INFUSION OF TRITIATED PROSTAGLANDIN $F_{2\alpha}$ INTO THE ANTERIOR UTERINE VEIN OF THE EWE: ABSENCE OF LOCAL VENOUS-ARTERIAL TRANSFER

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(Received 13th February 1973)

Summary. A tracer dose of the presumptive uterine luteolytic factor ($PGF_{2\alpha}$) was introduced into the anterior uterine vein of twelve ewes, either as a bolus or as a 40- to 60-min infusion ($0.2 \mu\text{Ci/min}$). The ipsilateral ovary was left attached to or disconnected from its ligaments. Neither a bolus nor an infusion of $[9\text{-}^3\text{H}]PGF_{2\alpha}$ led to any transfer of radioactivity in the ovarian artery under controlled experimental conditions. These results show conclusively that diffusion, filtration, counter-current exchanges and active transport do not occur between the anterior uterine vein, the utero-ovarian vein and the ovarian artery. A direct and local luteolytic action of $PGF_{2\alpha}$ is therefore questionable.

INTRODUCTION

The regression of the corpus luteum is delayed by hysterectomy in several mammalian species including sheep (Anderson, Bland & Melampy, 1969). In these species, an intact uterus or a uterine graft appears to produce a luteolytic factor(s) and this factor is thought to act locally upon the ovary. It has been suggested that prostaglandin $F_{2\alpha}$ might be the luteolytic factor since it is present in the uterus in considerable quantities (Eglinton, Raphael, Smith, Hall & Pickles, 1963; Pickles, Hall, Best & Smith, 1965). This presumptive specificity remains controversial, however, since prostaglandins are ubiquitous in animal tissues and are found, for example, in particularly high concentrations in seminal fluid which has no rôle in luteolysis (Bergström, 1966). The compounds also have an amazingly wide range of pharmacological actions (Ramwell & Shaw, 1970). Several experiments have shown that $PGF_{2\alpha}$ exhibits a luteolytic effect in the ewe when infused into the ovarian artery (McCracken, Glew & Scaramuzzi, 1970; Goding, Baird, Cumming & McCracken, 1972), or into the uterine vein ipsilateral to the ovary bearing a corpus luteum (Goding *et al.*, 1972). Also in the ewe, prostaglandin $F_{2\alpha}$ has been identified in the plasma

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of the uterine vein (Bland, Horton & Poyser, 1971), in the peripheral blood (Caldwell, Tillson, Brock & Speroff, 1972; Coudert, Phillips, Palmer & Faïman, 1972), in the uterine fluid (Harrison, Heap, Horton & Poyser, 1972) and in the uterine endometrium (Wilson, Cenedella, Butcher & Inskeep, 1972).

If one accepts the phenomenon of a local luteolytic control by $\text{PGF}_{2\alpha}$, how can a substance that has free access to the systemic circulation act in a local manner or at least show a preference for a localized activity? Using a technique of specific cannulation of utero-ovarian vessels, no vascular communication could be shown which would enable direct local transfer of humoral agents from the uterus to the ovary (Coudert, Phillips, Faïman, Chernencki & Palmer, 1974). In the course of these experiments, tritiated prostaglandin $\text{F}_{2\alpha}$ was administered to investigate the possibility of local transfer such as might occur by passive transport, active transport or a counter-current exchange mechanism. The results of these studies are reported below.

MATERIALS AND METHODS

Animals and surgical techniques

The techniques used for specific cannulation of the utero-ovarian vessels and the experimental procedure have been described previously (Coudert *et al.*, 1974). Some of the animals previously described were used in the present study. For clarity, the same number and code for identification of animals as already used will be quoted here.

Radioisotope and analytical methods

Tritium-labelled prostaglandin $\text{F}_{2\alpha}$ ($[9\text{-}^3\text{H}]\text{PGF}_{2\alpha}$, sp.act. = 14 Ci/mmol) was bought from New England Nuclear Corporation, and diluted with ethanol : water (70 : 30). The $[9\text{-}^3\text{H}]\text{PGF}_{2\alpha}$ was made up either in saline or in heparinized blood and was infused at a rate of 0.2 $\mu\text{Ci}/\text{min}$ by means of a constant infusion pump (Harvard Apparatus, model 201). Samples of arterial blood were collected manually at 1-min intervals. A 1-ml aliquot from each sample was added to 10 ml absolute ethanol, mixed thoroughly and centrifuged for 10 min at 2000 rev/min. Two ml of the supernatant were added to 10 ml of Bray's scintillation solution and counted for 10 min in a liquid scintillation spectrophotometer (Nuclear-Chicago, Mark I). The counting efficiency was 20%. In preliminary trials, known amounts of radioactivity were added to 5-ml samples of saline and blood. Three 1-ml aliquots were processed as described above. The overall recovery was 89% (range 87 to 91%) for the saline samples and 94% (range 90 to 96%) for the blood samples. It was noted that acidified saline samples (0.1 ml 1N-HCl in 5.0 ml of saline) yielded a higher recovery (95 to 98%). However, similar acidification of blood resulted in haemolysis and considerable quenching. This procedure was discarded. Duplicate 1-ml aliquots of utero-ovarian venous blood pooled during each infusion period were also processed as above to check for recovery.

EXPERIMENTAL PROTOCOL

A total of fourteen ewes was used in two separate experiments. Specific

cannulation of the utero-ovarian vessels were successful in twelve animals. Two different types of experiments were carried out as follows.

Experiment A

The aim of this experiment was to check the transfer, if any, of tritiated $\text{PGF}_{2\alpha}$ with the ovary left *in situ* with all its connections with the broad ligament, the mesosalpinx and the mesovarium. In addition, low resistance to arterial blood flow was achieved by introducing a K-50 connecting tube to the short

Table 1. Reproductive status and blood flow in ewes under different experimental conditions for administration of [^3H] $\text{PGF}_{2\alpha}$

Experiment	Sheep No. Code	Reproductive status	Blood flow (ml/min)	Method of delivery of radioactivity
A	5 S-1	—	O.A.: 10	Bolus
	6 S-2	—	—	Infusion (blood)
	7 S-3	—	—	Infusion (saline)
	8 S-4	L.O.: no visible CL or follicle R.O.: 1 big CL + 2 big follicles	O.A.: 10 to 11 U.O. vein: 20	Infusion (saline)
	9 S-5	L.O.: 1 big recent CL + 2 follicles R.O.: idem	O.A.: 17 U.O. vein: 30	Infusion (saline)
	10 S-6	L.O.: 1 big recent CL R.O.: no visible CL or follicle	O.A.: 12 U.O. vein: 20	Infusion (blood)
	11 S-7	L.O.: 1 follicle + 1 old CL R.O.: idem	O.A.: 4 U.O. vein: 17	Infusion (blood)
B	12 D-1	—	—	Failure of <i>in-vitro</i> perfusion
	13 D-2	L.O.: small, old CL R.O.: 1 recent CL	O.A.: 12 U.O. vein: 22	Infusion (blood)
	14 D-3	L.O.: 1 old CL R.O.: 1 old CL	O.A.: 7 U.O. vein: 14 to 15	Infusion (blood)
	15 D-4	—	—	Failure: coagulation of artery
	16 D-5	L.O.: 1 big recent CL + 2 small fresh CL R.O.: no visible CL or follicle	O.A.: 7 U.O. vein: 8	Infusion (blood)
	17 D-6	L.O.: 1 big recent CL R.O.: 1 small recent CL	O.A.: 7 U.O. vein: 18	Infusion (blood)
	18 D-7	L.O.: follicles R.O.: idem	—	Infusion (blood)

Experiment A: low resistance to blood flow; ovary left *in situ*. Experiment B: low resistance to blood flow; ovary disconnected.

Abbreviations: O.A., ovarian artery; U.O. vein, utero-ovarian vein; L.O., left ovary; R.O., right ovary; CL, corpus luteum.

catheter. Table 1 shows that adequate blood flow values were obtained in this way. However, it was decided to work under the same conditions and the sampling pump was set at 7 ml/min whenever possible.

Experiment B

This experiment was designed to test the effects of disconnecting the ovary from all surrounding tissues on the transfer of tritiated $\text{PGF}_{2\alpha}$. Collateral drainage is important while the ovary is maintained *in situ* with all its different

attachments. It seemed probable, therefore, that the specificity and efficiency of direct transfer, if any, would be increased if such routes for losses were avoided. Again, the arterial blood flow was set at 7 ml/min whenever possible.

Table 1 summarizes the distribution of animals in the two experiments and gives additional information as to their reproductive status, blood flow and types of infusions.

RESULTS

Neither a bolus injection nor a 40- to 60-min infusion of tritium-labelled prostaglandin $F_{2\alpha}$ into the anterior uterine vein led to the appearance of radioactivity in the ovarian artery under controlled experimental conditions. The results obtained in the two experiments described above were as follows.

Experiment A

Sheep S-1 was injected in preliminary trials with three different boluses of $[9-^3H]PGF_{2\alpha}$ (0.1 μCi , 0.5 μCi and 5 μCi). Blood samples were collected every

TEXT-FIG. 1. Absence of transfer of radioactivity into the ovarian artery of Sheep S-1 after bolus injection or short infusion of $[9-^3H]PGF_{2\alpha}$ into the anterior uterine vein.

10 sec. A 0.5-ml aliquot of ovarian arterial blood was extracted (or 1/2 of the output in 10 sec) and 2 ml supernatant were counted (or 2/11 of the extract), thus the proportion of the 10-sec output counted was $1/2 \times 2/11 = 1/11$. The background activity averaged 20 ct/min. It can be demonstrated that the net minimum detectable activity in a sample should be greater than three times the standard deviation of the background (Chase & Rabinowitz, 1962), i.e. $N_s > 3\sqrt{20}$ or $N_s > 13$ ct/min. This additional 13 ct/min above background represents 1/11 of the ovarian artery output or 134 ct/min or 142 ct/min when

TEXT-FIG. 2. Apparent transfer of radioactivity into the ovarian artery of Sheep S-2 during a 60-min infusion of [^3H]PGF $_{2\alpha}$ into the anterior uterine vein. Kinking of the utero-ovarian tube occurred during this experiment.

corrected for a 94% extraction. Since three different boluses were injected in less than 5 sec and were, respectively: 44,000 ct/min; 220,000 ct/min and 2,200,000 ct/min, the amount injected was, therefore, $44,000/142 = 310$, 1550 and 15,500 times the minimum amount of radioactivity needed to demonstrate a rapid transfer of [^3H]PGF $_{2\alpha}$ from the venous to the arterial compartment. The results obtained from three trials in Sheep S-1, however, show conclusively that no measurable radioactivity above background was detected in the ovarian artery (Text-fig. 1). In the same animal, an infusion of [^3H]

TEXT-FIG. 3. Absence of transfer of radioactivity into the ovarian artery of five ewes (S-3, S-4, S-5, S-6, and S-7) during a 40- to 60-min infusion of $[9\text{-}^3\text{H}]\text{PGF}_{2\alpha}$ into the anterior uterine vein. Contamination of control samples occurred in Sheep S-3 and Sheep S-4. The ovary was left attached *in situ* (Exp. A). The solid circle with vertical lines represents mean and range of ct/min.

$\text{PGF}_{2\alpha}$ at a rate of $0.2 \mu\text{Ci}/\text{min}$ (about 88,000 ct/min for a 20% efficiency) during a short period (5 min) also failed to result in any transfer of radioactivity into the ovarian artery (Text-fig. 1).

Sheep S-2 received an infusion of $[9\text{-}^3\text{H}]\text{PGF}_{2\alpha}$ for 60 min. This infusion apparently resulted in a transfer of radioactivity into the ovarian artery approximately 28 min after starting (Text-fig. 2). The transfer did not, however, present a continuous pattern but exhibited many peaks reading up to 770 ct/min above an average background of 36 ct/min. These data are suspect

however, since it was found late in the course of the experiment that the free drainage of blood from the utero-ovarian vein was impaired by the kinking of the tube inserted into it. It was noted that there was oedema in the tissue surrounding the vascular pedicle, presumably due to increasing pressure effects in the vein.

TEXT-FIG. 4. Absence of transfer of radioactivity into the ovarian artery of five ewes (D-2, D-3, D-5, D-6, and D-7) during a 45- to 60-min infusion of [^3H]PGF $_{2\alpha}$ into the anterior uterine vein. Apparent transfer of radioactivity occurred after clotting in Sheep D-3. The ovary was disconnected from its ligaments (Exp. B). The solid circle with vertical lines represents mean and range of ct/min.

Five other sheep (S-3, S-4, S-5, S-6 and S-7) received an infusion of [^3H]PGF $_{2\alpha}$ under careful conditions to avoid kinking of the utero-ovarian catheter. None of these animals showed any transfer of radioactivity (Text-fig. 3). In Sheep S-3 and S-4, there were unexpectedly high values for blood samples taken as background samples. It was found that this represented a methodological error

due to contamination by the automatic pipetting device from samples of pooled blood containing high amounts of radioactivity, which had been pipetted just before the background samples.

In all the above six animals (S-1, S-3, S-4, S-5, S-6 and S-7), no recirculation of radioactivity was noted since only background counts were detected in samples of blood taken from the aorta at 10-min intervals (Text-figs 1 and 3).

Experiment B

In five sheep with successful cannulation of the vessels (D-2, D-3, D-5, D-6 and D-7; Table 1), infusion of [$9\text{-}^3\text{H}$]PGF $_{2\alpha}$ was carried out for 60 min at the same rate as above. With the exception of the last 15 min of the infusion in Sheep D-3, none of these animals exhibited any transfer of radioactivity in the ovarian artery, since only background counts were detected (Text-fig. 4). Although there is no certain explanation for the discrepancy in Sheep D-3, it may have been due to procedural factors. Clotting occurred in the infusion syringe at about 48 min after the start. This infusion was stopped, more heparin was added with mixing into the syringe and the infusion was resumed. The pattern of infusion during the first 45 min was entirely similar to that observed in the other animals of this experiment.

In Sheep D-1, D-2, D-5, D-6 and D-7, no recirculation of radioactivity was noted since only background counts were detected in samples of blood taken either from the aorta or the jugular vein at 10-min intervals (Text-fig. 4). In Sheep D-3, no recirculation was observed in the jugular vein blood until about 50 min after starting the infusion.

CALCULATION OF THE MINIMUM DETECTABLE TRANSFER OF RADIOACTIVITY DURING THE INFUSION OF [$9\text{-}^3\text{H}$]PGF $_{2\alpha}$

Since 1-ml aliquots of ovarian arterial blood were extracted (or 1/7 of the output) and 2 ml of supernatant were counted (or 2/11 of the ethanolic extract), the proportion of a minute's output which was counted was $1/7 \times 2/11 = 1/38$. The rate of infusion was $0.2 \mu\text{Ci/min}$ or $4.4 \times 10^5 \text{ d/min}$ or $88,000 \text{ ct/min}$ for a 20% counting efficiency. The background averaged 25 ct/min. Using the same convention as previously described, the minimum detectable activity is 15 ct/min above background. This additional 15 ct/min above background represents 1/38 of the ovarian artery output or 570 ct/min in 1 min or 600 ct/min when corrected for a 94% extraction. The minimum transfer of radioactivity which could be detected with our experimental system was, therefore $(600/88,000) \times 100 = 0.7\%$.

DISCUSSION

Previous experiments, including the use of radioactive xenon and radioiodinated human serum albumin (RISA), have demonstrated that there is no direct anatomical connection between the utero-ovarian vessels which would enable a local transfer of uterine humoral agents to the ovarian artery to take place (Coudert *et al.*, 1974). The present study shows conclusively that a tracer dose of the presumptive luteolytic factor, PGF $_{2\alpha}$, injected as a bolus or infused for

40 to 60 min in the form of [$9\text{-}^3\text{H}$]PGF $_{2\alpha}$, did not result in any transport of radioactivity into the ovarian artery of eleven ewes. This confirms the absence of a functional shunt between the utero-ovarian vessels.

The transfer of substances between compartments can occur, however, either as passive processes, such as diffusion, filtration, counter-current exchanges, or as active processes such as active transport. We have previously shown that ^{133}Xe can diffuse to some extent to the ovarian artery. By contrast, $^{131}\text{RISA}$ repeatedly failed to diffuse across the anterior uterine vein of the ovarian artery. This is understandable since $^{131}\text{RISA}$ is a very large molecule. Although [$9\text{-}^3\text{H}$]PGF $_{2\alpha}$ (mol.wt. = 354) is just about two and a half times the weight of ^{133}Xe , our results make it clear that simple diffusion of this substance did not occur.

The process of filtration implies a difference in hydrostatic pressure on the two sides of a porous membrane. Since, however, the venous compartment (anterior uterine vein and utero-ovarian vein) is a system of low pressure as compared to the arterial system (ovarian artery), it follows that filtration cannot occur here.

A counter-current system is a more attractive hypothesis since the ovarian artery runs in general in an opposite direction to that of the anterior uterine vein and utero-ovarian vein. Such a system can only operate as a simple exchanger or as a multiplier (Smith, 1959). Counter-current exchangers are extensively used in engineering and are widespread among various species to conserve body heat (Schollander, 1957). In many mammals, including sheep, the mother and the fetus share some of their blood substances by means of a counter-current exchange system in the placenta (Ramsey, 1960; Assali, 1968), though such an exchange system only enables the transfer of heat, gases and electrolytes to take place. Other molecules such as glucose and amino-acids are coupled with active transport (Assali, 1968).

A counter-current multiplier system requires both a semi-permeable membrane and an 'Einzeleffekt' or primary osmotic pressure difference produced by the hairpin arrangement of the system (Hargitay & Kuhn, 1951). These specific requirements do not exist between the utero-ovarian vessels.

A third possibility would involve coupling a multiplier system with an active transport of PGF $_{2\alpha}$, thus drawing an analogy with the situation for electrolytes in the loop of Henle in the kidney (Wirz, Hargitay & Kuhn, 1951). The utero-ovarian vessels, however, do not fit the hairpin arrangement of the vasa recta and there is no such structure in the ovarian vascular pedicle which resembles the pattern of the loop of Henle and which can be held responsible for the production of a concentration gradient.

Thus, any forms of the counter-current mechanism examined so far do not appear to be applicable to the utero-ovarian circulation. This is well supported by the results of the present experiments in eleven ewes. A major advantage of our technique is to prevent recirculation of radioisotopes by draining the utero-ovarian venous blood out of the abdomen. In this way, any appearance of radioactivity in the ovarian artery cannot be accounted for by contamination due to recirculation but must be due to some local transfer mechanism. Our technique permits the detection of a transfer, if any, in excess of 0.7% of the

infused [$9\text{-}^3\text{H}$]PGF $_{2\alpha}$, yet the data obtained under controlled experimental conditions in eleven ewes leave no doubt that PGF $_{2\alpha}$ does not cross from the anterior uterine vein and the utero-ovarian vein to the ovarian artery. The existence of a major peak of circulating PGF concentrations comparatively early in the luteal phase in sheep (Day 12 after the peak of LH) at a time when progesterone levels remain stable (Coudert *et al.*, 1972), casts additional doubt on the relevance of PGF $_{2\alpha}$ as the physiological luteolysin.

ACKNOWLEDGMENTS

One of us (S.P.C.) is most grateful to Dr A. R. Downs, Head of the Section of Vascular Surgery, Winnipeg General Hospital, for personal help and encouragement and to Dr A. Naimark, Dean of the Faculty of Medicine, for facilities and encouragement. We thank Mrs K. Gordon for preparing radioisotopes. The technical assistance of Mr Ed. Desharnais, Mr B. Dunford, Mr K. Lobchuck, Mr D. Powell, Mr R. Rankin, Mr H. Silverman, Mr E. Stearns and Mr R. Taylor is appreciated. This study was supported in part by the M.R.C. Canada Grant MA-2997, the Manitoba Department of Agriculture and the National Research Council Grant A-6251.

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