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ORIGINAL ARTICLE

Involvement of Beta-adrenoceptors in a Central Regulation of the Ovarian Progesterone Release in Rats

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Abstract

The intracerebroventricular (i.c.v.) injection of epinephrine modifies ovarian progesterone (P) release in rats on diestrus day 2 (D2).

OBJECTIVES: To investigate the characteristic of a central adrenergic effect on the ovarian P release on D2. Also, the function of the superior ovarian nerve (SON) is re-examined.

METHODS AND RESULTS:P concentrations were measured using radioimmunoassay techniques. The i.c.v. injection of 5 μg isoproterenol (beta-adrenergic agonist) in SON-intact rats on D2, decreased the P levels in ovarian vein blood from 1 to 25 min after injection ($p<0.05$). Similar treatment in SON-transected rats did not modify the P concentrations in ovarian vein blood between 1 and 25 min after injection. After 5 μg propranolol (beta-adrenergic antagonist) i.c.v. injection in SON-intact rats, the P levels in ovarian vein blood increased from 2 to 4 min ($p<0.05$). Similar treatment in SON-transected rats did not change the P concentrations in ovarian vein blood during 25 min after injection. The i.c.v. injection of 5 μg phenylephrine (alpha-adrenergic agonist) in SON-intact or SON-transected rats, did not modify the P levels in ovarian vein blood between 1 and 25 min after injection. After 5 μg phentolamine (alpha-adrenergic antagonist) i.c.v. injection in SON-intact or SON-transected rats, the P concentrations in ovarian vein blood did not change during 25 min.

CONCLUSIONS: These results suggest the participation of central beta-adrenergic receptors in the neural regulation of the ovarian P release in rats on D2, and, furthermore, that the central beta-adrenergic input is conduced almost entirely through the superior ovarian nerve.

Abbreviations and units

i.c.v.	intracerebroventricular
P	progesterone
D2	diestrus day 2
SON	superior ovarian nerve
p	statistical probability
LH	luteinizing hormone
i.p.	intraperitoneal
w/v	weight in volume
SEM	standard error medium
min	minute
h	hour
g	grams
µg	micrograms
mg	milligrams
°C	centigrade grades
mm	millimeters
cm	centimeters
U	units
ml	milliliters
µl	microliters

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 SON which is associated with the suspensory ligament [1, 2]. Also, the neurons from which the SON originates are located in para- and prevertebral ganglia [3]. Microscopic studies show that the SON fibers end directly on the secondary interstitial cells and near the theca interstitial cells, while the fibers coming from the ovarian plexus nerve are associated with the ovarian vascular system [2, 4]. Beta-adrenergic receptors have been characterized in different populations of ovarian cells [5]. The occupation of such receptors with adrenergic agonists induces ovarian P and androgens release *in vitro* [4, 5, 6].

Transection of the SON causes a drop in the number of the ovarian beta-adrenergic receptors 48 hs later [7], while transection leads to an increase of these receptors after 7 days [8]. Additionally, the electrical stimulation or the acute transection of the SON performed in live anaesthetized animals enhances or inhibits the P release from the ovary, respectively [9].

Furthermore, evidence of communication between brain neurons and the ovary has been provided by electrical stimulation of the central nuclei of rats [10, 11], producing changes in both the release and synthesis of estrogen and progesterone by the ovary.

The i.c.v. injection of epinephrine increases the P concentration in ovarian vein blood of rat – in short times – on diestrus day 1, and decreases it on D2 [12]. The epinephrine effect is not mediated by LH and is driven almost entirely through the SON. Also, this central adrenergic stimulus competes with LH in ovarian P and androstenedione response on D2 [13].

Although important advances have been made in this field, the physiological role of the ovarian innervation in modulating steroids release remains incomplete. The objective of this work was to investigate the characteristic of the central adrenergic effect on the ovarian progesterone release in rats on D2. Also, the participation of the SON was re-examined.

Material 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*] and water. They were housed in cages under controlled light (lights on from 07:00 to 19:00 h) and temperature (24 ±2 °C). In all the procedures we followed the Revised Guide for the Care and Use of Laboratory Animals [14].

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*] 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 D2, the animals were used for the experimental procedures described below. All the following procedures were carried out under chloral hydrate [*Merck*] anaesthesia (35 mg/100 g of body weight, i.p.) in animals bearing chronic intracerebroventricular cannula.

Ovarian vein cannulation: On the day of the experiment, shortly before the 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^{-1} heparin [*Abbot Lab.*] in 0.9% w/v NaCl [*Mallinckrodt*]) was injected. The needle was then removed and the left ovarian vein cannulated with the teflon tube, as described previously [12, 15]. The left uterine vein was ligated to avoid blood draining into the left ovarian vein.

SON transection: The procedure was described in previous reports [1, 8]. Briefly, before the i.c.v. injection, the suspensory ligament that contains the left 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 experimental groups of animals,

the procedure used was the same, but the suspensory ligament with the SON was not transected.

I.c.v. injections and blood samples collection: After the ovarian vein cannulation, the rats were injected i.c.v. Each adrenergic agent (5 μg) was dissolved in 5 μl vehicle (ascorbic acid [*Sigma*] solution, 0.1 mg ml^{-1} in 0.9% w/v saline). The doses were injected slowly into the right lateral ventricle. The injection lasted 1 min and the conclusion was considered time zero. After this, ovarian vein blood samples were collected from the cannula 15 times during 25 min, once per min during the first 5 min, and then every 2 min. The blood was collected with heparinized capillaries. In each of the 15 times, one capillary was filled and then the cannula was obliterated until the following one. After centrifugation, the plasma was collected and stored frozen until P determination.

Experimental protocols: The following experiments were performed on D2 between 16:00 and 18:00 h. The SON- transected rats were included in the protocol to study the differences in the effects of i.c.v. injections when the SON is intact and when this neural pathway is disabled.

Four groups of 5 SON-transected rats were respectively injected i.c.v. with: 5 μg isoproterenol [*Sigma*], 5 μg propranolol [*Ayerst*], 5 μg phenylephrine [*Sigma*] and 5 μg phentolamine [*Ciba-Geigy*]. Also, five groups of 5 SON-intact rats were respectively injected i.c.v. with: 5 μg isoproterenol, 5 μg propranolol, 5 μg phenylephrine, 5 μg phentolamine, and 5 μl vehicle.

Hormone assays: P concentration was measured by radioimmunoassay technique. The antisera were kindly provided by Dr R. P. Deis (Laboratorio de Reproducción y Lactancia, Mendoza, Argentina). This assay has been previously validated [12, 13].

Data analysis: Results are presented as mean ± SEM in each group. Differences between groups were analyzed with Student's *t*-test when two groups were compared. The analysis of the variance followed by Duncan's multiple range test was used for several comparisons [16]. A value of $p<0.05$ was considered statistically significant.

Results

Ovarian vein P basal levels on D2 after injecting i.c.v. vehicle (16:00–18:00 h)

The P concentrations in ovarian vein blood when SON-intact rats were injected i.c.v. with 5 μl vehicle are shown in Figure 1. The P levels did not change significantly between 1 and 25 min after injection.

Effect of isoproterenol i.c.v. injection on ovarian vein P levels on D2 (16:00–18:00 h)

Figure 2 shows the concentrations of P in ovarian vein blood when 5 μg isoproterenol (beta-adrenergic agonist) were injected i.c.v. at time zero in SON-intact and SON-transected rats.

Comparison between these two groups of animals with basal P values with vehicle i.c.v. showed that in SON-intact rats, the P levels decreased between 1 and 25 min after isoproterenol i.c.v. injection reaching minimal values at 3 min ($p<0.05$ at 1, and between 13 and 25 min; $p< 0.01$ between 2 and 11 min). In SON-transected rats the P concentration did not change significantly between 1 and 25 min after injection.

Effect of propranolol i.c.v. injection on ovarian vein P levels on D2 (16:00–18:00 h)

The levels of P in ovarian vein blood when SON-intact and SON-transected rats were injected i.c.v. with 5 μg propranolol (beta-adrenergic antagonist) are shown in Figure 3. Comparison with basal P values indicated that in SON-intact animals, the P levels increased only between 2 and 4 min after i.c.v. injection ($p<0.05$ at 2 and 4 min; $p<0.01$ at 3 min). In SON-transected animals the P concentration did not change significantly between 1 and 25 min after injection.

Effect of phenylephrine i.c.v. injection on ovarian vein P levels on D2 (16:00–18:00 h)

Figure 4 shows the concentrations of P in ovarian vein blood when 5 μg phenylephrine (alpha-adrenergic agonist) were injected i.c.v. at time zero in SON-intact and SON-transected rats. Comparison between these two groups of animals with basal P values, at each time, showed no changes in the P concentrations in SON-intact and SON-transected rats between 1 and 25 min after injection.

Effect of phentolamine i.c.v. injection on ovarian vein P levels on D2 (16:00–18:00 h)

The levels of P in ovarian vein blood when SON-intact and SON-transected rats were injected i.c.v. with 5 μg phentolamine (alpha-adrenergic antagonist) are shown in Figure 5.

Comparison between these two groups of animals with basal P values showed no changes in the P concentration in SON-intact and SON-transected rats between 1 and 25 min after injection. Regardless of the treatment, the P release in the SON-transected rats was always similar to that in SON-intact rats injected i.c.v. with vehicle.

Discussion

Our previous investigations have shown that central injection of epinephrine increases the ovarian P release in rat on diestrus day 1, and decreases it on D2 between 1 and 25 min after injection [12]. This rapid central effect of epinephrine is not mediated by LH and the SON drives the adrenergic input at least partly [12]. These results would indicate that the adrenergic input is transmitted by a neural direct

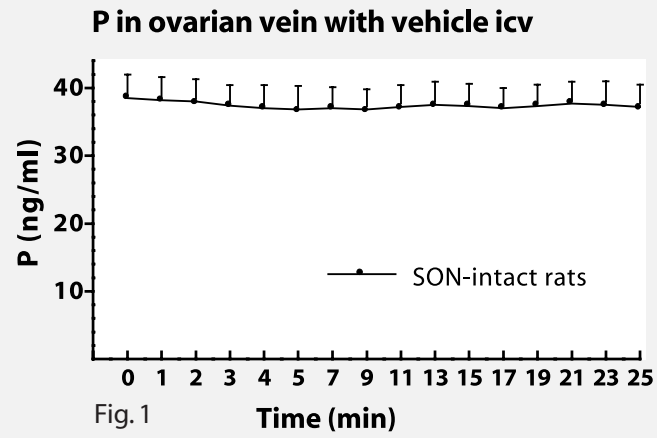

Fig. 1

Fig.1. P Concentration in ovarian vein blood in SON-intact rats on D2, injected i.c.v. with 5 µl vehicle in time 0. The values are means ± SEM, n=5.

Fig. 2. Concentration of P in ovarian vein blood in SON-intact and SON-transected rats on D2, injected i.c.v. with 5 µg isoproterenol in time 0. The values are means ± SEM, n=5. * p<0.05; ** p<0.01.

Fig. 3. P concentration in ovarian vein blood in SON-intact and SON-transected rats on D2, injected i.c.v. with 5 µg propranolol in time 0. The values are means ± SEM, n=5. * p<0.05. ** p<0.01.

Fig. 4. Concentration of P in ovarian vein blood in SON-intact and SON-transected rats on D2, injected i.c.v. with 5 µg phenylephrine in time 0. The values are means ± SEM, n=5.

Fig. 5. P concentration in ovarian vein blood in SON-intact and SON-transected rats on D2, injected i.c.v. with 5 µg phentolamine in time 0. The values are means ± SEM, n=5.

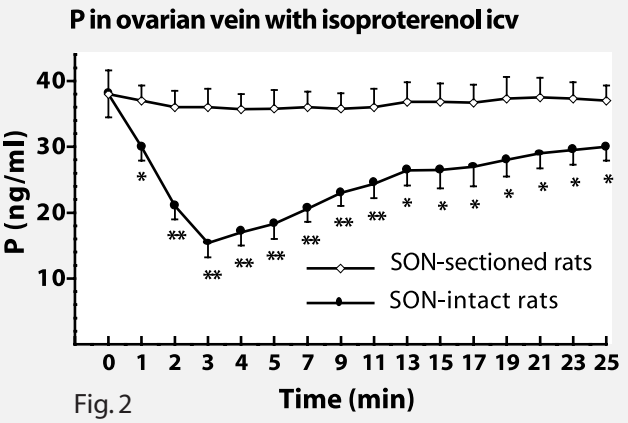

Fig. 2

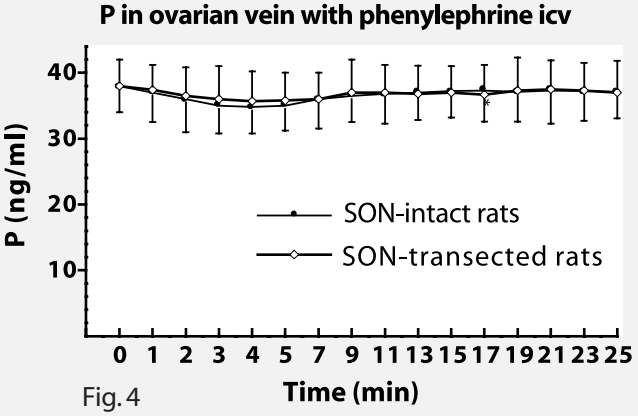

Fig. 4

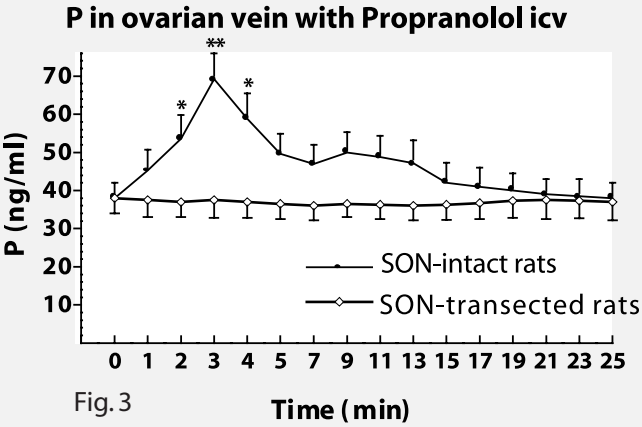

Fig. 3

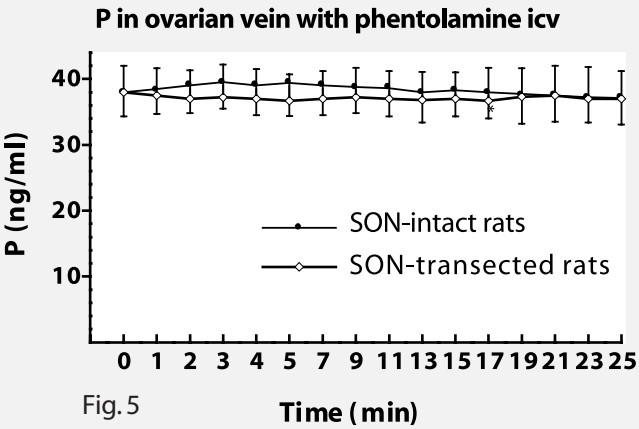

Fig. 5

pathway. Moreover, this central adrenergic stimulus, which arrives at the ovary through the SON, competes with LH on the release of ovarian P on D2 [13].

One of the issues that remains to be investigated is the characterization of this central adrenergic effect on the ovarian P release, particularly on D2. Precisely on this cycle stage, it was previously observed that 0.5 μg epinephrine i.c.v. injection decreases the P concentration in ovarian vein blood without increasing the levels of circulating LH [12]. This would provide evidence that the receptors involved in the neural effect, which is driven by the SON, are different from those involved in the endocrine regulation (LH) of the ovarian P release. In this respect, Tsukamura et al. [17] demonstrates that central alpha-adrenergic receptors are involved in the regulation of the LH secretion – hormonal regulation of ovarian P – and that the injection of isoproterenol in the paraventricular nucleus does not affect LH secretion.

In this work, when a beta-adrenergic agonist (isoproterenol) was injected i.c.v., the effect was very similar to that caused by an epinephrine i.c.v. injection on the ovarian P release [12], using the same dose (5 μg). Therefore, this central effect of isoproterenol was not observed when the SON was previously transected. The increase of ovarian P levels observed when inject-

ing i.c.v. a beta-adrenergic antagonist (propranolol) had an antagonistic effect to that obtained with epinephrine or with isoproterenol i.c.v. This antagonistic neural effect was not observed when the SON was transected. These results suggest that beta-adrenergic receptors are involved in the described central effect by regulating the ovarian P release, and, furthermore, that the beta-adrenergic input is driven almost entirely through the SON.

The fact that a beta-adrenergic antagonist produced an effect suggests the presence of a central beta adrenergic tone in the system under study. Propranolol injected i.c.v. would inhibit this tone and consequently increase the basal levels of ovarian P. A similar phenomenon has been described in the neural ganglion using an integrated system *in vitro* [18, 19].

The above discussed results were confirmed by central injection with either an alpha-adrenergic agonist (phenylephrine) or an alpha-adrenergic antagonist (phentolamine), since none of these treatments led to significant variations in the ovarian P release.

In agreement with this, but in male rats, Ogilvie and Rivier [20] found a neural, catecholamine-dependent pathway that connects the brain and the testes independently of the pituitary, using a central injection of the beta-adrenergic agonist isoproterenol, as well as of norepinephrine.

Finally, we should be cautious as regards the probable regulation of beta-adrenergic receptors after i.c.v. treatment with either adrenergic agonists or antagonists, considering the short time in which ovarian P answer was obtained (1 to 25 min). In the future, performing these experiments in diestrus 1 and in proestrus could clarify the role of the central beta-adrenoceptors in the oestrous cycle. Nevertheless, the results here obtained with adrenergic agonists and antagonists on D2 may provide an important contribution to the understanding of the role of central adrenergic receptors in the ovarian P release regulation.

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