Delayed luteolysis and suppression of testosterone secretion after recombinant ovine interferon treatment in goats (Capra hircus)

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Oxytocin at a dose of 100 µg injected s.c. daily into goats (Capra hircus) between day 3 and day 6 of the oestrous cycle caused a significant increase in testosterone secretion and luteolysis compared with saline-treated animals. Intrauterine administration of recombinant ovine interferon tau (80, 160 or 320 µg day \(^{-1}\)) between days 12 and 18 of the oestrous cycle, or concomitantly (80 µg day \(^{-1}\)) with oxytocin between day 3 and day 7, delayed luteolysis and blocked the increased release of testosterone. It is suggested that recombinant ovine interferon tau can act as an antiluteolytic agent in goats.

Introduction

In goats (Homeida and Cooke, 1982) and most other species (guinea-pigs, cows, ewes) in which the nonpregnant uterus has a luteolytic action (Anderson et al., 1969; Martal, 1981), the presence of the embryo prevents luteolysis. The antiluteolytic factors secreted by sheep, goat and cattle conceptuses are closely related structurally to \(\alpha\)-interferons (IFN-\(\alpha\)) and are known as ovine, caprine and bovine trophoblast protein-1, respectively (Gnatek et al., 1989; Plante et al., 1990; Ott et al., 1991). These embryonic interferons bind to IFN-\(\alpha\) receptors, have antiviral and antiproliferative properties and can influence the production of uterine PGF\(_{2\alpha}\) and proteins (Bazer et al., 1987; Roberts, 1989). Intrauterine administration of IFN-\(\alpha\) can extend the interoestrus interval and luteal lifespan in cows (Plante et al., 1991) and sheep (Stewart et al., 1992). Significant improvement in the pregnancy rate has been observed in unilaterally ovariectomized ewes treated with IFN-\(\alpha\) (Nephew et al., 1990). An increase in embryonic survival and the promotion of maternal recognition of pregnancy in sheep (Schalke-Francis et al., 1991) are also among the many biological activities of trophoblast interferons.

Another process by which luteal regression is elicited in the goat is via testosterone release (Homeida and Cooke, 1984; Homeida, 1986). Testosterone may be released in response to PGF\(_{2\alpha}\) secreted by the uterus, since both hormones are synchronously released during luteal regression and are further stimulated by oxytocin and blocked by oxytocin antagonists during oxytocin-induced luteolysis (Homeida and Khalafalla, 1990).

The objective of the present experiment was to determine the effects of recombinant ovine interferon on luteal function and on natural and oxytocin-induced testosterone secretion in goats.

Materials and Methods

Animals

Twenty-eight Awassi goats (2–4 years of age, and with a normal oestrous cycle of 19–20 days) were used. They were housed in individual pens under conditions of natural day-length and temperature. The oestrous cycles were synchronized with two 5 mg injections i.m. of a PGF\(_{2\alpha}\) analogue (Lutalyse: Upjohn Ltd, Crawley) given 11 days apart; when they exhibited oestrus (determined by a fertile buck), they were randomly allocated to the groups described below.

Experiment 1

In group A, four goats were injected daily with oxytocin s.c. at a dose of 100 µg between day 3 and day 6 of the oestrous cycle (oestrus = day 0). In group B, four goats were treated like those in group A but were given saline instead of oxytocin [0.9% (w/v) NaCl]. In group C, four goats were treated like those in group A, but in addition recombinant ovine interferon tau (rIFN-\(\tau\)) (a gift from Professor F. W. Bazer, Texas) was infused i.w. at a dose of 40 µg twice per day, divided equally between the two horns. The protein was mixed with 3 mg BSA and saline; it was about 90% pure and had \(0.45 \times 10^8\) antiviral units mg \(^{-1}\) (Ott et al., 1991). Animals were anaesthetized i.v. with sodium thiopentone and maintained with thiopentone and oxygen (Homeida and Khalafalla, 1990). At surgery (Fincher et al., 1986), a sterile polyvinyl catheter was inserted 30 mm into the anterior lumen of each uterine horn. Catheters were secured to the flank with suture.

Experiment 2

In group D, the uteri of four goats were infused with 3 mg BSA in saline between day 12 and day 18 of the oestrous cycle. In groups E, F and G (n = 4 goats in each group), the animals were treated like those in group D but were infused twice a day with 40, 80 and 160 µg rIFN-\(\tau\), respectively.

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Table 1. Mean (±sd) plasma concentration of progesterone and testosterone in goats (n = 4 per group) treated with oxytocin (group A), saline (group B) and oxytocin and recombinant ovine interferon tau (group C) on days 3–7 of the oestrous cycle

<table>
<thead>
<tr>
<th>Day of oestrous cycle</th>
<th>Group A Progesterone (ng ml⁻¹)</th>
<th>Group A Testosterone (µg ml⁻¹)</th>
<th>Group B Progesterone (ng ml⁻¹)</th>
<th>Group B Testosterone (µg ml⁻¹)</th>
<th>Group C Progesterone (ng ml⁻¹)</th>
<th>Group C Testosterone (µg ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0.7 ± 0.2</td>
<td>55 ± 10</td>
<td>0.8 ± 0.2</td>
<td>76 ± 7</td>
<td>0.6 ± 0.2</td>
<td>66 ± 8</td>
</tr>
<tr>
<td>4</td>
<td>1.3 ± 0.2</td>
<td>60 ± 10</td>
<td>1.4 ± 0.2</td>
<td>60 ± 8</td>
<td>0.9 ± 0.2</td>
<td>62 ± 6</td>
</tr>
<tr>
<td>5</td>
<td>0.8 ± 0.2*</td>
<td>360 ± 40*</td>
<td>2.0 ± 0.2</td>
<td>65 ± 10</td>
<td>1.8 ± 0.2</td>
<td>57 ± 8</td>
</tr>
<tr>
<td>6</td>
<td>0.4 ± 0.1*</td>
<td>520 ± 60*</td>
<td>2.5 ± 0.3</td>
<td>60 ± 9</td>
<td>2.4 ± 0.3</td>
<td>68 ± 7</td>
</tr>
<tr>
<td>7</td>
<td>0.2 ± 0.1*</td>
<td>630 ± 90*</td>
<td>3.0 ± 0.3</td>
<td>55 ± 8</td>
<td>2.9 ± 0.3</td>
<td>70 ± 8</td>
</tr>
</tbody>
</table>

*Value is significantly different from control (saline-treated) animals (P < 0.001).

All animals were observed for oestrus at least twice a day. Jugular vein blood (5 ml) was collected three times a day by venepuncture using 23-gauge needles. Blood was collected into heparinized tubes, centrifuged at 2000 g for 10 min and plasma was stored at −20°C until analysed.

Radioimmunoassay of hormones

Plasma progesterone (0.1 ml) and testosterone (0.5 ml) were measured by radioimmunoassay, as described by Homeida (1986) and Homeida et al. (1988). Progesterone antibody (provided by H. Dobson, Liverpool) was raised in rabbits against progesterone-11-succinyl-BSA, and used at a final dilution of 1:7000; crossreactios were 100% with progesterone and < 0.1% with corticosterone, desoxyxocorticosterone and ketocorticosterone. The intra-assay and interassay coefficients of variation for progesterone were 4.6% (n = 25) and 11.6% (n = 20), respectively, for a plasma sample of low progesterone concentration (0.8 ng per tube) and 4.2% (n = 25) and 11.6% (n = 20), respectively, for a plasma sample of high concentration (5 ng per tube). The sensitivity of the assay was 48 pg per tube. Extraction efficiency was 85.1 ± 5% (mean ± sd), and the results were corrected for extraction losses.

Testosterone crossreactions obtained were 100% for testosterone, 50% for dehydrotestosterone, 7% for androstanediol and < 0.02% for progesterone and oestradiol. The intra-assay coefficient of variation for testosterone was 11.9% (n = 15) for a plasma sample of low testosterone concentration (60 pg per tube) and 12.9% (n = 15) for a plasma sample of high testosterone concentration (900 pg per tube), and the assay sensitivity was 13 pg per tube. The efficiency of radioactive hormone recovery was 75 ± 2% and values were corrected for extraction losses.

Statistical analyses

Data were expressed as means ± sd. Analysis of variance (ANOVA) for repeated measures using the general linear model (GLM) procedure of the statistical analysis system (SAS, 1985) was used to test the effect of saline, oxytocin or roIFN-τ. Comparison of means in different groups was made by Duncan’s multiple-range test.

Results

Oxytocin administered to goats (group A) between day 3 and day 7 of the oestrous cycle induced luteal regression, indicated by a significant decrease in the progesterone concentration and an increase in the testosterone concentration (P < 0.001), and caused oestrus compared with control saline-treated animals (group B) (Table 1). Co-administration of roIFN-τ to animals in group C completely blocked oxytocin-induced luteolysis and the rise in testosterone secretion (Table 1).

Administration of roIFN-τ to goats at doses of 80 µg (group E), 160 µg (group F) and 320 µg (group G) between day 12 and day 18 of the oestrous cycle significantly (P < 0.01) delayed luteolysis and increased the duration of the cycle in a dose-dependent manner to 23.2, 25.2 and 27.5 days in groups E, F and G, respectively, compared with 20 days for group D; roIFN-τ also significantly (P < 0.001) inhibited the rise in testosterone in animals in groups E, F and G compared with those in group D (Table 2).

Discussion

The use of roIFN-τ (previously called ovine trophoblast protein-1) in this experiment rather than caprine trophoblast protein-1 was based on a number of similarities between them. Both are acidic proteins of low molecular masses, both have identical physical characteristics and are immunologically related (Gnalek et al., 1989).

Intrauterine administration of roIFN-τ into the goat at the time of luteal regression delays luteolysis. Similarly, luteolysis is delayed in sheep and cattle following intrauterine infusion of ovine and bovine trophoblast interferons or conceptus protein (Knickerbocker et al., 1986; Vallet et al., 1988; Thatcher et al., 1989; Garverick et al., 1992). Co-administration of roIFN-τ and oxytocin also blocked oxytocin-induced luteolysis, giving further support to the hypothesis that roIFN-τ can behave as a luteotrophic agent in goats.

In control animals (groups B and D), luteal regression – whether natural or induced – was associated with a significant increase in testosterone secretion (Homeida and Cooke, 1984; Homeida, 1986; Homeida and Khalafalla, 1990), which was blocked by roIFN-τ. The exact mechanism whereby roIFN-τ inhibits testosterone secretion is unknown. Serum testosterone
concentrations in men decrease after injection of IFN-α (Orava et al., 1986). Pretreatment with IFN-α also reduces hCG-stimulated testosterone secretion from cultured porcine Leydig cells (Orava, 1989). Systemic administration of IFN-α to women reduces the concentration of oestradiol and progesterone in the blood (Kaupilla et al., 1982). Systemic effects of intrauterine rolIFN-γ on the ovary can also be expected, since it has been reported that antiviral activity of ovine trophoblast-1 of the conceptus can occur in uterine venous serum, presumably after absorption from the uterine lumen (Schalke-Francis et al., 1991). The decrease in testosterone secretion induced by rolIFN-γ could be a direct effect or it could occur via the inhibition of release of PGF2α, the uterine luteolysis (Horton and Poyser, 1970). Addition of PGF2α to incubations of corpora lutea increases testosterone synthesis (Shemesh et al., 1975). Testosterone and PGF2α are released on days 13 and 14 of the oestrous cycle of goats (Homeida and Cooke, 1982, 1984). Recombinant bovine and ovine interferons have been shown to decrease the release of uterine PGF2α in vitro (Barros et al., 1990; Ott et al., 1992) and in vivo (Plante et al., 1991; Ott et al., 1992). Furthermore, bovine conceptus secretory protein induces prostaglandin inhibitor activity (Cross and Roberts, 1997).

The effect of rolIFN-γ on luteal function and testosterone secretion in the goat mimics that of an oxytocin antagonist (Homeida and Khalaafalla, 1990). Both substances delay luteolysis and block natural and oxytocin-induced testosterone secretion that precedes oestrus. The oxytocin antagonist binds to oxytocin receptors inhibiting phospholipase A2, the arachidonic acid cascade and the production of prostaglandins (McCracken et al., 1984; Bazer et al., 1986; Homeida and Al-Eknah, 1992); rolIFN-γ can also inhibit production of PGF2α (Ott et al., 1992) via inhibition of oxytocin receptor expression (Flint et al., 1992). However, the goats in groups A–G must have had functional oxytocin receptors (Roberts et al., 1976) at the time of interferon administration; the fact that rolIFN-γ still prevented luteolysis suggests that it also has effects that do not involve the oxytocin receptor.

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Table 2. Mean (±SD) plasma concentration of progesterone and testosterone in goats (n = 4 per group) treated with BSA (group D) or recombinant ovine interferon tau at a total dose of 80 μg (group E), 160 μg (group F) and 320 μg (group G) on days 12–18 of the oestrous cycle

<table>
<thead>
<tr>
<th>Day of oestrous cycle</th>
<th>Group D</th>
<th>Group E</th>
<th>Group F</th>
<th>Group G</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Progesterone (ng ml⁻¹)</td>
<td>Testosterone (pg ml⁻¹)</td>
<td>Progesterone (ng ml⁻¹)</td>
<td>Testosterone (pg ml⁻¹)</td>
</tr>
<tr>
<td>12</td>
<td>4.6 ± 0.4</td>
<td>75 ± 12</td>
<td>4.3 ± 0.3</td>
<td>70 ± 12</td>
</tr>
<tr>
<td>13</td>
<td>4.1 ± 0.4</td>
<td>110 ± 20</td>
<td>4.3 ± 0.3</td>
<td>75 ± 10</td>
</tr>
<tr>
<td>14</td>
<td>4.2 ± 0.3</td>
<td>720 ± 20</td>
<td>4.5 ± 0.3</td>
<td>73 ± 15*</td>
</tr>
<tr>
<td>15</td>
<td>3.2 ± 0.4</td>
<td>460 ± 80</td>
<td>4.8 ± 0.4</td>
<td>65 ± 10*</td>
</tr>
<tr>
<td>16</td>
<td>2.2 ± 0.2</td>
<td>160 ± 20</td>
<td>4.2 ± 0.3*</td>
<td>70 ± 9*</td>
</tr>
<tr>
<td>17</td>
<td>1.6 ± 0.2</td>
<td>75 ± 10</td>
<td>4.1 ± 0.4*</td>
<td>60 ± 9</td>
</tr>
<tr>
<td>18</td>
<td>0.6 ± 0.1</td>
<td>65 ± 10</td>
<td>4.2 ± 0.3*</td>
<td>75 ± 10</td>
</tr>
</tbody>
</table>

*Value is significantly different from control (P < 0.001).

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