

Research

Open Access

The role of the muscarinic system in regulating estradiol secretion varies during the estrous cycle: the hemiovariectomized rat model

María E Cruz*¹, Angélica Flores¹, María T Palafox¹, Griselda Meléndez¹, Jorge O Rodríguez¹, Roberto Chavira² and Roberto Domínguez¹

Address: ¹Unidad de Investigación en Biología de la Reproducción. FES Zaragoza. UNAM, México and ²Instituto Nacional de Ciencias Médicas y de la Nutrición "Salvador Zubirán," México

Email: María E Cruz* - mecbloy@yahoo.com.mx; Angélica Flores - angy1414@yahoo.com.mx; María T Palafox - angy1414@yahoo.com.mx; Griselda Meléndez - angy1414@yahoo.com.mx; Jorge O Rodríguez - angy1414@yahoo.com.mx; Roberto Chavira - angy1414@yahoo.com.mx; Roberto Domínguez - rdcasala@hotmail.com

* Corresponding author

Published: 21 August 2006

Received: 03 May 2006

Reproductive Biology and Endocrinology 2006, 4:43 doi:10.1186/1477-7827-4-43

Accepted: 21 August 2006

This article is available from: <http://www.rbej.com/content/4/1/43>

© 2006 Cruz et al; licensee BioMed Central Ltd.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/2.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Abstract

There is evidence that one gonad has functional predominance. The present study analyzed the acute effects of unilateral ovariectomy (ULO) and blocking the cholinergic system, by injecting atropine sulfate (ATR), on estradiol (E₂) serum concentrations during the estrous cycle. The results indicate that ULO effects on E₂ concentrations are asymmetric, vary during the estrous cycle, and partially depend on the cholinergic innervation.

Perforation of the left peritoneum resulted in lower E₂ serum concentrations in the three stages of the estrous cycle. At proestrus, unilateral or bilateral perforation of the peritoneum resulted in lower E₂ serum concentrations.

ULO of the right ovary (left ovary *in situ*) resulted in significantly higher E₂ concentrations than animals with ULO of the left ovary (right ovary *in situ*). ATR treatment to ULO rats on D1 resulted in a significant drop of E₂ serum concentrations. ULO rats treated with ATR on D2 or P, resulted in an asymmetrical E₂ secretion response; when the right ovary remained *in situ* an increase in E₂ was observed, and a decrease when the left ovary remained *in situ*.

The results obtained in the present study suggest that each ovary's ability to compensate the secretion of E₂ from the missing ovary is different and varies during the estrous cycle. The results also suggest that the cholinergic system participates in regulating ovarian E₂ secretion. Such participation varies according to the ovary remaining *in situ* and the stage of the estrous cycle of the animal.

The results agree with previously stated hypothesis of a neural pathway arising from the peritoneum that participates in regulating E₂ secretion, and also supports the idea of cross-talk between the ovaries, via a neural communication, that modulates E₂ secretion.

Background

Estradiol secretion is regulated by pituitary [follicle stimu-

lating hormone (FSH) and luteinizing hormones (LH), prolactin, and adrenocorticotropin (ACTH)]. The effects

of these hormones are modulated by neurotransmitters released by the intrinsic ovarian innervation near the follicular wall. Acetylcholine produced by the follicle may be one of the neurotransmitters participating in modulating the effects of pituitary hormones on the follicle [1-3].

Evidence suggesting that one gonad has functional predominance in mammals and birds have been published [1,4-8]. In previous studies we have shown that unilateral ovariectomy (ULO) modifies progesterone and/or testosterone serum concentrations, and that the effects of ULO depend on both, the stage of the estrous cycle when ULO was performed and the ovary (left or right) remaining *in situ* [9-11].

Asymmetry in ovarian functions has been explained by differences in the ovarian innervation participating in modulating the effects of gonadotropin on the ovarian follicles [1,6]. Kawakami et al [12] showed that electrical stimulation of the medial basal pre-chiasmatic area, the ventro-medial hypothalamus, and the areas in the mesencephalon of hypophysectomized and adrenalectomized female rats resulted in a significant increase of estradiol (E_2) and progesterone (P_4) plasma concentrations in the contra-lateral ovarian venous blood. In turn, stimulating the dorsal hippocampus, the lateral amygdala, and the mesencephalic areas resulted in lower E_2 and P_4 concentrations. Ovarian denervation of rats in proestrus stage blocks E_2 secretion induced by stimulating the medial basal pre-chiasmatic area. In addition, the electrochemical stimulation in proestrus day of the medial basal pre-chiasmatic area of untreated rats increased E_2 and P_4 concentrations in serum. This effect was not observed when stimulation was applied to the pre-optic supra-chiasmatic area. According to the authors' interpretation of the results, the efferent neural system connecting the brain and the ovaries is supplementary to the brain-pituitary-ovarian hormonal mechanisms regulating ovarian steroid secretion, and the system may be required for adjusting ovarian responsiveness and sensitivity to gonadotropins [12,13].

Gerendai et al. [14] described a multi-synaptic neural pathway between the central nervous system and the ovaries, with the vagus nerve being one of the main neural pathways. In ULO treated rats, bi-lateral sectioning the vagus nerve (ventral or dorsal) results in lower compensatory ovarian hypertrophy. The effects of sectioning the left vagus nerve depend on the remaining ovary *in situ*: rats with the left ovary *in situ* had a larger proportion of ovulating animals, compensatory ovarian hypertrophy and number of ova shed. In turn, rats with the right ovary *in situ* showed a decrease in all parameters studied [15].

Based on available information, the present study aims to analyze if changes in E_2 secretion by the left and right ovaries vary during the estrous cycle, using the unilateral ovariectomized animal as a model of study.

We also investigated if, throughout estrous cycle diestrus 1 (D1), diestrus 2 (D2) and proestrus (P), the cholinergic system modulates E_2 secretion in an asymmetric way. For this purpose, we analyzed the effects of injecting ATR at 13.00 h to rats on D1, D2 or P with or without unilateral or bilateral ovariectomy.

Materials and methods

The study was performed with virgin adult female rats (195–225-g body weight) of the CIIZ-V strain from our own stock. Animals were kept under controlled lighting conditions (lights on from 05:00 to 19:00 h), with free access to food (Purina S.A., Mexico) and tap water; following NIH Guide parameters for the care of laboratory animals. The Committee of the Facultad de Estudios Superiores Zaragoza approved the experimental protocols.

Estrous cycles were monitored by daily vaginal smears. Only rats showing at least two consecutive 4-day cycles were used in the experiment. All surgeries were performed under ether anesthesia, between 13:00–13:15 hours. Rats were sacrificed by decapitation one hour after treatment.

Experimental groups

Rats were randomly allotted to one of the experimental groups described below. Animals from different experimental groups were treated simultaneously and sacrificed one hour after surgery. The number of animals used in each experimental group is presented in Tables 1, 2 and 3.

Control group ($N = 48$). Non-treated cyclic rats sacrificed at 14:00 h on D1 (17 rats), D2 (19 rats) and P (12 rats).

Ether anesthesia ($N = 24$): Groups of rats, on specific stages (D1, D2 or P) of the estrous cycle, were anesthetized for 10 min and sacrificed one hour later.

Unilateral peritoneal perforation (sham operation) ($N = 53$): A unilateral incision was performed 2-cm below the last rib; affecting skin, muscle, and peritoneum. The ovaries were not injured or manipulated. After surgical procedures the wound was sealed.

Bilateral peritoneal perforation (sham operation 2) ($N = 27$). A bilateral incision below the last rib, including skin and muscle, was performed. The ovaries were not injured or manipulated. After surgical procedures the wound was sealed.

Table 1: Effects of left, right or bilateral peritoneum perforation (PP) performed at 13:00 h of diestrus 1, diestrus 2 or proestrus on estradiol serum levels (pg/ml) measured one hour after surgery. Data are represented by means ± SEM.

Group	N	Diestrus 1	N	Diestrus 2	N	Proestrus
Control	18	55.3 ± 8.0	20	59.1 ± 7.9	15	158.4 ± 10.8
Left PP	8	25.0 ± 4.5*	10	29.1 ± 8.2*	9	111.1 ± 15.3*
Right PP	11	46.7 ± 7.5	9	109.3 ± 5.5*	10	78.9 ± 8.3*
Bilat. PP	8	76.3 ± 6.5	10	63.3 ± 7.1	9	93.7 ± 16.8*

* p < 0.05 vs. control group (ANOVA followed by Tukey's test)

Unilateral ovariectomy (ULO) (N = 50): A unilateral incision below the last rib, including skin and muscle was performed, and the right or left ovary was extirpated. The wound was subsequently sealed.

Bilateral ovariectomy (N = 23): A bilateral incision below the last rib, including skin and muscle was performed, and the ovaries removed. The wound was subsequently sealed.

Blocking the cholinergic system

To analyze the effects of blocking the cholinergic system, groups of animals were injected with atropine sulfate (ATR, Sigma Chem. Co. St. Louis, Mo.). ATR was injected one hour before surgery at doses known to block ovulation: in D1, 100 mg/kg body weight (b.w.); in D2, 300 mg/kg b.w.; and in P, 700 mg/kg b.w. [16].

One hour after ATR treatment, rats were randomly allotted to one of the following treatments: unilateral peritoneal perforation, bilateral peritoneal perforation, ULO, or bilateral ovariectomy. All animals were sacrificed one hour after surgery. For control purposes, untreated rats, on D1, D2 or P, were injected with ATR in the same dose as in their corresponding treatment group. The animals were sacrificed two hours after treatment.

Autopsy procedures

Animals were sacrificed by decapitation. The blood of the trunk was collected in a test tube, allowed to clot at room temperature for 30 minutes and centrifuged at 3,000 rpm for 15 minutes. Serum was stored at -20°C, until E₂ concentrations were measured.

Hormone assay

Concentrations of E₂ in serum were measured by Radio-Immuno-Assay (RIA); using kits purchased from Diagnostic Products (Los Angeles, CA). Results are expressed in pg/ml. The Intra- and inter-assay variation coefficients were 6.9% and 10.8 %, respectively.

Statistics

Data on hormonal concentrations in serum were analyzed using multivariate analysis of variance (MANOVA) followed by Tukey's test. Differences in serum hormone concentrations between two groups were analyzed by Student's t-test. A probability value of less than 5% was considered significant.

Results

Effects of ether anesthesia and unilateral or bilateral perforation of the peritoneum

In the control group, animals sacrificed on P showed significantly higher E₂ serum concentration than animals sacrificed on D1 or D2 (D1: 55.3 ± 8.0; D2: 59.1 ± 7.9; P: 158.4 ± 1.8). Compared to the control group, ether anesthesia treatment did not modify E₂ serum concentrations (D1: 62.9 ± 8.4; D2: 69.5 ± 12.0; P: 164.1 ± 17.6). Since ether anesthesia did not modify E₂ serum concentrations, treatment results are compared to their respective control group.

The effects on E₂ serum concentrations of unilaterally or bilaterally perforating the peritoneum depended on the side of the peritoneum and the stage of the estrous cycle when perforation surgery was performed. Perforating the left peritoneum on D1 resulted in lower E₂ serum concen-

Table 2: Means ± SEM of estradiol serum concentration (pg/ml) in animals with bilateral perforation of the peritoneum (PP) or bilateral ovariectomy (CAS) performed at 13:00 h on Diestrus 1, Diestrus 2 or Proestrus, sacrificed one hour later

Group	N	Diestrus 1	N	Diestrus 2	N	Proestrus
Bilateral PP	8	76.3 ± 6.5	10	63.3 ± 7.1	9	93.7 ± 16.8
CAS	7	20.2 ± 3.7*	10	17.3 ± 2.6*	6	14.7 ± 5.4*

* p < 0.05 vs. bilateral PP group (Student's t test)

Table 3: Means ± SEM. of estradiol serum concentration (pg/ml) in control and animals injected with atropine sulfate (ATR) at 12:00 h on Diestrus 1, Diestrus 2 or Proestrus. The animals were sacrificed at 14:00 h.

Group	N	Diestrus 1	N	Diestrus 2	N	Proestrus
Control	17	55.3 ± 8.0	20	61.7 ± 4.9	12	158.4 ± 10.8
ATR	8	9.0 ± 1.8*	8	67.5 ± 2.7	7	51.7 ± 9.8*

* p < 0.05 vs. non-ATR treated group (Student's t test)

trations (55%), while bilateral perforation, or perforating the right side of the peritoneum, had no apparent effects (Table 1).

Perforating the right side of the peritoneum on D2 day resulted in E₂ concentration increases (184%), while perforating the left side resulted in a decrease (51%) of E₂ serum concentrations. Bilateral perforation had no apparent effects on hormone concentrations. Perforating the peritoneum on P phase (left, right or bilateral) resulted in hormone serum concentration decreases (Left 30%; Right 50%; Bilateral 41%). Results are summarized in Table 1.

Effects of unilateral or bilateral ovariectomy

When surgery was performed on D1, no significant differences in E₂ serum concentrations were observed between rats with ULO (animals with intact left or right ovary *in situ*) or perforation of the peritoneum (Figure 1). Animals with the left intact ovary *in situ* showed significantly higher E₂ serum concentrations than animals with the right intact ovary *in situ* (61.5 ± 9.4 vs. 17.3 ± 4.3, p < 0.05 Student's t test).

Compared to animals with unilateral perforation of the peritoneum, animals with right ULO (left ovary *in situ*) performed on D2 had lower E₂ serum concentrations (55%). Such differences were not observed in rats with left ULO (Figure 1). As in rats treated on D1, E₂ serum concentrations were significantly higher in animals treated on D2 with the left ovary *in situ* (right ULO) than in animals with the right ovary *in situ* (49.5 ± 10.8 vs. 26.0 ± 6.9).

In animals treated on P, right ULO (left ovary *in situ*) resulted in higher E₂ (180%) serum concentrations than in animals with unilateral peritoneum perforation. ULO performed on the left side (right ovary *in situ*), resulted in significantly lower (45%) E₂ serum concentrations compared to rats with a unilateral perforation of the peritoneum (Figure 1). As observed in rats treated on D1 or D2, when the intact left ovary remains *in situ*, estradiol serum concentrations were significantly higher than in animals with the intact right ovary *in situ* (142.0 ± 14.1 vs. 61.5 ± 6.0). Compared to animals with a bilateral perforation of the peritoneum, bilateral ovariectomy resulted in significantly lower E₂ serum concentrations, regardless of the

stage of the estrous cycle surgery performed (D1 74%; D2 73%; P 84%). Results are summarized in Table 2.

Effects of blocking the cholinergic system

Injecting ATR on D1 or P resulted in E₂ serum concentrations decreases (84% and 67%, respectively), and had no apparent effects on E₂ serum concentrations when injected on D2 (Table 3).

Figure 2 shows that the effects of blocking the cholinergic system of rats with unilateral perforation of the peritoneum depended on both, the side (left or right) and the phase of the estrous when surgery was performed. Injecting ATR on D1 or D2 resulted in a significant drop in E₂ serum concentrations in animals with sham treatment on the right side. Blocking the cholinergic system of rats with left side peritoneum perforation on D1 or P resulted in a drop in E₂ serum concentrations (52%; 47%, respectively), while the same treatment performed on D2 resulted in a significant E₂ concentrations increase (157%).

Figure 3 shows the effects of blocking the cholinergic system of rats with ULO. ATR treatment on D1 or P stages performed on rats with the left ovary *in situ* resulted in a significant drop of E₂ serum concentrations (65%; 62% respectively). Such effects were not observed in rats treated on D2. When ATR treatment was performed on rats with the right ovary *in situ* on D1, E₂ serum concentrations were lower (48%) than in ULO animals. Blocking the cholinergic system on D2 resulted in E₂ serum concentrations increase (159%). When the treatment was performed on P, no significant differences in E₂ serum concentrations were observed.

Compared to bilateral treatment, perforation of the peritoneum or bilateral ovariectomy, ATR treatment on D1 resulted in significant E₂ serum concentrations decreases, 90% in bilateral peritoneal perforation and 60% in bilateral ovariectomized animals.

Blocking the cholinergic system on D2, to rats with bilateral perforation of the peritoneum or bilateral ovariectomy resulted in E₂ serum concentrations increases (159% and 253% respectively), while injecting ATR to animals treated on P had no apparent effects (Figure 4).

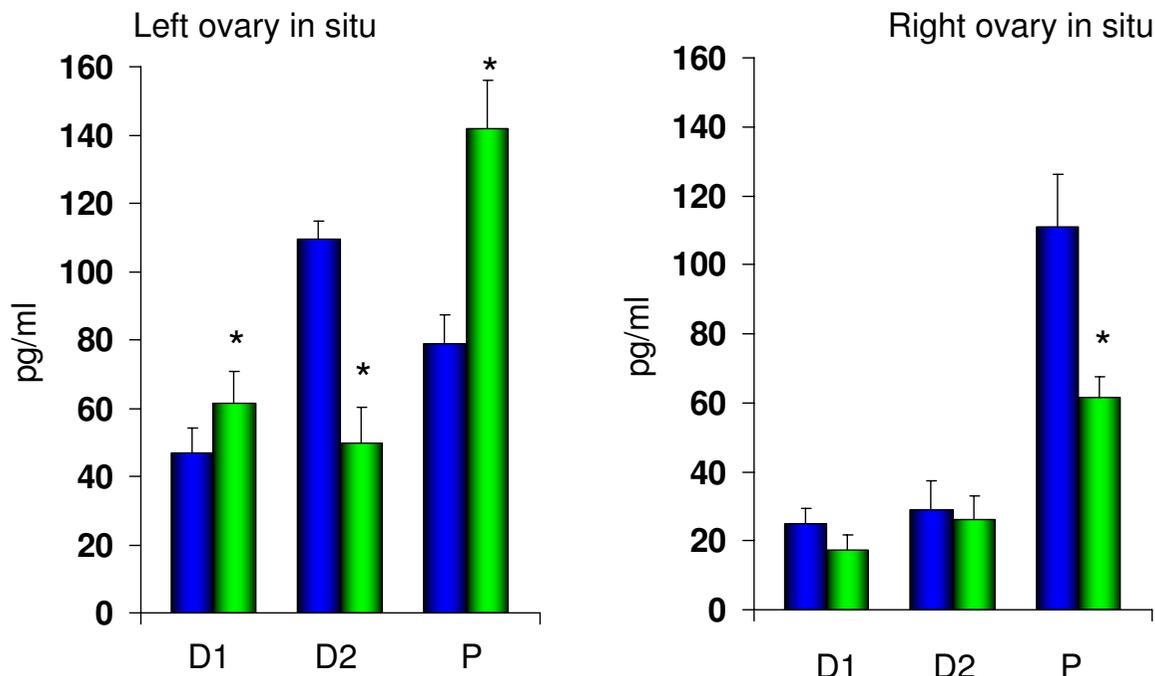


Figure 1
Effects of unilateral perforation of the peritoneum (PP) (blue) and unilateral ovariectomy (ULO) (green) on estradiol serum levels (pg/ml) performed at 13:00 h of diestrus 1 (D1), diestrus 2 (D2) or proestrus (P), sacrificed one hour later. * p < 0.05 vs. PP (Student's t test).

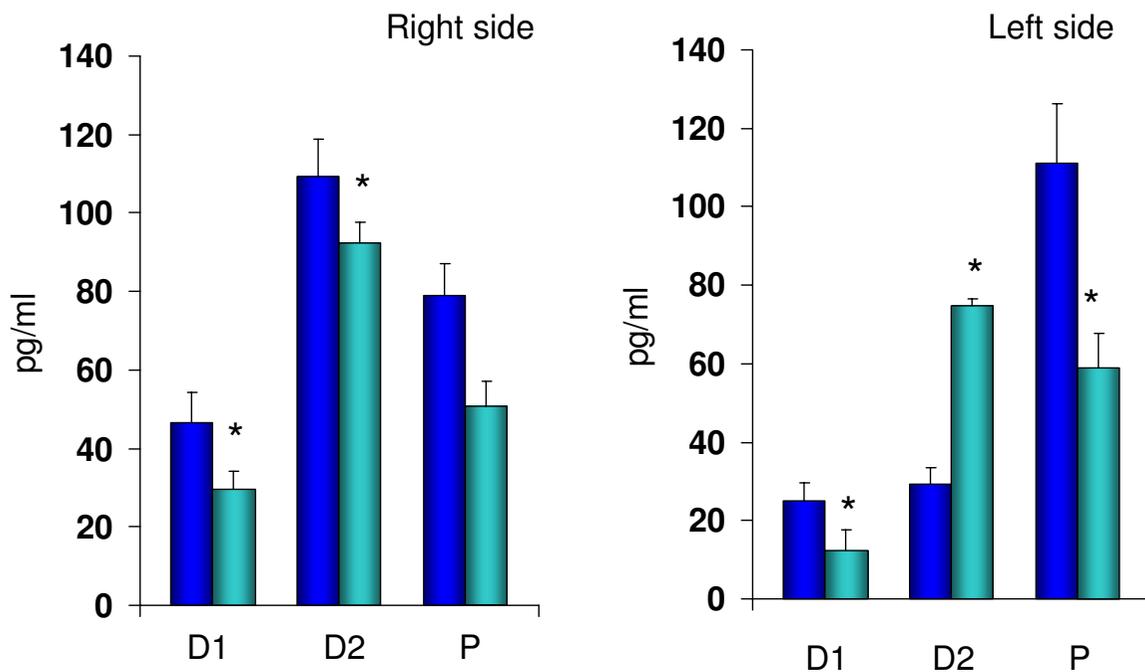


Figure 2
Comparative effects of atropine sulfate injection (blue) at 12:00 hours on estradiol serum levels (pg/ml), to rats with unilateral perforation of the peritoneum (sky blue) performed at 13:00 of diestrus 1 (D1), diestrus 2 (D2) or proestrus (P) sacrificed one hour after surgery. * p < 0.05 vs. unilateral perforation of the peritoneum (Student's t test).

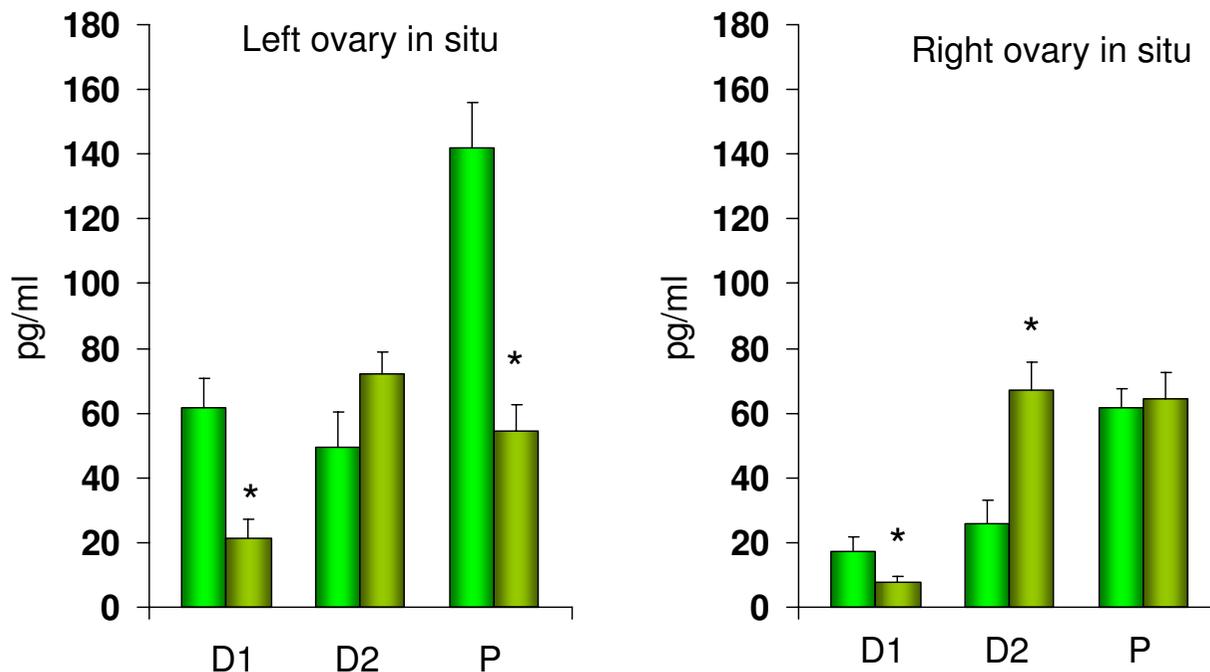


Figure 3
Comparative effects of atropine sulfate injection at 12:00 hours on estradiol serum levels (pg/ml) to rats with unilateral ovariectomy (olive green) performed at 13:00 of diestrus 1 (D1), diestrus 2 (D2) or proestrus (P) sacrificed one hour after surgery. * p < 0.05 vs. unilateral ovariectomy (Student's t test).

Discussion

The results obtained in the present study suggest that the ability to compensate the secretion of E₂ by the missing ovary is different between the right and left ovaries and varies during the estrous cycle. Similarly, our results suggest that the cholinergic system participates in regulating E₂ secretion by the ovary, and that such participation varies depending on the ovary remaining *in situ* and the stage of the estrous cycle when the surgical procedure was performed.

Previously, we suggested the existence of a neural pathway arising from the peritoneum that participates in regulating E₂ [9], P₄ [10] and testosterone secretion [11]. In the rat, the sensory information arising from the peritoneum is sent to the nucleus tractus solitarius and stimulates neurokinine-B receptors [17]. Since perforating the peritoneum unilaterally on each day of the estrous cycle changed E₂ serum concentrations, we think that each side of the peritoneum sends different neural information through the superior ovarian nerve (SON) to the ovary and the central nervous system, perhaps reaching nuclei related to the vagus nerve.

A study analyzing the distribution of sensory neurons innervating the peritoneum showed that when tracer was placed on the area where the peritoneum covers the abdominal wall, labeled neurons were observed only in the ipsilateral dorsal root ganglia [18]. The authors suggest that most of the parietal peritoneum receives sensory nerves from dorsal root ganglia, and visceral peritoneum from both, the spinal and vagus nerves.

According to Stener-Victorin et al. (19) repeated electroacupuncture treatments in rats with polycystic ovary syndrome (PCO), induced by a single injection of estradiol valerate, resulted in lower nerve growth factor (NGF) concentrations at the ovarian level than in non-electroacupuncture treated PCO rats. In our experiments, perforating the peritoneum affected the same somatic segments employed by Stener-Victorin et. al. [19].

We presume that peritoneum surgery resulted in an increase of NGF concentrations at the ovarian level, which in turn induced hyper-androgenism, as observed in women with PCO [20]. Previously, we showed that the unilateral perforation of the peritoneum results in a sig-

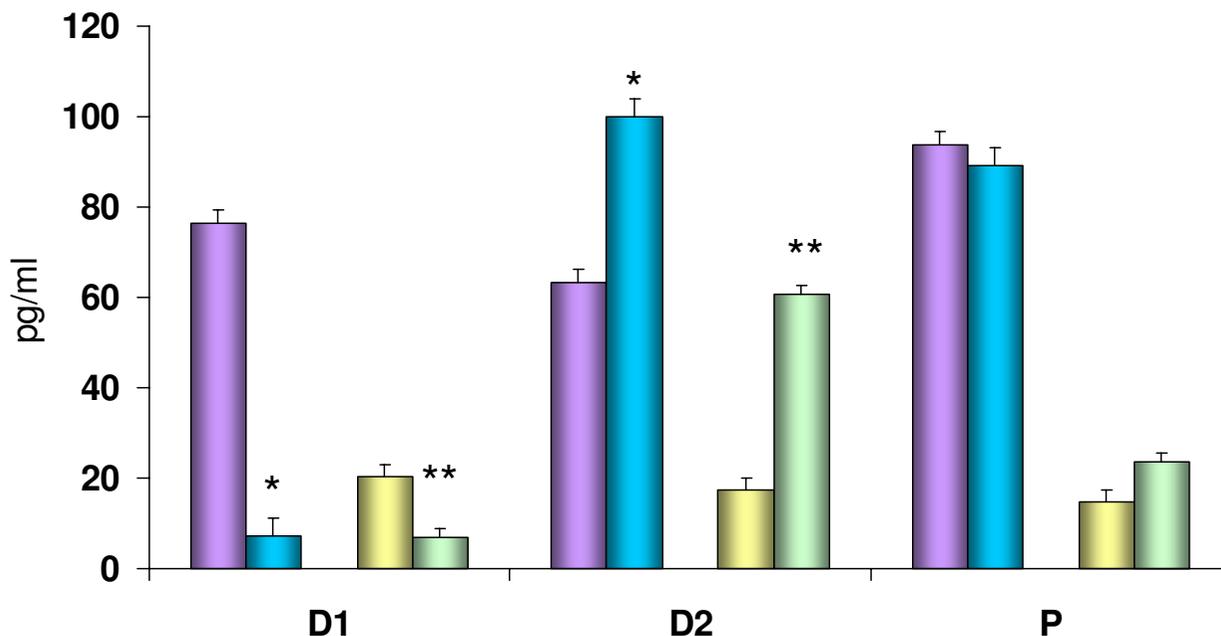


Figure 4

Comparative effects of atropine sulfate (blue, green) injection at 12:00 hours on estradiol serum levels (pg/ml) to rats with bilateral perforation of the peritoneum (purple) or bilateral ovariectomy (yellow) performed at 13:00 of diestrus 1 (D1), diestrus 2 (D2) or proestrus (P) sacrificed one hour after surgery. * $p < 0.05$ vs. bilateral perforation of the peritoneum (Student's t test); ** $p < 0.05$ vs. bilateral ovariectomy (Student's t test).

nificant increase in testosterone serum concentrations [11]. Because E_2 serum concentrations did not increase after left or bilateral perforation of the peritoneum, we suppose that the neural information originating from the peritoneum inhibits the mechanisms regulating aromatase activity within the follicle.

One of the ovaries' sources of catecholamines arrives through the SON. In the ovary, the SON fibers are mainly distributed in the peri-follicular theca layer, and in close relation with the cells of the theca interna [21,22]. Sectioning the SON of rats in P results in a sudden drop of P_4 and E_2 concentrations in the ovarian vein effluent [23], while the same procedure on estrus did not modify E_2 concentrations [24]. Therefore, it is possible that perforating the peritoneum modifies the type and/or rate of information arriving to the ovary via the SON. Another possibility is that perforating the left side of the peritoneum results in an increase release of ovarian gamma amino butyric acid (GABA), and a subsequent increase of E_2 concentrations.

According to Erdö, et. al. [25] and Laszlo, et. al. [26], injecting GABA into pseudo-pregnant rats increases E_2 concentration in the blood. Present results indicate that injecting ATR, before unilateral or bilateral perforation of the peritoneum, to rats in D1 or P, results in lower E_2 serum concentrations; leading us to think that some of the neural fibers present in the peritoneum are muscarinic. It is also possible that blocking the cholinergic innervation, by ATR treatment, results in lower adrenaline and norepinephrine release by the adrenal medulla.

Our results, and those of others, suggest that stimulating on D2 the sensory receptors located on the left side of the peritoneum triggers an E_2 secretion inhibitory mechanism, that the sensory pathway arising from the right side has a stimulatory effect, and that both are mediated by the cholinergic muscarinic system.

Previously, we showed that injecting ATR to rats in D2 results in increases of P_4 serum concentrations originating from the adrenals [8], without having apparent effects on

testosterone serum concentrations [11]. Since P₄ and androgens are precursors in the synthesis of E₂, we presume that this mechanism may explain the increase in E₂ serum concentrations observed in rats with peritoneum perforation previously injected with ATR.

Another possibility explaining the differences on E₂ secretion regulation during the estrous cycle is that the effects of the cholinergic system take place through changes at the celiac ganglion level. According to Aguado and Ojeda [23], acetylcholine inhibits P₄ secretion in the celiac ganglion-SON-ovary preparation obtained from rats in D1 or D2, while the preparation obtained from rats in P resulted in only a moderate stimulation. Since there is evidence that fibers from the vagus nerve innervate neurons in the celiac ganglion [27], we presume that the cholinergic system modulates the sympathetic post-ganglionic activity and the secretory ability of the ovaries through the SON.

Conclusion

Based on the differences in E₂ serum concentrations in rats with ULO, present results suggest that the capacity to release E₂ by the left and right ovaries varies during the estrous cycle. We presume that the left ovary releases more E₂ than the right one. As previously proposed, another possibility is that neural communication between the ovaries modulates E₂ secretion.

Acknowledgements

We want to thank A. Domínguez González M.Sc for the revision of the English manuscript. This work was supported by CONACyT, grant 40-300/Q, DGAPA-UNAM grants IN 200405 and IN 201005 and PASPA-UNAM to M.E. Cruz.

References

1. Domínguez R, Cruz ME, Chavez R: **Differences in the ovulatory ability between the right and left ovary are related to ovarian innervation.** In *Growth Factors and the Ovary Volume 39*. Edited by: Hirshfiel AN. Plenum Press; New York; 1988:321-325.
2. Mayerhofer A, Dimitrijevic N, Kunz L: **The expression and biological role of the non-neuronal cholinergic system in the ovary.** *Life Sci* 2003, **72**:2039-2045. Review
3. Fritz S, Wessler I, Breitling R, Rossmannith W, Ojeda SR, Dissen GA, Amsterdam A, Mayerhofer A: **Expression of Muscarinic Receptor Types in the Primate Ovary and Evidence for Nonneuronal Acetylcholine Synthesis.** *J Clin Endocrinol Metab* 2001, **86**:349-354.
4. Bleier WJ, Ehteshami M: **Ovulation following unilateral ovariectomy in the California leaf-nosed bat (*Macrotus californicus*).** *J Reprod Fertil* 1981, **63**:181-183.
5. Rao ChV, Edgerton LA: **Dissimilarity of corpora lutea within the same ovaries or those from right and left ovaries of pigs during the oestrus cycle.** *J Reprod Fertil* 1984, **70**(1):61-66.
6. Domínguez R, Morales L, Cruz ME: **Ovarian Asymmetry.** *Ann Rev Biomed Sci* 2003, **5**:95-104.
7. Wittwoch V: **Lateral asymmetry and gonadal differentiation.** *Lancet* 1975, **1**:401-402.
8. Marut EL, Hodgen GD: **Asymmetric secretion of principal ovarian venous steroids in the primate luteal phase.** *Steroids* 1982, **39**:461-469.
9. Barco AI, Flores A, Chavira R, Damián-Matzumura P, Domínguez R, Cruz ME: **Asymmetric effects of acute hemiovariectomy on steroid hormone secretion by the *in situ* ovary.** *Endocrine* 2003, **21**(3):209-215.
10. Flores A, Meléndez G, Palafox MT, Rodríguez J, Barco AI, Chavira R, Domínguez R, Cruz ME: **The participation of the cholinergic system in regulating progesterone secretion through the ovarian-adrenal crosstalk varies along the estrous cycle.** *Endocrine* 2005, **28**(2):145-152.
11. Flores A, Rodríguez JO, Palafox MT, Meléndez G, Barco A, Chavira R, Cruz ME, Domínguez R: **The acute asymmetric effects of hemiovariectomy on testosterone secretion vary along the estrous cycle. The participation of the cholinergic system.** *Reproductive Biology & Endocrinology* 2006, **4**:11.
12. Kawakami M, Kubo K, Uemura T, Nagase M, Hayashy R: **Involvement of ovarian innervation on steroid secretion.** *Endocrinology* 1981, **109**:136-145.
13. Kawakami M, Kubo K, Uemura T, Nagase M: **Evidence for the existence of extra-hypophyseal neural mechanisms controlling ovarian steroid secretion.** *J Steroid Biochem* 1979, **11**:1001-1005.
14. Gerendai I, Toth IE, Boldogkoi Z, Medveczky I, Halász B: **CNS structures presumably involved in vagal control of ovarian function.** *J Auton Nervous* 2000, **80**:40-45.
15. Chávez R, Cruz ME, Domínguez R: **Differences in the ovulation rate of the right and left ovary in unilaterally ovariectomized rats: Effects of ipsi- and contra lateral vagus nerves on the remaining ovary.** *J Endocrinol* 1987, **113**:397-401.
16. Domínguez R, Riboni L, Zipitria D, Revilla R: **Is there a cholinergic circadian rhythm throughout the oestrous cycle related to ovulation in the rat?** *J Endocrinol* 1982, **95**(2):175-180.
17. Chen LW, Guan ZL, Ding YQ: **Neurokinin B receptor (NK3)-positive neurons expressing c-fos after chemical noxious stimulation on the peritoneum: a double staining study in the nucleus tractus solitarius of the rat.** *Journal of Hirnforschung* 1997, **38**:363-367.
18. Tanaka K, Matsugami T, Chiba T: **The origin of sensory innervation of the peritoneum in the rat.** *Anat Embriol* 2002, **205**:307-313.
19. Stener-Victorin E, Lundeberg T, Waldenström U, Manni L, Aloe L, Gunnarsson S, Janson PO: **Effects of electro-acupuncture on nerve growth factor and ovarian morphology in rats with experimentally induced polycystic ovaries.** *Biology of Reproduction* 2000, **63**:1497-1503.
20. Franks S: **Polycystic ovary syndrome.** *Arch Dis Child* 1997, **77**:89-90.
21. Baljet B, Drukker J: **The extrinsic innervation of pelvic organ in the female rat.** *Acta Anat* 1980, **107**:241-267.
22. Lawrence IE Jr, Burden HW: **The origin of the extrinsic adrenergic innervation to the ovary.** *Anat Rec* 1980, **196**:51-59.
23. Aguado LI, Ojeda SR: **Ovarian adrenergic nerves play a role in maintaining preovulatory steroid secretion.** *Endocrinology* 1984, **114**:1944-1946.
24. Aguado LI: **Role of the central and peripheral nervous system in the ovarian function.** *Microsc Res Tech* 2002, **59**:462-473.
25. Erdő SL, Varga B, Horvath E: **Effects of local GABA administration on rat ovarian blood flow, and on progesterone and estradiol secretion.** *Eur J Pharmacol* 1985, **111**:397-404.
26. Laszlo A, Villanyi P, Zolnai B, Erdő SL: **Gamma-aminobutyric acid, is related enzymes and receptor-binding sites in the human ovary and fallopian tube.** *Gynecol Obstet Invest* 1989, **28**:94-97.
27. Berthoud HR, Powley TL: **Interaction between parasympathetic and sympathetic nerves in prevertebral ganglia morphological evidence for vagal efferent innervation of ganglion cells in the rat.** *Microsc Res Tech* 1996, **35**:80-86.