Transport of uterine PGF_{2α} to the ovaries by systemic circulation and local lymphovenous–arterial diffusion during luteolysis in sheep

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The theory of countercurrent vascular transfer of PGF_{2α} during luteolysis was examined. In the first experiment, pulmonary clearance of PGF_{2α} was determined to re-examine whether the total amount of PGF_{2α} was degraded in the lungs after one passage. Cardiac output was measured by the Fick method and PGF_{2α} by radio-immunoassay before and after vascular lung supply, using pulmonary catheterization and the interventional radiology method in ten anaesthetized ewes on day 16 of the oestrous cycle. Cardiac output remained stable (7156 ± 439 ml min⁻¹). Infusion of 5 iu oxytocin resulted in an increase in plasma PGF_{2α} concentrations at 30 min in the uterine vein and the pulmonary and femoral arteries (3811 ± 806 , 224 ± 55 and 18 ± 4 pg ml⁻¹, respectively). The PGF_{2α} concentrations decreased exponentially and the half-time decreases were 27 ($r = 0.99$), 16 ($r = 0.99$) and 18 ($r = 0.98$) min, respectively. Pulmonary clearance of PGF_{2α} was estimated at 6338 ± 451 ml min⁻¹. In a second experiment, an arterio–arterial gradient of plasma PGF_{2α} concentrations was analysed between the proximal and distal segments of the ovarian artery to verify whether the total amount of PGF_{2α} flowing to the ovary was from the local venous–arterial countercurrent pathway. Surgical catheterization techniques were performed on 11 ewes on day 16 of the oestrous cycle. The ovarian arterial blood flow was measured by the implantable Doppler method (8 ± 1 ml min⁻¹). The maximum plasma PGF_{2α} concentrations in the femoral and distal ovarian arteries were 23 ± 6 and 42 ± 11 pg ml⁻¹ ($P < 0.05$), respectively. Plasma PGF_{2α} decreased exponentially in the femoral artery and the half-time decrease was 26 min ($r = 0.98$), and in the distal ovarian artery close to the ovary PGF_{2α} decreased linearly and the half-time decrease was 108 min ($r = 0.96$). Consequently, the arterio–arterial diffusion gradient of PGF_{2α} concentrations was extended to 3 h. These experiments showed that the PGF_{2α} flow rate in the pulmonary artery was 42.275 ± 10.793 µg per 150 min ($n = 10$) and the systemic arterial PGF_{2α} flow rate was 5.359 ± 1.658 µg per 150 min ($n = 10$). Therefore, 12% of the PGF_{2α} was not oxidized by the lungs. The proximal ovarian PGF_{2α} flow rate was 6.909 ± 2.341 ng per 150 min, while the distal flow rate was 21.003 ± 5.703 ng per 150 min ($n = 11$). Thus, 33% of the PGF_{2α} was transported rapidly to the ovary via the systemic route, while 67% was transported by slow local countercurrent diffusion, which extended the duration of luteolytic activity to four times that of the PGF_{2α} surge. These results indicate both rapid systemic transport of PGF_{2α} to the ovaries and a slower buffer mechanism involving a local diffusion pathway, rather than a direct countercurrent system.

Introduction

The endocrine theory of local vascular countercurrent transfer of the luteolysin PGF_{2α} corresponds to a mechanism of local PGF_{2α} transfer from the uterine venous blood to the ovarian arterial blood during the oestrous cycle (Barrett *et al.*,

1971; McCracken *et al.*, 1972, 1981; Land *et al.*, 1976). Uterine cyclic production of PGF_{2α} is responsible for the ovarian cyclicity in most mammals, including ruminants (sheep, goats, cows), pigs, horses, rodents and guinea-pigs (Anderson *et al.*, 1969; Mayer *et al.*, 1973; Ginther, 1974), with the exception of humans and other primates (Neill *et al.*, 1969; Beling *et al.*, 1970). In most species, separation of the uterus and one of the two ovaries results in prolongation of the lifespan of the ipsilateral corpus luteum, whereas the

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contralateral corpus luteum regresses at the normal time (Mapletoft and Ginther, 1975). In horses and rabbits, no local effect can be demonstrated and luteal regression occurs even if the ovary is relocated outside the pelvic cavity (Scott and Rennie, 1970; Ginther and First, 1971). However, plasma $\text{PGF}_{2\alpha}$ is almost completely inactivated by oxidation in the lungs after one passage in most species, including guinea-pigs, rats, cats, dogs and sheep (Piper *et al.*, 1970; Piper and Vane, 1971; McCracken *et al.*, 1972; Baird, 1978; Einer-Jensen, 1988). Therefore, it is thought that $\text{PGF}_{2\alpha}$ crosses from the utero-ovarian vein into the ovarian artery by a local direct countercurrent transfer. This was first proposed in sheep (Barrett *et al.*, 1971; McCracken *et al.*, 1972; Land *et al.*, 1976), and later in guinea-pigs (Poyser, 1976) and pigs (Lindloff *et al.*, 1976; Kotwicka, 1980; Krzymowski *et al.*, 1986). In sheep, as in a number of species including monkeys (Ginther *et al.*, 1974) and humans (Bendz, 1977), an anatomical feature reinforces the possibility of local vascular transfer originating in the abdominal aorta: the ovarian artery is tortuous and in close apposition with the surface of the utero-ovarian vein, a result of the confluence of the uterine vein and the ovarian and oviductal veins near the ovarian hilus (Del Campo and Ginther, 1973). The walls of the utero-ovarian vein and ovarian artery are thinnest at the sites at which the vessels adjoin, although no direct anastomosis between vein and artery has been demonstrated (Coudert *et al.*, 1974a,b; Del Campo and Ginther, 1974; Lee and O'Shea, 1975). Indeed, any such anastomosis would lead to a shunting of blood down the pressure gradient from artery to vein, making the local transfer of $\text{PGF}_{2\alpha}$ from uterus to ovary particularly difficult (Baird, 1978). Moreover, a fine network of utero-ovarian lymphatics adheres closely to the venous and arterial vessels in the broad ligament (Staples *et al.*, 1982), and contributes to $\text{PGF}_{2\alpha}$ transfer (Heap *et al.*, 1985, 1989).

Although the theory of local countercurrent transfer is well recognized (Leymarie and Martal, 1993), it remains controversial (Thorburn and Mattner, 1971; Baird and Land, 1973; Palmer, 1974; Coudert *et al.*, 1974b; Alwachi *et al.*, 1981; Kotwicka *et al.*, 1983). In engineering countercurrent systems for heat exchange, the efficiency of transfer can be close to 100%, whereas it is less than 2% for $[^3\text{H}]\text{PGF}_{2\alpha}$ (McCracken *et al.*, 1972). The industrial and physiological heat or gas countercurrent transfer systems correspond to a very fast exchange (Waites and Moule, 1961; Einer-Jensen, 1974a,b) as opposed to $\text{PGF}_{2\alpha}$ transfer, which can take 30 min in sheep (McCracken *et al.*, 1972, 1981; Land *et al.*, 1976) and in cattle (Hixon and Hansel, 1974). Finally, whereas the utero-ovarian vein plays a key role in transporting $\text{PGF}_{2\alpha}$ from the uterus to the ovary, it is widely accepted that, in sheep, the means by which it reaches the adjacent ovary remains unknown (Baird, 1978).

This study was conducted to determine the validity of the $\text{PGF}_{2\alpha}$ countercurrent theory. The aim was to determine whether $\text{PGF}_{2\alpha}$ catabolism is almost complete in the lungs and whether the systemic vascular $\text{PGF}_{2\alpha}$ input to the ovary is effectively insignificant, as indicated in the literature. The interventional radiology method was used and a direct ovarian arterio-arterial differential analysis after *in situ* physiological $\text{PGF}_{2\alpha}$ secretion stimulated by oxytocin at

oestrus. Native uterine $\text{PGF}_{2\alpha}$ was analysed with a sensitive radioimmunoassay to avoid artificial $[^3\text{H}]\text{PGF}_{2\alpha}$ infusion.

Materials and Methods

Rationale

Complete $\text{PGF}_{2\alpha}$ transfer from the utero-ovarian vein to the ovarian artery by venous-arterial countercurrent mechanism implies that (i) $\text{PGF}_{2\alpha}$ is not present in systemic blood perfusing the proximal ovarian artery because the $\text{PGF}_{2\alpha}$ discharged into the blood supply is totally oxidized by the pulmonary filter; and (ii) the total amount of $\text{PGF}_{2\alpha}$ transported to the ovary is apparent along the length of the ovarian artery.

Pulmonary clearance of PGF_2 was determined to verify whether the total amount of $\text{PGF}_{2\alpha}$ is inactivated by the lungs. Cardiac output and $\text{PGF}_{2\alpha}$ concentrations were measured before and after vascular lung supply using the pulmonary catheterization procedure.

The presence of an arterio-arterial gradient in plasma $\text{PGF}_{2\alpha}$ concentrations between the proximal segment of the ovarian artery (before the vascular area exchange) and the distal segment (close to the ovary, according to the nomenclature in cardiovascular physiology) was investigated to determine whether the total amount of $\text{PGF}_{2\alpha}$ flowing to the corpus luteum and the ovary is provided by venous-arterial countercurrent transfer. Data pertaining to the plasma $\text{PGF}_{2\alpha}$ concentrations perfusing the beginning of the ovarian artery and the end of this artery were required. These studies were performed using surgical catheterization techniques on the reproductive tract supply.

$\text{PGF}_{2\alpha}$ flow rates were calculated in different vessels as the product of the mean $\text{PGF}_{2\alpha}$ blood concentration during the experiment and the blood flow to characterize $\text{PGF}_{2\alpha}$ exchange and yields. Cardiac output was determined by the Fick method and the ovarian arterial blood flow by the implantable Doppler method. $\text{PGF}_{2\alpha}$ concentrations in the vessels under study were also determined.

Animals

The study was performed on 28 multiparous ewes of the Préalpes du Sud breed aged 3 ± 1 years and weighing 53 ± 4 kg. All the animals were fasted for 36 h before anaesthesia. Each protocol was carried out on day 16 of the oestrous cycle. After oxytocin stimulation, a uterine $\text{PGF}_{2\alpha}$ blood surge was obtained between natural endogenous releases in 21 of the 28 ewes.

Anaesthetic administration

Anaesthesia was induced by a 20 ml mixture of Pentotal® (2% (w/v), sodium thiopental; Abbott, France), Nembutal® (0.6% (w/v), sodium pentobarbital; Sanofi, France) and atropine sulfate (0.003% (w/v); Aguetant, France) per ewe administered i.v. (external jugular vein), and maintained by

inhalation of a mixture of 2–3% halothane (Fluothane®; Zeneca Pharma, Cergy, France) and 97–98% oxygen. As soon as the animal was anaesthetized sufficiently, tracheal cannulation and ventilation with the anaesthetic gas mixture were performed and the ewe was placed supine on the operating table. The animal was heparinized (75 iu kg^{-1} ; Choay, France). One of the jugular veins was cannulated with a catheter (G18, Cathelon®; Critikron-France, Chatenay) for saline solution and drug infusions.

Pulmonary artery catheterization protocol

An introducer (F7; Terumo, Japan) was placed into the right femoral vein and a catheter (G20, Cathelon®; Critikron-France, Chatenay) into the right femoral artery. A Swan-Ganz catheter (F6) was introduced into the femoral vein through the desilet, and advanced through the inferior vena cava, the right auricle and ventricle to the pulmonary artery. The catheterization procedure was performed under X-ray control to verify the position of the Swan-Ganz catheter during the entire experiment (Stenoscopy 9600®; General Electric, France).

Blood samples (1 ml) were obtained from pulmonary and femoral arteries for the determination of the blood gases, pH, haemoglobin concentration, and percentage of saturated haemoglobin in O_2 on a radiometer haemoximeter (ABL 3®; Radiometer, Copenhagen). Blood samples (5 ml) from pulmonary and femoral arteries were collected for further measurements of plasma $\text{PGF}_{2\alpha}$ concentrations (Fig. 1). Meclofenamic acid ($50 \mu\text{l}$, 0.1% (w/v)) was added to each blood sample immediately after collection. Blood samples were collected 15 min before and just before infusion of 5 iu oxytocin (Syntocinon®; Sandoz, Rueil-Malmaison) into the jugular vein. Blood samples were taken every 15 min for 150 min. After collection of each blood sample, all catheters were filled with heparinized saline (100 iu ml^{-1}).

Surgical protocol

The uterus and annexes were exposed by laparotomy along the alba linea. The ovaries and uterine and ovarian vessels were examined and the side in which the corpus luteum was present was noted. The uterus was fixed in place with a nylon thread to maintain it on the exterior of the peritoneal cavity. On the side containing the corpus luteum, the ovarian artery was isolated and dissected. This artery was carefully separated from the utero-ovarian vein and a segment of 0.1–0.2 cm was isolated 5–6 cm before the ovarian hilus for application of the Doppler microdevice. Catheters were carefully introduced into the uterine vein between the uterus and the ovary under study (G22, Cathelon®; Critikron-France, Chatenay), into the distal and straight segment of the ovarian artery just at the ovarian hilus (G24, Cathelon®; Critikron-France, Chatenay), and into one of the femoral arteries, for collection of blood samples and determination of plasma $\text{PGF}_{2\alpha}$ concentrations (G20, Cathelon®; Critikron-France, Chatenay) (Fig. 1). All catheters were placed and advanced 1–2 cm retrogradely without any

Fig. 1. Sites of cannulation and collection of blood samples for the study of the transfer of $\text{PGF}_{2\alpha}$ from the uterus to the ovary through the vascular system in ewes.

ligation to maintain arterial and venous circulation in the reproductive tract supply. After collection of each blood sample, all catheters were filled with heparinized saline ($100 \text{ units ml}^{-1}$).

Mean velocity and diameter of the ovarian artery were monitored with an 8 MHz Doppler ultrasonic crystal applied directly to the vessel. The Doppler crystal was a piezo-electric crystal energized by a pulsed Doppler unit (Echovar® AL 40; Alvar, Montreuil). The repetition frequency of pulse was set at 31.25 kHz (Beloucif *et al.*, 1990).

Doppler measurements from the ovarian artery and blood samples from the uterine vein and the ovarian and femoral arteries in sequence were performed 15 min before and just before oxytocin infusion. The measurements and blood samples were continued every 15 min for 150 min after infusion.

$\text{PGF}_{2\alpha}$ measurements

Plasma $\text{PGF}_{2\alpha}$ concentrations were determined by radioimmunoassay without extraction as described by Andrianakis *et al.* (1989) with the following modifications. Briefly, $500 \mu\text{l}$ plasma sample was added to specific anti- $\text{PGF}_{2\alpha}$ antiserum (Institut Pasteur®, Paris) at a final dilution of 1:12 000 and to tritiated $\text{PGF}_{2\alpha}$ ($[5,6,8,9,11,12,14,15\text{-}^3\text{H(N)}]$)-

PGF_{2α}, NEN; Dupont De Nemours®, Les Ulis). Immune complexes were incubated for 2 h at room temperature and then overnight at 4°C. Free and antibody bound hormone were separated by precipitation using 2 ml 20% (w/v) polyethylene glycol 6000 and centrifugation at 3000 g for 20 min (Heraeus Christ®, Cryofuge 6-6). Centrifugation pellets were solubilized with 200 µl NaOH (0.1 mol l⁻¹) and radioactivity was measured in a 460C Packard counter (Packard®, Rungis). Plasma samples were run in duplicate. The limit of sensitivity was 2 pg ml⁻¹ and intra- and interassay coefficients of variation were 9 and 15%, respectively. The immunological crossreactivities of the PGF_{2α} antiserum at 50% displacement were: 12% with PGF_{1α}; 3% with PGD-2 and DH-PGF_{2α} (13,14-dihydro PGF_{2α}); 2% with 19 OH-PGF_{2α} (19-hydroxy-PGF_{2α}); 0.4% with PGD-1 and DH-PGF_{1α} (13,14-dihydro-PGF_{1α}); 0.03% with PGE-1 and PGE-2; 0.04% with 6-keto-PGF_{1α}; and < 0.01% with DHK-PGF_{2α} (13,14-dihydro-15-keto-PGF_{2α}), PGA-1, PGA-2, PGB-1, K-PGE_{1α} (15-keto-PGE_{1α}), DHK-PGF_{1α} (13,14-dihydro-15-keto-PGF_{1α}), K-PGE-2 (15-keto-PGE-2), K-PGF_{2α} (15-keto-PGF_{2α}) and TXB-2.

Measurements and calculations

PGF_{2α} pulmonary clearance was calculated according to the following formula (Lockhart, 1983a):

$$Cl_{PGF_{2\alpha}} = Q_c \times (1 - C_{fa}/C_{pa})$$

where $Cl_{PGF_{2\alpha}}$ is the PGF_{2α} pulmonary clearance (ml min⁻¹), Q_c represents the cardiac output (ml min⁻¹), C_{fa} is the femoral artery PGF_{2α} concentration, and C_{pa} is the pulmonary artery PGF_{2α} concentration (both pg ml⁻¹).

Cardiac output was calculated using the Fick equation (Lockhart, 1983b):

$$Q_c = V_{O_2}/DAV_{O_2}$$

where V_{O_2} is the oxygen consumption (ml min⁻¹) estimated by the product of the body weight in kg and the mean O_2 consumption for the ewe (3.66 ml min⁻¹ per kg) (Ruckebusch, 1977), and DAV_{O_2} is the arterio-venous difference in O_2 content calculated from the femoral and the pulmonary arteries (ml per 100 ml). The blood O_2 contents were calculated as the sum of the O_2 bound by haemoglobin and O_2 solubilized in plasma. Owing to the anaesthetic gas mixture used in the study, all animals were submitted to hyperoxia, which involved a marked increase in the plasma O_2 concentration.

Haemoglobin binding O_2 content was calculated according to the following equation:

$$O_2 \text{ content} = Sa_{O_2} \times Hb \times 1.36 \text{ (ml per 100 ml)}$$

where Sa_{O_2} is the percentage haemoglobin O_2 saturation, Hb is the haemoglobin concentration (g per 100 ml), and 1.36 is the haemoglobin O_2 binding capacity (ml g⁻¹) (Even, 1983).

Non-haemoglobin binding O_2 content was calculated according to the formula of Henry's Law (Even, 1983):

$$O_2 \text{ content} = (P_{O_2}/P_{atm}) \times 0.023 \times 100 \text{ (ml per 100 ml)}$$

where P_{O_2}/P_{atm} is the O_2 fraction in the blood and 0.023 is the O_2 coefficient solubility in plasma.

Ovarian artery blood flow was calculated using the following formula (Lockhart, 1983b):

$$Q = \pi (D/2)^2 \times V_{\text{mean}} \times 60$$

where Q is the blood flow (ml min⁻¹), D represents the diameter of the vessel (cm), and V_{mean} is the mean velocity (cm s⁻¹), both determined by Doppler techniques.

PGF_{2α} flow rates were calculated according to the formula (Lockhart, 1983b):

$$PGF_{2\alpha} \text{ flow rate} = c \times bf \times t$$

where c is the mean PGF_{2α} blood concentration during the observation period, bf is the blood flow and t is the time of the observation period (150 min). The PGF_{2α} blood concentrations were calculated as the product of plasma PGF_{2α} concentrations and a factor $(1 - Ht)$ where Ht is the haematocrit (%). PGF_{2α} flow rates were determined in the pulmonary artery, the aorta, the proximal ovarian artery and the distal ovarian artery. For the pulmonary artery, the pulmonary PGF_{2α} blood concentrations and the cardiac output were used for the calculation. This pulmonary arterial PGF_{2α} flow rate was representative of the total PGF_{2α} produced in the uterus during stimulation and transported to the lungs. PGF_{2α} blood concentrations in the femoral artery and cardiac output were used for calculation of the post-pulmonary PGF_{2α} flow rate, which represents PGF_{2α} not oxidized by the lungs that could flow by systemic circulation to the organs, especially the ovaries. For the proximal ovarian artery, the ovarian blood flow and PGF_{2α} blood concentrations in the femoral artery, similar to concentrations at the beginning of the artery, were used. For the distal ovarian artery, PGF_{2α} blood concentrations determined from blood samples taken from the distal ovarian artery at the ovarian hilus (Fig. 1) and the ovarian blood flow were used.

Statistical analysis

An ANOVA for repeated measurements was applied to compare data recorded before and during PGF_{2α} stimulation and a paired Student's t test was used to compare data recorded at the same stage of the protocol. The PGF_{2α} kinetics of decrease were assessed using linear and exponential regressions and half-time decrease was calculated (STATVIEW, Power Macintosh). The level of significance was set at $P < 0.05$. All values represent mean $\pm$ SEM unless otherwise specified.

Results

The measured blood gases, haemoglobin concentration, haemoglobin O_2 saturation percentage and O_2 content remained stable throughout the pulmonary catheterization procedure (Table 1). The calculated cardiac mean output was 7156 ± 439 ml min⁻¹ for the ten catheterized ewes. Pre-treatment (t_0) plasma PGF_{2α} concentration in the uterine vein, at the moment of luteolysis, that is, on day 16 of the oestrous cycle, was 1429 ± 444 pg ml⁻¹ (Table 2). Infusion of oxytocin

Table 1. Haemoglobin concentration, O_2 partial pressure and percentage O_2 saturation in haemoglobin, cardiac output O_2 consumption and O_2 content in pulmonary and femoral arteries during catheterization procedures in a study of uterine $\text{PGF}_{2\alpha}$ transport in ewes

Femoral artery oxygen content ($n = 10$ ewes)	
Haemoglobin ($\text{g } 100 \text{ ml}^{-1}$)	10.68 ± 0.05
Haemoglobin O_2 saturation (%)	99.64 ± 0.10
Haemoglobin binding O_2 content ($\text{ml } 100 \text{ ml}^{-1}$)	14.83 ± 0.34
O_2 partial pressure (mm Hg)	389 ± 35
Non-haemoglobin binding O_2 content ($\text{ml } 100 \text{ ml}^{-1}$)	1.12 ± 0.10
O_2 content ($\text{ml } 100 \text{ ml}^{-1}$)	15.95 ± 0.34
Pulmonary artery oxygen content ($n = 10$ ewes)	
Haemoglobin O_2 saturation (%)	88.36 ± 1.28
Haemoglobin binding O_2 content ($\text{ml } 100 \text{ ml}^{-1}$)	12.92 ± 0.34
O_2 partial pressure (mm Hg)	75 ± 4
Non-haemoglobin binding O_2 content ($\text{ml } 100 \text{ ml}^{-1}$)	0.23 ± 0.01
O_2 content ($\text{ml } 100 \text{ ml}^{-1}$)	13.15 ± 0.35
Cardiac output ($n = 10$ ewes)	
Arterio-venous difference in O_2 content ($\text{ml } 100 \text{ ml}^{-1}$)	2.80 ± 0.21
O_2 consumption (ml min^{-1})	191 ± 5
Cardiac output ($\text{ml } 100 \text{ ml}^{-1}$)	7156 ± 439

Table 2. Plasma $\text{PGF}_{2\alpha}$ concentration in the uterine vein, femoral artery (proximal ovarian artery) and distal ovarian artery before and after oxytocin stimulation in ewes

Time (min)	Uterine vein $\text{PGF}_{2\alpha}$ concentration (pg ml^{-1})	Femoral artery $\text{PGF}_{2\alpha}$ concentration (pg ml^{-1})	Distal ovarian artery $\text{PGF}_{2\alpha}$ concentration (pg ml^{-1})	Difference in distal – proximal ovarian artery $\text{PGF}_{2\alpha}$ concentrations (pg ml^{-1})
-15	2192 ± 583	7 ± 3	9 ± 6	2 ± 5
0	1429 ± 444	5 ± 2	14 ± 7	9 ± 6
15	$3285 \pm 670^*$	$20 \pm 8^*$	$31 \pm 12^*$	11 ± 7
30	$3811 \pm 806^*$	$23 \pm 6^*$	$42 \pm 11^*$	20 ± 9
45	$3347 \pm 649^*$	$17 \pm 6^*$	$43 \pm 10^*$	$26 \pm 8^*$
60	$2664 \pm 626^*$	$15 \pm 6^*$	$35 \pm 8^*$	21 ± 6
75	1760 ± 444	6 ± 3	$36 \pm 13^*$	$30 \pm 11^*$
90	1105 ± 318	5 ± 4	$31 \pm 9^*$	$26 \pm 6^*$
105	784 ± 305	4 ± 3	22 ± 5	18 ± 4
120	433 ± 133	3 ± 2	21 ± 6	19 ± 6
135	256 ± 64	1 ± 1	22 ± 7	21 ± 7
150	$226 \pm 68^*$	1 ± 1	15 ± 5	14 ± 5

Measurements were taken 15 min before, just before ($t = 0$), and every 15 min for 150 min after, oxytocin stimulation ($n = 11$ ewes). $\text{PGF}_{2\alpha}$ concentration reached a maximum at 30 min and then decreased.

* $P < 0.05$ compared with values at $t = 0$.

resulted in an increase in the concentration to $3811 \pm 806 \text{ pg ml}^{-1}$ at 30 min, which remained stable for 15 min and then decreased according to an exponential function (half-time decrease of 27 min, $r = 0.99$) (Fig. 2).

In the pulmonary artery (Table 3), maximum plasma $\text{PGF}_{2\alpha}$ concentration as a result of oxytocin stimulation was obtained at 30 min ($224 \pm 55 \text{ pg ml}^{-1}$), and the concentration then decreased according to an exponential function (half-time decrease of 15 min 47 s, $r = 0.99$) (Fig. 3). Maximum plasma $\text{PGF}_{2\alpha}$ concentration in the femoral artery was $18 \pm 4 \text{ pg ml}^{-1}$ at 30 min (Table 3) and decreased exponentially and the half-time decrease was 18 min ($r = 0.98$) (Fig. 3). The

lungs oxidized about 88% of the $\text{PGF}_{2\alpha}$ secreted by the uterus and measured in the pulmonary artery for 150 min after oxytocin stimulation (Fig. 1), and $\text{PGF}_{2\alpha}$ pulmonary clearance was estimated at $6338 \pm 451 \text{ ml min}^{-1}$ (Table 4).

In the surgical experiments, ovarian blood flow remained stable at $8 \pm 1 \text{ ml min}^{-1}$ (Table 4). The maximum plasma $\text{PGF}_{2\alpha}$ concentration in the proximal ovarian artery (that is, the femoral artery) was $23 \pm 6 \text{ pg ml}^{-1}$ at 30 min and decreased according to an exponential function (half-time decrease of 26 min, $r = 0.98$) (Table 2, Fig. 4). In the distal ovarian artery close to the ovary, the maximum value was higher than in the proximal segment of the artery (42 ± 11

Fig. 2. Plasma PGF_{2α} concentrations in the uterine vein after injection of 5 iu oxytocin (OT) into the jugular vein in ewes (*n* = 11). The concentration decreased 30 min after injection according to an exponential function and the half-time decrease was 27 min (*r* = 0.99).

Table 3. Plasma PGF_{2α} concentration in the pulmonary and femoral arteries before and after oxytocin stimulation in ewes

Time (min)	Pulmonary artery PGF _{2α} concentration (pg ml ⁻¹)	Femoral artery PGF _{2α} concentration (pg ml ⁻¹)	Percentage of PGF _{2α}
-15	100 ± 24	10 ± 3	10
0	98 ± 23	8 ± 4	8.2
15	158 ± 42	12 ± 4	7.6
30	224 ± 55*	18 ± 4*	8
45	107 ± 30	10 ± 2	9.3
60	66 ± 17	9 ± 3	13.6
75	36 ± 15	5 ± 2	13.8
90	21 ± 11*	2 ± 1	9.5
105	11 ± 5*	1 ± 1	9.1
120	5 ± 3*	0	—
135	2 ± 1*	0	—
150	1 ± 1*	0	—

Measurements were taken 15 min before, just before (*t* = 0), and every 15 min for 150 min after, oxytocin stimulation (*n* = 11 ewes). PGF_{2α} concentration reached a maximum at 30 min and then decreased.
**P* < 0.05 compared with values at *t* = 0.

versus 23 ± 6 pg ml⁻¹, *P* < 0.05) (Table 2), and decreased linearly. The half-time decrease was 108 min and thus the concentration remained higher in the distal segment than in the proximal segment during the observation period (Fig. 4). The difference between distal and proximal plasma PGF_{2α} concentrations increased from the moment of stimulation to reach a maximum at 75 min and then decreased (Table 2, Fig. 4).

Total PGF_{2α} secreted in the circulatory supply was

calculated as the pulmonary artery PGF_{2α} flow rate, 42.275 ± 10.793 µg per 150 min (Table 4). The flow rate of PGF_{2α} not oxidized by the lungs and flowing in the systemic arterial supply was calculated as 5.359 ± 0.836 µg per 150 min, and PGF_{2α} clearance was stable throughout the experiments (Table 4). The proximal ovarian PGF_{2α} flow rate was 6.906 ± 2.341 ng per 150 min, while the distal rate was 21.003 ± 5.703 ng per 150 min (Table 4, Fig. 1).

Thus, total PGF_{2α} flowing in the systemic veins to the

Fig. 3. Plasma $\text{PGF}_{2\alpha}$ concentrations in (□) the pulmonary artery and (■) the femoral artery after injection of 5 iu oxytocin (OT) into the jugular vein in ewes ($n = 10$). The concentration in the pulmonary artery decreased 30 min after injection according to an exponential function and the half-time decrease was 15 min 47 s ($r = 0.99$). The concentration in the femoral artery also decreased according to an exponential function and the half-time decrease was 18 min ($r = 0.98$).

Table 4. $\text{PGF}_{2\alpha}$ flow rates for the pulmonary artery, the arterial blood flow, the proximal ovarian artery and the distal ovarian artery after oxytocin stimulation in ewes

Results from pulmonary artery catheterization procedure ($n = 10$)	
Cardiac output (ml min^{-1})	7156 ± 439
$\text{PGF}_{2\alpha}$ pulmonary clearance (ml min^{-1})	6338 ± 451
$\text{PGF}_{2\alpha}$ flow rate in pulmonary artery over 150 min ^a (μg)	42.275 ± 10.793
$\text{PGF}_{2\alpha}$ flow rate after the lungs over 150 min ^b (μg)	5.359 ± 1.658
Percentage $\text{PGF}_{2\alpha}$	12.28 ± 1.68
Results from surgical procedure ($n = 11$ ewes)	
Ovarian blood flow (ml min^{-1})	8 ± 1
$\text{PGF}_{2\alpha}$ flow rate in proximal ovarian artery over 150 min ^c (ng)	6.906 ± 2.341
$\text{PGF}_{2\alpha}$ flow rate in distal ovarian artery over 150 min ^d (ng)	21.003 ± 5.703

^aFor the pulmonary artery, the plasma $\text{PGF}_{2\alpha}$ concentrations, the haematocrit and the cardiac output were used for calculation of $\text{PGF}_{2\alpha}$ flow rate. This pulmonary arterial $\text{PGF}_{2\alpha}$ flow rate was representative of the total $\text{PGF}_{2\alpha}$ produced by the uterus after oxytocin stimulation and transported to the lungs.

^bFor the arterial blood flow, the plasma $\text{PGF}_{2\alpha}$ concentrations in the femoral artery and the cardiac output were used for calculation of the flow rate. This post-pulmonary $\text{PGF}_{2\alpha}$ flow rate was representative of $\text{PGF}_{2\alpha}$ not oxidized by the lungs that could flow by systemic circulation to the organs and ovaries.

^cFor the proximal ovarian artery, the ovarian blood flow and $\text{PGF}_{2\alpha}$ concentrations in the femoral artery, similar to concentrations at the beginning of the artery, were used to calculate flow rate.

^dFor the distal ovarian artery, concentrations used for calculation of the flow rate were determined from blood samples taken from the distal ovarian artery at the ovarian hilus.

Fig. 4. Plasma $\text{PGF}_{2\alpha}$ concentrations in (●) the proximal ovarian artery and (□) the distal ovarian artery, 30 min after injection of 5 iu oxytocin (OT) into the jugular vein in ewes ($n = 11$). The concentration in the proximal ovarian artery (that is, the femoral artery) decreased according to an exponential function and the half-time decrease was 26 min ($r = 0.98$). The concentration in the distal ovarian artery (close to the ovary) decreased according to a linear function and the half-time decrease was 108 min ($r = 0.96$). The difference between the plasma $\text{PGF}_{2\alpha}$ concentration in the proximal and distal ovarian arteries (■) described a square function ($r = 0.89$).

lungs was partially degraded, permitting infusion of the ovarian artery with non-degraded $\text{PGF}_{2\alpha}$: 33% of the $\text{PGF}_{2\alpha}$ was transported to the ovary by systemic circulation and 67% by diffusion along the artery.

Discussion

When considering the supply pathways to the genital tract in ewes, which includes a coiled ovarian artery close to the utero-ovarian vein, four compartments were identified: (i) an area for synthesis and release of $\text{PGF}_{2\alpha}$: the uterus; (ii) an area of transfer: the broad ligament containing the vessels; (iii) an area of $\text{PGF}_{2\alpha}$ catabolism: the lungs; and (iv) an effector organ: the ovary containing the corpus luteum. The area of $\text{PGF}_{2\alpha}$ transfer has two inputs: the uterine vein and the proximal ovarian artery; and two outputs: the distal ovarian artery close to the ovary and the utero-ovarian vein.

With regard to the two inputs and the area of degradation, the kinetics of $\text{PGF}_{2\alpha}$ decrease described exponential functions, that is, in the uterine vein, the proximal ovarian artery and the pulmonary artery. The half-time decreases were lower during the $\text{PGF}_{2\alpha}$ pulmonary experiments (approximately 16 min in the pulmonary artery and 18 min in the femoral artery) than in the ovarian $\text{PGF}_{2\alpha}$ arterio-arterial gradient experiments (27 min in the uterine vein and 26 min in the femoral artery). This discrepancy could be due

to the use of two different ventilatory apparatuses, one located in the catheterization room and the other in the surgical room. The hyperoxia in the surgical room was lower (arterial P_{O_2} of 250 mmHg) than that in the catheterization room (arterial P_{O_2} of 389 mmHg). Indeed, $\text{PGF}_{2\alpha}$ degradation in the lungs consists of oxidation by prostaglandin 15 hydroxydeshydrogenase and prostaglandin Δ^{13} reductase in the 13,14 dihydro-15 keto- $\text{PGF}_{2\alpha}$ (Piper *et al.*, 1970; Sun and Armour, 1974). Although it is possible that degradation of $\text{PGF}_{2\alpha}$ was accelerated in the pulmonary artery catheterization experiments, $\text{PGF}_{2\alpha}$ pulmonary clearance of about 90% was observed, permitting infusion of the systemic arterial supply with about 10% of the total $\text{PGF}_{2\alpha}$ secreted by the uterus, especially the proximal ovarian artery. Taking into account the hyperoxia during these experiments, it is possible that the $\text{PGF}_{2\alpha}$ pulmonary clearance was overestimated and, consequently, that the systemic $\text{PGF}_{2\alpha}$ arterial flow rate to the ovary was underestimated. Indeed, the calculated $\text{PGF}_{2\alpha}$ flow rate to the ovary (6.906 ng per 150 min) was higher in the experiments in which the arterio-arterial gradient of plasma $\text{PGF}_{2\alpha}$ concentrations was determined than that estimated from $\text{PGF}_{2\alpha}$ concentrations in the femoral artery during determination of $\text{PGF}_{2\alpha}$ pulmonary clearance (5.359 ng per 150 min). These results therefore indicate an increase in $\text{PGF}_{2\alpha}$ pulmonary catabolism due to hyperoxia.

With regard to the two output areas of $\text{PGF}_{2\alpha}$ transfer, in the utero-ovarian vein, the kinetics of $\text{PGF}_{2\alpha}$ decrease

Fig. 5. $\text{PGF}_{2\alpha}$ systemic and local vascular pathway between the uterus and ovary in sheep. $\text{PGF}_{2\alpha}$ transfer from the uterus to the ovary is achieved by systemic circulation and local diffusion. Of the $\text{PGF}_{2\alpha}$ secreted by the uterus during 150 min (approximately 42 μg), 0.05% reaches the ovary and corpus luteum (approximately 21 ng). One-third of the $\text{PGF}_{2\alpha}$ is transported to the ovary by systemic circulation and the proximal ovarian artery (approximately 7 ng). $\text{PGF}_{2\alpha}$ secreted by the uterus returns to the lungs in the venous system. About 12% of the $\text{PGF}_{2\alpha}$ passes through the lungs without degradation and flows by the arterial systemic circulation, in particular by the ovarian artery, to the ovary. This represents a rapid transport of $\text{PGF}_{2\alpha}$ to the ovary and a maximum blood surge 30 min after oxytocin stimulation, corresponding to the maximum secretion in the uterine vein. The kinetics of the decrease in $\text{PGF}_{2\alpha}$ follow an exponential function and the half-time decrease is 26 min. Two-thirds of the $\text{PGF}_{2\alpha}$ is transported to the ovary by diffusion from the utero-ovarian vein (one-third, approximately 7 ng) and the uterine lymphatics (one-third, approximately 7 ng) to the ovarian artery, which is tortuous and in close proximity to the vein and the lymphatics. This represents slow transport of $\text{PGF}_{2\alpha}$ to the ovary and the estimated maximum duration is 75 min. In addition, the arterial systemic circulation and local diffusion from the utero-ovarian vein and the uterine lymphatics prolong the transfer of $\text{PGF}_{2\alpha}$, as the kinetics of the decrease in the transfer to the ovary follow a linear function and the half-time decrease is 108 min.

followed the same exponential function as in the uterine vein because a very small amount of $\text{PGF}_{2\alpha}$ diffuses out of the vein, and in the distal ovarian artery close to the ovary, the kinetics of decrease followed a linear regression and the half-time decrease was extended considerably (108 min). Moreover, the ovarian arterio-arterial difference in $\text{PGF}_{2\alpha}$ was maximal in the 90 min after oxytocin stimulation, 60 min after the maximum $\text{PGF}_{2\alpha}$ concentration was reached in the ovarian artery. This delay implies a mechanism of slow diffusion between the vessels under consideration and prolongs the $\text{PGF}_{2\alpha}$ infusion of the ovary to more than 216 min. This delay was determined from the intersection of the linear regression of $\text{PGF}_{2\alpha}$ concentrations in the distal ovarian artery and the time axis. The calculated $\text{PGF}_{2\alpha}$ arterio-arterial differential gradient therefore represents the rate of $\text{PGF}_{2\alpha}$ diffusion through the vessel walls and broad ligament. It is probable that hyperoxia plays a role in the

tissue catabolism of $\text{PGF}_{2\alpha}$, particularly in the broad ligament.

The calculated relative rate of $\text{PGF}_{2\alpha}$ transported to the ovary by the systemic circulation is 33%. Two research teams (Abdel-Rahim *et al.*, 1983, 1984; Heap *et al.*, 1985, 1989) have demonstrated that, in sheep, diffusion in the area of $\text{PGF}_{2\alpha}$ transfer derives from both the utero-ovarian vein (about 50%) and the uterine lymphatics (about 50%). Therefore, it is proposed that about one-third of the total $\text{PGF}_{2\alpha}$ secreted by the uterus is transported to the ovary by arterial systemic circulation, one-third by diffusion from the utero-ovarian vein and one-third by diffusion from the uterine lymphatics (Fig. 5).

Therefore, in ewes, $\text{PGF}_{2\alpha}$ transfer by arterial systemic circulation represents a rapid route to the ovary. $\text{PGF}_{2\alpha}$ transfer by local diffusion represents a slow route that prolongs the stimulation to four times that of the duration of

a native $\text{PGF}_{2\alpha}$ surge (half-life in the distal ovarian artery of 108 min versus $\text{PGF}_{2\alpha}$ half-life in the proximal ovarian artery of 26 min). Thus, $\text{PGF}_{2\alpha}$ delivery to the ovary is considerably extended with regard to the original vascular network in the genital tract. The impact of the general and local vascular organization on the biodistribution and delivery to the effector organ of a hormone $\text{PGF}_{2\alpha}$ is clearly demonstrated. The original local vascular pathway functions as a buffer mechanism. Nevertheless, the yield of this type of mechanism is very low since only 0.05% (Fig. 5) of the total $\text{PGF}_{2\alpha}$ released by the uterus reaches the ovary through both arterial systemic and local diffusion pathways. The efficiency of local $\text{PGF}_{2\alpha}$ diffusion is about 0.035%, as reported by Heap *et al.* (1989), who found that the estimated percentage transfer of $[\text{H}]\text{PGF}_{2\alpha}$ from the uterine vein into the ovarian artery was less than 0.1%. In a study by McCracken *et al.* (1972) on the local countercurrent transfer of infused tritiated $\text{PGF}_{2\alpha}$, it was calculated that >2% of the total radioactivity infused into the uterine vein. With regard to the estimation of $[\text{H}]\text{PGF}_{2\alpha}$ including extraction, reverse-phase partition chromatography, silicic acid chromatography and GLC-mass spectrometry, the discrepancy in results between the two methods is small. In the present study, the sensitive radioimmunoassay used to determine $\text{PGF}_{2\alpha}$ concentrations is much more precise. Furthermore, the release of uterine $\text{PGF}_{2\alpha}$ by oxytocin mimics physiological conditions more closely than infusion of radioactive $\text{PGF}_{2\alpha}$. Walsh *et al.* (1979) reported a local diffusion countercurrent transfer for $[\text{H}]\text{progesterone}$ in ewes and an estimated efficiency rate of about 0.035%, compared with 0.5–1% reported by Einer-Jensen and McCracken (1981).

The observation that one-third of the $\text{PGF}_{2\alpha}$ returns to the ovary by the systemic arterial circulation is significant, since it corresponds to one-third of the total amount of $\text{PGF}_{2\alpha}$ that is transported to the ovary. In addition, local transfer of $\text{PGF}_{2\alpha}$ from the utero-ovarian vein to the ovarian artery is by a normal local diffusion pathway from both venous and lymphatic vessels into the ovarian artery. It has been proposed that this mechanism is a countercurrent transfer for the following reasons: (i) the close apposition of the ovarian artery and the ovarian vein; (ii) the existence of extensive coils in the ovarian artery that increase the potential area of exchange; and (iii) the opposing direction of blood flow in the two vessels (Einer-Jensen and McCracken, 1981). However, a number of observations indicate that it is not a local countercurrent system but a local vascular diffusion pathway. Firstly, the time course of $\text{PGF}_{2\alpha}$ transfer from the utero-ovarian vein to the ovarian artery is particularly short and has a time-lag of 15–30 min and a duration of several hours (McCracken *et al.*, 1972; Land *et al.*, 1976; Moore *et al.*, 1986). In the present study, a new approach was used in the arterio-arterial analysis to demonstrate both the time-lag and the very slow diffusion of local transfer from the utero-ovarian vein to the ovarian artery. Secondly, the efficiency rate of $\text{PGF}_{2\alpha}$ transfer is particularly low in all studies, indicating a mechanism of slow diffusion rather than a countercurrent system.

The term 'countercurrent system' should not be used to avoid confusion, with the exception of when it is associated with the word diffusion, as in 'countercurrent diffusion

system' (Free and Jaffe, 1975). In a number of reports alternative definitions have been used, including the terms 'local steroid concentrating mechanism' (Walsh *et al.*, 1979), 'local veno-arterial pathways' (Ginther, 1974) or 'lymphatic-vascular transfer of $\text{PGF}_{2\alpha}$ ' (Heap *et al.*, 1985, 1989).

Vascular countercurrent transfer from the utero-ovarian vein to the ovarian artery has also been reported for heat, xenon, krypton, tritiated water, progesterone, testosterone and small peptides such as relaxin and oxytocin (Waites and Moule, 1961; Schramm *et al.*, 1986a,b; Einer-Jensen, 1988).

Heap *et al.* (1989) compared the mechanisms of lymphatic-vascular transfer of $[\text{H}]\text{PGF}_{2\alpha}$ in sheep, 8–15 days after ovulation, with transfer of $[\text{H}]\text{Na}$, which diffuses rapidly, and $[\text{H}]\text{mannitol}$, which is transferred by the paracellular route. Transfer occurred according to the sequence $^{36}\text{Cl} > ^{14}\text{C} > ^3\text{H}$, indicating that $[\text{H}]\text{PGF}_{2\alpha}$ is not transferred by rapid diffusion as is $[\text{H}]\text{Na}$, nor by a paracellular route as is $[\text{H}]\text{mannitol}$, but by a slower process possibly involving facilitated diffusion. The estimated percentage transfer of $[\text{H}]\text{PGF}_{2\alpha}$ from the uterine afferent lymph into the utero-ovarian vein plasma was 27% and into the ovarian artery was 1%, whereas less than 0.1% of the total amount infused from the uterine vein into the ovarian artery (Heap *et al.*, 1989). It is noteworthy that the proportion of labelled $\text{PGF}_{2\alpha}$ transferred by the lymphatic-arterial pathway was greater (1%) than that observed for transfer between utero-ovarian vein and ovarian artery (0.1%). The latter rate was similar to that (0.1–0.56%) reported by Land *et al.* (1976) and (0.3%) by Heap *et al.* (1985). Therefore, $\text{PGF}_{2\alpha}$ can pass from the lymph vein to the utero-ovarian vein, in which the blood flow is concurrent, or to the ovarian artery, in which blood flow is countercurrent. The discrepancy in the percentage transfer of $[\text{H}]\text{PGF}_{2\alpha}$ from the lymph into the utero-ovarian vein and directly into the ovarian artery also indicates that $\text{PGF}_{2\alpha}$ is not first transferred into the utero-ovarian vein and then into the ovarian artery, but probably occurs into both simultaneously. Estimation of the total amount of $[\text{H}]\text{PGF}_{2\alpha}$ transferred from the lymph into the ovarian venous blood gave a value similar to that for transfer from a uterine vein to the ovarian artery (0.4% versus 0.3%) (Heap *et al.*, 1985). There is no evidence for retrograde lymph flow between the uterus and ovaries, but the close proximity of the utero-ovarian lymphatics, utero-ovarian vein and ovarian artery provide pathways for countercurrent diffusion of $\text{PGF}_{2\alpha}$ or progesterone, for instance.

The duration of $\text{PGF}_{2\alpha}$ diffusion observed in this present study (150 min) is compatible with that of physiological $\text{PGF}_{2\alpha}$ pulsatile release during luteolysis (Martal *et al.*, 1997). Zarco *et al.* (1988a,b) showed that the average interpulse interval for $\text{PGF}_{2\alpha}$ pulses associated with luteolysis was 7.7 h and each one lasted about 3 h.

It would be interesting to determine the importance of the systemic route for transfer of $\text{PGF}_{2\alpha}$ in non-anaesthetized ewes on day 16 of the oestrous cycle. The significance of this pathway is of particular interest in species such as pigs, horses and rabbits. In rabbits, $\text{PGF}_{2\alpha}$ catabolism in the lungs is largely dependent on the physiological state (Sun and Armour, 1974), especially during pregnancy. In horses and

rabbits, in which a unilateral luteolytic effect has not been demonstrated and the ovarian artery and ovarian vein are independent, the systemic PGF_{2α} pathway is probably particularly predominant. Finally, in primates, and particularly in humans, even if control of luteolysis is not dependent on uterine PGF_{2α}, the extensive area of contact between the utero-ovarian vein and the ovarian artery implies that several lympho-veno-arterial diffusion pathways, for example, of PGF_{2α} and progesterone, may be important.

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