Differential Threshold of Progesterone Required for Maintenance of Diestrus Smear, Pseudopregnancy and Pregnancy in Rats (37254)

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Introduction. The ability of the corpus luteum of the intact rat to secrete progesterone is known to vary depending upon the physiological state of the animal. Thus, the ovary of the pregnant rat secretes relatively greater amounts of progesterone than that of the pseudopregnant or cycling animal (1). The often-confirmed observation that prolactin alone can maintain in hypophysectomized rats certain physiological events—like vaginal diestrus smear and decidual cell reaction —that are dependent upon active progesterone synthesis in the corpus luteum, has led to the belief that this hormone is the sole luteotropin in the rat (2, 3). However, recent investigations from our laboratory, using an antiserum to LH, has shown that LH is involved in the regulation of the corpus luteum function in the rat (4–6). Particularly during pregnancy when the need for progesterone is maximal, deprival of LH support has been shown to result in a marked reduction in ovarian progesterone output (5). Further, while prolactin administration is known not to support pregnancy in hypophysectomized rats (7), LH administered alone has been shown to sustain gestation (8, 9).

In light of this, it is conceivable that prolactin's luteotropic activity is demonstrable in situations where the threshold for progesterone is low. Thus, an attempt has been made in the present study to establish the variation, if any, in the progesterone threshold for maintaining different physiological states.

Materials and Methods. Adult, virgin, female albino rats of our Institute colony weighing about 160–200 g were used in these studies. Estrus cycles were observed by daily vaginal smears for about 2 weeks and only those rats showing regular cycles were used. The rats were ovariectomized on the first day the vaginal smear became dominated by leukocytes (diestrus I) and separated into different groups for daily treatment with varying doses of progesterone together with a constant level of 0.01 μg of estradiol-17β. In another experiment, rats ovariectomized on diestrus I were primed for 5 consecutive days with 0.02 μg of estradiol-17β per day. After a continuous estrus phase (as observed by vaginal smears) was established, the rats were supplemented daily with varying doses of progesterone, the estradiol administration being continued throughout the experiment. The end point of both these experiments was the continuous maintenance of vaginal diestrous smear in the face of a daily challenge dose of estrogen.

Pseudopregnancy in adult virgin rats was induced on the day following successful matings with males of proven fertility (Day 1 being the day of sperm detection) by ligating the uterine horns at the utero-tubal junction and thus preventing the ova from passing into the horns. Decidualization was then induced in the ligated horns by injecting into each horn 20 μg of histamine dihydrochloride (Sigma Chemical Company, USA) in 0.01 ml of distilled water, at 10 AM on Day 5 of pseudopregnancy, the time of maximal uterine sensitivity to trauma. Prior to inducing trauma, the animals were ovariectomized on Day 5 of pseudopregnancy. These were then supplemented with varying doses of progesterone daily, from Days 5–9. No estrogen was administered as it was assumed that sufficient estrogen would be available from

the "surge" on Day 4.

The rats were autopsied on Day 10 and the uterus and ovaries removed, cleaned of adhering tissue, and weighed to the nearest 10th of a mg. The formation of decidual tissue was checked by visual and histological examination, as well as by estimating glycogen in 100 mg portions of the decidual tissue by the method of Seifter *et al.* (10).

In another experiment, pregnant rats were ovariectomized on different days of pregnancy (Days 5, 8, 12, and 16) and pregnancy maintained by supplementing with varying doses of progesterone for the next 4 days. Twenty-four hours after cessation of progesterone treatment, the animals were autopsied and the nature and number of viable implantation sites noted.

Progesterone and estradiol-17β (Sigma Chemical Company, USA) were injected subcutaneously, using refined peanut oil as the vehicle. Progesterone was administered at the dose levels of 20, 50, 100, 150, 250, and 500 μg in 0.1 ml of refined peanut oil for the experiment with cycling rats and at dose levels of 250, 500, 1000, 2000, 3000, and 4000 μg in 0.1 ml peanut oil for the experiments with pregnant and pseudopregnant rats.

Results. Determination of progesterone threshold in cycling rats. It can be seen, from the results presented in Table I, that the minimum effective dose (m.e.d.) of progesterone required to extend the duration of leukocytic smears in ovariectomized rats, in the face of a constant supply of estrogen (0.01 μg/day/rat), was found to be between 50–100 μg/day/rat. Increasing doses of progesterone (100, 250, 500 μg/day) gradually prolonged the duration of leukocytic smears. Daily treatment with 20 μg of progesterone was unable to overcome the effect of estradiol administration and a prolonged estrus phase was induced, as indicated by the cornification of the vaginal epithelium. Establishment of a continuous estrus phase prior to the start of treatment with progesterone, shows that the m.e.d. of the latter needed to change the vaginal smear to diestrus is increased from 50–100 μg to 100–150 μg/day (Table I). This increase in the m.e.d. of progesterone, perhaps reflects a build-up in estrogen concentration during the first phase of the experiment when no progesterone was given, resulting thereby in an incrased need for progesterone to overcome the estrogen effect on vaginal smear. As in the case of the first experiment, increasing the daily dose of progesterone over the m.e.d. led to prolongation of the duration of the leukocytic smear.

TABLE I. Minimum Effective Dose of Progesterone Required to Maintain the Vaginal Diestrus Smear in Cycling Rats Castrated on Diestrus I.

Expt	Daily progesterone treatment (μg)	Number of rats	N/ND[a]	% Diestrus
A[b]	20	3	18/0	0
	50	5	45/23	51
	100	5	52/34	65
	250	5	43/37	86
	500	3	17/15	88
B[c]	50	3	21/0	0
	100	3	33/18	55
	150	3	21/12	57
	250	3	24/15	63
	500	3	21/18	86

[a] N = Total number of observations. ND = Total number of diestrus smears seen.

[b] A = Daily 0.01 μg estradiol-17β together with progesterone.

[c] B = Priming with 0.02 μg estradiol-17β daily for 5 days prior to start of progesterone treatment. Estradiol injections continued till end of experiment.

Minimum effective dose of progesterone required to maintain decidualization. Traumatization of the uteri of castrated rats on Day 5 of pseudopregnancy with 20 μg of histamine and with no progesterone supplementation did not induce decidualization, as seen by the uterine weight taken at autopsy on Day 10, and also by the amount of total glycogen present in the uterine tissue (see Table II). Daily administration of progesterone at increasingly higher doses (100, 200, 500, 1000, 3000 μg per rat) resulted in a gradual increase in the decidual weights and glycogen content of the tissue. This is similar to the results obtained by Yochim and De Feo (11); 250 μg/day/rat of progesterone brought about a doubling in

TABLE II. Minimum Effective Dose of Progesterone Required to Maintain Decidualization in Pseudopregnant Rats Castrated on Day 5.

Progesterone treatment (mg)	Number of rats	Average uterine weight at autopsy (mg ± SE)	Average glycogen (μg)	
			Total	100 mg tissue
Control[a]	4	204.0 ± 25.5	45.3 ± 15.11	21.92 ± 4.7
0.10	4	224 ± 2.3	63.32 ± 33.3	28.52 ± 15.2
0.25	5	775 ± 164.3	760.2 ± 195.8	89.94 ± 7.07
0.50	5	836.6 ± 77.7	1029.3 ± 328.5	121.03 ± 30.3
1.00	5	1814.8 ± 423.5	933.2 ± 88.2	57.75 ± 12.5
3.00	4	1713.3 ± 178.5	1452.6 ± 434.8	88.9 ± 31.6

[a] No progesterone.

the uterine weights and also showed a dramatic increase in the total glycogen content. Maximal response was obtained with dosages of 1 and 3 mg of progesterone.

Minimum effective dose of progesterone required to maintain gestation in rats castrated on different days of pregnancy. From the data presented in Table III it appears that, in rats castrated on Day 5 of pregnancy, a minimum of 2–4 mg of progesterone/day/rat, from Days 5–9, is required to maintain implanted sites in a viable state. Estrogen already present in the system from the "surge" on Day 4 is perhaps sufficient to synergize with progesterone in maintaining implantation. Dosage of progesterone below 1 mg/day did not support implantation. Since at the dose level of 2 mg/day, implantation sites could still be seen on Day 10 (though these were significantly of smaller size), the m.e.d. for successful maintenance of implantation sites during this period may be around 3.0 mg/day. The period of Day 8 to 12 seems to be most sensitive to progesterone dosage since reducing the level from 3 to 2 mg per day resulted in resorption. Progesterone requirement to maintain gestation seems to be drastically reduced after Day 12 (Table III). The m.e.d. thus shifts from 3 mg/day during Days 8–12 to 2 mg/day during Days 12–15 and to 250–500 μg/day during the final phase of gestation, *viz*, Days 16–19.

Discussion. The data presented in this study indicates that there is a significant difference in the threshold requirement of progesterone for the maintenance of pregnancy, decidual cell reaction and vaginal

TABLE III. Minimum Effective Dose of Progesterone Required to Maintain Pregnancy.[a]

Progesterone dosage (μg)	Duration of treatment							
	Days 5–9		Days 8–12		Days 12–16		Days 16–19	
	No. of rats	Av. no. sites NV/N[b]	No. of rats	Av. no. sites NV/N	No. of rats	Av. no. sites NV/N	No. of rats	Av. no. sites NV/N
250	4	0/0	3	0/0	—	—	3	7/7
500	3	0/0	3	0/0	3	0/0	3	8/8
1000	4	0/0	3	0/0	3	0/0	3	6/6
2000	4	10/10[c]	3	0/0	4	6/6	—	—
3000	—	—	3	8/8	—	—	—	—
4000	4	8/8	—	—	—	—	—	—

[a] Animals were ovariectomized on the day of start of progesterone therapy. Autopsy was done 24 hr after cessation of treatment.

[b] NV = Number of viable sites. N = Total number of sites.

[c] Sites appeared much smaller in size when compared to normal.

diestrus smear, the latter two physiological events requiring lesser amounts than that needed for pregnancy. Further, even in pregnancy the progesterone requirement gradually reduces with the advancement of gestation (from 4 mg to 250 μg/day). This is in keeping with Wiest's observation that the levels of ovarian and plasma progesterone reach a peak by Day 11 and gradually decline thereafter (12).

It thus appears that the success earlier workers have had in demonstrating the leuteotropic activity of prolactin has been due to the choice of physiological parameters which happen to be dependent upon low progesterone thresholds. Prolactin has been shown to contribute to luteal function by maintaining normal levels of the enzymes cholesterol ester synthetase and esterase, involved in cholesterol turnover (13) and by inhibiting the enzyme 20 α-steroid dehydrogenase, involved in the conversion of progesterone to its 20 α-dihydro-derivative (14). Assuming that the luteal tissue devoid of any tropic support has still an inherent capacity to produce progestins, even though at a low level, prolactin could then perhaps maintain the luteal activity at a low key for a protracted time, the progesterone produced under such an action being sufficient to exhibit a diestrus smear and decidual cell reaction. It may be pertinent to refer here to the work of Macdonald, Moudgal, and Greep (in press) who showed that ovaries of rats bearing autotransplanted pituitaries and treated with LH antiserum are still capable of producing enough progesterone to exhibit a diestrus smear in the face of an estrogen challenge.

Obtaining maximal decidual cell response or maintaining early pregnancy, however, is only possible with optimal progesterone levels. As has been shown earlier (5) amplification of luteal functionality (in producing progesterone at optimal rates) is dependent upon LH stimulus. The fact that threshold for progesterone to maintain viability of the fetuses is lowered with the progress of gestation, coupled to the earlier observation that progesterone output of ovaries of late pregnancy are relatively less effected by LH deprival (5) may explain the ineffectiveness of the LH antiserum, when given from Day 12 onwards to disrupt pregnancy.

Summary. The threshold of progesterone required for maintenance of diestrus smear, pseudopregnancy and pregnancy in ovariectomized rats has been shown to differ significantly. Whereas, vaginal diestrus can be maintained in ovariectomized rats, in the face of constant estradiol challenge, with as little as 50–100 μg of progesterone, the amounts needed for induction of decidualization and maintenance of early pregnancy are respectively 250 μg and 3 mg. In pregnancy itself, the progesterone need continuously varies, from a high of 3–4 mg of progesterone/day during the first 12 days to as little as 250 μg during the last 5 days of gestation. This differential threshold for progesterone may perhaps be responsible for the observed luteotropic activity of prolactin and LH in varying physiological situations.

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