Prolactin Replacement Fails to Inhibit Reactivation of Gonadotropin Secretion in Rams Treated with Melatonin under Long Days

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ABSTRACT

This study tested the hypothesis that prolactin (PRL) inhibits gonadotropin secretion in rams maintained under long days and that treatment with melatonin (s.c. continuous-release implant; MEL-IMP) reactivates the reproductive axis by suppressing PRL secretion. Adult Soay rams were maintained under long days (16L:8D) and received 1) no further treatment (control; C); 2) MEL-IMP for 16 wk and injections of saline/vehicle for the first 8 wk (M); 3) MEL-IMP for 16 wk and exogenous PRL (s.c. 5 mg ovine PRL x daily) for the first 8 wk (M+P). The treatment with melatonin induced a rapid increase in the blood concentrations of FSH and testosterone, rapid growth of the testes, an increase in the frequency of LH pulses, and a decrease in the LH response to N-methyl-D,L-aspartic acid. The concomitant treatment with exogenous PRL had no effect on these reproductive responses but caused a significant delay in the timing of the sexual skin color and growth of the winter pelage. These results do not support the hypothesis and suggest that PRL at physiological long-day concentrations, while being totally ineffective as an inhibitor of gonadotropin secretion, acts in the peripheral tissues and skin to maintain summer characteristics.

INTRODUCTION

In sheep and other seasonal breeding species that have a mating season in autumn or winter, there is an inverse relationship between prolactin (PRL) and gonadotropin secretion, with high circulating PRL concentrations in summer and during the sexually inactive phase. This temporal association occurs under natural conditions outdoors [1–4], under artificial light cycles designed to drive the seasonal cycles [5, 6], and under free-running conditions of constant long days [7], and it supports the generalized hypothesis that PRL is inhibitory to gonadotropin secretion [8]. The demonstrations that PRL receptor mRNA/protein is expressed in the sheep retrochiasmatic and periventricular hypothalamic areas [9]—regions that are known to control GnRH secretion [10]—and is expressed selectively by the gonadotrophs in the anterior pituitary gland [11] indicate that PRL may act within both the hypothalamus and pituitary gland to inhibit gonadotropin secretion.

The possibility that photoperiod dictates the timing of the seasonal reproductive cycle, at least in part, through the regulation of PRL secretion has been investigated in many studies using the long-acting dopamine (DA) receptors agonist, bromocriptine, to chronically suppress PRL release. In some species, treatment with bromocriptine during the spring and summer induces the premature reactivation of gonadotropin secretion and gonadal activity (white-tailed deer [12]; arctic fox [13]) or delays the onset of seasonal infertility (red deer [14]), which is consistent with an inhibitory role of PRL. In other species, however, including sheep, bromocriptine is ineffective at altering the seasonal reproductive cycle [15–17] and may actually inhibit LH and/or FSH secretion [1, 3, 4, 15]. These observations, combined with other data, have been used to refute the importance of PRL in the regulation of seasonal breeding [16, 18].

One major limitation in the use of bromocriptine is that the DA agonist, besides blocking PRL secretion, also acts directly in the hypothalamus to suppress GnRH release. In sheep, DA neural pathways make direct synaptic contact with GnRH nerve terminals in the median eminence [19], and the administration of DA receptor antagonists (specific for the D-2 subtype) induces acute pulsatile release of LH, indicative of the inhibitory role of DA in the control of gonadotropin secretion [20, 21]. In addition, the DA system in the mediobasal hypothalamus appears to be activated under long days to mediate suppressive effects of photoperiod on the reproductive axis [22–25]. Since chronic treatment with bromocriptine is likely to mimic the effect of this central inhibitory system, this will negate any positive response that may occur due to the withdrawal of PRL. This renders the systemic approach of little value in assessing the role of PRL, unless a minimal dose of bromocriptine can be used to block PRL secretion without affecting the central control of GnRH secretion. This approach has been used in the study of lactational regulation of gonadotropin secretion (e.g., rhesus monkey [26]), but not in relation to the control of seasonality.

The aim of the current study was to use a PRL replacement strategy to definitively test the hypothesis that PRL is inhibitory to gonadotropin secretion in sheep. This involved treating sexually inactive rams housed under long days with a continuous-release implant of melatonin to activate the full spectrum of short-day responses (a decrease in PRL and increase in gonadotropin secretion), and simultaneously administering exogenous PRL to assess its specific functions. Previous studies have shown that the introduction of an s.c. implant of melatonin to rams under long days induces a very rapid decrease in the circulating concentrations of PRL (latency of 1–3 days) and a more delayed increase in the concentrations of FSH and LH (latency of 2–3 wk [27–29]). This sequence is consistent with the hypothesis that PRL is inhibitory to gonadotropin secretion under long days and that melatonin activates gonadotropin secretion by initially inhibiting PRL secretion (removal of inhibition). The prediction in the current study was that if PRL is inhibitory to gonadotropin secretion, exogenous PRL would block or attenuate the gonadotropin response induced by the melatonin signal. In addition to the reproductive parameters, changes in wool and horn growth were measured, since these features provide a bioassay for the action of PRL [30–32]. Preliminary results of this study have been reported in an abstract form [33].
MATERIALS AND METHODS

Animals

Adult rams (2 yr old) of the Soay breed of feral sheep, which show pronounced photoperiodically regulated cycles in testicular activity, coat growth, and other seasonal characteristics [2, 34], were used in this study. The animals (mean body weight 34.9 kg at start of experiment) were housed permanently in light-controlled rooms and exposed to alternating 16-wk periods of long days (16L:8D) and short days (8L:16D) to entrain the long-term endocrine cycles. Light intensity was approximately 160 lux at eye level of the animals. The time of lights-on was constant (0800 h), and adjustments in photoperiod were achieved by abruptly changing the time of lights-out by 8 h. The animals received a maintenance diet of dried grass nuts, with hay and water ad libitum.

Experimental Manipulations

The experiment was initiated at 10 wk into long days (16L:8D), when the rams were sexually inactive with high blood concentrations of PRL; the protocol is summarized in Figure 1. Fifteen animals were assigned to three equal groups balanced by body weight (n = 5 rams/group). These were treated as follows: 1) group C (control) received no hormonal manipulations; 2) group M received an s.c. silicone elastomer implant containing melatonin that was left in place for 16 wk, and were treated with saline/vehicle (3× daily s.c. injections) for the first 8 wk (see below); 3) group M+P received an s.c. silicone elastomer implant of melatonin that was left in place for 16 wk, and were treated with ovine PRL (3× daily s.c. injections 5 mg ovine PRL) for the first 8 wk. All groups were maintained under long days throughout the treatments and for a further 10 wk after removal of the melatonin implants to monitor the recovery after treatments (total experiment: 26 wk, Fig. 1).

The melatonin implants consisted of a sealed envelope (surface area 32 cm²) of 0.127-mm Silastic sheeting (Bibby Sterlin, Aberbargoed, Mid Glamorgan, UK) containing 850 mg melatonin (Genzyme, Haverhill, Suffolk, UK). The implants were incubated overnight in a large volume of 0.9% saline and washed before implantation to prevent an initial high release of melatonin. Such implants have previously been shown to maintain the circulating concentrations of melatonin 2–3 times higher than the normal nocturnal concentrations observed in untreated rams [28] and to induce a long-term sequence of testicular changes similar to that induced by exposure to short days [27]. Each animal received a local anesthetic, and a single implant was placed beneath the skin over the rib cage. All implants were removed after 16 wk using a local anesthetic.

The ovine PRL (4.5 g, LER-1790) was purified from sheep pituitary glands by Professor L.E. Reichert (Department of Biochemistry and Molecular Biology, Albany Medical College, Albany, NY). The potency, determined by the pigeon crop sac assay, was 26.4 U/mg (95% confidence limits = 17.9–38.9). Contamination with FSH was < 0.004 NIH-FSH-S1 U/mg (FSH-augmented ovarian weight-gain method), with LH was < 0.004 NIH-LH-S1 U/mg (ovarian ascobic acid-depletion assay), with thyroid-stimulating hormone (TSH) was < 0.004 NIH-TSH-S1 U/mg, and with growth hormone was < 0.010 IU/mg (hypoxy rat weight-gain assay; L.E. Reichert, personal communication). Aliquots of freeze-dried PRL (75 mg) were dissolved daily in 17 ml 0.9% saline + 0.2 ml 0.2 M NaOH, and once in solution, the pH was adjusted to near neutrality by the careful addition of 0.2 M HCl. Each ram in group M received 1.0 ml solution (5 mg PRL) s.c. on the side of the neck three times daily (0800, 1600, and 2300 h); the site of injection was varied to prevent local inflammation. This dose was selected on the basis of a preliminary trial that demonstrated that 5 mg PRL (3× daily) maintained the circulating concentrations of PRL continuously within or above the physiological range for rams housed under long days (> 100 ng/ml plasma NIH-PRL-S13; [28]). In the experiment, the dose was progressively increased during the first 12 days of treatment (from 2 mg to 5 mg 3× daily) to compensate for the progressive fall in endogenous PRL secretion induced by the melatonin implant based on the previously observed latency of this response [27]; thus the animals in the M+P group were never hypoprolactinemic during the first 8 wk of the experiment (phase 1, see below). Rams in group M received injections of the vehicle to provide a melatonin/vehicle positive control. The animals tolerated the 3× daily treatments with no deleterious side effects.

Routine Measurements

To record the long-term endocrine and morphological changes during the 26-wk experiment, blood samples were collected from the jugular vein by venipuncture twice weekly from each ram during the light phase (between 1000 and 1200 h). The samples were heparinized, and the plasma was separated by centrifugation within 30 min and stored at −20°C until the concentrations of PRL, FSH, and testosterone were measured by RIA. Every 2 wk the diameter of the testes was measured, and the intensity of the sexual skin coloration in the inguinal region was visually scored as an index of androgen secretion [35]. The pattern of growth (short: < 0.6 cm; medium: 0.7–1.4; long: > 1.5 cm) and molting of the wool on the scrotum (% molt/ease of plucking), and the growth of the horns [30] were also recorded to correlate with the changes in PRL secretion. The acute changes in pulsatile LH secretion were mea-
As measured in all animals on one occasion at 8 wk into the experiment (end of PRL treatment). This involved the collection of 2- to 3-ml blood samples every 10 min on 3 consecutive days. On Day 1 (Day 56 of study), samples were collected for 16 h to measure the spontaneous pattern of hormone secretion (samples 1–97, PRL injections given after samples 4 and 52 in group M+P). On Day 2, samples were collected for 4 h after the i.v. injection of N-methyl-D,L-aspartic acid (NMDA; Sigma-Aldrich, Poole, Dorset, UK) (samples 98–122, 2 mg/kg given after sample 101) to stimulate GnRH/LH secretion; this provided an index of hypothalamic responsiveness. On Day 3, sampling was continued for 4 h after the i.v. injection of GnRH (samples 123–149, 250 ng/ram given after samples 126 and 138) to stimulate LH secretion; this provided an index of pituitary responsiveness. NMDA was dissolved in 0.9% saline (0.2 mg/ml) and administered i.v. slowly over a period of 1 min; the dose was selected to be close to the minimum effective dose shown to elicit LH secretion in sexually inactive, not sexually active, Soay rams [36]. GnRH (Cambridge Research Biochemicals, Northwich, Cheshire, UK) was dissolved in 0.9% saline; the dose was selected to induce an acute increase in LH of amplitude similar to that of a spontaneous LH pulse [37]. To facilitate the repeated collection of blood samples, a polyethylene cannula was inserted into the jugular vein on the day before the start of the intensive study, and samples were collected via a 3-way tap using heparinized saline to maintain patency of the indwelling cannula. The heparinized blood samples were placed onto water ice, and the plasma was separated within 30 min and stored at −20°C until LH concentrations were measured by RIA.

### RIAs

The concentrations of PRL and FSH in the weekly plasma samples and the concentrations of LH in the frequent serial samples were measured using routine RIAs validated for sheep plasma for PRL [38], FSH, and LH [39]. The PRL assay had a lower limit of detection (10% decrease in binding relative to Bo) of 0.5 ng NIH-PRL-S13/ml plasma, and intra- and interassay coefficients of variation (CV) of 7.0% and 10.0%, respectively, based on low-, medium-, and high-quality control samples measured in 12 assays. The corresponding values for the FSH assay were 0.2 ng/ml NIH-DK-FSH-RP2, 9.3% and 10.2%, respectively, and for the LH assay were 0.2 ng/ml NIH-LH-S18, 8.0% and 12.0%, respectively. All samples from a single animal were measured within the same assay. The concentrations of testosterone in the weekly plasma samples were measured by RIA with solvent extraction and the use of iodinated tracer [40]. The sensitivity of the assay was 0.1 ng/ml, and the mean intra- and interassay CVs were <12%.

### Statistical Analysis

ANOVA was used for between-group comparisons of the plasma hormone concentrations based on the routine blood samples for all animals for two different phases of the experiment, i.e., phase 1: 0–8 wk (group C: no treatment; group M: treated with melatonin; group M+P: treated with melatonin and PRL), and phase 2: 9–16 wk (group C: no treatment; group M: treated with melatonin; group M+P: treated with melatonin but no PRL). The times of maximum testis diameter, maximum plasma FSH concentrations, and maximum testosterone concentrations, and times to minimum rate of horn growth were assessed for each animal using a 3-point moving average for each parameter, and the values were expressed in weeks relative to the start of the experiment as a group mean ± SEM. These values were also compared between groups by ANOVA. For analysis of the LH profiles based on frequent blood sampling, significant LH pulses were identified using the criteria of two consecutive high values, at least one of which exceeded the mean of the preceding two values by twice the intraassay CV [41]. The LH response to the injection of NMDA and GnRH was calculated as the mean concentration of LH from 0 to 60 min after the treatment minus the mean concentration of LH in the pretreatment sample, based on 10-min samples. In all cases, the differences between groups in mean concentrations and LH pulse amplitude were compared by ANOVA. The effects of treatments on LH pulse frequency were examined by the Kruskal-Wallis test.

### RESULTS

#### PRL Concentrations in Peripheral Blood

The changes in the daytime (1000–1200-h) blood plasma concentrations of PRL in the three treatment groups throughout the 26-wk experiment are illustrated in Figure 2. At the start, PRL concentrations were high at 80–100 ng/ml in all groups, as expected for rams exposed to long days. In group C, the concentrations gradually decreased throughout the experiment because of the development of long-day refractoriness for the PRL axis. Treatment with melatonin in group M induced a rapid and sustained decrease in the concentrations of PRL throughout 16 wk, while the administration of exogenous PRL in group M+P maintained PRL concentrations throughout the first 8 wk above pretreatment values. The statistical analysis (Table 1) revealed that during phase 1 (1–8 wk), PRL concentrations were significantly reduced in group M (suppressive effect of the melatonin implant) and increased in group M+P (successful replacement therapy) compared with group C. During phase 2 (Wk 9–16), PRL concentrations were similar in groups M and M+P and were significantly (p < 0.01) reduced compared with group C (suppressive effect

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Experimental phase</th>
<th>C</th>
<th>M</th>
<th>M + P</th>
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<tbody>
<tr>
<td>PRL (ng/ml)</td>
<td>1</td>
<td>7.16 ± 6.30</td>
<td>14.38 ± 3.41</td>
<td>194.96 ± 26.08</td>
</tr>
<tr>
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<td>2</td>
<td>41.80 ± 4.56</td>
<td>6.37 ± 0.77</td>
<td>10.58 ± 1.41</td>
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<tr>
<td>FSH (ng/ml)</td>
<td>1</td>
<td>0.39 ± 0.13</td>
<td>2.26 ± 0.22</td>
<td>2.57 ± 0.43</td>
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<tr>
<td></td>
<td>2</td>
<td>2.29 ± 0.31</td>
<td>3.10 ± 0.36</td>
<td>4.10 ± 0.35</td>
</tr>
<tr>
<td>Testosterone (ng/ml)</td>
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<td>2.23 ± 0.33</td>
<td>2.68 ± 0.09</td>
<td>2.01 ± 0.26</td>
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<tr>
<td></td>
<td>2</td>
<td>2.94 ± 0.18</td>
<td>4.69 ± 0.51</td>
<td>5.31 ± 0.59</td>
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</table>

*a, * Corresponding values denoted by a different superscript denote values that are significantly different (p < 0.01).
of the melatonin implant and no posttreatment effect of PRL).

**FSH Secretion and Testicular Activity**

The effects of the experimental manipulations on the long-term changes in the blood plasma concentrations of FSH and in testis growth are illustrated in Figure 3, and summarized in Tables 1 and 2. At the start of the experiment, all rams were sexually inactive with low concentrations of FSH and regressed testes. In group C, there was a gradual reactivation of the reproductive axis due to the development of long-day refractoriness. Treatment with melatonin in groups M and M+P induced a significantly earlier increase in the plasma concentrations of FSH and testosterone, growth of the testes, and redevelopment of the sexual skin coloration compared with those of group C (Table 2). These changes were all reversed after the removal of the melatonin implant (Fig. 3).

The statistical analysis on FSH concentrations confirmed that during phase 1, FSH values were significantly higher (p < 0.01) in groups M and M+P than in group C (stimulatory effect of the melatonin implant and no effect of treatment with PRL). During phase 2, FSH concentrations were similar in groups M and M+P and were increased (p < 0.01) compared with those of group C (stimulatory effect of the melatonin implant).

There was no significant difference in mean FSH concentrations, or in the timing of the melatonin-induced testicular cycle, between groups M and M+P (Tables 1 and 2). There were differences, however, in the mean diameter of the testes (reduced in the M+P group compared with group M in phase 2, p < 0.05) and in the timing of the onset of the sexual skin coloration (significantly delayed in the M+P group compared with group M; p < 0.001, Table 2).

**Pulsatile LH Secretion**

The patterns of pulsatile LH secretion, and the short-term fluctuations in the plasma concentrations of PRL at

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**TABLE 2. Summary of the timing of the reproductive responses in groups of Soay rams housed under long days (mean ± SEM; n = 5).**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Time interval to maximum value* (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
</tr>
<tr>
<td>Plasma FSH concentrations</td>
<td>18.50 ± 1.17</td>
</tr>
<tr>
<td>Plasma testosterone concentrations</td>
<td>21.10 ± 1.27</td>
</tr>
<tr>
<td>Testis diameter</td>
<td>21.60 ± 1.17</td>
</tr>
<tr>
<td>Sexual skin coloration</td>
<td>17.00 ± 0.45</td>
</tr>
<tr>
<td>Horn growth rate</td>
<td>22.80 ± 0.49</td>
</tr>
</tbody>
</table>

*Except horn growth rate, which is interval to minimum value.

Values denoted by a different superscript are significantly different (p < 0.01).
FIG. 4. Short-term fluctuations in the blood plasma concentrations of LH (open circles) and PRL (solid circles) in two representative animals each for the three groups of Soay rams. The animals were studied after 8 wk of treatment, and blood samples were collected every 10 min for 16 h. The timing of the s.c. injections of 5 mg PRL are shown for group M+P. The significant LH pulses are indicated by arrowheads.

Wk 8 of the experiment are illustrated in Figure 4 and summarized in Figures 5 and 6. At this stage (end of phase 1), there was a significant effect of treatments on mean LH concentrations, LH pulse frequency, and LH response to NMDA, but not on LH pulse amplitude (marginal effect $p = 0.052$) or the LH response to GnRH (Figs. 5 and 6). Treatment with melatonin in groups M and M+P increased mean LH concentrations and LH pulse frequency compared with those in group C, while the LH response to NMDA was decreased (modulatory effect of the melatonin implant). In group M+P, mean LH concentrations were significantly ($p < 0.05$) higher compared with those in group M (Fig. 5).

Wool and Horn Growth

The effects of the experimental manipulations on the long-term changes on growth and molting of the pelage, and on growth of the horns are illustrated in Figure 7. At the start of the study, the animals were developing the sparse summer coat on the scrotum, having recently molted the old pelage in response to long days. In group C, the summer-type pelage was maintained through most of the experiment. In group M, the treatment with melatonin induced a rapid development of denser, long winter-type pelage. This was later molted 6–10 wk after the removal of the melatonin implants (Fig. 7). In group M+P, the summer-type pelage was retained throughout the period of treatment with PRL, and the development of the winter-type pelage was significantly delayed compared with that in group M (Table 2). At 8 wk into the experiment, at the end of the PRL-treatment phase, the weight of pelage harvested from a standardized area on the scrotum was 27.0 ± 3.0, 78.5 ± 3.8, and 37.4 ± 3.6 mg for groups C, M, and M+P, respectively (M vs. C and M+P, $p < 0.001$). The timing of the molt induced by withdrawal of melatonin occurred at the same time in both melatonin-treated groups.

In group C, horn growth declined progressively during the study to a minimum at about 22 wk. In groups M and M+P, treatment with melatonin induced a significantly more rapid decline in horn growth (Table 2), and this was reversed after the removal of the melatonin implants. There was no significant difference in the pattern of horn growth between the M and M+P groups.

DISCUSSION

This study used a hormone-replacement strategy to test the hypothesis that in sheep PRL is inhibitory to gonadotropin secretion under long days and that a short-day melatonin signal activates the reproductive axis by suppressing PRL secretion (removal of inhibition). The prediction was that administration of exogenous PRL to rams treated with melatonin to maintain high circulating concentrations of
PRL would block or delay the associated reactivation in gonadotropin secretion. The results, however, revealed no such effect. The introduction of a continuous-release implant of melatonin induced the expected activation of FSH secretion and an increase in the frequency of LH pulses, but the simultaneous administration of exogenous PRL failed to attenuate these responses. Indeed, the LH mean concentrations observed after 8 wk were marginally increased in the group treated with PRL compared with the corresponding positive control group.

The PRL used in this study was a highly purified preparation derived from ovine pituitary glands, and the treatment maintained circulating concentrations continuously in the high physiological range for 8 wk. This was despite the full suppression of endogenous PRL as evidenced in the group treated with melatonin alone. Since the pulsatile pattern of LH secretion was unaffected by exogenous PRL, and this closely reflects the pulsatile pattern of GnRH secretion from the hypothalamus [42, 43], the results clearly indicate that PRL per se is not suppressive to GnRH secretion. In addition, the LH response to NMDA was reduced in the same manner in both melatonin-treated groups, as occurs in sexually active rams [36], confirming that the exogenous PRL had not modified the central mechanisms regulating GnRH secretion. These results are also consistent with previous findings that the acute intracerebral ventricular (icv) injection of ovine PRL does not affect pulsatile LH secretion in ovariectomized, estradiol-implanted ewes [44], and the chronic icv infusion of ovine PRL in sexually active Soay rams is also ineffective at suppressing gonadotropin secretion [33].

This negative result appears to contrast the situation in rodents, in which there is clear evidence for a suppressive role of PRL on GnRH/gonadotropin secretion. For example, experimentally induced hyperprolactinemia in rats has been shown to be associated with reduced concentrations of GnRH in the portal blood [45], reduced gonadotropin secretion [46, 47], and an attenuated postcastration rise in LH release [48]. Treatments with PRL have also been shown to suppress GnRH secretion from hypothalamic explants [49] and from GT1 neuronal cell lines [50]. Since PRL receptors are expressed in several areas of the rat hy-
Values for horn growth are the biweekly mean intensity of color (reddening due to local hyperemia) treated rams receiving constant testosterone replacement; the reappearance of the sexual skin coloration in rams treated with PRL may also be due to a direct effect of PRL on the androgen-responsive vasculature in the skin.

PRL receptors are expressed in the dermal papillae of the hair follicles and the sebaceous glands in sheep [32], and it is well established in other species that seasonal changes in PRL secretion regulate the timing of the molt in spring and autumn, and the type of pelage developed in summer and winter [31, 61–64]. In the current study, the patterns of pelage and horn growth were measured to provide a biological index of PRL concentrations. In rams treated with exogenous PRL, the pelage retained the summer characteristics throughout the period of treatment, consistent with an action of PRL in the hair follicles. Furthermore, the development of the long, coarse winter coat occurred when circulating PRL concentrations were suppressed because of the presence of a melatonin implant, and a conspicuous molt followed the resurgence of endogenous PRL secretion after removal of the implant, as expected if PRL directly dictates these responses. The pattern of horn growth was suppressed by the introduction of melatonin, but this was not affected by the concomitant treatment with exogenous PRL. This is contrary to the observation that PRL stimulates the horn growth in spring [30, 31]. In the current study, however, the introduction of the melatonin implant reactivated the testicular axis along with an increase in testosterone secretion, and testosterone is known to inhibit horn growth before the mating season [65, 66]. Thus, any trophic effect of PRL on the tissues producing the horn may be blocked by the action of the androgen.

In conclusion, to our knowledge this is the first study, in any species, that has employed a long-term hormone replacement strategy using physiological concentrations to investigate the hypothesis that PRL is an inhibitor of gonadotropin secretion. Our results do not support the hypothesis and lead to the conclusion that the PRL and gonadotropin systems are independently regulated by photoperiod in the sheep. This is consistent with the most recent evidence that melatonin acts primarily in the pituitary gland to mediate the effects of photoperiod on PRL secretion [67] but acts primarily in the mediobasal hypothalamus to mediate the effects of photoperiod on gonadotropin secretion [68–71]. While PRL appears to be totally ineffective as an inhibitor of GnRH-induced gonadotropin secretion in the ram, the hormone has important actions in the testis, skin, and other peripheral tissues to induce the characteristic physiology of summer.

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