Effect of prostaglandin F-2 α on plasma levels of progesterone and pregnenolone in the hysterectomized guinea-pig

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Summary. Repeated injections of PGF-2 α were given to hysterectomized guinea-pigs. Within 12 h after the first injection, plasma progesterone levels were significantly reduced ($P < 0.001$) and declined to <1 ng/ml by the 4th day after the end of PGF-2 α treatment. A decline in plasma levels of pregnenolone paralleled that of progesterone. PGF-2 α treatment did not affect the metabolic clearance rate of [3 H]pregnenolone, but the conversion of [3 H]pregnenolone to [3 H]progesterone was reduced by about 50%.

Introduction

Evidence is accumulating which suggests that prostaglandin (PG) F-2 α is the uterine luteolytic hormone in the guinea-pig. Both hysterectomy and indomethacin, an inhibitor of prostaglandin synthesis, have been shown to lengthen the oestrous cycle of the guinea-pig (Loeb, 1923; Marley, 1973). Active immunization against PGF-2 α also prolonged the length of the oestrous cycle in treated guinea-pigs (Horton & Poyser, 1974). The guinea-pig uterus has been found to release PGF-2 α after bead insertion (Poyser, Horton, Thompson & Los, 1971) and oestrogen treatment (Blatchley *et al.*, 1971). In addition, Poyser (1972) and Blatchley, Donovan, Horton & Poyser (1972) have shown that the synthesis and release of PGF-2 α from the uterus increase toward the end of the oestrous cycle when natural luteal regression occurs.

In previous studies the effect of PGF-2 α on the length of the oestrous cycle and on the onset of oestrus have been examined. However, the effect of PGF-2 α on plasma progesterone levels as an indicator of luteal function has not been determined. Plasma progesterone and pregnenolone levels were therefore studied after PGF-2 α treatment. The metabolic clearance rate of pregnenolone was also determined so that the effect of PGF-2 α on progesterone and pregnenolone synthesis could be examined. Hysterectomized guinea-pigs were used because the corpus luteum of the guinea-pig is refractory to the luteolytic effects of PGF-2 α until Day 9 of the oestrous cycle (Dwyer, 1977; Tso & Tam, 1977).

Materials and Methods

Animals

Adult female guinea-pigs, selected for 16–18-day oestrous cycles and weighing 800–1000 g were housed under constant temperature (20°C) and a lighting regimen of 12 h light and 12 h dark. Water, to which ascorbic acid had been added, and food were always available. Oestrous cycles were monitored by daily examination for vaginal opening and by lavage smears when the vagina was perforate. The day of oestrus (Day 0) was taken to be the day of maximal

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cornification of the vaginal smear before an influx of leucocytes. All animals were hysterectomized on Day 5 of the oestrous cycle. Chronic jugular cannulae, inserted on Day 13 of the oestrous cycle, were used for blood sampling. Starting 15 days after oestrus, animals were injected i.p. twice daily (09:00 and 15:00 h) for 3 days with 125 µg PGF-2α (free acid equivalent of Prostin F_{2α}; Upjohn Co.) in 0.5 ml saline (9 g NaCl/l: 10 animals) or with 0.5 ml saline (10 animals) and blood samples (1.5 ml) were collected in heparinized tubes three times daily (09:00, 15:00 and 21:00 h) on Days 1 and 2 of treatment, twice daily (15:00 and 21:00 h) on the 3rd day of treatment and once daily (09:00 h) thereafter for 5 days. The blood samples were centrifuged at 1500 g and 4°C for 10 min and the plasma was stored at -20°C until assay.

Steroid assays

Plasma samples (0.5 ml) were extracted twice with 5 ml diethyl ether after the plasma volume was made up to 1 ml with saline and the pooled ether extracts were evaporated to dryness under nitrogen. Progesterone and pregnenolone were separated by thin-layer chromatography on silica gel using a solvent mixture of ether:benzene (2:1, v/v). Steroid standards (progesterone, pregnenolone and 20α-hydroxypregn-4-en-3-one; Sigma Chemical Co.) were run in parallel to help locate the steroids in the samples, by u.v. absorption (progesterone) and by streaking with sulphuric acid:ethanol (2:1, v/v) (pregnenolone). The steroids were eluted from the chromatogram with 5 ml ether and the ether extracts were evaporated to dryness under nitrogen. Recovery rates of progesterone and pregnenolone after separation were 73.4% and 69.3% ($n = 10$) respectively.

Progesterone and pregnenolone concentrations were determined by a radioimmunoassay (Lindner, Perel, Friedlander & Zeitlin, 1972) using rabbit anti-progesterone-11α-BSA serum (Miles-Yeda Ltd). The sensitivity of the assay is such that 5–10 pg progesterone can be detected. The antiserum is highly specific and has little cross-reaction (<1%) with pregnenolone and 20α-hydroxypregn-4-en-3-one. Pregnenolone was converted to progesterone before assay by using the method of Strott, Bermudez & Lipsett (1970) involving 3β-hydroxysteroid dehydrogenase (*Pseudomonas testosteronii*; Worthington). Average conversion of pregnenolone to progesterone was found to be $92.3\% \pm 1.73$ ($n = 10$).

Metabolic clearance rate

The metabolic clearance rate of pregnenolone was determined in PGF-2α and saline treated hysterectomized guinea-pigs by constant infusion of [³H]pregnenolone. Starting 15 days after oestrus (and 10 days after hysterectomy), 4 animals were injected i.p. with 125 µg PGF-2α in 0.5 ml saline twice daily for 3 days, and 4 control animals received 0.5 ml saline twice daily for 3 days. On the day following the last injection, the animals were anaesthetized using sodium pentobarbitone (Diabutol: Diamond Laboratories Inc., 30 mg/kg body weight), and 1.5 µCi [³H]pregnenolone (sp. act. 17.2 Ci/mmol: New England Nuclear) in 0.75 ml sterile saline containing 1% ethanol was introduced through a jugular cannula as a priming dose to shorten the time necessary to attain a constant plasma concentration of labelled pregnenolone. Infusion of steroid was started 30 min after the priming dose and infused at a rate of 50 µl/min (2 µCi/ml) for 2 h. A blood sample (2.5 ml) was withdrawn between 12:00 and 15:00 h from a carotid cannula every 15 min starting 90 min after the start of infusion and 3 blood samples were taken from each animal. The blood volume was replaced by 5% dextrose. Blood samples were centrifuged at 1500 g and 4°C for 10 min and stored at -20°C until assay. Progesterone and pregnenolone were extracted and separated as described above. Following thin-layer chromatography the progesterone and pregnenolone areas were cut out and scraped into vials to which 0.5 ml ether was added to extract the steroids from the silica gel before addition of 8 ml scintillation fluid (16 g Omnifluor in 20 l toluene and 1.0 l Triton X-100) for counting. The

metabolic clearance rate (MCR) was derived by the method of Tait & Burnstein (1964) and is given by:

$$\text{MCR (l/day)} = \frac{\text{infusion rate } (\mu\text{Ci/min}) \times 1.44}{\text{plasma conc. of } [^3\text{H}]\text{pregnenolone } (\mu\text{Ci/ml})}$$

Results

Cornification followed by an influx of leucocytes in the vaginal smear occurred between 4 and 6 days after the end of PGF-2 α treatment, and ovulation was assumed.

The effect of PGF-2 α administration on plasma progesterone levels in the hysterectomized guinea-pig is illustrated in Text-fig. 1(a). Progesterone concentrations had declined by 25% at 6 h after the start of treatment and were significantly reduced ($P < 0.001$, t test) by 12 h. There was then a slight rise on the following day, but after the next PGF-2 α injection progesterone levels declined again. Concentrations then remained low and were < 1 ng/ml by 4 days after the end of PGF-2 α treatment. The results for one animal were excluded because the plasma steroid levels

Text-fig. 1. Mean $\pm$ s.e.m. concentrations of (a) progesterone and (b) pregnenolone in jugular venous plasma of guinea-pigs after treatment (arrows) with PGF-2 α (---, N = 9) or saline (—, N = 10).

differed greatly from those of the other 9 PGF-2 α treated animals, and there had been no signs of oestrus or ovulation when vaginal smears were taken during the blood sampling.

The effect of PGF-2 α treatment on plasma pregnenolone levels are shown in Text-fig. 1(b). The decline in plasma pregnenolone levels paralleled that of plasma progesterone levels, being significantly reduced ($P < 0.005$) after the second day of PGF-2 α treatment and remaining low thereafter.

The MCR of pregnenolone was 1388.0 ± 167 (s.e.m.) $\text{l} \cdot \text{day}^{-1} \cdot \text{kg}^{-1}$ in the 4 control guinea-pigs and 1408.6 ± 68 $\text{l} \cdot \text{day}^{-1} \cdot \text{kg}^{-1}$ in the 4 animals treated with PGF-2 α for 3 days. However, the peripheral conversion of pregnenolone to progesterone was about 50% lower in the PGF-2 α -treated guinea-pigs ($18.18 \pm 1.63\%$ versus $44.57 \pm 13.25\%$).

Discussion

The experiments reported in this paper are part of a study on the biochemical events involved in PGF-2 α induced luteolysis. The decline in plasma progesterone levels after PGF-2 α treatment was accompanied by a decrease in plasma levels of pregnenolone, a precursor in progesterone synthesis, but there was no change in the metabolic clearance rate of pregnenolone. Therefore, the decline in pregnenolone levels after PGF-2 α treatment would seem due to a decrease in the synthesis and release of pregnenolone. The reduction in the conversion *in vivo* of [^3H]-pregnenolone to [^3H]-progesterone after PGF-2 α treatment suggests that PGF-2 α may reduce the activity of 3β -hydroxysteroid dehydrogenase, the enzyme responsible for the conversion of pregnenolone to progesterone, as was suggested by Hoppen, Williams & Findlay (1976) after a similar study in the ewe.

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