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BRIEF COMMUNICATION

Administration of Gonadotropin-Releasing Hormone to Pregnant Rats Inhibits Postpartum Sexual Behavior[1]

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DUNLAP, K. D. AND R. SRIDARAN. *Administration of gonadotropin-releasing hormone to pregnant rats inhibits postpartum sexual behavior.* PHARMACOL BIOCHEM BEHAV 31(3) 725–728, 1988.—Administration of gonadotropin-releasing hormone (GnRH) can both facilitate sexual behavior in estrogen-primed, ovarietomized rats and induce numerous antifertility effects in pregnant rats. The present study was undertaken to determine the effect of GnRH treatment on 1) sexual behavior of rats during late pregnancy and at postpartum and 2) plasma levels of dihydrotestosterone (DHT) and progesterone (P), two hormones implicated in the regulation of sexual behavior during pregnancy. Rats were given either 4 μg or 12 μg GnRH or no treatment on day 18 of pregnancy. On days 18 (4 hr posttreatment) and 19 of pregnancy, the day of parturition and the day following parturition, each rat was tested for lordosis response to male mounts and subsequently bled from the jugular vein for determination of plasma DHT and P levels by radioimmunoassay. Both doses of GnRH significantly inhibited lordosis at postpartum estrus and failed to induce lordosis on days 18 and 19 of pregnancy and the day following parturition. DHT was suppressed on day 19 and at postpartum; P was elevated on the day following parturition. Our data demonstrate that as little as 4 μg GnRH can induce postpartum "behavioral antifertility" effects without apparent detriment to the pregnancy.

Gonadotropin-releasing hormone Sexual behavior Postpartum Pregnancy Dihydrotestosterone Progesterone

ADMINISTRATION of gonadotropin-releasing hormone (GnRH) to female rats can both stimulate reproductive behavior and disrupt reproductive physiological function. The ability of GnRH to regulate ovarian steroid production and ovulation through its control of gonadotropin secretion and independently stimulate lordosis behavior in ovariectomized, hypophysectomized (ovx/hypox) rats (14) has led to the conclusion that GnRH acts to synchronize the endocrine and behavioral events necessary for successful reproduction (13). However, GnRH administration to rats can also induce numerous antifertility effects including inhibition of steroidogenesis and ovulation, and pregnancy termination [for review, see (12)]. Our study was undertaken to determine the effect of GnRH treatment on sexual behavior in late pregnancy.

Pregnant rats show virtually no lordosis behavior at any time (15). Progesterone (P) and dihydrotestosterone (DHT) have been implicated as endogenous inhibitors of sexual behavior during pregnancy (1). GnRH is known to have a suppressive effect of P in vivo and in vitro (12,17) and androgens in vitro (4). In the present study it was hypothesized that GnRH may facilitate sexual behavior during pregnancy by its direct stimulation of neural tissues and/or its suppression of these two putative behavioral inhibitors.

On the evening following parturition the females of many polyestrus mammalian species ovulate and become sexually receptive. Although this postpartum estrus may well be the period when conception usually occurs among rats in the wild (5), relatively little is known about the hormonal regulation of postpartum sexual behavior. Prompted by an endogenous pulse of GnRH, gonadotropin levels surge shortly after parturition to initiate postpartum ovulation (5). In this study we examined whether exogenous administration of GnRH would affect postpartum sexual behavior.

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TABLE 1

LORDOSIS QUOTIENTS OF RATS GIVEN GnRH OR NO TREATMENT AT 1830 HR ON DAY 18 OF PREGNANCY[a]

Treatment	Pregnancy D18	Pregnancy D19	Postpartum	Day Following Parturition
Control	0	0	97 ± 2*	0
GnRH (4 μg)	0	0	77 ± 14*	0
GnRh (12 μg)	0	0	26 ± 11*	0

*All values significantly different from each other ($p<0.05$).
[a]All behavioral tests were conducted between 2200 and 2300 hr.

METHOD

Twenty-three pregnant Sprague-Dawley rats were obtained from Holtzman Company (Madison, WI). The day of insemination was designated day 1 of pregnancy. Animals were housed one per cage under 14 L:10 D lighting conditions. The room temperature was maintained at 25–27°C, and food and water were provided ad lib throughout the experiment.

Ten to twenty minutes before the onset of dark on day 18 of pregnancy, 0.75 ml of blood was collected by jugular venipuncture from 17 rats under ether anesthesia. While still anesthetized, 8 animals were injected SC with 4 μg GnRH (U.S. Biochem No. 40553) in 0.25 ml saline, and 9 animals received 12 μg GnRH in 0.75 ml saline. Six control animals received no treatment.

Three to four hours after the onset of dark on days 18 and 19 of pregnancy, the day of parturition and the day following parturition, each female was placed in a 38×42×20 cm testing arena lighted only by a 25 W red light. Lordosis responses to male mounts were scored according to Hardy and DeBold (11). Only responses receiving scores of 2 and 3 were included in calculating the lordosis quotient (number of lordosis responses × 100/number of mounts by male). The observer was not aware of the subject's treatment while collecting behavioral data. Behavioral tests were terminated after 8–10 mounts. Immediately following each behavioral test, the female was anesthetized with ether and 0.75 ml of blood was collected again. Blood was kept at 4°C until centrifuged. Plasma was stored at −20°C.

Plasma levels of DHT were measured by radioimmunoassay (RIA) using a highly specific antiserum (Radioassay System Laboratories, Carson, CA, catalogue No. 155, Lot R-140). The antiserum cross-reacts 8% with testosterone and none with P, estradiol, and corticosterone. The assay was described in detail by Sridaran and Gibori (19). Tritiated DHT [2,4,5,6,7,-^{3}H(N) dihydrotestosterone] was obtained from New England Nuclear. The standard curve ranged from 0.005 to 1.0 ng, and all working standards were in a volume of 0.5 ml. The sensitivity of the assay was 10 pg/assay tube. Plasma P was also measured by RIA as described in detail previously (9).

All litters were examined within 8 hours of parturition. To determine whether GnRH treatment affected lactation or offspring development, each litter of pups was weighted to the nearest 1.0 g by triple beam balance on 3, 5, 9, 13, 17, and 21 days after parturition. All pups were allowed to suckle immediately after birth and were only removed from the mother during postpartum behavioral tests and periodic

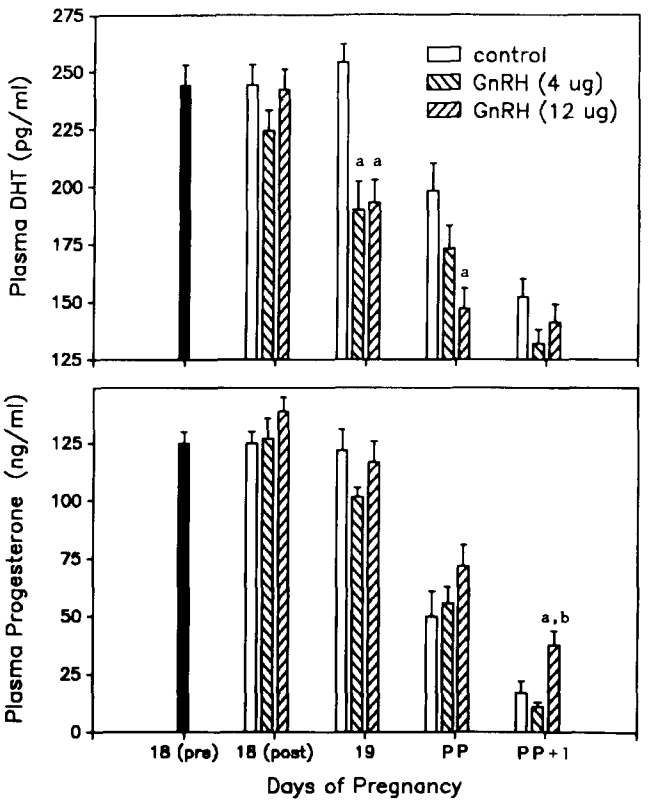

FIG. 1. The effects of GnRH treatment on plasma levels of dihydrotestosterone (DHT) and progesterone. Each bar represents mean±SEM of hormone concentrations in 6–9 rats. Experimental rats were bled just prior to treatment on day 18 [solid bar, 18(pre)] as an internal control. Immediately after pretreatment bleeding, rats received 4 or 12 μg GnRH and were bled after behavioral testing 4 hr later [18(post)]. Blood samples from experimental and control rats were also collected immediately after behavioral tests on day 19 of pregnancy, the day of parturition (PP) and the day following parturition (PP+1). The solid bar at 18(pre) and the open bar at 18(post) represent the same data. [a]Significantly different from the respective control group; [b]significantly different from GnRH (4 μg) group.

weight measurements. The data were analyzed for differences by one-way analysis of variance followed by the Newman-Keuls test when differences were significant. A value of $p<0.05$ was considered significant.

RESULTS

Both doses of GnRH treatment significantly inhibited postpartum lordosis behavior ($p<0.05$; Table 1). No rat, regardless of treatment, showed lordosis on days 18 and 19 of pregnancy or the day following parturition.

Both doses of GnRH significantly depressed plasma levels of DHT on day 19 ($p<0.05$; Fig. 1). Although both GnRH-treated groups had lower levels of DHT at postpartum, levels were only statistically different from those of controls in the 12 μg GnRH group. GnRH treatment did not affect plasma P until the day after parturition, when the 12 μg GnRH group showed significantly higher levels of P ($p<0.05$). Neither plasma DHT nor P showed any response to GnRH treatment within 4 hours.

Among the 9 rats given 12 μg GnRH, one litter had one

stillborn pup, one litter had three stillborn pups, and one pregnancy failed completely (vaginal bleeding was observed on day 22). It appeared that 12 μg GnRH had a partial and inconsistent detrimental effect on the pregnancies. All other pups were unaffected by treatment as determined by visual examination on the day of parturition. Treatment did not affect lactation, timing of parturition or offspring development (as measured by litter weight gain). The mean litter weight of each treatment group did not differ significantly at 3, 5, 9, 13, 17 or 21 days after birth.

DISCUSSION

Our data demonstrate that GnRH administration inhibits the expression of postpartum lordosis behavior. Numerous hormonal changes occur in the final days of pregnancy which not only regulate the physiological events of parturition, postpartum ovulation and lactation, but also direct the behavioral transition from sexual inactivity during pregnancy through postpartum receptivity to maternal care and renewed sexual inactivity during lactation (13). Given the quantity and extent of these changes, it is difficult to determine where and when GnRH treatment exerts its inhibition of postpartum sexual behavior.

Several lines of evidence indicate that GnRH can influence reproductive behavior by its independent action in the brain. GnRH stimulates lordosis behavior for several hours when injected SC in ovx/hypox rats (14) or infused directly into the brain (16), indicating that GnRH has behavioral effects that are not mediated by the pituitary. However, since the circulatory half-life of GnRH is 5–10 minutes in the rat (2) and the behavioral effects in this study were not expressed until 5 days posttreatment (Table 1), it is unlikely that GnRH inhibited postpartum lordosis by its direct action on neural tissue.

Exogenous GnRH is also known to influence gonadal hormones which regulate postpartum lordosis behavior. In one of the few studies on the physiological basis of postpartum behavior, Connor and Davis (5) reported that a rise and fall of P coincides with the expression of sexual receptivity. The temporal association between P and receptivity at postpartum is similar to the pattern found during the period of estrus in the cycling rat, when P facilitates lordosis. Many studies have demonstrated that GnRH can inhibit P production during pregnancy (12,17), but the doses administered in the present study had no effect on P until the day after postpartum estrus when it actually elevated plasma P (Fig. 1).

The suppression of DHT at postpartum suggests that GnRH may inhibit postpartum lordosis by acting on ovarian steroidogenesis. Fox and Smith (8) showed that the cervical stimulation involved in parturition may directly stimulate hypothalamic secretion of GnRH which in turn initiates the preovulatory surge of gonadotropins occurring several hours postpartum. The administration of GnRH on day 18 may have prematurely induced ovulation and altered plasma estradiol concentrations, though this possibility was not tested. Wallen *et al.* (20) showed that chronic treatment of a GnRH agonist to cycling rhesus monkeys inhibits female initiated sexual behavior while simultaneously suppressing steroidogensis and ovulation.

Virtually all studies which demonstrate the inhibitory action of GnRH on female reproductive physiological function in rats have used either chronic treatment regimens or single injections of very high doses (200–1000 μg) [for review, see (12)]. Sridaran (17) was unable to terminate pregnancy with as much as 100 μg/day administered continuously during mid-pregnancy. While the higher dose (12 μg) in the present study exerted some antifertility effects, the lower dose (4 μg) appeared to have no detrimental effects on the pregnancy. Nevertheless, these relatively small doses of GnRH acted as an agent of "behavioral antifertility" by interrupting full induction of postpartum sexual behavior. This reduction in receptivity may be particularly significant since a greater degree of copulatory stimulation is required to initiate pregnancy at postpartum than during the estrus cycle (6).

Although GnRH influenced postpartum behavior, it did not induce lordosis behavior during pregnancy as hypothesized (Table 1). GnRH must be accompanied by estrogen priming to have behavioral potency in ovx rats. Although the subjects in our study were not estrogen-primed, two observations indicated that the failure of GnRH to stimulate lordosis did not result from inadequate estrogen titers: 1) estradiol levels at day 18 of pregnancy (3) are equivalent to peak levels reached at proestrus of the estrous cycle, when GnRH stimulates sexual behavior, and 2) in a preliminary study, we found no lordosis response among 8 rats treated with 2 μg estradiol on days 13 and 17 and GnRH on days 14 and 18.

The hormonal mechanisms that inhibit receptivity during pregnancy are not known, though elevated levels of DHT have been suggested (1). GnRH suppresses androgen production in vitro (4) but has no apparent affect on testosterone in vivo during pregnancy (18). Our study demonstrates that, although GnRH significantly reduced plasma levels of DHT on day 19 (Fig. 1), lordosis was not expressed (Table 1). While not conclusive evidence, these data support the findings by Erskine *et al.* (7) and deGreef *et al.* (10) that DHT does not act as an endogenous inhibitor of sexual behavior during pregnancy.

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