Assessment of the luteolytic potency of various prostaglandins in the pseudopregnant rabbit

S. J. Kehl and J. C. Carlson

Biology Department, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1

Summary. The ability of systemic infusion of arachidonic acid and various prostaglandin (PG) compounds to induce luteolysis was examined in the Day 9 pseudopregnant rabbit. Administration of PGF-2α (25 µg/h for 6 h) elicited a decline in plasma progesterone from mean ± s.e.m. pretreatment levels of 8.83 ± 0.54 to 0.54 ± 0.05 ng/ml on the following day (P < 0.01). A shorter infusion (25 µg/h for 3 h) or a lower hourly dose rate (12.5 µg/h for 6 h) of PGF-2α was ineffectual, suggesting that a dose/duration regimen exists for PGF-2α-mediated luteolysis. PGE-2 (25 µg/h for 6 h) or sodium arachidonate (667 µg/h for 6 h) did not significantly affect luteal function. Of the PGF-2α metabolites examined, 13,14-dihydro-PGF-2α was the most effective: it was approximately 4-fold more potent as a luteolysin than PGF-2α since functional regression occurred with infusion of 6.25 µg/h for 6 h. These data illustrate that systemic infusion of PGF-2α is capable of inducing luteolysis in the rabbit. The metabolite 13,14-dihydro-PGF-2α may also be involved luteolytically in this species.

Introduction

That the uterus exerts a regulatory effect on the life-span of the corpus luteum (CL) in a number of species is demonstrated by the delay of normal luteolysis following complete hysterectomy (Anderson, Bland & Melampy, 1969). In the unilaterally hysterectomized ewe, luteolysis is observed only in the CL ipsilateral to the uterine horn (Caldwell, Rowson, Moor & Hay, 1969). Additionally, autotransplantation of an ovary to the neck in unilaterally ovariectomized ewes prolongs luteal function (Baird, Goding, Ichikawa & McCracken, 1968). The latter observation may be attributed to the disturbance of the normal utero-ovarian interrelationship such that local countercurrent transfer of prostaglandin (PG) F-2α, the putative uterine luteolysin in the sheep, from the utero-ovarian vein to the ovarian artery is prevented (McCracken et al., 1972). This local transfer system avoids the problem of systemic dilution and pulmonary prostaglandin dehydrogenase inactivation (Ferreira & Vane, 1976).

In the rabbit, however, there is no anatomical basis or experimental evidence for local transfer of a uterine luteolysin (Del Campo & Ginther, 1972; Einer-Jensen, 1974); unilateral hysterectomy does not result in a differential gravimetric change in the CL of either ovary, the CL in both ovaries regress synchronously (Hunter & Casida, 1967); the CL autotransplanted to the kidney capsule have a life-span similar to that of CL in situ (Scott & Rennie, 1970). These experimental observations suggest that if there is a luteolysin of uterine origin in the rabbit, it must reach the ovary by a systemic route.

PGF-2α is luteolytic in the rabbit when administered in large, supra-physiological doses (Gutknecht, Duncan & Wyngarden, 1972; Bruce & Hillier, 1974). The observation that arachidonic acid, the fatty acid precursor of PGF-2α, is also luteolytic indicates that uterine
arachidonic acid released into the systemic circulation might act as the physiological stimulus for CL regression by conversion to PGF-2α at the CL (Hoffman, 1974; Carlson & Gole, 1978).

The purpose of this experiment was to compare the luteolytic potency of systemically infused arachidonic acid, PGE-2, PGF-2α and some of its metabolites in the pseudopregnant rabbit to aid in the identification of the uterine luteolysin in this species.

Materials and Methods

Female New Zealand rabbits (3–4 kg), housed individually under controlled conditions, were supplied daily with rabbit chow and water. Pseudopregnancy was induced by injection of 50 i.u. hCG (Pregnyl: Organon) in 0.3 ml saline (9 g NaCl/l) into the marginal ear vein on Day 0 (Scott & Rennie, 1970; Hoffman, 1974). On Day 7 the animals were anaesthetized with pentobarbionate sodium (Nembutal: Abbott) and a cannula of polyethylene (PE50) was introduced into the right jugular vein and coursed caudally approximately 8–10 cm into the posterior vena cava. The free end of the cannula was then threaded subcutaneously to an exit point at the back of the neck.

All experimental treatments began between 08:00 and 09:00 h on Day 9 of pseudopregnancy. Serial blood samples (1.5 ml), taken at regular intervals on Days 9 and 10 from conscious, restrained animals, were mixed with 0.2 ml heparinized saline (1000 USP units/ml) and centrifuged. The plasma was stored at −10°C. No gross change in the haematocrit was observed during blood collection. The compounds to be examined for luteolytic potency were dissolved in saline and infused via the indwelling cannula with a constant-rate (2.8 ml/h) infusion pump (Harvard Apparatus). Infusions were interrupted at hourly intervals for approximately 1 min to allow withdrawal of blood samples.

The 50 pseudopregnant rabbits were subdivided into 7 groups (Table 1). Group 1 animals received sodium arachidonate (Sigma, Grade 4-S) in saline at a rate of 667 µg/h for 6 h. In Groups 2a, 2b and 2c PGF-2α was infused at 25 µg/h for 6 h, 12.5 µg/h for 6 h or 25 µg/h for 3 h, respectively. The prostaglandin metabolite 13,14-dihydro-15-keto-PGF-2α was administered to Group 3 animals at 25 µg/h for 6 h. Similarly, Group 4 animals received 15-keto-PGF-2α at

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rabbits</th>
<th>Progesterone conc. (ng/ml)</th>
<th>Pretreatment†</th>
<th>Post-treatment‡</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>10.51 ± 0.33</td>
<td>7.39 ± 0.83</td>
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<tr>
<td>2a</td>
<td>5</td>
<td>8.83 ± 0.54</td>
<td>0.54 ± 0.05*</td>
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<tr>
<td>2b</td>
<td>5</td>
<td>10.77 ± 0.53</td>
<td>6.55 ± 0.76</td>
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<tr>
<td>2c</td>
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<td>8.45 ± 0.70</td>
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<tr>
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<tr>
<td>5a</td>
<td>9</td>
<td>10.02 ± 0.42</td>
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<tr>
<td>5b</td>
<td>5</td>
<td>7.87 ± 1.19</td>
<td>0.62 ± 0.04*</td>
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</tr>
<tr>
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<td>2.68 ± 0.35</td>
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<tr>
<td>6</td>
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<td>7.28 ± 0.77</td>
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<tr>
<td>7</td>
<td>5</td>
<td>7.13 ± 0.73</td>
<td>7.42 ± 0.53</td>
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</table>

Values are mean ± s.e.m.
* Significantly different from pretreatment values: \( P < 0.01 \)
(ANOVA and Student–Newman–Keuls test).
† Samples taken at −1, −0.5 and 0 h.
‡ Samples taken at 24, 26 and 28 h.
25 μg/h for 6 h. The effects of three different dose levels of 13,14-dihydro-PGF-2α were examined: 25 μg/h for 6 h, 6·25 μg/h for 6 h and 3·13 μg/h for 6 h (Groups 5a, 5b and 5c, respectively). In Group 6, PGE-2 was administered at 25 μg/h for 6 h, while the controls in Group 7 received saline only.

Radioimmunoassay

Progesterone concentrations were determined by the radioimmunoassay described by Carlson & Gole (1978). The efficiency of the ether extraction ranged from 80 to 95%. The accuracy of the assay was determined by the recovery of 75, 150 and 225 pg progesterone added to 0·1 ml rabbit plasma treated with charcoal to remove endogenous steroids; estimated levels (mean ± s.e.m.) were 71·3 ± 3·65, 152·4 ± 5·76 and 198·7 ± 8·68 pg progesterone, respectively (n = 15). A pool of normal rabbit plasma containing 3·03 ± 0·25 ng progesterone/ml gave intra- and inter-assay coefficients of variation of 7·96 and 7·79% respectively (n = 48). The assay sensitivity was 50 pg/ml plasma (Rodbard, Rayford, Cooper & Ross, 1968).

Statistical analysis

Differences between means were assessed by one-way analysis of variance (ANOVA) and Student–Newman–Keuls test (Sokal & Rohlf, 1969). When the variance was heterogeneous, logarithmic transformation of the data was performed.

Results

The mean concentrations of plasma progesterone before and on the day following experimental treatments are shown in Table 1; the pretreatment levels of progesterone are similar to those reported for mating-induced pseudopregnancy (Carlson & Gole, 1978). Functional luteal regression normally occurs in the pseudopregnant rabbit between Days 13 and 15. Complete CL regression was interpreted as a decline in plasma progesterone to the levels observed in the oestrous rabbit, i.e. 1·0 ng/ml or less. There was no significant change in peripheral progesterone concentrations in Groups 1 (Text-fig. 1a), 2b, 2c, 3, 5c, 6 (Text-fig. 1b), and 7 (Text-fig. 1c), but in Group 2a there was significant (P < 0·05) decrease in progesterone levels by 8 h after the start of the infusion (Text-fig. 1d).

A decline in progesterone concentrations was observed in Group 4 by 4 h after the start of treatment (P < 0·05), but the effect was transitory, since progesterone levels on the following day were not significantly different from the pretreatment means (Text-fig. 1e). Complete luteal regression occurred in Groups 5a and 5b (Text-fig. 1f): plasma progesterone concentrations declined significantly 2 h after treatment began (P < 0·05) and were still low on the following day.

Discussion

In this study direct posterior vena caval infusion was employed to reflect the postulated natural delivery route and thereby expose the compounds tested for luteolytic potency to the same systemic (e.g. pulmonary) modulation. Additionally, this mode of administration allowed more precise control, compared to injection regimens, over both the amount of the compound entering the circulation and the duration of the treatment.
The effect of a 6-h systemic infusion (horizontal bar) of (a) 667 μg sodium arachidonate/h (Group 1); (b) 25 μg PGE-2/h (Group 6); (c) vehicle only (Group 7); (d) 25 μg PGF-2α/h (Group 2); (e) 25 μg 15-keto-PGF-2α/h (Group 4); or (f) 6-25 μg 13,14-dihydro-PGF-2α/h (Group 5) on progesterone concentrations in posterior vena cava plasma in Day-9 pseudopregnant rabbits. Values are mean ± s.e.m. for (a) 3, (b) 3, (c) 5, (d) 5, (e) 4 and (f) 5 rabbits.

The induction of luteal regression by treatment with PGF-2α and the anti-luteolytic effect of the prostaglandin synthetase inhibitor indomethacin (O'Grady, Caldwell, Auletta & Speroff, 1972) have been presented as evidence supporting PGF-2α as the uterine luteolysin in the rabbit, as well as a number of other non-primate mammals. However, a clearcut increase in circulating PGF levels before or during a decline in peripheral progesterone concentrations in the pseudopregnant (Carlson & Gole, 1978) or the pregnant (Challis, Davies & Ryan, 1973) rabbit has not been demonstrated. To explain this apparent paradox, it may be argued that the doses of PGF-2α employed in preliminary studies (Gutknecht et al., 1972; Bruce & Hillier, 1974) were unnaturally large and that extrapolation to PGF-2α involvement in spontaneous regression was therefore spurious. Our results show that treatment with PGF-2α at a dose level of 25 μg/h for 6 h did produce a rapid decline in circulating progesterone to basal levels. However, a dose of 12.5 μg/h for 6 h produced no significant effect and suggests that PGF-2α is indeed luteolytic at high levels only. Moreover, the dose of 25 μg PGF-2α/h was luteolytic when infused for 6 h but not for 3 h. In addition to a critical concentration, there appears to be a threshold exposure time for PGF-2α-mediated luteolysis. In the autotransplanted sheep ovary, the minimum effective luteolytic dose of PGF-2α administered by constant rate infusion into the ovarian artery was approximately 19 μg (Chamley et al., 1972). However, McCracken, Schramm, Barcikowski & Wilson (1981) have demonstrated that the same effect can be produced with a total dose of only...
300 ng PGF-2α, if it is administered as a series of injections over a 24-h period. This approach is thought to be a more accurate replication of the pulsatile release of uterine PGF-2α that occurs in the sheep (Thorburn, Cox, Currie, Restall & Schneider, 1972).

Intermittent injection of PGF-2α may also be a more effective luteolytic regimen in the rabbit. A pulse of uterine PGF-2α might swamp the lung inactivation mechanism and allow the escape of some luteolytically active PGF-2α into arterial blood. Pulsatile release of uterine PGF-2α might also explain the absence of a detectable increase in PGF prior or concomitant to CL regression in some studies. Nathanielsz, Abel & Smith (1972) induced parturition, which was attributed to CL regression rather than a myometrial effect, in pregnant rabbits on Day 21 by extended intra-aortic infusion of a total dose of 102 ng PGF-2α. That such a low dose of PGF-2α was luteolytic was probably due to the mode of administration which by-passed initial pulmonary PG metabolism. Nonetheless, the results of Nathanielsz et al. (1972) demonstrate that rabbit luteal tissue is very sensitive to PGF-2α and small amounts of PGF-2α left unmetabolized by the lungs could act luteolytically. Because 15-keto-PGF-2α induced luteal regression in the rhesus monkey (Wilks, 1977) the effect of PGF-2α metabolites in the rabbit was examined. The experimental results show that of the three metabolites examined, 15-keto-PGF-2α, 13,14 dihydro-15-keto-PGF-2α and 13,14 dihydro-PGF-2α, only the last induced a sustained, significant decline in plasma progesterone levels and the dose required for a 6 h constant rate of infusion was 75% less than that required with PGF-2α, suggesting that it is a possible luteolysin in the doe. However, although this metabolite has been detected in some other species (Hamberg & Samuelsson, 1971; Granstrom, 1972; Aizawa, Inazu & Kogo, 1980), peripheral levels in the rabbit need to be measured. This metabolite is also luteolytic in the cow, albeit at levels similar to those required with PGF-2α (Milvae, 1981).

It seems unlikely that systemic conversion of PGF-2α to 13,14 dihydro-PGF-2α is the basis for the luteolytic effect of PGF-2α in the rabbit, since infusion of the same amount of an intermediate metabolic product of PGF-2α inactivation (13,14 dihydro-15 keto-PGF-2α) did not produce a significant effect on luteal function. If 13,14 dihydro-PGF-2α is involved in spontaneous regression it may be released as such from the uterus. The increase in uterine prostaglandin dehydrogenase (PGDH) and Δ13 PG reductase activity consequent to progesterone treatment in the rabbit (Bodkhe & Harper, 1979) implies that the pseudopregnant uterus has the enzyme capability for synthesis of 13,14 dihydro-PGF-2α, but data concerning the rates of uterine PGF-2α 13,14 reduction and 15 oxidation during spontaneous regression are unavailable.

Treatment with arachidonic acid results in luteal regression in the rabbit (Hoffman, 1974; Carlson & Gole, 1978). In addition, there is evidence that rabbit luteal tissue is capable of de novo synthesis of PGF-2α (Wilks, Hunter & Norland, 1972). In the present study, however, systemic infusion of 4 mg sodium arachidonate did not produce a significant change in circulating progesterone levels. It therefore seems unlikely that arachidonic acid plays a role as the systemic luteolysin in this species, unless it can be demonstrated that there is a significant increase in systemic plasma levels before luteolysis.

Progestosterone levels were also unaffected by PGE-2 administered at a dose/duration rate at which PGF-2α was luteolytic. PGE-2 (2 mg) administered in Silastic capsules has been shown to impair luteal function in the rabbit (Saksena, Lau & Chang, 1975), an effect which was attributed to its conversion to PGF-2α by 9-keto-reductase. Clearly, in our study an insufficient amount of the infused PGE-2 was converted to PGF-2α to lead to luteal regression. The efficacy of PGF-2α and 13,14 dihydro-PGF-2α, but not PGE-2, suggests that luteolytic activity requires the presence of a hydroxyl group at both the C9 and C15 position of the molecule.

We thank Dr S. Smith for assistance with the statistical analysis; Dr J. E. Pike, the Upjohn Company, for the PGs; and Dr G. D. Niswender, Colorado State University, for the progesterone antiserum. This work was supported by the National Science and Engineering Research Council of Canada.
References


Received 7 July 1980