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Ovarian Responses to Perphenazine-Induced Prolactin Secretion in the Rat: Effect of Ovulation, Stress, and Steroids (38752)

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(Introduced by J. J. Biezenski)

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Physiological effects of progesterone including vaginal mucification during estrogen treatment (1), inhibition of estrus (2, 3), and growth of deciduomata after uterine traumatization (4) were shown over 30 yr ago to occur after administration of prolactin to normal mature female rats. Although prolactin without LH is insufficient to maintain progesterone secretion for more than 6 days in rats that have received cervical stimulation (5–7), prolactin alone is adequate to maintain progesterone secretion during postpartum lactation (8) or in unmated rats whose pituitaries have been autotransplanted beneath the kidney capsule 1 or 2 days after ovulation (9, 10).

Since prolactin apparently acts at an earlier step in the steroid biosynthetic pathway than does LH (11–14) and because LH (15) but not prolactin (16) promotes ovarian steroidogenesis during a 2–3 hr incubation *in vitro*, it was of interest to investigate the time-course of the marked rise in peripheral serum progesterone that occurs (17) when serum prolactin is elevated by daily perphenazine administration. The 17-fold increase in serum progesterone in rats treated with perphenazine is presumed to be due to increased steroidogenesis and not to decreased 20α-reduction of progesterone because serum 20α-dihydroprogesterone was not altered by the treatment (17).

Since perphenazine administration has been shown to elevate serum prolactin as much as 10-fold within 30 min (18, 19), the time required for an ovarian response can be related to this rapid rise in serum prolactin. Also, since continued daily injections of perphenazine result in continued elevation of serum prolactin (20), perphenazine administration provides a useful means of assessing the ovarian response to relatively continuous and high levels of prolactin *in vivo*.

The purpose of this study was to examine the time-course of the serum progesterone response to elevated prolactin levels and to study the influence of ovulation, stress, estrogen, and cortisol on this response.

Materials and Methods. Animals. Female rats, weighing between 180 and 230 g, obtained from Hormone Assay, Chicago, were housed in an air-conditioned room lighted daily from 05.00 to 17.00 hr. Vaginal smears obtained by lavage were examined daily for at least two complete estrous cycles before treatment was begun. Perphenazine (Schering Corp.) was dissolved in 0.03 *N* HCl to concentrations of 2.5, 5.0 or 10.0 mg/ml, and 0.1 ml/100 g body wt was administered subcutaneously daily.

Procedure 1. Perphenazine treatment was begun at 12.00 hr on the day of estrus and was continued for a total of five daily injections. Blood samples of between 1.5 and 2.0 ml were collected from the cut tip of the tail of rats anesthetized with ether immediately before the first, second, and fourth injections of perphenazine. Bleeding was stopped by ligating the tail 3 mm from the cut end. The last blood sample was collected from the severed vessels after decapitation 24 hr after the last injection of perphenazine. Control rats received the same dose of the 0.03 *N* HCl vehicle daily and were bled on the same schedule.

Procedure 2. Rats received injections on the same schedule with 2.5 or 5.0 mg perphenazine/kg body weight, but blood was collected only after decapitation. No blood samples were obtained from the tails before or during perphenazine treatment. Control rats on this schedule received injections of the 0.03 *N* HCl vehicle.

Procedures 3 and 4. The same regimen as in Procedure 2 with daily 5.0 mg perphenazine/kg injections was employed except

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TABLE I. SERUM PROGESTERONE CONCENTRATIONS DURING DAILY PERPHENAZINE TREATMENT OF SERIALLY ANESTHETIZED AND BLED RATS.

Daily dose of perphenazine	Duration of treatment (days)			
	0	1	3	5
(mg/kg body wt)	ng/ml			
None	9 ± 5	17 ± 5	18 ± 4	20 ± 7
2.5	14 ± 2	13 ± 2	36 ± 7	58 ± 4
5.0	9 ± 4	15 ± 7	27 ± 9	55 ± 5
10.0	9 ± 3	10 ± 3	34 ± 9	59 ± 12

TABLE II. PERIPHERAL SERUM CONCENTRATIONS OF PROGESTERONE AND 20α-DIHYDROPROGESTERONE (20α-OHP) AFTER 5 DAILY INJECTIONS OF PERPHENAZINE STARTING ON ESTRUS (DAY 0) WITH OR WITHOUT ETHER ANESTHESIA AND TAIL BLEEDING ON DAYS 0, 1, AND 3.

Daily dose of perphenazine	With prior anesthesia and bleeding		Without prior anesthesia or bleeding	
(mg/kg body wt)	Progesterone (ng/ml)	20α-OHP (ng/ml)	Progesterone (ng/ml)	20α-OHP (ng/ml)
	Procedure 1		Procedure 2	
0.0	20 ± 7	45 ± 12	6 ± 2	111 ± 22
2.5	58 ± 4	73 ± 6	62 ± 7	33 ± 4
5.0	55 ± 5	134 ± 15	110 ± 7	48 ± 9
10.0	59 ± 12	107 ± 24	—	—

that rats in Procedure 3 received 0.5 μg estradiol-17β in 0.2 ml of sesame oil daily s.c. and rats in Procedure 4 received 0.2 ml of an aqueous preparation containing 2 mg cortisol acetate, 1.8 mg NaCl, and 1.0 mg methyl cellulose s.c. daily in addition to the perphenazine injections.

Procedure 5. Perphenazine injections, 5.0 mg/kg body wt, were begun between 11.00 and 12.00 hr in proestrus to inhibit ovulation, and were continued daily for a total of six injections. Blood was collected only after decapitation, 24 hr after the last perphenazine injection.

Procedure 6. Rats were given injections subcutaneously daily beginning at 11.00 hr on proestrus or estrus with 5.0 mg perphenazine/kg body wt for 2, 4, or 6 days. No blood samples were taken. Vaginal smears were examined daily for a minimum of 20 days to ascertain the effect of the drug on subsequent estrous cycles.

Analyses. Blood was allowed to clot for 1 hr at room temperature. Serum was separated by centrifugation and was stored in sealed vials at −20° for subsequent steroid assays. Progesterone and 20α-hydroxy-4-pregnen-3-one (20α-OHP) were analyzed after purification by thin-layer chromatography by competitive protein-binding assays described in detail previously (17). Each value in Tables I and II is the mean steroid concentration for a group of between four and eight rats with its standard error.

Ovaries obtained at necropsy were removed from the bursa and fixed in 4% formaldehyde for subsequent paraffin embedding and staining with hematoxylin and eosin. Discontinuous serial sections (8 μm thick) were examined for evidence of ovulation, luteal maintenance, and follicular development.

Results. Serum Progestin responses. Serum progesterone concentrations obtained on estrus and at several intervals during perphenazine treatment are presented in Table I. All eight control rats that had 5-day estrous cycles before the bleeding sequence and s.c. injections with 0.03 *N* HCl were begun had one additional day of diestrus while on the sampling regimen. The apparent increase in serum progesterone during the sampling regimen was not, however, statistically significant. By Analysis of Variance

and Duncan's multiple range test, all groups receiving perphenazine had significantly greater serum progesterone than the control group, but no significant differences were found among groups of rats treated with 2.5, 5.0, or 10.0 mg perphenazine/kg body wt. Therefore, for further analysis the data for each time interval were pooled among the three dose regimens. No increase in serum progesterone was found 1 day after the first injection of perphenazine. However, significant differences occurred between days 1 and 3, and between days 3 and 5. Thus, perphenazine treatment, independent of the dose tested, resulted in a gradual and continuing increase in serum progesterone over the course of 5 days.

The serum progesterone (P) and 20α-OHP concentrations (Table II) were compared 24 hr after the fifth perphenazine injection in rats bled before and during treatment (Procedure 1) and in rats bled only at the end of the treatment regimen (Procedure 2). In control rats without tail bleeding the P/20α-OHP ratio was significantly lower than in rats subjected to anesthesia and bleeding from the tail. Total serum progestin ($P +$ 20α-OHP) was similarly increased by increasing doses of perphenazine in stressed (Procedure 1) and unstressed (Procedure 2) rats; but in the presence of stress perphenazine stimulated relatively less progesterone and more 20α-OHP. The serum progesterone concentration in rats receiving the 2.5 mg dose of perphenazine was significantly increased to a similar extent in rats subjected to Procedures 1 and 2. Higher doses of perphenazine did not increase serum progesterone in stressed rats (Procedure 1), but increasing the dose of perphenazine from 2.5 to 5.0 mg/kg in rats bled only once resulted in a highly significant increase in serum progesterone.

Among rats bled once, the increase in serum progesterone observed in rats receiving the 2.5 mg dose of perphenazine over that of the control group can be accounted for by decreased reduction of progesterone to 20α-OHP, since total serum progestin ($P +$ 20α-OHP) concentrations were similar. However, the increase between groups receiving 2.5 and 5.0 mg perphenazine was associated with a highly significant increase in total serum progestin concentration.

When 0.5 μg of estradiol-17β was injected with perphenazine (Procedure 3), serum progesterone was significantly ($P < .01$) reduced from 110 $\pm$ 7 to 68 $\pm$ 5 ng/ml. Daily administration of 2 mg cortisol acetate (Procedure 4) with perphenazine, however, had no effect; the progesterone concentration was 94 $\pm$ 13 ng/ml. When perphenazine administration was begun in proestrus in order to block ovulation (Procedure 5), serum progesterone was significantly lower ($P < .01$) than that observed in rats that received perphenazine first in estrus; progesterone and 20α-OHP were 25 $\pm$ 8 and 44 $\pm$ 5 ng/ml, respectively. The serum progesterone was, however, greater ($P < .02$) than in control animals (6 $\pm$ 2 ng/ml) and the P/20α-OHP ratio was considerably increased.

Physiological Responses. Ovarian histology confirmed the effectiveness of the perphenazine treatment in inhibiting ovulation. Very little luteal tissue could be identified in ovaries from these rats. No mature follicles were present and the effect of estrogen secretion, as inferred by cornified cells in the vaginal washings, was not observed. Ovaries of all other groups had large corpora lutea and no mature follicles. No corpora hemorrhagica were observed in any ovaries examined.

When 5.0 mg perphenazine/kg body weight was injected 2 days starting on estrus and vaginal smears were examined daily for 26–30 days without blood sampling (Procedure 6), four of five rats exhibited an extended period of diestrus of 14.5 $\pm$ 1.6 days. Subsequent periods of vaginal cornification occurred at regular 4- to 5-day intervals. The one rat that failed to respond also failed to respond when tested 3 wk later. Two rats that received injections beginning on estrus for 4 days had diestrus periods of 14 and 15 days, respectively. However, when the same dose of perphenazine was injected daily for 2–4 days starting at 11.00 hr on proestrus, regular 4–5 day estrus cycles were uninterrupted. However, when treatment was extended to 6 days, 9- to 17-day periods of diestrus ensued before regular cycles resumed.

Discussion. Since prolactin is elevated within minutes after perphenazine administration (18, 19) to levels comparable to or greater than the peak concentrations present during pregnancy (21) or pseudopregnancy (22), administration of perphenazine is a useful method for ascertaining effects of prolactin on ovarian and mammary gland function. Administration of 2.5 or 5.0 mg perphenazine/kg body wt daily for 5 days results in serum progesterone concentrations similar to those of rats 5 days pregnant or pseudopregnant (23). In fact, when the 5.0 mg dose of perphenazine was administered only during the first 2 days after ovulation, a period of diestrus typical of pseudopregnancy was induced. The hormonal milieu of target organs during continued daily administration of perphenazine, however, is not the same as that of pseudopregnancy; the mammary gland response is distinctly greater in the perphenazine-treated rats (24).

Several lines of evidence indicate that gonadotropin secretion is inhibited during perphenazine administration: absence of nucleated or cornified cells in the vaginal smears, absence of ovarian follicular growth, and (17) low serum estrogen concentration. Yet, perphenazine administration results in significant elevation of serum progesterone. Consistent with the failure to observe an increase in progesterone biosynthesis during a 3-hr incubation of ovarian tissue slices with prolactin (16), serum progesterone did not rise until after 24 hr from the time of perphenazine administration. Although the stress of serial anesthesia and bleeding of rats during perphenazine treatment reduced the response at higher dose levels (by comparison with rats bled once after five injections) the serum progesterone response at the 2.5 mg/kg dose level was relatively unaffected and, therefore, is considered to provide a reliable estimate of the time required for an ovarian response to this stimulus. In the absence of stress the 5.0 mg dose of perphenazine was previously shown (17) to increase serum progesterone to 138 $\pm$ 11 ng/ml on day 3. The response to this dose in the absence of stress may, therefore, be considerably more rapid than that to the 2.5 mg dose. Earlier studies (8–10) have shown that prolactin may be luteotropic in the absence of gonadotropins, particularly when the corpora lutea have not been exposed to prolonged LH secretion after ovulation as occurs after mating (25, 26) or cervical stimulation (26). Although the increase in serum progesterone in the absence of stress after five daily injections of 2.5 mg perphenazine/kg body wt could be the result of a decrease in the activity of ovarian 20α-hydroxysteroid dehydrogenase, the increase observed when the 5.0 mg dose was administered cannot, since both serum progesterone and 20α-OHP increased. Although a decrease in progesterone clearance cannot be ruled out, an increase in steroidogenesis is more consistant with the observations since the 5.0 and 10.0 mg doses of perphenazine did not increase serum progesterone in the presence of stress. That the serum progesterone was primarily of ovarian origin was shown by the inability of perphenazine to increase progesterone in rats in which ovulation had been inhibited. The small but significant increase in serum progesterone over control levels in these rats may be due to increased adrenal secretion since perphenazine treatment has been shown to increase significantly serum corticosterone (17), and prolactin administered to ovariectomized rats increases serum progesterone (27).

Stress has been shown by Stern *et al.* (28) to decrease serum prolactin when prolactin secretion is normally elevated. In pseudopregnancy the diurnal, but not the nocturnal, rise in prolactin is inhibited (22). In the present study the progesterone/20α-OHP ratio but not total serum progestin was reduced in stressed relative to unstressed rats treated with perphenazine, suggesting that luteal support particularly that of prolactin was declining (29, 30). However the progressive increase in mammary gland response to increasing doses of perphenazine observed in the same rats (24) is inconsistant with a decrease in prolactin. This suggests that in the presence of stress some factor other than decreased prolactin limited the rise in serum progesterone.

Interactions of estradiol-17β and cortisol were studied to attempt to explain this paradoxical result. Estradiol decreased the response significantly, but there was no evidence for increased estrogen formation in

stressed rats; the decrease with estradiol is probably a result of less efficient stimulation of prolactin secretion by perphenazine because of competition between estradiol and perphenazine for a common hypothalamic receptor site (31). Cortisol acetate administration had no significant effect on serum progesterone, although Ben-David *et al.* (20) have shown that the prolactin response to perphenazine is somewhat greater after adrenalectomy. The effect of stress on the ovarian response evidently is not mediated by adrenal stimulation. The best explanation is that stress decreases prolactin secretion, particularly high secretory rates (28), and that mammary tissue is more sensitive to small or transitory increments in prolactin secretion than is the ovary.

On the other hand, the stimulation of prolactin secretion by stress when prolactin secretion is low (28, 32) was also observed. In stressed control rats the ratio of progesterone to 20α-OHP was increased, indicative of prolactin secretion (33), and diestrus was extended by 1 day.

Summary. A single injection of 2.5 mg perphenazine (PH)/kg body wt to rats on the day of estrus (day 0) did not result in increased serum progesterone 24 hr later. Continued daily injections, however, resulted in a 2.5-fold increase in serum progesterone between days 1 and 3 and a 1.6-fold increase between days 3 and 5 to a final concentration of 58 $\pm$ 4 ng/ml on day 5 in serially anesthetized and bled rats. Neither daily administration of 5.0 nor 10.0 mg PH/kg body wt to rats subjected to the stressful conditions of this regimen resulted in further increases in serum progesterone, but the 5.0 mg dose of PH in unstressed rats bled only on day 5 resulted in a highly significant increase in serum progesterone to 110 $\pm$ 7 ng/ml.

In unstressed rats the increase in serum progesterone over control values after five daily injections of 2.5 mg PH/kg body wt could be attributed to decreased 20α-reduction of progesterone, but when the dose of PH was increased to 5.0 mg/kg, a highly significant increase in both progesterone and total progestins occurred indicating that prolactin can increase steroidogenesis as well as reduce 20α-hydroxysteroid dehydrogenase activity.

After inhibition of ovulation, the 5.0 mg daily dose of PH resulted in serum progesterone of only 25 $\pm$ 8 ng/ml on day 5 in unstressed rats. Thus, serum progesterone in ovulating rats treated with PH originated primarily in the corpora lutea. Perphenazine, 5.0 mg/kg, administered only on estrus and the first day of diestrus was sufficient to induce pseudopregnancy of 14.5 $\pm$ 1.6 days. No evidence for gonadotropin stimulation of the ovaries of any rats was observed. The effect of stress on the progesterone response was not mimicked by administration of cortisol acetate and is assumed to be mediated by suppression of prolactin secretion.

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