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Photoperiodic Effects on Steroid Negative Feedback in Female Prairie Voles (*Microtus ochrogaster*)

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Breeding in prairie voles is mainly restricted to the autumn and winter of most years. The organization of estrus in female prairie voles is unusual because behavioral estrus is induced by chemosensory stimuli from the urine of adult conspecific males. Isolated females exhibit undetectable levels of estradiol and never display estrous behavior, yet exposure to male urine causes a cascade of endocrine changes that evoke estrogen secretion from the ovaries and estrous behavior within 24 hr. In the prairie vole, the extreme dependence of estrus on chemosensory stimuli raises the possibility that their ovaries may be less prominent in the regulation of gonadotropin secretion than in species with more endogenously organized estrous cycles. The present study examined the contribution of the ovaries in luteinizing hormone (LH) regulation in prairie voles. Females were maintained for 9 weeks in either long (LD 16:8) or short (LD 8:16) photoperiodic conditions, a blood sample was obtained, and then animals were either ovariectomized or received a sham procedure. Another blood sample was obtained a week later and assayed for serum LH. Blood serum LH levels were significantly reduced in short-day voles, compared to long-day animals. After ovariectomy both long-day and short-day voles exhibited equivalent elevations in LH levels. This study provides evidence that photoperiod is measured in female voles and the ovaries appear to produce sufficient steroids to suppress LH release.

Female prairie voles (*Microtus ochrogaster*) do not exhibit estrous cycles and have extremely low circulating luteinizing hormone (LH) and estrogen levels (Richmond and Stehn, 1976; Carter *et al.*, 1980, 1989). Blood LH and estrogen levels increase after social interactions with an adult male conspecific or after application of male urine to the upper lip or external nares of females (Richmond and Conaway, 1969; Carter *et al.*, 1980; Cohen-Parsons and Carter, 1987; Dluzen *et al.*, 1981; Moffatt *et al.*, 1995). A single drop of adult male urine applied to the upper lip of female prairie voles increases gonadotropin releasing hormone (GnRH) content in the olfactory bulbs after 1 hr. Serum LH increases after 1 min of treatment, remains elevated at 30 min, and returns to baseline levels by 60 min (Dluzen *et al.*, 1981). The chemosignal that induces estrus does not appear to be airborne because females, separated from males by a double-wire mesh screen that prevents direct physical contact, remain anestrus (Carter *et al.*, 1980).

These characteristics suggest that the ovaries of prairie voles remain functionally quiescent until they are activated by a male-induced surge in serum LH levels. If this assumption is correct, then basal LH and estrogen blood levels in prairie voles may be regulated by steroid-independent mechanisms. The hypothesis that a negative-feedback mechanism may not be involved in the regulation of serum LH levels in female prairie voles, as it is in other species thus

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far examined (Everett, 1988), was tested in the present experiment by examining postovariectomy LH levels. If ovarian estrogen plays a role in regulating gonadotropin secretion and maintaining basal LH levels in prairie voles, then removal of the ovaries should cause a significant elevation in circulating LH levels. Alternatively, if the ovaries do not play a role in regulating gonadotropin secretion and maintaining basal LH, then removal of the ovaries should not affect LH levels.

Throughout much of their range, prairie voles breed primarily between March and October (Nelson, 1987). Nontropical rodent species that display similar seasonal reproductive patterns typically rely on photoperiod to phase breeding activities. Males and females undergo gonadal involution with the advent of autumnal short day lengths (Bronson and Heideman, 1994). Changes in day length evoke alterations in the release of LH in many species. These changes in LH secretion presumably represent changes that occur in the mechanisms governing the release of LH from the anterior pituitary. In Syrian hamsters (*Mesocricetus auratus*), photoperiod-induced changes in LH secretion are produced by a combination of steroid-dependent and steroid-independent mechanisms (Tamarkin *et al.*, 1976; Turek, 1977, 1979). That is, sex steroids have enhanced suppressive effects on LH secretion in short photoperiods relative to long photoperiods (steroid-dependent mechanism) (Tamarkin *et al.*, 1976; Turek, 1977); however, in the absence of the suppressive effects of sex steroids, LH levels are lower in short days as compared to long days (steroid-independent mechanism) (Turek and Campbell, 1979). If a steroid-independent mechanism regulates seasonal alterations in LH responses in prairie voles, then females maintained in long days should exhibit higher LH levels than females maintained in short days after ovariectomy. Thus, the present study had three goals: (1) to discover the extent to which basal LH levels in female prairie voles were maintained by a steroid-independent mechanism, (2) to determine the existence of photoperiod-induced changes in the LH levels of female prairie voles, and (3) to describe the extent to which any photoperiodic changes in LH levels were produced by a steroid-independent mechanism.

METHODS

Adult female prairie voles (*Microtus ochrogaster ochrogaster*) (>50 days) were used in this study. Animals were individually housed in polycarbonate cages (28 × 17 × 12 cm) with *ad libitum* access to food (Agway ProLab 2000; Syracuse, NY) and tap water throughout the study. All of the animals were born and maintained in long day lengths [LD 16:8; lights on 0700 hr Eastern Standard Time (EST)] until the start of the experiment. An ambient temperature of 21 ± 2° and a relative humidity of 50 ± 5% were maintained throughout the study.

At the start of the experiment approximately half of the animals ($n = 24$) were transferred to short day lengths (LD 8:16; lights on 0900 hr EST) and half ($n = 22$) remained in long days (LD 16:8). After 9 weeks, all of the animals were anesthetized with methoxyflurane vapors (Metofane; Pitman–Moore, Mundelein, IL) and a blood sample was obtained from the retro-orbital sinus (Riley, 1960). Voles then received either a bilateral ovariectomy or a sham procedure. All of the females were anesthetized 7 days later under methoxyflurane vapors and another blood sample was obtained.

Samples were stored at room temperature (20°) for 1 hr, and clots were removed. Blood samples were centrifuged for 1 hr at 3500 rpm at 4°; the supernatant was placed into a microcentrifuge tube and then stored at −80° until assayed for LH. Serum LH was determined using a competitive enzyme-linked immunosorbant assay (Assay Research, College Park, MD). Ninety-six-well immunoplates (Nunc, MaxiSorp) were incubated for 2 hr at room temperature with 100 μl/well of an ovine polyclonal antibody against rat LH diluted 1:1000 in a carbonate/bicarbonate buffer (0.1 *M*, pH 9.6). The plates were then washed four times with PBS (0.05 *M*, pH 7.4) containing 0.05% Tween-20 and 0.001% NaN_3 using an automatic microplate washer (Bio-Rad, Model 1550). A standard curve (upper limit, 1000 ng/ml; lower limit, 0.1 ng/ml) was prepared using purified rat LH diluted in a solution of 50% human serum. The standards (50 μl/well in triplicate) and samples of vole serum (25 μl/well in duplicate) were then placed in wells on the plates. Fifty microliters of biotinylated LH-diluted 1:1000 in standard diluent was then added to each well containing vole serum. The plates were incubated overnight at room temperature (21°) and then washed, and 100 μl of streptavidin-conjugated alkaline phosphatase diluted 1:1000 in standard diluent was added to each well and incubated at room temperature for 2 hr. The plates were washed again and 100 μl of substrate buffer [0.1 m*M* *p*-nitrophenyl phosphate in diethanolamine buffer (0.1 *M*, pH 9.5) containing 5 m*M* $MgCl_2$] was added to each well. The plates were incubated for 30 min and the optical density of the resulting colored product in each well was measured at 405 nm using an automated microplate reader (Bio-Rad, Model 450). The concentration of LH in the samples was determined relative to the standard curve.

The data were analyzed using repeated measures analyses of variance with the Systat statistical program employing day of blood sample as a within-subject variable. Differences among means were considered statistically significant if $P < 0.05$.

RESULTS AND DISCUSSION

Ovariectomized prairie voles exhibit higher circulating LH levels than sham-operated animals 7 days after surgery ($P < 0.001$) (Fig. 1). This response indicates that the ovaries of female prairie voles are usually an integral part of the mechanism regulating basal LH levels.

Day length affects serum LH levels of female prairie voles. Overall, females maintained in long day lengths display higher LH levels than females maintained in short day lengths ($P < 0.05$). There is no difference in the LH levels of females maintained in long and short day lengths 7 days after surgery ($P > 0.05$).

The ovaries of prairie voles play an important role in the regulation of circulating LH levels. Removal of the ovaries causes a dramatic increase in blood serum LH levels of females housed in both long and short day lengths, implying a release of negative feedback. This so-called "castration" response is consistent with the hypothesis that circulating LH levels are regulated by a negative feedback mechanism (Martin, 1985). These data suggest that, despite very low levels of circulating estrogen, the regulation of LH levels in female prairie voles is similar to that of females of more widely studied mammalian species.

Day length affects the basal LH levels of female prairie voles. Females maintained in short days displayed lower serum LH levels than females maintained in long-day-length conditions. This response to short day lengths is similar to that exhibited by male Syrian hamsters, ovariectomized female Syrian hamsters, or female field voles (*Microtus arvalis*) maintained in short photoperiods (Bittman *et al.*, 1992; Ebling *et al.*, 1992; Jetton *et al.*, 1991; Martinet and Meunier, 1975; Urbanski, 1992). No evidence from the present experiment indicated that a steroid-independent mechanism contributed to the reduction in LH levels exhibited by females in short day lengths. Short- and long-day animals exhibited comparable postovariectomy elevations in serum LH. If a steroid-independent mechanism was responsible for the short-day reduction in serum LH levels, then short-day females should have exhibited a smaller postovariectomy rise in LH levels than long-day animals. Presumably, the reduction in

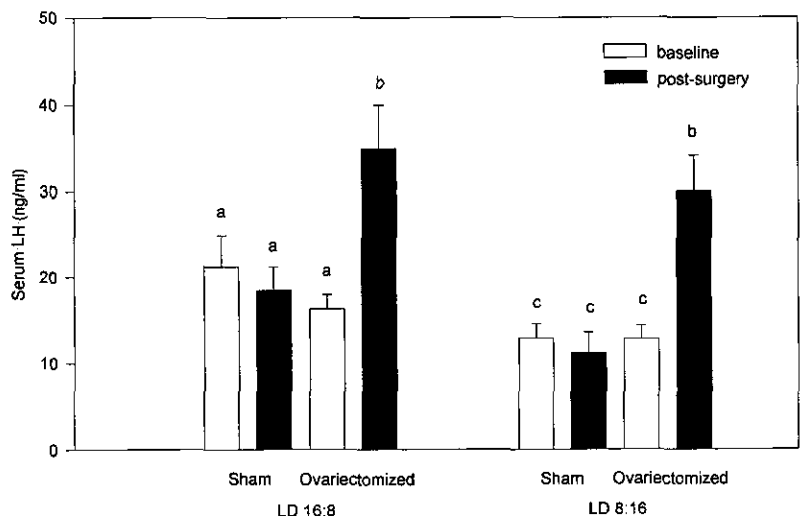

FIG. 1. Mean [±standard error of the mean (SEM)] blood serum LH levels (ng/ml) of female prairie voles that were maintained in either long (LD 16:8) or short (LD 8:16) day lengths. The animals were either ovariectomized or sham-ovariectomized. The baseline LH levels were measured in serum samples obtained immediately before surgery, and the postsurgical samples were obtained 7 days after the surgery. Columns sharing superscripts are statistically equivalent.

circulating LH levels exhibited by short-day voles could be the result of increased sensitivity of the negative-feedback system regulating LH levels. It is also possible that photoperiod-mediated alterations in LH turnover or GnRH responsiveness contribute to the present results.

Additional studies are required to test the hypothesis that alterations in the sensitivity of the negative-feedback mechanisms regulating LH are mediated by photoperiod. An experiment to determine the amount of estrogen necessary to suppress LH levels in ovariectomized voles maintained in long or short photoperiods is necessary. If a steroid-dependent mechanism is responsible for the observed difference in basal LH levels, then the serum LH levels of ovariectomized voles maintained in short days should be suppressed by lower doses of estrogen than the dose required to suppress the LH levels of ovariectomized females maintained in long-day conditions. The role of the adrenal glands in gonadotropin regulation should also be examined in order to understand the unusual regulation of estrus in prairie voles.

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