Participation of intraluteal progesterone and prostaglandin $F_{2\alpha}$ in LH-induced luteolysis in pregnant rat

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Abstract

We examined the participation of the intraluteal levels of progesterone (P_4) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in the induction of luteolysis by LH and its relationship with the induction of the 20α -hydroxysteroid dehydrogenase activity (20α -HSD). Subcutaneous administration of four doses of 10 μ g ovine LH (oLH) at 0800, 0900, 1000 and 1100 h on day 19 of pregnancy induced a decrease in the activity of the enzyme 3β -HSD 24 and 48 h after treatment and an increase in luteal 20α -HSD activity 48 h after oLH treatment when compared with control rats. Intraluteal and serum P_4 levels were lower than control values 24 and 48 h after oLH treatment, with a significant increase in luteal $PGF_{2\alpha}$ content and a decrease in corpus luteum (CL) weight 48 h after oLH treatment. Intrabursal ovarian (IB) treatment with an inhibitor of PG's biosynthesis (diclofenac) (70 μ g/ovary) or P_4 (3 μ g/ovary) on day 20 of pregnancy, prevented the increase in 20α -HSD

activity observed 48 h after oLH treatment, without any effect on 3β -HSD activity. The IB administration of P_4 prevented the increase in intraluteal $PGF_{2\alpha}$ content induced by oLH treatment and the increases in 20α -HSD activity and intraluteal $PGF_{2\alpha}$ content observed in control animals on day 21 of pregnancy. The inhibition of PG biosynthesis also prevents the decrease in intraluteal and serum P_4 level induced by oLH. These results provide good evidence of the important participation of intraluteal P_4 and $PGF_{2\alpha}$ on the oLH-induced luteolysis in pregnant rats. We also found that P_4 produced by the CL is involved, in part, in the regulation of luteal PG synthesis. Thus, the early decline in 3β -HSD activity and the consequent fall in intraluteal P_4 content, may trigger the synthesis of PGs and thereafter the increase in luteal 20α -HSD activity to establish luteolysis.

Journal of Endocrinology (1998) **156**, 253–259

Introduction

In rats, the corpus luteum (CL) plays an important role throughout pregnancy. The most important function of CL appears to be the secretion of progesterone (P_4), which is required for the establishment and maintenance of pregnancy. A decrease in serum P_4 concentration is necessary for the initiation of parturition (Niswender & Nett 1994). The mechanism responsible for the regression of the CL function and consequently the decrease in serum P_4 levels remains unknown. The role of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) as the endogenous mediator of luteolysis in many species, including rats, is well established (Rothchild 1981, Olofsson *et al.* 1990, Olofsson & Leung 1994). Administration of $PGF_{2\alpha}$ induces abortion (Deis 1971), and induction of 20α -hydroxysteroid dehydrogenase (20α -HSD) activity (Pharriss & Wyngarden 1969, Strauss & Stambaugh 1974, Lamprecht *et al.* 1975, Bussmann & Deis 1979), an enzyme that converts P_4 into a derivative devoid of progestational activity and considered a marker of luteolysis (Wiest 1968, Wiest *et al.* 1968). The role of $PGF_{2\alpha}$ as an inducer of the physiological luteolysis in the rat is corroborated by the demonstration of specific recep-

tors for $PGF_{2\alpha}$ on luteal membranes (Wright *et al.* 1979, Bussmann 1989). Locally synthesised PGs have an important role in CL regression as indicated by an increase in the content of $PGF_{2\alpha}$ in the CL during spontaneous luteolysis (Olofsson & Selstam 1988). Furthermore, in rats the ability to synthesise $PGF_{2\alpha}$ increases with the age of the CL accompanying the decline in P_4 production (Olofsson *et al.* 1992).

Since luteal cells contain high levels of P_4 , this local steroid may exert a paracrine or autocrine regulation of luteal $PGF_{2\alpha}$ synthesis. An inverse relationship between P_4 production and luteal PG content as hypothesised by Rothchild (1981), has been corroborated by studies from several groups (Olofsson *et al.* 1990, 1992, Cao & Chan 1993). P_4 alters the synthesis of luteal PGs (Pate 1988), which may be modulators of luteal steroidogenesis (Olofsson & Leung 1994). On the other hand, in luteolysis preceding normal parturition (Wiest *et al.* 1968, Lacy *et al.* 1976), or induced by $PGF_{2\alpha}$ (Bussmann 1989) or LH (Stocco & Deis 1996), the decrease in P_4 content in luteal cells precedes the increase in 20α -HSD activity.

The enzyme 3β -HSD is required for oxidation and isomerisation of the precursor pregnenolone for P_4

synthesis. Recent studies suggest that the expression of this enzyme is hormonally modulated (Chedrese *et al.* 1990, Couët *et al.* 1990, Martel *et al.* 1990, Hawkins *et al.* 1993) and could well play an important role in the luteolytic process induced by prolactin (PRL) (Martel *et al.* 1994) or LH (Stocco & Deis 1996). The objective of this study was to examine the participation of the intraluteal levels of P_4 and $PGF_{2\alpha}$ in the induction of luteolysis by ovine luteinising hormone (oLH) and its relationship with the induction of the enzyme 20 α -HSD.

Materials and Methods

Animals

Virgin female rats 3–4 months old (200–220 g) bred in our laboratory and originally Wistar strain were used. The rats were kept in a light- (lights on 0600–2000 h) and temperature- (22–24 °C) controlled room; rat chow (Nutric, Córdoba, Argentina) and tap water were available *ad libitum*. Vaginal smears were taken daily. Rats were caged individually with fertile males in the night of pro-oestrus, and the presence of spermatozoa was checked in the vaginal smears the following morning. This day was designated day 0 of pregnancy. In our laboratory rats usually give birth on day 22. The experiments were performed in accordance with The Guidelines on the Handling and Training of Laboratory Animals, The Biological Council, UFAW 1992.

Experimental procedures

Pregnant rats received a s.c. administration of 10 μ g oLH (NIDDK-oLH-26, NIDDK Bethesda, MD, USA) or vehicle at 0800, 0900, 1000 and 1100 h on day 19 of pregnancy. The animals were killed 24 or 48 h after the last dose of oLH.

In order to determine if P_4 prevents the luteolytic process induced by oLH, the steroid was injected intrabursally to oLH-treated rats. To minimise leakage from the bursa, 1 ml drug solution was mixed with 40 mg methyl cellulose (MC) (Sigma Chemical Co., St Louis, MO, USA) giving a final concentration of 4% MC. Groups of oLH-treated animals (day 19) received intrabursally a single dose of 30 μ l MC or MC plus 3 μ g progesterone, according to the method described by Tellería & Deis (1994), on day 20 of pregnancy between 1000 and 1100 h or between 1900 and 2000 h. The animals were killed on day 21 of pregnancy at 1100 h.

To prevent the biosynthesis of $PGF_{2\alpha}$, groups of animals treated with oLH s.c. on day 19 of pregnancy were injected intrabursally with diclofenac (an inhibitor of PG biosynthesis, Ciba-Geigy, Buenos Aires, Argentina). Groups of oLH-treated animals (day 19) received intrabursally a single dose of 30 μ l MC or MC plus 70 μ g

diclofenac between 1000 and 1100 h on day 20 of pregnancy. The animals were killed on day 21 of pregnancy at 1100 h.

All rats were killed by decapitation, trunk blood collected and allowed to clot at room temperature and the serum separated and stored frozen at –30 °C until assayed for P_4 . Both ovaries were removed from each rat and immediately placed on ice to prevent PG synthesis, trimmed from surrounding fat, and the CLs enucleated and stored at –70 °C until determination of enzymatic activities or luteal P_4 and $PGF_{2\alpha}$ content.

Radioimmunoassay of progesterone

Serum P_4 was measured using an RIA developed in our laboratory (Bussmann & Deis 1979) with an antiserum raised against progesterone-11-BSA conjugate in rabbits. The sensitivity of the assay is less than 5 ng/ml of serum and the inter- and intra-assay coefficients of variation were less than 10%. The P_4 content of the CLs was measured according to the methodology described by Tellería *et al.* (1995b).

Radioimmunoassay of prostaglandin $F_{2\alpha}$

$PGF_{2\alpha}$ was quantified by RIA using rabbit antiserum from Sigma Chemical Co. according to the methodology described by Motta *et al.* (1995) with slight modifications. The CL were thawed and homogenised in 1 ml 100% ethanol, centrifuged at 1000 g for 15 min and the supernatant was transferred to polypropylene tubes. The pellet was washed with the same volume of absolute ethanol, centrifuged again and both supernatants were pooled. The extracts were evaporated to dryness under nitrogen at room temperature. The tissue residue was reconstituted in PBS 0.01 M (pH=7.4) containing 0.1% BSA (Sigma Chemical Co.). Fractions (100 μ l) of standards or sample extracts were incubated with antiserum (500 μ l) for 30 min at 4 °C. After addition of tracer (100 μ l, specific activity 150–180 Ci/mmol; [5,6,8,9,11,12,14,15,(n)³H] $PGF_{2\alpha}$, Amersham Life Science, Amersham, Bucks, UK) incubation was continued for 60 min at 4 °C. Bound and free forms were separated by addition of 200 μ l dextran-coated beads (0.1–1%), incubated for 10 min at 0 °C, then centrifuged at 2000 g for 15 min at 4 °C. The supernatant from each tube was removed, and the amount of radioactivity was determined. Sensitivity of these assays was 10 pg/tube. The cross-reactivity of $PGF_{2\alpha}$ was 60% with $PGF_{1\alpha}$ and less than 0.1 % with other PGs. The results were expressed as $PGF_{2\alpha}$ equivalents. To our knowledge, there is no evidence in the literature of the ovarian concentration of $PGF_{1\alpha}$ during the luteolytic process in rats.

Enzyme activities

The activities of the enzymes were measured as described by Kawano *et al.* (1988) with slight modifications (Tellería

& Deis 1994). CLs from each animal were homogenised in 0.7 ml 0.1 mol Tris-HCl l^{-1} , 1 mmol EDTA l^{-1} (pH 8) at 0 °C with a glass homogeniser. The homogenates were centrifuged at 105 000 g for 60 min. The supernatant fluids were used for the assay of 20 α -HSD activity. The precipitates were rehomogenised with 0.7 ml 0.2 mol sucrose/ l and centrifuged at 800 g for 5 min. The supernatants were used as the enzyme solution for the assay of 3 β -HSD activity. Both enzyme activities were assayed spectrophotometrically, dependent on the increase in NADH or NADPH in 1 min at 37 °C and the values were expressed as mU/mg protein. The method of Lowry *et al.* (1951) was used for protein determination using BSA as standard.

Statistics

All data are presented as means $\pm$ S.E.M. Statistical evaluations of the results were done with unpaired Student's *t*-test to assay significant differences between means of two groups. One way analysis of variance (ANOVA) followed by Tukey's test was used for multiple comparisons. The threshold of significance was set at $P < 0.05$.

Results

Effect of s.c. administration of four doses of 10 μ g oLH on luteal 3 β -HSD and 20 α -HSD activities, serum P_4 levels, P_4 and $PGF_{2\alpha}$ luteal concentrations and CL weight at the end of pregnancy

The s.c. administration of oLH on day 19 of pregnancy (0800–1100 h) decreased 3 β -HSD activity 24 and 48 h after treatment, when compared with control values ($P < 0.001$) (Fig. 1A). In control and oLH-treated rats a significant decrease ($P < 0.001$) in 3 β -HSD activity was observed on day 21 of pregnancy when compared with its respective values on day 20. Luteal 20 α -HSD activity was not detectable 24 h after treatment in both vehicle and oLH-treated groups, but 48 h after treatment a significant ($P < 0.001$) increase was observed in the oLH-treated group (Fig. 1B).

The administration of oLH induced a significant ($P < 0.001$) reduction in CL P_4 content 24 and 48 h after treatment when compared with control rats (Fig. 1C). Serum P_4 levels in oLH-treated animals were significantly lower ($P < 0.01$) than control values 24 and 48 h after treatment (Fig. 1D). In control and oLH-treated rats a significant decrease ($P < 0.01$) in intraluteal and serum P_4 level was observed on day 21 of pregnancy when compared with its respective values on day 20 of pregnancy.

The administration of oLH on day 19 of pregnancy did not modify the intraluteal $PGF_{2\alpha}$ content 24 h after treatment, but provoked a significant increase ($P < 0.001$) in intraluteal $PGF_{2\alpha}$ content when compared with control

Figure 1 Luteal 3 β -HSD (A) and 20 α -HSD (B) activities, intraluteal (C) and serum (D) P_4 concentration, intraluteal $PGF_{2\alpha}$ levels (E) and CL weight (F) at 1100 h on days 20 (24 h) and 21 (48 h) of pregnancy after s.c. administration of vehicle (□) or oLH ($4 \times 10 \mu$ g) (■) between 0800 and 1100 h on day 19 of pregnancy. Values are means $\pm$ S.E.M. of groups of six to nine animals. *** $P < 0.001$; ** $P < 0.01$ compared with control values; ●●● $P < 0.001$; ●● $P < 0.01$; ● $P < 0.05$ when compared with its respective values on day 20 of pregnancy. nd, not detectable.

values 48 h after treatment (Fig. 1E). In control ($P < 0.05$) and oLH-treated ($P < 0.001$) rats, a significant increase in the CL $PGF_{2\alpha}$ content was observed on day 21 of pregnancy when compared with its respective values on day 20.

There were no differences in CL weight between control and oLH-treated rats 24 h after treatment, but a significant ($P < 0.001$) decrease in this parameter was observed 48 h after treatment in oLH-treated rats (Fig. 1F).

Interestingly, in control and oLH-treated rats there was an inverse correlation between intraluteal P_4 and $PGF_{2\alpha}$ content 24 and 48 h after treatment.

Figure 2 Effect of a single intrabursal ovarian injection of progesterone (P_4), diclofenac (Dic) or methyl cellulose (MC) on luteal 3β -HSD (A), 20α -HSD (B) activities, intraluteal P_4 (C) and serum P_4 level (D) and intraluteal $PGF_{2\alpha}$ content (E); 48 h after administration of oLH (LH) or vehicle (SF) on day 19 of pregnancy. P_4 , Dic or MC were injected on day 20 of pregnancy between 1000 and 1100 h. Values are means $\pm$ S.E.M. of groups of six to nine animals. Columns with the same letter differ significantly ($^{a,b}P<0.001$; $^{c,d,e}P<0.01$; $^{f,j}P<0.05$).

Effect of a single intrabursal ovarian injection of P_4 (3 μ g per ovary) on day 20 of pregnancy to oLH-treated rats on luteal 3β -HSD and 20α -HSD activities, serum P_4 level and luteal $PGF_{2\alpha}$ content

In order to determine if the decrease in intraluteal P_4 content observed after oLH treatment may induce the increase in 20α -HSD activity, rats pre-treated with oLH on days 19 received intrabursally 3 μ g P_4 per ovary on day 20 at 1100 h (24 h after oLH treatment), before the increase in 20α -HSD activity. Intrabursal ovarian P_4 treatment did not prevent the decrease in 3β -HSD activity observed 48 h after oLH treatment (Fig. 2A), but prevented significantly ($P<0.001$) the increase in 20α -HSD activity (Fig. 2B). The intrabursal administration of P_4 on

day 20 of pregnancy also prevented ($P<0.05$) the physiological increase in 20α -HSD activity observed in control animals at 1100 h on day 21 of pregnancy (Fig. 2B). However, P_4 administered intrabursally to oLH pre-treated rats at 2000 h on day 20 of pregnancy did not affect the enzymatic changes induced by oLH (data not shown).

The intrabursal administration of P_4 increased significantly the intraluteal P_4 content when compared with vehicle and oLH-treated groups (Fig. 2C). However, the intrabursal administration of P_4 did not modify the decrease in serum P_4 level observed 48 h after treatment with oLH (Fig. 2D).

The intrabursal administration of P_4 prevented significantly ($P<0.01$) the increase in intraluteal $PGF_{2\alpha}$ content observed on day 21 of pregnancy 48 h after oLH treatment (Fig. 2E). Interestingly, the intrabursal ovarian administration of P_4 to vehicle-treated rats also induced a significant decrease ($P<0.05$) in intraluteal $PGF_{2\alpha}$ content when compared with the control group (Fig. 2E).

Effect of a single intrabursal ovarian injection of an cyclooxygenase inhibitor on day 20 to oLH pre-treated rats on luteal 3β -HSD and 20α -HSD activities, intraluteal and serum P_4 levels on day 21 of pregnancy

In order to confirm the participation of intraluteal PG on oLH induced luteolysis, an inhibitor of PG biosynthesis (diclofenac) was administered intrabursally to oLH-treated rats. Diclofenac did not modify the decrease in 3β -HSD activity induced by oLH (Fig. 2A), but significantly prevented ($P<0.001$) the increase in 20α -HSD activity observed 48 h after oLH treatment (Fig. 2B). The intrabursal administration of diclofenac on day 20 of pregnancy also prevented ($P<0.05$) the physiological increase in 20α -HSD activity observed in control animals at 1100 h on day 21 of pregnancy (Fig. 2B).

The inhibition of PG biosynthesis at the ovarian level prevented ($P<0.01$) the decrease in intraluteal P_4 content induced by the treatment with oLH (Fig. 2C) and also prevented ($P<0.05$) the decrease in serum P_4 level observed 48 h after oLH treatment (Fig. 2D). The intrabursal administration of diclofenac prevented significantly ($P<0.001$) the luteal oLH-induced PG synthesis (Fig. 2E).

Discussion

The present study describes the participation of intraluteal P_4 and $PGF_{2\alpha}$ in the spontaneous and oLH-induced luteolysis in pregnant rats. Our results may suggest that the decrease in serum and intraluteal P_4 concentration during luteolysis can be attributed in part to the previous decrease in 3β -HSD and not only to an increase in the activity of 20α -HSD as postulated by Wiest *et al.* (1968). The significant decrease in 3β -HSD activity occurred at least

24 h before significant changes in 20α -HSD activity, while the size of the CLs was apparently unchanged. Recent studies have shown that a decrease in 3β -HSD is correlated with the decrease in plasma P_4 level (Bassett *et al.* 1991, Tian *et al.* 1994). Martel *et al.* (1994) showed that the inhibition of 3β -HSD gene expression and activity in rat corpora lutea occurred early in the PRL-induced luteolytic process, even before gross morphological changes could be detected.

In the present and previous studies, the decrease in intraluteal and serum P_4 level precedes the increase in luteal 20α -HSD activity in oLH-induced luteolysis (Stocco & Deis 1996) and in the normal luteolysis preceding parturition (Wiest *et al.* 1968, Lacy *et al.* 1976). Similar changes were also observed during the luteolysis induced by $PGF_{2\alpha}$ (Bussmann & Deis 1979) or by RU-486 treatment (Tellería *et al.* 1995a). The intrabursal P_4 treatment given before the increase in 20α -HSD activity prevents partially the physiological or oLH-induced increase in this enzyme activity on day 21 of pregnancy. Bussmann & Deis (1979) observed that the effect of $PGF_{2\alpha}$ on 20α -HSD induction is conditioned to the circulating levels of P_4 . It has been also reported that neutralisation of LH by an antiserum, between days 8 and 12 of pregnancy induces an increase in 20α -HSD activity which is prevented by P_4 treatment (Loewit & Zambelis 1979). These results give good evidence that P_4 is in some way involved in the regulation of 20α -HSD expression at the end of pregnancy, and suggest that the decrease in intraluteal P_4 content produced by the fall in 3β -HSD activity may trigger the increase in 20α -HSD activity. These results also provide evidence of the role of P_4 as a modulator of its own production and secretion (Rothchild 1981, 1996, Tellería & Deis 1994). The effectiveness of intrabursal administration of P_4 without affecting serum P_4 levels suggests that the site of action of the steroid would be at the ovarian level. The failure of intrabursal ovarian P_4 administered 36 h after oLH treatment to prevent the increase in 20α -HSD activity, may indicate that, when the expression of the enzyme has been initiated, the regulatory effect of P_4 does not occur.

Although P_4 is the major secretory product of the CL, this gland also synthesises PGs (Olofsson *et al.* 1992) which may modulate luteal steroidogenesis (Olofsson & Leung 1994). Our results, indicating an increase in CL $PGF_{2\alpha}$ content in control rats at the end of pregnancy, correlate with the results reported by Olofsson & Selstam (1988), showing an increase in luteal $PGF_{2\alpha}$ content at the end of the luteal phase in pseudopregnant rats. We also show that the administration of oLH enhances the physiological increase in luteal $PGF_{2\alpha}$. These results are in agreement with those obtained by Olofsson *et al.* (1992) who observed that LH induced an increase in $PGF_{2\alpha}$ production during the luteolytic period in pseudopregnant rats. The intrabursal administration of the cyclooxygenase inhibitor (diclofenac) prevented the increase in 20α -HSD

activity observed in oLH-treated rats, indicating that intraluteal PGs may mediate, at least in part, oLH-induced luteolysis in late pregnant rats. We have recently shown that s.c. administration of several doses of diclofenac to pregnant rats treated with the antiprogesterone RU-486, delayed abortion and prevented the increase in 20α -HSD activity (Tellería *et al.* 1995a), indicating that PGs may mediate RU-486 induced luteolysis in late pregnant rats. Considering that the IB administration of diclofenac or P_4 prevents partially the increment in 20α -HSD activity induced by oLH, we may suggest that both a decrease in the intraluteal P_4 content and an increase in the synthesis of $PGF_{2\alpha}$ are necessary in the induction of the 20α -HSD activity at the end of pregnancy.

It is noteworthy that, in spite of the intraluteal level of $PGF_{2\alpha}$ on day 20 of pregnancy, the luteal function is not impaired. The increase in luteal $PGF_{2\alpha}$ content and 20α -HSD activity in control rats are correlated with a significant decrease in intraluteal P_4 levels. These changes may indicate that the high level of intraluteal P_4 content during pregnancy may protect the corpora lutea from the luteolytic action of endogenous $PGF_{2\alpha}$. Interestingly, an inhibition of [3H] $PGF_{2\alpha}$ binding to its receptors by P_4 in bovine CL has been demonstrated (Rao 1976). In recent studies (C O Stocco & R P Deis, unpublished observations), the intrabursal administration of 3 μ g P_4 to rats on day 19 of pregnancy prevented the well-known luteolytic action of $PGF_{2\alpha}$ administered i.p. It has been shown in pregnant rats (Vermouth & Deis 1975) that the abortive action of $PGF_{2\alpha}$ administered at the end of pregnancy is dependent on plasma and uterine P_4 concentration. Another factor to be considered is a change in the sensitivity of the CL to $PGF_{2\alpha}$ with advancing gestation (Vermouth & Deis 1975, Khan *et al.* 1979, Lahav *et al.* 1988). Recent reports indicate that the number of $PGF_{2\alpha}$ binding sites increased with the age of the CL (Bussmann 1989, Orlicky *et al.* 1992).

The mechanisms that initiate luteal synthesis of $PGF_{2\alpha}$ are unknown; however, we observed that the increase in luteal $PGF_{2\alpha}$ content is correlated with a previous decrease in luteal and serum P_4 levels. An inverse relationship between the production of P_4 and PG at the luteal level was proposed by Rothchild (1981) and corroborated by numerous studies (Weems 1979, Olofsson *et al.* 1990, 1992, Cao & Chan 1993). It has been demonstrated that P_4 inhibits the production of $PGF_{2\alpha}$ by bovine luteal cells (Pate 1988) and the synthesis and release of $PGF_{2\alpha}$ from the uterus in ruminants (Silvia *et al.* 1991) and in pseudo-pregnant rats (Motta *et al.* 1995) or stimulates the activity of the PG metabolising enzyme 15-hydroxy PG dehydrogenase in the uterus and oviduct (Flower 1977, Bodkhe & Harper 1979, Liggins 1979). We found that the intrabursal administration of P_4 inhibits the increase in intraluteal $PGF_{2\alpha}$ content in oLH-treated rats and diminished significantly the normal level observed in the vehicle-treated rat on day 21 of pregnancy. These results may indicate that in

rats, luteal $PGF_{2\alpha}$ synthesis could be partially regulated by P_4 .

Park-Sarge *et al.* (1995) have shown, with a very sensitive method, that rat luteal cells do not have detectable levels of P_4 receptor mRNA. Therefore, in rats P_4 does not affect CL function through an intracellular receptor but may affect it through other non receptor-mediated mechanisms. P_4 inhibits the metabolic activities of monocytes or macrophages (Grossman 1985), the production of tumor necrosis factor by macrophage (Polan *et al.* 1988) and superoxide radical production by the mononuclear phagocytes in the CL of pseudopregnant rats (Sugino *et al.* 1996). Considering that mononuclear phagocytes are increased in number in the CL during luteolysis (Adashi 1990), the decrease in intraluteal P_4 content may allow macrophages and $PGF_{2\alpha}$ to participate in CL regression.

In summary, we have shown that during oLH-induced luteolysis, the decrease in luteal 3β -HSD and P_4 concentration precedes the rise in 20α -HSD activity, whereas local administration of a single dose of P_4 on each ovary prevented significantly the increase of both 20α -HSD activity and $PGF_{2\alpha}$ content in the CL. In addition, local administration of a PG synthesis inhibitor prevents the rise in 20α -HSD. These results provide evidence that the oLH-induced and the physiological luteolytic processes, may be associated with an early decrease in luteal 3β -HSD activity, followed by a decrease in intraluteal P_4 content which may trigger the synthesis of $PGF_{2\alpha}$ and the increase in 20α -HSD activity. Previous studies (Tellería *et al.* 1995a, Stocco & Deis 1996) and the present results suggest that luteal regression may involve, not only the regulation of both the synthesis and degradation of P_4 , but also the regulation of luteal $PGF_{2\alpha}$ biosynthesis.

Acknowledgements

This work was supported by grant no. 3-122100/88 from CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina) and in part by grant no. PLI-222/95 from PLACIRH (Programa Latinoamericano de Capacitación e Investigación en Reproducción Humana). The authors are grateful to NIH for the generous supply of oLH and Ciba-Geigy (Argentina) for the gift of diclofenac.

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Received 26 November 1996

Revised manuscript received 9 June 1997

Accepted 26 August 1997