Dynamic studies of prostaglandin F-2 α in the utero-ovarian circulation of the sheep

R. B. Land, D. T. Baird* and R. J. Scaramuzzi*

A.R.C. Animal Breeding Research Organization, West Mains Road, Edinburgh, EH9 3JQ and *M.R.C. Unit of Reproductive Biology, 39 Chalmers Street, Edinburgh, EH3 9ER, U.K.

Summary. The concentration of prostaglandin (PG) F-2 α in ovarian arterial blood was compared to that in aortic and carotid blood at different stages of the ovine oestrous cycle, and its concentration measured in the ovarian artery following the infusion of radioactive PGF-2 α to the uterine vein.

In all cases the concentration of PGF-2 α increased during passage through the ovarian artery, and the increase was proportional to the log of the concentration in the uterine vein. No such change was observed in the concentration of progesterone in the ovarian artery or of PGF in the uterine artery.

It was concluded that PGF-2 α can be and is normally transferred from the uterine vein to the ovarian artery. These results are compatible with the hypothesis that PGF-2 α of uterine origin is the normal luteolytic agent in the sheep.

Introduction

The control of the corpus luteum by the ipsilateral uterine horn was elegantly demonstrated in the sheep by Moor & Rowson (1966). It has been suggested that this localized control is effected via the production of prostaglandin (PG) F-2 α by the uterus, its drainage to the utero-ovarian vein, and transfer to the ovarian artery by a countercurrent mechanism within the ovarian pedicle (McCracken, Baird & Goding, 1971; Goding, 1974), but this concept has been challenged (Coudert, Phillips, Faiman, Chernecki & Palmer, 1974).

In the present studies (1) the concentration of endogenous PGF was measured at different stages of the oestrous cycle in the blood vessels supplying and draining the ovary and uterus, and (2) the radioactivity associated with purified PGF-2 α was measured following the infusion of labelled PGF-2 α into the uterine vein.

Materials and Methods

Endogenous studies

These were conducted in two series. In the first, 4 animals of the Blackface or Finn-Dorset breed types were allocated for study on each of Days 8, 10 or 11, and 15 of the oestrous cycle.

On the day of study, the reproductive tract was exposed by mid-ventral laparotomy under pentothal-induced halothane anaesthesia, using normal aseptic surgical procedures. Blood samples for assay were then collected consecutively from the jugular vein, dorsal aorta, ovarian artery and uterine vein; the sample from the uterine vein being taken at least 2 cm above the junction of this vein with the ovarian vein. All samples except that from the ovarian artery were collected using syringes and 23-gauge needles. In each case, the object was to collect 20 ml blood, and this was facilitated by the i.v. administration of 5000 i.u. heparin to each animal after the collection of aortic blood. Ovarian arterial blood was sampled by dissecting approximately 1 cm of the artery free from the ovarian pedicle, so that it formed a short loop, 2–4 cm below the ovary. The distal end of this loop

was cut after collection of the aortic sample and the blood allowed to flow freely and directly from the cut end of the vessel into a sterile beaker. Particular attention was paid to the free and direct flow of blood. Samples were cooled immediately after collection, centrifuged at 4°C, and the plasma removed and frozen within 30 min of collection.

In the second series of experiments, 7 animals were studied on Day 15 of the oestrous cycle, some of them bilaterally. The sampling procedure was similar to that of the first series with the exception that samples were also taken from the uterine artery after collection from the uterine vein so that the effects of collection from small cut arteries could be studied. The procedure corresponded to that for the collection of ovarian arterial blood, the artery being dissected free from connective tissue before sampling.

Two different radioimmunoassays were used for measurement of PGF. The plasma samples in the first series of experiments were assayed using the method and antiserum of Caldwell, Tillson, Brock & Speroff (1972). After the addition of 3000 d/min (3 pg) tritiated PGF-2 α , 4 ml plasma were extracted twice with 8 ml freshly purified redistilled diethyl ether and the extract discarded. Extraction losses were estimated by counting the radioactivity in 0.1 ml of the final extract; the mean ($\pm$ S.E.M.) recovery for a typical assay was $74 \pm 8\%$ ($n=32$). Appropriate aliquots were then assayed in duplicate.

The intra-assay precision was 10% (143.7 ± 4.6 pg/ml; $n=10$) and the interassay precision over 10 assays was 13% (1642.1 ± 59.2 pg/ml; $n=13$). The mean water blank over 20 assays was 32.8 ± 4.8 pg/ml ($n=22$) and the mean plasma blank obtained from carotid arterial plasma from an ovariectomized hysterectomized ewe was 45.8 ± 3.8 pg/ml ($n=11$).

The samples in the second series of experiments were assayed using a more specific antiserum, saturated ammonium sulphate-calcium sulphate to separate bound and free fractions and the extraction of a smaller volume of plasma (0.5 ml) (Henman, Johnson, Newton & Collins, 1974). The interassay precision for the method was 8.9% (1012.2 ± 32.0 pg/ml; $n=8$), and the water blank was 33.5 ± 3.9 pg/ml ($n=8$).

Infusion studies

The transfer of [^{3} H]PGF-2 α from the utero-ovarian vein to the ovarian artery was investigated in two Finn-Dorset ewes on Day 14 of the oestrous cycle. The procedure, similar to that described by McCracken *et al.* (1972), was identical in the two experiments, and was to compare the concentration of PGF-2 α in plasma collected simultaneously from the ovarian and carotid arteries during the infusion of PGF-2 α into the uterine vein. A higher concentration of [^{3} H]PGF-2 α in the ovarian arterial samples indicated local transfer.

The reproductive tract was exposed as in the previous experiments and a branch of the middle uterine vein cannulated with teflon tubing (No. 6417, ultra-thin wall and i.d. 0.9 mm: Becton-Dickinson, Rutherford, New Jersey). The tip of the cannula was advanced so that it lay in the middle uterine vein about 3 cm above its junction with the utero-ovarian vein. An indwelling cannula was also placed in the carotid artery through a short skin incision. Throughout the experiment Dextravan 70 (Macrodex-Pharmacia) or blood was infused into the jugular vein at a rate sufficient to compensate for the blood loss due to sampling.

Heparin (10,000 i.u.) was injected intravenously to prevent clotting and the distal end of the ovarian arterial loop cut with sharp scissors. Two samples of ovarian arterial blood for measurement of endogenous PGF-2 α were collected into sterile beakers (25–50 ml) before the infusion of [^{3} H]-PGF-2 α was started. Ovarian arterial blood was collected as successive samples (50–100 ml) of approximately 5-min duration throughout the infusion which lasted 90 min and for a further 42 and 80 min in Exps 1 and 2 respectively (Text-figs 1a and b). Control 25-ml samples of carotid arterial blood corresponded to each sample from the ovarian artery. Samples were cooled immediately on ice, and the plasma was stored at -15°C until analysis.

The PG solution was made up by dissolving 180 μg (23×10^6 d/min) of [^{3} H]PGF-2 α (New England Nuclear Chemicals, Postfach 71, Germany) in 39 ml sterile normal saline. It was infused at a constant rate (approximately 10 ml/hr) such that 0.77 μg or 100,000 d/min were delivered each minute

into the uterine vein. The rate of infusion was checked by serial measurements of the amount of radioactivity delivered from the end of the teflon tubing before and after the experiment.

After extraction of the PGF from the plasma (Baird, Collet, Fraser, Kelly, Land & Wheeler, 1973) an aliquot of the ether extract was taken, and the total radioactivity counted. PGF-2 α was then separated from PGF-1 α by further thin-layer chromatography on silica gel plates (0.5 mm thick) impregnated with 10% (v/v) silver nitrate and run with the solvent mixture ethyl acetate:acetic acid:methanol:iso-octane:water (220:20:30:20:200 by vol.). The spot containing PGF-2 α was eluted with methanol. The eluate, redissolved in 1 ml ether:ethyl acetate (3:1 v/v) was washed with equal volumes of water to remove excess silver nitrate before counting in a liquid scintillation counter. The overall recovery estimated on separate plasma samples to which known amounts of [^3H]PGF-2 α had been added was $57 \pm 3\%$ after the first thin layer and $22 \pm 4\%$ after the second thin layer. The mean recovery factor was used to correct purified PGF-2 α results for procedural losses.

The amount of PGF-2 α present in selected samples of ovarian, carotid and jugular blood was also estimated by combined gas chromatography-mass spectrometry, using PGF-1 α as internal standard (Baird *et al.*, 1973).

Results

Endogenous studies

From the results in Table 1 it can be seen that the concentration of PGF in the ovarian artery at each stage of the oestrous cycle was higher than that in the aorta, indicating that the concentration of PGF increased as the blood passed from the aorta through the ovarian artery to the ovary. No such indication was, however, evident for the progesterone; although the drop in concentration was not statistically significant ($P > 0.05$; paired t test), it could be real, and have arisen from the elimination of the main blood supply to the ovary and corpus luteum during the collection of ovarian arterial blood. The individual change in concentration of PG during passage through the ovarian artery was independent of changes in concentration of progesterone ($r = 0.08$, $P > 0.05$).

Table 1. The mean concentrations of prostaglandin F and progesterone in the utero-ovarian vasculature on different days of the oestrous cycle of the ewe together with the mean and S.E. of log_e transformed data

	Day 8	Day 10 or 11	Day 15
No. of animals	4	4	4
PGF concentration/animal (pg/ml)			
(1) Jugular vein (mean of 2 samples)	80.5 (4.38 $\pm$ 0.07)	88.8 (4.45 $\pm$ 0.15)	113.8 (5.18 $\pm$ 0.61)
(2) Aorta	83.0 (4.39 $\pm$ 0.11)	83.3 (4.36 $\pm$ 0.19)	87.0 (4.45 $\pm$ 0.11)
(3) Ovarian artery	103.3 (4.59 $\pm$ 0.17)	104.8 (4.62 $\pm$ 0.15)	158.0 (4.98 $\pm$ 0.23)
(4) Uterine vein	159.3 (5.00 $\pm$ 0.20)	855 (6.33 $\pm$ 0.48)	12,147 (8.81 $\pm$ 0.83)
(5) Increase (3 - 2)	20.3	21.5	71.0
Progesterone (ng/ml)			
Aorta	6.4 (1.70 $\pm$ 0.36)	4.4 (1.38 $\pm$ 0.27)	3.8 (0.83 $\pm$ 0.56)
Ovarian artery	5.6 (1.63 $\pm$ 0.25)	2.9 (0.92 $\pm$ 0.28)	1.9 (0.48 $\pm$ 0.29)

In the second series of experiments, samples were obtained from both ovarian arteries in 5 of the 7 animals. The mean ($\pm$ S.E.M.) concentrations of PGF were: ovarian artery, 431 ± 100.7 ; dorsal aorta, 166 ± 12.9 ; uterine artery, 175 ± 28.5 ; and jugular vein, 288 ± 62.9 pg/ml plasma. In each animal, the mean of both or the concentration of PGF in the single artery sampled was greater than that in the aorta. The higher values in this series may have arisen from the different antibody used; the results from both series were combined by the use of least squares methods to allow for the nested structure of the data, and a logarithmic transformation to normalize the variation. This indicated that the difference in concentration between the two vessels was significant ($F = 8.82$; d.f. 1,23; $P < 0.01$). Over all stages of the oestrous cycle the least squares means indicated that the concen-

tration of PGF in the ovarian artery was 58% greater than that in the aorta. Although the difference in concentration between the aorta and the ovarian artery and its statistical significance is independent of the assay blank, this estimate of the increase in the ovarian artery relative to the aorta is not and it is therefore a minimum estimate of the relative increase.

The difference between the days of the oestrous cycle was not statistically significant, but the greater ovarian arterial concentration at Day 15 indicates that more PGF is transferred at this time. When the increase in concentration in the ovarian artery is related to that in the uterine vein, however, it can be seen that it is relatively lower on Day 15 than on Days 10 or 11 and 8. Taking 40 pg/ml as the best estimate of the assay blank, the ratio of concentrations increases from around 1:6 on Day 8 to 1:1.70 on Day 15, a 30-fold difference. The transformation of all concentrations to logarithms however gives an increase in the concentration in the ovarian artery which is closely related to the concentration in the uterine vein, the ratio on different days only differing by a factor of around 1.5; i.e.

$$\begin{aligned}\log[\text{ovarian artery}] - \log[\text{aorta}] &= k \cdot \log[\text{uterine vein}] \\ \therefore [\text{ovarian artery}]/[\text{aorta}] &= k \cdot \log[\text{uterine vein}]\end{aligned}$$

As the concentration in the aorta was relatively constant, the concentration of PG in the ovarian artery was therefore proportional to the log of the concentration in the uterine vein.

Infusion studies

The blood flow from the cut end of the ovarian artery remained fairly constant throughout both experiments (mean $\pm$ S.E.M.; 22.2 ± 1.9 ml/min, $n = 24$, in Exp. 1 and 13.2 ± 0.2 ml/min, $n = 28$, in Exp. 2). The concentration of total radioactivity in carotid and ovarian arterial blood increased immediately after the start of both infusions and declined rapidly after the infusion was stopped (Text-figs 1a and b). There was no significant difference in the concentration of total radioactivity in carotid or ovarian arterial blood.

The concentration of [^3H]PGF-2 α was much lower than the concentration of total radioactivity. Carotid arterial PGF-2 α concentration remained high from within 30 min of starting the infusion and did not show any trends until it declined rapidly when the infusion was stopped. The [^3H]PGF-2 α in ovarian arterial blood represented a greater proportion of the total radioactivity, and in both experiments the concentration of [^3H]PGF-2 α in ovarian arterial blood after 40 min was consistently higher than that in carotid arterial blood (12 of the last 13 samples in Exp. 1, and in all of the last 16 samples in Exp. 2). The concentration of [^3H]PGF-2 α declined more slowly after the infusion was stopped than did total radioactivity (Text-figs 1a and b), so that [^3H]PGF-2 α accounted for an increasing proportion of the total radioactivity.

In Exp. 2 the concentration of PGF-2 α in ovarian arterial plasma measured by mass spectrometry rose from 270 pg/ml before the infusion to 800 pg/ml by 90 min.

Discussion

The present studies demonstrate that the concentration of PGF in the arterial blood reaching the ovary is greater than that in the blood entering the ovarian artery from the aorta. The specificity of this increase to the ovarian artery is indicated by the absence of any increase in PG concentration in the samples collected from the uterine artery. Because of the very high concentration of PGF-2 α in the uterine vein, however, it is important to exclude the possibility of contamination of ovarian arterial blood with utero-ovarian venous blood. The fact that the concentration of progesterone in ovarian arterial plasma was no higher than that in aortic plasma makes this unlikely. Although the concentration of PGF in the uterine vein on Day 15 was similar in the present study to that reported by Pexton, Weems & Inskeep (1975), the concentration in ovarian arterial blood was much (10-fold) lower. In the absence of estimates of the concentration in the systemic circulation, however, it is difficult to compare the results further.

Text-fig. 1. The total radioactivity (— — —), and the radioactivity associated with PGF-2 α (—) in the carotid (●) and ovarian arteries during and after the infusion (shown by the horizontal bar) of labelled PGF-2 α into the uterine vein of two ewes (a) and (b).

The infusion studies supplement the observation of an endogenous increase in the concentration of PG with the demonstration that PGF-2 α itself can be transferred from the uterine vein to the ovarian artery without passing through the general circulation, confirming the findings of McCracken *et al.* (1972). The initial observations of McCracken, Baird & Goding (1971) were challenged by Coudert *et al.* (1974) who failed to demonstrate any significant transfer of [^3H]PGF-2 α using a similar experimental design. However, Coudert *et al.* measured only the total radioactivity, and this in a volume of blood equivalent to that reaching the ovary in one-tenth of a minute compared to the 5-min sample of the present experiments. Not only was the limit of detection approximately 50-fold less sensitive than in the present study therefore, but significant transfer might have remained undetected because PGF represents a smaller proportion of total radioactivity in carotid arterial than in ovarian arterial blood. Presumably over 90% of the radioactivity in carotid blood represents metabolites of PGF-2 α formed during circulation through the lungs (Piper, Vane & Wyllie, 1970).

The rate of transfer is difficult to estimate because a steady state is not achieved. An approximate estimate of the rate of transfer at 90 min is between 0.1 and 0.2%. A slightly higher figure of 0.56% is obtained when the calculations are based on the concentration of non-radioactive PGF-2 α . Although the uterus may secrete up to 0.5 μg PGF-2 α /min into the uterine vein towards the end of the cycle (Baird, Land, Scaramuzzi & Wheeler, 1976) the release is short-lived and the amount never reaches 46 $\mu\text{g/hr}$. Thus the relatively low rate of transfer observed in the present experiments could be due to saturation of the system.

The combined results of the endogenous and infusion studies strongly suggest that the extra PG observed in the ovarian artery was transferred to it from the uterine vein, supporting the hypothesis of a transfer system proposed and reviewed by Goding (1974). It is also evident, however, that the transfer of PG is not a linear process because the concentration in the ovarian artery increased more slowly than that in the uterine vein as the oestrous cycle progressed, and the concentration of PGF in the ovarian artery was more closely related to the logarithm of the concentration in the uterine vein. Progressive increases in the concentration of PG in the uterine vein are followed by progressively smaller changes in that of the ovarian artery, leading to smaller transfer rates. This endogenous decrease in transfer rate as the concentration in the uterine vein increases is paralleled by the results of infusion studies. The infusion of 0.2 µg PG/hr (McCracken *et al.*, 1972) led to an estimated transfer of 2%, whereas less than 0.2% of the 45 µg PGF-2α infused per hour in the present experiment was transferred to the ovarian artery. The sensitivity of the system used by Coudert *et al.* (1974), with a minimum detectable transfer of 0.7%, would have been too low to detect transfer following the infusion of 61 µg PGF-2α/hr.

The observation of a log relationship, and the delay before the start and end of transfer relative to the infusion, indicates some of the likely characteristics of the transfer system. In particular, the former indicates that entry to the system is limited and relatively inelastic, so that large changes in potential input are not reflected in the output, and the latter suggests that transfer is not simply a process of rapid diffusion, but involves a large pool of PG in transit. Such a pool could be associated with the membranes and spaces separating the two vessels. It is possible therefore that PG molecules pass slowly down a concentration gradient from vein to artery.

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