Photoperiodic history and a changing melatonin pattern can determine the neuroendocrine response of the ewe to daylength

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Summary. The reproductive neuroendocrine response of Suffolk ewes to the direction of daylength change was determined in animals which were ovariectomized and treated with constant release capsules of oestradiol. Two groups of animals were initially exposed to 16 or 10 h light/day for 74 days. On day zero of the study, when one group of ewes was reproductively stimulated (elevated LH concentrations) and the other reproductively inhibited (undetectable LH concentrations), half the animals from each group were transferred to an intermediate daylength of 13 h light/day. The remaining ewes were maintained on their respective solstice photoperiods to control for photo-refractoriness. LH concentrations rose in animals experiencing a 3 h decrease in daylength from 16L:8D to 13L:11D while LH concentrations fell to undetectable values in those that experienced a 3 h increase in daylength from 10L:14D to 13L:11D. The photoperiodic response of the Suffolk ewe, therefore, depends on her daylength history. Such a result could be explained if the 24-h secretory pattern of melatonin secretion, known to transduce photoperiodic information to the reproductive axis, was influenced by the direction of change of daylength. Hourly samples for melatonin were collected for 24 h 17 days before and three times after transfer to 13L:11D. The melatonin secretory profile always conformed to daylength. Therefore, the mechanism by which the same photoperiod can produce opposite neuroendocrine responses must lie downstream from the pineal gland in the processing of the melatonin signal.

Introduction

Daylength has been demonstrated to be an important cue for timing the period of reproductive activity of sheep as well as many other seasonally breeding species (Yeates, 1949; Hafez, 1952; Mauleon & Rougeot, 1962; Reiter & Follett, 1980). Typically, breeding begins in autumn, as daylength is decreasing and ends in late winter as the photoperiod is increasing. Sheep are, therefore, considered to be 'short-day breeders'. This is substantiated by laboratory studies which indicate that short days can be stimulatory and long days suppressive to reproduction (Yeates, 1949; Legan & Karsch, 1980).

Experiments in other seasonal breeders have led to the concept of a species specific 'critical daylength', a pivotal photoperiod on either side of which lies reproductive induction or inhibition (Elliott, 1976). In their outdoor environment, however, sheep may not use an absolute critical daylength to time reproductive transitions. Under natural conditions, the same daylength occurs twice per year; hence breeding might be expected to extend symmetrically about the solstices. This,

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however, is generally not the case. Most breeds of sheep are reproductively stimulated at one time of year but inhibited under the same photoperiod at the other (Hafez, 1952; Robinson & Karsch, 1984). Therefore, the concept of an absolute critical daylength may be untenable. Rather, as is the case in other seasonal breeders such as the Japanese quail, the wild European rabbit and certain rodents, the photoperiodic history, and therefore the direction of change of daylength, may be crucial in determining the reproductive state (Robinson & Follett, 1982; Horton, 1984; Stetson, 1985; Boyd, 1986; Hoffmann *et al.*, 1986).

In the present study, we sought to determine whether the reproductive response of the ewe to a given daylength depends upon her recent photoperiodic experience. Further, we examined whether the 24-h pattern of melatonin secretion is influenced by the direction of change in daylength. In this regard, melatonin is known to relay photoperiodic information to the reproductive axis of the ewe by virtue of being secreted only during the night (Lincoln & Short, 1980; Karsch *et al.*, 1984). Any discordant pattern of melatonin secretion, therefore, could constitute a mechanism whereby the same absolute daylength leads to different reproductive responses.

Materials and Methods

Animals and treatments. Experiments were conducted on 21 sexually mature Suffolk ewes maintained on a diet of hay. Before the study, animals were ovariectomized and treated with a subcutaneous Silastic capsule containing 3 cm of packed oestradiol-17 β (Karsch *et al.*, 1973). This treatment has been shown to maintain a physiological serum concentration of oestradiol (3–5 pg/ml: Legan *et al.*, 1977; Karsch *et al.*, 1980). Reproductive neuroendocrine responses were assessed from serum concentrations of LH in samples obtained twice a week by jugular venepuncture. Under stimulatory short daylengths, oestradiol has only a weak negative feedback action and LH concentrations are elevated; under inhibitory long daylengths oestradiol is a potent inhibitor of LH secretion and concentrations are low or undetectable (Legan *et al.*, 1977; Legan & Karsch, 1980). It is now well established that this change in response to oestradiol negative feedback constitutes the endocrine basis for seasonal fluctuations in ovarian function and that sustained rises and falls in LH in this animal model parallel seasonal changes in oestrous cyclicity (Karsch *et al.*, 1984). Specifically sustained increases in LH above 1 ng/ml occur at the same time as the onset of oestrous cycles; decreases in LH below 1 ng/ml are coincident with the onset of anoestrus (Robinson & Karsch, 1984; Robinson *et al.*, 1985; F. J. Karsch, unpublished).

Photoperiodic manipulations. Animals were housed in light-sealed rooms in which daylength was regulated automatically. Light was provided by fluorescent tubes which produced an intensity of 350 lux lateral to the sheep's head. A dim red light (<2 lux) remained on continuously to facilitate night-time blood collection. The experiment consisted of a 10-month priming period and a 3-month experimental period (Fig. 1). At the start of the priming period, all ewes were reproductively suppressed (mean LH concentrations undetectable). During this priming phase ewes were assigned to one of two groups. The first (N = 12) was to be reproductively suppressed at the start of the experimental period on a daylength similar to that of the summer solstice at Ann Arbor, Michigan (42°N) and was treated as follows: 8L:16D for 80 days, 16L:8D for 60 days, 8L:16D for 70 days, 16L:8D for 74 days. The other group (N = 9) was to be reproductively stimulated at the start on a daylength similar to that of the winter solstice and was treated as follows: 8L:16D for 100 days, 16L:8D for 110 days, 10L:14D for 74 days. Such manipulations also ensured that at the start of the experimental period all ewes were photosensitive.

The experiment began on 12 April 1984 when 6 ewes were given an abrupt 3-h decrease in photoperiod (16L:8D to 13L:11D). Simultaneously, 4 ewes experienced a 3-h increase in daylength to the same intermediate photoperiod (10L:14D to 13L:11D). Daylength changes were made by altering the time of lights off (lights on in all photoperiods at 06:00 h EST). The remaining ewes were maintained on their respective solstice photoperiods to control for photo-refractoriness, spontaneous changes in reproductive condition which occur during extended periods of stimulatory or inhibitory photoperiods (Robinson & Karsch, 1984; Robinson *et al.*, 1985; Karsch *et al.*, 1986). The 24-h secretory profile of melatonin was assessed before and three times after these photoperiodic shifts (Days –17 and +7, 41, 81; see M, Fig. 1). Samples were taken hourly; those at the light/dark transitions were collected within 15 min of lights on or off.

Assays. LH was assayed in duplicate in 25–200 μ l samples of serum according to the method of Niswender *et al.* (1969) modified as described by Hauger *et al.* (1977). Sensitivity averaged 0.6 ng/ml for 200 μ l serum. Inter- and intra-assay coefficients of variation (CV) for three serum pools determined 6 times in each assay were 9.3% and 6.5%, respectively (7 assays). Melatonin concentrations were determined in duplicate in extracts of 500 μ l serum as described by Rollag & Niswender (1976) with slight modifications (Bittman *et al.*, 1983). Sensitivity averaged 2.5 pg/ml. Values for a daytime serum pool measured 6 times in each assay averaged 56 ± 4 pg/ml; those for a night-time pool averaged 414 ± 13 pg/ml. Inter-assay CV for daytime and night-time serum pools averaged 29.8% and 13.4%, respectively.

Statistics. Data were analysed by comparing the proportion of ewes which responded with a rise or fall in LH by a specified time with that in the respective refractoriness control group. For this purpose, Fisher's exact probability test was used for animals initially maintained on 16L:8D; the Mann-Whitney two-sample rank-sum test was used for animals initially maintained on 10L:14D.

Fig. 1. Experimental design: daylengths to which 21 ovariectomized and oestradiol-treated ewes were exposed just before and during the 85-day experimental period (see text for details). M indicates the times of the 24-h sampling periods for melatonin. N = number of animals per group.

Results

Thirteen hours of light per day was regarded as stimulatory to reproductive neuroendocrine function in animals experiencing a 3-h decrease in daylength (from 16L:8D to 13L:11D; Fig. 2, left). About 40 days after this light shift, serum LH concentrations rose from <0.6 ng/ml to ~ 3.0 ng/ml. These LH responses were significantly different ($P < 0.01$, Fisher's exact test) from those in the refractoriness controls maintained on 16L:8D, in which LH values remained undetectable. The maximal concentration of LH achieved in animals exposed to the 3-h decrease in photoperiod, however, was less than the values we have observed in ewes which experienced a larger decrement in daylength (see Fig. 2, LH values above 10 ng/ml on Day 0 in ewes exposed to 10L:14D). The 3-h reduction in daylength to 13L:11D may therefore represent a marginally stimulatory photoperiod (see 'Discussion').

In marked contrast to the reproductive response following the shift from 16 to 13 h light/day, 13L:11D was regarded as inhibitory to reproductive neuroendocrine function by animals experiencing a 3-h increase in daylength from 10L:14D (Fig. 2, right). LH concentrations plunged from above 10 ng/ml and became undetectable while values remained maximal in refractoriness controls maintained on 10L:14D. Although LH concentrations subsequently fell as these controls became refractory to the stimulatory daylength, the fall occurred significantly later ($P < 0.005$, Mann-Whitney two-sample rank-sum test) than in experimental ewes. At the time LH in this control group became undetectable, animals had actually experienced 10L:14D for 142 days (74 pretreatment days plus 68 experimental days). This duration of exposure to an inductive photoperiod is similar to that which we have observed in several other studies (Malpaux *et al.*, 1985; Karsch *et al.*, 1986).

The 24-h patterns of circulating melatonin 17 days before transfer from the solstice photoperiods and after 7 days of 13L:11D are shown in Fig. 3. Without exception, the melatonin secretory profiles conformed to photoperiod, i.e. hormone concentrations were low during the light phase and elevated during darkness, and the period of increased melatonin secretion spanned the dark phase. Patterns determined after 41 and 81 days of 13L:11D (not shown) were essentially the same and melatonin release reflected daylength irrespective of photoperiodic history and the neuroendocrine state of the animals.

Fig. 2. Serum LH concentrations (mean $\pm$ s.e.m.) in ewes which experienced a 3-h decrease in daylength from 16L:8D to 13L:11D (solid symbols, left), or a 3-h increase in daylength from 10L:14D to 13L:11D (solid symbols, right). Controls for refractoriness remained on the solstice photoperiods of 16L:8D (open symbols, left) or 10L:14D (open symbols, right). M indicates the time of blood sampling for melatonin measurements.

Discussion

Our findings demonstrate that the reproductive neuroendocrine response of the Suffolk ewe to daylength depends on her photoperiodic experience. Further, they indicate that this species does not exhibit a rigid critical daylength for reproductive induction or inhibition, and raise the possibility that the ewe may be sensitive to the direction of photoperiodic change and alterations in the secretory pattern of melatonin. That photoperiodic history may be of widespread importance in seasonal reproduction is suggested by observations on Japanese quail, juvenile microtine voles, adult and juvenile Djungarian hamsters and rabbits (Robinson & Follett, 1982; Hoffmann, 1984; Horton, 1984; Stetson, 1985; Boyd, 1986; Hoffmann *et al.*, 1986).

The amplitude of the LH rise following the 3-h decrease in daylength was not as large as that which we had observed in ewes experiencing larger decrements in daylength (priming phase of the study; Legan & Karsch, 1980; Bittman *et al.*, 1983; Karsch *et al.*, 1986). This supports the previous conclusion that the magnitude of the reproductive neuroendocrine response of the ewe depends upon the strength of the photoperiodic signal (Robinson *et al.*, 1985) and that the 3-h reduction in daylength constituted a marginally stimulatory signal. It is not possible from the present study to assess whether the photoperiodic signal would have been sufficient to promote the onset of oestrous cycles had the ewes not been ovariectomized. However, under the natural and artificial

Fig. 3. Diurnal patterns of serum melatonin concentrations (mean $\pm$ s.e.m.) in ovariectomized-estradiol-treated ewes determined 17 days before transfer from the solstice daylengths (left panels), or 7 days after being placed on 13L:11D (right panels). Shaded areas depict the period of darkness. N = number of animals.

photoperiod conditions, there is a strong temporal association between the onset of oestrous cycles in ovary-intact ewes and LH concentrations above 1 ng/ml in ovariectomized-estradiol-treated animals. In this study LH concentrations rose to an average of 3 ng/ml.

Although the importance of photoperiodic history has been most thoroughly studied in the Japanese quail (Robinson & Follett, 1982) it has not proved possible to unravel the underlying mechanism in this species. This is, in part, because the photoneuroendocrine pathway in birds is not well understood. The sheep provides a good opportunity to begin to explore the underlying mechanism because the pineal gland and its light-regulated secretion of melatonin are known to be important components of the pathway, and secretory profiles of melatonin can easily be determined in individual animals. Thus, the generation of a melatonin profile which does not accurately reflect photoperiod could constitute a mechanism whereby the same photoperiod leads to opposite reproductive responses in photosensitive animals.

In the present study, however, the melatonin secretory pattern always conformed to daylength. Therefore, the mechanism whereby the same photoperiod can stimulate or inhibit LH secretion in ovariectomized-estradiol-treated ewes must lie downstream from the pineal gland, possibly in the processing of the melatonin signal. In this regard, two hypotheses may be forwarded: either the processing of the melatonin signal depends on the reproductive neuroendocrine state of the animals, or the response to this signal depends upon the preceding melatonin pattern. The latter possibility is particularly attractive because, under different conditions, the response of ewes to melatonin is influenced by prior exposure to this hormone (Karsch *et al.*, 1986).

The melatonin profiles in our study also have important implications concerning the critical feature of the melatonin pattern which carries photic information. At present there are two main conceptual models to explain how melatonin transduces daylength information in a photoperiodic species, one involving duration and the other phase of elevated melatonin secretion (Goldman *et al.*, 1982). According to the duration model, the length of the night dictates the length of time that

melatonin is elevated in each 24-h period and this provides the neuroendocrine code for daylength. In sheep this model is supported by observations that the duration of elevated melatonin secretion is proportional to the length of the night (Rollag *et al.*, 1978; Lincoln & Short, 1980; Arendt *et al.*, 1981; Kennaway *et al.*, 1983; Karsch *et al.*, 1985). Moreover, studies involving infusion of melatonin into pinealectomized ewes suggest that reproductive state can be dictated by the period that melatonin is raised each 24 h (Bittman & Karsch, 1984; Yellon *et al.*, 1985). Similarly, infusion studies of the Djungarian hamster provide strong evidence that duration of nightly melatonin is critical in that long-day breeder (Carter & Goldman, 1983; Goldman *et al.*, 1984). Despite this supporting evidence, our present finding that the same absolute duration of the melatonin rise provoked opposite neuroendocrine responses in the ewe cannot be accommodated by the duration hypothesis in its most rigid form. Should duration prove to be pivotal in determining the breeding condition of the ewe, then it must be the duration relative to the preceding melatonin pattern, and therefore whether the period of secretion each night is expanding or contracting. A recent study of the male Djungarian hamster has also led to this conclusion (Hoffman *et al.*, 1986).

The phase model is based on the hypothesis that there is a circadian rhythm of sensitivity to melatonin; the phase relationship of the melatonin rise to this sensitive period then dictates the reproductive response (Goldman *et al.*, 1982). Although this hypothesis has not been formally tested in sheep, supporting evidence has been obtained from experiments involving timed injections of melatonin into pinealectomized Syrian hamsters (Watson-Whitmyre & Stetson, 1983; Watson-Whitmyre, 1985). Our finding that a common melatonin pattern induces opposite neuroendocrine states could be explained by a phase model, if photoperiodic history were to influence the circadian time of the entrained melatonin sensitive phase. Although this remains to be studied, photoperiodic history is known to affect circadian rhythms in rodents under free-running conditions (Pittendrigh & Daan, 1976).

In summary, our findings demonstrate that the concept of an absolute critical day length for reproductive stimulation or inhibition in the Suffolk ewe is untenable; the photoperiodic threshold and the response to melatonin vary with recent photoperiodic experience. The adaptive significance of the resulting seasonal shift in the photoperiodic threshold in the ewe may be to permit this 'short day' breeder to become sexually active under the relatively long (but decreasing) daylengths of late summer. Births would therefore occur in early spring, enabling growing lambs to benefit from a maximal period of favourable environmental conditions. Furthermore, breeding would be curtailed by the relatively short (but increasing) daylengths of late winter, preventing births at times when lambs could not attain sufficient body weight to survive their first winter.

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