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Reversal by Progesterone of Barbiturate Blockade of Ovulation: Effect on Concentration of Serum LH.[1] (38098)

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In adult female rats, administration of pentobarbital early on the afternoon of proestrus prevents the ovulatory surge of gonadotrophin secretion (1) and ovulation (2) from occurring. However, if progesterone is injected on the morning of proestrus, ovulation will not be blocked by pentobarbital unless the time of pentobarbital administration is advanced by 2–3 hr (3). Measurement of plasma LH in adult rats shows that the progesterone treatment advances the time of ovulatory gonadotrophin secretion by 2–3 hr (4, 5), and the time of ovulation is also advanced by 2–3 hr (6). The capacity of progesterone to counteract barbiturate blockade of ovulation has also been demonstrated in hamsters (7, 8) and in immature rats pretreated with pregnant mare's serum gonadotrophin (PMSG) (9–12).

In the 32-day-old rat induced to precocious puberty with 8 IU of PMSG, progesterone administration decreases the capacity of phenobarbital (PhB) to block ovulation regardless of whether progesterone precedes, coincides with, or follows the time of PhB treatment (McCormack and Strauss, unpublished observations). This present investigation was undertaken to determine the blood levels of LH in PMSG-primed rats treated with progesterone and/or PhB in anticipation that this information might reveal part of the mechanism by which progesterone counteracts the barbiturate block of ovulation.

Materials and Methods. Weanling female rats weighing 45–55 g were obtained from the Holtzman Company of Madison, Wisconsin, at 22 days of age (day 22). They were maintained in an air-conditioned (24.5°–28°) room in which the lights came on at 0500 and went off at 1900. Rockland rat/mouse diet and tap water were provided ad lib. throughout the experiment. On day 30 at 1000, 8 IU of PMS (Antex, Leo Pharmaceutical Products) was injected sc in 0.5 ml of 0.9% NaCl solution (saline). On day 32 at 1000, some rats were injected sc with 0.5 mg of progesterone in 0.1 ml of corn oil; controls were injected with the corn oil vehicle. One hour later, some rats were injected sc with 10 mg PhB in 0.25 ml of water; controls were injected with saline.

Between 1040 and 1836 on day 32, blood samples were taken. In trials 1 and 2 of this experiment, a terminal sample of blood was taken from the abdominal aorta under ether anesthesia. A few rats from each treatment group were not bled and killed on day 32, but instead were killed on the morning of day 33 at which time their oviducts were examined for ova. In trial three, 0.75 ml of blood was taken by venipuncture (iliac or jugular vein) under ether anesthesia on day 32. These rats were then killed on the following morning and their oviducts were examined for ova. The blood samples were allowed to clot at 4°. The serum

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TABLE I: Effect of Progesterone and (or) Phenobarbital on Ovulation.

Treatment on Day 32		Proportion with oviductal ova on Day 33	Average no. ova per ovulating rat ± SEM
at 1000	at 1100		
Oil	Saline[a]	0/3	0
Oil	Saline	6/6	10.5 ± 0.7
Progesterone	Saline	5/5	10.2 ± 1.1
Oil	Phb	1/9[b]	10
Progesterone	PhB	6/9[c]	9.3 ± 1.2

[a] No PMSG was given to this group. All other groups received 8 IU of PMSG on day 30.
[b] This response differs significantly ($p < .05$) from all other groups given PMSG.
[c] Blood samples were taken by venipuncture on day 32 between 1340 and 1615 in three of the six ovulating rats. In each the serum LH was <15 ng/ml.

was then placed in vials and quickly frozen. It was stored at −30° until assayed for LH 1–4 wk later. The LH was assayed by the ovine: ovine radiommunoassay for rat LH using NIH-LH-S14 as the standard (13). Mean serum LH values for samples taken by venipuncture did not differ from those taken by arterial exsanguination; thus, the serum LH values for trials 1, 2, and 3 were pooled within treatment groups. Statistical significance was evaluated by Student's two-tailed *t* test and by the Fourfold contingency test (14).

Results. Ovulation data (Table 1). Without pretreatment with PMSG, ovulation did not occur, but with PMSG treatment all rats ovulated (compare oil–saline-groups, Table 1). Progesterone treatment at 1000 on day 32 did not interfere with PMS-induced ovulation. In rats not given progesterone, treatment with PhB at 1100 blocked ovulation in all but one of nine rats. Progesterone treatment significantly decreased the capacity of PhB to block ovulation; i.e., six of nine ovulated. The average number of ova per ovulating rat did not differ significantly between any of the groups (*t* test).

Serum LH concentrations (Fig. 1). In controls given oil and saline, LH was highest during the 1400–1600 time interval and remained quite high between 1600 and 1800. The same was true for rats given progesterone and saline. The abrupt increase in serum LH is somewhat concealed by the averaging nature of the time intervals in Fig. 1. More specifically, all oil–saline controls and all progesterone–saline treated rats sampled prior to 1330 had LH levels less than 25 ng/ml; all rats in the same two groups sampled between 1350 and 1600 had values in excess of 50 ng/ml. The LH values of the oil–saline and progesterone–saline groups did not differ significantly for any of the time intervals. Treatment with PhB prevented the serum LH from rising whether or not progesterone was given. Never-

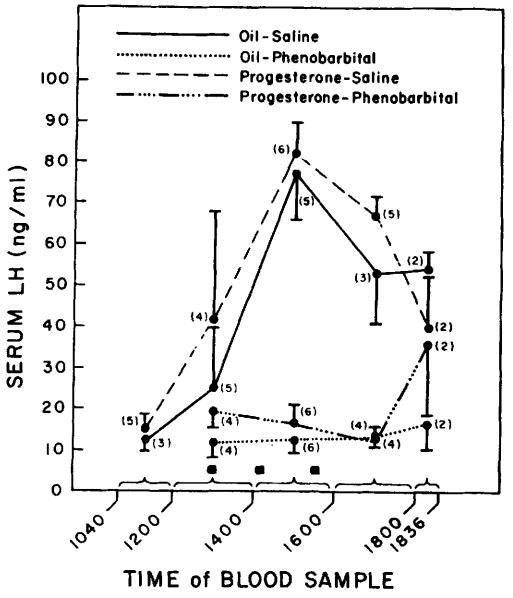

FIG. 1. Concentration of LH (NIH-LH-S14) in the serum of PMSG-treated rats during the afternoon preceding ovulation: Effect of progesterone and (or) phenobarbital. The mean serum LH from all rats (N is in parentheses) sampled during a given time interval (designated by enclosure marks on the abscissa) is plotted at the midpoint of that interval. The vertical bars represent the SEM. Progesterone or its corn oil vehicle was given at 1000; phenobarbital or isotonic saline was given at 1100. The serum LH values of three rats given no PMSG treatment are designated by the small squares near the abscissa.

theless, three of the rats in the progesterone–PhB group which had low LH levels (less than 15 ng/ml) when blood was taken by venipuncture between 1340 and 1615 on day 32, ovulated by the morning of day 33 (Table 1, footnote c). The elevation of LH between 1800 and 1836 in the progesterone–PhB group was due to one rat (exsanguinated at 1821) with a serum LH of 52 ng/ml.

Three rats were not given PMSG, progesterone, or PhB. The serum LH of these rats on the afternoon of day 32 was consistently low; thus, without PMSG-priming, no LH surge was occurring (Fig. 1).

Discussion. The possible mechanisms by which progesterone might act to counteract barbiturate blockade of ovulation can be grouped into two categories: *first,* by a "central" action on the brain and/or anterior pituitary, progesterone could (a) directly antagonize the inhibitory effect of barbiturates, or (b) advance the time of the ovulatory gonadotrophin surge, thus making barbiturate administration at the usual hour (i.e., 1400) ineffective; *second,* progesterone could act "peripherally" on ripe ovarian follicles to induce their rupture directly or to increase their sensitivity to the ovulatory action of LH.

In our experiments, ovulation which occurred in the progesterone–PhB group (Table 1) was not due to a breakthrough of LH secretion during the usual hours of the LH surge, nor to an early LH surge, since LH levels were low throughout the sample period of 1040–1800 (Fig. 1). Additional progesterone–PhB treated rats should be sampled for serum LH at 1800 or later to determine if an LH surge occurs late in the afternoon when the sedative effect of PhB wears off. Actually, the surge levels of LH may not be essential for induction of ovulation because in adult rats, serum LH values equivalent to only 14% of peak surge values are sufficient to induce ovulation (15). Information on the serum FSH levels in progesterone–PhB-treated rats would also be of interest, since FSH can induce ovulation in rats (16).

The failure of progesterone in these experiments to advance significantly the onset of the LH surge was surprising, because we have observed in this same PMSG-treated rat that progesterone advances ovulation by 1–2 hr (McCormack and Strauss, unpublished). Sridharan *et al.* (12), utilizing this same PMSG-treated rat, observed that if progesterone was administered at 0600–0700 on day 32, the peak in plasma LH was 4 hr earlier than in vehicle-injected controls. Ying and Meyer (10), using a younger PMSG-treated rat, found that progesterone treatment at 0530 advanced the LH surge and the onset of ovulation by 2–4 hr. The earlier hour of administration of progesterone by these investigators may account for the measurable advance in the onset of the LH surge in their rats.

The question arises as to why in adult rats given progesterone on the morning of proestrus, ovulation can be blocked by appropriately advancing the injection of barbiturate (3, 4), but in our PMS-treated rats, ovulation cannot be blocked consistently with barbiturate once progesterone has been given (McCormack and Strauss, unpublished). A possible explanation may lie in the fact that, thus far, those who have investigated progesterone facilitation of ovulation in the adult rat have utilized pentobarbital (3, 4) or atropine (17), as the blocker, whereas in our immature rats only PhB has been employed. Differences in the sedative and anesthetic properties of these drugs are well recognized (18).

Our experimental results neither substantiate nor rule out the possibility that progesterone counteracts PhB blockade of ovulation by sensitizing the ovarian follicles to the action of LH. Some workers (19) have hypothesized that locally secreted progesterone is essential for processes initiating follicular rupture; others (20) question this hypothesis. Earlier experiments of McCormack (21) have dealt with the possibility that progesterone and PhB act directly on the ovary. In PMSG-treated-immature rats, ovulation is prevented by hypophysectomy performed at noon on the second day after PMSG; however, iv injection of 0.75 IU of HCG 2 hr after hypophysectomy induces ovulation in all rats (21). If PhB is injected 1 hr after hypophysectomy (i.e., 1 hr prior to HCG), not all rats ovulate in response to the HCG, and those that do, have significantly fewer ova. These results show that PhB can inhibit ovulation in the PMSG-primed rat by some action that is independent of the pituitary. Progesterone treatment (2 hr prior to hypophysectomy) did not restore the ovulatory response to HCG of hy-

pophysectomized PhB-treated rats to that of hypophysectomized rats given HCG, but not PhB (21).

The following mechanism has recently been proposed to explain progesterone's capacity to counteract barbiturate blockade of ovulation (22, 23): Shortly after they are administered, barbiturates act centrally (i.e., CNS–pituitary) to decrease tonic LH secretion (24, 25), and in the face of diminished LH, ovarian steroid secretion is depressed (22, 23). Without a normal steroid environment, the hypothalamic–pituitary axis fails to secrete an ovulatory quota of gonadotrophin (23). Administration of progesterone corrects the steroid deficiency and allows an ovulatory quota of gonadotrophin to be secreted. Presumably, a similar mechanism might also explain why progesterone treatment decreases the capacity of other drugs [i.e., chlorpromazine (26) and atropine (17)] to block ovulation in adult rats and PMSG-primed immature rats (9).

Summary. In prepubertal rats injected with PMSG an ovulatory surge of LH was secreted between the hours of 1400 and 1800 on the second day after PMSG. Administration of phenobarbital at 1100 prevented the LH surge and ovulation from occurring; however, if progesterone was injected 1 hr prior to the phenobarbital, most rats ovulated. No consistent evidence of an LH surge was seen in rats treated with progesterone and phenobarbital even though they ovulated.

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