Neuroendocrine responsiveness to light during the neonatal period in the sheep

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ABSTRACT

Circulating prolactin concentrations were monitored during the early postnatal period in sheep to evaluate their response to photoperiod. In the first experiment, male and female lambs were exposed from 1 week of age, with their mothers, to either long days (16 h light:8 h darkness; $n=15$) or short days (8 h light:16 h darkness; $n=16$) to test whether they could discriminate different day lengths. In both sexes, serum prolactin concentrations were higher on long than on short days during the first 7 weeks after birth. In the second experiment, female lambs ($n=21$) were raised on long days from 2 weeks of age. The superior cervical ganglia were removed bilaterally at 4 weeks of age from 14 lambs to lesion the sympathetic innervation to the pineal gland, and thus ablate the nocturnal increase in pineal melatonin secretion. After surgery, serum prolactin concentrations on long days were significantly lower in ganglionectomized lambs than in the intact controls. In the third experiment, the amplitude of the night-time melatonin rise was artificially increased in female lambs ($n=8$) between 2 and 7 weeks of age to adult levels. Unrestrained lambs were infused during the 8-h dark phase of each day with melatonin by means of a self-contained, computerized syringe-pump. Concentrations of circulating prolactin did not differ from those in uninfused lambs ($n=8$) with lower endogenous nocturnal melatonin. These results reveal that the sheep can discriminate photoperiod cues during the early postnatal period, and suggest that the low-amplitude melatonin rhythm in the neonatal lamb is sufficient to mediate this response.


INTRODUCTION

Photoperiod is an important environmental cue, timing puberty in sheep (Foster, 1981; Foster, Karsch, Olster et al. 1986). In the spring-born female Suffolk lamb, a decrease in daylength is necessary for the initiation of reproductive cycles in the autumn (Yellon & Foster, 1985). However, if this decrease in daylength is experienced very early in life, for example in the first 10 weeks, then puberty does not occur on time (Foster, 1983; Yellon & Foster, 1985). It is not clear whether this failure to respond reproducitively results from an inability of the lamb to perceive changes in length of day early in life, or whether it results from the inability of the developing reproductive axis to respond to changes in photoperiod. The present study examined the first possibility. We determined if and how the lamb monitors changing daylength early in life by assessing responses to light which do not involve the reproductive axis. Another photoneuroendocrine system was chosen for study, namely that regulating prolactin secretion, because length of day is a major environmental cue regulating the seasonal pattern of this hormone in the adult sheep of both sexes (Pelletier, 1973; Lincoln, McNeilly & Cameron, 1978; Munro, McNatty & Renshaw, 1980), and light-induced alterations in its secretion have been observed in male lambs during the peripubertal period (from 18 weeks of age) (Forbes, Driver, El Shahat et al. 1975; Brown & Forbes, 1980).

The aims of the present study were first, to ascertain whether the neonatal female lamb can distinguish long from short photoperiods as determined from the level of prolactin secretion, and secondly, if this were
so, whether the pattern of melatonin secretion from the pineal gland transduces photoperiod information during this period to modulate prolactin secretion as it does in older sheep (Barrell & Lapwood, 1979; Lincoln, 1979; Brown & Forbes, 1980; Lincoln & Ebling, 1985; Poulton, English, Symons & Arendt, 1986). Our approach was to determine whether ablation of nocturnal melatonin secretion by lesioning the sympathetic innervation to the pineal gland of the neonate disrupted the photoperiodic prolactin response. Finally, we determined whether nightly supplementation of the nocturnal melatonin rise to adult levels altered prolactin secretion. The latter experiment tested the hypothesis that the failure of reproductive responses to occur after alterations in photoperiod during the first few weeks after birth is due to inadequate nocturnal melatonin secretion (i.e. failure to distinguish daylength because of sub-threshold nightly melatonin rises). A diurnal pattern of serum melatonin exists in the fetal (Yellon & Longo, 1987) and neonatal lamb (L. E. Claypool, R. I. Wood, F. J. P. Ebling & D. L. Foster, unpublished information) but the amplitude of the nocturnal rise is much less than that of adult sheep. A preliminary report of this work was presented at the 69th Annual Meeting of the Endocrine Society, Indianapolis, IN, U.S.A., June 1987 (Foster, Ebling & Claypool, 1987).

MATERIALS AND METHODS

General

Suffolk lambs born on April 4±1 (mean ± S.E.M.), range March 29–April 11 (experiments 1 and 3) or on March 26±3, range March 20–April 10 (experiment 2) were used. Lambs were born into a commercial sheep facility with dim continuous artificial light, and were moved into light-sealed controlled photoperiod rooms at The University of Michigan Sheep Research Facility during the first 2 weeks after birth. Fluorescent lights provided a daytime light intensity of approximately 350 lux. Lighting was controlled by electronic (System X10 appliance module; BSR, Miami, FL, U.S.A.) or electric (Tork 8001 Program Time Switch; Tork, Mt Vernon, NY, U.S.A.) time clocks. Constant dim red light of less than 2 lux was provided to aid blood sample collection during the dark period. Long-day treatment was 16 h light per day (16L:8D), lights on at 06.00 h Eastern Standard Time, and short-day treatment was 8L:16D, lights on at 06.00 h. The rooms were ventilated through a light baffle, but not air conditioned, so temperature changed seasonally. Temperature in the rooms recorded at the time of blood sampling was similar to external ambient temperature. Values for daily maximum and minimum temperatures were obtained from The University of Michigan Botanical Gardens, adjacent to the Sheep Research Facility. Lambs were housed with their mothers, and solid food consisting of alfalfa hay and a commercial pelleted diet (Lamb 18; Kent Feed Inc., Muscatine, IA, U.S.A.) was available ad libitum. They were weaned after completion of the experiments.

Experiment 1: effect of long and short photoperiod on prolactin secretion

This experiment was to determine whether the neonatal sheep is capable of a neuroendocrine response to daylength. Male and female lambs were housed together with their mothers on long days (n= seven male, n= eight female) or short days (n= eight male, n= eight female) from a mean age of 7±1 days. Serum prolactin concentrations were measured, as an index of photoperiod responsiveness, in samples collected by jugular venepuncture three times weekly (08.00–10.00 h) from 9 days of age (2 days after being placed in a controlled photoperiod) until 7 weeks of age.

Experiment 2: effect of superior cervical ganglionectionomy on prolactin secretion

This experiment was to determine whether the pattern of pineal melatonin secretion mediates the influence of photoperiod on prolactin secretion in the young lamb. Female lambs were housed with their mothers on long days (n= 21) or short days (n= 8) beginning at a mean age of 13±1 days. At 30±2 days of age, both superior cervical ganglia were surgically removed from 14 of the lambs exposed to long days using the technique of Appleton & Waites (1957), under halothane anaesthesia. The excised tissue was confirmed histologically to be sympathetic ganglion. Blood samples for prolactin determinations were collected by jugular venepuncture twice-weekly (08.00–10.00 h) from 2–5 weeks of age (5 days after being placed in a controlled photoperiod) until weaning at 10 weeks of age. Subsequently, hourly blood samples collected for 28 h confirmed the effect of cranial sympathtectomy on melatonin secretion in the lamb; daytime and nighttime serum melatonin concentrations were below the assay limit of detection (25 pmol/l). This is in agreement with our previous observations in ganglionectionized lambs at later ages (Foster, Yellon, Ebling & Claypool, 1988).

Experiment 3: effect of increased nocturnal melatonin amplitude on prolactin secretion

This experiment was to determine whether increasing the amplitude of the endogenous nocturnal melatonin
rise would modify the prolactin response to photoperiod, because the normal amplitude of the nighttime increase in melatonin in the neonate is much less than that of the adult. Sixteen lambs were placed in long days at 8±1 days of age. A programmable, battery-operated syringe pump (AS6MP; Autosyringe, Hooksett, NH, U.S.A.) contained in a portable backpack system permitted delivery of melatonin (Sigma, St Louis, MO, U.S.A.) into a jugular vein of freely moving, unrestrained nursing lambs. Eight of the lambs were infused nightly for 5 weeks during the 8-h dark phase of the long-day photoperiod (22.00–06.00 h). A canvas backpack was custom designed (Fox Tent and Awning, Ann Arbor, MI, U.S.A.) for carrying the syringe pump enclosed within a plastic container. The backpack was held in place entirely by tape which was replaced periodically as the lambs grew. Daily loading of the syringe with melatonin solution was facilitated by placement of the lamb in a canvas sling (Fox Tent and Awning). The backpacks were fitted at 11 days of age, and at 14 days of age infusions with heparinized (1000 units/l) 0·22% (v/v) ethanolic saline containing 270 nmol melatonin/8 h night (at a mean body weight of 8·1±0·5 kg) were begun. They were gradually increased to 490 nmol melatonin/8 h at the end of the experiment (body weight 18·3±1·0 kg). These infusion rates (3·3–4·2 nmol/h per kg) achieved circulating melatonin concentrations of 1000–1500 pmol/l, as determined each week in blood samples collected from the contralateral jugular vein 2 h after the start of infusion, and also collected at hourly intervals for 24 h on the last day of infusion. The remaining eight lambs served as controls and constituted the 'long-day' group in experiment 1. Four were fitted with a harness to determine whether any possible non-specific stress associated with the portable infusion apparatus might influence prolactin secretion. Blood samples for determination of prolactin concentrations were collected from all lambs three times weekly by jugular venepuncture between 08.00 and 10.00 h, beginning at 9 days of age (2 days after being placed in a controlled photoperiod).

There were no effects of the photoperiod treatments and experimental procedures described above on the growth of the lambs. For example, in experiment 1 the rates of liveweight gain in the females raised on long and short days were 2·18±0·08 and 2·21±0·10 kg/week respectively. In experiment 2, the rates of liveweight gain in intact and ganglionectionized females raised on long days were 1·85±0·31 and 1·74±0·13 kg/week respectively.

**Hormone assays**

Serum prolactin concentrations were measured in duplicate 20 μl samples using the radioimmunoassay developed by Davis, Reichert & Niswender (1971) with the following modifications: the tracer preparation (LER-860-2) was iodinated with 125I rather than 131I, phosphate-buffered saline containing 1·0% bovine serum albumin (Sigma) was used as the assay buffer, and a preprecipitated second antibody was used to separate bound antibody from free ligand (Midgley & Hepburn, 1980). Serial dilution of serum pools collected from female lambs at 4 and 17 weeks of age produced inhibition curves parallel to the standard. Assay sensitivity was 5 μg/l for 20 μl serum, expressed relative to NIH-P-S8. Mean interassay coefficient of variance (c.v.) based on duplicates of low, medium and high serum pools run in all assays, was 9·1%. Mean intra-assay c.v. was 12·3% for these pools (n=9 assays). Prolactin concentrations are expressed relative to standard NIH-P-S8. Melatonin was measured using the assay established by English, Poulton, Arendt & Symons (1986) with modifications for lamb serum as described by Foster et al. (1988). Mean intra-assay c.v. for duplicates of low and high serum pools was 6·0%; interassay c.v. for these pools was 13·7% (six assays). Mean limit of detection was 25 pmol/l for 350 μl extracted serum.

**Statistical analysis**

Serum prolactin concentrations were log-transformed, then smoothed by grouping into time-periods of 1 week (experiments 1 and 3) or 1·5 weeks (experiment 2). The summated values for each time-period were subject to analysis of variance with repeated measures (BMDP Statistical Software Inc., Los Angeles, CA, U.S.A.) followed by Tukey’s test to determine significant differences between particular time-periods (Miller, 1966). A value of P<0·05 was considered significant in all analyses.

**RESULTS**

**Experiment 1: effect of long and short photoperiod on prolactin secretion**

Serum prolactin concentrations in female and male lambs on long-day and short-day photoperiods in relation to ambient temperature are illustrated in Fig. 1. In females, serum prolactin concentrations differed significantly between the photoperiod treatments from 3 weeks after birth until the end of the experiment; in males, prolactin concentrations were significantly higher on long than short days on all sampling occasions. In both sexes, in the short-day photoperiod treatments there was a significant decrease in prolactin concentrations by 3 weeks of age relative to initial values.
Experiment 2: effect of superior cervical ganglionectomy on prolactin secretion

Serum prolactin concentrations in lambs raised on long days, on long days and ganglionectomized at approximately 4 weeks of age, or on short days, are illustrated in Fig. 2 in relation to ambient temperature. Circulating prolactin concentrations in the ganglionectomized, long-day group were significantly lower from 3-5 weeks of age (1-5 weeks after surgery) than in the intact long-day group, but did not differ significantly from those in the intact short-day group. On all sampling occasions, prolactin concentrations were significantly lower in the short-day treated lambs than in the intact long-day treated lambs. Thus prolactin concentrations in the ganglionectomized groups were intermediate between those in the intact long- and short-day groups.

Experiment 3: effect of increased nocturnal melatonin amplitude on prolactin secretion

Nocturnal melatonin levels, assessed by blood samples collected 2 h after lights off, rose from a mean concentration of 69±17 pmol/l at 1 week of age to 379±108 pmol/l at 7 weeks of age in uninfused control lambs (Fig. 3a), though there was considerable variation between individuals at older ages (range 52–818 pmol/l at 7 weeks of age). The night-time concentrations were always above daytime levels; the latter were usually undetectable (< 25 pmol/l). The timed melatonin infusions were effective in increasing nocturnal serum melatonin concentrations to the adult range (750–2000 pmol/l; Figs 3b and 4). Body weight increased from 8·1 ±0·5 kg at 2 weeks of age to 18·3 ±1·0 kg at 7 weeks of age. However, the adjustments in infusate concentrations maintained relatively constant serum melatonin concentrations over this period (Fig. 3b). Despite the greater nocturnal melatonin concentrations in the infused group compared with the control group, no significant differences in serum prolactin concentrations occurred between these groups (Fig. 3c). Within the control group there was no significant difference in serum prolactin concentrations between those lambs carrying infusion harnesses and those left untreated (overall group mean 475±53 vs 406±30 µg/l).

DISCUSSION

These studies reveal that photoperiod modulates the neuroendocrine system in the early postnatal Suffolk lamb. In both sexes, serum prolactin concentrations were found to be higher under a long-day photoperiod (16L:8D) than a short-day photoperiod (8L:16D).
These effects of photoperiod in the first 10 weeks of life are consistent with those previously observed in older lambs (>18 weeks of age) (Forbes et al. 1975; Brown & Forbes, 1980; Foster et al. 1988). It is not possible to determine the exact stage of development at which photoresponsiveness develops, because a latent period may exist between the time of exposure to a change in photoperiod and the resultant neuroendocrine response. In adult sheep transferred from long to short photoperiods, reports of the length of time for a significant decrease in serum prolactin concentrations to occur are of the order of 1–4 weeks (Lincoln et al. 1978; Poulton et al. 1986). However, the different prolactin concentrations in male (experiment 1) and female (experiment 2) lambs just 2 and 5 days respectively after being exposed to long- or short-day photoperiods, may indicate that the latent period is even shorter in neonatal sheep than in the adult. Our results raise the possibility that the lamb is able to perceive and respond to photoperiod from birth.

Prolactin secretion can be regulated by environmental factors other than photoperiod, one of which is temperature (young female cattle; Wettemann & Tucker, 1974; Tucker & Wettemann, 1976). Studies in adult male sheep subjected to changes in photoperiod, but uncontrolled temperature, also provide evidence that temperature can modulate prolactin secretion, since the circulating concentration of prolactin is considerably higher when long-day exposure coincides with warm ambient temperatures than when it coincides with cold temperatures (Lincoln, 1979; Ebling & Lincoln, 1987). In the current study, ambient temperature was not controlled. It is possible that the synchronous changes in prolactin concentrations which occurred from day to day in all groups within a particular experiment (e.g. weeks 9–10; Fig. 2) might reflect changes in ambient temperature. However, because temperature was similar in all treatment groups within an experiment, the differences in prolactin secretion between lambs are attributable to the differential photoperiods.

It is unlikely that the differences in prolactin secretion between groups are due to differences in

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**FIGURE 3.** (a) Serum melatonin concentrations in samples collected 2–4 h after lights on (open bars) and 2 h after lights off (solid bars) once per week in female lambs exposed to 16 h light; 8 h dark (n = 8). (b) Corresponding melatonin levels in female lambs that were infused nightly for 5 weeks with 3–4.2 nmol melatonin/kg per kg body weight for the duration of the dark phase, starting at 2 weeks of age, as indicated by the arrow (n = 8). (c) Serum prolactin concentrations in these two groups of lambs: broken line, non-infused controls (data are replotted from Fig. 1; n = 8); solid line, melatonin-infused group (n = 8). The start of infusion is indicated by the arrow. Values are means ± S.E.M. (except for non-infused controls in c, where means are shown).

**FIGURE 4.** Serum melatonin concentrations in samples collected at 1- to 4-h intervals over 24 h from 7-week-old female lambs on 16 h light: 8 h dark (□; n = 8), and from age-matched female lambs on the same photoperiod infused with 3–4.2 nmol melatonin/kg per kg body weight for the duration of the dark period (■; n = 8). The shaded area represents the dark period. Values are means ± S.E.M.
stress between groups. Although certain studies in sheep have suggested that plasma prolactin concentrations are increased by restraint and handling (Davis, 1972), other studies in castrated adult sheep have not detected increases in circulating prolactin in response to restraint and isolation stress, even though these procedures significantly increased plasma cortisol concentrations (Parrott, Thornton & Robinson, 1988). In the current study, the long-day and short-day treatments were carried out in rooms of similar size, and handling procedures were similar. Furthermore, prolactin concentrations in the lambs infused with melatonin, or in those carrying sham-infusion harnesses, did not differ from uninfused control lambs. Therefore, any stress associated with wearing the harnesses or with daily handling to replace the melatonin solution in the pumps did not influence prolactin secretion.

Normal pineal gland function is necessary for the photoperiodic response. This was determined in the present study upon measurement of circulating prolactin after bilateral superior cervical ganglionectionomy, which prevents the nocturnal rise in circulating melatonin concentrations (Yellon & Foster, 1986; Foster et al. 1988). In the assay system using the Guildhay antiserum (English et al. 1986), values for serum melatonin in ganglionectionomized lambs are below the limit of detection (Foster et al. 1988). Ablation of the nightly melatonin increase beginning at 4 weeks of age prevented the subsequent long-day stimulation of prolactin secretion in our study. This is consistent with previous studies in older (5-month-old) lambs in which pineal excision rendered the young males unresponsive to the stimulatory effects on prolactin secretion of long days and of light pulses interrupting the night (Brown & Forbes, 1980; Brinklow & Forbes, 1984), and with several studies in adult sheep and goats demonstrating pineal mediation of seasonal photoperiod information on prolactin secretion (Barrell & Lapwood, 1979; Lincoln, 1979; Maeda, Mori & Kano, 1986). Because our lambs were housed with their pineal-intact mothers, yet long day-induced prolactin secretion was prevented by ganglionectionomy, it is unlikely that maternal signals convey significant photoperiod information to the suckling lamb. Before birth, however, maternal cues could be important. Recent studies in rodents reveal that the fetus can respond to photoperiod in utero (Horton, 1984, 1985; Stetson, Elliott & Goldman, 1986). This might also be the case in ruminants because circadian variation in serum melatonin concentrations has been detected in the fetal lamb at 120 days of gestation (Yellon & Longo, 1987). It is presently unclear as to what extent this reflects maternal melatonin secretion because melatonin readily crosses the ovine placenta (Kennaway, Matthews & Seamark, 1981).

We considered whether the age-related increase in the amplitude of the night-time melatonin rise in the sheep might be important to the development of photoperiodic responses. The amplitude of the nocturnal increase in melatonin concentrations in the fetal lamb, and in lambs at 1, 3 and 6 weeks of age, is typically 200–300 pmol/l (Yellon & Longo, 1987; experiment 3, this study; L. E. Claypool, R. I. Wood, F. J. P. Ebling & D. L. Foster, unpublished observations). This is two- to fivefold less than that detected with the same assay system in adult sheep, which is typically 500–2000 pmol/l (Malpaux, Robinson, Brown & Karsch, 1987). Perhaps the failure of long-day to short-day changes in photoperiod to initiate puberty early in life (Yellon & Foster, 1985) might result from the inability of the lamb to transduce photoperiod information, and further that this inability results from inadequate secretion of melatonin at night. This is unlikely to be the case, as inferred from our study in which nocturnal melatonin was increased to adult levels through timed infusions, and prolactin secretion, a second photoperiodic response, was examined. Supplementation of nocturnal melatonin had no additional effect on this response. Therefore, other explanations must hold for the failure of long-day to short-day photoperiod transitions in the early postnatal period to induce premature puberty. These most likely relate to the inadequate somatic growth of the lamb. Reproductive maturation in the lamb can be delayed indefinitely if growth is retarded by means of restricted dietary intake (Foster & Olster, 1985). Thus we hypothesize that the initiation of oestrous cycles in response to photoperiodic cues can occur only if internal signals relating to growth and metabolism are permissive (Foster, Yellon & Olster, 1985). Presumably, these developmental requirements, as yet unknown, have not been fulfilled in the neonatal sheep. Thus at such an early stage of life the initiation of oestrous cycles in response to changing photoperiod is not possible, although the prolactin response to photoperiod is fully competent.

Finally, our results indicating that a low-amplitude melatonin rhythm is entirely adequate to produce a long- or a short-day prolactin response is consistent with other findings in both the sheep and the Djungarian hamster; it is the duration of the nocturnal melatonin rise that determines the photoperiodic response, rather than the amplitude of this rise (Goldman, Darrow & Yoge, 1984; Karsch, Bittman, Foster et al. 1984).

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