Luteotropic and Luteolytic Effects of Prostaglandins in the Hamster[1] (36663)

JANINA LUKASZEWSKA,[2] LAIRD WILSON, JR.,[3] AND WILLIAM HANSEL
Cornell University, Ithaca, New York 14850, and West Virginia University, Morgantown, West Virginia 26506

Hysterectomy prolongs the life span of the corpora lutea of pseudopregnancy in the hamster from 9 days in the intact animal to 17 days (1). While a threshold amount of uterine tissue may be necessary for normal involution of the corpus luteum of pseudopregnancy in the hamster (2), the nature of the uterine factor(s) in this species is not clear. Mazer and Wright (3) were able to extract an active luteolytic factor with saline. On the other hand, large amounts of a lipid, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), have been shown to be luteolytic in the hamster (4).

The present investigation was conducted to compare lipid and aqueous extracts of hamster uteri, collected at various times during pseudopregnancy, for their ability to induce involution of the corpus luteum. The minimum effective level of $PGF_{2\alpha}$ required to induce luteolysis when administered ip and directly into the ovarian bursa was also determined. Finally, an attempt was made to quantitate prostaglandins of the F series (PGF) extracted from hamster uteri at various times during pseudopregnancy.

Materials and Methods. A total of 154 female Golden Hamsters weighing 140–180 g each were housed 4–6 per cage and maintained on a daily lighting schedule of 14 hr light and 10 hr darkness (lights on at 7 AM and off at 9 PM Eastern Standard Time). Each animal was observed for three consecutive 4-day estrous cycles, Day 1 being designated as the day of the characteristic postovulatory vaginal discharge, before being assigned to this investigation. Hamsters were then hysterectomized through midventral incisions under Nembutal anesthesia. Three normal 4-day cycles were allowed to elapse before pseudopregnancy was induced by mating with intact males. Day 1 of pseudopregnancy was designated as the day sperm were observed in the vaginal smear.

Substances or extracts tested for luteolytic activity were administered either by ip or intrabursal injections in a volume of 0.5–1.0 ml or 20–30 μl of saline, respectively. Ip injections were initiated on Day 5 and carried through Day 8. Intrabursal injections were made under Nembutal anesthesia on Day 7 of pseudopregnancy through a flank incision. Animals were observed daily for termination of pseudopregnancy as estimated by postovulatory vaginal discharge and verified by the presence of ovulation points on the ovaries. Hamsters were sacrificed on the morning of the postovulatory discharge, or on Day 11 of pseudopregnancy; the corpora lutea were dissected out, weighed, and progesterone determined by competitive protein binding assay (5).

Hamster uteri to be extracted and tested for luteolytic activity were collected from animals in which the utero–tubal junctions were severed before mating with intact males. The uteri, collected under Nembutal anesthesia, were trimmed, split, washed briefly in saline, and stored frozen. Subsequently, these uteri were cut into small pieces, ground in saline with a virtis homogenizer and the saline extracts centrifuged at 750*g* for 20 min at 0°. The supernatant was divided into daily doses and frozen until injected. Lipid

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[2] Present address-Warsaw 86, Kielpinska 6m 41, Poland.

[3] Present address-West Virginia University, Morgantown, W. Va. 26506.

extracts were prepared from pooled uterine tissue (2–20 uteri for each day of collection). The essential steps of extraction included: (1) precipitation of proteins with acetone (1:3 by volume); (2) addition of pg amounts of tritiated $PGF_{2\alpha}$ to the supernatant; (3) extraction with hexane, which was later discarded; (4) extraction of the residue with diethyl ether after lowering the pH to 3.5 with citric acid; and (5) evaporation of the ether under nitrogen.

The samples from extracts of pooled uterine tissue were dissolved in ethyl acetate: chloroform, 85:15, then spotted on silicia gel coated plates and chromatographed in a system of chloroform:methanol:acetic acid, 90:10:1 (6). The area corresponding to $PGF_{2\alpha}$ was scraped and eluted with 70% methanol, the methanol evaporated under nitrogen and the lipid reextracted with diethyl ether. The ether was evaporated under nitrogen and the residue containing PGF was stored at —20°. Aliquots were assayed for PGF activity by the gerbil colon method (7). Additional aliquots, when counted, showed a recovery of 50–75% of the added $PGF_{2\alpha}$; reported values were corrected accordingly.

Investigations on the minimal effective luteolytic level of $PGF_{2\alpha}$ were carried out with material supplied by The Upjohn Company. $PGF_{2\alpha}$ was dissolved in methanol and refrigerated. Aliquots for injections were dried and redissolved in saline for injection.

Treatment means were compared by Tukey's honestly significant difference (HSD) procedure (8).

Results. Hysterectomy performed as late as Day 8 of pseudopregnancy was generally effective in maintaining pseudopregnancy beyond Day 9, the time at which pseudopregnancy was terminated in nonhysterectomized hamsters (Table I). Only 1 of 11 animals hysterectomized between 9 AM and 12 noon on Day 8 had a normal pseudopregnancy (9 days), and only 3 of 9 animals hysterectomized between 1 PM and 5 PM of Day 8 returned to estrus on Day 9. These observations suggest that functional involution of the corpus luteum in pseudopregnant hamsters is a rapid process limited to a few hours during a 24-hr period between 8 AM on Day 8 and 8 AM on Day 9. Nevertheless, saline extracts of uteri collected on Days 5, 7, and 8 of pseudopregnancy failed to exhibit luteolytic activity. The hamsters received 0.75 uterine equivalents, ip, per day for 4 days, and were sacrificed on Day 11. Weights and progesterone contents of corpora lutea from animals given these saline extracts of uteri did not differ from each other or from corpora lutea of animals receiving only saline (Table II).

Lipid extracts of uteri collected on Days 5, 7, and 8 and not subjected to thin layer chromatography were administered ip to pseudopregnant hamsters. No significant luteolytic activity was noted although corpus luteum weights and progesterone contents in animals treated with lipid extracts of Day 7 pseudopregnant uteri were reduced and one animal returned to estrus (Table III). When these extracts were assayed for PGF activity, the highest activity of 180 ng PGF per uterus was found on Day 8 of pseudopregnancy. Extracts of uteri from Day 7 animals contained detectable, but unmeasurable levels and extracts of Day 5 uteri showed no PGF activity. No prostaglandin E activity was detected in any of these preparations.

The results of experiments in which $PGF_{2\alpha}$ was administered ip to hysterectomized pseudopregnant hamsters (Table III) show that very high levels (10 μg/day) were luteolytic and estrus was observed in all animals tested after 2–4 days of treatment. One μg per day lowered corpus luteum weight and progesterone content, and 100 ng/day suppressed progesterone content without affecting corpus luteum weight.

Because of the high levels of $PGF_{2\alpha}$ required to induce luteolysis following ip administration, $PGF_{2\alpha}$ or PGF isolated by thin layer chromatography from lipid extracts of hamster uteri were administered in single injections directly into the ovarian bursae. The results demonstrate a distinct lutetropic effect of low levels of $PGF_{2\alpha}$ (2 and 4 ng), as indicated by the greatly increased progesterone content of the corpora lutea 4 days after the single injection (Table IV). When $PGF_{2\alpha}$ was injected into only one ovarian bursa, no difference was found in the proges-

TABLE I. Effects of Hysterectomy on the Length of Pseudopregnancy in the Hamster.

Day of hysterectomy	No. of animals	Mean duration of pseudopregnancy (days)	Range (days)
5	12	16	13–23
7	10	17	13–25
8 (9 AM and 12 noon)	11	15.6	9–21
8 (1 PM and 5 PM)	9	13.0	9–19
Nonhysterectomized	10	9.1	8–10

terone contents between the treated and nontreated ovaries (58 ± 5 and 61 ± 3 ng/CL, respectively). However, a small, but statistically significant ($p < 0.05$) difference was noted in weights of the treated and nontreated corpora lutea (0.74 ± .03 and 0.90 ± 0.02 mg/CL, respectively).

Larger amounts of $PGF_{2\alpha}$ (20, 50 and 100 ng) exerted a luteolytic effect. Mean luteal tissue weights were reduced when compared with either the control animals or those treated with 2 or 4 ng of $PGF_{2\alpha}$. Only one ovary was treated with 50 ng $PGF_{2\alpha}$ and luteal tissue weights were depressed in the treated ovaries when compared to the nontreated (0.52 ± 0.18 vs 0.81 ± 0.09 mg/CL). However, this difference was not significant ($p > 0.05$). Progesterone contents per CL were also nonsignificantly ($p > 0.05$) lower in the treated than in the nontreated ovaries (14 ± 5.0 vs 23 ± 9.4). Values for treated and nontreated ovaries were averaged for presentation in Table IV.

No change in mean corpus luteum weight or progesterone content was noted in hamsters that received PGF extracted from Day 5 pseudopregnant uteri (Table IV); these values were similar to those found in saline injected controls (Tables II and III) and in the bovine serum albumin-injected control animals. Bioassay of this preparation indicated the absence of any PGF activity. Extracts made from Day 7 pseudopregnant uteri, which assayed equivalent to 90 ng PGF, caused a small, nonsignificant ($p > 0.05$) decrease in corpus luteum weight and progesterone content when compared to extracts from Day 5.

Finally, animals treated with PGF-like material isolated from pseudopregnant uteri collected between 1 PM and 12 midnight on Day 8 had significantly ($p < 0.05$) reduced luteal tissue weights when compared to either the controls or the animals treated with Day 5 extracts. Progesterone content was not significantly reduced, but one hamster of this group returned to estrus. A bioassay for PGF activity indicated an amount equivalent to 255 ng per uterus. On the other hand, an extract obtained from uteri collected on the morning of Day 8 (8 AM–12 noon) contained the equivalent of only 100 ng per uterus, indicating that the PGF content of the uterus rises rapidly before the onset of luteal regression.

Discussion. The luteolytic activity of $PGF_{2\alpha}$, previously shown in pregnant and pseudopregnant hamsters, was also demonstrated in pseudopregnant hysterectomized hamsters in this investigation. Thus, the action of exogenous $PGF_{2\alpha}$ is probably not

TABLE II. Effects of Saline Extracts of Pseudopregnant Hamster Uteri Injected into Hysterectomized, Pseudopregnant Hamsters.

Preparation tested	Dose level equiv. of uterus/day[a]	No. of animals	Total luteal weight (mg)[b]	Weight/CL (mg)	Total progesterone (ng)	Progesterone/CL (ng)
Day 5 extract	0.75	4	15.1 ± 1.3	.85 ± .05	363 ± 46	20.3 ± 2.0
Day 7 extract	0.75	5	14.9 ± 1.3	.87 ± .05	405 ± 47	23.7 ± 1.8
Day 8 extract	0.75	4	12.9 ± 0.7	.72 ± .02	379 ± 81	20.9 ± 4.4
Control (saline)	—	8	11.3 ± 1.0	.72 ± .06	304 ± 58	19.0 ± 3.5

[a] Test substance injected ip on Days 5, 6, 7, and 8 of pseudopregnancy; animals sacrificed on Day 11 of pseudopregnancy.

[b] Means ± SE.

E III. Effects of Intraperitoneal Injections of Prostaglandin $F_2\alpha$ and a Lipid Extract of Pseudopregnant Hamster Uteri on Co
(CL) of Hysterectomized Pseudopregnant Hamsters.[a]

ration ted	Dose of $PGF_2\alpha$ (ng), or uterine (ut.) equiv.	PGF content per uterus (ng)	No. of animals	Mean total luteal tissue weight (mg)[bc]	Mean weight per CL (mg)[b]	Mean total progest. (ng)[bc]	Mean progest. per CL (ng)[b]
α	100	—	4	16.7 ± 1.2^x	$0.91 \pm .04$	171 ± 41^x	9 ± 2.0
α	1000	—	4	8.7 ± 2.2^y	$0.52 \pm .12$	153 ± 37^x	9 ± 2.1
α	10,000	—	4	4.6 ± 0.4^y	$0.27 \pm .01$	38 ± 6^y	2 ± 0.2
extract 5	0.75 ut. equiv.	ND[d]	4	15.7 ± 1.7^x	$0.93 \pm .02$	339 ± 45^x	20 ± 2.5
extract 7	0.75 ut. equiv.	D[e]	4	9.9 ± 2.2^x	$0.56 \pm .12$	257 ± 86^x	15 ± 4.6
extract 8	0.75 ut. equiv.	180	5	13.8 ± 2.1^x	$0.86 \pm .07$	369 ± 66^x	23 ± 3.4
ol	—		8	11.3 ± 1.0^x	$0.72 \pm .06$	304 ± 58^x	19 ± 3.5

test substances injected intraperitoneally on Days 5, 6, 7, and 8 of pseudopregnancy and animals sacrificed on Day 11 of pseudopr
n ± SE.
ns with different superscripts (x, y) are significantly ($p < 0.05$) different.
detectable.
ectable but not measurable.

IV. Effects of Single Intrabursal Injections of Prostaglandin $F_{2}\alpha$ and Prostaglandin F-like Materials Extracted from Pseudopreg ster Uteri on Corpora Lutea (CL) of Hysterectomized Pseudopregnant Hamsters.[a]

ration ted	Dose of $PGF_{2}\alpha$ (ng), or uterine (ut.) equiv.	PGF content per uterus (ng)	No. of animals	Mean total luteal tissue weight (mg)[bc]	Mean weight per CL (mg)[b]	Mean total progest. (ng)[bc]	Mean progest. per CL (ng)[b]
α	2	—	5	14.1 ± 0.7^{x}	$0.82 \pm .04$	1009 ± 49^{x}	59 ± 2.6
α	4	—	5	13.7 ± 4.9^{x}	$0.81 \pm .03$	678 ± 99^{y}	41 ± 6.7
α	20	—	5	8.5 ± 0.3^{y}	$0.57 \pm .01$	399 ± 70^{z}	27 ± 4.9
α	50	—	4	8.1 ± 1.3^{y}	$0.67 \pm .20$	271 ± 56^{z}	18 ± 11.0
α	100	—	4	8.5 ± 1.1^{y}	$0.60 \pm .05$	241 ± 15^{z}	17 ± 2.0
xtract 5	0.84 ut. equiv.	0	5	15.6 ± 0.6^{x}	$0.95 \pm .05$	344 ± 47^{z}	21 ± 3.3
xtract 7	0.98 ut. equiv.	90	5	11.7 ± 0.7^{x}	$0.74 \pm .03$	270 ± 54^{z}	17 ± 3.4
xtract 8	0.66 ut. equiv.	255	5	6.8 ± 1.2^{y}	$0.45 \pm .07$	151 ± 69^{z}	10 ± 4.6
l (BSA)[d]	—	—	10	12.7 ± 0.9^{x}	$0.95 \pm .03$	200 ± 31^{z}	15 ± 2.5

substances injected directly into both ovarian bursae on Day 7 of pseudopregnancy, except in the groups treated with 2 and 50 ng nly one ovarian bursa was injected; all animals were sacrificed on Day 11 of pseudopregnancy.
ns ± SE.
ns with different superscripts (x, y, z) are significantly different ($p < 0.05$).
ine serum albumin (5 mg).

mediated through the uterus. The minimum ip dose of $PGF_{2\alpha}$ that terminated pseudopregnancy in all animals was found to be 10 μg, a figure at least 10 times less than that employed by other investigators (4). Measurements of corpus luteum weights and progesterone contents served as sensitive indices of $PGF_{2\alpha}$ luteolytic activity.

Luteal progesterone contents were elevated by single injections into one or both ovarian bursae of either 2 or 4 ng of $PGF_{2\alpha}$, while single doses of 50 and 100 μg of $PGF_{2\alpha}$ were luteolytic. $PGF_{2\alpha}$ administered ip in very high doses for several days may affect synthesis and release of gonadotropins, although a direct action on the corpus luteum is also possible. The simulatory effect of very low doses of $PGF_{2\alpha}$ on synthesis of progesterone may also be mediated through the pituitary, or it may act directly on the luteal cells, as has been shown to be the case in incubation studies where $PGF_{2\alpha}$ was added directly to the incubation medium (9). However, the failure to obtain a difference between progesterone contents of treated and untreated ovaries argues against a direct luteotropic action on luteal cells in this case.

In contrast to the results of Mazer and Wright (3), these studies show that the luteolytic substance is present in lipid and not in saline extracts of pseudopregnant uteri. A most significant finding was the effectiveness of PGF-like material isolated from hamster uteri in suppression of corpus luteum weights in the experimental animals. Single injections of 128 ng of PGF extracted from 0.66 uterine equivalents of Day 8 pseudopregnant uteri exerted a luteolytic effect. The rapid increase noted in PGF content in hamster uteri on the afternoon of Day 8 supports the hypothesis that $PGF_{2\alpha}$ may be a physiological luteolysin in this species. However, it is possible that other lipids present in the extracts made from Day 7 and Day 8 pseudopregnant uteri may also play a luteolytic role.

Summary. Experiments were carried out in order to ascertain the effects of prostaglandin $F_{2\alpha}$ in pseudopregnant hysterectomized hamsters, and to compare these effects with those of lipid extracts of hamster uteri. All animals injected ip with 10 μg of $PGF_{2\alpha}$ returned to estrus within 2–4 days after the treatment. Single injections of 50 and 100 ng of $PGF_{2\alpha}$ into the ovarian bursae depressed corpus luteum weights. However, a remarkable stimulation in luteal tissue progesterone levels in both ovaries followed single injections of only 2 ng of $PGF_{2\alpha}$ into one ovarian bursa. A rapid increase in PGF content of pseudopregnant hamster uteri was found to occur between Days 7 and 8, and on Day 8 just prior to the time of corpus luteum regression.

1. Kent, G. C., Jr., and Atkins, G., Proc. Soc. Exp. Biol. Med. **101**, 106 (1959).

2. Caldwell, B. V., Mares, R. S., and Wright, P. A., Endocrinology **80**, 477 (1967).

3. Mazer, R. S., and Wright, P. A., Endocrinology **83**, 1065 (1968).

4. Gutknecht, G. D., Wyngarden, L. J., and Pharriss, B. B., Proc. Soc. Exp. Biol. Med. **136**, 1151 (1971).

5. Neill, J. D., Johansson, E. D., Datta, J. K., and Knobil, E., J. Clin. Endocrinol. **27**, 1167 (1967).

6. Wilson, L., Jr., Cenedella, R. J., Butcher, R. L., and Inskeep, E. K., J. Anim. Sci. **34**, 93 (1972).

7. Weeks, J. R., Schutz, J. R., and Brown, W. E., J. Appl. Physiol. **25**, 783 (1968).

8. Steel, R. G. D., and Torrie, J. H., "Principles and Procedures of Statistics," p. 305. McGraw–Hill, New York (1961).

9. Pharriss, B. B., Wyngarden, L. J., and Gutknecht, G. D., *in* "Gonadotropins" (E. Rosenberg, ed.), p. 121. Genon-x, Los Altos, CA (1968).

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