Prostaglandin F-2 α receptors in corpora lutea of pregnant rats and relationship with induction of 20 α -hydroxysteroid dehydrogenase

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Summary. Luteal receptors for PGF-2 α in the pregnant rat were characterized. No changes in the K_d were found during pregnancy, whereas capacity increased to a maximum on Day 19, decreasing thereafter. The decrease in binding sites seen from Days 20 to 22 may be due to down regulation of the receptor by its ligand, since it was prevented by inhibition of PG synthesis by indomethacin treatment. Likewise, in-vivo treatment with PGF-2 α reduced the apparent number of PG binding sites.

PG receptor concentration seems to be modulated by oestrogens since an increment was found on Day 19, associated with the known increase in plasma oestradiol concentrations, and since receptor concentration on Day 16 was significantly increased by oestradiol benzoate.

The uterus also had a negative influence on the appearance of the PG receptor, since hysterectomy on Day 16 increased the number of binding sites on Day 18. However, receptor concentration and 20 α -hydroxysteroid dehydrogenase induction by hysterectomy was not affected by indomethacin, indicating that these events are probably not related to prostaglandin withdrawal. However, treatment with hCG, which diminishes enzyme induction by hysterectomy, did not produce changes in receptor concentration.

The present results suggest that PGF-2 α , acting through a specific receptor site, is the physiological luteolytic signal. The consequence of its receptor binding seems to be the blockade of a gonadotrophic stimulus, which in turn determines (1) the decrease in progesterone synthesis and (2) the induction of 20 α -hydroxysteroid dehydrogenase.

Keywords: PFG-2 α ; receptors; rat; CL; 20 α -hydroxysteroid dehydrogenase

Introduction

A decrease in circulating progesterone concentrations must precede the initiation of parturition. At that time, corpus luteum regression occurs in spite of the presence of luteotrophic signals, such as placental lactogen and a luteotrophin with an hCG-like activity (Shiu *et al.*, 1973; Blank *et al.*, 1979). Therefore, a luteolytic signal must override those gonadotrophic stimuli. At the end of pregnancy, the induction of luteal 20 α -hydroxysteroid dehydrogenase (20 α -HSD, EC 1.1.1.149) is a sign of functional luteolysis and is responsible for the sharp decrease in circulating progesterone values that precedes parturition (Wiest *et al.*, 1968; Kuhn & Briley, 1970).

The luteolytic action of prostaglandin (PG) F-2 α in the rat was first demonstrated by Pharriss & Wyngarden (1969), who reported the increase in 20 α -dihydroprogesterone concentrations that follow PG treatment. Subsequently, several hypotheses were proposed for the mechanism of action

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of PGF-2 α (reviewed in Behrman *et al.*, 1979). Specific receptors for PGF-2 α have been described for corpora lutea (CL) of cows (Rao, 1975) and of cyclic and pseudopregnant rats (Wright *et al.*, 1979, 1980; Muller *et al.*, 1981). A physiological role of this PG at the end of pregnancy is indicated by the prevention of the normal induction of 20 α -HSD activity that is caused by indomethacin, an inhibitor of prostaglandin synthesis (Strauss & Stambaugh, 1974).

The rapid induction of 20 α -HSD activity after PGF-2 α treatment suggests an effect of the PGF-2 α on the synthesis of this enzyme (Bussmann & Deis, 1979). This is also indicated by the abolition to a different extent by indomethacin of the inductions of enzyme activity caused either in early pregnancy with 2-bromo- α -ergocriptine or in late pregnancy with aminoglutethimide or hCG (Hickman-Smith & Kuhn, 1976).

The experiments reported here were undertaken to study the participation of the PG receptor in the mechanism of luteolysis and 20 α -HSD induction in late pregnancy.

Materials and Methods

All chemicals were reagent grade obtained from Sigma Chemical Co. (St Louis, MO, USA) unless otherwise indicated. [5,6,8,9,11,12,14,15-³H]Prostaglandin F-2 α (sp. act. 150 Ci/mmol) and [1,2,6,7-³H]progesterone (sp. act. 90 Ci/mmol) were from New England Nuclear Corp. (Boston, MA, USA). Acetonitrile was from Merck (Darmstadt, West Germany) and Silastic tubing from Ducilo Argentina (Buenos Aires, Argentina). The following were generously donated: indomethacin phosphate (Montpellier Argentina, Buenos Aires, Argentina), PGF-2 α -Tris salt (the Upjohn Company, Buenos Aires, Argentina), 2-bromo- α -ergocriptine mesylate (Sandoz, Buenos Aires, Argentina), ovine prolactin NIH-S-11 (NIADDK, Baltimore, MD, USA).

Animals and general methodology. White nulliparous rats, about 3 months old (180–200 g) were used. They were caged with a male rat during the night after pro-oestrus. The next morning was taken as Day 1 of pregnancy if spermatozoa were found in the vaginal smear. In our colony, rats usually deliver on Day 23. The rats were kept in a constant-temperature room (24 $\pm$ 2°C) with a controlled light cycle (lights on from 06:00 to 20:00 h).

Hysterectomies were performed, under light ether anaesthesia, through a ventral incision; sham-operated animals received only the incision. The rats were killed by decapitation; blood was collected, allowed to clot and centrifuged. Serum was kept frozen at –30°C until assayed.

Ovaries were collected immediately after killing the rats and put in ice-cold saline (9 g NaCl/l) containing indomethacin phosphate (10 μ g/ml). The CL were dissected under a stereoscopic microscope by means of small forceps and a needle; they were counted and weighed before homogenization with an Ultra-Turrax in 3 ml ice-cold buffer (10 mM-Tris, 250 mM-sucrose, 1 mM-CaCl₂, 1 mM-dithiothreitol, 0.1% BSA, 0.1% Na₃N, 2 mM-indomethacin phosphate) pH 7.0 (TIS buffer) and centrifuged at 800 *g* for 10 min. The supernatant was centrifuged again at 20 000 *g* for 45 min. The supernatant was saved for 20 α -HSD determination in some cases. The pellet was resuspended in TIS buffer in a proportion of 20 μ l per 3 mg original tissue.

Protein determinations were performed, after digestion of an aliquant of membrane with 1 N-NaOH, by the method of Lowry *et al.* (1951) using bovine serum albumin as standard.

PGF-2 α receptor assay. PGF-2 α receptors were assessed as described by Wright *et al.* (1979), with minor modifications. Duplicate 20 μ l samples of luteal membrane preparation (160–220 μ g protein) were incubated with different concentrations of the tracer (1–80 nM), in a final volume of 50 μ l in plastic tubes. Incubation was stopped by placing the samples in an ice bath and adding 1 ml TBSA buffer (10 mM-Tris, 1 mM-CaCl₂, 1 mM-dithiothreitol, 0.1% BSA, 0.1% Na₃N) pH 7.0 and 1 mg celite. Membrane-bound [³H]PGF-2 α was separated by centrifugation at 6000 *g* for 30 min, washed once with 1 ml TBSA buffer, centrifuged again, and the pellet resuspended in 1 ml TBSA buffer. This was then transferred to a scintillation vial and counted in a Kontron 300 DPM (Kontron AG, Switzerland) with automatic quench correction using Bray's scintillation cocktail. Non-specific binding was assessed in each experiment, and for each [³H]PGF-2 α concentration, by incubating aliquants in duplicate with an excess of unlabelled PGF-2 α (1 μ M). The quantity of [³H]PGF-2 α bound in these samples (0.4%) was essentially the same as that in tubes in which there were no luteal membranes. The non-specific binding was subtracted from total binding to obtain specific binding.

The purity of the [³H]PGF-2 α was routinely checked by thin-layer chromatography on silica gel G using toluene-ethyl acetate-methanol-acetic acid (20:80:3:3, by vol.). When purity was <95%, the tracer was repurified before use in binding studies. Also, in the studies of association kinetics, after the incubations for the different times and temperatures the purity of the tracer was checked. Membrane-bound and free [³H]PG were separated by centrifugation and both fractions were acidified, extracted with ethyl acetate and chromatographed in the system described above.

Studies on the temperature dependence of binding were performed using a pool of CL from 20-day-pregnant rats. Aliquants were incubated at different temperatures in the presence of 7 nM-[³H]PGF-2 α alone or plus 1 μ M unlabelled PGF-2 α . At different times the incubation was stopped and processed as above. Dissociation analyses were done on a pool of CL from 20-day-pregnant rats, preincubated with 6 nM-[³H]PGF-2 α for 90 min at 30°C. At zero time 1 nmol

unlabelled PGF-2 α in 1 ml TIS buffer was added and the incubation was allowed to proceed for various times. Association (K_a) and dissociation (K_d) rate constants were assessed as described by Schrader (1975).

Saturation analysis was performed, using membrane preparations from 3 rats at the same stage of pregnancy, for each day studied. Samples were incubated with 9 [3 H]PGF-2 α concentrations ranging from 1 to 80 nM. Scatchard plots (Scatchard, 1949) were used to determine equilibrium constants (K). PGF-2 α receptor concentrations for the different days of pregnancy, which are shown in Fig. 2, were determined in luteal membranes from single rats (i.e. without pooling). Incubation with the tracer was done at 3 concentrations, 5, 20 and 80 nM.

20 α -HSD and hormone assays. Luteal 20 α -HSD enzymic activity was determined in a computerized model 101 Hitachi spectrophotometer (Hitachi Ltd, Tokyo, Japan) as previously described (Bussmann & Deis, 1979). The supernatant from the 20 000 g, 45 min centrifugation (50–500 μ l) was preincubated at 37°C for 10 min in a 1-ml cuvette with 0.35 μ mol NADP $^+$ in a final volume of 975 μ l. The reaction was started by the addition of 0.1 μ mol 20 α -dihydroprogesterone in 25 μ l ethanol. Change in extinction at 340 nm was recorded for 5 min. Enzyme activities are expressed in mU, one U being equal to the formation of 1 μ mol NADPH/min at 37°C.

Serum progesterone concentrations were assessed by radioimmunoassay and placental lactogen by a radioreceptor assay as described previously (Bussmann & Deis, 1979). All serum samples were measured in a single assay to eliminate interassay variations. The limit of sensitivity of the progesterone assay was 12.5 pg (per tube) and the mean intra-assay coefficient of variation was 6.5%.

Placental lactogen was determined using a membrane preparation from livers of female rats. The tracer used was 125 I-labelled oPRL (NIH-oPRL S12, 35 IU/mg) iodinated by the lactoperoxidase method (Thorell & Johansson, 1971). The same prolactin tracer was used for the standard curve, and serum of males was added in the same proportion that was present in the sample tubes. The sensitivity of the assay ranged from 0.4 to 50 ng/tube. Mean intra-assay coefficient of variation was 9%.

Intraluteal steroid concentrations were determined by h.p.l.c. using Waters equipment with a μ Bondapak column C18 (Rodriguez-Consword, Bernal, Buenos Aires, Argentina) and acetonitrile:water (50:50 v/v) as the mobile phase. Steroids were detected at 254 nm. The lowest detectable concentrations of progesterone and 20 α -dihydroprogesterone were 2 ng. Both ovaries were removed, trimmed of surrounding fat and homogenized in 0.5 ml buffer containing 50 mM-sodium phosphate, 150 mM-NaCl, 1 mM-EDTA, 0.1% gelatin, pH 7.4, and 10 000 c.p.m. [3 H]progesterone as internal standard. The homogenate was extracted twice with 2 ml ethyl acetate and the combined organic phases were filtered through a Millipore filter FHLF 13 mm (MTN, Buenos Aires, Argentina) and dried under nitrogen. The residue was dissolved in acetonitrile and portions were used for h.p.l.c. analysis. The recovery was estimated by counting the [3 H]progesterone in a liquid scintillation counter.

Statistical analysis. Results are given as means $\pm$ s.e.m., with the number of observations in parentheses. Differences between groups were assessed by Student's *t* test or Duncan's test for multiple comparisons.

Results

Characterization of the prostaglandin receptor in corpora lutea of pregnancy

The characterization of the PGF-2 α receptor was performed on luteal membrane preparations from Day 20 of pregnancy. As shown in Fig. 1(a) the association kinetics of [3 H]PGF-2 α to luteal membranes were temperature dependent. At 37°C association was fast, reaching a maximum of 45 min, and declining thereafter. As metabolism or degradation of the tracer was not found after incubation (94.7%, 94% and 92% of the radioactivity was recovered as [3 H]PG after incubation for 60, 90 and 120 min, respectively), this decline is interpreted as an increase in dissociation or receptor degradation. The binding at 30°C was complete by 90 min. At 24°C the binding was less rapid and increased throughout the time studied, without reaching the plateau. When the values were fitted to a hyperbolic function a time of 11.3 h was obtained to reach 95% of the $B_{\max}$ at 24°C. Very little binding was found when the incubation was performed at 0°C. The K_a values ($\text{M}^{-1} \text{sec}^{-1}$) were 1.18×10^5 , 1.09×10^5 , 3.39×10^4 and 1.82×10^3 at 37, 30, 24 and 0°C, respectively.

The dissociation of PGF-2 α at 30°C was rapid, so that by 15 min only 50% of the tracer was still bound, yielding a K_d of $2.06 \times 10^{-4} \text{ M sec}^{-1}$ (Fig. 1b). The dissociation at 0°C was very slow. The equilibrium constant (K_d) at 30°C, calculated as the ratio K_d/K_a , was $1.89 \times 10^{-9} \text{ M}$. In view of the above results the 90-min period and the 30°C temperature were chosen for all incubations at equilibrium.

Figure 1(c) shows the saturation curve for PGF-2 α with various concentrations of the tracer. Saturation was reached at 80 nM. Scatchard analysis (Fig. 1c, insert) indicated a single binding site

Fig. 1. Binding kinetics of the PGF-2 α receptor in CL of pregnant rats. (a) Association kinetics of PGF-2 α with luteal membrane binding sites. Membranes were incubated with 7 nM- $[^3\text{H}]\text{PGF-}2\alpha$ at 37°C (▲), 30°C (●), 24°C (■) and 0°C (○). (b) Dissociation kinetics of PGF-2 α . Luteal membranes were labelled with 6 nM- $[^3\text{H}]\text{PGF-}2\alpha$ for 90 min at 30°C, at zero time 1 ml Buffer TIS containing 1 nmol unlabelled PGF-2 α was added and the incubation allowed to proceed for different times at 30°C (●) and 0°C (○). (c) Saturation curve of PGF-2 α receptors. Luteal membranes were incubated for 90 min at 30°C. Insert shows Scatchard plot of $[^3\text{H}]\text{PGF-}2\alpha$ binding data. For more details see text.

with high affinity and low capacity (K_d $7.27 \pm 0.4 \times 10^{-9}$ M, $B_{\max}$ 236 ± 19 fmol/mg protein, $N = 3$). Almost identical affinities were found for pooled CL from different stages of pregnancy (K_d range 7.68×10^{-9} to 5.83×10^{-9} M). The binding capacity on the different days ranged between 170 and 280 fmol/mg protein.

Specificity of the PGF-2 α receptor was tested against PGF-2 α , cloprostenol (a PGF-2 α analogue), PGE-2, hCG and prolactin (data not shown). Only PGF-2 α and cloprostenol competed with $[^3\text{H}]\text{PGF-}2\alpha$ in almost a 1:1 ratio. Little competition was found with PGE-2. Prolactin and hCG, which are known to have specific receptors in luteal membranes, did not compete with the binding of the tracer.

Changes in PGF-2 α receptor concentrations during pregnancy

The binding of PGF-2 α to luteal membranes was studied on Days 14, 16, 18, 19, 20, 21 and 22 of pregnancy (Fig. 2). The levels of specific binding rose significantly on Day 19 of pregnancy, when compared with Days 14, 16 and 18. On Day 20 the binding was still significantly high, but it decreased on Day 21 to the initial levels. Some increase was seen on Day 22 of pregnancy, but the values were still similar to those of Days 16 and 18 of pregnancy.

To determine whether the decrease in binding of PGF-2 α that is seen on Days 21 and 22 was due to down regulation of the receptor by endogenous prostaglandin, the binding was measured in pregnant rats that were previously treated with indomethacin. Figure 2 shows that indomethacin did not affect the amount of receptor on Days 14, 16, 18 and 19. However, indomethacin effectively prevented the decline in PGF-2 α binding that was seen in untreated rats on Days 20, 21 and 22,

Fig. 2. PGF-2 α binding to luteal membranes of rats at different stages of pregnancy. Membranes were incubated for 90 min at 30°C with 5, 20 and 80 nM-[³H]PGF-2 α . Indomethacin (1 mg s.c.) was administered at 08:00, 20:00 and 08:00 h, starting the day before measurement. All rats were killed at 12:00 h. Values are means $\pm$ s.e.m. for the no. of observations in parentheses. Bars with the same letter(s) are not significantly different at $P < 0.05$.

leaving the receptor value on Days 19–22 significantly elevated over that on Days 14–18 of pregnancy. K_d values, measured on pooled preparations of ovaries from 3 rats, were unchanged.

PGF-2 α receptor concentration after treatment in vivo with PGF-2 α

To study further the down regulation of PGF-2 α receptor by its own ligand, 19-day-pregnant rats (6 rats each group), pretreated with indomethacin (1 mg s.c. every 12 h starting the day before experiment at 08:00 h) were injected with PGF-2 α (750 μ g s.c.) or vehicle. Rats were killed 2 h or 24 h after treatment and the receptors measured. The K values were determined in pooled preparations and no differences from the control group were found (data not shown). The receptor concentrations were significantly lower in both groups: PGF-2 α treatment 2 h before killing gave values of 215 ± 23 and 277 ± 21 fmol/mg protein for treated controls ($P < 0.01$) and treatment PGF-2 α 24 h before killing gave values of 193 ± 12 and 272 ± 10 fmol/mg protein in treated and controls ($P < 0.001$).

Regulation of PGF-2 α receptor by ovarian steroids and its relationship with the sensitivity for luteolysis

Since the sensitivity of CL to the luteolytic action of PGF-2 α increases with their age, accompanying a decrease in serum progesterone and an increase in serum oestrogen concentrations, the participation of both steroids on the concentration of PGF-2 α receptor was studied. Progesterone was injected s.c. (10 mg in oil) every 12 h on Days 16, 17, 18 and 19, and the rats killed on Day 19 at 12:00 h, or implanted s.c. on the back with a progesterone-filled Silastic tubing (40 mm long $\times$ 3.2 mm i.d.) on Day 17, and the rats killed on Day 20 of pregnancy at 12:00 h. None of these treatments produced a significant change in the concentration (mean $\pm$ s.e.m., 6 rats) of PGF-2 α binding sites: 270 ± 10 and 287 ± 17 fmol/mg protein for progesterone-injected rats and oil-injected controls, respectively, and 220 ± 10 and 240 ± 15 fmol/mg protein for progesterone-implanted and empty-implant rats, respectively.

Oestrogen treatment (oestradiol benzoate, 10 µg in oil s.c.) was given on Days 14 and 15 of pregnancy at 08:00 h, and the rats (6 per group) were killed on Day 16 at 12:00 h, when the luteal receptor concentration in untreated rats in previous experiments was still low (see Fig. 2). This treatment caused a significant increase in receptor concentration, which was not different from that observed on Day 19 (210 ± 8 and 191 ± 13 fmol/mg protein in oestradiol-treated and oil-injected rats, respectively; $P < 0.001$). In 6 additional rats, this treatment did not alter serum concentrations of progesterone (oestrogen-treated: 62.5 ± 4.8 ng/ml; oil-injected controls: 58.6 ± 6.5 ng/ml), or placental lactogen (oestrogen-treated: 975 ± 96 ng/ml; oil-injected controls: 1020 ± 105 ng/ml), suggesting that the placental support of luteal function was unaffected.

To find out whether this oestrogen-induced increase in PG binding sites has a physiological meaning, the sensitivity to PGF-2α was studied in rats treated with oestradiol benzoate (10 µg in oil s.c.). On Day 16, when in the previous experiment an increase of PGF-2α receptor to levels similar to those of Days 19–22 was seen, they were also treated with 750 µg PGF-2α s.c. The rats were killed 24 h later and 20α-HSD activity was measured. The enzyme activity was not detectable in control rats and treatment with oestrogen alone did not produce a large increase (9.44 ± 1.1 mU/min/2 ovaries, $N = 5$). Treatment with PGF-2α on Day 16, however, was followed by a larger increase in 20α-HSD activity 24 h later (53.76 ± 5.6 mU/min/2 ovaries, $N = 7$, $P < 0.001$ when compared with the previous value). When PGF-2α was administered to oestrogen-pretreated rats, i.e. with higher levels of PGF-2α receptor, the induction was found to be significantly greater (175 ± 21.1 mU/min/2 ovaries, $N = 7$, $P < 0.001$) when compared with the group treated with PGF-2α alone.

Progesterone and 20α-HSD in control and indomethacin-treated pregnant rats (Fig. 3)

Serum progesterone concentrations in control pregnant rats were generally high, although with a marked decrease on the last days of pregnancy. Luteal 20α-HSD activity in the same rats was not detectable until Day 19 of pregnancy, but increased sharply on Day 22. Indomethacin treatment completely prevented the fall of progesterone concentrations at the end of pregnancy, and markedly diminished the simultaneous induction of 20α-HSD activity. Moreover, none of a group of 6 rats that were treated with indomethacin from Day 21 until the morning of Day 24 had delivered by that night (when they were killed; data not shown in Fig. 3).

Ovarian steroid concentration after PGF-2α treatment

As shown in Table 1, at 2 h after treatment there was a decrease in both 20α-dihydroprogesterone and progesterone concentrations, although the ratios of these steroids were similar to those of the control rats.

By 3 h after treatment, progesterone had further decreased in luteal cells, but 20α-dihydroprogesterone started to increase, when compared with the 2 h concentration, reaching the control values. The total luteal progestagen remained essentially constant and so the 20α-dihydroprogesterone/progesterone ratio changed dramatically.

This result seems to show that the first action of PGF-2α on luteal steroidogenesis is the decrease of Δ^4 -3 oxo-steroid production, while the induction of 20α-HSD activity could be either a consequence of this decrease or at least a later effect of the PGF-2α on the luteal cell.

Induction of 20α-HSD activity by hysterectomy

To study whether the induction of 20α-HSD in hysterectomized pregnant rats is a PG receptor-mediated response, both receptor concentration and enzymic activities were measured after hysterectomy. Table 2 shows that treatment with indomethacin did not prevent the induction of 20α-HSD by hysterectomy, and that the receptor concentration did not change when compared

Fig. 3. Serum progesterone concentrations and ovarian 20 α -HSD at various times of pregnancy in the rat. (a) Untreated pregnant rats: $\square$, serum progesterone; $\blacksquare$, 20 α -HSD activities. (b) Indomethacin-treated pregnant rats: $\hatched$, serum progesterone; $\crossed$, 20 α -HSD activities. Indomethacin treatment was as indicated in Fig. 2. The rats were killed at 12:00 h. Values are means $\pm$ s.e.m. for the number of observations in parentheses. * P < 0.02, ** P < 0.01 when compared with the value of the preceding day. ND, non-detectable activities; NM, not measured.

with that of the hysterectomized, untreated rats. This was true whether the operation was performed on Day 19 of pregnancy and measurements made 24 h later or on Day 16 and measurements made 48 h later. On the other hand, hCG treatment after hysterectomy reduced the induced enzyme activity to 31% without affecting the receptor concentrations. PGF-2 α synthesis inhibition by indomethacin treatment to hysterectomized rats treated with hCG did not reduce further 20 α -HSD activity nor produced changes in receptor concentrations.

The effect of hCG on enzyme induction by hysterectomy on Day 16 of pregnancy was not dependent on prolactin, since the administration of this hormone (1 mg in saline s.c. every 12 h, beginning immediately after surgery) did not increase the effect of hCG on 20 α -HSD activity (hysterectomized hCG-treated 7.7 ± 0.6 mU/min/2 ovaries, $N = 6$; hysterectomized hCG + prolactin-treated 6.1 ± 0.5 mU/min/2 ovaries, $N = 8$). This lack of an effect of exogenous prolactin is not due to an increase of the endogenous one, which begins to increase 18–24 h after

Table 1. Luteal progestagens and serum progesterone concentration in rats after PGF-2 α treatment

	Luteal progesterone (ng/100 mg)	Luteal 20 α -dihydro- progesterone (ng/100 mg)	20 α -OHP: progesterone	Luteal progestagens (ng/100 mg)	Serum progesterone (ng/ml)
Control 2 h	1528 $\pm$ 120 (5)	1350 $\pm$ 221 (5)	0.88	2878	69.4 $\pm$ 4.4 (6)
Treated 2 h	1024 $\pm$ 73 (6)**	860 $\pm$ 62 (6)*	0.84	1884	52.4 $\pm$ 5.1 (6)*
Treated:control	0.67	0.64		0.65	
Control 3 h	1617 $\pm$ 117 (5)	1405 $\pm$ 139 (5)	0.87	3022	70.7 $\pm$ 4.9 (5)
Treated 3 h	784 $\pm$ 61 (6)***	1441 $\pm$ 174 (6)	1.84	2225	41.8 $\pm$ 4.9 (6)**
Treated:control	0.47	1.03		0.73	

Rats were injected at 10:00 h on Day 19 of pregnancy with 750 μ g PGF-2 α or vehicle and killed 2 h or 3 h later. Results are means $\pm$ s.e.m. for the no. of rats in parentheses.

* P < 0.05, ** P < 0.001, *** P < 0.001, compared with control value.

Table 2. PGF-2 α receptor concentrations and 20 α -HSD activities in hysterectomized pregnant rats

	Day of measurement	PGF-2 α bound (fmol/mg)	20 α -HSD (mU/min/2 ovaries)	
Normal pregnant	20	240 $\pm$ 15 (6)	10.8 $\pm$ 0.2 (6)	P < 0.001
Hysterectomy Day 19	20	295 $\pm$ 21 (6)	70.3 $\pm$ 7.9 (6)	
Normal pregnant + indomethacin	20	273 $\pm$ 10 (6)	ND	
Hysterectomy Day 19 + indomethacin	20	296 $\pm$ 24 (5)	69.1 $\pm$ 10.7 (7)	
Normal pregnant	18	180 $\pm$ 13 (6)	ND	P < 0.01
Hysterectomy Day 16	18	289 $\pm$ 26 (6)	32.9 $\pm$ 2.1 (9)	
Normal pregnant + indomethacin	18	198 $\pm$ 26 (6)	ND	P < 0.001
Hysterectomy Day 16 + indomethacin	18	328 $\pm$ 17 (6)	30.2 $\pm$ 3.7 (6)	
Hysterectomy Day 16 + hCG	18	266 $\pm$ 10 (6)	10.3 $\pm$ 0.6 (9)	
Hysterectomy Day 16 + indomethacin + hCG	18	288 $\pm$ 12 (6)	9.8 $\pm$ 0.7 (6)	

Indomethacin (1 mg) was given s.c. every 12 h starting at 08:00 h on Day 18, when surgery was performed on Day 19 and the measurement on Day 20 of pregnancy, or else on Day 15 at 20:00 h, when hysterectomy was done on Day 16 and the measurement on Day 18 of pregnancy. HCG (1.5 μ g) was administered every 12 h beginning immediately after surgery. Surgery was done between 08:00 and 10:00 h and the rats were killed 24 h (20-day groups) or 48 h (18 day groups) later. Results are means $\pm$ s.e.m. for the number of observations in parentheses. ND, not detectable.

hysterectomy, inasmuch as the treatment with bromocriptine (1.5 mg/kg, every 12 h starting the day before surgery) did not prevent or diminish the luteotrophic effect of the hCG (7.6 $\pm$ 0.8 mU/min/2 ovaries).

Discussion

The observations reported here on the binding kinetics of the PGF-2 α to its receptor during the last stages of pregnancy are in agreement with previous reports on pseudopregnant (Wright *et al.*, 1979, 1980) and cyclic (Muller *et al.*, 1981) rats. A correlation was found between the luteolytic action of PGF-2 α and its receptor concentration in the CL. It has been reported (Strauss & Stambaugh, 1974; Vermouth & Deis, 1975; Rodway & Kuhn, 1975a) that as the corpus luteum ages its sensitivity towards PGF-2 α increases. In agreement with these findings, the present study revealed that the concentration of binding sites reached a maximum on Day 19 of pregnancy. The subsequent decrease observed from Day 20 to 22 of pregnancy was probably due to down regulation of the receptor, since the assay measured the total amount of binding sites, and since indomethacin treatment prevented the decrease in binding normally seen at the end of pregnancy. Also, the treatment with PGF-2 α on Day 19 of pregnancy caused a decrease in its luteal receptor concentration.

Previous studies of pseudopregnant rats found no changes in the concentrations of the ovarian receptor on different days of pseudopregnancy (Wright *et al.*, 1980). This is not surprising in view of the differences between the two reproductive states. The CL of pseudopregnancy are under pituitary control and, at the stage of pregnancy in which this study was done, control had already changed from the CL to the placenta (Morishige & Rothchild, 1974).

The rise in PGF-2 α receptor concentration on Day 19 seems to be associated with an increase of oestrogen (Shaikh, 1971) and a decrease of serum progesterone at the end of pregnancy (Morishige *et al.*, 1973; Busmann & Deis, 1979). Both steroids are known to be involved in the synthesis and metabolism of PGs in the uterus and ovary (Carminati *et al.*, 1975). The present results of steroid treatments suggest also that, whereas progesterone does not affect the increase in PGF-2 α receptor, oestradiol may actually advance it, and hence make the corpus luteum more responsive to the luteolytic action of PGF-2 α .

Although these results show that prostaglandin and its receptor are an essential part of the luteolytic process at the end of pregnancy, the fact that 20 α -HSD induction occurs in the hysterectomized indomethacin-treated rats indicates that the induction by hysterectomy is due to the removal of luteotrophic substances arising from the uterus, as proposed earlier (Wiest *et al.*, 1968; Rodway & Kuhn, 1975a; Hickman-Smith & Kuhn, 1976). In addition, no change in PGF-2 α receptor was found in the hysterectomized groups without indomethacin treatment, either after 20 α -HSD induction (i.e. down regulation) or after the partial abolition of it by hCG treatment (i.e. increase in receptor concentration), indicating that prostaglandins are not involved in enzyme induction due to hysterectomy.

The increase in receptor concentration on Day 20 that follows hysterectomy is not different from that seen in indomethacin-treated unoperated rats on the same day of pregnancy. These similar values in receptor concentrations would seem to indicate that uterine rather than luteal prostaglandins cause down-regulation of receptors, at least on Day 20 of pregnancy. Also some substance arising from the uterus may be inhibiting the expression of binding sites since the values on Day 18 in hysterectomized rats were higher than those in the unoperated group.

Although there is evidence for a participation of pituitary lactogenic hormones in restraining this enzyme during early pregnancy (Rodway & Kuhn, 1975b) and, also, a rat placental lactogen has been characterized (Shiu *et al.*, 1973), prolactin treatment was not able in this study to enhance the luteotrophic action of hCG in rats that were hysterectomized on Day 16. Nor did the administration of bromocriptine diminish the hCG effect on these animals. The induction of 20 α -HSD by hysterectomy, and the decrement of the induction by hCG treatment but not by indomethacin treatment, may be interpreted as suggesting that the luteolytic action of PGF-2 α is exerted through a blockade of the action of a placental luteotrophin with hCG-like activity; nevertheless, its molecular structure should be rather different from that of LH, since attempts to find an mRNA encoding for the α - or the β -subunit of an LH/hCG-like hormone have failed (Carr & Chin, 1985).

The hypothesis of the abrogation of a luteotrophic stimulus is supported by the finding of a decrease in luteal binding of ^{125}I -labelled hCG (Behrman & Hichens, 1976) that rapidly follows the treatment *in vivo* with PGF-2 α , and also by the PGF-2 α -induced decrease in the accumulation of cAMP in luteal cells that is produced by LH, fluoride, noradrenaline or cholera toxin (Lahav *et al.*, 1976; Khan & Rosberg, 1979; Dorfinger & Behrman, 1979). There is evidence that the action of PGF-2 α may involve changes in membrane fluidity, and a possible resulting loss of gonadotrophin-binding sites or their uncoupling from the adenylate cyclase system (Carlson *et al.*, 1982). On the other hand lactogenic hormones seem to counteract the action of PGF-2 α on the luteal membrane (Behrman *et al.*, 1978; Buhr *et al.*, 1983).

In luteolysis preceding normal parturition (Wiest *et al.*, 1968), or in that caused by PGF-2 α (this paper), the decrease in progesterone content in the luteal cells precedes the increase of 20 α -dihydroprogesterone. This may indicate that 20 α -HSD is under the restraint of placental luteotrophic hormones (either directly, or indirectly through the maintenance of high levels of intraluteal progesterone). It is therefore suggested that luteolysis at the end of pregnancy is the result of the blockade, at the luteal cell membrane, of the luteotrophic signals coming from the placenta, i.e. placental lactogen, which has a permissive action on progesterone biosynthesis, and a molecule with hCG-like activity, which maintains the high rates of this biosynthetic pathway. The blocking of luteotrophic signals is effected by an action of PGF-2 α on a PGF-2 α membrane receptor. The decrease in intraluteal progesterone thus produced would be the signal for 20 α -HSD induction. The increasing oestrogen concentrations at the end of pregnancy would be responsible for the crucial appearance of PGF-2 α binding sites in the corpora lutea and for the stimulation of PGF-2 α biosynthesis.

This work was supported by grants from CONICET and the BID-CONICET programme. I thank Dr A. Castro-Vazquez and Dr N. J. Kuhn for critical reading of the manuscript and Lederle Laboratories of Argentina for facilities.

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Received 25 November 1987