The Effect of Prostaglandin $F_{2\alpha}$ on the Progestogen Content of Ovaries from Pseudopregnant Rats (33495)

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As a possible explanation for the local uterine cornual control exerted in some species over corpora lutea of an adjacent ovary, a concept of an indirect mechanism of action was conceived. This hypothesis was concerned with the possibility that ovarian blood flow could be restricted by a venoconstricting secretion of the uterus that would affect the venous drainage common to the ovary and uterus. Under this restriction of blood flow, corpora lutea could succumb to conditions such as ischemia, limited substrate availability or accumulation of metabolites. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) which is a potent and selective venoconstrictor (1) and the major prostaglandin associated with endometrium (2) was selected as a test agent for this concept.

Experimental Procedure. Mature (200–300 g) female Spartan Sprague-Dawley rats exhibiting regular estrous cycles were prepared with a right heart or right uterine cornu indwelling catheter (3). One end of the catheter was inserted 10 mm into the right heart or uterus and held in place by a suture, and the other end was exposed at the back of the neck. After recovery, animals were again checked for regularity of cycles, and when in proestrus were stimulated vaginally with an electric probe to induce pseudopregnancy (4). Vaginal cytology was examined daily to ascertain pseudopregnancy, and on the morning of day 5 of pseudopregnancy either $PGF_{2\alpha}$ in saline or just saline infusions were initiated. The volume infused was 2.06 ml/day, and the daily $PGF_{2\alpha}$ dose was 1 mg/kg. After 48 hr of constant infusion, ovaries were removed and frozen in 1 ml of 2.5% NaOH. This investigation was run in two separate parts. In each experiment the procedure was similar except that in the first trial only uterine infusions were done while in the second, uterine and heart cannulations were used.

Gas chromatography was employed to determine the progesterone and 20α-hydroxypregn-4-ene-3-one (20α dihydroprogesterone) levels in ovaries (5). Ovaries were homogenized in 2.5% NaOH. Nonsaponified lipids were extracted with ether and subjected to two dimensional thin-layer chromatography. After progesterone and 20α-dihydroprogesterone spots were eluted, these steroids were assayed using an F and M model 1609 gas chromatograph (1% silicone rubber SE-30 on F&M Diatoport S, 80-100 mesh column). Tritiated steroids were added at homogenization to enable correction for percentage recovery.

In another study 28 pseudopregnant rats were divided into two groups. Eleven were given 0.5 ml of saline subcutaneously b.i.d. for 8 days; the other 17 females were given 0.8 mg of $PGF_{2\alpha}$ each injection following the same regimen. Vaginal smears were recorded daily until two normal estrous cycles were recorded.

Results. Infusion of $PGF_{2\alpha}$ on days 5 and 6 of pseudopregnancy caused a dramatic shift in the steroid character of the ovaries of infused animals. Control ovaries, harvested on day 7 of pseudopregnancy (Table I) in the first experiment, contained 10 times as much progesterone as 20α-reduced progesterone/mg of ovary. Treatment with $PGF_{2\alpha}$ at 1 mg/kg/day for 2 days, however, resulted in a shift in the ratio of progesterone to its reduced form so that the 20α-dihydroprogesterone was approximately 4 times as concentrated as progesterone (3.6 vs 16.2 and 2.1 vs. 7.8 μg/g of tissue).

The direction and magnitude of effect of $PGF_{2\alpha}$ were similar in both trials, but initial values were somewhat different for each. In saline controls of the second study the ratio of progesterone to 20α-dihydroprogesterone was close to unity (4.4 vs 5.2 and 6.7 vs 4.6 μg/g of tissue). Infusion with $PGF_{2\alpha}$ either

TABLE I. Effect of $PGF_{2\alpha}$ Infusion on the Concentration of Progesterone and 20α-Dihydroprogesterone in Ovaries of Pseudopregnant Rats.

Treatments	No. of rats	Ovarian wt. (mg)	Steroid concentration[a] (μg/g of tissue)		P/OHP ratio
			Progesterone	20α-Prog.[b]	
Expt. 1					
Saline infusion (2.06 ml/day) in uterus	2	137	11.7	1.2	9.75
$PGF_{2\alpha}$ infusion (1 mg/kg/day) in uterus	3	281	3.6	16.2	0.22
	2	184	2.1	7.8	0.27
			$\bar{x} = 2.8$	12.0	
Expt. 2					
Saline infusion (2.06 ml/day) in uterus	3	474	4.4	5.2	0.85
	3	484	6.7	4.6	1.46
			$\bar{x} = 5.6$	4.9	
$PGF_{2\alpha}$ infusion (1 mg/kg/day) in uterus	3	296	0.70	11.4	0.06
	3	426	0.64	8.7	0.07
			$\bar{x} = 0.67$	10.1	
$PGF_{2\alpha}$ infusion (1 mg/kg/day) in right heart	3	631	0.32	5.5	0.06
	2	274	0.89	8.3	0.11
			$\bar{x} = 0.61$	6.9	

[a] Each value is the average of duplicate determinations on pooled ovaries from 2 or 3 rats.
[b] 20α-Prog. = 20α-dihydroprogesterone.

via the uterus or heart resulted in a shift to the reduced steroid. In uterine infusions, progesterone concentration dropped while the reduced steroid content increased. Again, the combined concentrations of the two steroids were similar in the uterine infusions (10.5 and 10.8 μg/g of tissue); however, animals perfused via the right heart had a somewhat lower concentration of combined steroid (7.5 μg/g). Nevertheless the $PGF_{2\alpha}$ induced shift from progesterone to its 20α-reduced form was similar in both uterine and heart infusions.

In the study where $PGF_{2\alpha}$ was injected daily and vaginal smears followed until return to estrus, control animals exhibited pseudopregnancy lasting from 13 to 20 days (mean = 17 days) while $PGF_{2\alpha}$ treated animals returned to vaginal estrus in 5–13 days (mean = 8 days).

Discussion. $PGF_{2\alpha}$ infusion for 48 hr can significantly alter relative concentrations of ovarian progesterone and 20α-dihydroprogesterone, and it is quite likely that this shift is indicative of luteal degeneration. This conclusion is strengthened by the ability of injected $PGF_{2\alpha}$ to appreciably shorten pseudopregnancy as witnessed by vaginal estrus. In untreated pseudopregnant rats, maximal levels of progesterone secretion are reached about day 4 of pseudopregnancy and are maintained at a high level until just before termination of pseudopregnancy on days 13–15 (6). 20α-Dihydroprogesterone on the other hand is quite low in comparison to progesterone during pseudopregnancy but predominates when no active corpora lutea are present (7). Furthermore a shift to predominance of 20α-dihydroprogesterone while the combined concentration of progesterone and the 20α-reduced form has not changed is indicative of early luteal degeneration (8).

Preliminary studies in this laboratory indicate that progesterone synthesis is not hindered when pseudopregnant rat ovaries are incubated with $PGF_{2\alpha}$ *in vitro*. This preliminary information, in conjunction with the

data reported in Table I, presents evidence for an indirect mechanism for $PGF_{2\alpha}$ in terminating pseudopregnancy. The obvious answer to this proposal so far is that hypothalamo–pituitary–gonadal relationships are involved. This may well be the case, and $PGF_{2\alpha}$ may be causing a chemical hypophysectomy, but the information could also be evidence for the hypothesis presented earlier—that of venous involvement. Our hypothesis is further supported by a recent report (9) wherein the necessity of normal vascular channels between the uterus and ovary for luteolytic expression of the uterus has been demonstrated. Whether $PGF_{2\alpha}$ works by actions on the venous drainage from the ovary or via other mechanisms is unknown, but further studies should resolve the validity of our hypothesis.

Summary. Prostaglandin $F_{2\alpha}$ was infused into pseudopregnant rats for 2 days (days 5 and 6) at 1 mg/kg/day. The infusion tubing was placed either in the uterine lumen or the right heart. The progestogen content of the ovaries of these animals was compared to that of animals receiving only saline; progesterone levels were decreased and 20α-dihydroprogesterone concentrations were increased. Vaginal smear records of pseudopregnant rats receiving $PGF_{2\alpha}$ subcutaneously showed a shortening of pseudopregnancy to 7 days from a normal of 14 days. This information supports an indirect mechanism for local luteal control by uterine tissue.

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