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Epinephrine intracerebroventricular stimulation modifies the LH effect on ovarian progesterone and androstenedione release

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Abstract

This study investigates the interaction between the effect of epinephrine intracerebroventricular (i.c.v.) injection and LH on the progesterone concentration in ovarian vein blood (Po) in vivo, and also, on the release of ovarian progesterone and androstenedione in vitro, in rats on dioestrus day 2. When 2 mg ovine LH were injected in vein (i.v.), Po increased reaching 120 ± 12.2 and 151 ± 17.5 ng ml^{-1} at 22 and 25 min, respectively. Another group of rats was injected intracerebroventricular with 5 μg epinephrine at time zero, and with 2 mg ovine LH i.v. 3 min later. This time Po decreased during the first 3 min, then increased, reaching 64 ± 7.1 ng ml^{-1} at 25 min, lower than the Po obtained 22 min after LH i.v. injection only ($P < 0.01$). Moreover, rats were injected i.v. with 2 mg ovine LH at time zero, and 7 min later with epinephrine intracerebroventricular. Po increased during the first 7 min, decreased until the 13th minute and reached 70 ± 8.9 ng ml^{-1} at 25 min, lower than the Po obtained 25 min after LH i.v. injection only ($P < 0.01$). In other experience, rats with one (either right or left) superior ovarian nerve transected (SON-t), were injected intracerebroventricular with epinephrine. Five minutes later, the ovaries were removed and incubated in vitro with LH. Both ovaries (right or left) in which the SON was intact at time of epinephrine i.c.v. injection, showed a reduction of progesterone and androstenedione released in vitro ($P < 0.05$). These results suggest that, on dioestrus day 2, the central adrenergic stimulus competes with LH in the release of ovarian progesterone. Also, the neural input that arrives at the ovary through the SON would antagonize the ovarian progesterone and androstenedione response to LH.

Keywords: Epinephrine; Progesterone; Androstenedione; Ovary LH

1. Introduction

The rat ovary has been described as receiving 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 [1,2].

Microscopic studies show that the SON fibres end directly on the secondary interstitial cells and near the theca interstitial cells, while the fibres coming from the ovarian plexus nerve are associated with the ovarian vascular system [2–4].

Evidence of communication between brain neurons and the ovary has been provided by electrical stimulation of central nuclei of rats [5–7], producing changes in both the release and synthesis of estrogen and progesterone by the ovary. In agreement, the intracerebroventricular (i.c.v.) injection of epinephrine increases the progesterone concentration in ovarian vein blood of the rat on dioestrus day 1, and decreases it on dioestrus day 2 [8]. In this paper the participation of the SON in these effects was also demonstrated.

At the ovarian level, the effect of norepinephrine and VIP (found in the SON) is to increase these steroids' release [4,9,10]. On the other hand, the occupation of GnRH receptors in luteal and granulosa cells decreases the LH-induced progesterone release [11–14].

The secretion of ovarian androstenedione from the interstitial cells is stimulated by LH [4,15], as well as by norepinephrine and beta adrenergic agonists [9,15,16].

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In opposition, GnRH and oestradiol inhibit the androstenedione production of the ovarian interstitial cells [17–20].

Although important advances have been made in this field, our comprehension of the role of the innervation in ovarian physiology remains incomplete. The objective of this work was to investigate the relationship between the effect of epinephrine i.c.v. injection and of LH i.v. injection on the progesterone concentration in ovarian vein blood in vivo. Also, the interaction between the central adrenergic stimulation and LH on the in vitro release of ovarian progesterone and androstenedione was studied.

2. Material and methods

2.1. Animals

Virgin Holtzman strain female rats weighing 250–300 g were used in all the experiments. Animals had free access to food (Cargill, Buenos Aires, Argentina) and water. They were housed in cages under controlled light (lights on from 07:00 to 19:00 h) and temperature (24 ± 2°C).

2.2. Cerebral 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 i.c.v. microinjections. Ether (Tetrahedron, Buenos Aires, Argentina) 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 presurgical body weight. After that, on the following dioestrus day 2, the animals were used for the experimental procedures described below.

All the following procedures were carried out under chloral hydrate (Merck Química Argentina, Buenos Aires) anaesthesia (35 mg/100 g of body weight, i.p.).

2.3. SON transection

The procedure has been described in previous reports [1,10]. Briefly, before the i.c.v. injection, the suspensory ligament that contains the SON was isolated by passing a suture thread under it, and was carefully lifted and severed not less than 1 cm from the ovary.

The suspensory ligament is clearly visible and the SON travels along it. Thus, the transection of the suspensory ligament results in transection of the SON.

In the sham operated rats, the procedure used was the same, but the suspensory ligament and the SON were not transected.

2.4. Intracerebroventricular injection

All the i.c.v. injections were performed in rats on D2 between 16:00 and 18:00 h.

5 μg epinephrine bitartrate (Sigma, St Louis, MO, USA) were dissolved in 5 ml vehicle (ascorbic acid Sigma solution, 0.1 mg ml^{-1} in 0.9%w/v NaCl, Mallinckrodt, USA) and injected slowly into the right lateral ventricle. The injection lasted 1 min and the conclusion of this injection was considered time zero.

2.5. Ovarian vein cannulation

On the day of the experiment, shortly before i.c.v. or i.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^{-1} heparin, Abbot Lab, Buenos Aires, Argentina, in 0.9% w/v saline) was injected. The needle was then removed and the left ovarian vein cannulated with the teflon tube, as described previously [21]. The left uterine vein was ligated to avoid blood draining into the left ovarian vein.

In all experiences, ovarian vein blood samples were collected from the cannula during 25 min, once per min during the first 5 min, and then every 2 min. After centrifugation, the plasma was collected and stored frozen until progesterone determination.

2.6. Interaction between epinephrine i.c.v. and LH i.v. injection on ovarian vein progesterone

In these experiences, the effect of epinephrine injected i.c.v. was challenged by the effect of LH injected i.v. in rats on D2, between 16:00 and 18:00 h.

In the first step, doses of 0.5, 1, or 2 mg of ovine LH (Sigma, St Louis, MO, USA) in 0.2 ml saline were injected into the jugular vein of different groups of rats, to produce an important increase of the progesterone released to the ovarian vein during a time period of 25 min. The results obtained showed that 2 mg ovine LH was the dose which produced the highest Po levels during this time. Because of that, in subsequent steps this was the LH dose used.

In the second step, one group of five rats was injected i.c.v. with 5 μg epinephrine (time zero), and 3 min later, 2 mg ovine LH in 0.2 ml saline were injected into the jugular vein. The LH injection was made at that time due to the Po values having been at the lowest point recorded in our previous report [8].

In the third step, five rats were injected in the jugular vein with 2 mg of ovine LH in 0.2 ml saline (time zero), and 7 min later, 5 μg epinephrine were injected i.c.v.

In these experiences, the measurements of Po were performed at time zero (basal level), and during 25 min as was above described.

2.7. Ovarian incubations

These experiments were conducted between 16:00 and 18:00 h on D2, using two groups of 5 rats. In group I, only the left SON was transected and in group II, only the right SON was transected. Immediately, the two groups of animals were injected i.c.v. with 5 μg epinephrine, and 5 min later the ovaries were taken out, cleaned, and the oviducts removed. The ovaries were then incubated in halves in a Dubnoff metabolic bath (37°C, with 95% v/v O_2 and 5% v/v CO_2), while being continuously agitated. The in vitro incubations were carried out using 2 ml Krebs Ringer bicarbonate buffer, pH 7.4, as previously described [9,22–27], added with 50 ng of ovine LH (Sigma, St Louis, MO) per ml of incubation buffer. To retain the in vivo neural influence caused by the epinephrine i.c.v. injection, the preincubation period was shortened to 15 min. The levels of progesterone and androstenedione showed that the system becomes stable during this time (data not shown). Samples of the incubation buffer (250 μl) were taken out at 30, 60, 120 and 180 min for measuring progesterone and androstenedione. The results were expressed as ng/mg of ovary.

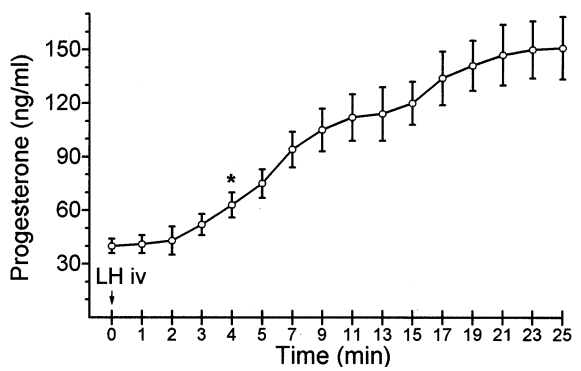

Fig. 1. Concentration of progesterone in the ovarian vein in rats on dioestrus day 2 after i.v. injection of LH. The values represent the means ± SEM of five experiments. *$P < 0.05$ compared with basal values at time 0.

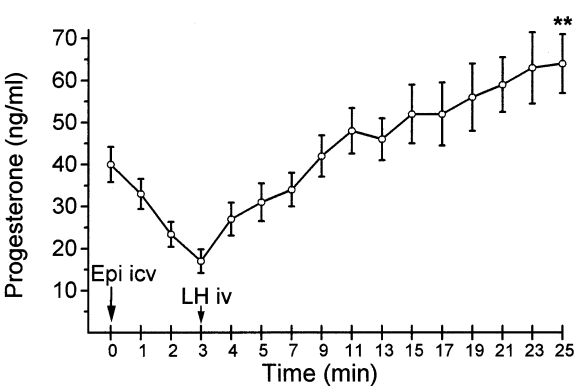

Fig. 2. Concentration of progesterone in the ovarian vein in rats on dioestrus day 2 after i.c.v. administration of epinephrine (Epi) at time 0 and i.v. injection of LH at 3 min. The values represent the means ± SEM of five experiments. **$P < 0.01$ compared with the 22nd min value of Fig. 1.

2.8. Hormone assays

P and androstenedione concentrations were measured in duplicate by radioimmunoassay. Plasma was extracted as previously described [28]. The antisera were kindly provided by Dr R. P. Deis (Laboratorio de Reproducción y Lactancia, Mendoza, Argentina). These assays have previously been validated [29,30].

2.9. Data analysis

Results are presented as mean ± SEM in each group of five or six rats. 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 [31]. In the in vitro experiences, the left ovaries vs the right ones of each animal were compared. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Interaction between epinephrine i.c.v. and LH i.v. injection on ovarian vein progesterone levels on D2 (16:00–18:00 h)

Fig. 1 shows the concentrations of progesterone in ovarian vein blood when 2 mg ovine LH were injected i.v. at time zero in rats on D2. The basal progesterone value was 40 ± 4.1 ng ml^{-1}. It increased from the 4th min ($P < 0.05$ vs. basal level), reaching concentrations of 120 ± 12.2 ng ml^{-1} and 151 ± 17.5 ng ml^{-1} at 22 and 25 min, respectively.

The levels of progesterone in ovarian vein blood when epinephrine (Epi) was injected i.c.v. at time zero and ovine LH was injected i.v. 3 min later were shown in Fig. 2. The progesterone concentrations decreased until the 3rd min, then increased, reaching 64 ± 7.1 ng ml^{-1} at 25 min. This Po value was lower ($P < 0.01$) than 120 ± 12.2 ng ml^{-1}, which was obtained 22 min after LH i.v. injection only (see Fig. 1).

Fig. 3 shows the concentrations of progesterone in ovarian vein blood when LH was injected i.v. at time zero and epinephrine was injected i.c.v. 7 min later. The progesterone levels increased until the 7th min, then decreased until the 11th min, reaching the highest value

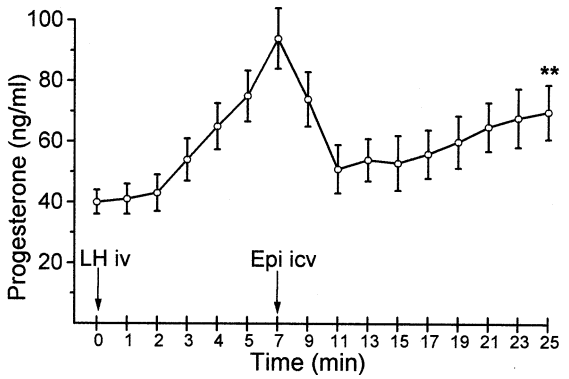

Fig. 3. Concentration of progesterone in the ovarian vein in rats on dioestrus day 2 after i.v. injection of LH at time 0 and i.c.v. administration of epinephrine (Epi) at 7 min. The values represent the means ± SEM of five experiments. $^{**}P < 0.01$ compared with the 25th min value of Fig. 1.

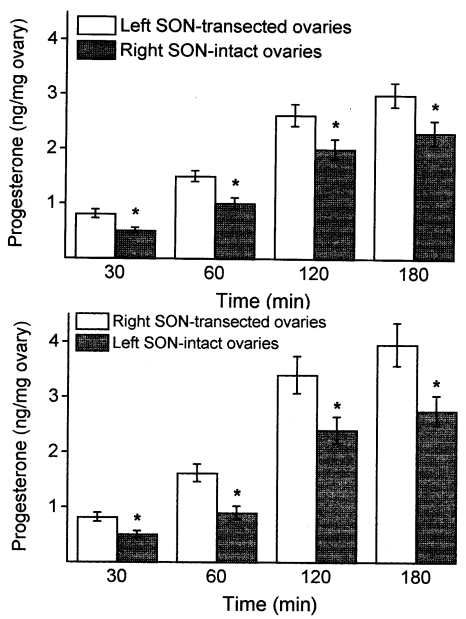

Fig. 4. Top panel: Concentration of progesterone released per mg ovary incubated with LH, after in vivo i.c.v. injection of epinephrine to rats on dioestrus day 2 with the left superior ovarian nerve (SON) transected. The values represent the means ± SEM of five experiments. $^{*}P < 0.05$ (left SON-transected vs right SON-intact ovaries). Bottom panel: Concentration of progesterone released per mg ovary incubated with LH, after in vivo i.c.v. injection of epinephrine to rats on dioestrus day 2 with the right superior ovarian nerve (SON) transected. The values represent the means ± SEM of five experiments. $^{*}P < 0.05$ (right SON-transected vs left SON-intact ovaries).

(70 ± 8.9 ng ml^{-1}) at 25 min. This Po value was lower than 151 ± 17.5 ng ml^{-1}, which was obtained 25 min after LH i.v. injection only ($P < 0.01$).

3.2. In vitro ovarian incubation after the i.c.v. injection of epinephrine on D2 (16:00–18:00 h)

Ovaries were removed after i.c.v. injection of 5 μg epinephrine and were incubated in vitro with LH.

Group I: The right ovaries that had the intact SON (when the i.c.v. injection was done) released less progesterone during the incubation than the left ovaries that had the SON transected (Fig. 4, top). The progesterone release was different between both groups at 30, 60, 120 and 180 min of incubation ($P < 0.05$).

Group II: The left ovaries that had the intact SON (when the i.c.v. injection was done) released less progesterone during the incubation than the right ovaries that had the SON transected (Fig. 4, bottom). The progesterone release was also different between both groups at 30, 60, 120 and 180 min of incubation ($P < 0.05$).

The analysis of *androstenedione* in group I, revealed that the right ovaries that had the intact SON (when the i.c.v. injection was done) released less steroid during the incubation than the left ovaries which had the SON transected (Fig. 5, top). The androstenedione release was different between both groups at 30 and 60 min of incubation ($P < 0.05$). At 120 and 180 min there was a similar difference but without statistical significance.

In group II, the left ovaries that had the intact SON (when the i.c.v. injection was done) released less androstenedione during the incubation than the right ovaries that had the SON transected (Fig. 5, bottom).

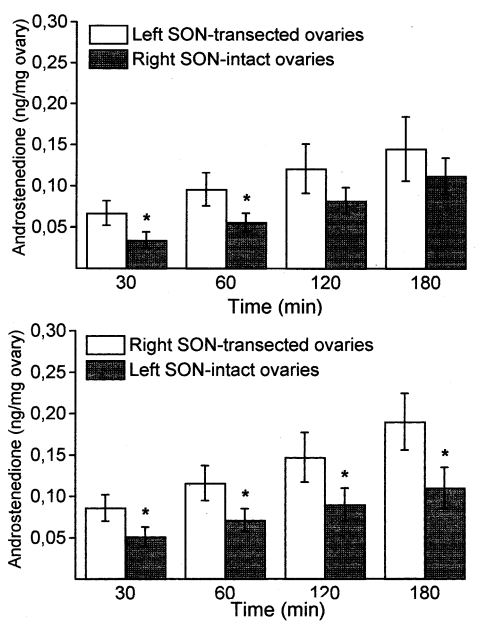

Fig. 5. Top panel: Concentration of androstenedione released per mg ovary incubated with LH, after in vivo i.c.v. injection of epinephrine to rats on dioestrus day 2 with the left superior ovarian nerve (SON) transected. The values represent the means ± SEM of six experiments. $^{*}P < 0.05$ (left SON-transected vs right SON-intact ovaries). Bottom panel: Concentration of androstenedione released per mg ovary incubated with LH, after in vivo i.c.v. injection of epinephrine to rats on dioestrus day 2 with the right superior ovarian nerve (SON) transected. The values represent the means ± SEM of five experiments. $^{*}P < 0.05$ (right SON-transected vs. left SON-intact ovaries).

The androstenedione release was different between both groups at 30, 60, 120 and 180 min of incubation ($P < 0.05$).

4. Discussion

It had been proven in a previous paper [8] that the i.c.v. administration of epinephrine reduces ovarian progesterone release on dioestrus day 2 for a short period of time. The dose of 5 μg epinephrine had a more extended effect on the ovarian progesterone release than the dose of 0.5 μg. Also, the pituitary LH levels increased 3 min after 5 μg epinephrine i.c.v. injection. This rise of LH did not produce an increase in ovarian vein progesterone, therefore the neural effect would have prevailed over the endocrine effect of LH. In the mentioned paper [8] we have demonstrated that the i.c.v. injection of the vehicle used for epinephrine does not produce effects in the progesterone blood concentrations at the ovarian vein and also, that 5 μg epinephrine i.c.v. injected do not modify the ovarian vein blood flow. The studied time was 25 min because in the control rats the progesterone levels were maintained without variations only during this time [8].

In the present paper the antagonistic effect of epinephrine i.c.v. and LH i.v. on the release of ovarian progesterone in rats on dioestrus day 2 was shown. To challenge this antagonistic effect in vivo, a dose of ovine LH was sought, which when injected i.v., would produce an important increase of progesterone in the ovarian vein within the subsequent 25 min.

This optimal dose of LH was 2 mg, and was injected i.v. 3 min after the i.c.v. administration of epinephrine. The LH injection was done at 3 min because we had previously demonstrated that at this time, Po values were the lowest observed within the 25 min [8].

The progesterone in the ovarian vein rose after the LH injection, but this increase was lower than that observed when only LH was injected, suggesting an inhibition by the previous adrenergic administration. When LH was injected first, epinephrine was administered i.c.v. 7 min later, at which time progesterone in the ovarian vein was clearly increased. In this case, the LH effect on progesterone release was also inhibited by the epinephrine i.c.v. injection.

The obtained results suggest a competition between the peripheral LH and the central adrenergic stimulus on the regulation of ovarian progesterone release, thus the prevalence of one or the other could contribute to determining the blood levels of the steroid in the oestrous cycle.

When the release of ovarian progesterone and androstenedione was studied in in vitro experiments, the incubation of rat ovaries on dioestrus day 2 served to confront the effects of epinephrine injected i.c.v. with the effects of a known quantity of LH. The ovarian incubations were made in an environment free of any rat humoral factors, such as pituitary or adrenal hormones, which could interfere with the results. A neural impulse that travels through the SON produces the release of neurotransmitters at the ovarian level, which could modify progesterone release by the corpora lutea or granulosa cells and also androstenedione release by the interstitial cells [4,12,14,19,32,33]. The 5 min from the time of epinephrine i.c.v. injection until the time of extraction of the ovaries from the rats, was considered sufficient time for releasing neurotransmitters and studying their effects on the ovarian progesterone and androstenedione release in vitro.

Rats were used with the right or left SON transected, and the contralateral one intact, to compare the effects of epinephrine i.c.v. injection on each ovary. In all cases, ovaries in which the SON was intact at the time of epinephrine i.c.v. injection, showed a reduction of progesterone and androstenedione released in vitro, in spite of the stimulating presence of LH in the incubation medium. These facts suggest that: (a) the central adrenergic stimulus arrives at the ovary through the SON; (b) the epinephrine i.c.v. injection has an inhibitory effect on the in vitro release of ovarian progesterone and androstenedione on dioestrus day 2; (c) the observed inhibitory effect is manifested in spite of the antagonistic effect of LH.

The central adrenergic effect on ovarian progesterone and androstenedione release was similar, which coincides with the described effects of neurotransmitters such as GnRH or norepinephrine on the ovarian release of those steroids [4,12–14,19,20].

The results presented suggest the existence of a peripheral neuroendocrine interaction between central adrenergic stimuli and peripheral LH which affects the release of ovarian progesterone and androstenedione.

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