Steroid transfer from the ovarian vein to the ovarian artery in the sow

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Summary. The ovary and its vascular pedicle were isolated and transferred under a stereomicroscope to a heated surface (40°C). The ovary was supplied with the blood from the middle uterine artery through a cannula. On different days of the oestrous cycle [³H]testosterone, [³H]progesterone or [³H]oestradiol-17β were infused for 30 min into the ovarian vein 1–2 cm below the ovary. During and 30 min after the infusion radioactivity was found in ovarian arterial blood and ovarian tissue. Recovery of [³H]testosterone, [³H]oestradiol and [³H]progesterone infused into the ovarian vein from ovarian venous blood collected during 60 min of experiment was (mean ± s.e.m.) 26.8 ± 6.8%, 20.1 ± 6.1% and 23.7 ± 6.2% respectively. When ⁵¹Cr-labelled blood cells were infused in the same fashion as the steroids there was no radioactivity in arterial blood or ovarian tissue. These results indicate the existence of a countercurrent transfer mechanism and retention of the hormones in tissue(s) other than blood in the ovarian pedicle.

Introduction

We have previously demonstrated the existence of a countercurrent mechanism for testosterone transfer in the ovarian vascular pedicle of the sow (Krzymowski, Kotwica & Stefańczyk, 1979, 1981b). Individual differences were found in the amounts of [³H]testosterone which were being transferred from the ovarian vein to the ovarian artery and we suggested that it was the influence of dilatation or constriction of the ovarian artery during the experiments that caused the irregular flow of blood through the ovarian vascular pedicle. To eliminate these factors we have now studied the isolated ovary and its ovarian pedicle together with a constant blood pressure and constant blood flow rate through the ovarian vasculature. Venous–arterial countercurrent exchange of testosterone, oestradiol and progesterone occurs in the ovarian vascular pedicle of the sow (Krzymowski, Kotwica, Stefańczyk, Dębek & Czarnocki, 1981a). In the present study, we have examined the transfer of these steroids from the ovarian vein to the ovarian artery in more detail.

Materials and Methods

Animals

Pregnant gilts of the Polish Large White breed weighing 120–150 kg farrowed and were allowed to nurse their litters for 28 days before weaning. Oestrus was observed 6–7 days after weaning and was designated Day 0.

Surgery procedure

On Days 5, 10, 12, 13, 14, 17, 18, 19 or 20 of the oestrous cycle, laparotomies were performed under general anaesthesia induced by i.v. administration of pentobarbitone sodium (12 mg/kg body weight) and maintained with a mixture of ether and oxygen in a closed-circuit with soda lime included to remove CO₂. The ovary and its associated vasculature in the pedicle were isolated by many double ligatures. Just before transection of the ovarian preparation 10⁴ i.u. heparin were injected intravenously.

The isolated ovary and the ovarian pedicle were transferred onto a wire net and then placed just over the surface of a water bath (40°C) under a stereomicroscope ($\times 12.5$ magnification). The tissues were covered by a layer of cotton saturated with saline (9 g NaCl/l) at 40°C. The temperature of the preparation was regulated at a physiological level by electric lamps. Throughout the experiment there was no change in the normal colour, volume or consistency of the ovary and its pedicle.

The cut ovarian artery was connected with the middle uterine artery by a polyethylene cannula. This cannula was passed through a peristaltic miniflow pump (type 304 ELMED; Unipan, Poland) and a water bath (40°C). In this way it was possible to establish the constant flow rate of the blood supplying the ovarian artery. The blood flow rate was 7.36 ± 0.43 (s.e.m.) ml/min ($n = 28$). The blood pressure in the ovarian artery was 80–100 mmHg (10.64–13.3 kPa). Heparin was infused continuously at 50 i.u./min into the blood flowing through the cannula to prevent clotting. Just before the cannula was inserted the oviduct was ligated and cut off. Cannula 1 (5 mm i.d.) was inserted into the incised utero-ovarian vein and the blood flowing out of this vein was collected in a vessel.

Steroid infusions

Cannula 2 (0.2 mm i.d.) supplied with a steel-wire stiffener, was inserted into the ovarian vein through the wall of Cannula 1 which had been inserted earlier into the utero-ovarian vein.

The end of Cannula 2 was placed in the ovarian vein about 1–2 cm below the ovary hilus and the other end, after removing the steel-wire stiffener, was connected to an infusion pump through which one of the tritiated steroid hormones was infused. [1,2,6,7-³H]Testosterone (sp. act. 81 Ci/mmol), [1,2,6,7-³H]progesterone (sp. act. 110 Ci/mmol) and [2,4,6,7-³H]oestradiol-17 β (sp. act. 92 Ci/mmol) were purchased from the Radiochemical Centre (Amersham, Bucks, U.K.) and 1 day before the study were purified on thin-layer chromatography plates (Merck) in a benzene:acetone (6:1, v/v) system. Five minutes before the hormone infusion into the ovarian vein, 5×10^7 c.p.m. of steroid were dissolved in 3 ml saline (9 g NaCl/l). The amounts of testosterone, progesterone or oestradiol used, 156, 129 and 136 ng respectively, were infused for 30 min at a rate of 0.1 ml solution/min.

Control infusions with ⁵¹Cr-labelled red blood cells

In 3 experiments, at 30 min after the hormone infusion and in the same conditions without changing the place of the ovary and its vascular pedicle, 3 ml autologous red blood cells labelled with ⁵¹Cr and suspended in saline (1:1 v/v) were infused. Erythrocytes were labelled with ⁵¹Cr by the method of Grey & Starling (1950) using Na₂⁵¹CrO₄ (sp. act. 176 mCi/mg Cr: Atomic Energy Institute, Swierk, Poland). The labelled red blood cells ($\sim 2 \times 10^6$ c.p.m.) were infused into the ovarian vein through Cannula 2 exactly as were the steroid hormones.

Sample collection

On the dorsal surface of the ovarian pedicle, 2–3 cm below the ovary, a thin layer of muscles was cut 1 cm long with an electric knife under stereomicroscope control. After careful unveiling

of the surface arterial network lying on the ovarian vein, blood samples were collected under stereomicroscope control as follows. A branch of the ovarian artery was picked up by a ligature above the surface of the arterial network. A small gap was made in the wall by a microelectric coagulator. The stream of blood from the gap was immediately collected by a thin elastic cannula. The end of the cannula was brought near the surface of the gap and blood leaking from the incised ovarian artery branch was aspirated into the cannula by a tube connected to a vacuum. The blood was sampled in this way during the infusion (30 min) and for 30 min afterwards. Depending on the amount of blood flowing out, different quantities of samples were collected. To present the results of radioactivity measurements during and after infusion of ^3H -labelled steroids, the mean value from all the samples was calculated for every 10 min. The collected samples were stored at 4°C and at the end of the experiment the plasma was separated by centrifugation (3000 g for 10 min) and stored at -20°C until assay. Immediately after the blood sample collections the ovaries were excised and stored at -20°C .

Estimation of the radioactivity

Steroids were extracted from the plasma by shaking 0.5 ml plasma with 8 ml distilled diethyl ether. The ovary was homogenized (14 000 r.p.m., 1 min; Type 302 homogenizer, Unipan, Poland) and extracted by the method described by Shemesh & Hansel (1976). One half of the ovarian or plasma extract was transferred to the scintillation vials, evaporated under a stream of nitrogen to dryness and dissolved in dioxane scintillation fluid. The recovery in the control samples was $88 \pm 1.2\%$ ($n = 12$) for plasma and $69 \pm 0.6\%$ ($n = 9$) for tissue. The results were not corrected for extraction losses but they were corrected for background counts. The other half of the ovarian or plasma extract was placed on thin-layer chromatography plates which were developed three times in a mixture of ethyl acetate:cyclohexane (1:1 v/v) and once in a mixture of chloroform:ethanol (95:5 v/v) at a temperature of $17 \pm 1^\circ\text{C}$. The radioactivity of the ^3H -labelled steroids was determined in a liquid scintillation counter (LS-100C Beckman) with an efficiency of counting of 52% for tritium. Radioactivity of ^{51}Cr was measured in a gamma scintillation counter (Polon, ZNN 44, Poland). For the gamma radiation measurement the samples were prepared by mixing 0.5 ml whole blood and 0.5 ml distilled water.

Results

In 28 experiments in which tritium-labelled testosterone ($n = 12$), oestradiol- 17β ($n = 8$) or progesterone ($n = 8$) was infused into the ovarian vein below the ovarian hilus, there was increased radioactivity in the arterial blood collected from the incised ovarian artery (Table 1). Differences amongst some of the samples were large and there was no obvious association with the days of the oestrous cycle. However, radioactivity was present in all samples taken in the 28 experiments. In the 3 experiments in which chromium-labelled autologous red blood cells were infused into the ovarian vein (Exps 5, 6 and 11) no radioactivity could be determined in the blood samples taken from the same ovarian artery branch.

The results in Table 1 give mean values for each of the 10-min periods. During each period one or more blood samples with a volume of ≥ 1 ml were collected. After prolonged constriction of an incised artery branch it was sometimes impossible to collect 1 ml of blood during a 10-min period. In such experiments the period of blood sampling was extended to the next 10 min interval and the results given for the whole period of 20 min (Table 1). The radioactivity in the blood plasma samples collected from the incised branch of the ovarian artery was high even 30 min after the infusion was stopped. In relation to the value of the last sample during the infusion (30 min) the radioactivity in the last sample of the experiment (i.e. 60 min after start of infusion)

Table 1. Radioactivity (c.p.m./ml corrected for background counts) in the plasma of blood samples collected from an incised branch of the ovarian artery during and after infusion of ^3H -labelled steroids into the ovarian vein of sows

Exp.	Day of oestrous cycle	During infusion of 1.66 × 10 ⁶ c.p.m./min			After infusion		
		10 min	20 min	30 min	10 min	20 min	30 min
^[3H] Testosterone							
1	10	550	3716	1650	4838	1843	920
2	10	134 340	78 000	78 000	30 050	9400	6820
3	10		9233	7111	3479	2508	1158
4	12	105	98	132	112	130	86
5	12	104	130	147	162	152	163
6	13	87	58	61	64	50	30
7	13	182	197	263	282	215	256
8	13		408	2176	400	545	508
9	13	1062	1062	1000	596	434	256
10	17		306	238	252	203	239
11	20	4375	3193	4139	3750	4350	1317
12	20	464	145 214	151 406	8200	5164	3910
^[3H] Progesterone							
13	5	40 000		36 800		4300	1360
14	10	264		3641	1440	—	—
15	12	75	63	74	—	—	—
16	13	304	260	200	620	1500	1593
17	17	130	191	743	432	342	358
18	18	11 700	11 300	3540	1800	1094	680
19	18	1260	622	731	669	494	480
20	20	212	238	235		190	178
^[3H] Oestradiol-17β							
21	10	3600	81 600	48 069		12 390	6378
22	10	900	33 210	15 600	7000	3350	2800
23	13	178	378	2194		266	194
24	14	473	458	604	447	456	447
25	14	1000	3695	2930	3633	1605	4767
26	19	2249	2867	6910	6410	2435	9377
27	20	208	145	134	—	—	—
28	20	636	99 550		2300	3860	1035

decreased in 20 experiments, increased in 4 and did not change in 1 experiment. The decrease was by 80–90% in 9 experiments, 50–80% in 5; 20–50% in 5 and <20% in 1.

The radioactivity in the ovary tissue after infusion of the testosterone ($n = 6$), progesterone ($n = 5$) and oestradiol-17 β ($n = 6$) was (mean $\pm$ s.e.m.) 408 ± 111 , 1942 ± 1538 and $14\,892 \pm$

Table 2. Recovery of steroid hormones infused into the ovarian vein of the sow from ovarian venous blood collected during 60 min and from the ovary

Hormone	Total amount of tritiated hormone infused into ovarian vein over 30 min (ng)	Recovery from blood collected during 60 min (%)	Recovery from ovary after end of exp.
Testosterone	156	26.81 ± 6.77	0.006 ± 0.002
Oestradiol	129	20.08 ± 6.10	0.23 ± 0.21
Progesterone	136	23.69 ± 6.23	0.26 ± 0.33

Values are mean $\pm$ s.e.m.

9552 c.p.m./g tissue respectively. The percentage of the steroid hormones recovered from the ovary and from the total ovarian venous blood collected during experiment is shown in Table 2.

Thin-layer chromatography analysis indicated that 64–69% of the radioactivity in the ovary and blood plasma extracts corresponded to the tritiated steroid hormone infused into the ovarian vein. This indicates that most of the radioactivity present in the arterial blood samples or in the ovarian tissue corresponds to the infused hormone. Possible metabolites of the infused hormones were not investigated.

Discussion

The present experimental system was an improvement over that used by Krzymowski *et al.* (1981b), because site of blood collection could be controlled much more precisely. Use of an electrocoagulator to incise the muscle covering the arterial network also prevented any bleeding before the incision of the ovarian artery branch. The failure to recover radioactivity in the samples of arterial blood taken from an incised branch of the ovarian artery after infusion of ^{51}Cr -labelled red blood cells into the ovarian vein (3 experiments) proved that there was no contamination by venous blood of the arterial blood collected as described. Vascular connections between the ovarian vein and ovarian artery in the ovarian vascular pedicle have not been demonstrated by studies with ^{51}Cr -labelled erythrocytes (Krzymowski *et al.*, 1981b) or ^{131}I -labelled serum albumin (Coudert, Phillips, Faiman, Chernecki & Palmer, 1974; McCracken & Einer-Jensen, 1976) or by histology (Del Campo & Ginther, 1974).

There were considerable differences in the amount of steroid hormone transferred in the various experiments, even when the sows were at the same stage of the oestrous cycle (Table 1). This variation could be due to the course of the incised ovarian artery branch and the distance between the site of incision and the ovarian vein. Other factors which could influence the radioactivity level in the ovarian artery would be the reaction of the artery when incised and constriction of ovarian artery branches during blood sampling. Although a constant blood flow rate was maintained through the ovarian artery and ovary, different branches of the ovarian artery constricted to various degrees during the experiment. Although no correlations with different days of the oestrous cycle could be shown, the high levels of radioactivity in the ovarian arterial blood 30 min after the end of the infusion stopped (Table 1) confirm our previous suggestion (Krzymowski *et al.*, 1981b) that the ovarian pedicle contains a compartment, perhaps the interstitial fluid, which may act as a depot.

The hormone concentration in the ovarian blood plasma may be a factor influencing the countercurrent transfer. Free & Jaffe (1975) demonstrated that the concentration of testosterone and the flow of blood in the testicular vein of rats were the major factors influencing testosterone transfer in the pampiniform plexus. However, the very small increase (8.5%) of progesterone concentration in the ovarian vein (Kotwica *et al.*, 1981) and the marked progesterone transfer from the ovarian vein into the ovarian artery (Table 1) indicate that concentration of the steroid hormones was not the major factor which prompted the countercurrent transfer in the ovarian pedicle.

The recovery of infused ^3H -labelled steroids from the ovarian venous effluent collected during the whole experiment (60 min) was only 20.08–26.81% (mean), indicating that at least 75–80% of infused steroids diffused from the ovarian vein blood and were retained mainly in the ovarian pedicle. The recovery of the steroid hormones in these in-vitro type experiments was lower than that recorded with the ovaries *in situ* (47%) (Krzymowski *et al.*, 1981a). However, in the present experiments the blood flow rate through the ovarian vein was nearly 2-fold lower (7.36 ml/min instead of 13.1 ml/min) while the sows weighed about 25% less and, moreover, the temperature conditions were not regulated quite so precisely. However, the lower blood flow rate ensured a blood pressure in the ovarian artery at a physiological value of not higher than 80–100

mmHg. Our results for countercurrent transfer of all three steroid hormones in the ovarian vascular pedicle of the sow are similar to those for experiments with male rats (Free, Jaffe, Jain & Gomes, 1973) and rams (Ginther, Mapletoft, Zimmerman, Meckley & Nuti, 1974). When testosterone was infused into the testicular vein testosterone continued to appear in the testicular artery for 30 min after infusion had been stopped (Free *et al.*, 1973). A basically similar mechanism of transfer from veins to arteries could occur in males and females.

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