Blockade of the Proestrous LH Surge in Cyclic Rats by Barbiturate Administration on Diestrus[1] (37147)

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Administration of barbiturates immediately prior to the proestrous critical period of cycling rats delays ovulation and blocks the proestrous surge of LH (1–3). Administration of barbiturates early on the afternoon of the day before proestrus also may delay ovulation but apparently does not prevent the release of gonadotrophin responsible for ovarian steroid secretion (4, 5).

In this study, we report that pentobarbital (PB) administered at 1330 hr on the day of diestrus (D_2) in 4-day cyclic rats blocks the proestrous surge of LH, thereby inhibiting the subsequent events of ovulation.

Methods. Sprague–Dawley (Madison, Wisc.) rats, 250–290 g, were acclimated to laboratory conditions and maintained on a 14 light/10 dark lighting schedule with midnight the midpoint of the dark period. An indwelling cannula was implanted into the sinus venosus of each 4-day cyclic rat by way of the jugular vein (6) on the morning of metestrus or diestrus. One full cycle was allowed to complete before initiating treatment and blood sampling for serum LH determinations.

Pentobarbital (Nembutal®) 30 mg/kg ip in saline or a saline volume control was injected on 1330 hr of diestrus during the second full cycle following cannula implantation. Serial blood sampling (0.6 ml) of unanesthetized animals was carried out at hourly intervals from 1330–1830 hr on proestrus. All values for LH were determined using NIH-LS-S14 as the standard by an ovine:ovine radioimmunoassay as previously described (7).

Results. Table I confirms prior reports of the ability of PB to block ovulation in 4-day cyclic rats when administered in the early afternoon of diestrus. The degree of uterine ballooning, as indicated by intralumenal water which was still present at "estrous" autopsy in PB-treated rats, provides further support for the hypothesis that administration of a short acting barbiturate earlier than proestrus in the estrous cycle does not reduce gonadotrophin release enough to prevent estrogen secretion (8). The prolongation of the large amount of intralumenal water to the day of estrus in four of five treated rats also suggests that the usual progesterone secretion was blocked at proestrus (8).

TABLE I. Autopsy Data on the Day of Expected Estrus.

Animal No.	Uterine wet wt., mg.	Uter. lumenal water, mg.	Ovulation +,–
1. Pentobarbital Administered at 1330 hr—Diestrus			
DX 15	466.0	301.0	–
DX 40	449.5	133.9	–
DX 66	643.8	161.0	–
DY 40	524.8	346.2	–
EB 144	341.0	39.0	partial
2. Saline Administered at 1330 hr—Diestrus			
DX 56	440.6	57.2	+
DY 13	447.9	29.5	+
DY 29	362.5	45.1	+
DY 61	475.1	34.6	+
ED 27	—	20.6	+
EB 116	426.0	33.2	+

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Histological examination of the ovaries of PB-treated animals revealed large follicles with no fresh corpora lutea in 4 of 5 animals, with the remaining animal showing only a partial ovulation in one ovary.

Figure 1 illustrates, on an individual basis, the reduction of the proestrous LH surge to near background levels by barbiturate administration on 1330 hr of diestrus. These data resemble closely data from our laboratory following PB injection on 1330 hr of proestrus (3).

Discussion. These data (Fig. 1) provide evidence for a central, rather than an ovarian (9), site of action of the barbiturate inhibition of the proestrous LH surge and support the concept of the existence of an endogenous neural timing center stimulating LH release (8, 10). Inhibition of ovarian progesterone synthesis as a site of action of a barbiturate effect on ovulation would appear to be negated since circulating progesterone levels are usually minimal during diestrus and do not begin to rise until the late afternoon of proestrus, as the result of normal secretion of the LH surge (11).

If inhibition of the proestrous LH surge were at the hypophyseal, rather than the neural level, it would be difficult to reconcile inhibition of the proestrous LH surge with administration of a short-acting barbiturate 24 hr earlier. The cell membrane depolarization following an increase in permeability to K^+ ion after barbiturates, which might affect LH release, is transient and dependent on the drug's wash-out time (12). Systemic clearance of PB is fairly rapid (13) and circulating levels 24 hr post injection are far below that normally used to block the

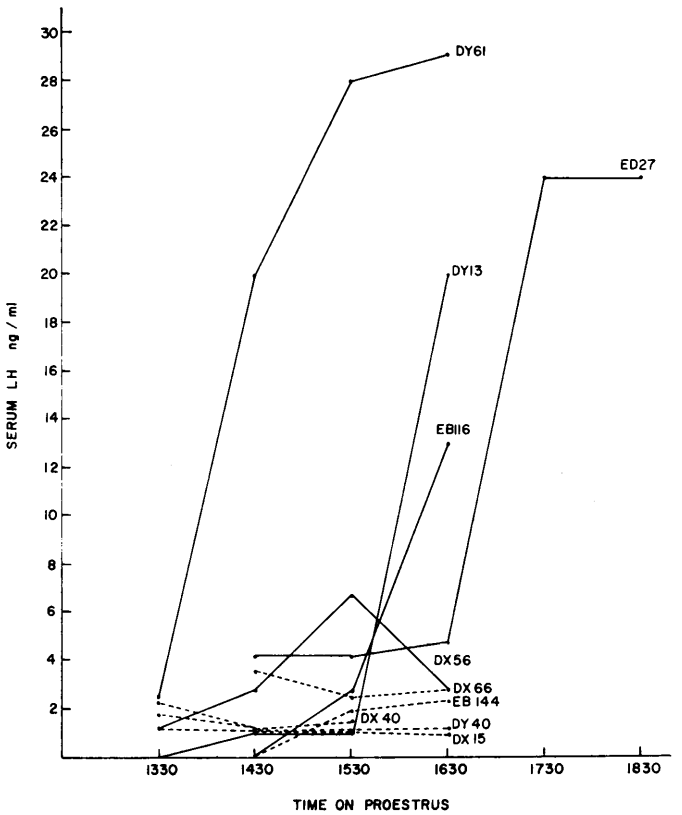

FIG. 1. The effect of pentobarbital or saline, administered at 1330 hrs. of diestrus, on serum LH at proestrus. Data from individual animals are plotted: — saline; - - - Pentobarbital 30 mg/kg, ip.

proestrus LH surge (1). The possibility of a temporary inhibition of hypophyseal gonadotrophin release interrupting in a significant way gonadal steroid secretion would appear to be remote, as uterine ballooning was not prevented in our animals or those of Schwartz and Lawton (5).

Rather, if barbiturates elevate the threshold of an endogenous neural timing center stimulating a daily, yet tonic, LH release, then the consistent 24-hr delay in ovulation seen after acute barbiturate administration may be partially explained (10). We have recently observed (14) that PB reduces the endogenous tonic and surge release of LH present in castrated female rats, lending further support to the above hypothesis.

Summary. Ovulation and the proestrous LH surge of cycling 4-day rats was blocked by barbiturate administration at 1330 hr of diestrus. Uterine ballooning was not blocked in the majority of animals injected with pentobarbital. These results suggest that barbiturates, administered in the early afternoon of any day of the estrous cycle of the 4-day rat, inhibit ovulation by a central block of LH release.

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