Adrenergic intracerebroventricular stimulation affects progesterone concentration in the ovarian vein of the rat: participation of the superior ovarian nerve

M A De Bortoli, M H Garraza and L I Aguado

Laboratorio de Biología de la Reproducción (LABIR), Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, (5700) San Luis, Argentina

(Requests for offprints should be addressed to L I Aguado, Universidad Nacional de San Luis, Chacabuco 917, (5700) San Luis, Argentina)

Abstract

The present study investigates the acute consequences of central adrenergic stimulation on the release of steroids from the ovary. The influence of the superior ovarian nerve (SON) and the relationship between the neural effect and peripheral LH levels were also examined.

The intracerebroventricular (i.c.v.) injection of 5 µg epinephrine in SON-intact rats on day 1 of dioestrus (D1) increased progesterone levels in ovarian vein blood from 7 to 21 min after injection but the same injection in SON-intact rats on day 2 of dioestrus (D2) decreased progesterone levels in ovarian vein blood from 1 to 25 min. A smaller dose (0.5 µg) of epinephrine injected i.c.v. in SON-intact rats produced a decrease in progesterone levels in ovarian vein blood of shorter duration. In SON-transected (SONt) animals, 0.5 µg epinephrine i.c.v. caused a smaller decrease in progesterone levels compared with SON-intact rats ($P < 0.05$). On the other hand, in SON-intact rats on D2, the i.c.v. injection of 0.5 µg epinephrine did not modify the peripheral LH levels during 25 min, but 5 µg epinephrine injected i.c.v. raised the peripheral LH level from the third minute after

injection ($P < 0.05$). Oestradiol levels in the ovarian vein blood did not change after epinephrine i.c.v. injection in rats on D2.

To avoid any humoral influence, SONt and SON-intact rats on D2 were injected i.c.v. with 5 µg epinephrine or with vehicle, and 5 min later the ovaries were incubated *in vitro* with or without LH. Under these conditions, it was demonstrated that the previous injection of epinephrine in SON-intact rats resulted in a diminished release of progesterone from ovaries incubated with or without LH.

These results suggest that a central adrenergic stimulus increases progesterone release from the ovary on D1 and decreases it on D2. Also, this neural input would arrive at the ovary through the SON, and would condition the ovarian response to LH on D2. Ovarian progesterone changes could be attributed to signals coming from ganglionic neurons, which are affected by the central adrenergic stimulation.

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Introduction

The rat ovary receives innervation from two main sources: the ovarian plexus nerve that travels along the ovarian artery, and the superior ovarian nerve (SON) which is associated with the suspensory ligament (Lawrence & Burden 1980, Burden 1985). Microscopical studies show the SON fibres innervating thecal and interstitial cells, and also fibres coming from the ovarian plexus nerve associated with the vascular ovarian system (Baljet & Drukker 1980, Burden 1985). Receptors of the beta adrenergic type have been characterized in ovarian membranes (Jordan 1981, Aguado *et al.* 1982) and have been found in different populations of ovarian cells (Condon & Black 1976, Hsueh *et al.* 1984, Norjavaara *et al.* 1989). Additionally, the occupation of such receptors with adrenergic agonists

produced progesterone and androgens release in the whole ovary *in vitro* (Aguado *et al.* 1982), and in luteal, granulosa and interstitial cells in culture (Condon & Black 1976, Aguado & Ojeda 1984a, Hsueh *et al.* 1984, Erickson *et al.* 1985, Norjavaara *et al.* 1989).

Transection of the SON 48 h previously results in a drop in the number of ovarian beta adrenergic receptors (Marchetti *et al.* 1987), while transection 7 days previously raises them (Aguado & Ojeda 1984b). Additionally, electrical stimulation or the acute transection of the SON performed in live anaesthetized animals enhances or inhibits respectively the progesterone release from the ovary (Weiss *et al.* 1982, Aguado & Ojeda 1984a).

The neurons from which the SON originate are located in the para- and prevertebral ganglia (Klein & Burden 1988). Evidence of the neural connection between the

central neurons and the ovary has been provided by electrical stimulation of central nuclei (Kawakami *et al.* 1972, 1981, Chiappas *et al.* 1977).

Although important advances have been made in this field, the role of the innervation in ovarian physiology remains obscure. In the present work, the effect of a central adrenergic stimulation on progesterone release from the ovary was studied in two cycle stages in which the corpus luteum is predominant in ovarian physiology. Also, the participation of the SON and the relationship between the neural effect and the level of luteinizing hormone (LH) were analysed.

Materials and Methods

Animals

Virgin Holtzman strain female rats weighing 250–300 g were used in all the experiments. Animals had free access to food (Cargill SACI, Saladillo, Provincia de Buenos Aires, Argentina) and water. They were housed in cages under controlled light (lights on from 0700 to 1900 h) and temperature ($24 \pm 2^\circ\text{C}$).

Ventricle cannulation

Only rats showing at least two consecutive 4-day oestrous cycles were used. By means of a stereotaxic apparatus, a 22-gauge stainless steel guide cannula was implanted chronically. The tip of the cannula was located within the right lateral cerebral ventricle (4.0 mm ventral to the bregma and 1.5 mm lateral to the midline) and was later used for the intracerebroventricular (i.c.v.) microinjections. Ether was used as the anaesthetic, and the animals were maintained in individual boxes after the completion of the surgery. All rats were allowed to recover for 1 week after the stereotaxic surgery in order to recover their pre-surgical body weight. After that, on the following day 1 of dioestrus (D1), or day 2 of dioestrus (D2), the animals were used for the experimental procedures described below.

Ovarian vein cannulation

On the day of the experiment, shortly before i.c.v. injection, the left ovarian vein was cannulated as follows: a needle covered with a teflon tube (Abbocath-T 24-G) was passed through the left renal vein and 0.2 ml heparin saline solution (200 U/ml heparin in 0.9% NaCl; Abbot Lab, Sarmiento, Buenos Aires, Argentina) was injected. The needle was then removed and the left ovarian vein cannulated with the teflon tube, as described previously (Shaikh & Shaikh 1975). The left uterine vein was ligated to avoid blood draining into the left ovarian vein.

All the vein cannulations and blood sample collections were carried out under chloral hydrate (Merck Química,

Roseti, Buenos Aires, Argentina) anaesthesia (35 mg/100 g body weight, i.p.).

SON transection

The procedure was described in previous reports (Lawrence & Burden 1980, Aguado & Ojeda 1984b). Briefly, before ovarian vein cannulation was completed, the suspensory ligament that contains the left SON was isolated by passing a suture thread under it. Cannulation of the ovarian vein was then completed, and the suspensory ligament was carefully lifted and severed not less than 1 cm from the ovary. In the sham-operated group, the procedure used was the same, but the SON was not transected.

Jugular vein cannulation

In another group of animals also carrying the ventricular cannula, the jugular vein was cannulated as previously described (Harms & Ojeda 1974, Aguado & Ojeda 1984c). After each blood extraction, samples were centrifuged and the plasma was frozen until LH determination. The original blood volume was recuperated by adding saline solution to the red cells and this was quickly returned to the animal through the jugular cannula.

Ovarian incubations

In the first experiment, two groups of rats were used: the sham-operated (SON-intact), and the SON transected (SONt). These two groups were injected i.c.v. with $5\ \mu\text{g}$ epinephrine, and 5–6 min later ovaries were removed, cleaned, and the oviducts were removed. The ovaries were then incubated in halves in a Dubnoff metabolic bath (37°C , with 95% O_2 and 5% CO_2), while being continuously agitated.

When testing the effect of LH, the ovaries were obtained only from animals with intact SON. Two groups were injected i.c.v. with $5\ \mu\text{l}$ vehicle or $5\ \mu\text{g}$ epinephrine respectively. The ovaries were incubated with ovine LH ($50\ \text{ng/ml}$ incubation buffer).

The *in vitro* incubations were carried out using 2 ml Krebs Ringer bicarbonate buffer, pH 7.4, as previously described (Selstam *et al.* 1976, Advis & Ojeda 1978, Advis *et al.* 1979, Aguado *et al.* 1982, Lara *et al.* 1990a,b, Ferruz *et al.* 1991). To retain the *in vivo* neural influence received by the epinephrine i.c.v. injection, the preincubation period was shortened to 15 min. The determinations of progesterone showed that the system becomes stable during this time (data not shown). Samples of the incubation buffer ($200\ \mu\text{l}$) were taken at 30, 60, 120 and 180 min. The results were expressed as ng progesterone/mg ovary.

Experimental protocols

The following experiments were performed on D1 between 1800 and 1900 h, or on D2 between 1600 and

1800 h. On D1, the experiments were performed after 1800 h in order to study the ovarian sensitivity to the neural inputs in coincidence with the progesterone rise described at this time (Uchida *et al.* 1969, Smith *et al.* 1975).

Blood flow determination

The blood flow was assessed in the ovarian vein, cannulated as described above, allowing the free flow of blood. Animals were divided into two groups: one group received no treatment (control) and the other group was injected i.c.v. with 5 µg epinephrine at time zero. Blood samples were collected during 25 min, once per min during the first 5 min, and then every 2 min. The volume of each sample was assessed by means of a set of calibrated tubes.

Basal values of progesterone

At the time of the experiments, basal values of progesterone in the ovarian vein blood were measured during 25 min following the i.c.v. injection of the vehicle (5 µl ascorbic acid Sigma solution, 0.1 mg/ml in 0.9% saline) (Ba).

The effect of SON transection on the basal levels of progesterone in the ovarian vein was tested in blood samples collected after surgery (Bson). The means of the basal progesterone values obtained in each case were considered as 100% for future experiments.

Intracerebroventricular injections

After ovarian or jugular vein cannulation, the rats were subjected to i.c.v. administration of epinephrine under chloral hydrate anaesthesia.

Two doses, 5 or 0.5 µg, of epinephrine bitartrate (Sigma, St. Louis, MO, USA) were used in different groups of animals. The drug was dissolved in 5 µl vehicle (ascorbic acid Sigma solution, 0.1 mg/ml in 0.9% saline). Each dose was injected slowly into the right lateral ventricle during 1 min. The end of this injection was considered as time zero. After this, blood samples were collected from the ovarian vein for 25 min according to the schedule described for blood flow determination. After centrifugation, the plasma was collected and stored frozen until progesterone determination.

Jugular vein blood was collected in other animal groups at 3, 10, 17 and 25 min after epinephrine i.c.v. injection. The volume of plasma necessary to measure LH (100 µl) required the extraction of at least 0.5 ml blood in each sample. The preservation of the physiological condition of the animals thus made it impossible to obtain samples every minute. Therefore, we chose the times at which major changes in progesterone concentration in the ovarian vein occurred. After centrifugation, the plasma was collected and stored frozen until LH assay.

Hormone assays

Progesterone and oestradiol were measured by RIA. Plasma was extracted as previously described (Jahn *et al.* 1995). The antisera were kindly provided by Dr R P Deis (Laboratorio de Reproducción y Lactancia, Mendoza, Argentina). The assays have previously been validated (Bussmann & Deis 1979, Donoso 1988).

LH was measured by RIA, using a kit provided by the NIADDK of NIH (Dr Raiti, NIADDK Rat Pituitary Hormone Distribution Program), as recommended. The results were expressed in terms of LH-RP-2 NIH standard preparation.

Data analysis

Results are presented as means $\pm$ S.E.M. in each group. Differences between groups were analysed with Student's *t*-test when two groups were compared. The analysis of the variance (ANOVA) followed by Duncan's multiple range test was used for several comparisons (Snedecor & Cochran 1976). A value of $P < 0.05$ was considered statistically significant.

Results

Blood flow

To detect if central adrenergic stimulation affects the ovarian vessels, the blood flow was measured under experimental conditions ($n=7$). The volume of blood collected per minute from the ovarian vein of both control and epinephrine i.c.v. injected animals was analysed. When both groups were compared, no statistical differences could be detected. The values obtained were: control rats, 89.4 ± 8.7 µl/min; epinephrine i.c.v. injected rats, 83.9 ± 9.8 µl/min.

Effect of epinephrine i.c.v. injection on ovarian vein progesterone levels on D1 (1800–1900 h)

Figure 1 (top panel) shows the levels of progesterone concentration in ovarian vein blood when vehicle or 5 µg epinephrine was injected i.c.v. at time zero. When both groups of animals were compared at each time point the concentration of progesterone increased between 7 and 21 min ($P < 0.02$ at 7, 9 and 17 min; $P < 0.01$ at 11, 13 and 15 min; $P < 0.05$ at 19 and 21 min) in the rats injected with epinephrine.

Effect of epinephrine i.c.v. injection on the ovarian vein progesterone levels on D2 (1600–1800 h)

Figure 1 (bottom panel) shows the concentration of progesterone in ovarian vein blood when the vehicle or 5 µg epinephrine was injected i.c.v. at time zero. When both groups of animals were compared, the progesterone

Figure 1 Concentration of progesterone in the ovarian vein: basal values after i.c.v. injection of vehicle (○) or 5 µg epinephrine (Epi) (△) to rats on dioestrus day 1 (D1) (top panel) and on dioestrus day 2 (D2) (bottom panel). The values represent the means $\pm$ S.E.M. of five experiments. Top panel: * $P < 0.05$, ** $P < 0.02$, *** $P < 0.01$; bottom panel: * $P < 0.025$, ** $P < 0.01$ compared with basal values at the same time points.

values decreased between 1 and 25 min after the i.c.v. injection of epinephrine reaching minimal values at 3 min ($P < 0.025$ at 1, 21, 23 and 25 min; $P < 0.01$ between 2 and 19 min).

Figure 2 (top panel, circles) shows the progesterone concentration in ovarian vein blood when SON-intact rats were injected i.c.v. with 0.5 µg epinephrine. In these animals, the concentration of progesterone (as a percentage of Ba, where $Ba = 37.5 \pm 1.62$ ng progesterone/ml) decreased between 1 and 13 min after i.c.v. injection ($P < 0.05$ at 1 and 13 min; $P < 0.01$ between 2 and 11 min). Basal progesterone concentrations were reached at 25 min. In these animals the decrease in progesterone levels between 19 and 25 min was less than in animals injected i.c.v. with 5 µg epinephrine ($P < 0.05$).

In SON-transected (SONt) animals (Fig. 2, top panel, triangles), the values of progesterone (as a percentage of Bson, where $Bson = 35.0 \pm 1.49$ ng progesterone/ml) decreased between 2 and 7 min ($P < 0.05$ at 2, 4 and

Figure 2 Top panel: concentration of progesterone in the ovarian vein after i.c.v. injection of 0.5 µg epinephrine (Epi) to SON-intact (○) or to SONt (△) rats on D2. The values are the percentage of the basal value, Ba, for SON-intact rats and the percentage of the basal value, Bson, for SONt rats respectively, and represent the means $\pm$ S.E.M. of five experiments. * $P < 0.05$, ** $P < 0.025$, *** $P < 0.01$ (vs Ba or Bson respectively). ^a $P < 0.05$ for comparison of SON-intact vs SONt rats at each time point. Bottom panel: peripheral LH levels after i.c.v. injection of 0.5 µg (○) or 5 µg (△) epinephrine (Epi) to rats on D2. The values represent the means $\pm$ S.E.M. of five experiments. * $P < 0.05$, ** $P < 0.01$ comparing each value of both doses vs basal values.

7 min; $P < 0.025$ at 3 and 5 min) and basal levels were reached at 9 min. When SON-intact and SONt rats injected i.c.v. with 0.5 µg epinephrine were compared, the former group had lower progesterone values than the latter group ($P < 0.05$ between 2 and 13 min).

Effect of epinephrine i.c.v. injection on circulating LH levels on D2 (1600–1800 h)

The concentrations of LH in jugular vein blood of rats (with intact SON) after 0.5 or 5 µg epinephrine i.c.v. injection are shown in Fig. 2 (bottom panel). LH basal values were 0.56 ± 0.08 ng/ml. This experiment allows the study of LH levels when the central adrenergic stimulation affects progesterone secretion from the ovary *in vivo*. When the lower dose (0.5 µg) of epinephrine was

Figure 3 Top panel: concentration of progesterone released per mg ovary incubated after *in vivo* i.c.v. injection of epinephrine (Epi) to SON-intact and SON-transected rats on D2. The values represent the means $\pm$ S.E.M. of five experiments. $*P < 0.05$ (SON-intact vs SON-t rats). Bottom panel: concentration of progesterone released per mg ovary incubated with LH, after *in vivo* i.c.v. injection of vehicle or epinephrine to SON-intact rats on D2. The values represent the means $\pm$ S.E.M. of five experiments. $*P < 0.05$ (Epi vs vehicle i.c.v. injected rats).

administered i.c.v. there was no difference in the circulating levels of LH compared with basal values. However, when the higher dose (5 μ g) of epinephrine was administered, the levels of LH increased from 3 to 25 min ($P < 0.05$ at 3, 10 and 17 min; $P < 0.01$ at 25 min).

In vitro ovarian incubation after the i.c.v. injection of epinephrine on D2 (1600–1800 h)

Ovaries were removed after i.c.v. injection of 5 μ g epinephrine and were incubated *in vitro*. The ovaries from sham-operated rats with intact SON (Fig. 3, top panel), released less progesterone than the ovaries from SON-transected animals. Progesterone release was different ($P < 0.05$) between both groups at 30 min (0.65 ± 0.12 vs 0.30 ± 0.07 ng/mg ovary) and at 60 min (1.07 ± 0.20 vs

0.45 ± 0.12 ng/mg ovary). No significant differences were observed at 120 and 180 min.

In SON-intact animals, in which the central stimulus would arrive at the ovary, progesterone release was inhibited compared with rats previously SON-transected.

When ovaries from rats (all with intact SON) injected i.c.v. with vehicle or 5 μ g epinephrine were incubated *in vitro* with LH, different amounts of progesterone were released (Fig. 3, bottom panel). Thus, the ovaries from animals injected i.c.v. with epinephrine released less progesterone than ovaries obtained from rats injected i.c.v. with vehicle ($P < 0.05$ at 30–180 min).

Effect of epinephrine i.c.v. injection on oestradiol levels in the ovarian vein on D2 (1600–1800 h)

Ovarian granulosa and luteal cells possess beta adrenergic receptors but are not directly innervated. This experiment was conducted to study the effect of central adrenergic stimulation on ovarian oestradiol release, the principal steroid produced by granulosa cells.

After i.c.v. injection of 5 μ g epinephrine, oestradiol levels in the ovarian vein blood did not change significantly during 25 min, compared with its own basal levels (262 ± 51 pg/ml) on D2 ($n = 7$) (data not shown).

Discussion

Evidence has been provided supporting a neural connection between the central nervous system (CNS) and the ovary. Thus, electrical stimulation of some central nuclei affect progesterone and oestradiol release from the ovary (Kawakami *et al.* 1981) without any changes in gonadotrophins (Kawakami *et al.* 1972) or gonadotrophin-releasing hormone (GnRH) release (Chiappas *et al.* 1977).

There are nervous fibres descending from the hypothalamus to the spinal cord that influence the peripheral neurons of the autonomic system (Saper *et al.* 1976). Descending projections of the hypothalamic paraventricular nucleus and the lateral hypothalamic area to sympathetic and vagal preganglionic neurons have been identified in the rat (Hosoya *et al.* 1991, Loewy 1991). In addition, sympathetic neurons reaching the ovary have been detected in para- and prevertebral ganglia by means of a retrograde tracer applied on the SON (Klein & Burden 1988).

On the other hand, the presence of beta adrenergic receptors has been reported in the hypothalamus (Wilkinson *et al.* 1979, Wanaka *et al.* 1989) and in the hypothalamic GnRH neurons (Findell *et al.* 1993), and the challenge of these receptors by an agonist stimulates the release of GnRH *in vitro* (Martínez de la Escalera *et al.* 1992). The presence of alpha adrenergic receptors has also been reported in the hypothalamic paraventricular, supraoptic and arcuate nuclei (Pascual *et al.* 1992, Rosin *et al.* 1996).

Furthermore, epinephrine injected i.c.v., travels from the cerebral spinal fluid (CSF) to the hypothalamus and produces an increase in circulating LH; it is more effective than norepinephrine during a period of 30 min (Krieg & Sawyer 1976, Vijayan & McCann 1978, Gallo & Drouva 1979, Leung *et al.* 1982), which is why epinephrine was used in this study.

Considering all this evidence together, we can assume that epinephrine injected i.c.v. reaches the hypothalamus, impacting upon adrenergic receptors and affecting the autonomic function. Thus, we hypothesize that the central stimulation produced by epinephrine modifies ovarian function.

The effect of epinephrine on gonadotrophin release (McCann 1975, Krieg & Sawyer 1976, Gallo & Drouva 1979, Donoso 1988), allows the study of peripheral neuroendocrine integration between the hormones of the pituitary and the neural inputs to the ovary, which is the primary objective of this paper.

Release of progesterone was assessed because it appears to be the steroid most responsive to neural stimulation (Aguado *et al.* 1982, 1984a), and consequently two of the cycle stages in which the corpus luteum predominates in the ovary were selected. Thus the progesterone released by the ovary comes mainly from the corpus luteum and not from the granulosa cells in our experimental design.

In this animal preparation, changes in steroid concentration in the ovarian vein produced by i.c.v. injection of epinephrine, can be checked almost continuously during 25 min, making the system appropriate for the study of neural effects. The blood collection times used agreed with the electrical registers in the median eminence and in the arcuate nucleus after catecholamine i.c.v. administration (Weiner *et al.* 1971, Krieg & Sawyer 1976). These registers were affected immediately after injection and for up to 40 min (Sawyer 1979).

A point of discussion is the neural effect on ovarian blood flow. Here it has been demonstrated that, in the period studied, blood flow in the ovarian vein was not affected by i.c.v. epinephrine administration. Thus, it was assumed that changes in progesterone concentration in the ovarian vein were not due to variability in the blood volume draining the ovary. Additionally, the basal levels of progesterone in the ovarian vein in rats injected i.c.v. with vehicle remains unaffected on D2. Perfusing the ovary of rats with adrenergic agonists produces a fall of 30% in the blood flow rate but this does not modify progesterone release from the corpus luteum (Massa & Bruce 1994). In our case, the fact that i.c.v. administration reversed the effect on progesterone release on D1 compared with D2 under the same experimental conditions, led us to think that the small but not significant changes in the blood flow did not influence steroid output.

When adrenergic agonists act directly on the ovary in the *in vitro* systems (Adashi & Hsueh 1981, Aguado *et al.* 1982, Aguado & Ojeda 1984b) or by systemic admin-

istration (Bahr *et al.* 1974, Ojeda & Aguado 1985), progesterone release always increases. In the present experiments, the drop in progesterone output observed on D2 led us to consider that the effect produced by the i.c.v. administration of epinephrine could not be accounted for by modifications in the circulating catecholamines levels.

Additionally, the fact that the release of progesterone was modified in opposed sense at D1 and D2 suggests that some other neurotransmitter distinct from norepinephrine is affecting the ovary in D2 (Dissen *et al.* 1993).

When a dose of 0.5 µg epinephrine was injected i.c.v. into D2 animals, the fall in progesterone release observed in SON-transected animals was less pronounced and shorter than that observed in SON-intact rats. This finding supports the idea that SON participates, at least in part, in the neural pathway which links the CNS and the ovary. The remaining suppression of progesterone release that occurred in SON-transected animals could have been produced by the effect of epinephrine i.c.v. injection on the intact plexus ovarian nerve (Burden *et al.* 1981). Doses of 4 to 25 µg norepinephrine or epinephrine administered i.c.v. usually produce an LH surge in peripheral blood after 10 to 20 min (Vijayan & McCann 1978, Krieg & Sawyer 1976, Rance *et al.* 1981, Wise *et al.* 1981, Donoso 1988). In this study, the smaller dose (0.5 µg) of epinephrine did not modify LH levels, but a larger amount of epinephrine (5 µg) injected i.c.v., as by these authors, raised the gonadotrophin level from 3 to 25 min in the jugular vein blood. Perhaps this increase in LH masks a more pronounced neural effect on ovarian progesterone output on D2. Otherwise, it is possible that the enhanced progesterone release observed on D1 after i.c.v. epinephrine injection, was affected by a similar LH increase found on D2. Anyway, the progesterone peak that occurs on the afternoon of D1 in cyclic rats has been described as independent of gonadotrophin action (Uchida *et al.* 1969, Barraclough *et al.* 1971, Smith *et al.* 1975).

Considering that both doses of i.c.v. injected epinephrine inhibited the release of ovarian progesterone in rats on D2, we conclude that the neural effect is overwhelming the endocrine LH effect, and opens the possibility that the neural inputs could modulate ovarian sensitivity to the endocrine stimulations. In fact, an interrelationship between gonadotrophins and norepinephrine effects has been reported at the ovarian level (Aguado & Ojeda 1984a, 1986, Ferruz *et al.* 1991). The clear effect found on progesterone release by epinephrine i.c.v. injection, however, contrasts with the lack of effect observed on oestradiol output. This finding agrees with previous results which suggest that oestradiol is modified by neural inputs only at pro-oestrus (Aguado & Ojeda 1984c).

To take advantage of the pronounced effect on progesterone release shown after the 5 µg epinephrine i.c.v. injection on D2, we carried out ovarian incubation shortly thereafter. This *in vitro* system has been widely validated previously (Hamberger & Ahrén 1967, Selstam *et al.* 1976,

Advis & Ojeda 1978, Advis *et al.* 1979, Aguado *et al.* 1982, Lara *et al.* 1990a,b). In this condition, ovaries from rats previously injected i.c.v. with epinephrine were less responsive to the LH action in terms of progesterone release. If animals were previously SON transected, the inhibiting effect of epinephrine i.c.v. injection on ovarian progesterone output is less pronounced. These results confirm the neural nature of the effect shown on progesterone release; the idea of the participation of the SON also receives strong additional support. Experiments carried out by direct manipulation of the SON have suggested that the nerve maintains or even enhances progesterone output from the ovary (Weiss *et al.* 1982, Aguado & Ojeda 1984c). However, as we have seen, adrenergic i.c.v. administration increases the progesterone release on D1 and inhibits it on D2. A similar pattern was observed when the celiac ganglion was stimulated by adrenergic agonists in an *in vitro* system (Z Sosa & LI Aguado, unpublished observations). In view of this fact, we hypothesize that the i.c.v. injection of epinephrine primarily affects the central neurons which, in turn, stimulate other neurons that reach the ovary. The location of the former neurons remains unknown, but the location of the latter neurons could be in the paravertebral ganglia and especially the preaortic ganglia (Messenger *et al.* 1994). Of great interest is the fact that the effect produced in the ovary by central adrenergic stimulation is reversed between the D1 and D2 stage. This different action could be attributed to the central effect of the first small peak of progesterone occurring on D1 (Uchida *et al.* 1969), or to the rise in the oestradiol levels which starts on the afternoon of D1 (Shaikh & Shaikh 1975). In fact, the influence of the CNS on the reproductive realm is modified by ovarian steroid in different cycle states and in ovariectomized estrogen–progesterone primed rats (Freeman 1994).

Finally, more investigation is needed to understand better the neural influence on the ovary, and particularly on the corpus luteum.

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