Venous–arterial counter-current transfer of [³H]testosterone in the vascular pedicle of the sow ovary

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Summary. [³H]Testosterone or ⁵¹Cr-labelled red blood cells were infused for 30 min through a cannula inserted into the ovarian vein below the hilus of the ovary of cyclic sows. The whole of the vascular pedicle was isolated by ligations from the systemic circulation except for the ovarian artery supplying the ovary. During and after the testosterone infusion, radioactivity was found in ovarian arterial blood and ovarian tissue, but not in peripheral blood. When ⁵¹Cr-labelled blood cells were infused in the same fashion there was no radioactivity in arterial blood or ovarian tissue. These results indicate the existence of a counter-current transfer for testosterone in the sow ovarian vascular pedicle.

Introduction

In the ewe prostaglandin (PG) F-2 α , a luteolytic substance, is believed to pass from the uterine vein to the associated ovarian artery and hence to the ovary (Barrett *et al.*, 1971; Goding, Baird, Cumming & McCracken, 1972; McCracken *et al.*, 1972; Del Campo & Ginther, 1973). The experimental results of Caldwell & Moor (1971), Ginther & Bisgard (1972), Goding (1974), Hixon & Hansel (1974) and Land, Baird & Scaramuzzi (1976) have supported this suggestion and similar evidence of utero-ovarian local transfer of prostaglandin in the pig has been given by Gleeson (1974), Lindloff, Holtz, Elsaesser, Kreikenbaum & Smidt (1976), Krzymowski, Kotwica, Okrasa, Doboszyńska & Zięcik (1978) and Kotwica (1980).

Study of the local transfer of inert radioactive gases (Einer-Jensen, 1974; Einer-Jensen & McCracken, 1977; Bendz, Einer-Jensen, Lundgren & Janson, 1979) has shown that xenon-133 or krypton-85 infused into the uterine horn of the mouse, hamster and guinea-pig or into the utero-ovarian vein of sheep or women reached higher concentrations in the adjacent than in the opposite ovary. A counter-current transfer of hormone in the ovarian pedicle has been described for progesterone in sheep (McCracken & Einer-Jensen, 1976; Walsh, Yutzenka & Davis, 1979), and for testosterone for sows (Krzymowski, Kotwica & Stefańczyk, 1979) and a local steroid-concentrating mechanism in the female reproductive vasculature seems likely. In the present study, we have examined the transfer of testosterone from the ovarian vein to the ovarian artery in the sow in more detail.

Materials and Methods

Animals and surgical preparation

The 13 gilts of the Polish Large White breed, weighing 150–200 kg, were allowed to farrow and nurse their litters for 28 days before weaning. Oestrus was observed 6–7 days after

weaning and was designated Day 0. On different days of the oestrous cycle laparotomies were performed under general anaesthesia induced by i.v. administration of pentobarbitone sodium (0.2 ml/kg body weight) and maintained with a mixture of ether and oxygen in a closed circuit with soda lime included to remove CO₂.

To isolate the ovary and its vascular pedicle about 10 ligatures were placed between the ovary and oviduct and through the broad ligament of the uterus along the ovarian and utero-ovarian vein (Text-fig. 1). The ligatures ensured complete isolation of the ovarian pedicle and ovary circulation from the rest of the blood vasculature in the broad ligament. The ovarian artery supplying the ovary and the ovarian nerve remained intact. If there was radioactivity detected in blood taken from the jugular vein during infusion of [³H]testosterone or ⁵¹Cr-labelled red blood cells the animal was eliminated from the experiment and the results were not included.

Text-fig. 1. Illustration of the experimental design to study the counter-current transfer of [³H]testosterone in the ovarian vascular pedicle of the sow. [³H]Testosterone was infused into the ovarian vein through Cannula 2. Blood samples from the incised branch of the ovarian artery or from an incised corpus luteum were collected.

The uterine vein was ligated at a distance of 15–20 cm from the ovary without interfering with the ovarian artery and nerve. The utero-ovarian vein was transected near its junction with the external iliac vein and Cannula 1 was inserted to divert blood flowing from the ovary for extracorporeal collection of samples.

In order not to damage the wall of the ovarian vein, Cannula 2 (0.2 mm i.d.) with a temporary steel-wire stiffener was inserted into the ovarian vein through the wall of Cannula 1 and through the utero-ovarian vein. The end of this cannula was placed in the ovarian vein about 3–5 cm below the ovary hilus. Its peripheral end after removing the steel-wire stiffener was connected to the infusion pump through which the [^3H]testosterone or ^{51}Cr -labelled red blood cells were infused into the ovarian vein. Throughout the experiment the ovary was suspended 5–10 cm above the abdominal cavity, but the flow of blood from the ovary through Cannula 1 into the glass bottle placed below the surgery table was quite free. Heparin (5000 i.u.) was injected i.v. before the infusion to prevent clotting. All the sows had had 2 or 3 litters and the broad ligament was very flexible, allowing this degree of withdrawal of the tissues without traction.

Infusion of [^3H]testosterone

[1,2,6,7- ^3H]Testosterone (sp. act. 81 Ci/mmol: Radiochemical Centre, Amersham, England) was purified on thin-layer chromatography plates (Merck) by elution with benzene:acetone (6:1 v/v) before the infusion. Samples of the purified testosterone (100 μCi , 1×10^8 c.p.m. = 332 ng testosterone) were evaporated to dryness under N_2 and dissolved in 3 ml saline (0.154 M-NaCl) containing heparin (1500 i.u.) 5 min before the infusion. The isotope was infused into the ovarian vein through Cannula 2 (Text-fig. 1) for 30 min at a rate of 0.1 ml/min.

Infusion with ^{51}Cr -labelled red blood cells

In 5 experiments at 20 min after the testosterone infusion and in the same conditions without changing the place of the ovary and its vascular pedicle, a 3 ml suspension of autologous red blood cells labelled with ^{51}Cr in saline (1:1 v/v) was infused. The erythrocytes were labelled with ^{51}Cr by the method of Grey & Starling (1950) using $\text{Na}_2^{51}\text{CrO}_4$ (sp. act. 176 mCi/mg Cr) produced at the Atomic Energy Institute in Swierk (Poland). The suspension of total radioactivity 18×10^5 c.p.m./ml was infused into the ovarian vein through Cannula 2 exactly as described above for the [^3H]testosterone infusion. Blood samples were collected from the incised corpora lutea (Sows 1, 6, 7) or from the ovarian artery branch (Sows 11, 12) used earlier for blood sample collection during the testosterone infusion.

Blood sample collection

Before the infusion of [^3H]testosterone blood samples were taken from the ovarian vein to measure the endogenous testosterone concentrations. During and 10 min after the end of infusion blood samples were taken from the jugular vein to check whether there was any radioactivity in the systemic blood. If there was a superficial loop of an ovarian artery branch on the ovarian vein surface, 2–3 cm below the ovary, it was incised under $\times 3$ magnification to avoid damage to any other vessels. The blood was collected for not more than 5 min, by a small pipette or immediately into a vacuum tube. If there was no suitable superficial vessel, the corpus luteum of the ipsilateral ovary was incised and the blood samples were collected as above. In 5 experiments blood samples were collected from incised corpora lutea of the ipsilateral ovary and from the contralateral ovary.

The collected samples were stored at 4°C and at the end of the experiment the plasma was separated after centrifuging (3000 g for 10 min) and stored at -20°C before assay. Immediately after the blood sample collections, the ovaries were excised and stored at -20°C .

Estimation of radioactivity

The blood samples were centrifuged and 0.5 ml plasma was mixed with 8 ml ethyl ether and shaken. The extract was collected in scintillation vials, evaporated to dryness under N₂ and dissolved in scintillation fluid for assessment by liquid scintillation counting (LS-100C Beckman).

The radioactivity in the ovary after the testosterone infusion was also measured in 5 experiments. Both ovaries were excised 20 min after the infusion, homogenized in liquid nitrogen and the [³H]testosterone was extracted by the method described by Shemesh & Hansel (1976). The recovery of [³H]testosterone after blood and ovary extraction was 87% and 80% respectively. Subsequent results were not corrected for extraction losses. The amount of ⁵¹Cr in plasma and ovarian tissue was measured after the control infusion in a gamma scintillation counter (Polon, ZNN 44, Poland).

Assay of endogenous androgen (testosterone and 5 α -dihydrotestosterone)

A radioimmunoassay method without chromatography was used. The antibody (provided by Professor R. Rembiesa, Institute of Pharmacology, Polish Academy of Science, Krakow) was produced in rabbits against testosterone-3-CMO-BSA. This antibody showed cross-reactivity with 5 α -dihydrotestosterone (86%), 5 α -androstane-3 α ,17 β -diol (7.1%), androstenedione (2.9%), androst-5-ene-3 β ,17 β -diol (1.4%), epitestosterone (0.7%), 5 α -androstane-3,17-dione (0.6%) and androsterone, dehydroepiandrosterone, progesterone, pregnenolone, corticosterone, oestradiol-17 β and hydrocortisone (<0.01%). The recovery of radioactive testosterone was 89.68 $\pm$ 3.75%. The sensitivity of the assay was 2 pg/ml. The coefficient of variation was < 1.84% within assays and < 4.98% between assays.

Results

In all the experiments with [³H]testosterone infused into the ovarian vein radioactivity was found in the arterial blood flowing into the ipsilateral ovary, i.e. in blood from incised corpora lutea (Table 1) and in blood collected from an incised branch of the ovarian artery near the ovarian hilus (Table 2). There were large differences in the values obtained but there was no obvious correlation with the number of corpora lutea or day of the oestrous cycle. Although radioactivity

Table 1. Radioactivity in blood samples collected from incised corpora lutea of the ipsilateral ovary during infusion of [³H]-testosterone or ⁵¹Cr-labelled red blood cells into the ovarian vein of sows

Animal	Day of oestrous cycle	Radioactivity in the blood plasma (c.p.m./ml)	
		During testosterone infusion*	During RBC infusion
1	13	1100 $\pm$ 584	0.0
2	15	1490 $\pm$ 1835	—
3	17	340 $\pm$ 196	—
4	17	980 $\pm$ 115	—
5	17	900 $\pm$ 598	—
6	18	118 $\pm$ 57	0.0
7	19	400 $\pm$ 375	0.0

* Values are mean $\pm$ s.e.m. for 4–9 samples.

was present in all the ovarian blood samples none was detected in the systemic blood samples taken every 5 min from the jugular vein during and after the infusion. In the 5 experiments (Sows 3, 4, 5, 6, 7) in which blood samples were collected from the incised corpora lutea of the contralateral ovary, no radioactivity was found. Large fluctuations in the blood flowing through the ovary vessels were observed during testosterone infusion. The mean $\pm$ s.e.m. flow was 13.1 ± 4.1 ml/min from the ovarian vein but spontaneous dilatation or constriction of the ovary vessels appeared every few minutes, causing irregular blood flow from the ovarian vein and the incised branch of the ovarian artery.

Radioactivity was detected in the ovarian arterial blood samples 1–2 min after the start of infusion and was still present 5 and 10 min after the end of infusion although the values were generally lower than during the infusion (Table 2).

Table 2. Radioactivity in blood samples collected from a branch of the ipsilateral ovarian artery during and after infusion with [^3H]testosterone or ^{51}Cr -labelled red blood cells into the ovarian vein of sows

Radioactivity in the blood plasma (c.p.m./ml)					
Testosterone infusion					
Animal	Day of oestrous cycle	During*	After		During RBC infusion
			5 min	10 min	
11	3	87 862 ± 57 983	896	1 135	0.0
12	13	28 933 ± 314	30 000	25 548	0.0
13	13	111 ± 74	0	0	—
14	14	5 845 ± 349	4 700	3 780	—
15	18	53 390 ± 53 972	7 512	4 000	—
16	18	16 444 ± 18 626	5 000	1 000	—

* Values are mean $\pm$ s.e.m. for 6–11 samples.

The radioactivity in the ovary 20 min after the testosterone infusion stopped was 9253 ± 5604 (s.e.m.) c.p.m./g ipsilateral ovary tissue. There was no detectable radioactivity in the contralateral ovary tissue.

In all infusions with the ^{51}Cr -labelled red blood cells, no radioactivity was determined in the blood samples taken from the incised corpora lutea (2 expts) or from an incised branch of the ovarian artery (3 expts).

The endogenous androgen (testosterone and 5α -dihydrotestosterone) concentration in the blood plasma from the ovarian vein was 0.885 ± 0.09 (s.e.m.) ng/ml ($N = 13$).

Discussion

These results indicate that [^3H]testosterone was transferred from the ovarian vein to the ovarian artery. The mechanism of this pathway is unknown. Bendz *et al.* (1979) have suggested that the transfer of krypton-85 in women is based on passive diffusion aided by the close relationship between the ovary vein and the tortuous artery. In sows, extensive surface contact between the ovarian vein and artery would aid direct passage of testosterone, but we suggest that more than direct diffusion is involved because although the radioactivity in arterial blood decreased, it continued to appear in the ovarian artery 10 min after the infusion was stopped. During the 30 min infusion of 1×10^8 c.p.m. [^3H]testosterone into the ovarian vein and the next 10 min, only 47% of the radioactivity was recovered from the ovary and the ovarian artery vessel. The rest of the radioactivity remained in the ovarian pedicle tissue, perhaps the interstitial (intercellular) fluid, thus acting as a component of the veno-arterial pathway. The hormone concentration in

the venous blood plasma could influence the mechanism of the testosterone transfer. Free & Yaffe (1975) demonstrated that the concentration of the testosterone and the flow of the blood in the testicular vein of the rat were the major factors influencing testosterone transfer in the pampiniform plexus. In our experiments the infusion of tritiated testosterone (11.06 ng/min) resulted in an increase of testosterone concentration in the ovarian vein. The blood flow through the ovarian vein was estimated to be 13.1 ± 4.7 ml/min and the testosterone concentration in the ovarian vein rose from 0.885 to 2.75 ng/ml. Such an increase could probably affect the rate of the counter-current transfer but is unlikely to have altered the transfer characteristics or the transfer mechanism.

The possibility of minute vascular channels between the ovarian vein and ovarian artery has been considered, but such structures were not found in sheep by a histological study of the utero-ovarian vascular pedicle (Del Campo & Ginther, 1974) or by Coudert, Phillips, Faiman, Chernecki & Palmer (1974) and McCracken & Einer-Jensen (1976) in experiments with ^{131}I -labelled serum albumin. The failure of ^{51}Cr to appear in the blood samples taken from the incised ipsilateral corpus luteum or ovarian artery branch in our experiments also indicates the lack of anastomoses and vascular communications between the ovarian vein and ovarian artery. These results also confirmed that incision of the ovarian artery branch did not damage the wall of the ovarian vein.

The counter-current transfer of $[^3\text{H}]$ testosterone was observed on different days of the oestrous cycle. The testosterone concentration in the jugular vein of sows varies with the oestrous cycle, reaching the highest level before or during oestrus (Krzymowski, Krzymowska & Nowicka, 1979). There are also differences in the testosterone content of the follicular fluid of sows on different days of the oestrous cycle (6.99–25.98 ng/ml) (Krzymowski, Stefańczyk, Krzymowska, Lipski & Olejniczak, 1979). The individual large differences in the amounts of testosterone transferred into the ovarian artery blood may be related to the day of the oestrous cycle, but they are more likely to be due to anatomical differences between animals and to the observed irregularities of blood flow.

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