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Progesterone Inhibits Female Courtship Behavior in Domestic Canaries (*Serinus canaria*)

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We studied copulation solicitation display (CSD) responses to playback in photostimulated female canaries given systemic injections of progesterone. Eight females received injections of 0.1 mg of progesterone dissolved in olive oil during their first breeding cycle and were untreated during their second breeding cycle; eight females received only the oil vehicle during their first breeding cycle and received no treatment during their second breeding cycle. The injections were performed every second day during 15 days, after the onset of nest building. Progesterone treatment resulted in a significant increase of plasma progesterone which in turn provoked an inhibition of females' CSDs and decreased the size of the clutch. During the first breeding cycle, progesterone-treated females had lower CSDs and egg-laying scores than did control females. During the second breeding cycle, when females received no treatment, no differences emerged between the two groups. The suppressive effect of progesterone on female sexual responses was observed as soon as 48 h after the beginning of the treatment. We propose that progesterone plays a key role in mediating the transition from active female courtship behavior to sexual refractoriness in this species. Suppressive effects of progesterone on female sexual behavior have been previously described in lizards as well as in rodents. Our data are consistent with the hypothesis of Godwin *et al.* (J. Godwin, V. Hartman, M. Grammer, and D. Crews, *Horm. Behav.* 30, 138–144, 1996) which proposed that the decrease in sexual behavior following plasma progesterone increase represents an evolutionarily conserved mechanism in the regulation of female sexual behavior.

Key Words: **progesterone; courtship behavior; female canary.**

In mammals, sequential release of the ovarian steroid hormones estradiol and progesterone during the breeding season coordinates the behavioral transitions of the female's reproductive cycle. Gonadal steroids exert modulating influences upon the central nervous system, resulting in physiological as well as behavioral changes (Mani, Blaustein, and O' Malley, 1997). In rodents, progesterone initially facilitates and later inhibits sexual receptivity (Blaustein and Wade, 1977; González-Mariscal, Melo, and Beyer, 1993).

Experimental studies on hormonal regulation of reproductive behavior in female birds are scarce. Nest building is an estrogen-dependent behavior in female domestic canaries (*Serinus canaria*) (Hinde and Steel, 1976, 1978; Hinde, Steel, and Follett, 1974). Estradiol is also involved in the regulation of courtship behavior in female passerine birds, with the main evidence coming from field and laboratory studies showing that conspecific song playback elicits copulation solicitation displays (CSDs) in wild female songbirds primed with estradiol (Moore, 1982, 1983; O'Loghlen and Beecher, 1997; Searcy, 1992; Searcy and Capp, 1997; Searcy and Marler, 1981). Female canaries which breed in captivity produce displays without estradiol priming (Leboucher, Kreutzer, and Dittami, 1994). However, the emergence of CSDs is delayed in female domestic canaries whose plasma estradiol is artificially reduced at the very beginning of the breeding period, emphasizing the critical role of estradiol in sexual soliciting (Leboucher, Béguin, Mauget, and Kreutzer, 1998).

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In birds, progesterone is generally associated, in both sexes, with incubation readiness (Askew, Georgiou, Sharp, and Lea, 1997; Logan and Wingfield, 1995; Sockman and Schwabl, 1999). Exogenous progesterone treatment has little or no additional stimulatory effect on the sexual receptivity of estradiol-primed female Japanese quails (Adkins and Adler, 1972; Deville and Balthazart, 1987; Noble, 1972). In contrast, the inhibitory effect of pharmacological dosages of progesterone on male sexual behavior was demonstrated, a long time ago, by the use of systemic and brain implants (Riddle and Lahr, 1944; Komisaruk, 1967). A suppressive effect of progesterone on female sexual behavior was incidentally observed in a study dealing with the control of nesting in ovariectomized turkey hens (*Meleagris gallopavo*) (El Halawani, Silsby, Behnke, and Fehrer, 1986); in this study, estradiol-primed hens showed sexual squatting whereas females treated with estradiol and progesterone failed to show sexual activity. Suppressive effects of progesterone on female sexual behavior have been clearly established in lizards (Godwin, Hartman, Grammer, and Crews, 1996) as well as in rodents (Blaustein and Wade, 1977; González-Mariscal *et al.*, 1993).

In birds, progesterone is mainly produced by mature follicles in the few days before ovulation (Bahr, Wang, Huang, and Calvo, 1983; Lipar, Ketterson, Nolan, and Casto, 1999). In female canaries, progesterone increases sharply 1 day before the first egg is laid and maintains a high plateau through 2 days after the onset of laying (Sockman and Schwabl, 1999). In the majority of bird species, copulation is frequent 1 day before the first egg is laid and decreases abruptly after the first egg is laid (Birkhead and Møller, 1992); canaries share this general pattern (Leboucher *et al.*, 1994; and unpublished observations).

We hypothesized that the rise in progesterone, which normally occurs during egg laying, might be responsible for the transition of female canaries from active courtship to sexual refractoriness. In this study, we used progesterone treatment in order to inhibit the development of female courtship behavior in domestic canaries.

METHODS

Subjects and Housing

The subjects of the experiment were 16 one-year-old female domestic canaries (*Serinus canaria*), without reproductive experience and weighing 23.1 ± 1 g. Before the experiments, all females were housed during 3 months in single-sexed groups in aviaries on a short daylight schedule (LD 9:15). Under these conditions the birds show no reproductive activity. Eight days before the beginning of the experiments, the subjects were housed in individual cages (38 × 33 × 26 cm). Reproductive activity was then stimulated by exposure to a photostimulatory lighting regime (LD 15:9). The day before the beginning of the experiments, individual cages were fitted with nest bowls (10-cm diameter) and each bird was supplied with 8 g of cotton strings placed in a dispenser attached near a perch within its reach. Females were given seeds, fresh vegetables, and water on a daily basis.

Experimental Groups

Females were randomly assigned to one of the two experimental groups. The eight females of the progesterone-treated group (P group) received injections of progesterone dissolved in olive oil during their first breeding cycle and were untreated during their second breeding cycle. The eight females of the control group (C group) received only the oil vehicle during their first breeding cycle and received no treatment during their second breeding cycle. We chose to administer hormonal treatment during the first breeding cycle rather than during the second cycle because sexual displaying tends to be more pronounced during a first cycle than during subsequent cycles (authors' unpublished data).

Behavioral Observations

CSDs. Female sexual responsiveness to male songs was assessed in two tests each day, one in the morning (11:00 to 12:00) and one in the afternoon (15:00 to 16:00). During song test sessions, individual cages were placed in a separate room, in glass-enclosed sound attenuation chambers (52 × 50 × 40 cm inside, 80 × 72 × 70 cm outside). Female canaries were presented with four different playback songs. Songs were extracted from domestic canary repertoires; each song lasted 10 s, a duration that is within the normal range for male canaries. Two consecutive songs were separated by a pause of 9 s. All the females were tested with the same songs. Stimuli were played back by a tape recorder (50–14,000 Hz) connected to a speaker (100–18,000 Hz) placed within the attenuation chamber. The degree of a female's sexual response was measured by the number of complete CSDs. We con-

sidered that a complete display had occurred when the female crouched and arched her back while vibrating her wings that she held away from her body. Each complete display was scored as a unique event.

Measures of building and laying activities. Every day, between 16:00 and 17:00, females were supplied with 8 g of cotton strings placed in a dispenser. During the same time, the dispensers provided the day before were removed. The weight of cotton strings gathered (i.e., removed from dispensers) was taken, and the number of eggs was recorded in each nest.

First Breeding Cycle—Hormonal Treatment

Treatment began after the onset of nest building and lasted 15 days. Estradiol is considered to exert a permissive effect on progesterone through the induction of progesterone receptors (Askew *et al.*, 1997; Balthazart, Blaustein, Cheng, and Feder, 1980); thus, estradiol priming is generally required before progesterone treatment. As previously mentioned, nest building is an estrogen-dependent behavior in female domestic canaries (Hinde and Steel, 1976); we considered that actively building females had adequate plasma estradiol and could receive progesterone injections without estradiol priming. In a previous study (Leboucher *et al.*, 1994), we observed that nest building occurred about 5 days before the laying of the first egg, whereas courtship behavior was displayed from about 3 or 4 days before the beginning of egg laying until the third day after the first egg was laid. Thus, the period of treatment clearly overlapped the period of sexual receptivity.

From the onset of the experiment, females were checked daily for nest building and egg laying and tested twice daily for sexual responsiveness. When a female began to build a nest, she was randomly assigned to one of the two experimental groups. We considered that a female began to build a nest when she gathered at least 4 g of nesting material and placed it in her nest cup without scattering the strings on the floor of her cage. When a female reached this criterion, she was no longer monitored for nest building. Treatment began on day 0, determined as the day when a female reached the criterion for nest building. Females which began to display CSDs before the onset of nest building were treated after the first test during which they displayed sexual responses. Eight females of the P group received subcutaneous injections of 0.1 mg of progesterone (Sigma) dissolved in 0.1 ml of pure olive oil (about 4.3 mg/kg of progesterone). Eight females of the C group only received subcutaneous injections of 0.1 ml pure olive oil. The injections were performed every second day between 17:00 and 18:00. Treatment lasted 15 days and each bird received a total of eight injections (on days 0, 2, 4, 6, 8, 10, 12, and 14). Each female was tested for sexual responsiveness until the 12th day after the beginning of the hormonal treatment, but egg laying was monitored until the end of the hormonal treatment.

Second Breeding Cycle

At the end of the first reproductive cycle, the females were allowed to incubate their unfertilized eggs for 10 days. Then, the incubation was disrupted by nest withdrawal. Two days after nest withdrawal, each female was given another nest bowl and a new dispenser of cotton strings (8 g) to stimulate the start of a second reproductive cycle. During this second reproductive cycle, females were checked daily for egg laying and were tested twice a day for sexual responsiveness, as previously described. During this second reproductive cycle, the females of the two groups did not receive any hormonal treatment.

Blood Samples and Hormone Assays

During the first breeding cycle, blood samples (approximately 350 μl) were collected by venepuncture from the wing vein using a heparinized syringe and immediately centrifuged. The first blood sample was collected 1 h before the first injection; the second sampling was performed 24 h after the last injection. Plasma was stored at -20°C until assayed for progesterone.

Plasma progesterone was measured in ether-extracted samples (50 μl) by radioimmunoassay using a specific antiserum (supplied by Dr. J. Fiet, Hôpital St. Louis, France). As shown for other birds (Mauget, Jouventin, Lacroix, and Ishii, 1994), we confirmed that the assay is reliable without celite chromatography following extraction. Duplicate aliquots (100 μl) of the extracts were incubated overnight at 4°C with 8000 cpm of [^{3}H]progesterone (Amersham Pharmacia Biotech, France) and antiserum. The bound and free progesterone was separated by adding dextran-coated charcoal. After centrifugation the bound fraction was counted in a liquid scintillation counter. Minimal detectable progesterone levels were 0.1 ng/ml. The coefficients of variation intra- and interassay were 6 and 12%, respectively. However, in this study, to exclude

interassay variance, all samples were measured in a single radioimmunoassay.

Statistical Analysis

Parametric statistics were used to analyze data (Winer, 1971). Analyses were computed using Sigmastat 2.03 (SPSS Inc., Chicago, IL). A *t* test was used to estimate the effect of hormonal treatment on progesterone plasma. To compare the total number of CSDs and the total number of eggs in the two groups during the two successive breeding cycles, we used a two-way ANOVA for repeated measures. The development of CSDs and egg laying during the first breeding cycle was also analyzed by a two-way ANOVA for repeated measures. ANOVAs were followed by post-hoc multiple-comparisons tests (Bonferroni *t* tests). CSDs data were $\log_{10}$-transformed so that groups of data with different variances could be compared parametrically.

RESULTS

Before the beginning of treatment, the mean level of progesterone was similar for the two groups (1.54 ± 0.19 ng/ml for the P group and 1.11 ± 0.28 ng/ml for the C group; *t* test, $t = 1.54$, $df = 14$, $P > 0.10$). One day after the last injection, there was an increase of plasma progesterone within the physiological range in seven of the eight progesterone-treated females. These seven progesterone-treated females had higher concentrations of plasma progesterone than did control females (1.78 ± 0.17 ng/ml for the P group vs 1.07 ± 0.26 ng/ml for the C group; *t* test, $t = 2.25$, $df = 13$, $P < 0.05$). Only one progesterone-treated female had a supraphysiological increase of plasma progesterone (40.21 ng/ml). All the results of this female were discarded from further analyses.

During the first breeding cycle, only one control and one progesterone-treated female began to display CSDs before the beginning of nest building. The CSDs emitted before treatment (16 for the P female group and 4 for the C female group) were not included in any of the results.

Comparison of the Two Groups during the Two Breeding Cycles

During the first breeding cycle, females of the C group exhibited more CSDs and laid more eggs than did females of the P group. In contrast, during the second breeding cycle, when animals received no treatment, no significant differences were found between the two groups. Detailed results and statistical analysis are presented in Table 1.

TABLE 1

Copulation Solicitation Displays (CSDs) and Egg Laying in Female Canaries during Two Consecutive Breeding Cycles

	First cycle (treatment)	Second cycle (no treatment)
CSDs[a]		
P group ($n = 7$)	3.0 ± 2.21	7.43 ± 2.35*
C group ($n = 8$)	13.38 ± 3.28*	8.63 ± 3.67*
Number of eggs[b]		
P group ($n = 7$)	2.14 ± 0.63	3.57 ± 0.37*
C group ($n = 8$)	4.0 ± 0.19*	3.88 ± 0.26*

Note. During the first breeding cycle, females of the P group were treated with progesterone; females of the C group only received the vehicle. During the second breeding cycle, the two groups were not treated. Statistical analyses: two-way ANOVA for repeated measures.

[a] CSDs: group effect, $F_{(1)} = 3.91$, $P = 0.07$; repetition, $F_{(1)} = 0.39$, $P = 0.54$; interaction, $F_{(1)} = 10.82$, $P = 0.006$.

[b] Number of eggs: group effect, $F_{(1)} = 4.80$, $P = 0.05$; repetition, $F_{(1)} = 6.57$, $P = 0.025$; interaction, $F_{(1)} = 9.81$, $P = 0.009$.

* Indicates a significant difference (Bonferroni *t* test, $P < 0.05$) with the progesterone-treated females (P group, first cycle).

Development of CSDs and Egg Laying in the Two Groups during the First Breeding Cycle

We analyzed the development of CSDs and egg laying during the first breeding cycle. Control females displayed more CSDs than did progesterone-treated females on days D2, D3, D4, and D5. Moreover, control females laid more eggs than did progesterone-treated females on days D9 to D12. Detailed results and statistical analysis are presented in Fig. 1.

Finally, in the P group, two females failed to lay, two females laid only two eggs, one female laid three eggs, and two females laid normal clutches of four eggs; in the C group, one female laid five eggs, six females laid four eggs, and one female laid three eggs.

DISCUSSION

Progesterone treatment resulted in a significant increase of plasma progesterone which in turn provoked an inhibition of females' courtship behavior and a

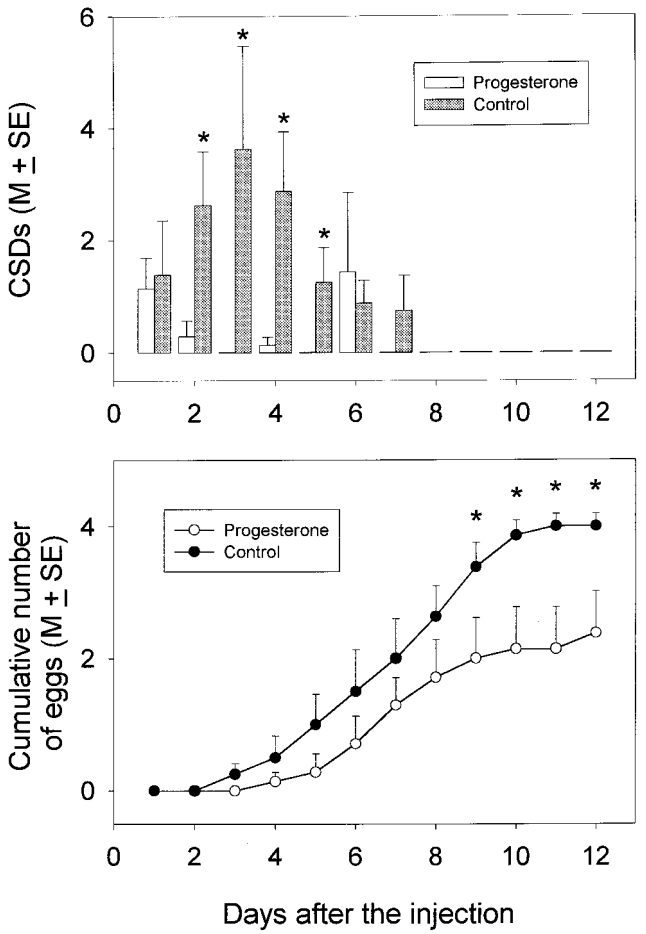

FIG. 1. Development of CSDs and egg laying during the first breeding cycle in progesterone-treated (open bars, open circles) and control females (gray bars, solid circles). Statistical analyses: two-way ANOVA for repeated measures. CSDs: group effect, $F_{(1)} = 8.35$, $P = 0.013$; repetition, $F_{(11)} = 3.70$, $P < 0.001$; interaction, $F_{(11)} = 2.43$, $P = 0.008$. Number of eggs: group effect, $F_{(1)} = 4.41$, $P = 0.056$; repetition, $F_{(11)} = 40.46$, $P < 0.00001$; interaction, $F_{(1)} = F_{(11)} = 2.94$, $P = 0.002$. * indicates a significant difference (Bonferroni t test, $P < 0.05$) between the two groups.

reduction of egg laying. The inhibitory effect of progesterone on egg laying was not unexpected because long-lasting treatments with progesterone are known to depress plasma luteinizing hormone (LH) and to delay the onset of lay in prepubertal pullets (Johnson, 1983); progesterone implantation also provokes a depression of egg production in turkey hens (Mashalay, Proudman, and Wentworth, 1979).

In light of these previous results, the suppressing effect of progesterone on female courtship could be regarded as the consequence of the inhibitory effect of long-lasting progesterone treatment on the release of gonadotropic hormones. The inhibition of gonadotropin release would provoke a depression of follicular development, a decrease of estradiol secretion (Harvey, Scanes, and Phillips, 1986; Sharp, 1996), and a consecutive inhibition of courtship behavior. However, it must be pointed out that as early as day 2, control females showed more CSDs than did progesterone-treated females (Fig. 1). At this time, treated females received only a single injection of 0.1 mg of progesterone on day 0; the second injection occurred on day 2, after behavioral testing. Thus, the effect of progesterone on sexual behavior cannot be considered as the result of a long-lasting treatment.

In a previous study, we found that sexually active female canaries treated with Fadrozole, an inhibitor of the aromatization of androgens to estrogens, showed a reduction of plasma estrogen and a pronounced inhibition of egg laying; despite these marked effects, treated females had normal sexual responses. We suggested that only a threshold concentration of plasma estradiol was required to activate the neural circuitry mediating the courtship response (Leboucher *et al.*, 1998). In the present study, progesterone treatment began after the onset of nest building, an estrogen-dependent behavior in female domestic canaries (Hinde and Steel, 1976), and we can consider that actively building females had adequate estradiol in the plasma at the beginning of treatment. Moreover, five of the seven progesterone-treated females laid reduced or normal clutches; these females required adequate concentrations of plasma LH at the time of ovulation (Harvey *et al.*, 1986; Sharp, 1996).

Thus, it seems unlikely that progesterone modifies the courtship response through an indirect effect on ovarian activity; our data rather suggest that progesterone works directly at the brain level to inhibit sexual behavior. Moreover, our results are consistent with the hypothesis that the rise in progesterone, which normally occurs during egg laying, is responsible for the transition of female canaries from active courtship to sexual refractoriness.

Our experiment gives no information about the cellular mechanisms mediating the suppressing effect of progesterone on sexual behavior. Previous experiments suggested that downregulation of estrogen receptors represented a fundamental mechanism of progesterone action in birds as well as in mammals (Selcer and Leavitt, 1988). Selcer and Leavitt (1988) showed that a downregulation of estradiol receptors in chick oviduct occurred 4 h after acute progesterone treatment.

In whiptail lizards (*Cnemidophorus* sp.), progesterone was found to inhibit female-typical receptive behavior and to decrease estrogen and progesterone receptor expression in the hypothalamus (Godwin *et al.*, 1996).

It is noteworthy that progesterone was found to exert a suppressive effect on female sexual behavior in reptiles (Godwin *et al.*, 1996) as well as in birds (El Halawani *et al.*, 1986; and present study) and mammals (Blaustein and Wade, 1977; González-Mariscal *et al.*, 1993). The consistency of progesterone effects among species of different vertebrate classes supports the hypothesis of Godwin and co-workers (1996) that the decrease in sexual behavior following plasma progesterone increase might represent an "evolutionarily conserved mechanism in the regulation of female sexual behavior."

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