PHOTOPERIODIC CONTROL OF GONADOTROPHIN SECRETION IN THE RAM: A DETAILED STUDY OF THE TEMPORAL CHANGES IN PLASMA LEVELS OF FOLLICLE-STIMULATING HORMONE, LUTEINIZING HORMONE AND TESTOSTERONE FOLLOWING AN ABRUPT SWITCH FROM LONG TO SHORT DAYS

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SUMMARY

Six adult Soay rams were housed under artificial lighting conditions of long days (16 h light: 8 h darkness) for 4 months and this caused the animals to lapse into a state of reproductive quiescence with low levels of gonadotrophins in the circulation and regressed testes secreting very low amounts of testosterone. The photoperiod was changed abruptly to short days (8 h light: 16 h darkness) to induce a resurgence of sexual activity, and a detailed study was made of the pituitary and testicular responses over the first 100 days.

Plasma levels of LH and FSH first began to increase between days 6 and 12 of short days, and rose progressively until days 33–54 before declining again. Testicular growth of the rams began on days 19–26 and continued for most of the remaining period of study. Plasma testosterone levels rose in parallel with the growth of the testes, and were greatly increased by day 100 when gonadotrophin levels were reduced.

At most stages there were short-term fluctuations in the plasma levels of FSH, LH and testosterone indicative of episodic secretion. Peaks in plasma levels of LH were especially conspicuous and from the changes in frequency and amplitude of these peaks it was possible to predict the way in which photoperiod influenced gonadotrophin secretion by its effect on hypothalamic LH-RH secretion. A slight 24 h rhythm in the plasma levels of all three hormones was observed, and the significance of this in relation to the photoperiodic response is discussed.

INTRODUCTION

Rams of the Soay breed are seasonal in their reproductive physiology and behaviour: gonadotrophin secretion and testicular activity vary dramatically throughout the year (Lincoln & Davidson, 1977) and in the wild the animals show conspicuous rutting behaviour in the autumn (Grubb & Jewell, 1973). The annual cycle of varying daylength provides the principal environmental cue that dictates the timing of the reproductive changes; decreasing daylengths of the autumn stimulate activity, and increasing daylengths of the spring have the reverse effect. The normal physiological changes associated with seasonal breeding in the ram can be induced experimentally using an artificial lighting regimen (Lincoln, 1976a; Lincoln & Davidson, 1977; Lincoln, Peet & Cunningham, 1977).

In the present study the photoperiodic response of the ram was investigated by exposing animals to a sudden change in daylength.
MATERIALS AND METHODS

Animals

Six adult Soay rams were housed in a light-proof building and provided with artificial light from four, white fluorescent strip lights (Crompton, U.K.) providing illumination of 140–180 lux at floor level (Lincoln et al. 1977). The rams received alternating 4 month periods of long days (16 h light:8 h darkness; 16L:8D) and short days (8L:16D). The lighting change was abrupt and consisted of advancing or retarding the time of ‘lights out’ by 8 h; ‘lights on’ occurred at 08.00 h throughout. The rams were preconditioned to this artificial lighting régime for nearly 2 years, and the present study deals with a period of just 18 weeks when the animals experienced a change from long to short days. The temperature in the animal house was not controlled, but followed the normal seasonal pattern for Edinburgh over the period April to July.

Each week the testicular size of the rams was measured and the stage of the sexual cycle monitored by observation of the sexual flush on the exposed ventral skin, and by making a quantitative record of aggressive behaviour (Lincoln & Davidson, 1977). On eight occasions during the study, jugular blood samples were collected at hourly intervals for 24 h from each animal (Fig. 1) using an indwelling cannula inserted on the day before sampling (Lincoln et al. 1977).

Fig. 1. Testicular diameter (mean ± S.E.M.) of six adult Soay rams following a change from long (16 h light:8 h darkness, 16L:8D) to short days (8L:16D). Also shown are the times when 24 h serial blood collections were made (black arrows) and the stages of sexual maturation (see text above).
Radioimmunoassays

Plasma gonadotrophin levels were measured by specific double antibody radioimmunoassays using the method of Scaramuzzi, Caldwell & Moor (1970) for luteinizing hormone (LH) and the method of Lincoln et al. (1977) for follicle-stimulating hormone (FSH). Plasma testosterone levels were determined by a specific radioimmunoassay not involving chromatography (Corker & Davidson, 1977).

RESULTS

Long-term changes in plasma gonadotrophin levels and testicular activity

At the beginning of the study the rams had been living under long days (16L:8D) for 16 weeks and gonadotrophin secretion and testicular activity had declined to a low level. The sudden change to short days (8L:16D) stimulated reproductive development, and the sequence of events at the level of the pituitary gland and testis after the change in lighting is illustrated in Figs 2 and 3, and summarized in Table 1.

Plasma FSH levels began to rise from day 12 after the initiation of short days, and had increased markedly by days 33 and 54; subsequently the levels declined and were low by day 100. Testicular growth in the rams commenced during the period of rapidly rising FSH levels (generally days 19–26), and continued until day 100 when the testes were fully grown (Figs 2 and 3). Plasma LH levels began to rise as early as day 6 of short days, and rose progressively until days 33 or 54 before declining, thus showing a long-term pattern of change similar to that of FSH. Plasma testosterone concentrations increased only slightly during the early part of the study, but began to rise markedly by day 54, when LH levels were high, and were greatly increased by day 100 when LH concentrations had decreased (Figs 2 and 3).

The changes in testosterone were accompanied by the predicted responses of the androgen target organs; the sexual flush first began to develop on the exposed ventral skin of the animals at day 42 of short days (Stage 1, Fig. 1). The rams showed the first signs of increased aggressiveness at day 77 (Stage 2, Fig. 1) and they reached a state of maximum sexual and aggressive activity by day 110 of short days (Stage 3, Fig. 1).

Short-term variations in plasma levels of FSH, LH and testosterone

Within each 24 h sampling period there were short-term variations in the plasma levels of all the hormones measured, apparently related to episodic release. Transitory peaks in the levels of LH and testosterone in the blood were especially noticeable, while fluctuations in FSH were less obvious. Each peak in LH was generally associated with a small rise in plasma FSH, and was invariably followed by a peak in testosterone within 1–3 h; the short-term fluctuations in the plasma level of all three hormones were thus temporally linked (Fig. 4).

The change from long to short days caused an increase in the frequency of episodic LH peaks and there were corresponding changes in the short-term fluctuations of FSH and testosterone. The frequency of LH peaks was as low as one/24 h at the beginning of the study, but increased progressively throughout the period of short days (starting to increase by day 6 and being significantly increased by day 12, \( P < 0.05 \) by Student's \( t \)-test, Table 1). The amplitude of the LH peaks increased in parallel with the increase in frequency until days 33–54 (starting to increase by day 12 and significantly increased by day 19, \( P < 0.05 \) by Student's \( t \)-test, Table 1; see maximum LH value/24 h), and then decreased again by day 100 when the frequency was greatest.

The change in frequency of the plasma testosterone peaks accurately reflected the change in frequency of LH peaks throughout the study (compare Figs 2 and 3). However, the
Table 1. Summary of the changes in plasma levels of FSH, LH and testosterone and testicular diameters (means ± S.E.M.) in six adult Soay rams exposed to a sexually stimulatory photoperiod

<table>
<thead>
<tr>
<th>Number of days of exposure to short days</th>
<th>0</th>
<th>6</th>
<th>12</th>
<th>19</th>
<th>26</th>
<th>33</th>
<th>54</th>
<th>100</th>
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<tbody>
<tr>
<td>Testicular diameter (mm)</td>
<td></td>
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<tr>
<td>Plasma testosterone (daily mean ng/ml)</td>
<td></td>
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<tr>
<td>Plasma FSH (daily mean ng NIH-FSH-S10/ml)</td>
<td>18.5 ± 3.6</td>
<td>20.6 ± 3.2</td>
<td>29.6 ± 3.6*</td>
<td>58.7 ± 6.6</td>
<td>133.8 ± 9.5</td>
<td>204.4 ± 15.2</td>
<td>193.1 ± 35.6</td>
<td>37.4 ± 8.2</td>
</tr>
<tr>
<td>Plasma LH (daily mean ng NIH-LH-S14/ml)</td>
<td>0.59 ± 0.08</td>
<td>0.66 ± 0.09</td>
<td>0.94 ± 0.14*</td>
<td>1.18 ± 0.17</td>
<td>1.43 ± 0.19</td>
<td>1.52 ± 0.18</td>
<td>1.14 ± 0.14</td>
<td>0.67 ± 0.08</td>
</tr>
<tr>
<td>Plasma LH: Frequency of plasma peaks/24 h</td>
<td>1.0 ± 0.3</td>
<td>1.8 ± 0.3*</td>
<td>2.3 ± 0.4</td>
<td>2.7 ± 0.4</td>
<td>3.3 ± 0.4</td>
<td>3.2 ± 0.4</td>
<td>3.7 ± 0.7</td>
<td>approx. 5.9</td>
</tr>
<tr>
<td>Maximum value/24 h (ng/ml)</td>
<td>3.22 ± 1.1</td>
<td>3.57 ± 0.8</td>
<td>4.86 ± 1.0*</td>
<td>6.75 ± 1.0</td>
<td>8.17 ± 1.1</td>
<td>9.55 ± 1.6</td>
<td>4.62 ± 1.4</td>
<td>1.68 ± 0.4</td>
</tr>
<tr>
<td>Minimum value/24 h (ng/ml)</td>
<td>0.33 ± 0.02</td>
<td>0.35 ± 0.02</td>
<td>0.42 ± 0.04*</td>
<td>0.45 ± 0.04</td>
<td>0.53 ± 0.06</td>
<td>0.58 ± 0.06</td>
<td>0.61 ± 0.07</td>
<td>0.42 ± 0.03</td>
</tr>
</tbody>
</table>

* Day when more than half of the rams first showed a significant increase in the parameter compared with day 0.
Fig. 2. Plasma levels of FSH (□) and LH (●) in two adult Soay rams (S3 and S5) sampled hourly for 24 h on eight occasions during the study. The number of days of exposure to short days following the change from long days is shown on the x axis, along with the timing of the light and dark phases for each 24 h period.
Fig. 3. Plasma levels of testosterone (○) in one adult Soay ram (S5) sampled hourly for 24 h on eight occasions following the change in photoperiod to short days. Also shown is the testicular diameter (■) of the ram over the same period. The number of days of exposure to short days following the change from long days is shown on the x axis, along with the timing of the light and dark phases for each 24 h period.
Fig. 4. Temporal relationship between the plasma levels of FSH (□), LH (●) and testosterone (○) based on plasma samples collected from rams on days 26 and 33 of short days. Mean changes in plasma levels (± S.E.M.) were determined by combining values for all animals showing three episodic peaks of plasma LH during each day. The LH peaks were designated A, B or C according to their timing during the day, and the mean changes in LH values relating to these peaks were calculated. Having synchronized the plasma profiles according to the timing of the LH peaks, the corresponding changes in the values for FSH and testosterone were determined. In all cases the changes in plasma values were calculated by reference to the mean plasma value during the 3 h preceding the LH peak and these mean basal values are shown.

The amplitude of the testosterone peaks changed independently of the pattern for LH peaks, particularly at the end of the period of short days when the size of the LH peaks was reduced, and yet the amplitude of the testosterone peaks was greatly increased.

The changes in the short-term fluctuations in plasma FSH were difficult to compare with those of LH due to their smallness and variability; as far as could be judged the frequency
and amplitude of the FSH peaks changed in parallel with the changes observed for LH peaks. Whereas the short-term fluctuations in plasma FSH were small, the long-term changes in basal levels throughout the study were dramatic, in complete contrast to the findings for LH.

**Twenty-four hour cycle in plasma levels of FSH, LH and testosterone**

At all stages throughout the study plasma levels of FSH, LH and testosterone were generally lowest early in the daylight phase of the 24 h cycle, and highest during darkness (Fig. 5). The timing of this daily rhythm remained roughly consistent throughout, while the amplitude of the changes varied; the cycle for FSH and LH was most conspicuous from days 19 to 54 of short days when gonadotrophin secretion was raised.

The daily rhythm in the plasma hormone levels was dictated by the short-term episodic fluctuations. With LH, for example, the peaks occurred least frequently in the early part of the light phase of the 24 h cycle and most frequently later in the day (Fig. 6a). The amplitude of these peaks also changed during the 24 h (Fig. 6b).

The relationship between plasma LH and FSH levels was influenced by the time of day; early in the day the LH peaks were associated with relatively large and sustained rises in FSH, while later in the day the rises in FSH concentration were small and transient (Fig. 4). The relationship between plasma LH peaks and testosterone peaks was unaffected by time of day (Fig. 4).

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**Fig. 5. Summary of the changes in the plasma levels of FSH (□) and LH (●) in six adult Soay rams following a change from long to short days based on samples collected at hourly intervals for 24 h on eight occasions during the study. Four-hourly mean values (± S.E.M.) were calculated and data for some sampling days were combined (i.e. days 6 and 12; days 19 and 26; days 33 and 54). The period of darkness for each 24 h period is shown by stippling.**
DISCUSSION

Inferred changes in LH-RH secretions

Luteinizing hormone releasing hormone (LH-RH) stimulates the secretion of both LH and FSH in the ram (Hopkinson, Pant & Fitzpatrick, 1974; Lee, Cumming, de Kretser, Findlay, Hudson & Keogh, 1976). Changes in the release of this single hypothalamic hormone are likely to be responsible for the changes in gonadotrophin secretion observed in the present study. Direct measurement of LH-RH secretion during changes in photoperiod have not been made in the ram, although Pelletier (1971) has demonstrated that the hypothalamic...
content of LH-RH is influenced by photoperiod, and rams living under natural daylength have increased LH-RH activity in their hypothalami before the autumn mating season at the time when the testes are increasing in size and activity.

In the absence of direct measurement, it is necessary to postulate the manner in which photoperiod dictates LH-RH secretion from a close study of the changes in the plasma gonadotrophin profiles. The plasma levels of LH may be especially useful in this respect since it is probable that the episodes of LH secretion which cause the transitory peaks in the hormone levels in the blood are the result of episodes of LH-RH secretion (Katongole, Naftolin & Short, 1974; Lincoln, 1976b); the pituitary gland does not appear to possess an intrinsic capacity to cause episodic gonadotrophin release (Osland, Gallo & Williams, 1975; Bremner, Findlay, Cumming, Hudson & de Kretser, 1976). Using the LH profiles as the indirect indicator of hypothalamic activity it appears that the change from long to short days stimulates LH-RH secretion mainly by increasing the frequency of the episodes of release. The quantity of LH-RH liberated at each episode may also be enhanced under these conditions as suggested by the increased amplitude of the plasma LH peaks which occurs under short-day lighting; this change is more difficult to predict since the pituitary gland changes its responsiveness to LH-RH under these conditions (Lincoln, 1977).

**Relationship between plasma levels of LH and FSH**

The similarity in the overall cycle between plasma LH and FSH levels, and the short-term relationship between peaks of plasma LH and FSH described in this study, support the notion that a single hypothalamic hormone (LH-RH) regulates the secretion of both gonadotrophins. The explanation for the non-parallel fluctuations in the plasma levels of the two hormones appears to lie in differences in the way the pituitary gland secretes the gonadotrophins, as well as differences in the systemic clearance rates following secretion. The significance of the long half-life of FSH in the circulation compared with that of LH has been commented on previously (Lincoln et al. 1977), yet the importance of the different patterns of release has not been emphasized. A single injection of synthetic LH-RH given to a ram induces an immediate and substantial rise in plasma levels of LH but the effect is of short duration. In contrast, a similar dose of LH-RH has a less dramatic immediate effect on plasma levels of FSH, although the effect is more sustained (Hopkinson et al. 1974; G. A. Lincoln, unpublished results using 1 and 5 μg LH-RH). A close inspection of the plasma profiles in Fig. 2 (summarized in Fig. 4) shows that in the natural situation the release of LH and FSH occurs in much the same way - LH secretion occurs in pulses of short duration while FSH secretion occurs more gradually.

Some insight into the causes of this difference in secretion pattern is provided by work on the rat, in which it has been shown that RNA-dependent synthesis of protein precedes the secretion of gonadotrophins which occurs in a delayed fashion following treatment with LH-RH, while no such synthesis precedes the more immediate release (de Koning, van Dieten & Van Rees, 1976; Vilchez-Martinez, Arimura & Schally, 1976). Since there are quantitative differences in the timing of LH and FSH release following LH-RH administration, it appears that the mechanisms of synthesis/storage/release for the two hormones are subtly different; some LH is always available in a readily releasable reserve but little FSH is present in this form. It is interesting to look to Fig. 2 and envisage how an increase in frequency of LH-RH release due to photoperiod dictates the changes in LH and FSH.

**Relationship between testicular activity and gonadotrophin secretion**

The Leydig cells and Sertoli cells are the important target tissues in the testes for LH and FSH, and presumably they are adapted to receive either episodic or continuous stimulation according to the cell type. The episodic release of LH causes transitory stimulation of the
Leydig cells and this results in episodic testosterone secretion (Katongole et al. 1974; Sanford, Winter, Palmer & Howland, 1974; Lincoln, 1976b; Schanbacher & Ford, 1976). The temporal relationship between LH and testosterone is illustrated in the present study. Since the testis increases its sensitivity to LH under prolonged stimulation (Lincoln, 1976b), testosterone secretion becomes greatly enhanced after many weeks of short days. At this time the high levels of steroid in the blood may have feedback effects on LH secretion either modifying the pituitary response to LH-RH (Hopkinson et al. 1974; Pelletier, 1974) or suppressing hypothalamic LH-RH production (Pelletier, 1970; Lincoln, 1977). While the amplitudes of the plasma LH peaks are depressed markedly at this time, the frequency continues to increase suggesting that hypothalamic neuronal activity may remain raised in spite of the high testosterone levels.

There is no easily measured Sertoli cell secretion which provides a sensitive indicator of FSH release, in the same way that testosterone monitors LH release. The high plasma level of FSH in the ram following a change to short days clearly stimulates testicular growth, mediating its effect on the Sertoli cells and hence spermatogenesis (Courrot, 1971). Once testicular development becomes maximal, plasma FSH values decline rapidly to a level only slightly above that seen in the sexually regressed animals. If the secretion of the putative ‘inhibin’ (Franchimont, Chari & Demoulin, 1975) is maximal at this time it could account for this decline.

**Timing of the photoperiodic response**

In the present study the initial change in gonadotrophin secretion occurred by day 6 following the switch to a stimulatory daylength, and by days 12 and 19 the gonadotrophin levels in the blood were markedly increased. The speed of this photoperiodic response may be comparable to that found in other seasonally breeding mammals such as the hamster (Turek, Elliott, Alvis & Menaker, 1975), but is relatively sluggish compared with that found in birds where gonadotrophin secretion may be augmented within 1 day of exposure to a stimulatory daylength (Nicholls, Scanes & Follett, 1973; Nicholls & Follett, 1974). Pelletier & Ortavant (1964) have claimed that very rapid changes can occur in the content of pituitary LH and FSH in rams following an abrupt change in photoperiod, but these results are not compatible with the present findings.

**Twenty-four hour rhythms and photoperiodism**

Throughout the present study a daily rhythm in gonadotrophin secretion was apparent, similar to that reported previously (Lincoln et al. 1977). This rhythm has been tentatively linked with the daily activity cycle of the rams (sleep/wake cycle) with gonadotrophin release at a maximum when the animals are resting, and vice versa. In the present study it was apparent that the timing of the 24 h cycle of gonadotrophin release remained roughly constant throughout the change in photoperiod, i.e. the acrophase of the cycle remained at the same time of day. This may support the notion that the activity rhythm is involved since the timing of ‘dawn’ and feeding time were kept constant throughout, while only the timing of ‘dusk’ was altered. Thus the period of most intense activity was at the same time each day at all stages.

In a previous communication (Lincoln et al. 1977), we speculated that the 24 h cycle of gonadotrophin secretion may reflect a daily rhythm in photosensitivity, with the peak period of episodic release occurring during the photosensitive phase. This view originated from the observation that the peak period for episodic LH release during the early response to stimulatory photoperiod (short days) was 9–11 h after dawn which would be the predicted time of such a sensitive phase. This observation is not entirely borne out in the present study since the peak time for episodic LH release was slightly earlier in the day at the end of the
light phase. The reason for a preponderance of LH peaks at this time may be related to an overall daily rhythm in the hypothalamo-pituitary activity, rather than any effect of a photosensitive phase. Indeed, the pattern of FSH secretion indicates a more gradual change throughout the 24 h characteristic of a circadian rhythm (Fig. 5).

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REFERENCES


