Contribution of increasing and decreasing daylength to the photoperiodic control of LH secretion in the Ile-de-France ram

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Summary. Sexually mature Ile-de-France rams were exposed to an 8-month light regimen in which the daily light increment and decrement were constant and equal to 7 min/day. Daylength therefore varied from 6 to 20 h. The animals were allotted to two groups of 12 rams each and submitted to the same light regimen but 4 months out of phase. Blood was collected every 40 min for 10 h, on 15 occasions at various intervals during the second light cycle. Plasma LH and on some occasions plasma testosterone concentrations were measured by radioimmunoassay. In both groups the number of LH pulses increased significantly as daylength increased from 11:40 to 20:00 h ($P < 0.01$) but because their amplitude was low the mean plasma LH increase, although significant, was moderate. As daylength started to decrease (from 20:00 to 18:30 h), the frequency of LH pulses further increased ($P < 0.05$) and the pulse amplitude doubled so that mean plasma LH values increased abruptly and remained high until the decreasing light photoperiod reached 11:40 h. Thereafter, the frequency and amplitude of LH pulses decreased and the mean plasma LH dropped to one-fourth of its maximal value. Mean plasma testosterone concentrations were low during most of the 8-month cycle but increased steeply when daylength decreased from 11:40 to 6:00 h. These results indicate that increasing daylength provides a slow stimulation of LH release by an increase in the number of LH pulses, while a long decreasing photoperiod (20:00 to 11:40 h) provides a further and strong stimulation of both LH pulse frequency and amplitude in rams submitted to this regimen. The abrupt increase of LH release before and after the shift from increasing to decreasing daylength indicates that this increase is largely dependent on the decreasing daylength *per se* and not just the result of a photostimulation initiated in increasing daylength. The opposite patterns of plasma LH and testosterone secretions when daylength decreased from 11:40 to 6:00 h also suggest a possible cause-to-effect relationship between the high testosterone and the low plasma LH values recorded at that time.

Introduction

Photostimulation of LH release in the ram occurs when daylength decreased under natural light conditions (Sanford, Palmer & Howland, 1974), under a 6-month light regimen, i.e. reproducing in 6 months the normal annual changes in daylength (Pelletier & Ortavant, 1975), or after an abrupt change from long to short days (Lincoln & Peet, 1977). By comparison, LH release was decreased when daylength increases (Pelletier & Ortavant, 1975; Schanbacher & Lunstra, 1976; Barrell & Lapwood, 1978). However, the onset of LH increase is often observed before the summer solstice under natural conditions (Barrell & Lapwood, 1978; Pelletier, Garnier, de Reviers, Terqui &

Ortavant, 1982) and even under a 6-month light regimen a moderate but significant increase has been observed in long days, suggesting an earlier stimulation (Lindsay, Pelletier, Pisselet & Courot, 1984). In the ewe, it has been shown that the onset into anoestrus was proportional to the value of the daylight increment (Ducker & Bowman, 1970) and conversely that the return to cyclicity to that of decrement (Ducker, Thwaites & Bowman, 1970). Similarly, the greater the light decrement the faster the increase in LH pulsatility in the ovariectomized ewe (Robinson, Radford & Karsch, 1985). These results provide evidence that the magnitude of daily change in daylength is an important parameter for the photoperiodic drive of LH secretion and reproduction. Consequently, in the above mentioned sinusoidal regimens, the decrease in the light increment before the summer solstice may be related to the shift of gonadotrophic activity which, low in increasing daylength, becomes high in decreasing daylength. The present study was therefore designed to compare the effects of increasing and decreasing daylengths by imposing a constant daily change of daylight duration along the photoperiodic cycle.

Materials and Methods

General rationale. The experiment was undertaken with three points in mind: (1) the daily light increment and decrement were equal and constant, (2) these changes were twice those observed at the time of equinoxes in order to amplify the effects of changing daylength, and (3) since in rams (Lincoln & Short, 1980) and ewes (Robinson *et al.*, 1985) the LH release tends to 'escape' the initial influence of any imposed photoperiod (photorefractoriness), a light cycle period was chosen that was 8 months in duration so that this phenomenon could be taken into account.

Animals. Two groups of 12 sexually mature Ile-de-France rams (2–3 years old) were submitted to the artificial 8-month light regimen. Animals were housed in a light-proof building in 4 rooms of 10 m² each where the onset and end of lighting was provided by an electronic clock. The experiment began on 2 January 1982. Animals of Group I were transferred abruptly from ambient lighting conditions to a 6-h artificial daylength then from 6 to 20 h light daily in 4 months. Animals of Group II were transferred initially to 20 h light daily and the regimen was then similar to that of Group I with a 4-month shift (Fig. 1a). These two groups being out of phase precluded attribution to a given photoperiod of the effect of any uncontrolled factor. The daily increment and decrement in daylength was equal to 7 min and affected both 'dawn' and 'dusk'. During a first 8-month light cycle, the animals were allowed to adjust to their new light regimen. During the second cycle, bleedings via acute puncture of the jugular vein were done (one bleeding every 40 min for 10 h) when daylength was 6:00, 7:30, 9:00, 11:40, 14:20, 17:00, 18:30 or 20:00 h. These bleeding times were chosen so that the hormonal changes around the 'summer' and 'winter' solstices could be well defined. Plasma samples were kept frozen at –14°C until radioimmunoassays for LH were carried out.

LH assay. LH was measured in duplicate samples according to the method previously described (Pelletier *et al.*, 1982) but with the following modification: plasma samples were incubated for 3 days with the antibody alone and then the labelled LH (about 24 000 d.p.m.) was added for an additional 1·5 days. This procedure allowed an improved sensitivity which was 50 pg/ml. The intra-assay coefficient of variation was 4·5% and the interassay coefficient was 8·5%. In the course of the present experiment some LH pulses appeared so low (about 300 pg/ml) that plasma testosterone measurements were required to verify their existence.

Testosterone assay. Testosterone concentration was measured directly in 0·05 ml plasma by the technique described by Garnier, Cotta & Terqui (1978) in four rams of each group when increasing daylength was 7:30, 14:20, 18:30 or 20:00 h and when decreasing daylength was 18:30, 14:20, 11:40, 9:00, 7:30 and 6:00 h. Sensitivity of the assay was 0·625 ng/ml. The intra-assay coefficients of variation were 5·9 and 8·2% for 2·5 and 10·0 ng/ml respectively, and the interassay coefficient of variation was 9·4%.

Data analysis. Pulses of LH in individual profiles were identified according to Goodman & Karsch (1980). The number of pulses where the maximum was greater than 2 ng/ml was also considered. This arbitrary threshold, generally not reached during pulses recorded during the shortest days, was chosen as a marker of the magnitude of the LH release. As peak values within a testosterone pulse did not adequately describe the phenomenon of secretion under the longest and the shortest days since the time of secretion frequently exceeds the pulse interval at this period (see Figs 3a & 3c), the frequency of plasma testosterone concentrations greater than 5 ng/ml was considered in addition to the mean plasma level. The effects of daylength on the number of LH pulses or the mean LH and testosterone concentrations were tested statistically by the non-parametric test of Mann & Whitney (1947). Comparisons between frequencies of LH pulses > 2 ng/ml or testosterone values > 5 ng/ml were made by χ^2 analyses.

Fig. 1. Daylength variations, months of experiment and synopsis of male sexual activity in Ile-de-France rams (a), mean plasma LH concentrations (b), number of LH pulses/ram/10 h (c), and percentage of LH pulses > 2 ng/ml (d) in Groups I and II.

Results

Variations of plasma LH concentrations according to the light treatment

Basically, mean plasma LH values were high in both groups when daylength was decreasing, and low when it was increasing (Fig. 1b). Mean plasma values were minimal when increasing light photoperiod was equal to 7:30 h for Group I (0.40 ± 0.057 ng/ml) and 9:00 h for Group II (0.29 ± 0.041 ng/ml). Conversely, mean plasma values were maximal when the decreasing light photoperiod was equal to 14:40 h for Group I (2.31 ± 0.491 ng/ml) and 18:30 h for Group II (1.38 ± 0.247 ng/ml). The ratios of the highest to the lowest mean LH levels were 578 and 474% for Groups I and II. However, in both groups, a progressive but significant increase ($P < 0.001$) occurred in plasma LH when daylength varied from 7:30 to 20:00 h. In Group I, a further increase took place as soon as the light duration decreased (20:00 vs 18:30 h, $P < 0.05$). Conversely, the LH values were significantly reduced ($P < 0.001$) before the decreasing daylength reached 6:00 h.

Number of LH pulses

Within the 336 LH profiles studied 1193 LH pulses were identified, 569 in Group I and 624 in Group II. The number of LH pulses was minimum when increasing daylength was 11:40 h (1.16 and 2.16/ram/10 h in Groups I and II respectively) (Fig. 1c). The number of LH pulses increased signifi-

Fig. 2. Daylength variations and cycle of testicular weight (a), mean LH (●—●) and testosterone (○—○) concentrations (b) and frequencies of LH pulses > 2 ng/ml and testosterone concentrations > 5 ng/ml (c) in rams of Groups I and II pooled.

cantly when daylength progressed from 11:40 to 20:00 h ($P < 0.01$) but increased again in Group I when daylength returned from 20:00 to 18:30 h ($P < 0.05$). It was maximum on two occasions in both groups when decreasing daylength was 18:30 and 9:00 h. Indeed, in Group II, the number of LH pulses was consistently high between 11:40 and 6:00 h, after which it declined abruptly. During increasing daylength, the mean number of LH pulses/ram/10 h was 2.41 and 2.73 in Groups I and II respectively while it was 4.36 and 4.06 when daylength decreased.

Number of LH pulses > 2 ng/ml

When daylength was 6:00 or 7:30 h very few LH pulse values were > 2 ng/ml (8/251, 3.1%). When daylength varied from 6:00 to 20:00 h the frequency of these pulses increased from about 2 to 30% in both groups. As soon as daylength decreased from 20:00 to 18:30 h the frequency of pulses > 2 ng/ml increased, particularly in Group I in which this frequency doubled (62%) and remained high between 18:30 and 11:40 h (range 62–82.9%) (Fig. 1d). Finally, the mean occurrence of pulses > 2 ng/ml in Groups I and II was 17.7% when daylength increased and 38.5% when it decreased. The overall mean maximum value for LH pulses was 2.69 ng/ml when daylength was 18:30 h ($n = 114$) but was only 0.74 ng/ml when daylength was 6:00 h ($n = 89$).

LH: testosterone relationships during increasing and decreasing daylength

The above results do not indicate important differences between Groups I and II so the data were pooled for each equivalent increasing or decreasing daylength studied. Mean plasma LH and testosterone concentrations are given (Fig. 2b) according to treatment (Fig. 2a). In these conditions ($N = 24$) plasma LH was maximal when daylength was long but decreasing. A return to low LH

Fig. 3. Plasma LH and testosterone pulses in Ram 2 when daylength was (a) 6 h, (b) 14:20 h (decreasing daylength) and (c) 20 h. ●, Pulse values of LH.

concentrations was observed when daylength varied from 11:40 to 6:00 h. The pattern of the mean plasma testosterone value was clearly different. The testosterone concentration was practically constant when daylength increased from 11:40 to 20:00 h and then decreased from 20:00 to 14:20 h. It increased abruptly thereafter when daylength reached 11:40 h ($P < 0.001$) and remained high until daylength was 6:00 h.

The frequency of plasma testosterone values > 5 ng/ml had a distribution also different from that of the LH pulses of > 2 ng/ml (Fig. 2c). The frequency of these LH pulses was high when daylength was decreasing from 20:00 to 14:20 h at a time when the high values of plasma testosterone were negligible. Both frequencies were high when daylength was 11:40 h and, when it was 6:00 h, LH pulses of > 2 ng/ml had practically disappeared while 78.9% of testosterone concentrations were high. Examples of LH:testosterone pulse relationships under different daylengths are given in Fig. 3.

Discussion

In spite of 8 months of acclimatization to photo-treatment so that animals might adjust to their new environment, rams of Group II, at the beginning of the experiment exposed to a change in daylength from 8 to 20 h, presented LH variations of less amplitude than did those of Group I. This finding was also previously observed in groups of rams kept in opposite 6-month light regimens (Lindsay *et al.*, 1984). It can presumably be related to the fact that animals that have been exposed to a large abrupt change in daylength need more than two light cycles to be fully adapted to their new regimen. However, as the variations in both groups were parallel, the experimental conclusions derived from combining the data of the two groups of rams can be considered valid.

The close LH:testosterone pulse relationship indicates that the low LH pulses are not grossly underestimated due to the schedule of bleedings even if it cannot be ascertained that none have been missed. Similarly, if the amplitude of LH pulses is not known with precision because of the interval between blood collections, the number of LH pulses recorded here, more than 1100, justifies the use of a threshold value (2 ng/ml) which effectively discriminates pulsatility between long and short days.

Under the present protocol in which daylength can be far below that observed at the winter solstice at the latitude of Nouzilly, both the basal plasma LH concentrations and the maximum values of pulses were found at values much lower than those encountered when rams are under normal daylength conditions (Pelletier *et al.*, 1982). LH pulses detected within the definition of Goodman & Karsch (1980) were sometimes so low (250–300 pg/ml) that it seemed necessary to verify their existence by measuring plasma testosterone. Indeed, for the low LH pulses observed, particularly for 6:00 and 7:30 h of daylight, the greatest testosterone pulses were detected, related to the maximal testicular enlargement observed at that time (J. Pelletier, M. Blanc, C. Pisselet & J. P. Ravault, unpublished results: see Fig. 2a), a finding also found for Soay rams (Lincoln & Short, 1980).

The result of the experiment is clear: in spite of a high and constant daylength increment, there is an increase both of plasma LH concentrations and the pulsatility before the longest days. Under natural light conditions, therefore, and also under a 6-month light regimen (Lindsay *et al.*, 1984), the decrease in the daily light increment before the summer solstice is probably not a major source of stimulation of the hypothalamo-hypophysial system before the time of decreasing daylength. Similarly, in the present experiment, the decrease in LH release occurred before the 'winter' solstice and hence cannot be attributed to a diminution of the light decrement.

The second interesting finding of this study is the steep increase of the plasma LH concentrations, pulse frequency and amplitude shown as soon as the daylength was decreasing from 20:00 to 18:30 h. At that time there was no opposition between amplitude and frequency of LH pulses as shown in rams under normal conditions (Sanford, Beaton, Howland & Palmer, 1978;

Lincoln & Short, 1980) since both were augmented. The abrupt increase of both parameters makes unlikely that it was merely the result of a previous stimulation in increasing daylength. On the contrary, it suggests a mechanism different from that involved before the shift took place. Furthermore, according to the results of Schanbacher & Ford (1979) this mechanism would not be dependent on a previous exposure to long days. The exact nature of this mechanism is largely unknown although the pineal gland is probably involved (Lincoln, 1979; Lincoln & Almeida, 1981; Bittman & Karsch, 1984). This stimulation occurring during decreasing daylength is perhaps related to a time when the interval between successive dawns is less than 24 h, but this time is unique when the protocol involves an abrupt long-day to short-day change which produces a prolonged stimulation of LH release. The present results, and also protocols involving quick changes in daylength both in rams (Lincoln, 1979; D'Occhio, Schanbacher & Kinder, 1984) and in ewes (Ducker *et al.*, 1970), suggest that this mechanism is not related to steroid feedback even if, as discussed by Robinson *et al.* (1985), it may not be completely unrelated. However, increased LH release was shown in castrated male (Pelletier & Ortavant, 1975) and female (Goodman, Bittman, Foster & Karsch, 1982) sheep when daylength was decreasing. Most of the evidence therefore suggests that the stimulation in LH release observed when daylight is long and decreasing represents mainly the effect of a direct photoperiodic drive.

It was not the primary objective of this experiment to study the LH–testosterone relationships. However, it seems likely that the photorefractoriness to decreasing daylength which results in the decline of LH release, could be related at least in part, to the progressive increase in the amplitude of testosterone pulses even if, as pointed out by Lincoln & Short (1980), the negatively correlated hormonal patterns did not constitute a formal cause-to-effect proof. Similar clear opposite patterns were shown in Finnish Landrace (Sanford *et al.*, 1978) and Soay (Lincoln & Short, 1980) rams. In addition, in Finnish Landrace rams implanted with capsules filled with testosterone, the LH pulse frequency was clearly related to the mean plasma testosterone concentration (D'Occhio *et al.*, 1982).

Similarly, we can speculate that photorefractoriness to increasing daylength, which produces an augmentation of LH release, can have two sources which are not mutually exclusive: (1) the low testosterone circulating values, which correspond to a moderate negative feedback, allow a reactivation of the hypothalamo-hypophysial system and (2) there is, in long days (> 14:20 h), a lengthening of the photoinducible phase (Pelletier *et al.*, 1981). However, the respective contributions of these two distinct mechanisms are still unknown.

In summary, this experiment shows that a high daily light increment does not prevent the augmentation of LH pulsatility which takes place in long increasing daylength. Whatever the mechanism(s) involved it presents a slow effectiveness on the stimulation of LH release. By contrast, the decreasing daylength appears to be a distinct, quick activatory mechanism which seems partly independent both of steroid or long-day exposure.

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