Daily Changes in Concentrations of Prolactin in Serum of Prepubertal Bulls Exposed to Short- or Long-Day Photoperiods[1] (42886)

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Abstract. **Early temporal changes in concentrations of prolactin (PRL) in serum after a sudden change in photoperiod and daily responsiveness to PRL-releasing and inhibiting factors were investigated in prepubertal Holstein bull calves exposed to different photoperiods. In calves switched from 8-hr light:16-hr dark to 16-hr light:8-hr dark, there was no observable change in the daily pattern of serum concentrations of PRL after 1, 2, or 4 days. On the other hand, in animals switched from 16-hr light:8-hr dark to 8-hr light:16-hr dark, there was a consistent increase in serum PRL from 33.4 ng/ml on Day 0 to maximum values of 57.3, 62.7, and 78.9 ng/ml between 14 and 18 hr after onset of light on Days 1, 2, and 4, respectively. Thus, absence of light allowed expression of a daily rhythm in serum concentrations of PRL that persisted for at least 4 days after the photoperiod switch. There were no differences in L-dopa inhibition of PRL release in animals exposed to 16-hr light:8-hr dark at 3 or 15 hr after onset of light. However, thyrotropin-releasing hormone-induced release of PRL was greater 3 hr after onset of light (11 hr after onset of dark) compared with release at 9, 15, and 21 hr after onset of light in animals exposed to 16-hr light:8-hr dark, but not in bulls exposed to 8-hr light:16-hr dark. The results provide evidence that the cue for the putative photosensitive period of PRL secretion in cattle may be more closely associated with onset of dark, not onset of light.** [P.S.E.B.M. 1989, Vol 191]

Photoperiodic stimulation of circannual physiologic phenomena often takes weeks or months before changes are observed. In cattle, 6–8 weeks elapse before significant increases in body growth rate can be detected following a shift from 8 to 16 hr of light daily (1, 2). It takes 7–10 days before significant increases are observed in basal concentrations of prolactin (PRL) (3, 4). In birds, on the other hand, photoperiodic stimulation of at least one hormone, luteinizing hormone (LH), is established within 20–22 hr after shifting from 8 hr of light:16 hr of dark to 20-hr light:4-hr dark (5). Furthermore, successive increases occur for several days until gonadotropin secretion remains consistently high (6). In cattle, early temporal changes in a photoperiodically responsive trait, such as PRL, after a sudden change in photoperiod have not been studied.

Photoperiodic stimulation of body functions probably results from modification of neuroendocrine events set in motion by an internal biologic clock. Bunning advanced the hypothesis that photoperiodic time measurement is based on an endogenous circadian rhythm of photosensitivity (7). Later, Pittendrigh and Minis (8) suggested that light entrains the proposed cycle of endogenous photosensitivity, and if light coincides with the period of photosensitivity, a physiologic response occurs. In fact, a skeleton photoperiod of 6-hr light:8-hr dark:2-hr light:8-hr dark is as effective as 16-hr light:8-hr dark in stimulating PRL secretion in cattle (9). Similar observations were made in sheep (10). Cattle maintained for prolonged periods on repeating photoperiods of 8-hr light:16-hr dark or 16-hr light:8-hr dark do not express daily rhythmicity in basal concentrations of PRL in serum (9). In this study, our objectives were to determine early temporal changes in

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concentrations of PRL in cattle after an abrupt change in photoperiod and to determine daily responsiveness to PRL-releasing and inhibiting factors after prolonged exposure to different photoperiods.

Materials and Methods

Management of Animals and Blood. In each experiment, prepubertal Holstein bull calves (1–3 days of age) were housed in a light-controlled chamber for 6 to 8 weeks and exposed to 8-hr light:16-hr dark. Calves were individually penned in 1.2- × 1.8-m stalls. Cool-white fluorescent lamps emitting a median of 210 lux approximately 1 m above the floor were used. All calves were weaned from milk at approximately 6 weeks of age. After weaning, groups of four bulls each were relocated into one of two temperature- and light-controlled chambers as previously described (3). Calves were given a complete pelleted diet, alfalfa hay, trace mineralized salt, and water *ad libitum*. Blood samples were collected from a polyvinyl cannula inserted in a jugular vein 1 day prior to collection. Calves were undisturbed during sampling and at night samples were collected within 1 min or less with the aid of a flashlight (≤11 lux at 0.5 *M* from the animal). We (Peters and Tucker, unpublished data) previously showed that prolactin concentrations were similar when collected in total darkness or with short-term exposure to ≤11 lux of light. Blood samples were allowed to clot for 2 to 6 hr at room temperature; then samples were kept at 4°C for approximately 24 hr before centrifugation at 1000*g* for 20 to 30 min. Sera were decanted and stored at −20°C until assayed for PRL (11).

Experiment 1. The objective of this experiment was to measure the early changes in concentrations of PRL following an abrupt change in photoperiod. For this purpose, two groups of four bulls each were exposed for 7 weeks to 8 or 16 hr of cool-white fluorescent light daily (lights on at 0800 hr). Then, on a single day, light periods for each group were shifted from 8 to 16 hr or from 16 to 8 hr per day for an additional 4 days. Collection of blood started at 1500 hr the day before changes in the photoperiod were initiated (Day 0). Samples of blood were taken at 30-min intervals for 35 hr until 0200 hr on Day 1. Blood collection resumed at 30-min intervals for 6 hr beginning 12 hr after dawn (at 2000 hr) on Days 2 and 4. Based on our previous research (9), this sampling period should encompass the putative photosensitive period of PRL secretion.

Experiment 2. In this experiment, our objective was to measure concentrations of PRL after injections of thyrotropin-releasing hormone (TRH) at two different times of the day in animals exposed to 16-hr light:8-hr dark. Accordingly, seven bull calves were exposed to 16-hr light:8-hr dark for 6 weeks (lights on at 0300 hr). Then, on a single day, all animals received intravenously TRH (33 μg/100 kg body wt; 12) 3 and 15 hr after onset of light (0600 and 1800 hr). This dose of TRH maximizes PRL secretion (12). Blood samples were collected 120, 90, 60, 30, 15, 5, and 0 min before injection and at injection of TRH.

Experiment 3. In view of results obtained in Experiment 2, we decided to investigate further the daily variations of TRH-induced release of PRL in bull calves. Two groups of four bulls each were subjected to either 8 or 16 hr of light daily (lights on at 0800 hr) for 6 weeks. Then, each bull received on a single day a single intravenous injection of a half-maximal dose of TRH (16.5 μg/100 kg body wt; 12) at either 3, 9, 15, or 21 hr after onset of light. A 4 × 4 Latin square design balanced for residual effects (13) was used such that, on any given day, each of the four injection times were tested but each bull received TRH only once a day and 1 day was allowed between each injection. Each bull was tested at 3, 9, 15, or 21 hr after onset of light. Blood samples were collected 30, 15, 10, 5, and 0 min before injection and at 4, 6, 8, 10, 15, 20, 25, 30, 45, and 60 min after injection of TRH.

Experiment 4. In this experiment, our objective was to determine the effects of L-dopa, the immediate precursor of dopamine, on PRL release at two different times of the day in bull calves exposed to 16-hr light:8-hr dark. Dopamine is a well-known inhibitor of PRL secretion. L-dopa was used at a dose of 1.5 mg/100 kg body wt. In a preliminary trial, this dose was determined to suppress basal PRL concentrations 20–40%. After 6-week exposure to 16-hr light:8-hr dark (lights on at 0300 hr), on a single day, bulls ($n = 8$) received L-dopa intravenously 3 and 15 hr (0600 and 1800 hr) after onset of light. Blood was sampled as described in Experiment 2 plus additional samples were collected 10 min before and 90, 120, and 180 min after injection of L-dopa.

Statistical Analyses. Analyses were performed on data transformed to natural logarithms to reduce heterogeneity of variance of PRL. Transformed data were analyzed by split-plot analysis (13) with repeat measure over time in all experiments. Least-squares means reported, however, are untransformed. Differences among least-squares means were tested by the Bonferroni *t* test, a procedure designed for nonorthogonal, a priori comparisons (13).

Results

Experiment 1. Between 1500 hr on Day 0 and 1600 hr on Day 1, PRL averaged 22.0 and 36.4 ng/ml of serum and was different ($P < 0.05$) in bulls exposed to 8 or 16 hr of light daily, respectively. Beginning at 1630 hr on Day 1 (initial sampling time when both groups would experience a different photoperiod) and extending through 0200 hr, there was no observable change in mean concentration of PRL in animals switched from 8-hr light:16-hr dark to 16-hr light:8-hr dark (Fig. 1). Moreover, concentrations of PRL did not change in this group ($P > 0.05$) through Day 4 (Fig. 1),

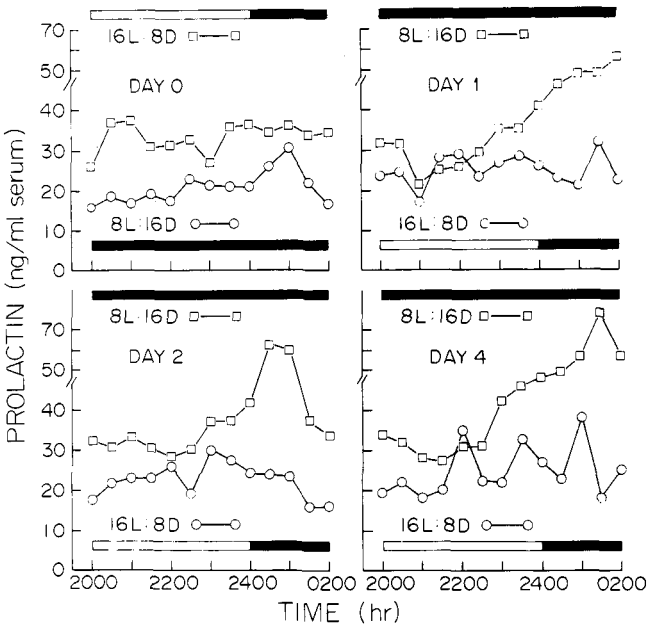

Figure 1. Basal PRL in serum of prepubertal bulls between 2000 and 0200 hr on the day before (Day 0) and 1, 2, and 4 days after switching photoperiod from 8-hr light:16-hr dark to 16-hr light:8-hr dark (open circles) or from 16-hr light:8-hr dark to 8-hr light:16-hr dark (open squares). Pooled SE was 1.4 ng/ml. Open horizontal bars indicate lighted times and closed bars dark times. Onset of lights was 0800 hr.

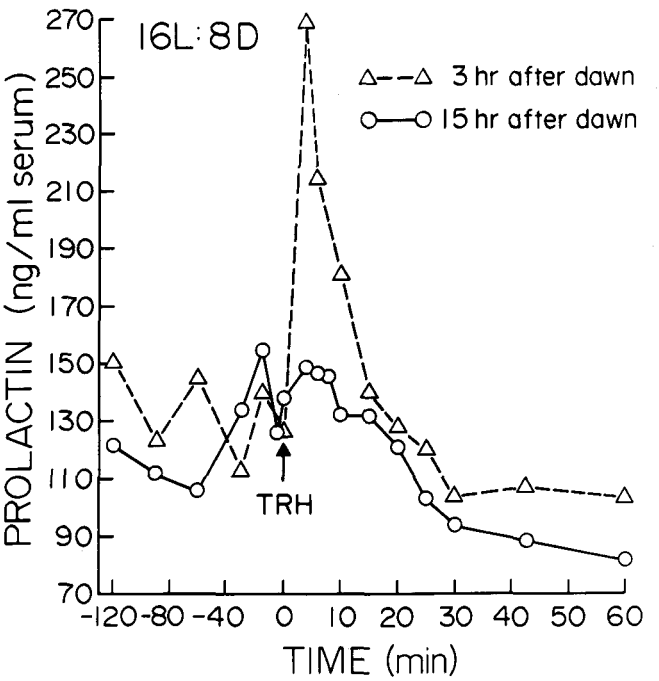

Figure 2. TRH-induced release of PRL in serum of prepubertal bulls exposed for 6 weeks to 16-hr light:8-hr dark. Pooled SE was 43.7 ng/ml. TRH was injected at (time 0) 3 (open triangles) and 15 hr (open circles) after onset of light. Onset of lights was 0300 hr.

even though there seemed to be greater pulsatility in PRL in these animals on Day 4.

In sharp contrast, in bulls switched from 16-hr light:8-hr dark to 8-hr light:16-hr dark, PRL decreased ($P < 0.05$) during the early dark period to a minimum of 21.6 ng/ml at 2100 hr and then increased ($P < 0.01$) to 57.3 ng/ml at 0200 hr. The nocturnal increases in concentrations of PRL recurred on Days 2 and 4 with PRL reaching peak values of 62.7 and 78.9 ng/ml of serum at 0030 and 0100 hr, respectively (Fig. 1). These nocturnal increases on Days 1, 2, and 4 were consistently greater ($P < 0.01$) than respective concentrations at similar times on Day 0.

Experiment 2. In calves exposed to 16-hr light:8-hr dark (Fig. 2), peak PRL in serum attained after TRH injection at 3 hr after onset of light (268.9 ng/ml) was greater ($P < 0.001$) than at 15 hr after onset of light (149.1 ng/ml).

Experiment 3. In animals exposed to 16-hr light:8-hr dark, more ($P < 0.01$) PRL (peak and area under the curve) was released in response to TRH 3 hr after onset of light than at 9, 15, and 21 hr after onset of light (Fig. 3). However, TRH-induced release of PRL did not vary in a daily pattern in animals exposed to 8-hr light:16-hr dark (Fig. 4). At every time tested, TRH-induced release of PRL was greater ($P < 0.01$) for animals subjected to 16 as compared with 8 hr of light daily (Figs. 3 and 4).

Experiment 4. There was no difference ($P > 0.05$) in the ability of L-dopa to inhibit PRL secretion at 3 or

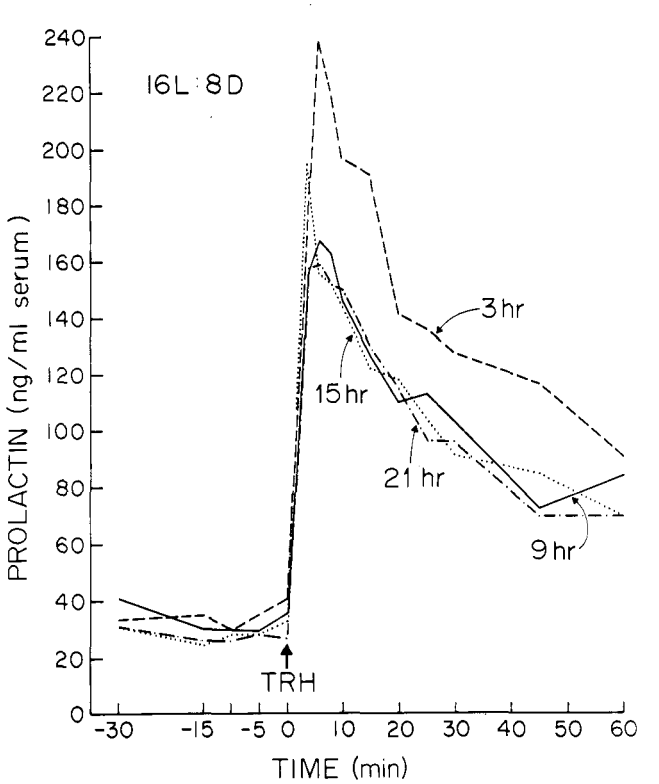

Figure 3. TRH-induced release of PRL in serum of prepubertal bulls exposed for 6 weeks to 16-hr light:8-hr dark. Pooled SE was 20.9 ng/ml. TRH was injected at (time 0) 3 (dash line), 9 (solid line), 15 (dotted line), and 21 hr (dashed-dotted line) after onset of light. Onset of lights was 0800 hr.

15 hr after onset of light (Fig. 5). Maximal inhibition ($P < 0.05$) was similar ($P > 0.05$) in both treatments (26.2 and 28.3%) and occurred within 6 min after injection of L-dopa (Fig. 5).

Discussion

A daily rhythm of photosensitivity in serum concentrations of PRL was reported in cattle (9), and a daily pulse of light between 14 and 16 hr after onset of light (6-hr light:8-hr dark:2-hr light:8-hr dark) was as effective in stimulating PRL as a continuous block of 16 hr of light (16-hr light:8-hr dark). In this study, a clear increase in concentrations of PRL was observed between 14 and 18 hr (2200 and 0200 hr) after onset of light (0800 hr) in animals switched from 16 to 8 hr of light daily; this increase in PRL coincided with the putative photosensitive period of PRL secretion. However, this effect was not observed in animals switched from 8 to 16 hr of light daily. Thus, it was absence of light which allowed expression of a daily rhythm in serum PRL concentrations in animals previously exposed to 16-hr light:8-hr dark.

In agreement with previous data (3, 4), photoperiod-induced stimulation of serum PRL concentrations was a relatively sluggish response. Four days after switching from 8 to 16 hr of light daily, there was still no significant increase in average PRL concentrations. PRL concentrations would be expected to increase within 7–10 days and attain maximal concentrations after 3–6 weeks of exposure to 16-hr light:8-hr dark (3,

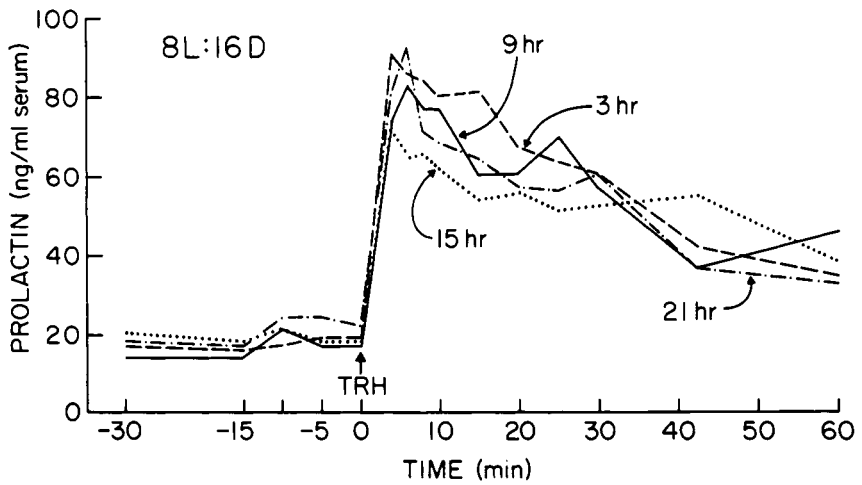

Figure 4. TRH-induced release of PRL in serum of prepubertal bulls exposed for 6 weeks to 8-hr light:16-hr dark. Pooled SE was 20.9 ng/ml. TRH was injected at (time 0) 3 (dash line), 9 (solid line), 15 (dotted line), and 21 hr (dashed-dotted line) after onset of light. Onset of lights was 0800 hr.

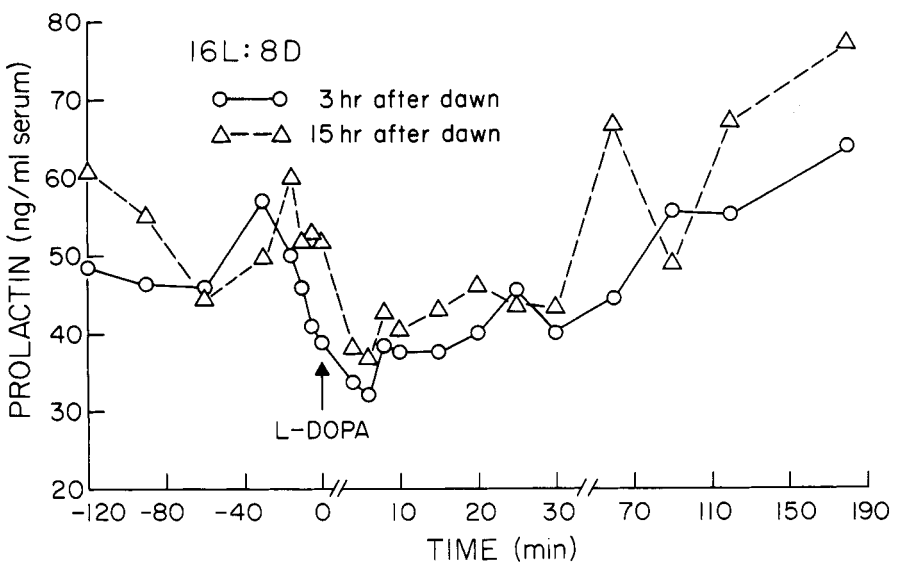

Figure 5. L-dopa-induced inhibition of PRL release in serum of prepubertal bulls exposed for 6 weeks to 16-hr light:8-hr dark. Pooled SE was 13.5 ng/ml. L-dopa was injected at (time 0) 3 (open circles) and 15 hr (open triangles) after onset of light. Onset of lights was 0300 hr.

4). The failure of PRL to change immediately after change in daily duration of light in cattle is in sharp contrast with results obtained in birds concerning photoperiodic stimulation of LH secretion (5). For example, 1 day after switching from 8-hr light:16-hr dark to 20-hr light:4-hr dark in birds, there is a significant increase in LH concentrations between 20 and 22 hr after onset of light. The cause of the relative sluggishness of PRL in cattle to respond to photoperiodic stimulation is not known. Furthermore, it is not known if this represents a difference between PRL and LH or between cattle and birds.

For the first time in cattle, we report that L-dopa inhibits PRL secretion, but this effect did not vary at 3 or 15 hr after onset of light in animals exposed to 16-hr light:8-hr dark.

TRH stimulates release of PRL in cattle (12, 14) and this response is markedly enhanced when cattle are exposed to 16-hr light:8-hr dark or 6-hr light:8-hr dark:2-hr light:8-hr dark as compared with 8-hr light:16-hr dark (3, 9). In this study, we confirmed these observations. In addition, we showed in two experiments using two different doses of TRH that release of PRL was greatest 3 hr after onset of light (or 11 hr after onset of dark) in animals exposed to 16-hr light:8-hr dark. In sheep, greatest concentrations of PRL in serum occurs when a 1-hr pulse of light is inserted 9–10 hr after onset of dark (15) and the cue for the photosensitive period of PRL secretion was related to onset of dark, not to onset of light. In other words, if darkness persists for more than 9–10 hr, animals exhibit a short-day response. In the present study, maximal release of PRL after TRH challenge did not coincide with the putative photosensitive period which occurs 14–16 hr after onset of light but occurred very close to the photosensitive period which Ravault *et al.* (15) postulated to be present 9–10 hr after onset of dark. Consequently, we suggest that these results favor the hypothesis that photoperiodic control of PRL secretion is associated with onset of dark, not onset of light. On the other hand, in bulls exposed to 8-hr light:16-hr dark, there was little variation in PRL responses after injection of TRH 1, 7, 13, and 19 hr after onset of dark. However, none of these challenges with TRH were sufficiently close to the putative photosensitive phase to produce maximal release of PRL. Thus, these data also support the concept that the cue for the putative photosensitive period is associated with onset of dark, not onset of light.

In cattle, daily variations in secretions from the pineal gland are probably not involved in mediating daily rhymicity in prolactin concentrations because pinealectomy does not prevent photoperiod-induced changes in serum prolactin (16, 17). Furthermore, administration of melatonin to mimic short or long days does not affect concentrations of prolactin in cattle (18). Dopamine is a known prolactin-inhibiting factor (19). In fact, administration of a dopaminergic receptor blocker (20) or sectioning of the pituitary stalk (21) potentiates TRH-induced release of PRL. Therefore, decreased dopamine input to the pituitary gland could account for increased secretion and enhanced TRH-induced release of PRL in cattle exposed to 16-hr light:8-hr dark photoperiod. On the other hand, a greater amount of TRH reaching the pituitary gland, an increased number of TRH receptors or a greater content of PRL in the pituitary gland could also explain these results. Clearly, more research will be needed to understand the neuroendocrine events involved in photoperiodic control of PRL secretion.

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