Effects of Ether and Pentobarbital on Serum Prolactin and LH Levels in Proestrous Rats (35113)

W. WUTTKE[1] AND J. MEITES[2]

Department of Physiology, Michigan State University, East Lansing, Michigan 48823

A sharp increase in LH release was reported in rats on the afternoon of proestrus between 4 and 8 p.m., with subsequent ovulation later at night (1–3). Atropine or pentobarbital (P), administered shortly before the critical time before LH release during proestrus, were shown to block spontaneous ovulation (4, 5). This blocking action was overcome by electrical stimulation of the arcuate nucleus–median eminence complex or of the medial preoptic area (6–8). Terasawa and Sawyer (9) confirmed these observations, and also found related changes in electrical activity in the arcuate nucleus after electrochemical stimulation of the medial preoptic area. Since administration of LH could induce ovulation in rats treated with these drugs and hypophysectomized early in proestrus, it was concluded that atropine and P inhibited LH release (10).

During the estrous cycle of rats, the highest serum prolactin levels measured by radioimmunoassay (RIA) were found during proestrus and estrus (11–14). The effects of ether or P on serum prolactin values have not been determined, although these drugs are routinely used by us and others to collect blood from rats for RIA. The present study reports the effects of ether and P on serum prolactin and LH levels before and after the critical period on the day of proestrus.

Materials and Methods. A total of 108 female Sprague-Dawley rats, weighing between 160–180 g (Spartan Animal Farms, Haslett, Mich.), were used. The rats were kept on a light schedule of 14 hr light and 10 hr dark (light from 7 a.m. to 9 p.m.) and fed *ad libitum*. At least 2 normal estrous cycles were followed by vaginal smears and only 4-day cycling rats were used. On the day of proestrus, the animals were treated as follows:

1. *Short-term effects of ether or P.* a. A total of 20 control rats were guillotined at 1 or 2 p.m., and blood was collected from the trunk.

b. Ten individual rats were bled under light ether anesthesia starting at 1 p.m. and every 10 min thereafter until 2 p.m. Before each bleeding, the rats were placed in an ether chamber for about 0.5 min.

c. Rats were bled under P anesthesia at the same time as in (1b). P Na was injected in distilled water in a dose of 31.5 mg/kg ip and the animals remained anesthetized for the duration of the experiment.

2. *Long-term effects of P.* a. A pretreatment serum sample was collected at noon, P was given at 1:45 p.m., and the rats were bled at 3, 4, 5, and 6 p.m. The rats remained anesthetized for the duration of the experiment.

b. A pretreatment serum sample was collected at 3:30 p.m. P was given immediately thereafter and serum samples were collected at 5:30, 6:30, 7:30, and 8:30 p.m.

c. The same bleeding schedule was followed as in (2a), except that the rats were not given P but were placed in an ether chamber for 0.5 min each time prior to bleeding.

d. Rats were bled under light ether anesthesia only, as in (2b).

Prolactin and LH RIA. The fresh blood from each rat was placed in a refrigerator for 24 hr at 35°F, centrifuged at 2000 rpm for 10 min; and the serum was removed and kept frozen until the time of assay. Prolactin and LH were measured in duplicate or tripli-

[1] Postdoctoral Research Fellow of the Max Kade Foundation, New York, N.Y.

[2] This work was supported in part by NIH grants AM 07484 and CA 10771.

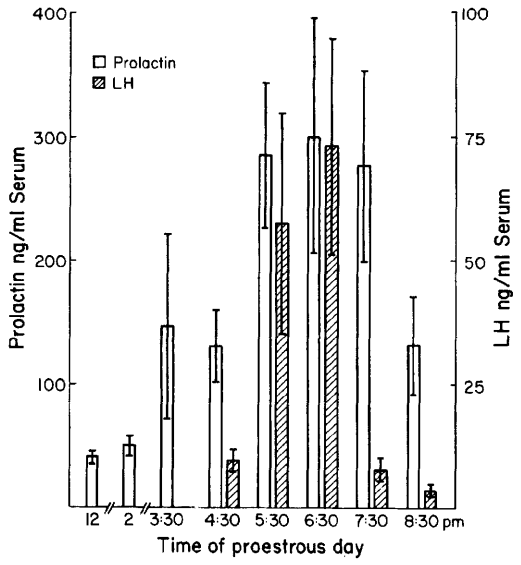

FIG. 1. Normal serum prolactin and LH levels during proestrus. Blood was obtained from the same animals (30 proestrous rats) after placing them under ether for about 0.5 min prior to each bleeding. Upper lines represent SE.

cate by methods described previously (11, 12). Purified rat prolactin was used as a reference preparation (HIV-8-C, biological potency = .77×NIH-P-Bl, kindly supplied by Dr. S. Ellis, NASA, Ames Research Center, Moffett Field, Calif.). The LH used for labeling (LER10506 C2) was kindly provided by Dr. L. Reichert, Emory University, Atlanta, Ga., and the LH-antibody by Drs. Niswender, Gay and Midgley, U. of Michigan, Ann Arbor. LH reference standard (NIAMD-Rat LH-RP-1) had a biological potency equal to .30×NIH-LH-Sl, and values are reported here in terms of the latter hormone. The data were analyzed by Student's *t* test and analysis of variance. Standard errors (SE) of the means are given on all blood values.

Results. Normal prolactin and LH values on the day of proestrus are shown in Fig. 1. Prolactin levels at 12 a.m. and at 2 p.m. were 40.6 ± 6.9 ng/ml and 50.1 ± 5.6 ng/ml, respectively. A significant increase was found at 3:30 and 4:30 p.m. (146.6 ± 76 ng/ml and 130.2 ± 30.1 ng/ml, respectively) ($p < .02$). Prolactin rose to peak values at 5:30 p.m. (284 ± 59.4 ng/ml), 6:30 p.m. (299.8 ± 969 ng/ml) and 7:30 p.m. (276.5 ± 78.4 ng/ml) ($p < .005$). By 8:30 p.m., serum prolactin fell to 133.9 ± 40.8 ng/ml ($p < .05$). LH was barely detectable (0.9 to 1.2 ng/ml) until 4:30 p.m. when a moderate increase occurred (to 9.6 ± 2.4 ng/ml, $p < .05$). A sharp increase to peak levels appeared at 5:30 and 6:30 p.m. (57.6 ± 22.3 ng/ml, and 73.8 ± 22.5 ng/ml, respectively) ($p < .001$). LH fell to lower levels by 7:30 and 8:30 p.m.

Figure 2 compares serum prolactin values collected from rats given ether or P with rats killed by guillotine. At 12:45 p.m., control proestrous rats were guillotined and 2 experimental groups were bled under ether anesthesia. No differences between the 3 groups was found. At 10 min after injection of P, prolactin rose to 133.1 ± 17.4 ng/ml, which was significantly higher than serum levels from ether treated rats (59.7 ± 19.8 ng/ml, $p < .01$). At 20 and 30 min after P injection, serum prolactin levels decreased slowly but were still higher than in ether treated rats. At 60 min after administration of P, prolactin appeared to be lower than in ether-treated or guillotined rats. Placement of rats under ether repeatedly for about 0.5 min produced no effect on serum prolactin values, which were comparable with those of guillotined rats.

The long term effects of P on prolactin are

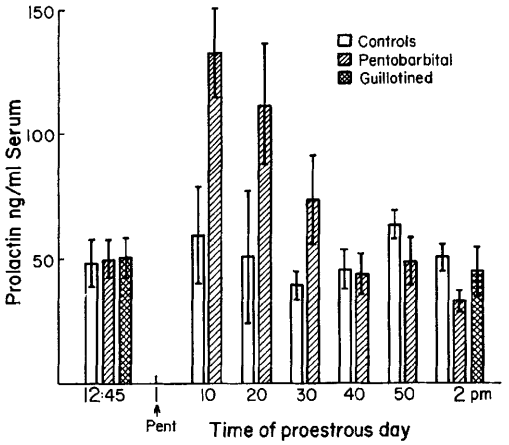

FIG. 2. Short-term effects of ether and pentobarbital (P) on serum prolactin. P was given at 1 p.m. only. Note stimulatory effect of P on serum prolactin during first 30 min. Each group represents 10 proestrous rats. Upper lines represent SE.

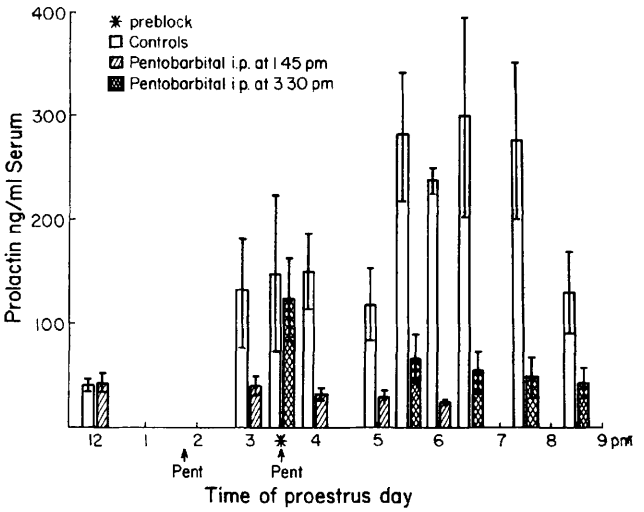

FIG. 3. Long-term effects of pentobarbital (P) on prolactin. Blood was removed from control rats under ether anesthesia (0.5 min), and shows normal proestrous prolactin values. P was given to one group at 1:45 p.m. (hatched bars) immediately after a pretreatment blood sample was taken. P was given to another group at 3:30 p.m. (crossed bars). As shown, P reduced serum prolactin levels in both groups. Each group represents 12 proestrous rats. Upper lines represent SE.

shown in Fig. 3. All prolactin values in P treated animals remained low compared to control rats given ether for 0.5 min. P prevented any rise in serum prolactin when given at 1:45 p.m. and reduced serum prolactin when given at 3:30 p.m. The absolute values in normal and P-treated animals, including the statistical differences, are shown in Table I.

The effects of P on LH are shown in Fig. 4. At 3:30 p.m. serum LH was very low. Immediately thereafter the rats were injected with P. The control rats given ether for 0.5 min showed a sharp increase in serum LH at 5:30 p.m. (57.6 ± 22.3 ng/ml) and at 6:30 p.m. (73.8 ± 22.5 ng/ml), whereas the LH rise was completely blocked by P. LH values in rats given P were 0.82 ± 0.15 ng/ml ($p < 0.05$) at 5:30 p.m., and 1.02 ± 0.16 ng/ml ($p < .01$) at 6:30 p.m., and remained low at 7:30 and 8:30 p.m.

Discussion. In early proestrus, prolactin

TABLE I. Long-Term Effect of Pentobarbital (P) on Serum Prolactin Levels on Day of Proestrus.[a]

Treatment	Prolactin (ng/ml)				
	12 a.m.	3 p.m.	4 p.m.	5 p.m.	6 p.m.
Controls (ether)	40.6 ± 6.9	132.4 ± 53.3	149.5 ± 36	167.2 ± 25.4	237.8 ± 12.6
P given at 1:45 p.m.	38.9 ± 9.3	39.4 ± 9	31.8 ± 5.5	28.8 ± 6.6	24.4 ± 2.8
$p <$		0.05	0.02	0.01	0.001
	3:30 p.m.	5:30 p.m.	6:30 p.m.	7:30 p.m.	8:30 p.m.
Controls (ether)	146.5 ± 76	384.6 ± 59.4	299.8 ± 96.9	276.5 ± 78.4	130.9 ± 40.9
P given at 3:30 p.m.	122.1 ± 40.7	69.3 ± 22	58.3 ± 29.4	48.8 ± 20.2	41.9 ± 15.3
$p <$		0.02	0.02	0.02	0.01

[a] p values indicate differences between control and P-treated rats at each time period; ± = SE.

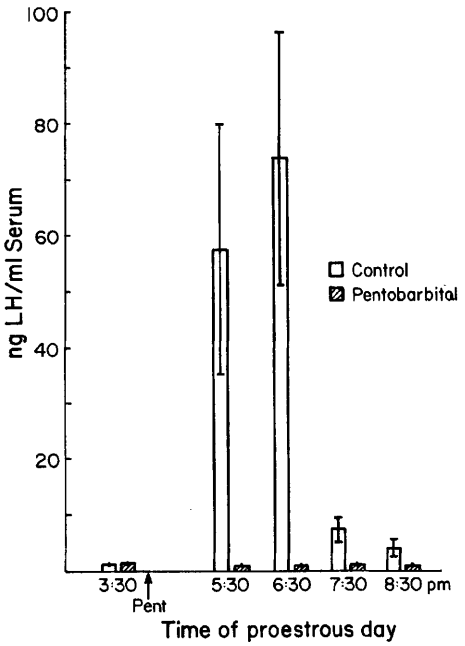

FIG. 4. Long-term effects of pentobarbital (P) on serum LH. The control group was bled under ether anesthesia (0.5 min) and the rats given P remained anesthetized for the duration of the experiment. Note suppression of LH rise. Each group represents 12 rats. Upper lines represent SE.

concentration in the serum is moderately elevated as compared to diestrous values (12, 13). In the present study, prolactin began to rise remarkably in the early afternoon of proestrus and reached peak values between 5:30 and 7:30 p.m. Serum LH values rose and fell more sharply than prolactin, but peak values were reached about the same time as prolactin.

Treatment of proestrous rats with sufficient P to maintain anesthesia for 5–7 hr completely blocked the LH rise, but produced about a 3-fold increase in serum prolactin 10 min after administering the drug. This rise in prolactin disappeared after 30 min and was then followed by very low values. The initial elevation in serum prolactin may be due to the early excitatory effects of P on the CNS. The inhibition by P of the serum LH rise on the afternoon of proestrus agrees with the early work of Everett and Sawyer (5) in which P was shown to block ovulation. Other workers (15) gave P to rats in lower doses and apparently found the LH peak to be unaffected. Therefore the dose of P administered may be an important factor in determining its ability to inhibit LH release. Ether given repeatedly for 0.5 min apparently had no effect on serum prolactin values, which were the same as in unanesthetized rats killed by guillotine. However, a more prolonged treatment with ether than used by us may increase serum prolactin values (Gay, personal communication).

The precise mechanism(s) of action of P on prolactin and LH release by the pituitary remains to be elucidated. P may initially depress hypothalamic PIF production which could account for the early rise in serum prolactin values. It may also inhibit hypothalamic LRF secretion, thereby reducing LH release from the pituitary. A direct effect of P on the anterior pituitary is also possible.

Summary. Serum prolactin and LH values were relatively low on the morning of proestrus, but increased remarkably on the late afternoon of proestrus. Peak values for both hormones were reached between 5:30–7:30 p.m., although prolactin began to rise earlier and declined later than LH. Placement of rats under ether anesthesia for about 0.5 min prior to withdrawing blood for radioimmunoassay did not change normal serum prolactin and LH trends, and prolactin values were the same as in unanesthetized rats killed by guillotine. Prolonged pentobarbital anesthesia completely blocked the late afternoon rise in serum prolactin and LH, but prolactin values showed a significant increase 10–30 min after injecting the drug.

1. Goldman, B. D., Kamberi, I. A., Siiteri, P. K., and Porter, J. C., Endocrinology **85**, 1137 (1969).

2. Ramirez, V. D., and McCann, S. M., Endocrinology **74**, 814 (1964).

3. Midgley, A. R., Jr., Gay, V. L., Caligaris, L. C. S., Rebar, R. W., Monroe, S. E., and Niswender, G. D., *in* "Gonadotropins" (E. Rosenberg, ed.), Vol. 68, p. 307. Geron-X, Inc., Los Altos, Calif. (1968).

4. Everett, J. W., Sawyer, C. H., and Markee, J. E., Endocrinology **44**, 234 (1949).

5. Everett, J. W., and Sawyer, C. H., Proc. Soc. Exp. Biol. Med. **71**, 696 (1949).

6. Everett, J. W., *in* "Control of Ovulation" (C. A. Villee, ed.). Pergamon, New York (1961).

7. Terasawa, E., and Sawyer, C. H., Endocrinology **84**, 918 (1969).

8. Critchlow, V., Amer. J. Physiol. **195**, 171 (1958).

9. Terasawa, E., and Sawyer, C. H., Endocrinology **85**, 143 (1969).

10. Redmond, W. C., Endocrinology **83**, 1013 (1968).

11. Monroe, S. E., Rebar, R. W., Gay, V. L., and Midgley, A. R., Jr., Endocrinology **85**, 720 (1969).

12. Niswender, G. D., Chen, C. L., Midgley, A. R., Meites, J., and Ellis, S., Proc. Soc. Exp. Biol. Med. **130**, 793 (1969).

13. Amenomori, Y., Chen, C. L., and Meites, J., Endocrinology **86**, 506 (1970).

14. Niswender, G. D., Midgley, A. R., Jr., Monroe, S. E., and Reichert, L. E., Proc. Soc. Exp. Biol. Med. **128**, 807 (1968).

15 Schneider, T., Piacsek, B., and Gay, V., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **29**, Abstr. 1101 (1970).

Received June 22, 1970. P.S.E.B.M., 1970, Vol. 135.