Relationship between blood flow and steroidogenesis in the rabbit corpus luteum*

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Summary. Blood flow in the corpus luteum of the pseudopregnant rabbit was measured with tracer-labelled microspheres before and at 1 and 3 h after saline treatment ($N = 8$) or after inhibition of progesterone synthesis with aminoglutethimide ($N = 10$). Before treatment luteal blood flow (29.5 ± 3.9 ml/min·g⁻¹ (mean $\pm$ s.e.m.)) was much higher than blood flow to other tissues (ovarian stroma = 2.9 ± 0.6 ; uterus = 0.5 ± 0.1 ; adrenal gland = 2.6 ± 0.2 ml/min·g⁻¹). Aminoglutethimide reduced serum progesterone by 60% within 1 h but luteal blood flow was unchanged (26.2 ± 3.5 ml/min·g⁻¹). At 3 h after aminoglutethimide, serum progesterone remained low and luteal blood flow was slightly reduced to 22.5 ± 3.4 ml/min·g⁻¹. This reduction was associated with a significant decline in mean arterial blood pressure which resulted in luteal vascular resistance being unaltered by aminoglutethimide treatment. Further analysis of these data indicated that serum progesterone concentration was not significantly correlated with blood flow to the corpora lutea or with blood flow to other tissues. In contrast, mean arterial blood pressure was highly correlated with blood flow to the corpus luteum ($r = 0.80$; $P < 0.001$) but not to the ovarian stroma ($r = 0.04$), or adrenal gland ($r = 0.06$). These results indicate that luteal blood flow is not acutely responsive to changes in luteal progesterone production and suggest that luteal blood flow changes passively with changes in arterial blood pressure.

Keywords: corpus luteum; ovary; blood flow; progesterone; aminoglutethimide; rabbit

Introduction

The mammalian corpus luteum has one of the highest rates of blood flow of any tissue or organ (Abdul-Karim & Bruce, 1973; Janson & Albrecht, 1975; Devoto *et al.*, 1977; Ford *et al.*, 1982), but the physiological regulation of luteal blood flow is not well understood. Because the corpus luteum produces progesterone, the hypothesis has developed that progesterone, at high concentrations, might be responsible for maintaining low vascular resistance in the corpus luteum (Ellinwood *et al.*, 1978). Serum progesterone is positively correlated to the rate of blood flow in the ovary containing luteal tissue (Niswender *et al.*, 1975; Ford *et al.*, 1979; Ford & Chenault, 1981; Janson *et al.*, 1981; Magness *et al.*, 1983), and progesterone treatment increases ovarian blood flow in pregnant rats (Waddell *et al.*, 1982). These observations are consistent with the hypothesis that progesterone leads to dilatation of the ovarian and perhaps the luteal vasculature. To test this hypothesis directly, we determined the acute effect of inhibiting progesterone synthesis on blood flow and

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vascular resistance in the corpus luteum, while simultaneously maintaining the serum concentration of the luteotrophic hormone, oestradiol-17 β (Keyes & Nalbandov, 1967; Bill & Keyes, 1983).

Materials and Methods

Animals and treatments. Virgin New Zealand White rabbits (Shankin Farms, MI; 2.8–3.4 kg) were individually caged in a room with 14 h of light per day, at $\sim 25^{\circ}\text{C}$. They were fed a daily ration of Purina rabbit chow and water was available *ad libitum*. All animals were injected with 40 or 50 i.u. PMSG (Sigma Chemical Co., St Louis, MO; G 4877) to ensure follicular development and 2 days later ovulation was induced by injection of 40 i.u. hCG (Sigma; CG-B). The day of injection of hCG was designated as Day 0 of pseudopregnancy. On Day 1 an oestradiol-filled Silastic implant (made previously in our laboratory) was inserted subcutaneously; the preparation and characteristics of these implants (3.35 mm i.d., 12 mm filled length) have been previously described (Holt *et al.*, 1975).

Radioimmunoassay. Serum progesterone concentration was measured by radioimmunoassay using a procedure developed by Niswender (1973), and modified for measurement in rabbit serum (Holt *et al.*, 1975). After addition of [^3H]progesterone, serum was extracted with petroleum ether (mean progesterone recovery $\pm$ s.d. = $78.7 \pm 9.9\%$). The 50% binding point was 156 pg and the intra- and interassay variability were 6 and 14% respectively.

Blood flow determination. On Day 9 of pseudopregnancy, all animals were anaesthetized by an intravenous injection of urethane (Sigma; U 2500, 1.5 g/kg). Lidocaine was also administered intradermally at sites of incisions. For injection of microspheres, a catheter (Intramedic, PE-90; Clay Adams, Becton Dickinson & Co., Parsippany, NJ) was inserted through the left carotid artery into the left ventricle. Correct placement of this catheter was verified by monitoring the characteristic waveform of the left ventricular pressure before and after microsphere injections. Another catheter was inserted through the right femoral artery into the abdominal aorta and this was used for the withdrawal of the reference sample and for monitoring of arterial blood pressure (with a Satham P23 Db transducer). Blood pressure was measured both before and immediately after the blood flow measurement but was not monitored during the 2-min period required to perform the microsphere injection.

Blood flow was measured with microspheres of $15 \pm 3 \mu\text{m}$ diameter, labelled with cerium-141, ruthenium-103, or scandium-46 (New England Nuclear, Boston, MA), using the reference withdrawal technique (Buckberg *et al.*, 1971; Bartrum *et al.*, 1974; Heymann *et al.*, 1977). Each batch of microspheres was mixed by ultrasonication for at least 30 min followed by vortex agitation for another 30 min immediately before injection. Before injection of microspheres, withdrawal of the reference blood sample was started via the catheter in the right femoral artery with a Sage withdrawal pump (model 351), at a continuous rate of 2.0 ml/min. Approximately 300 000 microspheres in 3 ml warmed saline (0.9% (w/v) NaCl) were infused into the left ventricle over 15 sec. The syringe and infusion catheter were then flushed twice with 3 ml warm saline to remove residual microspheres. The withdrawal of the reference sample was continued for 1 min after the first saline flush and this reference blood sample was dried in scintillation vials in a desiccator oven at 65°C . Second and third blood flow measurements were performed as described above utilizing microspheres labelled with a different radioisotope for each measurement.

At the end of the experiment, animals were killed by intraventricular injection of concentrated KCl and the ovaries, uterus, vagina, adrenal glands, and kidneys were removed. The corpora lutea were carefully dissected from the rest of the ovarian tissue and all tissues were stored in 10% formalin. Each tissue sample was weighed and placed in a vial for counting of radioactivity in a Tracor gamma scintillation counter (model 1185, TM Analytic, Elk Grove Village, IL). The counts per minute (c.p.m.) from the tissue samples and reference samples were corrected for background and overlap of radioactivity by simultaneous equations calculated on an Apple II plus microcomputer. Blood flow was then calculated (Heymann *et al.*, 1977) as: tissue blood flow (ml/min) = (c.p.m. in tissue/c.p.m. in reference sample) $\times$ withdrawal rate of the reference sample (ml/min). Flow per gram of tissue ($\text{ml}/\text{min}\cdot\text{g}^{-1}$) was calculated by dividing flow by the weight of the tissue sample. The tissue vascular resistance was calculated as: tissue vascular resistance ($\text{mmHg}/\text{ml}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$) = mean arterial pressure/tissue blood flow.

General experimental protocol. On Day 9 of pseudopregnancy, rabbits were anaesthetized and an initial blood flow measurement was performed as described above. A blood sample was then obtained from the abdominal aorta for the determination of pretreatment serum progesterone concentration. The animals were then treated with one of the following: (1) an injection of 300 mg aminoglutethimide phosphate/kg body weight (Ciba-Geigy, Summit, NJ) in 10 ml saline ($N = 5$); (2) an injection of 10 ml saline ($N = 4$); (3) an injection of 300 mg aminoglutethimide phosphate/kg followed by an infusion of 150 mg aminoglutethimide phosphate/kg $\cdot$ h $^{-1}$ for 3 h ($N = 5$); (4) a saline injection and subsequent saline infusion ($N = 4$). Subsequent blood flow determinations were performed and blood samples were taken at 1 and 3 h after the start of treatment in all animals.

Statistics. All statistical analyses were performed using the Statview 512+ statistics program for the Macintosh microcomputer. Differences between groups of animals were analysed by a repeated measures analysis of variance; within-animal changes in blood flow and vascular resistance during the aminoglutethimide or saline treatments were analysed by analysis of variance followed by Scheffe's F1 test. The correlation coefficients (Ott, 1977) were calculated for the natural log of the tissue blood flow versus the natural log of serum progesterone concentration or mean arterial pressure.

Results

Reliability of blood flow measurements

To document that the blood flow estimates in the corpora lutea were accurate, the number of microspheres trapped in the tissue was determined. The total luteal tissue from each animal received $\sim 24\,000$ microspheres, which corresponds to less than a 5% error in blood flow value due to random variability in microsphere distribution (Buckberg *et al.*, 1971). The difference in blood flow between the right and left kidneys was used as an index of adequate microsphere mixing (Heymann *et al.*, 1977). The correlation between blood flow to the right and left kidney was 0.98 with a regression coefficient of 1.016 (not significantly different from 1.0) and a y intercept of -0.062 . The mean ($\pm$ s.e.m.) blood flow to the right and left kidney cortex was almost identical (right = 5.82 ± 0.32 ml/min·g $^{-1}$; left = 5.77 ± 0.33). The average absolute difference between the blood flow to the right and left kidney was $7.7 \pm 0.9\%$ with only 2% of the values showing a difference of more than 20%. Therefore, by the time the microspheres had reached the renal arteries adequate mixing had occurred.

Blood flow and serum progesterone concentration

Before saline or aminoglutethimide treatment, tissue blood flows, mean arterial pressures, and serum progesterone values were not different for the two groups of animals (Table 1). The administration of saline did not alter serum progesterone values (Table 1); in contrast, treatment with aminoglutethimide caused a marked decrease in serum progesterone at 1 and 3 h ($P < 0.001$; Table 1).

Table 1. Serum progesterone (P_4), mean arterial pressure (MAP) and blood flow values at 1 and 3 h after aminoglutethimide (AG; N = 10 animals) or saline (N = 8) treatment

	P_4 (ng/ml)	MAP (mmHg)	Blood flow (ml/min·g $^{-1}$)					
			Corpus luteum	Ovarian stroma	Uterus	Vagina	Adrenal	Kidney
Before	34.6	76.4	29.5	2.9	0.5	0.5	2.6	6.8
AG	± 3.2	± 5.8	± 3.9	± 0.6	± 0.1	± 0.1	± 0.2	± 0.8
1 h	13.6	69.2	26.2	4.5	0.6	0.4	3.5	6.4
after AG	$\pm 2.2^*$	$\pm 3.6^*$	± 3.5	± 1.3	± 0.1	± 0.1	± 0.5	± 0.7
3 h	15.7	65.3	22.5	3.2	0.5	0.2	2.6	4.7
after AG	$\pm 2.2^*$	$\pm 5.1^*$	$\pm 3.4^*$	± 0.7	± 0.1	$\pm 0.04^*$	± 0.3	$\pm 0.5^*$
Before	38.7	74.0	29.2	4.0	0.7	0.5	2.7	6.0
saline	± 5.3	± 6.3	± 4.4	± 0.7	± 0.1	± 0.2	± 0.5	± 0.9
1 h	35.6	72.0	31.8	5.7	0.7	0.4	3.4	5.9
after saline	± 2.6	± 4.6	± 3.9	$\pm 1.0^*$	± 0.1	± 0.1	± 0.7	± 0.8
3 h	38.4	66.3	26.5	4.1	0.6	0.3	2.4	3.9
after saline	± 6.8	± 7.3	± 6.2	± 1.2	± 0.2	± 0.1	± 0.4	$\pm 0.8^*$

* $P < 0.05$ compared with pretreatment value.

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

Since blood flows and other measures were the same in animals treated by injection or infusion of saline, the data were pooled. Saline treatment had no significant effect on mean arterial pressure or blood flow to the corpus luteum, which remained at high levels at 1 and 3 h (Table 1). Significant changes were observed in ovarian blood flow at 1 h of saline administration, and in kidney blood flow at 3 h (Table 1).

Table 2. Correlation coefficients for natural log (ln) of serum progesterone concentration or mean arterial blood pressure versus tissue blood flow (*n* = 54 measurements in 18 rabbits)

	ln Blood flow (ml/min·g)					
	Corpus luteum	Ovarian stroma	Uterus	Vagina	Adrenal	Kidney
ln Serum progesterone conc. (ng/ml)	0.01	0.17	0.08	0.10	0.17	0.06
ln Mean arterial pressure (mmHg)	0.80*	0.04	0.61*	0.58*	0.06	0.63*

**P* < 0.001.

Blood flow and other measures were the same in animals treated by injection or infusion of aminoglutethimide and, therefore, the data were pooled. Aminoglutethimide treatment was accompanied by small, but significant, reductions in mean arterial pressures (Table 1). At 1 h there was no significant change in blood flow to the corpus luteum, but at 3 h the decreased mean arterial blood pressure was associated with a significant reduction in blood flow to the corpus luteum as well as to the vagina and kidneys (Table 1). Despite the diminution of blood flow to these organs, vascular resistance, which is the critical measure of vascular control, was unchanged and was not different from that in saline-treated animals (Fig. 1). Progesterone production was therefore reduced with aminoglutethimide, but this was not associated with significant alteration in vascular resistance of the corpus luteum.

Fig. 1. Effect of 3 h of aminoglutethimide (*N* = 10 animals) or saline (*N* = 8) treatment on tissue vascular resistance. Mean $\pm$ standard error of the mean.

The low vascular resistance in the corpus luteum, relative to that in other tissues (Fig. 1), was reflected in the high rate of luteal blood flow which was 10-fold greater than ovarian stromal blood

flow, 50-fold greater than uterine blood flow, 75-fold greater than vaginal blood flow, 10-fold greater than adrenal gland blood flow, and 4-fold greater than kidney blood flow (Table 1).

Table 2 shows that there was no significant correlation between serum progesterone concentration and blood flow to any of the tissues. In contrast, mean arterial pressure was significantly correlated with blood flow to the corpus luteum ($y = -3.94 + 1.68x$), uterus ($y = -7.25 + 1.54x$), vagina ($y = -8.54 + 1.72x$) and kidney ($y = -3.32 + 1.17x$; all values are for the natural log of the variable), whereas no significant correlation was observed between mean arterial pressure and blood flow to the ovarian stromal tissue or to the adrenal gland (Table 2).

Discussion

As reported previously (Abdul-Karim & Bruce, 1973; Janson & Albrecht, 1975; Devoto *et al.*, 1977), and confirmed here, the corpus luteum has a very high rate of blood flow. This high rate of blood flow suggests that the blood vessels of the corpus luteum exert a low resistance to blood flow. The objective of this study was to determine whether progesterone or other steroids might be responsible for maintaining a low vascular resistance in the corpus luteum. Progesterone has been shown to cause changes that are consistent with a role as a vasodilator: progesterone treatment increases ovarian blood flow in pregnant rats (Waddell *et al.*, 1982); progesterone decreases the contractile activity of myometrial (Allen & Reynolds, 1935) and gastrointestinal (Gill *et al.*, 1985) smooth muscle; progesterone pellets placed in the uterine horn cause an increase in the diameter of the ovarian and uterine vein in ovariectomized mice (Forbes & Glassen, 1972); and progesterone modifies the responsiveness of blood vessels to vasoactive agents (Orosz *et al.*, 1983; Skoog & Kenney, 1983). The inhibition of progesterone production by aminoglutethimide, however, did not change vascular resistance within the corpus luteum. The slight fall in luteal blood flow during aminoglutethimide treatment (Table 1) cannot be attributed to a direct effect of aminoglutethimide within the corpus luteum, because of the concomitant decline in mean arterial pressure in this group. Blood flow to the corpus luteum correlates closely with alterations in mean arterial pressure (Table 2), suggesting that the corpus luteum is operating on a linear pressure-flow curve.

Aminoglutethimide inhibits steroidogenesis by competing with cholesterol for the binding site on the mitochondrial P-450 enzyme that is responsible for cleaving the cholesterol side-chain (Uzgirir *et al.*, 1977; Simpson, 1979). Our results indicate that aminoglutethimide caused a 60% reduction in peripheral serum concentrations of progesterone within 1 h, and that this reduction was still present at 3 h. This interval was chosen as a reasonable period to expect a change in vascular resistance since oestradiol-17 β causes vasodilatation of the uterine vasculature within 30 min and has maximal effects by 2–3 h (for review see Meschia, 1983). Despite a marked decline in luteal progesterone production, the vascular resistance within the corpus luteum was not altered by the aminoglutethimide treatment. This finding leads to our main conclusion that vascular resistance in the corpus luteum is not acutely dependent upon the normal rates of luteal progesterone production at this stage of pseudopregnancy.

In other studies, serum progesterone concentration, and luteal or ovarian blood flow were significantly correlated (Niswender *et al.*, 1975, 1976; Ford *et al.*, 1979; Ford & Chenault, 1981; Janson *et al.*, 1981; Magness *et al.*, 1983), an observation that at first glance seems contradictory to the results reported here. However, these correlations were made throughout the entire luteal phase and include the effects of marked structural changes that occur in the luteal vasculature. The luteal vasculature is formed initially by multiplication and invasion of the thecal capillaries (for review see Findlay, 1986), leading to increased blood flow in the developing corpus luteum, which also has increased steroidogenic capacity. At the end of the luteal phase, in most species, luteal blood flow declines as serum progesterone values fall (Bruce & Moor, 1976; Niswender *et al.*, 1976; Devoto *et al.*, 1977; Wehrenberg *et al.*, 1978; Ford *et al.*, 1979; Hossain *et al.*, 1979; Magness *et al.*,

1983). Again this change in luteal blood flow is associated with structural changes in the vasculature, since the number of luteal endothelial cells declines during luteolysis (Niswender *et al.*, 1976; Azmi *et al.*, 1984; Farin *et al.*, 1986). Therefore, the lack of a correlation between serum progesterone concentration and luteal blood flow, in our experiments and in experiments of others (Bruce *et al.*, 1980; Bruce & Meyer, 1981), can be explained on the grounds that an experimental period of several hours is insufficient to observe structural changes in luteal blood vessels. It is also possible that a correlation between luteal progesterone production and luteal blood flow could not be detected because the measurement of peripheral serum progesterone concentration did not accurately reflect luteal progesterone production. However, if the half-life of progesterone was not altered (an assumption not verified in this study), then during a 3-h time period peripheral progesterone concentration should reflect luteal progesterone production.

If blood flow is not altered by acute changes in progesterone synthesis, what other hormones might affect blood flow? Oestradiol is a possible candidate, since this hormone is the primary luteotrophic hormone in the rabbit (Keyes & Nalbandov, 1967; Bill & Keyes, 1983) and has been shown to increase ovarian blood flow in sheep (Rosenfeld, 1980). In the present experiments, oestradiol was administered continuously in order to ensure that any observed changes in blood flow were not attributable to diminished availability of oestrogen. In a separate study (Wiltbank, 1987) we have found that neither oestradiol nor human chorionic gonadotrophin (hCG) alter luteal blood flow, despite marked changes in steroidogenesis induced by these hormones. Similar results have been reported by Norjavaara *et al.* (1987), who observed that the injection of LH or hCG did not produce an increase in blood flow to the rat corpus luteum. Based upon such observations, we have no compelling evidence that either progesterone or luteotrophic hormones acutely regulate blood flow in the corpus luteum.

The significant positive correlation between mean arterial pressure and luteal blood flow may offer some insight into the control of luteal blood flow. By calculating the coefficient of determination or R^2 (Ott, 1977), 65% of the variation in blood flow to the corpus luteum can be explained by variation in mean arterial pressure, whereas in other tissues, <40% of the variation is attributable to changes in mean arterial pressure. Other investigators have also reported a positive correlation between mean arterial pressure and luteal blood flow in anaesthetized rabbits (Janson & Albrecht, 1975; Janson *et al.*, 1981) and rats (Selstam *et al.*, 1985). At variance with the above, is a report by Bruce & Gibbs (1976) who observed a negative correlation between mean arterial pressure and luteal blood flow ($r = -0.425$) in unanaesthetized pregnant rabbits. However, these investigators observed that the unanaesthetized rabbits "were hyperventilating . . . [possibly] due to anxiety and disturbance of their catecholamine levels" (Bruce & Gibbs, 1976). An elevation in catecholamine concentrations could change the relationship between mean arterial pressure and luteal blood flow: catecholamines may increase mean arterial pressure but potentially decrease luteal blood flow (Selstam *et al.*, 1985; M. C. Wiltbank, K. P. Gallagher & P. L. Keyes, unpublished results).

In conclusion, the lack of responsiveness of the luteal vasculature to changes in hormonal milieu, and the positive correlation between blood flow and mean arterial pressure, suggest that the luteal vascular system is operating on a linear pressure-flow curve with a very low vascular resistance. The basis of low vascular resistance appears to be related to structural elements in the corpus luteum, such as high capillary density and lack of muscular arterioles (Dharmarajan *et al.*, 1985; Wiltbank, 1987) rather than to hormonal factors. This view is consistent with our knowledge of the luteal vasculature, which appears to consist of sinusoidal capillaries (Sobotta, 1897; Dharmarajan *et al.*, 1985), and which lacks autonomic nerves (Burden, 1972; Unsicker, 1974) or vascular smooth muscle (Wiltbank, 1987).

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