Inhibition of Ovulation by Inhibition of Steroidogenesis in Immature Rats[1] (35447)

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Both luteinizing hormone (LH) and human chorionic gonadotropin (HCG) induce a number of effects in the ovary, among these are: ovarian hyperemia (1), release of histamine (2), ovarian steroidogenesis (3, 4), ovulation (5), and corpus luteum formation (6). The mechanism by which LH or HCG induces ovulation in rats is still unknown. Since steroidogenesis is a prominent effect induced by these hormones, it is pertinent to examine the interaction between this action and ovulation (7).

To examine the relationship of steroids to ovulation, we have employed ethamoxytriphetol[3] (Mer-25), a postulated estrogen antagonist (8) and 2α-cyano-4,4,17α-trimethylandrost-5-en-17β-ol-3-one (cyanoketone)[3] a 3β-hydroxysteroid dehydrogenase inhibitor (9). Although Mer-25 had no effect on ovulation, cyanoketone caused complete inhibition of ovulation in immature rats pretreated with pregnant mare serum (PMS) and human chorionic gonadotropin (HCG).

Methods and Materials. Sprague-Dawley rats obtained at 22 days of age were maintained on a 14:10 hr light:dark regimen. All experiments were initiated at 4 p.m. on day 24 by the subcutaneous administration of 30 IU of PMS in 1 ml of saline. On the morning of the second day, or approximately 40–44 hr later, the animals were given 30 IU of HCG intravenously. Experimental animals received either Mer-25 or cyanoketone prior to receiving the HCG at stated intervals. Both compounds were dissolved in sesame oil in such concentrations that the volume of oil administered never exceeded 1 ml. The Mer-25 was administered in two equal doses but in 3 different amounts at 12 and 6 hr before the HCG. The amount of the drug given varied with the experiment. The dose of cyanoketone was, except when otherwise indicated, 3.52 mg/100 g of body weight. Since the animals averaged 50 g, they usually received a dose of 1.76 mg either in a single or in two divided doses. The cyanoketone was administered at 24 and 12, 12 and 6 or in single doses at 12, 6, or 3 hr before HCG.

The animals were killed by cervical disarticulation 24 hr after the HCG was administered. The ovaries and both horns of the uterus were removed in block, cleaned of fat, weighed, and the ova in the oviducts were counted. The data were analyzed by Dunnett's t statistic for comparing the means of several treatments with a control (10).

Results. Mer-25 prevented the uterine hypertrophy which usually results from the administration of HCG (Table I). The effectiveness of the Mer-25 as an inhibitor of uterine hypertrophy decreased as the dose increased, thus at a total dose of 20 mg the decrease from the control group was 36%, at 30 mg the decrease was 21% and at 40 mg it was only 9%. No effect of the Mer-25 at any dose level was apparent on ovulation. However, at the total dose of 40 mg there was a reduction in the number of ova shed of ap-

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TABLE I. Effect of Mer-25 and Cyanoketone on Ovulation and Uterine Weight.

Treatment	Mean ovum count	Reduction (%) in mean ovum count	No. ovulating/no. in group	Percentage ovulating	Mean uterine wt (mg)	Decrease (%)
Control	31.5 ± 3.2		6/6		92.7 ± 3.2	
Mer-25 (2 doses, 10 mg[b])	23.3 ± 3.9	26	6/6	100	59.4 ± 3.9[a]	36
Control	35.6 ± 3.2		6/6		85.6 ± 3.2	
Mer-25 (2 doses, 20 mg[b])	18.6 ± 3.7	48	6/6	100	78.6 ± 3.7[a]	9
Control	16.5 ± 2.3		6/6		75.0 ± 1.6	
Mer-25 (2 doses, 15 mg[b])	17.2 ± 4.3	—	6/6	100	59.2 ± 2.5[a]	21
Cyanoketone (2 doses, 0.88 mg[b])	4.0 ± 2.2[a]	76	3/6	50	88.8 ± 3.4	
Mer-25 (2 doses, 15 mg[b]) + cyanoketone (2 doses, 0.88 mg[b])	0.33± 0.33[a]	98	1/6	17	59.1 ± 1.8	

[a] At the 5% level of significance, $p \leq 0.05$.
[b] The dose noted is a single-dose quantity.

proximately 50%. When Mer-25 (30 mg) and cyanoketone were administered together an almost total inhibition of ovulation resulted.

Cyanoketone administered at 12 and 6, 24 and 12, 6, or 3 hr before HCG causes an inhibition of ovulation (Table IIA). In this series of experiments the drug was most effective when given 3 hr before HCG in a single

TABLE II. Inhibition of Ovulation by Cyanoketone.

Treatment	Mean ovum count	Reduction (%) in mean ovum count	No. ovulating/no. in group
	A. Effect of time of administration[a]		
Control	28.0 ± 5.6		6/6
Hr before HCG			
12, 6	0.33 ± 0.33[b]	99	1/6
12	3.5 ± 2.2	88	3/6
24–12	6.2 ± 3.7	78	3/6
Control	20.5 ± 4.5		6/6
12	4.5 ± 1.8	78	5/6
6	1.6 ± 0.92	92	4/6
3	0.33 ± 0.21	98	2/6
	B. Effect of dose[c]		
Control	20.5 ± 4.5	0	6/6
3.52	0.33 ± 0.2	98	2/6
Control	33.2 ± 4.0	0	6/6
1.76	0.5 ± 0.4	98	2/6
0.352	11.7 ± 2.4	64	6/6
0.176	21.7 ± 4.5	34	6/6

[a] Cyanoketone was given at dose of 3.52 mg/100 g of body weight.
[b] At the 1% level of significance, $p \leq 0.01$.
[c] Doses are indicated on the basis of mg/100 g of body weight.

TABLE III. Effect of Different Steroids on Cyanoketone-Induced Inhibition of Ovulation.

Treatment	Mean ovum count	Inhibition (%)	No. ovulating/ no. in group
Control	14.5 ± 4.8	0	4/4
Cyanoketone[a]	0.8 ± 0.4[e]	94	3/5
Progesterone + cyanoketone[b]	2.6 ± 1.6	82	4/5
Progesterone estradiol-17β +cyanoketone[c]	1.6 ± 1.1	89	3/5
Adrenalectomy[d]	11.6 ± 4.4	20	5/5

[a] Cyanoketone 3.52 mg/100 g of body weight in sesame oil, given 3 hr before HCG for all groups receiving the inhibitor.

[b] Progesterone 500 μg/0.1 ml of sesame oil given immediately before HCG and 4 hr later.

[c] Progesterone:estradiol-17β, 500 μg:μg/0.2 ml in sesame oil given immediately before HCG and 4 hr later.

[d] The adrenalectomy was performed 3 hr before the HCG was administered and the animals were given 1% NaCl solution and water until killed 24 hr later.

[e] At the 1% level of significance, $p \gtrsim 0.025$.

dose of 1.76 mg/100 g of body weight (Table IIB).

Since cyanoketone is an effective inhibitor of steroid biosynthesis, it seemed pertinent to examine the role of the adrenal gland in the ovulatory mechanism. Adrenalectomy performed (under ether anesthesia) 3 hr before HCG was administered caused slight (20%) reduction in the number of ova released but had no effect on the number of rats that ovulated (Table III). It should be noted that these rats had available a 1% NaCl solution as well as tap water for 24 hr.

Administration of progesterone (500 μg) or progesterone and estrogen (500 μg:1 μg) at the time HCG was administered and again 4 hr later did not reverse the inhibition of ovulation imposed by a dose of cyanoketone (3.52 mg/100 g of body wt) given 3 hr before the HCG (Table III).

Discussion. Ovulation is the final event in the life of the follicle. The events preceding ovulation are usually timed from the application of a gonadotropic stimulus (LH, HCG). These events usually last 10 to 12 hr in rats, rabbits, and hamsters and are terminated by rupture of the follicle. It is now apparent that in the rabbit (11, 12), hamster (13), mouse (14), rat (15), and frog (16) LH or HCG must act on the ovary for 2 to 4 hr. Subsequent changes are independent of continued exposure to gonadotropin.

As yet no data have appeared to explain how either LH or HCG induce ovulation. The direct action of progesterone on the follicle to induce ovulation has been examined in some detail with negative results (7, 17, 18); however, Forbes (19) did correlate the induction of gonadotropin secretion with an early rise in peripheral blood progesterone concentration in the rabbit. Uterine ballooning, an estrogen induced fluid secretion, subsides as a result of progesterone secreted by the ovary (20). Ferin *et al.* (21) have shown that antiprogesterone prevents the discharge of fluid from the ballooned uterus without preventing ovulation. Thus if progesterone induces ovulation its mechanism must be more subtle than present techniques can demonstrate.

Antiestrogen is able to block ovulation by interrupting the positive feedback circuit controlling LH secretion in immature rats (22). Ovulation in such animals follows administration of HCG, therefore the action of the antiestrogen is apparently not exerted on the ovary. Direct application of estrogen to the surface of immature rat ovaries causes an increase in the number of vesicular follicles and formation of small numbers of corpora lutea (23). Neither ovulation nor corpus luteum formation occurs in FSH-treated hypophysectomized immature rats after topical application of estradiol to the surface of one ovary (23). It is therefore unlikely that estrogens cause ovulation in the absence of LH and it appears unlikely that LH causes ovulation by causing estrogen secretion. The failure of Mer-25 to inhibit ovulation further supports the idea that although estrogen may affect ovulation by stimulation of reflex release of LH (24) it plays no direct role in the induction of ovulation. Cyanoketone, a 3-hydroxysteroid dehydrogenase inhibitor (25), is also an effective inhibitor of ovula-

tion when given 3 hr before administration of HCG. Since cyanoketone inhibits conversion of pregnenolone to progesterone, it effectively eliminates all the stages of steroidogenesis that follow the formation of pregnenolone, and indicates that one or several of the steroids in the biosynthetic chain may be responsible for the induction of ovulation.

The adrenal steroids probably have little effect on ovulation over a brief time, since adrenalectomy is without effect when the surgical procedure precedes the administration of HCG by 3 hr. The ovarian steroids progesterone and estradiol-17β in a regimen capable of inducing reflex ovulation (29) were ineffective in animals in which steroidogenesis was inhibited. Neither endogenous LH or exogenous HCG was able to cause ovulation in such ovaries. However this regimen does not exclude the possibility that high tissue concentration needs to be reached for the steroid(s) to be effective. Furthermore. the cyanoketone-inhibited ovary may not be a good model to test the direct action of steroids on ovulation.

Inhibitor studies suffer from the defect that they are negative observations. Unfortunately the positive evidence that a steroid precursor or a steroid is able to induce ovulation is not available. On the basis of present data, ovulation appears to be caused by steroids since inhibition of ovulation results when steroidogenesis is inhibited. However, it is also possible that the inhibitor may affect more than a single site and that both steroidogenesis and ovulation, although mediated by different pathways, are simultaneously inhibited.

*Summa*ry. Immature female rats treated with PMS on day 24 and HCG on day 26 ovulate and have ova in the oviduct on day 27. Treatment with ethamoxytriphetol (Mer-25) prior to the administration of HCG has no effect on ovulation but treatment with the 3β-hydroxysteroid dehydrogenase inhibitor 2α - cyano - 4,4,17α - trimethylandrost - 5 - en - 17β-ol-3-one (cyanoketone), also given before HCG, effectively inhibits ovulation. Adrenalectomy performed prior to administration of HCG does not prevent ovulation while neither progesterone (500 μg) nor progesterone:estrogen, 500 μg:1 μg overcome the cyanoketone block to ovulation. Ovarian steroidogenesis and ovulation are apparently related.

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