Acute increase of noradrenaline on vascular resistance in the corpus luteum of the pseudopregnant rat

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Summary. Noradrenaline infusion for 2 min (0.4 μg/min) in anaesthetized rats increased the vascular resistance in 6-day-old corpora lutea, but had no significant effect on the vascular resistance in young (2-day-old) or old corpora lutea (11 days old). The luteal blood flow of the control rats was higher in 6-day-old corpora lutea than in those of 2 and 11 days. The luteal blood flow apparently lacks autoregulation, since a linear relationship between blood flow and arterial blood pressure was registered. The present study shows that, besides the well known metabolic effects of catecholamines on corpus luteum function, catecholamines can exert acute vascular effects, but only on the corpus luteum of pseudopregnancy in the middle of its life span.

Introduction

The ovary has a high blood flow, a large proportion of which reaches the corpus luteum (Abdul-Karim & Bruce, 1973; Janson & Albrecht, 1975; Hossain, Lee, Clarke & O'Shea, 1979; Pang & Behrman, 1979; Damber et al., 1981). The role of the high luteal blood flow is at present under debate (Janson, Damber & Axen, 1981; Bruce, Meyer & Dharmarajan, 1984). Only prosta-glandin F-2α has been shown to influence the luteal blood flow (Nett & Niswender, 1981). Reports of hormonal effects on luteal blood flow are lacking and relationships between hormonal influences and indirect metabolic effects on the blood flow are at present difficult to interpret.

The catecholamines noradrenaline and adrenaline have direct effects in vitro on the cyclic AMP system and progesterone synthesis of the corpus luteum (see Selstam et al., 1984; Norjavaara, 1984). Noradrenaline infusion has also been shown to increase cyclic AMP production of the corpus luteum in vivo (Norjavaara, Selstam, Damber & Johansson, 1983). Besides these direct cellular metabolic effects, catecholamines can influence the myogenic tonus of the ovarian vasculature as shown for perfused ovaries in vitro of the rabbit (Selstam, 1975) and the human (Varga, Zsolnai & Bernard, 1979). It was therefore considered important to study a possible direct effect of noradrenaline on the vascular bed of the corpus luteum.

Materials and Methods

Animals. Mature, female Sprague–Dawley rats (225–300 g) were purchased from Anticimex (Stockholm, Sweden) when 3–4 months old. The rats were kept under controlled conditions with free access to food and water and with lights on from 05:00 to 19:00 h.

To obtain corpora lutea of pseudopregnancy of defined ages adult females were mated with vasectomized males. Three females were kept together with one male and vaginal smears were taken once daily around 09:00 h. Corpora lutea were designated as 1 day old on the day of oestrus if a vaginal plug was recovered. The length of pseudopregnancy was 13 ± 1 days (mean ± s.d.) as judged from plasma steroids and vaginal smears. Rats bearing 2-, 6- and 11-day-old corpora lutea of their first pseudopregnancy were used in this study.

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Experimental procedure. All rats were anaesthetized with an intraperitoneal injection of 40 mg pentobarbitone sodium/kg (Mebumal: ACO, Sweden). Thereafter they were placed in a supine position on a thermostat-regulated heating pad. Blood flow was determined with radioactive microspheres (Rudolph & Heymann, 1967) as modified for adult rats by Damber & Janson (1978) and Selstam & Damber (1983). Heparinized catheters (PE-10 or PE-50 plastic cannulas; Intramedic, Parsippany, NJ, U.S.A., with inner diameters of 0·4 and 0·6 mm respectively) were inserted into the right and left branchial arteries, the tail artery and the right carotid artery. The catheters were prefilled with a solution containing 500 i.u. heparin/ml saline (9 g NaCl/1). The left branchial arterial catheter was connected to a pressure transducer at the heart level (model 267A with amplifier model 311A; Sanborn Co., U.S.A.) and the mean systemic arterial blood pressure was recorded. Surgery took 20–40 min. Noradrenaline (4 µg/ml) or vehicle (0·1 mg ascorbic acid/ml saline) was infused into the right brachial artery at a rate of 0·1 ml/min. 1-Noradrenaline (Apoteksbolaget, Stockholm, Sweden) was kept on ice in the dark and diluted immediately before the start of infusion.

Radioactive carbonized microspheres with a diameter of 15 ± 0·3 µm (mean ± s.d.) labelled with 141 cerium suspended in 20% dextran were vigorously shaken in a small glass bottle. At 105 sec after the start of noradrenaline or vehicle infusion, 1 ml of the suspension containing ~150 000 microspheres was infused during 30 sec via the catheter in the carotid artery. The microsphere infusion was immediately followed by a short saline infusion. From 15 sec before until 15 sec after the microsphere infusion, blood was withdrawn at a constant rate (0·1 ml/min) from the tail artery (reference sample). Thereafter the rats were killed with an overdose of pentobarbitone sodium. The abdomen was opened and ovaries with surrounding tissues were removed and placed in saline under a stereomicroscope. The ovaries were trimmed and the corpora lutea were extirpated and weighed. The corpora lutea of pseudopregnancy at 2 days of luteal age were identified mainly by the vascular network around the ovariatory points and their more loose structure and at 6 and 11 days by their larger sizes and more reddish appearance compared to corpora lutea of earlier oestrous cycles. The kidneys, the adrenals and the lungs were also dissected free and weighed. The mean ± s.d. weight of all corpora lutea from each rat (N = 42) was 20·5 ± 0·35 mg and the mean weight for one kidney of each rat was 903 ± 64 mg. Radioactivity of tissue samples was determined in a gamma counter (Rackgamma, LKB-Wallac, Sweden). Blood flow (F) was calculated as

\[ F = Q_{\text{ref}} \times \frac{N_{\text{org}}}{N_{\text{ref}}} \]

where \( Q_{\text{ref}} \) = rate of withdrawal of the reference sample, \( N_{\text{org}} \) = radioactivity in the organ and \( N_{\text{ref}} \) = radioactivity in the reference sample. The accuracy and precision of blood flow measurement with the microsphere technique were calculated as described by Buckberg et al. (1971). The vascular resistance was calculated according to the following formula:

\[ \text{arterial blood pressure (mmHg)/organ blood flow (ml/100 g min}^{-1}) \].

The mean ± s.d. increase in blood pressure during the microsphere infusion was 4 ± 4 mmHg. Rats having a large decrease during microsphere infusion were not included. The microsphere method is based on the assumption that arterio-venous shunts do not exist. For the rabbit ovary in the follicular and luteal phases it has been shown that shunts of physiological significance do not exist (Ahren, Janson & Selstam, 1974). In a separate experiment radioactive microspheres were infused via the aorta into the rat ovary bearing corpora lutea of different ages in a preparation (Type III in-vivo perfusion, see Ahren et al., 1974) in which all connections to uterine vessels were ligated. Ovarian venous sampling of 4 rats revealed no shunting (G. Selstam, unpublished). It is well known that a small total body shunting mainly via arterio-venous shunts exists in the skin and total body shunting in this study, measured as percentage of injected microspheres in the lungs, was 1·0 ± 0·5% (mean ± s.d.).
Statistics. Values are presented as mean ± s.e.m. For comparisons between different luteal ages analysis of variance followed by Student–Neuman–Keul’s multiple range test was used. The effect of noradrenaline was tested with Student’s t test (Woolf, 1968).

Results

The relationship between arterial blood pressure and blood flow of 6-day-old corpora lutea of control rats is shown in Text-fig. 1. An almost linear relationship was found, indicating that the corpus luteum lacks autoregulation. Such a relationship was not found for the kidney, which is known to autoregulate its blood flow (data not shown). The effect of a 2-min infusion of noradrenaline on the arterial blood pressure is shown in Text-fig. 2(a). The increment was dependent on the initial blood pressure: low initial blood pressures resulted in large increments (Text-fig. 2b).

Infusion of noradrenaline for 2 min caused an increase in the vascular resistance of 6-day-old corpora lutea (Text-fig. 3a). Noradrenaline infusion had no significant effect on the blood flow of

**Text-fig. 1.** Relationship between arterial blood pressure and blood flow in 6-day-old corpora lutea of pseudopregnancy of control rats. Each point represents one rat. The linear regression is indicated in the figure. The correlation coefficient (r) was 0·83.

**Text-fig. 2.** Effect of noradrenaline infusion on the systemic arterial blood pressure in the rat. Noradrenaline was infused for 2 min at a rate of 0·4 µg/min via a catheter in the right branchial artery. The initial blood pressure was measured immediately before the start of infusion and the effect of noradrenaline was measured at end of infusion. (a) Blood pressures before and after infusion: the linear regression is indicated (r = 0·80). (b) The relative increase of the blood pressure of the same rats as in (a) in percentage of the initial blood pressure.
the corpus luteum (Text-fig. 3b) because noradrenaline increased the systemic arterial blood pressure. Noradrenaline had no significant effect on vascular resistance or the blood flow of 2- and 11-day-old corpora lutea. The vascular resistance of control rats differed for different ages of pseudopregnancy (Text-fig. 3a); 6-day-old corpora lutea had a lower vascular resistance and a higher blood flow than did 2- and 11-day-old corpora lutea. For corpora lutea from earlier cycles vascular resistance (0.13 ± 0.03, mean ± s.e.m. of 5 rats) and blood flow (1107 ± 227 ml/100 g min⁻¹) was in the same range as that for 2- and 11-day-old corpora lutea.

Text-fig. 3. Effect of noradrenaline infusion on the vascular resistance (a) and blood flow (b) in rat corpora lutea of different ages. The vascular resistance was calculated as:
- arterial blood pressure (mmHg)/organ blood flow (ml/100 g min⁻¹)
- Values are given as means ± s.e.m. for 6-8 rats. The arterial blood pressure before and after 2 min of noradrenaline infusion was: for 2 days, 108 ± 13 and 138 ± 7 mmHg; for 6 days, 120 ± 11 and 149 ± 8 mmHg; for 11 days, 108 ± 9 and 143 ± 12 mmHg, respectively.
- *Significantly different from value for saline-injected animals, P < 0.05 (Student's t test).
- **Significantly different from value for saline injected animals at Days 2 and 11, P < 0.05 (analysis of variance and Student–Newman–Keul's test).

As a comparison, the effect of noradrenaline on the kidney was determined. The vascular resistance increased and the blood flow of the kidney decreased significantly after noradrenaline infusion for 2 min (before: 0.23 ± 0.02 and 532 ± 28 ml/100 g min⁻¹, N = 22; after: 0.47 ± 0.05 and 364 ± 27 ml/100 g min⁻¹, N = 19).

**Discussion**

The present study demonstrates that in the adult pseudopregnant rat the corpus luteum in the middle of its lifespan (6 days of age) has a higher blood flow than do those of the early (2 days old) or late (11 days old) luteal phase. Only the blood vessels of the 6-day-old corpora lutea were responsive to noradrenaline, in that the vascular resistance was increased. The blood flow of the corpus luteum in control rats was higher at 6 days of luteal age than at 2 and 11 days. Since arterial blood pressure does not change throughout pseudopregnancy (Text-fig. 3) the high luteal blood flow at 6 days is therefore attributable to a decreased vascular resistance. At 6 days the steroid pro-
duction and metabolism of the corpus luteum is higher than at 2 days and metabolites may have induced vasodilatation. The high blood flow may also be an effect of gonadotrophins, since LH decreases vascular resistance (Janson, 1975) and possibly such an effect exists for the corpus luteum. Another possible explanation for the difference in blood flow between 2- and 6-day-old corpora lutea is that at Day 2 the luteinization process is not completed morphologically (Pederson, 1951) and the luteal vasculature is not fully developed (Bassett, 1943; Niswender, Reimers, Diekman & Nett, 1976), a difference which may also explain the lower luteal blood flow in young corpora lutea. In an earlier study (Damber et al., 1981) with immature rats stimulated by PMSG, such an increase between Days 2 and 6 was not found. Luteinization may be faster in rats stimulated with PMSG because a high luteal blood flow was already apparent on Day 2. In this study the luteal blood flow at 11 days was lower than that for 6-day-old corpora lutea. This is in accordance with the results of Pang & Behrman (1979) for adult pseudopregnant rats and of Damber et al. (1981) for PMSG-stimulated immature rats. This decrease in blood flow may be a reflection of a decreased metabolism or a decreased luteotrophic support. Determinations of the blood flow of the corpus luteum in pregnant rats with the microsphere method show that the luteal blood flow is similar on Days 4, 7 and 10 of pregnancy, after which time the blood flow increases (Bruce et al., 1984). Factors produced by the uterus/placenta/embryonic unit are the most likely causes of the difference between the luteal blood flow in pseudopregnant and pregnant rats during this period. It is unlikely that the decrease in luteal blood flow after Day 6 of pseudopregnancy is of importance for the initiation of luteolysis. In different experimental models, functional luteolysis as measured by progesterone production has actually been shown to precede the decrease in luteal blood flow (see Hossain et al., 1979; Pang & Behrman, 1979; Damber et al., 1981). The decreased luteal blood flow is therefore believed to be a part of the luteolytic process, but not the initiator.

Our results demonstrate a linear relationship between mean arterial blood pressure and luteal blood flow (Text-fig. 1). This is interpreted as a lack of autoregulation of the blood flow for the rat corpus luteum in the blood pressure range studied. It is in line with microsphere studies on corpora lutea of the rabbit ovary: based on experiments in which the aorta was constricted, Janson et al. (1981) concluded that the rabbit corpus luteum lacks autoregulation, while the stromal tissue of the ovary does autoregulate its blood flow.

The present study shows that acute administration of noradrenaline can increase the vascular tonus of 6-day-old corpora lutea. Such a direct effect of noradrenaline on the corpus luteum has to our knowledge not been shown earlier. Noradrenaline can increase the vascular tonus of whole ovaries as shown for perfused rabbit follicular-phase ovaries devoid of luteal tissue in vitro (Selstam, 1975) or in vivo (Janson & Albrecht, 1975), for near-term sheep ovaries with as well as without corpora lutea (Peronnet & Rankin, 1978) and for whole ovaries of pregnant guinea-pigs during late gestation (Mårtensson & Carter, 1982). Other adrenergic agonists can also increase the vascular resistance in whole ovaries as shown for human (Varga et al., 1979) and rat (Zsolnai, Varga & Horvath, 1982) ovaries. However, noradrenaline did not exert an acute effect on vascular tonus in 2- and 11-day-old corpora lutea in this study. The lack of effect of noradrenaline in 2-day-old corpora lutea could be due to the fact that the vessels are ‘immature’ as mentioned above. Under similar experimental conditions, infusion of noradrenaline increased the cyclic AMP levels in corpora lutea of PMSG-treated rats (Norjavaara et al., 1983). Since in the present study no significant effect was observed on vascular resistance or luteal blood flow in 2-day-old corpora lutea after infusion, this is a further indication that the in-vivo effect on the luteal cyclic AMP system is a direct effect on the luteal cells.

The lack of significant effect of acute infusion of noradrenaline on the vascular tonus in 11-day-old corpora lutea is difficult to understand. However, there is an increase in luteal content of noradrenaline at the end of the luteal period in PMSG-treated rats (unpublished) that may be responsible for the change in luteal vascular response to noradrenaline. The increased luteal content may represent a completed innervation of the luteal vasculature and therefore endogenous release of catecholamines may have caused refractoriness.
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References


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