Journal of Reproduction and Fertility (1994) **102**, 139–145

Increased LH receptor mRNA and extended corpus luteum function induced by prolactin and indomethacin treatment *in vivo* in hysterectomized pseudopregnant rats

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To assess the effects of prostaglandins and prolactin on corpus luteum function and regression, sterile-mated adult pseudopregnant rats hysterectomized on day 5 after mating were injected with indomethacin or prolactin. Daily samples of blood were collected via the tail, from day 12 to day 21, and assayed for serum concentrations of progesterone, 20α-dihydroprogesterone and LH, whereafter corpora lutea and the remainder of ovaries were separated and the tissue content of $PGF_{2\alpha}$, PGE_2 and LH receptor mRNA were measured. Injections of prolactin (8 iu) s.c. or a low dose of indomethacin (200 $\mu g\ kg^{-1}$) s.c. were administered twice a day, beginning on day 13 after mating. Both indomethacin and prolactin significantly increased serum progesterone concentrations ($P < 0.05$; $n = 8$), and extended the period of functional corpora lutea when compared with controls. Indomethacin, but not prolactin, lowered the concentration of serum 20α-dihydroprogesterone. In the corpora lutea of indomethacin-treated animals, collected on day 21, both prostaglandins measured were reduced in concentration by 50% or more, compared with controls ($P < 0.05$; $n = 8$), whereas prolactin had no effect. Both prolactin and indomethacin treatment caused a substantial (tenfold) increase in the concentration of LH receptor mRNA, confined solely to the luteal compartment. These findings *in vivo* provide further evidence for a luteolytic role of locally synthesized prostaglandins in the rat ovary. Furthermore, prolactin can sustain corpus luteum function by exerting a luteotrophic effect during the late luteal phase, as judged by the stimulation of progesterone synthesis and the expression of LH receptors.

Introduction

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) has a decisive role in the functional regression of the corpus luteum in most species, including rats (Olofsson and Leung, 1994). Thus, administration of $PGF_{2\alpha}$ or its analogues causes a premature regression of the corpus luteum. In large domestic animals, the uterus is the most likely source of luteolytic $PGF_{2\alpha}$. However, in many species, such as rats and humans, evidence is lacking for a local countercurrent mechanism of exchange between the uterus and ovaries, as demonstrated in sheep (McCracken *et al.*, 1971; Heap *et al.*, 1985). An important role of locally synthesized prostaglandins in the rat corpus luteum is indicated by a high degree of immunostaining for prostaglandin endoperoxide synthase (Curry *et al.*, 1991); this enzyme has also been detected by immunoblots (Olofsson *et al.*, 1991). Specific binding sites for $PGF_{2\alpha}$ in the corpus luteum have been identified using ligand binding assays (Wright *et al.*, 1979; Bussmann, 1989) and immunohistochemical localization (Orlicky *et al.*, 1992). Furthermore, it has been demonstrated that the rat corpus luteum has an increased capacity to synthesize prostaglandins *in vitro* towards the end of the luteal phase and that further stimulation of prostaglandin synthesis by arachidonic acid exerts an antigonadotrophic effect during this stage (Olofsson *et al.*, 1992).

The increased formation of prostaglandins occurs concomitantly with an increased tissue concentration in the corpus luteum, when measured *in vivo* (Weems, 1979; Olofsson and Selstam, 1988), and occurs before the decrease in peripheral progesterone concentrations during unmanipulated pseudopregnancy, as well as after hysterectomy or decidualization on day 5 of the same pseudopregnancy cycle (Olofsson *et al.*, 1990; Olofsson and Norjavaara, 1990). Concentrations of prostaglandins are kept low when progesterone production by the corpus luteum is kept high, that is, during pregnancy or after decidualization (Olofsson *et al.*, 1990; Olofsson and Norjavaara, 1990). However, little is known about the regulation of prostaglandin production in the rat corpus luteum.

Cervical stimulation during mating induces a biphasic circadian secretion of prolactin (Butcher *et al.*, 1972; Freeman *et al.*, 1974) and thereby rescues the corpus luteum from undergoing luteolysis (Smith *et al.*, 1976). During the early

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Received 15 February 1994.

luteal phase, luteal LH receptors depend on prolactin (Richards and Williams, 1976) and Gåfvels *et al.* (1992) demonstrated that this is also true for LH receptor gene expression. Moreover, inhibition of prolactin secretion on day 7 of pseudopregnancy decreases the number of luteal LH receptors and the concentration of adenylate cyclase, whereas the opposite occurs after decidualization (Gibori *et al.*, 1984). Prolactin can inhibit the induction of luteal 20α-hydroxysteroid dehydrogenase *in vitro* (LaHav *et al.*, 1977), the enzyme known to convert progesterone into its inactive metabolite 20α-dihydroprogesterone. Furthermore, this enzyme is induced by exogenous $PGF_{2\alpha}$ (Strauss and Stambaugh, 1974).

In this study, we explored the hypothesis that supplementation with prolactin or inhibition of prostaglandin synthesis by indomethacin treatment will maintain the functional lifespan of the corpus luteum for an extended period. Experiments were performed using hysterectomized pseudopregnant rats, thereby ruling out any effect mediated by uterine prostaglandins, and treatments began just before the end of the diurnal and nocturnal peaks of serum prolactin on day 16 (Freeman, 1979).

Materials and Methods

Animals and Experimental Procedures

Female Sprague-Dawley rats were purchased from Møllegaard Ltd (Ejby). They were kept under controlled environmental conditions (22°C, 45–55% humidity, light period between 06:00 and 18:00 h), and had free access to pellets (type R34 from Lactamin Ltd, Stockholm) and tap water. Pseudopregnancy was induced at 2 months of age (bodymass of 170–200 g) by mating with vasectomized males, as described by Norjavaara *et al.* (1987).

Twenty-four rats were hysterectomized on day 5 of pseudopregnancy, after recovery of a vaginal plug on day 1, a procedure known to prolong the duration of pseudopregnancy to approximately 18 days (Anderson *et al.*, 1969). The animals were anaesthetized with 0.6 mg fluanison kg^{-1} and 0.12 mg fentanyl-dihydrogencitrate kg^{-1} (Janssen Co, Beerse) i.p., following premedication with 0.05 mg atropine kg^{-1} bodymass (KabiVitrum Ltd, Stockholm) s.c. and 2.5 mg diazepam kg^{-1} (Dumex Ltd, Copenhagen) i.p. After recovery, the rats were randomly allocated to three groups and allocated to the different treatment regimens beginning on day 13 and continuing up to day 21.

One group of eight animals received 8 iu prolactin s.c. at 10:00 h and 22:00 h daily, to mimic the secretion pattern of prolactin (Gunnet and Freeman, 1983). A total injection volume of 0.5 ml per animal was used and ovine prolactin (NIADDK, Rockville, MD) was prepared before each injection, with some modifications to the procedure described by Gåfvels *et al.* (1992). Prolactin was dissolved in 0.25 ml buffer (0.03 mol $NaHCO_3$ l^{-1}, 0.15 mol NaCl l^{-1}, pH 9.5) and thoroughly emulsified with an equal volume of 45% (w/v) polyvinylpyrrolidone (PVP, M_r = 160 000, Fluka, Buchs) by the use of a double cannula.

Indomethacin (Dumex Ltd), diluted in sterile NaCl (0.15 mol l^{-1}) to a final concentration of 0.5 mg ml^{-1}, was given to eight animals in a volume of 0.1 ml s.c. twice a day, at the same times when prolactin was injected.

Control animals (n = 8) received vehicle (PVP plus buffer) alone. In a previous pilot experiment, no differences in serum progesterone or 20α-dihydroprogesterone concentrations were found between animals given PVP plus buffer or saline injections.

Daily blood samples were drawn (approximately 300 µl) before the morning injections from day 12 onwards (with the single exception for day 13, when samples were collected 1 h after treatments) by cutting the tip of the tail on day 12, and gently removing the blood clot on following days. On day 21, rats were decapitated and trunk blood was collected. Ovaries were rapidly removed and chilled in ice-cold 0.15 mol NaCl solution l^{-1} containing 14.0 µmol indomethacin l^{-1} for the left ovary (for prostaglandin determinations) and in 0.15 mol NaCl l^{-1} without additives for the right ovary (for LH receptor mRNA determinations). The corpora lutea of pseudopregnancy were identified (and distinguished from corpora lutea of earlier oestrous cycles) under a stereomicroscope using criteria described by Olofsson and Selstam (1988), and dissected free from the remainder of the ovary. Tissues were quickly blotted on filter paper, weighed and immediately immersed in liquid nitrogen. Samples were kept at − 70°C until analyses were performed. For comparison, four intact pseudopregnant rats were killed 8 days after mating, corpora lutea were excised and the concentration of LH receptor mRNA was determined as described below. The experimental protocols were approved by the Umeå University Animal Experimentation Ethical Committee.

Determinations of serum progestins and LH

Serum was extracted with diethyl ether and assayed by radioimmunoassay for concentrations of progesterone or 20α-dihydroprogesterone as described by Norjavaara *et al.* (1987) and Olofsson *et al.* (1990). Intra-assay and interassay coefficients of variation were 8.1% and 10.4% for progesterone and 5.0% and 6.1% for 20α-dihydroprogesterone, respectively.

Serum concentrations of LH in samples taken on days 18 and 21 were measured in a single assay by competition with ^{125}I-labelled rabbit-LH binding to rabbit anti-LH antibodies using kit RPA 552 (Amersham International, Amersham), in which the sensitivity was 0.08 pg per 100 µl and the coefficient of variation was 7.1%. The assay standard was rat LH, which had been calibrated against the NIH-RP2 reference standard preparation of the National Institute of Diabetes and Digestive and Kidney Diseases National Hormone and Pituitary Program, Baltimore, MD.

Determination of prostaglandins

Tissue concentrations of $PGF_{2\alpha}$ and PGE_2 were determined by methods described by Olofsson *et al.* (1990). Briefly, corpora lutea and the remainder of the ovary from the left side were homogenized on ice in a 5 ml glass/glass homogenizer (Waltergraf Co, Wertheim) after adding 2.0 ml PBS (10 mmol l^{-1}, acidified to pH 4.0 with 1.0 mol HCl l^{-1} to prevent *de novo* synthesis of prostaglandins. Samples were extracted by loading the solution on to a C18-LRC solid phase extraction cartridge (Bond-Elute, Analytichem Inc.,

Table 1. Daily serum concentrations of progesterone and 20α-dihydroprogesterone on different days after treatment from day 13 and onwards with vehicle (control), prolactin or indomethacin in pseudopregnant rats hysterectomized on day 5

Treatment	Days after mating 13	14	15	16	17	18	19	20	21
Progesterone (nmol l^{-1})									
Control	127 ± 18	94 ± 15	91 ± 10	62 ± 14	61 ± 9	39 ± 6	34 ± 4	29 ± 5	30 ± 9
Prolactin	138 ± 12	124 ± 10	137 ± 12[a]	115 ± 12[a]	109 ± 9[a]	95 ± 9[a]	76 ± 9[a]	56 ± 8[a]	45 ± 7
Indomethacin	128 ± 11	146 ± 10[a]	112 ± 5	88 ± 7	93 ± 8[a]	69 ± 10[a]	68 ± 8[a]	50 ± 8[a]	57 ± 6[a]
20α-Dihydroprogesterone (nmol l^{-1})									
Control	58 ± 5	48 ± 7	58 ± 11	54 ± 7	46 ± 5	56 ± 13	43 ± 9	56 ± 22	25 ± 9
Prolactin	61 ± 6	66 ± 9	69 ± 9	61 ± 7	59 ± 7	54 ± 5	57 ± 8	75 ± 12	59 ± 12
Indomethacin	35 ± 6[a]	36 ± 5	28 ± 4[a]	28 ± 3[a]	33 ± 4	35 ± 4	38 ± 6	38 ± 5	57 ± 5[a]

Values are means ± SEM; $n = 8$ for each group.
[a]For each parameter, value is significantly different from ($P < 0.05$) the control value on the same day.

Harbor City, CA). Proteins and neutral lipids were eluted with 5.0 ml filtered water and 5.0 ml HPLC-grade 10% (v/v) acetonitrile (Ratburn Chemicals, Walkerburne) at a constant flow rate of approximately 500 μl min^{-1} using a vacuum-station (Vac-Elute SPS 24, Analytichem Inc.). Prostaglandins were then recovered with 8.0 ml HPLC-grade methanol (Merck Co, Darmstadt). After evaporation under nitrogen, the dried extract residue was redissolved in 1.0 ml Tris–HCl [10 mmol l^{-1}, pH 7.4, containing 0.1% gelatin (w/v)], vortexed, and radioimmunoassays for the respective prostaglandins performed using specific antibodies purchased from Advanced Magnetics Inc. (Cambridge, MA) and tritiated prostaglandins from New England Nuclear (Boston, MA). All samples were measured in a single assay in which the coefficient of variation was 14.2% for $PGF_{2\alpha}$ and 14.0% for PGE_2. Sensitivity was 2.7 pg in 100 μl for both assays. Tissue concentrations of prostaglandins are expressed in pmol mg^{-1} protein, as assayed according to the method of Lowry *et al.* (1951) using BSA as standard.

Determination of LH receptor mRNA by solution hybridization

Total nucleic acid preparations from the corpora lutea of the right ovary and the remainder of the ovary were performed according to the method of Durnam and Palmiter (1983), as described by Gåfvels *et al.* (1992). Briefly, tissues were homogenized with a polytron in buffer containing 1% SDS, 20 mmol Tris–HCl l^{-1} (pH 7.5) and 10 mmol EDTA l^{-1}. The homogenates were then treated with Proteinase K and total nucleic acids were extracted with phenol–chloroform. A fragment of cDNA corresponding to the extracellular domain (nucleotides 212–510) of the LH receptor (kindly donated by H. Rajaniemi, University of Oulo) was used as a template to synthesize an antisense RNA probe using T7 RNA polymerase in the presence of [^{35}S]CTP (Amersham).

To measure the concentration of the LH receptor, the labelled RNA probe (about 20 000 c.p.m. per sample) was hybridized for 18 h at 70°C with total nucleic acid samples, in 60 mmol NaCl l^{-1}, 20 mmol dithiothreitol l^{-1} and 25% (v/v) formamide. The samples were then treated with 40 μg ribonuclease (RNase) A and 2 μg RNase T_1 (Boehringer, Mannheim) for 45 min at 37°C. RNase-protected probe was precipitated with trichloroacetic acid at 0°C. The precipitate was collected using Whatman GF/C filters (Whatman, Clifton, NJ) and a vacuum-filtration manifold (Schleicher and Schuell, Keene, NH), and washed twice with 5% (v/v) trichloroacetic acid in 5 mmol sodium pyrophosphate l^{-1} and twice in 96.5% (v/v) ethanol. The ribonuclease-protected material was solubilized from the filters by adding 900 μl Soluene 350 (Packard Instruments Co.) and 100 μl water. Scintillation fluor (Opti-Phase, LKB, Rockville, MD) was then added and the radioactivity in the samples measured in a liquid scintillation counter (Rackbeta, LKB-Wallac, Turkku). The relative amount of LH receptor mRNA present in each sample was expressed as c.p.m. μg^{-1} DNA, where the DNA content of the total nucleic acid preparations was assessed by fluorometry (Hoefor TKO 100, San Francisco, CA) according to the method of Labarca and Paigen (1980). Northern blots run in parallel experiments have previously verified changes in LH receptor mRNA abundance in the corpus luteum as detected by solution hybridization (Segaloff *et al.*, 1990; Gåfvels *et al.*, 1992).

Statistical analyses

Values are presented as means ± SEM. For comparisons between days and experimental groups, a two-way analysis of variance was used and a two-tailed Mann–Whitney U test was used to confirm the results. Differences between groups were considered significant for P values of < 0.05.

Results

Effects on serum progestins and corpus luteum composition

Administration of exogenous prolactin in a way that mimicked the pattern of pituitary prolactin release earlier in the luteal phase resulted in a 1.5–2.5-fold increase in peripheral progesterone concentrations from day 15 to day 20 (Table 1) and extended the luteal phase, as measured by the ratio of progesterone: 20α-dihydroprogesterone concentrations when compared with controls (Fig. 1). However, prolactin treatment did not cause a significant alteration in the serum

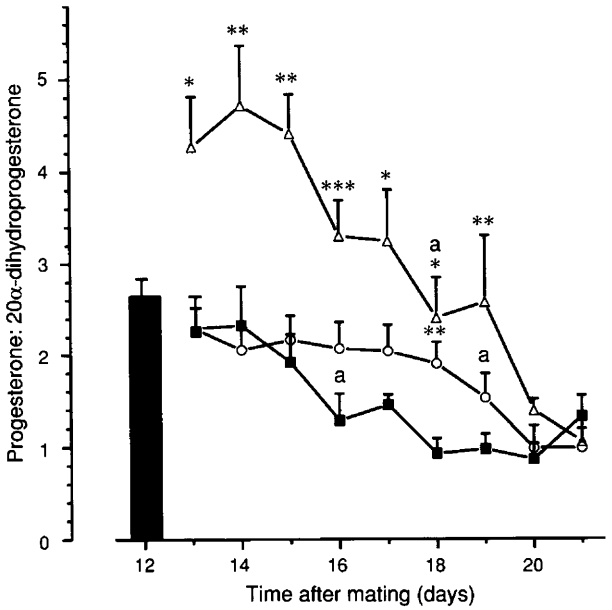

Fig. 1. Mean (± SEM) ratio of individual progesterone: 20α-dihydroprogesterone concentrations in pseudopregnant rats hysterectomized on day 5 and treated with vehicle alone (■), prolactin (○) or indomethacin (△). The black bar indicates the pretreatment concentration on day 12. Treatments were begun on day 13 after mating. Values are means ± SEM; $n = 8$ for each group. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ compared with control values on the same day. Within each treatment group, 'a' denotes the first day with a ratio significantly lower than that on day 13 ($P < 0.05$).

concentration of 20α-dihydroprogesterone, whereas treatment with indomethacin caused a 40% decrease in 20α-dihydroprogesterone concentrations 1 h after injection on day 13 when compared with controls, and these concentrations remained low up to day 20 (Table 1). Furthermore, in the indomethacin-treated group, progesterone concentrations were increased to values similar to those measured in prolactin-treated animals (Table 1). Progesterone production by the corpus luteum was extended by a similar amount in prolactin-treated and indomethacin-treated animals (Fig. 1). The different treatment regimens had no effect on corpus luteum mass, protein or DNA content, as measured after autopsy on day 21 (Table 2).

Effects on serum LH, ovarian tissue prostaglandin concentrations and LH receptor mRNA

In samples from indomethacin-treated animals immunoreactive serum concentrations of LH were reduced by 30% on day 18 of pseudopregnancy compared with controls (Table 3). Moreover, in the indomethacin-treated group, LH concentrations remained suppressed on day 21, when peripheral LH concentrations were further increased in both control as well as prolactin-treated animals (Table 3). Whereas indomethacin treatment significantly reduced luteal $PGF_{2\alpha}$ and PGE_2 tissue concentrations by more than 50% (Table 2), no adverse reactions such as gastrointestinal bleeding were detected upon autopsy, as is commonly seen when higher doses of this compound are used. In the remaining tissues of the ovaries, there was considerable variability between individual samples assayed for $PGF_{2\alpha}$ and PGE_2 concentrations. However, in indomethacin-treated animals, concentrations were markedly lower for both $PGF_{2\alpha}$ (control: 0.27 ± 0.18 pmol mg^{-1} protein; indomethacin: 0.04 ± 0.0003 pmol mg^{-1} protein; $P = 0.063$; $n = 8$) and PGE_2 (control: 3.9 ± 1.9 pmol mg^{-1} protein; indomethacin: 0.7 ± 0.05 pmol mg^{-1} protein; $P < 0.05$; $n = 8$), although these reductions were statistically significant for PGE_2 only. Prolactin had no effect on either $PGF_{2\alpha}$ or PGE_2 concentrations in corpora lutea (Table 2) or in the remainder of the ovarian compartment (data not shown).

LH receptor mRNA concentrations were barely detectable in the corpora lutea of control animals (Fig. 2); however, a tenfold increase in LH receptor mRNA was seen in the corpora lutea of prolactin-treated and indomethacin-treated groups (Fig. 2). This effect was confined to the luteal ovarian compartment only, since no changes were seen in the remainder of the ovaries between the different groups.

Discussion

The findings in the present study confirm and extend those of previous reports that both prolactin (Gunnet and Freeman, 1983) and indomethacin treatment prolong the functional lifespan of the rat corpus luteum (Lau *et al.*, 1975; Sànchez-Criado and Lopéz, 1986). Furthermore, the results suggest that inhibiting ovarian prostaglandin synthesis by indomethacin stimulates and extends the capacity of the corpus luteum to produce progesterone by exerting its effects via at

Table 2. Effect of control conditions, prolactin and indomethacin on corpus luteum wet mass, protein and DNA content and prostaglandin concentrations after 21 days of treatment in pseudopregnant rats that had been hysterectomized on day 5

Parameter	Control	Prolactin	Indomethacin
Wet mass (mg per corpus luteum)	1.8 ± 0.1	2.0 ± 0.1	1.7 ± 0.1
Protein content (μg mg^{-1} wet mass)	70.5 ± 9.3	71.3 ± 9.4	65.6 ± 6.2
DNA content (μg mg^{-1} wet mass)	2.5 ± 0.4	2.7 ± 0.2	2.7 ± 0.3
$PGF_{2\alpha}$ content (pmol mg^{-1} protein)	0.17 ± 0.04	0.20 ± 0.05	0.08 ± 0.007[a]
PGE_2 content (pmol mg^{-1} protein)	2.63 ± 0.18	1.74 ± 0.29	0.92 ± 0.07[a]

Values are means ± SEM; $n = 8$ for each group.
[a]For each parameter, value is significantly different from ($P < 0.05$) the control value on the same day.

Table 3. Concentrations of LH in serum on days 18 and 21 after mating in prolactin-treated, indomethacin-treated and control pseudopregnant rats that had been hysterectomized on day 5

	Control	Prolactin	Indomethacin
Serum LH, day 18 (ng ml^{-1})	2.09 ± 0.11	1.75 ± 0.11	1.47 ± 0.13[a]
Serum LH, day 21 (ng ml^{-1})	8.72 ± 3.96	9.74 ± 4.97	1.67 ± 0.08[a]

Values are means ± SEM; $n = 8$ for each group.
[a]For each parameter, the value is significantly different ($P < 0.05$) from the control value on the same day.

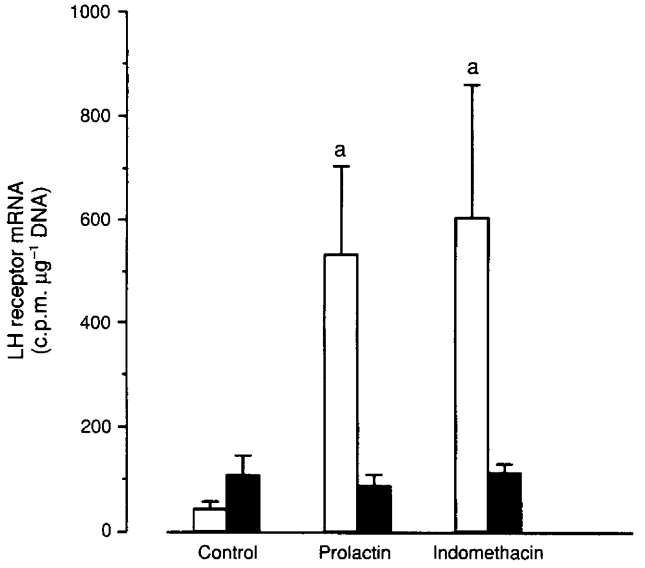

Fig. 2. Tissue concentrations of LH receptor mRNA (mean ± SEM) in rat corpus luteum (□) and the remainder of the ovary (■) on day 21 after treatment with control, prolactin or indomethacin. LH receptor mRNA concentrations were measured by solution hybridization from total nucleic acid preparations. For comparison, the mean concentration in the corpora lutea of uterine intact pseudopregnant rats 8 days after mating, run in the same assay, was 1656 ± 129 c.p.m. μg^{-1} DNA ($n = 4$). [a]For the respective tissues, value is significantly different ($P < 0.05$) from the control value.

least two different mechanisms. First, a drastic suppression of the concentration of 20α-dihydroprogesterone occurred only 1 h after a single injection of indomethacin, while progesterone concentrations were only significantly increased 1 day later. Although the reason why a corresponding rapid increase in progesterone concentrations does not occur is unknown, such a suppressive effect on 20α-dihydroprogesterone concentrations is consistent with the known regulation of 20α-hydroxysteroid dehydrogenase by $PGF_{2\alpha}$ (Strauss and Stambaugh, 1974). Second, as the effects of indomethacin became more pronounced during the second half of the treatment period, when progesterone concentrations remained higher on each day compared with controls, it is possible that this effect was mediated through an increased number of LH receptors, the stimulation of which can maintain corpus luteum function (Rothchild, 1981; Gibori, 1993).

Indomethacin does not alter the synthesis of prolactin by the rat pituitary (Burton *et al.*, 1975; Canonico *et al.*, 1983; Koike *et al.*, 1986; Junier *et al.*, 1990). The luteotrophic effect of indomethacin treatment is therefore not likely to be exerted at the pituitary level, since the serum concentration of LH was suppressed. This finding is not surprising, since it is known that PGE_2 is an important stimulatory mediator of hypothalamic GnRH production, and subsequently of LH release in pituitary gonadotrophs (Ojeda *et al.*, 1989). Such a decreased hypothalamic PGE_2 synthesis and thus diminished LH support of the corpus luteum may explain why progesterone concentrations continued to decline in indomethacin-treated animals in this study, despite increased LH receptor gene expression. In control animals, the low peripheral progesterone concentrations and a low concentration of LH receptor mRNA are indicative of functional luteolysis. Structural luteolysis had not yet occurred in these animals, as changes in neither corpus luteum mass protein nor DNA content were evident when compared with the other groups. This conclusion was also reached by Wang *et al.* (1993), who used a similar model to that reported here and showed that glucocorticoids can block luteal regression. Whether the decrease in the concentration of LH receptor transcripts is due to changes in transcription rate or mRNA stability cannot be ascertained by findings in the present study. It has recently been demonstrated that the decrease in LH receptor mRNA after hCG treatment is due to decreased stability of the transcripts (Lu *et al.*, 1993), but the physiological relevance of this remains to be elucidated during spontaneous luteolysis in rats.

Inhibiting prolactin secretion during the early luteal phase induces a decrease in the binding capacity of LH (Grinwich *et al.*, 1976; Holt *et al.*, 1976) as well as the expression of mRNA encoding LH receptors (Gåfvels *et al.*, 1992). During late pseudopregnancy and also in pregnant rats, pituitary prolactin secretion ceases, but there are at least seven known rat placental hormones, secreted during mid pregnancy or late pregnancy, that could bind to the prolactin receptor (Robertson and Friesen, 1975, 1981; Jayatilak *et al.*, 1985; Duckworth *et al.*, 1986, 1988; Deb *et al.*, 1991a, b). Thus, in the corpora lutea of prolactin- and indomethacin-treated animals in this study, the concentration of LH receptor mRNA was similar to that reported towards the end of unmanipulated pseudopregnancy (Gåfvels *et al.*, 1992), a time when the corpus luteum can still respond to LH by an increased progesterone output (Olofsson *et al.*, 1992), albeit at a subliminal concentration compared with the midluteal period. It has been reported that prolactin *in vitro* or *in vivo* inhibits the induction of 20α-hydroxysteroid dehydrogenase in superluteinized rat ovaries (LaHav *et al.*, 1977; Torjesen *et al.*, 1978; De la Llosa-Hermier *et al.*, 1979). However, we were not able to detect an effect on peripheral 20α-dihydroprogesterone concentrations during prolactin

supplementation in the study reported here. A possible explanation for this is that 20α-dihydroprogesterone secreted from the corpora lutea of earlier oestrous cycles contributed significantly to the serum concentrations measured. Indeed, this has been demonstrated to be of importance when comparing luteal and serum concentrations of 20α-dihydroprogesterone in oestrous cyclic rats (Uilenbroek *et al.*, 1989).

In summary, exogenous prolactin extended the duration of luteal LH receptor gene expression and corpus luteum progesterone secretion beyond the time when luteolysis normally occurs. We conclude that stimulation of the prolactin receptor elicits a luteotrophic action during the late luteal phase. Furthermore, the results of the present study strongly point towards a role of locally synthesized prostaglandins in luteolysis, the inhibition of which will increase and extend the function of the corpus luteum in terms of progesterone production.

The authors thank the National Hormone and Pituitary Program/ NIADDK, Rockville, MD, for the gift of prolactin, and E. Norjavaara for helping to develop the serial blood sampling procedure. The LH receptor plasmid was generously provided by H. Rajaniemi, University of Oulu, Finland. We also thank P. C. K. Leung for helpful comments during the preparation of this manuscript. This work was supported by grants (and a scholarship to J. I. Olofsson) from the Swedish Medical Research Council (5663), The Jeansson Foundation, The Medical Faculty at the University of Umeå and The Swedish Society of Medicine.

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