
6	28	3	28	dead

in six rabbits 28.8 ± 2.0 h after injection of trilostane. There were no differences during the course of labor and delivery. Injection of vehicle (DMSO) alone did not induce delivery and had no significant effect on concentrations of progesterone, PGFM and estradiol in maternal plasma.

The concentrations of progesterone were 18.8 ± 2.2 ng/ml (mean $\pm$ SE, $n=6$) before treatment, significantly ($p < 0.05$) decreased to 7.6 ± 1.0 ng/ml at 30 min after treatment, then remained low until premature delivery in the 6 rabbits which occurred at about 28 h after the administration of trilostane. Concentrations of estradiol were 2.9 ± 0.3 pg/ml before treatment, and they did not change until delivery. Concentrations of PGFM were 3.6 ± 1.0 ng/ml before treatment. Although they remained there until 6 h after treatment, they rose significantly ($p < 0.05$) to 7.3 ± 0.7 ng/ml at delivery. Concentrations of progesterone, estradiol and PGFM before and after trilostane administration in each rabbit are shown in Fig. 1.

Fig. 1. Concentrations of (1) progesterone, (2) 13, 14-dihydro-15-oxo-prostaglandin F_{2α} (PGFM) and (3) 17β-estradiol in the peripheral plasma of six rabbits before and after administration of trilostane (shown by arrows) on days 28 of gestation. The time of delivery is shown by the symbol d.

Discussion

The concentrations of progesterone in the peripheral plasma of the pregnant rabbits rapidly decreased at 30 min after trilostane administration, and remained low until delivery. The increase in the PGFM concentrations in the peripheral plasma may reflect increased synthesis of PGF2 α in the tissue of the placenta and uterine muscle. The concentrations of PGF2 α were reported by Makimura *et al.* (1981) to increase in the tissue of the placenta and uterine muscle in pregnant rabbits at delivery. Therefore, progesterone withdrawal seems to initiate delivery by stimulating PG synthesis in the tissue of the placenta and uterine muscle in rabbits, though the mechanism accounting for the stimulatory effect of progesterone withdrawal on the PG synthesis is not clear. Trilostane, given once on day 10 of gestation, disrupted pregnancy in 6 of 7 rats at a dose of 250 mg/kg, but concomitant administration of progesterone (4 mg/rat) prevented the disruption of pregnancy (Potts *et al.* 1978, Shane *et al.*, 1979). Exogenous progestin prevented the interceptive action of trilostane if given as much as 8 h later. However, if progestin administration was delayed 12 h, pregnancy was disrupted. In view of these findings, Shane *et al.* (1979) suggested that the progesterone reduction induced by trilostane was the cause, not the result, of the termination of pregnancy. Intravenous administration of trilostane to late-pregnant sheep resulted in a rapid decrease in circulating progesterone concentrations, and an increase in circulating PGFM concentrations (Taylor *et al.*, 1982). At the time of premature delivery, concentrations of PGFM were high, but concentrations of progesterone were close to the pretreatment values. In the present study, concentrations of progesterone were low at the time of delivery in the rabbits. The difference between the levels of progesterone at the time of delivery in our

results and those in another reported study (Taylor *et al.*, 1982) may be due to the different species used. Concentrations of estradiol were presently increased immediately before delivery or remained low until delivery. This might be due to the complete suppression of steroidogenesis by trilostane. Anderson *et al.* (1981) also reported that premature delivery occurred in sheep of late pregnancy treated with trilostane, though plasma estradiol did not increase. However, administration of estrogens in late pregnancy could lead to increased uterine activity both in sheep and human. Infusion of DHA into pregnant ewes resulted in increased maternal plasma concentrations of estrone and estradiol, and led to significant rises in blood flow to the endometrium, myometrium, cervix and vagina (Anderson *et al.*, (1981). Therefore estrogens might play a physiologically important role in the control of blood flow to the organs vitally involved in parturition. Flint *et al.* (1974) reported that in 4 out of 6 sheep in late pregnancy, utero-ovarian venous plasma PGF levels rose at the same time as estrogen levels, and that in the 2 animals where estrogen did not increase before the second stage of labor, the rise in PGF levels followed a drop in progesterone, with a consequent change in the estrogen: progesterone ratio. Further, Liggins *et al.* (1979) reported that progesterone: estrogen ratio efficiently decreased before parturition and the release of PG was promoted, although the concentration of estradiol remained low. Therefore, the decrease in the progesterone: estrogen ratio, but not the absolute levels of estrogen and progesterone per se, may be of importance in stimulating the synthesis of PG. In conclusion, reduced ovarian progesterone formation in the rabbits of late pregnancy treated with trilostane resulted in premature delivery. At delivery, the concentration of PGFM increased. This may reflect increased release and/or synthesis of PGF2 α in the placenta and uterine muscle.

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