Influence of adrenergic receptors on ovarian progesterone secretion in the pseudopregnant cat and oestradiol secretion in the oestrous cat

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Summary. The infusion of isoprenaline or propranolol into the abdominal aorta of the pseudopregnant cat caused an increase or decrease respectively in the ovarian progesterone secretion rate. These observations suggest that the sympathetic innervation of the ovary has a physiological influence on normal progesterone secretion, and this mechanism may explain stress-related increases in progesterone concentrations. The infusion of isoprenaline or propranolol after the stimulation of follicular growth had no consistent or convincing effect on oestradiol secretion.

Introduction

There is a substantial extrinsic innervation of the mammalian ovary (Neilson *et al.*, 1970) associated with blood vessels, forming plexuses around primary follicles, entering the membrana granulosa of maturing and mature follicles, and associated with the smooth muscle cells in the theca externa of mature follicles (Fink & Schofield, 1971); these nerves are predominantly adrenergic, with some cholinergic. It has been demonstrated that stimulating or inhibiting β -adrenergic neurotransmitter receptors affects progesterone steroidogenesis in terms of ovarian tissue content or secretion *in vitro* (cow: Condon & Black, 1976; sheep: Jordan *et al.*, 1978; rat: Ratner *et al.*, 1980) and peripheral blood concentrations (human: Fylling, 1971; sheep: Flint *et al.*, 1974). However, some experiments measuring blood progesterone concentrations in pregnant women (Carlström *et al.*, 1978; Hanssens *et al.*, 1983) and luteal tissue secretion *in vitro* (Richardson & Mason, 1980) have yielded contradictory results. The only information on such influences on ovarian progesterone secretion rates, which might better indicate possible physiological roles for the ovarian innervation, was obtained in the rat (Zsolnai *et al.*, 1982), in which the ovary is not particularly densely innervated (Burden & Lawrence, 1980).

It has been suggested that the ovarian innervation is involved in the growth and maturation of follicles (Fink & Schofield, 1971), and is responsible for the compensatory hypertrophy after unilateral ovariectomy (Burden & Lawrence, 1977) and the unequal ovulatory activity of the left and right ovaries (Wheeler, 1978). We therefore examined the influence of the β -adrenergic receptors on oestrogen secretion rates (as an indicator of follicular activity) in animals with preovulatory follicles.

We chose the cat as the experimental animal because the comparatively dense innervation of its ovaries (Burden & Lawrence, 1980) suggested a complementary abundance of neurotransmitter receptors that we could influence, and its size makes it suitable for acute experiments of this type. Although ovulation in the cat is induced by copulation (Longley, 1910), the mechanical stimulation of the vagina and the vaginal afferent nerves results in an increased LH concentration that induces

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ovulation (Glover *et al.*, 1985), as in spontaneous ovulators; the ovarian innervation plays no apparent role in ovulation in induced ovulators (Lambertsen *et al.*, 1976). The results of stimulating and blocking ovarian adrenergic receptors in the cat should therefore be generally applicable to other species, both induced and spontaneous ovulators.

The adrenergic receptor agonistic and antagonistic drugs that we intended to investigate could be expected to alter the performance of the cardiovascular system (e.g. heart rate, stroke volume, venous tone, vasoconstriction/dilatation); we therefore monitored arterial blood pressure and heart rate to identify and assess any such changes. The drugs were infused into the aorta cranially to the origins of the ovarian arteries to maximize the ovarian arterial blood concentrations of the drugs relative to the lower peripheral concentrations. Timed aliquants of the continuous flow of ovarian venous blood were collected so that the secretion rate for the period represented by each aliquant could be calculated, the secretion rate being the most sensitive indicator of any variation in ovarian secretion. These requirements suggested an acute experiment on an anaesthetized animal with euthanasia without recovery at the completion of the experiment.

Materials and Methods

Experimental procedure. Intact anoestrous cats were treated i.m. with 50 i.u. PMSG daily for 3 days (Folligon: Intervet International, Boxmeer, The Netherlands) to stimulate ovarian follicular development and on the following day 100 i.u. hCG (Chorulon: Intervet) was given i.m. to stimulate ovulation. Between 1 and 3 weeks later the animals were collected before feeding and anaesthetized with ketamine hydrochloride (70 mg i.m.; Ketalar: Parke-Davis, Caringbah, N.S.W.) and later pentobarbitone sodium (6 mg i.v.; Sagatal: May & Baker, West Footscray, Victoria). Further doses of anaesthetic were administered as required; this typically consisted of a further 20 mg ketamine hydrochloride and 6 mg pentobarbitone sodium after 1–2 h. The animals were weighed (Tables 1–3).

A saphenous vein and the two femoral arteries were cannulated (polyethylene; 0.86 mm internal diameter, 1.27 mm external diameter; Dural Plastics, Dural, N.S.W.). The intra-venous cannula was short and used for the administration of pentobarbitone sodium and anticoagulant. The end of one intra-arterial cannula was positioned in the thoracic aorta; this cannula was connected to a transducer for the continuous recording of arterial blood pressure. The end of second intra-arterial cannula was positioned in the upper-abdominal aorta so as to be cranial to the origin of the ovarian arteries; this cannula was used for the infusion of isotonic saline and the experimental drugs. A corrugated rubber tube fastened around the upper abdomen and connected to a transducer allowed the recording of respiratory movements.

The abdomen was opened through the mid-line central incision and, with as little handling as possible, the two ovaries were inspected. When corpora lutea were present on both ovaries one was selected at random; on that side the uterine branches of the ovarian vein (del Campo & Ginther, 1974) were ligated. Anticoagulant (2500 i.u. heparin i.v.; Sigma, St Louis, MO, U.S.A.) was administered and the ovarian vein (now carrying only ovarian venous blood) was cannulated near its origin from the posterior vena cava (right) or renal vein (left) in the short distance that this vein is separate from its complementary ovarian artery. To minimize any kinking of the vein, the cannula was led under the descending colon and out of the abdomen through a stab wound in the body wall on the other side; the cannula was then trimmed to minimize its length. The abdominal wound was repaired to minimize the trauma and heat loss.

The ovarian venous outflow was collected continuously into calibrated 10 × 75 mm glass tubes; at 1 or 2 ml intervals the time was noted and the collection tube changed. This allowed the ovarian blood flow to be calculated for each collection tube. Every fourth or fifth tube a sample of the blood was taken for packed cell volume (PCV) determination. The blood was centrifuged and the plasma stored at –20°C.

Saline (9 g NaCl/l) or the experimental drug was infused through the intra-arterial cannula ('Perfusor VI' adapted to use 1 ml syringes; B. Braun Melsungen AG, Melsungen, West Germany) at about 1 ml per 17 min.

At the completion of each experiment the animal was killed with pentobarbitone sodium and the volumes of the infusion and collection cannulae were noted.

Progesterone secretion rates. The antiserum (11P27; Fantl & Wang, 1984) had cross-reactions of 100% with progesterone, 96% with 11 α -hydroxyprogesterone, 11% with 5 α -pregnane-3,20-dione, 4% with 6 β -hydroxyprogesterone, 1% with pregnenolone, and <1% with 20 β -dihydroprogesterone, 17-hydroxyprogesterone and androstenedione; no other steroid cross-reacted at >0.1%. The progesterone radioimmunoassay method has been described elsewhere (Wheeler & Blackshaw, 1986). The efficiency of extraction was 80.6 ± 2.28% ($n = 38$). The repeated assay of ovarian venous plasma from a pseudopregnant cat gave a mean value of 219.2 ± 4.87 ng/ml ($n = 20$). The repeated assay of aliquants of plasma from an ovariectomized cat to which known amounts of progesterone had been added to give samples containing 0, 1.25, 2.5, 5 and 10 ng/ml gave data from which a regression line of $y = 0.381 + 1.116x$ (where y is the assayed progesterone concentration and x the added concentration, both in ng/ml) and a standard deviation of the regression coefficient of 0.049 ($t_{(112)} = 23.0$; $P < 0.001$) were calculated (Snedecor & Cochran, 1967). The intra- and inter-assay coefficients of variation (Dighe & Hunter, 1974) were 9.12 and 12.50% respectively.

Table 1. Progesterone secretion rates (ng/min) before, during and after the infusion of isoprenaline, and the maximum secretion rate and its time of occurrence

Cat no.	Body wt (kg)	Isoprenaline infusion rate ($\mu\text{g}/\text{min}$)	Pre-infusion	Infusion	Peak secretion		Post-infusion
					Rate (ng/min)	Time (min)*	
38	3.2	2.9	160 $\pm$ 13.0 (12)	236 $\pm$ 18.8 (8)	296	8.9	160 $\pm$ 10.9 (10)
39	2.5	11.8	196 $\pm$ 24.6 (9)	216 $\pm$ 10.5 (2)	—†		192 $\pm$ 17.5 (5)
46	1.7	2.8	920 $\pm$ 85.6 (9)	1523 $\pm$ 71.0 (2)	1594	15.0	508 $\pm$ 148.0 (3)
49	2.8	3.2	588 $\pm$ 40.9 (16)	791 $\pm$ 65.2 (5)	2025	4.2	518 $\pm$ 26.2 (8)
55	3.4	2.9	4170 $\pm$ 430.1 (12)	1989 $\pm$ 136.6 (5)	—‡		2246 $\pm$ 216.5 (18)
58b	3.3	2.9	932 $\pm$ 23.5 (5)	1093 $\pm$ 147.6 (4)	1508	1.5	1075 $\pm$ 87.1 (10)
73a	—	2.9	1393 $\pm$ 251.4 (19)	387 $\pm$ 36.2 (6)	—‡		1732 $\pm$ 249.8 (26)
73b	—	2.9	1802 $\pm$ 240.7 (26)	2387 $\pm$ 272.6 (6)	3268	1.5	1759 $\pm$ 233.2 (13)
84	3.0	5.6	660 $\pm$ 54.5 (10)	917 $\pm$ 260.3 (4)	1405	12.8	1103 $\pm$ 331.6 (6)
127	2.9	6.6	707 $\pm$ 70.5 (12)	1137 $\pm$ 378.6 (5)	2438	15.6	751 $\pm$ 128.5 (13)

Values are mean $\pm$ s.e.m. for the no. of samples in parentheses.

*From start of infusion.

†No significant variation in the progesterone secretion rate.

‡Progesterone secretion rate decreased during the infusion period.

Table 2. Progesterone secretion rates (ng/min) before, during and after the infusion of propranolol, and the time taken for the secretion rate to decrease to less than 50% of the pre-infusion mean

Cat no.	Body wt (kg)	Propranolol infusion rate ($\mu\text{g}/\text{min}$)	Pre-infusion	Infusion	Time for decrease to <50% of pre-infusion rate* (min)	Post-infusion
36	2.4	1.2	1355 $\pm$ 65.6 (9)	915 $\pm$ 107.5 (3)	18.3	366 $\pm$ 33.9 (5)
53	2.8	1.2	2238 $\pm$ 219.2 (13)	1376 $\pm$ 53.1 (4)	20.1	266 $\pm$ 193.3 (5)
58a	3.3	1.2	1435 $\pm$ 76.9 (11)	1079 $\pm$ 93.8 (8)	11.7	932 $\pm$ 23.5 (5)
60	2.9	1.2	325 $\pm$ 77.9 (16)	560 $\pm$ 32.8 (18)	—†	407 $\pm$ 68.6 (21)
109b	2.2	3.0	5627 $\pm$ 462.6 (13)	2373 $\pm$ 972.9 (6)	4.2	268 $\pm$ 69.5 (2)
126	3.2	2.8	3745 $\pm$ 157.5 (12)	2226 $\pm$ 173.6 (8)	7.3	1748 $\pm$ 114.2 (10)

Values are mean $\pm$ s.e.m. for the no. of samples in parentheses.

*From start of infusion.

†Progesterone secretion rate increased.

Table 3. Follicular oestradiol secretion rates (pg/min) before, during and after the infusion of isoprenaline or propranolol

Cat no.	Body wt (kg)	Infusion rate ($\mu\text{g}/\text{min}$)	Pre-infusion	Infusion	Post-infusion	Analysis of variance
Isoprenaline						
88	2.0	6	234 $\pm$ 8.0 (9)	214 $\pm$ 2.3 (3)	228 $\pm$ 13.4 (9)	$F_{(2,18)} = 0.45$; $P > 0.05$
94	2.6	12	328 $\pm$ 20.9 (15)	451 $\pm$ 30.9 (9)	345 $\pm$ 20.2 (8)	$F_{(2,29)} = 7.21$; $P < 0.01$
Propranolol						
97	2.5	3.4	2511 $\pm$ 124.2 (13)	3186 $\pm$ 149.3 (6)	2366 $\pm$ 131.9 (10)	$F_{(2,26)} = 7.58$; $P < 0.01$

Values are mean $\pm$ s.e.m. for the no. of samples in parentheses.

The plasma progesterone concentrations were corrected using the PCVs and blood flows to obtain the ovarian progesterone secretion rates (the arterial blood concentrations of progesterone were disregarded as negligible), each secretion rate being the mean for the period over which that volume of blood was collected. This procedure reduced the variation in the progesterone secretion rates.

Follicular oestradiol secretion rates. On the day on which the animals would otherwise have had their PMSG-stimulated follicles ovulated with hCG, isoprenaline or propranolol were infused to identify their effects on the ovarian follicular oestradiol.

The oestradiol immunoassay was conducted as described for progesterone (Wheeler & Blackshaw, 1986) with the following differences: 200 μ l aliquants of plasma were extracted with 2 ml diethyl ether, the standards ranged from 1 to 1000 pg/tube (oestradiol-17 β ; Sigma) and the radioactive label was [2,4,6,7-³H]oestradiol (Amersham International, Sydney, N.S.W.; 3.37 TBq/mmol). The antiserum used (6EI), described by Fantl & Wang (1984), bount to oestradiol (100%), 6 α -hydroxyoestradiol (45%), 16-oxo-oestradiol (5%), oestrone (0.04%) and oestriol (1%); no other steroid investigated bound at > 0.01%. The final dilution of the antiserum was 1 in 90 000.

The oestradiol secretion rates were calculated from the plasma concentrations, PCVs, times and volumes as described for the progesterone secretion rates.

Statistical analyses. Using the measured cannula volumes and flow rates, both the infusion and secretion times were corrected to the times that infused drugs arrived at the ovary and the ovary secreted the steroid so as to be comparable. This correction did not take into account the unknown volumes of the aorta and ovarian artery, and the ovarian vein; although these errors would invalidate any consideration of precise time relationships they were considered to be small (of the order of 1–2 min) and not prejudicial for the present purposes.

The significances of any differences between and pre-infusion, infusion and post-infusion periods were examined using analysis of variance (Sokal & Rohlf, 1981).

Results

The total volume of blood collected during these experiments varied from 22.9 to 84.4 ml (mean $\pm$ s.e.m: 54.4 ± 4.02 ml; $n = 19$). These volumes represented 16.4–44.4% ($28.8 \pm 1.89\%$; $n = 17$) of the estimated total blood volumes (66.7 ml blood per kg body weight; Spink *et al.*, 1966). The PCV at the beginning of the experiments was between 21 and 47.5% ($34.1 \pm 1.72\%$; $n = 19$) and decreased by the end to between 14 and 38% ($25.6 \pm 1.65\%$; $n = 19$).

The pre-drug infusion mean progesterone secretion rates for pseudopregnant cats varied between animals from a minimum of 160 ± 13.0 ng/min in Cat 38 to a maximum of 5626 ± 462.6 ng/min in Cat 109; the median was 1078 ± 75.1 ng/min ($n = 26$).

The pre-infusion progesterone secretion rates were unrelated to the numbers of corpora lutea present in the ovary on the side cannulated ($r_{(13)} = 0.234$; $P > 0.05$).

The effect of a continuous saline infusion with continuous ovarian venous blood collection on the ovarian progesterone secretion rate, blood flow, heart rate and arterial blood pressures are shown in Fig. 1. Except for the omission of a drug the protocol was the same as that followed in the other experiments. There was no significant variation in the progesterone secretion rates during three arbitrarily chosen periods corresponding to the pre-infusion, infusion and post-infusion periods ($F_{(2,19)} = 0.82$; $P > 0.05$).

Within 1.5 to 18.8 min of the start of the infusions of isoprenaline in 7 cats (Table 1) the progesterone secretion rates increased ($P < 0.05$) to a maximum rate between 162 and 398% of the pre-infusion mean rates; a typical response is shown in Fig. 2. After the peak in all instances the progesterone secretion rate decreased to a rate similar to that measured during the pre-infusion periods. The post-infusion mean for Cat 84 is considerably higher than the pre-infusion mean because the peak secretion rate after the start of the infusion was not reached until the first sample of the post-infusion period in this experiment. One additional infusion (Cat 39) had no apparent effect on the progesterone secretion rate, and two other infusions (Cats 55 and 73a) were associated with lesser progesterone secretion rates.

All the isoprenaline infusions resulted in a lower ovarian blood flow, the mean for the 10 experiments decreasing from a pre-infusion value of 0.898 ± 0.141 ml/min to 0.557 ± 0.075 ml/min, and remained low at 0.582 ± 0.099 ml/min during the post-infusion period. Isoprenaline had a small,

Fig. 1. Ovarian progesterone secretion rate (□ — □) and blood flow (● — ●), heart rate (■ — ■), PCV (× — ×) and systolic and diastolic arterial blood pressures (○ — ○) during continuous saline infusion in the pseudopregnant cat (Cat 42).

variable effect on the heart rate (from 97 to 127% of the pre-infusion rates) and consistently decreased arterial systolic (to 76.9%) and diastolic (to 64.7%) blood pressures. There was no consistent effect on the respiratory rates.

Of the 6 propranolol infusions (Table 2), 5 produced a marked decrease in the progesterone secretion rates, to <50% of the pre-infusion mean with 4.2–20.1 min of the start of the infusion. This low secretion rate was sustained in each case for the remainder of the experiment. The results for Cat 109 are shown in Fig. 3. One infusion, Cat 60 (an animal in which the progesterone secretion rate was already low), caused an increased secretion rate.

In response to propranolol the ovarian blood flow similarly declined, except in Cat 60 in which the blood flow increased. With this exception the pre-infusion mean for the 5 experiments was 1.207 ± 0.227 ml/min, the infusion being associated with a decrease to 0.778 ± 0.151 ml/min

Fig. 2. Ovarian progesterone secretion rate (□—□) and blood flow (●—●), heart rate (■—■) and systolic and diastolic arterial blood pressures (○—○) before, during and after the infusion of isoprenaline (Cat 38).

with a further decrease to 0.387 ± 0.115 ml/min during the post-infusion period. There were no significant or consistent effects on the heart rate, arterial blood pressures or respiratory rate.

The oestradiol secretion rate was measured in one animal during the induced luteal phase and the mean for the pre-infusion period was 1.12 ± 0.042 pg/min ($n = 13$; Cat 38)

In the oestrous animals one of the two isoprenaline infusions (Cat 94), and the propranolol infusion (Cat 97), were associated with significant variations in the follicular oestradiol secretion rate (Table 3). The patterns of the variation in the oestradiol secretion rate for these two animals is shown in Fig. 4.

Two experiments were rejected due to the ovarian oestradiol secretion rates being particularly low: 18.1 ± 0.59 ($n = 7$) and 8.1 ± 1.03 ($n = 10$) pg/min; these data were interpreted as indicating that the ovarian follicles were not as mature as had been expected.

Fig. 3. Ovarian progesterone secretion rate (□ — □) and blood flow (● — ●), heart rate (■ — ■) and systolic and diastolic arterial blood pressures (○ — ○) before, during and after the infusion of propranolol (Cat 109b).

Discussion

These results indicate that isoprenaline, a β -adrenergic agonist, enhanced ovarian luteal progesterone secretion rates, while propranolol, an adrenergic antagonist, has a depressive effect. Since propranolol has no direct action, and works rather by blocking the adrenergic receptor from stimulation by the endogenous neurotransmitter or administered agonists, the reduction in the progesterone secretion rate in response to propranolol described here suggests that the measured pre-infusion secretion rate was itself the consequence of endogenous, physiological adrenergic stimulation. This agrees with the earlier observation of decreased peripheral progesterone concentrations in sheep with denervated ovaries (Wheeler & Blackshaw, 1986). Presumably therefore, variation in the activity of the autonomic sympathetic nervous system has an effect similar to that produced by the drugs used here, increasing sympathetic tone increasing adrenergic neurotransmitter (principally noradrenaline) and adrenal medulla hormone (principally adrenaline) release and consequently ovarian progesterone secretion.

These observations extend earlier findings that adrenergic stimulation increases progesterone concentrations produced by the human (Fylling, 1971) and sheep (Flint *et al.*, 1974) placenta,

Fig. 4. Ovarian follicular oestradiol secretion rate before, during and after the infusion of propranolol (Cat 97; ● — ●) and isoprenaline (Cat 94; □ — □).

secretion *in vitro* by bovine (Condon & Black, 1976), ovine (Jordan *et al.*, 1978) and rat (Ratner *et al.*, 1980) luteal tissue and human placental tissue (Caritis *et al.*, 1983), and rat ovarian secretion (Zsolnai *et al.*, 1982). Most research on the effects of adrenergic neurotransmitters and sympathomimetic drugs on progesterone secretion has been conducted on pregnant women or sheep, or luteal or placental tissue *in vitro*. Apart from that reported by Zsolnai *et al.* (1982) this seems to be the only study on luteal progesterone secretion. Further, apart from the similar finding by Godkin *et al.* (1977) who used bovine luteal tissue *in vitro* this is the only demonstration that a β -adrenergic antagonist (propranolol) can reduce the progesterone secretion rate.

There is evidence that stress, and presumably increased sympathetic tone (Selye, 1950), stimulates increased progesterone concentrations. Undernutrition of the sheep results in greater peripheral progesterone concentrations (Cumming *et al.*, 1971). Similarly, extreme (stressful) ambient temperatures, both low (ewes: Lamond *et al.*, 1972; cows: Rosenberg *et al.*, 1982; buffalo cows: Rao & Pandey, 1982) and high (cows: Stott & Wiersma, 1973; ewes: Wheeler & Blackshaw, 1986), have been associated with increased peripheral progesterone concentrations. Further evidence is provided by the decrease in the plasma progesterone concentration during a 3-h intravenous infusion of saline into early pregnant women (Fylling, 1971), presumably due to the subjects acclimatising to the unfamiliar procedure with a consequent decreasing sympathetic stimulation of steroid secretion.

There may be a relationship between changes in the sympathetic tone induced by the emotional anxiety and physiological stress in female prisoners of war and their observed secondary amenorrhea (Whitacre & Barrera, 1944). Similarly, the stress of leaving the family home has been blamed for the regression to juvenile-type anovulation menstrual cycles in young ladies (Metcalf *et al.*, 1983). These are just two of the many effects of stress on reproduction that are difficult to explain (Ramaley, 1981), but may be mediated by the autonomic innervation of the gonad.

The depression of endogenous progesterone secretion by β -antagonists (Godkin *et al.*, 1977) has possibilities as a post-coital contraceptive administered either early in the luteal phase of the cycle to interfere with the transport of the fertilized ovum (Black & Asdell, 1959), late in the luteal phase to induce luteal regression (McCracken, 1980; Okulicz *et al.*, 1981). A post-coital contraceptive would fill a useful gap in the range of contraceptives currently available, all of which

require a certain amount of premeditation and therefore do not cater for occasional and infrequent sexual contact.

The rapid and temporary nature of the isoprenaline-stimulated increases in the progesterone secretion rates presumably reflects the similar pattern found in the concentration of the mediator of this effect, cyclic adenosine monophosphate (Norjavaara *et al.*, 1983) and the brief half-life of the drug (Weiner, 1980). The sustained depression caused by propranolol is presumably due to this drug's long half-life (4 h; Kornhauser *et al.*, 1978).

In all the experiments using pseudopregnant cats except two (Cats 60 and 124) the ovarian blood flow during the infusion period was less than that during the preceding period. Since this decrease occurred irrespective of the drug infused, this change in the ovarian blood flow was presumably due to a general systemic vasoconstriction by the animal in response to the decreasing blood volume caused by the continuous collection of the ovarian venous blood.

Although one of the two isoprenaline infusions and the propranolol infusion appeared to have statistically significant effects on the follicular oestradiol secretion rate, the pattern of neither variation is convincing, and the β -adrenergic agonist (isoprenaline) and antagonist (propranolol) apparently similarly increased the oestradiol secretion rate. Therefore, although there appear to be no differences between follicular and luteal β -adrenergic receptor concentrations (Jordan, 1980), there was no convincing influence of the β -adrenergic receptors on ovarian follicular activity, agreeing with earlier findings for the rat (Adashi & Hsueh, 1981) and disagreeing with a subsequent report (Zsolnai *et al.*, 1982). Nevertheless, this observation is disappointing since the oestradiol secretion rate is generally presumed to reflect ovarian follicular growth and development, which in turn is a necessary pre-requisite for ovulation. These observations fail to support earlier evidence that the ovarian innervation influences follicular growth and maturation in hypophysectomized mice (Grob, 1974) and ovulation in hens (Ferrando & Nalbandov, 1969), rabbit (Virutamasen *et al.*, 1971) and rat (Peppler & Jacobs, 1976; Kannisto *et al.*, 1985), and that denervation results in increased oestradiol concentrations (Chihai *et al.*, 1976). These conflicting findings regarding the influence of ovarian innervation on follicular oestradiol secretion, growth and maturation and ovulation leave unresolved proposals that such ovarian innervation may be the mechanism by which compensatory hypertrophy following unilateral ovariectomy (Burden & Lawrence, 1977) and the inequality of ovulatory activity between the left and right ovaries (Wheeler, 1978) might be produced.

The variability of the progesterone secretion rates may have been due to transitory kinking of the blood vessels and cannula that we were unaware of, or a reflection of the pulsatile nature of progesterone secretion (Bäckström *et al.*, 1982).

The low oestradiol secretion rates in 2 of the 5 animals were surprising since these cats had all been treated with PMSG in the same way as the other animals that subsequently received hCG and invariably ovulated and formed corpora lutea.

The progressive and sustained decrease in the arterial blood pressures observed during the saline-only infusion (Fig. 1) are presumably similarly due to the decreasing blood volume as the ovarian venous outflow was collected. This effect is therefore probably present in all the experiments.

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