PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 172, 478–481 (1983)

Evidence for Photosensitive Regulation of Prolactin Secretion in Prepubertal Bulls[1] (41590)

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Abstract. Changing daily exposure of prepubertal bulls from 8 hr of light: 16 hr of dark (8L:16D) to 16L:8D or 6L:8D:2L:8D increased basal secretion of prolactin 418% 6 weeks later. When daily exposure was changed from 8L:16D (6 weeks) to 6L:14D:2L:2D (6 weeks), basal secretion of prolactin increased only 173%. Among photoperiod exposures, prolactin released into blood after injection of 33 μg/100 kg body weight of thyrotropin-releasing hormone paralleled the changes described for basal conditions. There was no repeatable diurnal secretory pattern for secretion of prolactin. The data support the hypothesis that cattle possess a photosensitive rhythm for secretion of prolactin.

According to the hypothesis of Bunning (1), animals measure time from the degree of coincidence between a daily endogenous rhythm and the exogenous rhythm of photoperiod. Pittendrigh and Minis (2) suggested that light entrains the proposed cycle of endogenous photosensitivity, and if the period of light coincides with the endogenous rhythm of photosensitivity, physiological responses occur. Increasing exposure of calves from a single continuous block of 8 hr of light to 16 hr of light per day increased concentrations of prolactin in serum four to eight-fold (3, 4). Similar observations have been made in sheep (5). Hence, in terms of secretion of prolactin in ruminants, there should be an endogenous daily rhythm in sensitivity to light. Indeed, a photoperiod of 7L:9D:1L:7D [light (L), dark (D)] was as effective as 16L:8D in stimulating secretion of prolactin in adult rams and ewes (5, 6). Insertion of an 1-hr pulse of light at other times during the scotoperiod was ineffective in stimulating secretion of prolactin. Furthermore, in sheep, there is a marked diurnal increase in secretion of prolactin at the beginning of the scotoperiod (5, 7, 8). Thus, there is good evidence in sheep of a photosensitive phase for secretion of prolactin. The objective of the present study was to determine if a photosensitive phase for secretion of prolactin occurs in cattle, a species in which there is no obvious pattern of changes (other than random) in the daily secretion of prolactin.

Materials and Methods. *Routine management of animals.* Prepubertal Holstein bulls approximately 6 weeks of age at the beginning of the experiments were housed at 20° ± 1°C. Cool-white fluorescent lamps emitting a median of 500 lux at eye level of calves were used. Calves were fed *ad libitum* a complete pelleted ration, alfalfa hay, and trace mineralized salt with free access to water.

Experiment 1. Two groups of four bulls each were exposed daily to 8L:16D for 6 weeks. Following this control period, one group was subjected to 16L:8D, while the other received 6L:8D:2L:8D for 6 additional weeks (through Week 12 of experiment). Arbitrary dawn for the 8-, 16- and 6-hr intervals of light was 0700 hr each day. The 2-hr interval of light occurred 14–16 hr after arbitrary dawn.

Experiment 2. During the first 6 weeks of this experiment, two groups of four bulls were maintained on 8L:16D. After this exposure, one group was exposed to 6L:8D:2L:8D, while the other received 6L:14D:2L:2D for 6 additional weeks (through Week 12 of experiment). Arbitrary dawn for the 8 and 6 hr intervals of light was 0700 hr. The 2-hr intervals of light occurred 14–16 or 20–22 hr after arbitrary dawn.

Collection of blood. Blood was collected from a polyvinyl cannula inserted into a jugular vein approximately 15 hr before collection of blood samples. At the end of Weeks

[1] Michigan Agricultural Experiment Station Journal Article No. 10643. This research was supported in part by USDA Grant 901-15-2 and USPHS Grant HD 09883.

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0037-9727/83 $1.50

6 and 12 in both experiments, animals were bled every 30 min for 24 consecutive hr. Thyrotropin releasing hormone (TRH, 33 μg/100 kg body weight) was administered at 0930–1000 (Experiment 1) or at 1100 (Experiment 2) hr following each 24-hr sampling period, and a series of blood samples were collected for an additional 45 (Experiment 1) or 60 (Experiment 2) min.

Blood was allowed to clot for 2 to 6 hr at approximately 21°C, then stored at 4°C for approximately 24 hr before centrifugation at 2000*g* for 20 to 30 min. Sera were decanted and stored at −20°C until assayed for prolactin (9).

Statistical analysis. Analyses were performed on data transformed to natural logarithms to reduce heterogeneity of variance of prolactin means. The transformed data were analyzed as a double split plot (10). Means and standard errors presented are not transformed.

Results. *Experiment 1.* At the end of 6 weeks of 8L:16D, basal prolactin was similar in both groups of calves, averaging 8.3 and 8.5 ng/ml of serum (Fig. 1). Following 6 weeks of 16L:8D or 6L:8D:2L:8D, basal prolactin increased ($P < 0.01$) to averages of 42.0 and 37.3 ng/ml of serum, respectively. These latter concentrations were not different from each other ($P > 0.10$). There was no apparent diurnal pattern of change in secretion of prolactin.

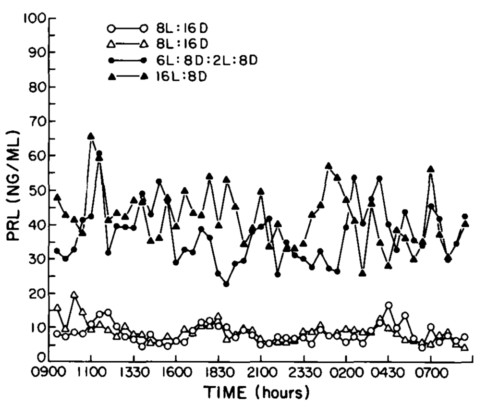

FIG. 1. Basal prolactin in serum of prepubertal bulls exposed for 6 weeks to 8L:16D (open symbols), then switched to 16L:8D or 6L:8D:2L:8D for 6 additional weeks (closed symbols). Onset of lights during the primary period of lighting was 0700 hr. Pooled SE of means during Weeks 6 and 12 were 0.4 and 3.3 ng/ml, respectively.

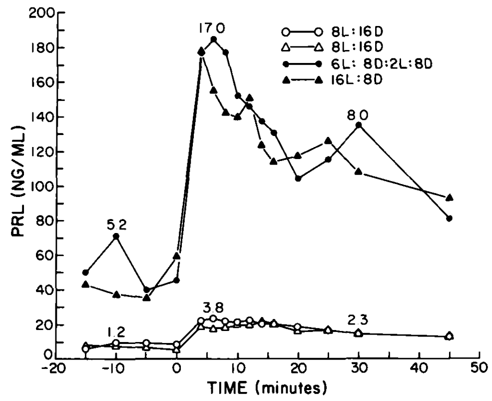

FIG. 2. TRH-induced release of prolactin in serum of prepubertal bulls exposed for 6 weeks to 8L:16D (open symbols), then switched to 16L:8D or 6L:8D:2L:8D for 6 additional weeks (closed symbols). TRH was injected at time 0 (0930 to 1000 hr). Onset of lights during the primary period of lighting was 0700 hr. Pooled SE of mean concentrations of prolactin measured between −15–0, 4–15, and 20–45 min appear above response curves.

At Week 6, prolactin averaged 11.8 ng/ml during intervals of light and 12.4 ng/ml during intervals of dark. At Week 12, prolactin averaged 43.7 and 52.6 ng/ml during light, and 38.5 and 47.2 ng/ml during dark for animals exposed to 16L:8D and 6L:8D:2L:8D, respectively. There were no differences in concentrations of prolactin during light versus dark periods ($P > 0.10$).

Greater quantities of prolactin were released following TRH when bulls were exposed to 16L:8D or 6L:8D:2L:8D then when exposed to 8L:16D (Fig. 2; $P < 0.05$). However, neither peak height nor shape of the response curve following TRH differed between the two groups of bulls within Weeks 6 or 12 ($P > 0.10$).

Experiment 2. Basal prolactin averaged 11.4 and 13.0 ng/ml of serum after 6 weeks of 8L:16D in the two groups of bulls (Fig. 3). Subsequently, prolactin increased ($P < 0.05$) to averages of 49.0 and 22.5 ng/ml following 6 weeks of 6L:8D:2L:8D or 6L:14D:2L:2D, respectively. Concentration of prolactin in bulls given 6L:8D:2L:8D was greater ($P < 0.05$) than for bulls maintained on 6L:14D:2L:2D. Diurnal variation in secretion of prolactin was not apparent. Prolactin averaged 8.8 ng/ml during light periods and 8.2 ng/ml during dark periods at Week 6. At Week

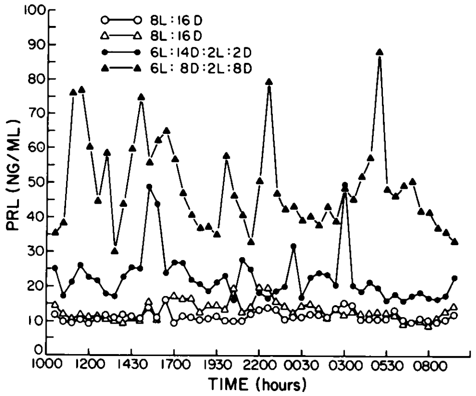

FIG. 3. Basal prolactin in serum of prepubertal bulls exposed for 6 weeks to 8L:16D (open symbols), then switched to 6L:8D:2L:8D or 6L:14D:2L:2D for 6 additional weeks (closed symbols). Onset of lights during the primary period of lighting was 0700 hr. Pooled SE during week 6 was 0.9 ng/ml; during week 12, pooled SE for 6L:14D:2L:2D and 6L:8D:2L:8D were 4.6 and 5.8 ng/ml, respectively.

12, prolactin averaged 38.7 and 23.7 ng/ml during the light periods, and 36.7 and 22.2 during dark periods for animals under 6L:8D:2L:8D and 6L:14D:2L:2D, respectively. Concentrations of prolactin during light periods did not differ from concentrations during dark periods in any treatment group ($P > 0.10$).

TRH-induced release of prolactin was greater ($P < 0.05$) in bulls given 6L:8D:2L:8D (4.9-fold) or 6L:14D:2L:2D (2.5-fold) than in bulls given 8L:16D (Fig. 4). Release of prolactin after TRH was greater ($P < 0.10$) in bulls given 6L:8D:2L:8D than in bulls exposed to 6L:14D:2L:2D.

Discussion. Results of this study confirm previous reports that increasing duration of daily light from 8 to 16 hr increases basal secretion and TRH-induced release of prolactin several fold in ruminants (3–5). In addition, we have now established that light does not need to be supplied as a continuous block of 16 hr since 6L:8D:2L:8D is as effective as 16L:8D in stimulating secretion of prolactin. This constitutes evidence that bull calves, similar to sheep (5, 6), possess a diurnal rhythm of sensitivity in terms of prolactin response to light. However, in cattle, this diurnal rhythm in photosensitivity appears to be unrelated to actual diurnal change in secretion of prolactin since they do not express any diurnal pattern of change in secretion of prolactin. In contrast, definitive increases in serum prolactin occur in sheep when lights are initially turned off (5, 7, 8).

The period of photosensitivity in adult sheep is a discrete period between 16 and 17 hr after dawn (5, 6). In contrast, results of the present study provide evidence that the period of photosensitivity for secretion of prolactin in prepubertal cattle extends at least from 14 to 22 hr after dawn. It should be noted, however, that insertion of a block of light between hours 20 and 22 after arbitrary dawn is much less effective in stimulating secretion of prolactin than insertion of light 14 to 16 hr after dawn. We speculate that the cycle of photosensitivity is waning by 20 to 22 hr after arbitrary dawn.

Ravault *et al.* (11) presented evidence in sheep that the phase of photosensitivity for secretion of prolactin may be related to onset of darkness, not to dawn. They observed greatest secretion of prolactin when a 1-hr pulse of light was inserted 9 hr after onset of darkness. Similarly, we obtained a greater increase in secretion of prolactin in bulls when a 2-hr pulse of light was given 8 hr as compared with 14 hr after onset of dark. Accord-

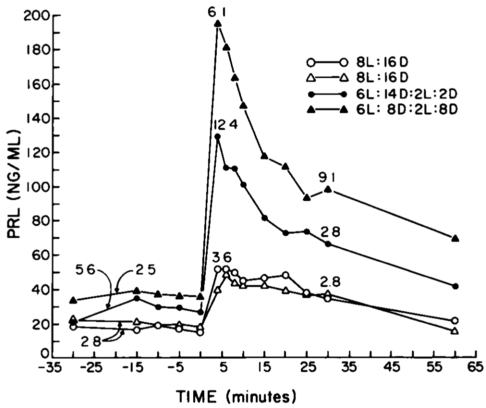

FIG. 4. TRH-induced release of prolactin in serum of prepubertal bulls exposed for 6 weeks to 8L:16D (open symbols), then switched to 6L:8D:2L:8D or 6L:14D:2L:2D for 6 additional weeks (closed symbols). TRH was injected at time 0 (1100 hr). Onset of lights during the primary period of lighting was 0700 hr. Pooled SE of mean concentrations of prolactin measured between −30–0, 5–15, and 20–60 min appear above response curves.

ing to this concept, duration of uninterrupted dark rather than light is the critical feature of a day in photoperiodic phenomena (2). Additional studies will be required to determine whether dawn or onset of dark more importantly affects secretion of prolactin in cattle.

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Received November 3, 1982. P.S.E.B.M. 1983, Vol. 172.