PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 155, 1-3 (1977)

Prolongation of the Proestrous LH Surge in the Rat with Cyanoketone (39731)

C. W. BEATTIE,*[1] C. H. RODGERS,† AND A. CORBIN*

* *Endocrine Section, Research Division, Wyeth Laboratories, Box 8299, Philadelphia, Pennsylvania 19101, and* †*Department of Psychiatry, University of Illinois College of Medicine, Chicago, Illinois 60680*

Progesterone's role in the initiation (1–3) or attenuation (4, 5) of the proestrous gonadotrophin surge in the intact cycling rat is a complex one. The temporal aspect of its secretion during the estrous cycle (6, 7), taken by itself, suggests that elevations in serum progesterone may be correlated with attenuation of the proestrous luteinizing hormone (LH) surge. Moreover, Ferin *et al.* (8) were unable to block ovulation or uterine ballooning in intact cycling rats with an antiserum to progesterone administered on diestrus or proestrus. However, these latter authors did not measure serum LH levels.

In contrast to these observations, cyanoketone (CK; 2-α-cyano-4,4,17α-trimethyl-androst-5-en-17-β-ol-3-one), a long acting inhibitor of 3β-hydroxysteriod dehydrogenase and Δ-5,3 ketosteroid isomerase (9), has been reported to block an LH-induced ovulation in PMS-primed immature rats (10–12).

These studies were designed to help resolve this dichotomy by administering CK on proestrus to intact 4-day cycling rats to determine whether the magnitude and/or duration of the proestrous LH surge was extended and ovulation was affected.

Materials and methods. Adult (200–250 g) female, Sprague-Dawley CD rats (Charles River) were acclimated to laboratory conditions and maintained on a 14-hr light/10-hr dark lighting schedule with midnight the midpoint of the dark period. Only those rats exhibiting at least two consecutive 4-day estrous cycles were used. CK (Sterling-Winthrop), 10 mg/rat (~40 mg/kg), was administered sc in corn oil at 1200 hr on proestrus. A control group received the corn oil vehicle alone. Each rat was sampled by substernal cardiac puncture (1 ml) at either 1630 or 2030 hr proestrus under light ether anesthesia for determination of serum LH and progesterone levels. At 0900 hr estrus, the vaginal smear was recorded, trunk blood was collected after decapitation for determination of serum LH and progesterone, and the uterus was weighed (luminal water expressed). Serum LH was assayed in duplicate 20-μl samples using the NIH rat LH kit. All values are expressed in terms of the NIAMDD rat LH-RP-1 standard (0.03 × NIH-LH-S-1). All serum LH levels were determined on samples assayed at the same time. Progesterone (p) was assayed in 200-μl serum samples utilizing an antiserum to 11α-hydroxy-11-hemisuccinate-BSA (No. P005) generated in rabbits, courtesy of Dr. Judith Weisz (Hershey Medical Center). Extracted samples were chromatographed on Sephadex LH-20 (Pharmacia) prior to assay. Cross-reactivity is illustrated in Table I. Appreciable cross-reactivity occurred only with 11α-hydroxyprogesterone and 17α-hydroxyprogesterone. Initial binding ranged from 40 to 53%. Recoveries ranged from 75 to 95%. All values reported are corrected. Sensitivity of the assay was 20 pg/ml. Tritiated P (sp act 313 dpm/pg) was purchased from New England Nuclear. Data were analyzed by means of Student's *t* test (13).

Results. Table II illustrates the effect of CK on the reproductive tract of the female rat. Administration of CK at 1200 hr on proestrus significantly increased the incidence of uterine ballooning (66% vs 0%) and uterine weight gain over control at estrous autopsy. There was no inhibition of vaginal cornification and no decrease in the incidence of ovulation or number of ova shed.

The data described in Table III demonstrate that proestrous administration of CK resulted in a significant increase in serum LH over control at 2030 hr proestrus. Al-

[1] Send reprint requests to C. W. Beattie, Ph.D., Department of Pharmacology, Bowman Gray School of Medicine, Winston-Salem, N. C. 27103.

ISSN 0037-9727

though large standard errors obscured the statistical significance of an increase in serum LH in CK-treated rats at 0900 hr estrus, the biological significance of such an increase is reflected by the increased incidence of uterine ballooning and increase in uterine wet weight on estrus. Table IV illustrates the effect of CK on proestrous/estrous serum progesterone levels. CK significantly reduced 2030-hr P levels, but did not reduce (~40%) circulating P to 0 or even diestrous levels. Estrous morning values were virtually identical to control levels.

Discussion. These data obtained with a purported inhibitor of progesterone synthesis confirm and extend the observations of Ferin *et al.* (8) of an increase in uterine ballooning on estrus with no decrease in ovulation after administration of an antiserum to P. The data reported herein also clearly demonstrate that the increase in uterine ballooning is accompanied not only by an actual increase in uterine wet weight but, perhaps more importantly, by a generalized increase in proestrous serum LH levels which may extend into the morning of estrus. These observations coupled with the partial decrease in late evening P levels strongly suggest that either enough estradiol (E_2) was present in CK-treated rats to stimulate the uterus and vagina and/or that P levels were reduced to the point that the biological effects of E_2 were not significantly antagonized. Recent reports suggest that P antagonizes the effect of E_2 at the level of

TABLE I. CROSS-REACTIVITY OF PROGESTERONE ANTISERA P005.

Steroid	Cross-Reaction at 50% displacement (%)
4-Pregnen-11-α-ol-3,20-dione (11α-hydroxypregn-4-ene-3,20-dione)	30.80
4-Pregnen-17-α-ol-3,20-dione (17α-hydroxypregn-4-ene-3,20-dione)	16.70
5β-Pregnan-3,20-dione	5.0
5β-Pregnan-3β-ol-20-one	0.20
4-Pregnen-20-α-ol-3-one (20α-dihydroprogesterone)	0.03
Corticosterone	0.02
Cyanoketone	0.02
5β-Pregnan-3β,20α-diol	0.01
Pregnenolone	0.01
Estrone	0.01
Estradiol	0.01
Estriol	0.01

TABLE IV. THE EFFECT OF CK (10 mg/RAT; 1200 hr; PROESTRUS) ON PROESTROUS (1630 AND 2030 hr) AND ESTROUS (0900 hr) SERUM PROGESTERONE LEVELS.

Treatment	Proestrus (ng/ml)		Estrus (ng/ml)
	1630	2030	0900
Oil	52.0 ± 3.2[a]	110.2 ± 15.0	38.1 ± 4.5
CK	62.0 ± 4.8	66.5 ± 5.4*	42.1 ± 5.0

[a] Mean ± SE.
* $P < 0.05$ vs oil control.

TABLE II. THE EFFECT OF CK (10 mg/RAT) ON THE REPRODUCTIVE TRACT OF THE FEMALE RAT.

Treatment	N	Number of ballooned uteri/total uteri	Number of cornified vaginal smears/total smears	Uterine weight (mg)	Number ovulating/total	Mean Number of ova
Oil	12	0/12	12/12	394.8 ± 18.6[a]	12/12	12.6 ± 0.9[a]
CK	12	8/12[b]	12/12	476.9 ± 19.1*	12/12	13.3 ± 0.8

[a] Mean ± SE.
[b] More than 100 mg of luminal fluid/uterus.
* $P < 0.05$ vs control.

TABLE III. THE EFFECT OF CK (10 mg/RAT; 1200 hr; PROESTRUS) ON Proestrous (1630 AND 2030 hr) AND ESTROUS (0900 hr) SERUM LH LEVELS.

Treatment	Proestrus (ng/ml)		Estrus (ng/ml)
	1630	2030	0900
Oil	1297.9 ± 479.8 (4)[a]	612.7 ± 167.1 (8)	346.0 ± 75.1 (12)
CK	1866.3 ± 213.1 (4)	1790.9 ± 339.5 (8)*	1065.1 ± 466.5 (12)

[a] Mean ± SE. Number of animals per group is in parentheses.
* $P < 0.01$ vs control.

the oviduct and uterus by interfering with the replenishment of cytoplasmic E_2 receptor. This secondarily reduces the number of receptor-E_2 complexes available for translocation to cell nuclei and results in a reduced tissue sensitivity to E_2 (14–17).

CK, in addition to its ability to produce a long-term inhibition of 3β-steroid dehydrogenase and Δ-5,3 ketosteroid isomerase, also binds to cytochrome *P*-450 and inhibits cytochrome oxidase *in vitro* (18). In spite of its ability to inhibit cholesterol side-chain cleavage and 3β-steroid dehydrogenase *in vitro*, either the dose of CK utilized in these *in vivo* studies or the time of administration on proestrus clearly was not able to entirely block P synthesis during the duration of the experiment. This of course implies that circulating E_2 was not reduced to negligible levels. Additionally, administration of CK at 1200-hr proestrus would for all practical purposes be too late to inhibit the preovulatory rise in E_2 via synthesis inhibition since Smith *et al.* (7) and Nequin *et al.* (6) have recently demonstrated that proestrous serum E_2 levels are already peaking at 1200 hr on proestrus.

It is tempting to speculate that the increase in magnitude and duration of the proestrous LH surge was the result of the partial reduction in proestrous serum P levels. A normal proestrous rise in serum P would therefore not only attenuate the proestrous surge of LH (4, 5), but elevated metestrous (diestrus 1) serum P values (6) may continue to suppress serum gonadotrophin levels prior to a subsequent diestrous (diestrus II) preovulatory rise in serum E_2 (7, 8, 18). Early diestrous administration of P or synthetic analogs to 4-day cycling rats is known to depress proestrous serum LH and FSH levels (1–3, 5). Similarly, administration of P prior to pentobarbital on proestrus (4) blocks the pentobarbital-delayed rise in serum LH (19) normally observed on the following day (estrus).

Collectively, these observations suggest that on proestrus, in the presence of high estrogen levels, a reduction in circulating P levels increases the magnitude and duration of the proestrous, preovulatory LH surge. Whether a reduction in serum P alters serum FSH levels and follicular recruitment is under investigation.

Summary. Administration of cyanoketone, an inhibitor of progesterone synthesis, just prior to the proestrous critical period significantly increased both the magnitude and duration of the proestrous LH surge and uterine weight without inhibiting ovulation in the 4-day cycling rat.

The authors would like to express their gratitude to J. Tracy and K. Koch for excellent technical assistance.

1. Everett, J. W., Endocrinology **43,** 389 (1948).
2. Zeilmaker, G. H., Acta Endocrinol. Copenhagen. **51,** 461 (1966).
3. Brown-Grant, K., Corker, C. S., and Naftolin, F., J. Endocrinol. **53,** 37 (1972).
4. Redmond, W. C., Endocrinology **83,** 1013 (1968).
5. Beattie, C. W., and Corbin, A., Endocrinology **97,** 885 (1975).
6. Nequin, L. G., Alvarez, J., and Schwartz, N. B., J. Steroid Biochem. **6,** 1007 (1975).
7. Smith, M. S., Freeman, M. E., and Neill, J. D., Endocrinology **96,** 219 (1975).
8. Ferin, M., Tempone, A., Zimmering, P., and Vande Wiele, R. L., Endocrinology **85,** 1070 (1969).
9. Goldman, A. S., Yakorac, W. C., and Bongiovanni, A. M., Endocrinology **77,** 1105 (1965).
10. Lipner, H., and Wendelken, L., Amer. Zool. **9,** 1087 (1969).
11. Lipner, H., and Wendelken, L., Proc. Soc. Exp. Biol. Med. **136,** 1141 (1971).
12. Lipner, H., and Greep, R. P., Endocrinology **88,** 602 (1971).
13. Snedecor, G. W., *in* "Statistical Methods." Iowa State University Press, Ames, Iowa (1956).
14. Brenner, R. M., Resko, J. A., and West, N. B., Endocrinology **95,** 1094 (1974).
15. Hseuh, A. J. W., Peck, E. J., Jr., and Clark, J. H., Nature (London) **254,** 337 (1975).
16. Hseuh, A. J. W., Peck, E. J., Jr., and Clark, J. H., Endocrinology **98,** 438 (1976).
17. Resko, J. A., Boling, J. L., Brenner, R. M., and Blandau, R. J., Biol. Reprod. **15,** 153 (1976).
18. Graves, P. E., Uzgiris, V. I., Querner, M., McIntosh, E. N., and Salhanick, H. A., "Program. Annual Meeting of the Endocrinology Society 58th," p. 131 (1976). [Abstract]
19. Schwartz, N. B., Amer. J. Physiol. **207,** 1251 (1964).

Received January 5, 1977. P.S.E.B.M. 1976, Vol. 155.