Effect of pinealectomy on the plasma concentrations of prolactin, cortisol and testosterone in sheep in short and skeleton long photoperiods

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ABSTRACT

Two experiments were carried out to investigate the effects of pinealectomy on the responses of prolactin, cortisol and testosterone to skeleton long photoperiods (7 h light: 10 h darkness: 1 h light: 6 h darkness; 7L: 10D: 1L: 6D) compared with short photoperiods (8L: 16D) in lambs. The first experiment included 23 female Suffolk cross sheep aged 10 months, of which six were pinealectomized. The skeleton long photoperiod significantly increased plasma levels of prolactin but this was blocked by pinealectomy; there was a peak around dusk and a trough around dawn and at the time of the 1-h period of light. There was no effect of either photoperiod or pinealectomy on plasma levels of cortisol. Testosterone was not measured in this experiment.

In the second experiment there were 12 intact males and 11 castrated males aged 3 months; six of the lambs in each group were pinealectomized. Prolactin was again greatly stimulated by skeleton long photoperiods and the effect was blocked by pinealectomy; there was a trough in plasma prolactin at dawn in all groups. In addition, castration increased prolactin levels on two of the four sampling days. Plasma cortisol concentrations were significantly lower under skeleton long photoperiods and this was also blocked by pinealectomy; there was no effect of castration. Testosterone was much higher in intact males. After 10 weeks of exposure, skeleton long photoperiods produced significantly lower concentrations than short photoperiods in the intact ram with pineal glands but not in those which were pinealectomized.


INTRODUCTION

The plasma concentrations of a number of hormones, including prolactin, cortisol and testosterone, have been shown to be dependent on the length of the daily photoperiod, with higher concentrations at certain times of the year or with different photoperiodic lengths. The plasma levels of prolactin in sheep are found to be higher in summer than in winter (Ravault, 1976; Kay, 1979) and in photoperiods of 16 h light per day compared with 8 h light per day (Forbes, Driver, El Shahat et al. 1975; Brown & Forbes, 1980; Lincoln & Short, 1980; Lincoln, Almeida, Klandorf & Cunningham, 1982). It has also been shown that prolactin is affected similarly by photoperiods of 16 h light and skeleton long photoperiods (7 h light: 9 h darkness: 1 h light: 7 h darkness; 7L: 9D: 1L: 7D), Ravault & Ortvant, 1977; 7L: 10D: 1L: 6D, B. R. Brinklow & J. M. Forbes, unpublished observations).

Photoperiodic effects on cortisol have not been detected by several authors (Peters, Chapin, Emery & Tucker, 1980; Kennaway, Obst, Dunstan & Friesen, 1981; Lincoln et al. 1982) though one report with prepubertal bulls (Leining, Tucker & Kesner, 1980) has shown a reduction in plasma cortisol levels in long photoperiods when compared with short photoperiods. Young lambs under skeleton long photoperiods show a similar change (B. R. Brinklow & J. M. Forbes, unpublished observations).

Testosterone fluctuates with photoperiod in rams in concert with the seasonal breeding cycle, showing peak levels in autumn and a nadir in spring. This phenomenon can be controlled by artificial photoperiods of 16L (inhibitory) or 8L (stimulatory) (Lincoln & Short, 1980).

The pineal gland is known to be important in the control of seasonal breeding and other photoperiodic mechanisms in several groups of mammals including...
various rodents (Reiter, 1980) and sheep (Lincoln & Short, 1980). It has been shown that the pattern of secretion of the pineal hormone melatonin is dependent on the length of the photoperiod in sheep and different patterns of secretion have been shown in short and long photoperiods (Lincoln et al. 1982) and in short and skeleton long photoperiods (B. R. Brinklow, J. M. Forbes & R. G. Rodway, unpublished observations). The present study was designed to examine the role of the pineal gland in the photoperiodic mechanisms controlling the secretion of prolactin, cortisol and testosterone in female, male and castrated male sheep.

MATERIALS AND METHODS

Animals and management

Two experiments were carried out in a building with two identical rooms with controlled lighting giving a light intensity of 80–100 lx at eye level in the sheep. In the first, 23 female Suffolk × (East Freisland × Blackface) lambs were used, 11 under short days and 12 under skeleton long days. They were born in March and the experiment started on 12 February of the following year after 3 weeks under 12L:12D. In mid-January 11 ewes were operated to remove the pineal gland but subsequent dissection and plasma melatonin assay showed that only six (three under each photoperiod) were completely pinealectomized. The animals were penned individually and given a complete pelleted feed at 70 g/kg live weight to the power 0.75 (metabolic live weight)/day.

Blood samples were taken by jugular puncture at approximately monthly intervals and in mid-March the animals were sampled intensively for 24 h through temporary jugular catheters.

For the second experiment 11 male Suffolk × Mule lambs were castrated at birth in March and 12 remained intact. They were used in a factorial experiment with two surgical treatments, two ‘sexes’ and two photoperiods with six of each group subjected to pinealectomy between 19 and 30 May, which in this experiment proved to be complete in every case, as judged by examination at autopsy and absence of melatonin from plasma. There were only two castrated sheep with intact pineal glands under the skeleton long photoperiod. The experiment started on 16 June; blood samples were again taken by puncture of the jugular vein at approximately 2 weekly intervals and, in late July, jugular catheters were fitted for intensive sampling for a 24-h period.

Pinealectomy

Pinealectomy was carried out under halothane general anaesthesia according to the method of Roche & Dziuk (1969), as used by Brown & Forbes (1980). Problems of swelling of the brain during surgery, which made pinealectomy difficult in experiment 1, were prevented in experiment 2 by the slow intravenous infusion of 15 ml/kg 20% sterile mannitol solution (Travenol, Thetford, Suffolk) between the induction of anaesthesia and the start of the operation, to increase the osmotic pressure of plasma.

Hormone assays

All plasma samples were assayed for prolactin using a double-antibody equilibrium method with an antiovine-prolactin antiserum raised in rabbits (R441BS) and kindly donated by Dr J. M. Chesworth, School of Agriculture, University of Aberdeen. Full details of the assay methodology, which followed the procedure of Follett, Scanes & Cunningham (1972), are given by Brinklow (1983). There was no cross-reaction with ovine luteinizing hormone (LH), follicle-stimulating hormone or thyroid-stimulating hormone at concentrations of 10, 10 and 8 mg/l respectively, while growth hormone cross-reacted only at concentrations over 1 mg/l, a level never encountered under physiological conditions. The interassay variance for ten assays for plasma containing 57 µg prolactin/l was 4.6%, while intra-assay variance was 5.6%. The mean lower detection limit was 7 µg/l.

The assay for cortisol was based on the competitive protein-binding method of Murphy (1967) using corticosteroid-binding protein from pooled human plasma, as described briefly by Thomas & Rodway (1983). The mean recovery of cortisol from the extraction procedure was 98.1 ± 6.1% (s.e.m., n = 9). Corticosterone cross-reacted strongly (90%) but levels in sheep plasma are usually less than 3 nmol/l (Ferguson & Cox, 1975); progesterone cross-reacted to a lesser extent (25%) but levels in males and prepubertal sheep are less than 2 nmol/l (Ferguson & Cox, 1975). Testosterone showed 6–5% cross-reactivity. The interassay variance for six assays for a plasma sample containing 42 µg cortisol/l was 15.3%, while intra-assay variance was 8.7%; the detection limit was 10 µg/l.

Testosterone was measured by radioimmunoassay, after ether extraction and charcoal separation, using antiserum MTB to a 3-carboxymethyl oxime conjugate as used by the Supraregional Assay Centre for Steroid Hormones, Leeds.

Statistical analysis

All hormone levels were subjected to logarithmic transformation before parametric analysis to equalize the variance between low and high values. Analysis of variance was used (Nie, Hull, Jenkins et al. 1975) for comparisons between photoperiods, sexes and surgical treatments.
To detect consistent fluctuations within 24-h profiles a non-parametric sign test was employed (Snedecor & Cochran, 1980), as used for comparable data by Lincoln et al. (1982). Individual values were assigned as being above, equal to or below the median value for the hormone during the whole sampling period for that animal. The times at which all (or all except one when there were six or more animals under a treatment) of the animals under each treatment had values above or below their median were determined and a series of at least two consecutive high or low values was taken to be a consistent peak or trough respectively.

RESULTS

In experiment 1 there were no differences between sham-operated and control animals and their results were pooled.

Prolactin

Table 1 shows that there was no difference between treatment groups during the pre-experimental period but that during the experiment there was a significant effect of photoperiod on plasma prolactin concentrations on both sampling dates with a significant interaction between the effects of photoperiod and pinealectomy; this was due both to a significant \((P<0.05)\) decrease in prolactin with removal of the pineal gland in the skeleton long photoperiod and to an increase in the short photoperiod. There was no difference between the values for pinealectomized animals under the two photoperiodic treatments. Using pre-experimental prolactin levels as covariate did not affect the significance of these effects.

The profiles in Fig. 1 show a consistent drop in plasma prolactin around the start of the 7-h light period and around the time of the 1-h ‘flash’, and emphasize the blocking effect of removal of the pineal gland on the photoperiodic control of prolactin.

In experiment 2 there was a significant \((P<0.05)\) effect of ‘sex’ with the castrated lambs having higher prolactin levels than the intact animals on 30 June and 28 August but not on the other dates.

Skeleton long photoperiods caused raised prolactin concentrations compared with short photoperiods, this effect being significant on three of the four dates; pinealectomy blocked this effect, the interaction between photoperiod and pinealectomy being significant \((P<0.05)\) on 14 July. On 30 June the levels were lower in pinealectomized sheep compared with controls under both photoperiodic treatments, whereas on 14 July there was a significant photoperiod \(\times\) pinealectomy interaction, as seen in experiment 1. Use of the pre-experimental prolactin values as covariate had little effect on the results. An interesting feature, however, was the higher levels during the 12L:12D pre-experimental period than during the experiment proper in either 8L:16D or 7L:10D:1L:6D; paired

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<th>TABLE 1. Geometric mean plasma prolactin concentrations (µg/l) in control (C) and pinealectomized (PNX) sheep under short (8 h light: 16 h darkness; 8L:16D) and skeleton long (7L:10D:1L:6D) photoperiods. Values are means; numbers of animals are shown in parentheses; standard errors are derived from the residual mean square of analysis of variance</th>
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<td>Prolactin (µg/l)</td>
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<td><strong>Experiment 1</strong></td>
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<td><strong>Experiment 2</strong></td>
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\(*P<0.05; **P<0.01; ***P<0.001:\) effect of photoperiod.
\(†P<0.05; ††P<0.01:\) effect of photoperiod \(\times\) pinealectomy.
\(‡P<0.05:\) effect of pinealectomy.
All comparisons tested by analysis of variance for main effects and two-way interactions.
FIGURE 1. Twenty-four hour profiles of plasma prolactin in female sheep (experiment 1). (a) Control sheep (n=8) and (b) pinealectomized sheep (n=3) kept under a short photoperiod, and (c) control sheep (n=9) and (d) pinealectomized sheep (n=3) maintained under a skeleton long photoperiod are shown. The solid bars indicate the periods of darkness and the vertical lines represent S.E.M. Arrows pointing downward indicate a peak and those pointing upward a trough, as defined in Materials and Methods.
FIGURE 2. Twenty-four hour profiles of plasma prolactin in male sheep (experiment 2). (a) Control sheep \((n = 6)\) and (b) pinealectomized sheep \((n = 6)\) kept under a short photoperiod, and (c) control sheep \((n = 5)\) and (d) pinealectomized sheep \((n = 6)\) maintained under a skeleton long photoperiod are shown. The solid bars indicate the periods of darkness and the vertical lines represent s.e.m. Arrows pointing downward indicate a peak and those pointing upward a trough, as defined in Materials and Methods.
t-tests showed this to be significant ($P<0.05$) in most cases.

The profiles in Fig. 2 again show a decrease in plasma prolactin concentrations at dawn and clearly show the lack of effect of the skeleton long photoperiod on prolactin levels in pinealectomized sheep.

### Cortisol

Cortisol was assayed in all samples taken via jugular catheters from 14 animals of the pinealectomized and unoperated groups of experiment 1. There were no significant effects of either photoperiod or pinealectomy, the mean concentration from the 24-h sampling periods being $25.7\pm1.8$ nmol/l.

In experiment 2 there was no evidence for any effect of castration on plasma cortisol so the data have been amalgamated. Control sheep had higher levels in short days (28·2 nmol/l) than in skeleton long days (21·9 nmol/l; $P<0.05$), with the pinealectomized animals being intermediate (25·1 and 27·9; s.e.m. of a treatment mean $\pm1.1$); the interaction was significant ($P<0.05$) when 'sex' was not included as a main effect in the analysis of variance.

### Testosterone

The highly significant difference between the intact and castrated animals in experiment 2 was expected. In the pre-experimental samples there were higher levels of testosterone in the unoperated animals, especially in those rams which were chosen at random for the skeleton long photoperiod (Table 2).

In the samples taken after 10 weeks of photoperiod treatment plasma concentrations were significantly higher in short days than in skeleton long days; in the uncastrated rams this effect was seen in those animals with intact pineal glands but not in the pinealectomized animals ($P<0.05$ for interaction). When the pre-experimental concentrations were used as co-variate in the analysis of variance the same levels of significance pertained.

The same pattern of effects was shown in samples taken by jugular catheter after approximately 6 weeks of treatment (Table 2) but the testosterone concentrations in the rams were very much lower. There were no effects of treatment in the castrated animals, all concentrations being close to the detection limit of the assay but tending towards increased testosterone in skeleton long photoperiods.

### DISCUSSION

The effects of photoperiod on plasma prolactin in intact animals were large and similar to those previously reported (Ravault & Ortavant, 1977; Schanbacher & Crouse, 1980). Pinealectomy blocked this effect of the nocturnal ‘flash’ as has also been shown for the effects of long days (Barrell & Lapwood, 1979; Brown & Forbes, 1980); pinealectomized animals had prolactin levels intermediate between those of intact animals under the two photoperiods, as seen in a comparison of the effects of short and long photoperiods (Brown & Forbes, 1980). This is in contrast to the observations of Munro, McNatty & Renshaw (1979) in which pinealectomy caused blood concentrations to be increased continuously to summertime levels. Experiment 2 started close to the longest day of the year and prolac-

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<th>Testosterone (nmol/l)</th>
<th>8L : 16D</th>
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<td>C</td>
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<td>INT</td>
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<td>16 June (pre-experimental)</td>
<td>(3) 1·66</td>
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<td>23/28 July</td>
<td>1·14</td>
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<td>28 August</td>
<td>15·9</td>
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* $P<0.05$: effect of photoperiod.
† $P<0.05$: effect of photoperiod × pinealectomy.
‡ $P<0.05$, †† $P<0.01$: effect of 'sex'.
§ $P<0.01$: effect of pinealectomy.
† $P<0.05$: effect of photoperiod × 'sex'.

All comparisons tested by analysis of variance for main effects and two-way interactions.

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tin levels declined subsequently, even in the skeleton long photoperiods. This decline might be due to a general decrease in environmental temperature, as recorded at the local meteorological station, which occurred during the course of this experiment; Wettmann & Tucker (1974) have found that prolactin levels are positively related to environmental temperature in cattle.

The profiles of plasma prolactin shown in the experiments reported here are similar to those obtained by Brown & Forbes (1980) with 8L:16D and 16L:8D treatments, with a peak associated with 'dusk' and a trough around 'dawn'; the peak was less obvious and the trough more so in the present experiment. Lincoln (1979) found that a small peak associated with 'dusk' remained at the same clock time when daylength was extended from 8 to 16 h in sheep which had been subjected to superior cervical ganglioneectomy, which prevents diurnal cyclical activity of the pineal gland. There was no evidence of such a peak in the pinealectomized animals of the present study, however.

Cortisol concentrations were not affected by photoperiod or pinealectomy in experiment 1, although there was a tendency for them to be lower in the skeleton long photoperiod than under the short photoperiod. In a previous experiment in which the conditions were very similar to those used in this experiment, but with younger lambs, this effect of photoperiod was significant (B. R. Brinklow & J. M. Forbes, unpublished results). In experiment 2, however, there was a significant interaction between the effects of photoperiod and pinealectomy, removal of the gland preventing the effect of photoperiod which was seen in the animals with pineal glands and causing a decrease in cortisol in skeleton long photoperiods and possibly stimulating it in short photoperiods. Other studies with sheep have not shown an effect of pinealectomy or superior cervical ganglionectionm on cortisol levels (Barrell & Lapwood, 1979; Kennaway et al. 1981; Lincoln et al. 1982), though in these cases the animals were older than those in experiment 2 and there was no main effect of photoperiod. Leining et al. (1980) working with young cattle did find significantly lower plasma cortisol levels in long days than in short days and it appears that the age of the animal is important in determining whether or not photoperiod affects corticosteroid secretion.

Skeleton long photoperiods had a similar effect to long days on the plasma concentrations of testosterone of young rams; pinealectomy blocked this effect (experiment 2). Conversely, Lincoln (1978) has shown that a skeleton short photoperiod (11L:1D:5L:7D) had the effect of increasing plasma testosterone. It would appear from this evidence that the control of testosterone secretion is by the pattern of lighting rather than the total amount of light, as is also the case with prolactin and cortisol. It is interesting, therefore, to note that the plasma levels of melatonin are high only during the first of the two daily dark phases in lambs kept under the same skeleton long photoperiods (B. R. Brinklow, J. M. Forbes & R. G. Rodway, unpublished results). This, together with the effect of pinealectomy, shows the dependence of the endocrine system on the pineal gland to relay information concerning photoperiod.

Although testosterone concentrations were very low in the castrated lambs, there was still a trend for photoperiod to affect testosterone in the same direction as in the intact males and parallel to the effects of photoperiod on plasma cortisol concentrations. This suggests that the adrenal cortex is sensitive to LH, which is assumed to be the link between the brain and the testis, and that it responds with both testosterone and cortisol secretion.

In both rams and wethers in experiment 2 there were higher levels of testosterone in blood in late August than in late July. This might indicate rising testosterone secretion as the breeding season approaches, although blood was taken through catheters in July and by venipuncture in August so that direct comparison might be invalid.

The control of the annual cycle of reproductive activity in sheep by photoperiod has been discussed in detail by Lincoln & Short (1980). One of our interests in possible effects of the hormonal changes demonstrated in this work was with effects of photoperiod on growth. There is ample evidence that long and skeleton long photoperiods stimulate growth in comparison with short photoperiods in several species (Forbes, 1982). In both experiments there was a negative correlation between rate of weight gain and mean plasma cortisol concentration. In experiment 2 the correlations between weight gain and prolactin and between weight gain and testosterone were both positive and significant, the latter due to the faster growth of uncastrated rams which also had higher testosterone levels (for further details and discussion see Brinklow, Jones & Forbes, 1984).

In conclusion, the work reported in this paper shows that skeleton long photoperiods stimulate prolactin secretion and depress plasma levels of testosterone and cortisol, all of which are blocked by removal of the pineal gland.

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