5α-Dihydroprogesterone Influence on Ovulation of Prepuberal Rats (36836)

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(Introduced by Roy O. Greep)

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Administration of pregnant mare's serum gonadotropin (PMSG) induces ovulation in the prepuberal rats (1). In these rats, ovulation occurs by activation of their own pituitary and subsequent release of ovulatory hormone (OH) approximately 52 hr following PMSG injection (2). Progesterone influences the release of OH and eventual ovulation depending upon the time of injection following PMSG treatment (3–6). In the present experiments the facilitation or inhibition effects of exogenous 5α- and 5β-dihydroprogesterone (DHP) on the PMSG induced ovulation of immature rats were demonstrated to be comparable with those of progesterone.

Materials and Methods. Prepuberal female rats (Charles River Laboratories) were housed under normal laboratory conditions of 14 hr of light (colony time 6 AM–8 PM) and were fed Purina Rat Chow and tap water *ad libitum*. At day 30 of age, they were administered 20 IU of PMSG (Equinex, Ayerst) dissolved in 0.2 ml of saline subcutaneously (sc) at 8 AM. They were then injected sc 0.5 or 2.0 mg crystalline progesterone, 5α-DHP (5α-pregnane-3,20-dione), or 5β-DHP (5β-prenane-3,20-dione) in 0.2 ml sesame oil at 24, 48, or 51 hr following PMSG (Fig. 1). The control rats were treated identically with gonadotropin and received sesame oil only. The rats were killed by an overdose of ether at time intervals of 24, 48, 54, 72, or 96 hr post-PMSG. Both oviducts were dissected out and pressed between two microscope slides and the number of eggs was noted.

Results and Discussion. PMSG (20 IU) alone normally induced ovulation of 20.3 ± 10.9 eggs/rat (mean ± SD of 10 rats) when

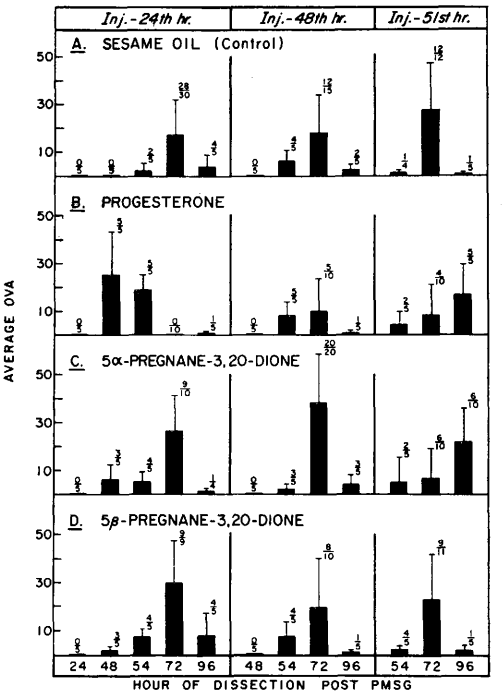

FIG. 1. Graph illustrates the effects of 2.0 mg/rat dose of progesterone, 5α-DHP, and 5β-DHP administered at 24, 48 and 51 hr post-PMSG on the average number of ova in oviducts dissected at various times. In the fraction, the numerator indicates the number of rats ovulated; the denominator, the number of rats used (av ova ± SD of all the rats used).

observed at 72 hr following its administration. Injection of 2.0 mg progesterone 24 hr post-PMSG caused advancement of the time of ovulation. The number of ova observed was 24.8 ± 17.8 and 18.6 ± 6.1 at 48 and 54 hr, respectively (Fig. 1B). Significantly smaller numbers of ova were seen in the

TABLE I. Influence of Progesterone and 5α- and 5β-DHP on PMSG-Induced Ovulation in 30–33-Day-Old Rats.[a]

Steroids	After PMSG (hr)	Steroid dose (mg)	Av ova/rat	Rats ovulated (%)	Av ova/ovulated rat
Sesame oil (control)	24	—	17.3 ± 14.4	93 (30)[b]	18.5 ± 14.1
	48	—	17.6 ± 15.9	80 (15)	22.1 ± 14.7
	51	—	27.3 ± 19.5	100 (12)	27.3 ± 19.5
Progesterone	24	0.5	10.0 ± 16.3	40 (10)	25.0 ± 17.3
		2.0	0.0 ± 0[c]	0 (10)	0.0 ± 0[c]
	48	0.5	22.7 ± 17.9	90 (10)	25.2 ± 17.0
		2.0	9.8 ± 13.7	50 (10)	19.6 ± 13.5
	51	0.5	25.0 ± 16.7	90 (10)	27.7 ± 15.1
		2.0	8.0 ± 13.0[c]	40 (10)	20.0 ± 13.8
5α-Pregnane-3,20-dione	24	0.5	28.9 ± 19.9[c]	90 (10)	32.1 ± 18.3[c]
		2.0	26.2 ± 14.5	90 (10)	29.5 ± 12.1[c]
	48	0.5	33.7 ± 14.5[c]	100 (10)	33.7 ± 14.5
		2.0	38.0 ± 20.7[c]	100 (20)	38.0 ± 20.7[c]
	51	0.5	15.3 ± 14.0	80 (10)	19.1 ± 13.1
		2.0	7.1 ± 12.7[c]	60 (10)	11.8 ± 15.0
5β-Pregnane-3,20-dione	24	0.5	19.4 ± 18.8	70 (10)	21.5 ± 18.5
		2.0	29.6 ± 17.2[c]	100 (9)	29.6 ± 17.2
	48	0.5	32.4 ± 20.9[c]	100 (10)	32.4 ± 20.9
		2.0	19.2 ± 20.5	80 (10)	24.0 ± 20.2
	51	0.5	17.3 ± 16.5	70 (10)	24.7 ± 14.0
		2.0	22.5 ± 19.7	80 (10)	27.5 ± 18.1

[a] Average ova ± SD at 72 hr post-PMSG.
[b] Number within parentheses is number of rats used.
[c] Significantly different from oil control $p < .05$.

groups autopsied at 72 and 96 hr, indicating that the eggs had moved out of the oviduct. Progesterone treatment at hour 48 or 51, presumably prior to or during the peak of OH release, caused no advancement of the time of ovulation, but gave a dose-dependent reduction in the percent rats ovulating and in the number of eggs shed per rat when observed at 72 hr (Fig. 1B, Table I). This may be due to progesterone interfering with the release of OH, thus causing subsequent delay of ovulation. The delay of ovulation was particularly marked in the group treated with progesterone at hour 51. These rats had a larger number of ova when autopsied at 96 hr, as compared to those at 72 hr, or to sesame oil controls (Fig. 1B).

The effect of progesterone on the advancement (3, 4) and facilitation (5, 6) of ovulation in the PMSG treated prepuberal rats (21–24 days old) had been demonstrated previously. Our present studies with 30–33-day-old rats indicated that administration of progesterone close to or during the time of OH release causes inhibition of ovulation. These results are comparable with adult 5 day cycling rats in which injection of progesterone at early diestrus day 3 induced premature release of gonadotropin and ovulation, while treatment at late diestrus prevented gonadotropin release and ovulation was inhibited (7–9).

Injection of 5α-DHP at hour 24 influenced ovulation by facilitation rather than by advancement as with progesterone. Administration of 5α-DHP at hour 48 caused ovulation in all the rats autopsied at 72 hr and ovulation of a significantly greater number of ova

than in the control rats (Fig. 1C, Table I). Treatment with 5α-DHP at hour 51 induced inhibition of ovulation analogous to progesterone. Perhaps 5α-DHP was also interfering with the release of OH and resulted in the shedding of fewer eggs when observed at 72 hr. At 96 hr, as in the progesterone treated rats, a larger number of ova was noted when compared with the 72 hr group. 5β-DHP caused ovulation in more rats and release of more ova if injected at hour 24 with 2 mg or at the hour 48 with 0.5 mg (Fig. 1D, Table I). No inhibitory effect of 5β-DHP was seen when administered at 51 hr.

Conversion of progesterone into 5α- or 5β-DHP is irreversible. These reductase enzymes, especially 5α, have been demonstrated in liver (10–12), hypothalamus (13, 14), and in the ovary (15–18) of rats. The circulating progesterone during its passage through these organs can be converted into ring A reduced products. It is possible that the ovulation facilitation or inhibitory effect produced by progesterone occurs after its reduction at ring A.

Summary. 5α-DHP administered 24 or 48 hr post-PMSG treatment to 30-day-old prepuberal rats stimulated ovulation, as indicated by a greater number of eggs in the oviducts at 72 hr and by a higher percentage of rats ovulating. 5α-DHP at 51 hr after gonadotropin stimulation caused inhibition of ovulation similar to the action of progesterone.

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