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Asymmetric Effects of Acute Hemiovariectomy on Steroid Hormone Secretion by the *In Situ* Ovary

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The acute effects of hemiovariectomy on progesterone, testosterone, estradiol, and luteinizing hormone (LH) concentrations in serum were studied in rats under the following experimental conditions: control, sham-operated (left or right), hemiovariectomized, bilateral adrenalectomized, and hemiovariectomized plus bilateral adrenalectomized. One-hour after surgery, the concentration of progesterone and testosterone in the serum of right-side sham-operated rats was significantly higher than in control animals. Testosterone concentration in serum in rats with the right ovary *in situ* was higher than in sham-operated animals; injecting atropine sulfate 1 h before surgery blocked such increase, while the same treatment to rats with the left ovary remaining *in situ* resulted in a significant increase of testosterone concentration. Adrenalectomy resulted in an increase of testosterone concentration, which was higher when atropine sulfate was injected before surgery. Our results support the idea that left and right ovaries play different roles in the regulation of hormone secretion, and that such differences are related to ovarian innervation.

Key Words: Asymmetry; estrus day; hemiovariectomy; adrenalectomy; steroid secretion.

Introduction

Hemiovariectomy is a time-honored procedure that has been useful in elucidating the follicular kinetics of different species. The effects of hemiovariectomy in mammals can be analyzed in terms of compensatory hypertrophy of the contralateral ovary (i.e., increased weight), enhanced follicular activity, and an increase in both the number of ova shed and the number of corpora lutea. The mechanisms involved in compensatory ovarian hypertrophy and compensatory ovulation in hemiovariectomized rats have been analyzed through changes in pituitary gonadotrophin secretion and ovarian steroids secretion *(1)*.

Many of the results obtained from hemiovariectomized rats cannot be solely explained on an endocrinological basis. For instance, compared to control values, follicle-stimulating hormone (FSH) concentration in plasma of 4-d cyclic rats increased during the first 6–12 h after hemiovariectomy, regardless of whether hemiovariectomy was performed before or after 08:00 h on the day of diestrus 2. In addition, in rats subjected to hemiovariectomy after 17.00 h of diestrus d 2, compensatory ovulation did not occur on the following day of estrus *(2)*. Another study comparing the effects of right- vs left-hemiovariectomy in rats showed that the ovulation rate diminished when the left ovary was left *in situ* (right hemiovariectomized rats), but remained normal in rats whose right ovary had been left *in situ* (left hemiovariectomized rats) *(3)*.

Sectioning both vagus nerves to right hemiovariectomized rats reduced compensatory ovarian hypertrophy. In turn, sectioning the left vagus nerve only induced different effects, depending on which ovary remained in situ. For instance, in right hemiovariectomized rats (left ovary *in situ*), the ovulation rate, the compensatory ovarian hypertrophy, and the number of ova shed increased after left-side vagotomy. In turn, the same procedure to left hemiovariectomized rats showed a decrease in all the enumerated parameters *(3)*.

The mammalian ovary is innervated by sympathetic and parasympathetic fibers, as well as by afferent sensory neurons whose perikarya are located in the lower thoracic and upper lumbar dorsal ganglia root *(4)*, as well as in the nodose ganglion of the vagus nerve *(5)*. According to Klein and Burden *(5)*, the right ovary receives a larger supply of sympathetic afferent fibers than the left one does. In human fetuses, as the gonads descend, the number of extra organic nerves increases, and so does innervation asymmetry between the right and left ovary. In addition, the number of neural fibers and trunklets in the plexus around the left ovarian artery are greater than on the right ovarian artery *(6)*.

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Table 1
Progesterone (P4), Testosterone (T), Estradiol (E), and LH Concentration in Serum of Control Rats and Rats Anesthetized with Ether, Sacrificed 1 h after Anesthesia[a]

Treatment	P4 (ng/mL)	T (pg/mL)	E (pg/mL)	LH (ng/mL)
Control	10.46 ± 2.37	<2.0	16.03 ± 3.35	1.66 ± 0.24
Ether anaesthesia	7.57 ± 1.59	<2.0	10.86 ± 2.12	0.41 ± 0.10*

[a]Data are represented by means ± SEM. Student's *t* test. *$p < 0.05$ compared to control group.

Table 2
Progesterone (P4), Testosterone (T), Estradiol (E), and LH Concentration in Serum of Control Rats, and Left-Side-Sham-Operated (L-sham), Right-Side-Sham-Operated (R-sham), and Bilateral-Sham-Operated Rats (B-sham), Killed 1 h after Treatment[a]

Group	P4 (ng/mL)	T (pg/mL)	E (pg/mL)	LH (ng/mL)
Control	10.46 ± 2.38	<2.0	16.03 ± 3.4	1.67 ± 0.2
L-sham	11.32 ± 2.9	<2.0	26.79 ± 5.2*	0.66 ± 0.1*
R-sham	20.31 ± 4.2*	131.6 ± 4.6*	16.32 ± 3.8	2.74 ± 0.6

[a]Data are represented by means ± SEM. ANOVA followed by Tukey's test. *$p < 0.05$ compared to control group.

Mammal species show side predominance of gonad functions *(7–11)*. To our knowledge, no data on steroid secretion differences between ovaries of hemiovariectomized rats have been reported. The aim of this work was to determine whether the acute changes in gonad steroid-hormone con-centrations after hemiovariectomy are dependent on the remaining ovary. Since evidence of the role played by cholinergic ovarian innervation in the regulation of ovarian functions has been reported *(12)*, the effects of cholinergic blockade by atropine sulfate, applied 1 h before experimental surgery, were also analyzed.

Results

Effects of Ether Anesthesia and Sham Operation on Hormone Concentration in Serum

Compared to the control group, the ether anesthesia group showed a decrease in luteinizing hormone (LH) serum concentration ($p < 0.05$). No apparent effects on progesterone, estradiol, or testosterone concentration in serum were observed. The concentration of testosterone in the serum of untouched control rats was below radioimmunoassay (RIA) sensitivity of 2.0 pg/mL (Table 1).

Table 2 presents the effects of sham operations (left side- or right side-) surgery on progesterone, testosterone, estradiol, and LH concentration in serum. Progesterone and testosterone concentration in serum of rats with right-sham operation was significantly higher ($p < 0.05$) than in control rats. The concentration of estradiol in rats submitted to left-sham operation was significantly higher than in rats with a right-sham operation ($p < 0.05$). A significant decrease in LH concentration in serum of rats with a left-sham operation ($p < 0.05$) was observed.

Because all sham operation treatments had an effect on progesterone, testosterone, estradiol, and LH concentration in serum, the effects of hemiovariectomy, with or without bilateral adrenalectomy, and atropine sulfate injection, were compared with the proper sham-operated group.

Effects of Atropine Sulfate Injection

Compared to untouched-control rats, injecting atropine sulfate caused a significant increase ($p < 0.05$) in progesterone, testosterone, and estradiol concentrations (Fig. 1, panel A).

Effects of Adrenalectomy

Compared to control rats, bilateral adrenalectomy resulted in a decrease ($p < 0.05$) in progesterone concentration in plasma and a significant increase ($p < 0.05$) in testosterone and estradiol serum concentrations (Fig. 1, panel B).

Effects of Left Hemiovariectomy (Rats with the Right Ovary Left In Situ)

Compared to left-sham operated rats, left hemiovariectomy caused no apparent changes in progesterone concentration. In turn, testosterone serum concentration increased, and estradiol concentration in plasma diminished significantly ($p < 0.05$) (Fig. 1, panel C).

Effects of Right Hemiovariectomy (Rats with the Left Ovary In Situ)

Compared to right-sham operated rats, no changes in progesterone, testosterone, or estradiol concentration in serum were observed (Fig. 1, panel D).

Effects of Atropine Sulfate Injection to Left-Hemiovariectomy Rats (Right Ovary In Situ)

Compared to rats injected with atropine sulfate and submitted to a left-sham operation, left-hemiovariectomized rats exposed to cholinergic blockade showed higher progesterone concentrations, a significant reduction of testosterone levels, and a non significant reduction in estradiol concentration in serum ($p < 0.05$) (Fig. 2, panel A).

Effects of Atropine Sulfate Injection to Right-Hemiovariectomized Rats (Rats with the Left Ovary In Situ)

The right-hemiovariectomized group of rats with a cholinergic blockade had similar progesterone concentration in serum ($p < 0.05$), and showed a significant increase in

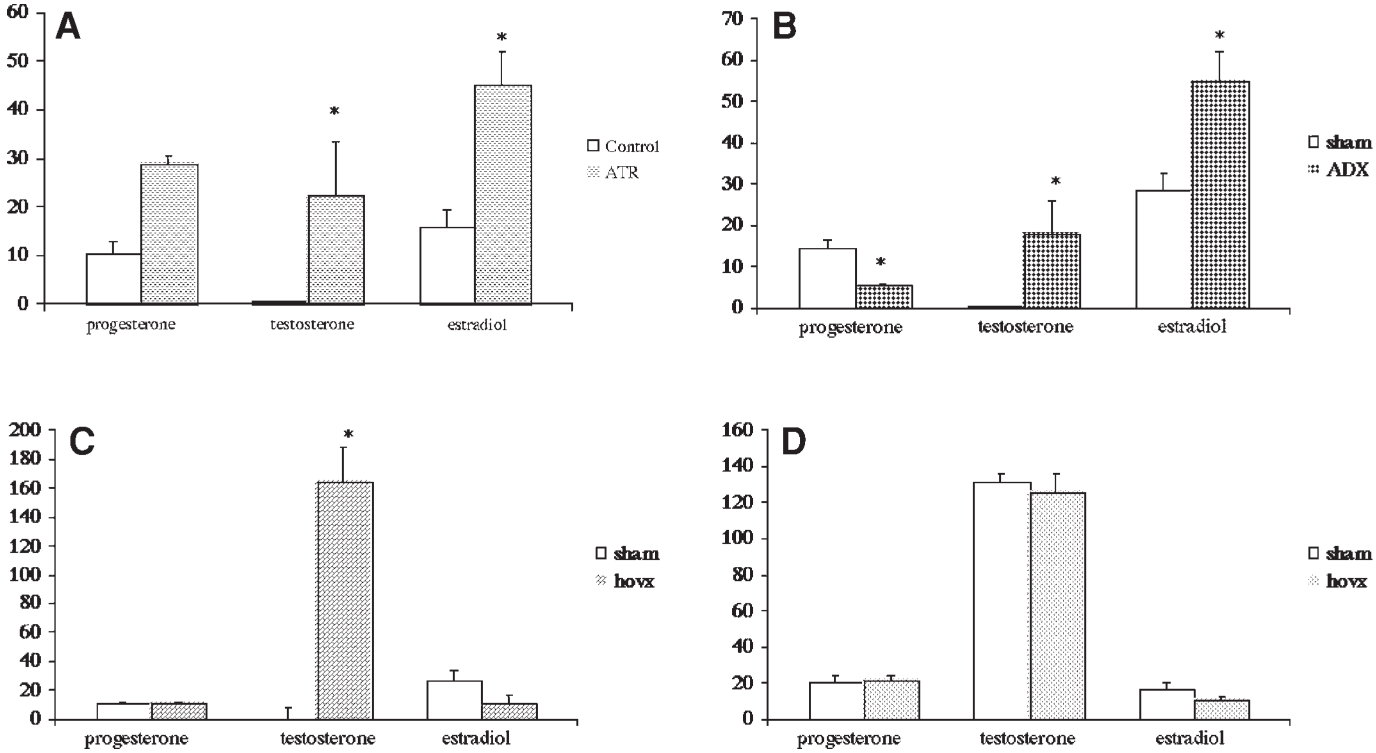

Fig. 1. (**A**) Progesterone (ng/mL), testosterone (pg/mL), and estradiol (pg/mL) serum concentration in control and atropine (ATR)-treated rats, killed 1 hr after treatment. (**B**) Progesterone (ng/mL), testosterone (pg/mL), and estradiol (pg/mL) serum concentration in control and adrenalectomized (ADX) rats, killed 1 h after treatment. (**C**) Progesterone (ng/mL), testosterone (pg/mL), and estradiol (pg/mL) serum concentration in sham-operated and hemiovariectomized (hovx) rats with the right ovary *in situ*, killed 1 h after treatment. (**D**) Progesterone (ng/mL), testosterone (pg/mL), and estradiol (pg/mL) serum concentration in sham-operated and hemiovariectomized (hovx) rats with the left ovary *in situ*, killed 1 h after treatment. $*p < 0.05$ in comparison with proper control (Sudent's *t* test).

testosterone and estradiol concentration in serum, compared to rats submitted to right-sham surgery and injected with atropine sulfate (Fig. 2, panel B).

Effects of Adrenalectomy to Rats with the Left Ovary Extirpated (Right Ovary In Situ)

In comparison to rats with bilateral adrenalectomy, left-hemiovariectomy resulted in a decrease of progesterone and testosterone concentration in serum ($p < 0.05$), without significant changes in estradiol serum concentration (Fig. 2, panel C).

Effects of Adrenalectomy to Rats with the Right Ovary Extirpated (Left Ovary In Situ)

In comparison to rats with bilateral adrenalectomy, right-hemiovariectomy resulted in a decrease in progesterone serum concentration, a significant increase in testosterone serum concentration ($p < 0.05$) and no apparent changes in estradiol serum concentration (Fig. 2, panel D).

Effects of Hemiovariectomy in LH Concentration in Serum

Hemiovariectomy did not have an apparent effect on LH concentration in serum, while all other treatments resulted in a significant decrease of LH ($p < 0.05$) (Table 3).

Discussion

The results presented herein support the idea that ovarian innervation modulates steroid secretion in a selective way, and that both ovaries have different capacities to react to the same type of stimuli *(3)*.

The increase in progesterone and testosterone concentration seems to be partially induced by an adrenal stress response, plausibly caused by the perforation to the peritoneum. We presume the existence of two different neural pathways: one, controlling the secretion of progesterone by the adrenals originating from the right side; and the other, originating from the left side, controlling secretion of estradiol from the left ovary.

This interpretation is based on the observed estradiol level increases in rats with left sham-surgery, and the absence of estradiol concentration increase in plasma when the left ovary was removed. These differences suggest the presence of a neural signal that reaches the left ovary and stimulates the secretion of estradiol. In this regard, it has been reported that pinching the adrenal pedicle stimulates DNA synthesis in the contralateral gland, concluding that an autonomic neural reflex mediate this rapid proliferative response *(13)*.

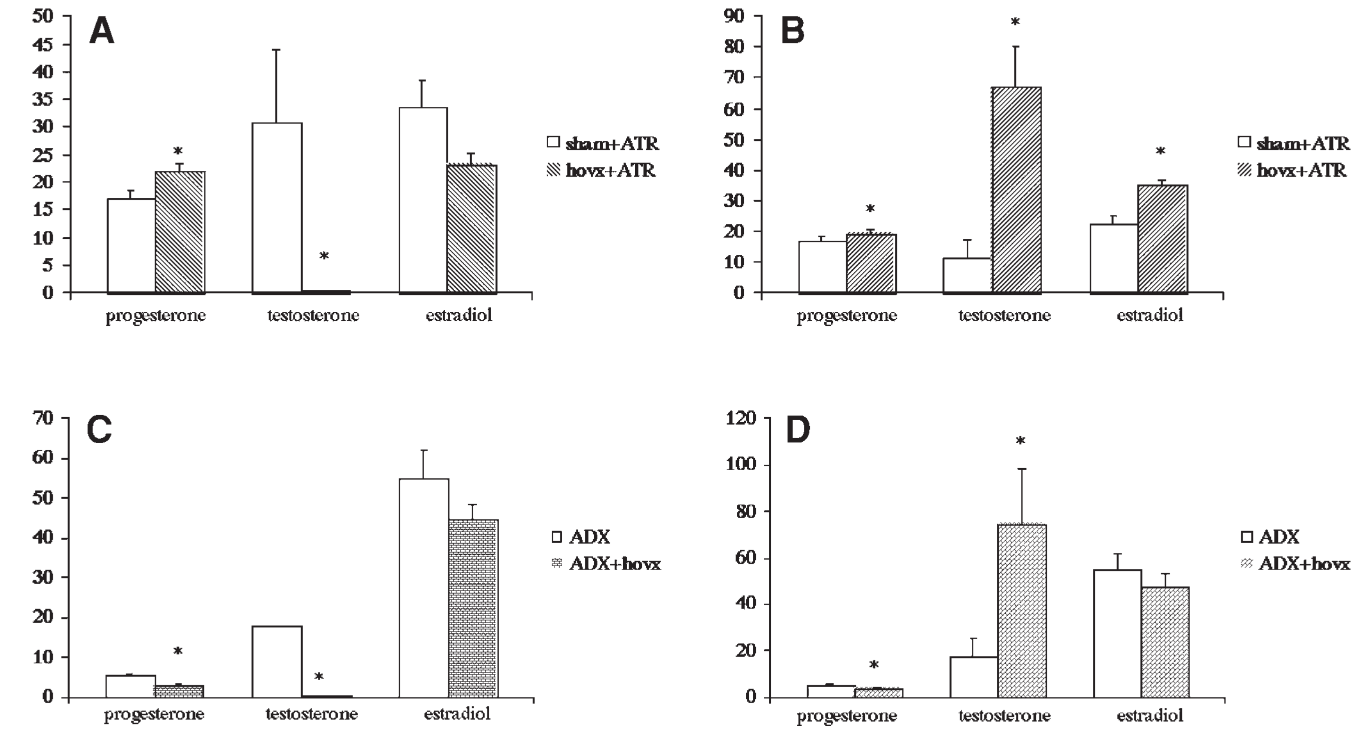

Fig. 2. (**A**) Progesterone (ng/mL), testosterone (pg/mL), and estradiol (pg/mL) serum concentration in sham-operated rats injected with atropine sulfate and hemiovariectomized (hovx) rats with the right ovary *in situ* treated with atropine sulfate, killed 1 h after treatment. (**B**) Progesterone (ng/mL), testosterone (pg/mL), and estradiol (pg/mL) serum concentration in sham-operated rats injected with atropine sulfate and hemiovariectomized (hovx) rats with the left ovary *in situ* treated with atropine sulfate, killed 1 h after treatment. (**C**) Progesterone (ng/mL), testosterone (pg/mL), and estradiol (pg/mL) serum concentration in adrenalectomized (ADX) and adrenalectomized+hemiovariectomy (ADX+hovx) rats with the right ovary *in situ*, killed 1 h after treatment. (**D**) Progesterone (ng/mL), testosterone (pg/mL), and estradiol (pg/mL) serum concentration in adrenalectomized (ADX) and adrenalectomized+hemiovariectomy (ADX+hovx) rats with the left ovary *in situ*, killed 1 h after treatment.

Table 3
LH (ng/mL) Concentration in Serum of Control Rats, and Hemiovariectomized (Hovx), Bilateral Adrenalectomized (ADX), Injected with Atropine Sulfate (ATR) and Bilateral Adrenalectomy in Hemiovariectomized (ADX+hovx) Rats, Treated or Not with Atropine Sulfate (ATR), Killed 1 h after Treatment[a]

Group	Left side (right ovary *in situ*)	Right side (left ovary *in situ*)
Control	1.66 ± 0.24	1.66 ± 0.24
Hovx	1.29 ± 0.10	1.39 ± 0.15
ADX	0.56 ± 0.09*	0.56 ± 0.09*
ADX + Hovx	0.62 ± 0.07*	0.57 ± 0.12*
ATR	0.63 ± 0.04*	0.63 ± 0.04*
ATR + Sham	0.47 ± 0.06*	0.44 ± 0.05*
ATR + Hovx	0.46 ± 0.06*	0.49 ± 0.04*
ATR + ADX	0.35 ± 0.04*	0.35 ± 0.04*

[a]Data are represented by means ± SEM. MANOVA followed by Tukey's test. *$p < 0.05$ compared to control group.

The proposed neural pathway between the peritoneum and the adrenals, and/or ovaries, seems to be very specific to the control of progesterone and testosterone secretion. One of the pathways involved in the regulation of progesterone secretion could not be mediated by muscarinic receptors, while the pathways mediating the regulation of testosterone secretion must have a muscarinic receptor linked to an inhibitory pathway. It is also possible that ovarian innervation participates in blood-flow regulation. There is evidence of both, an immediate and a delayed neural-mediated component that are influenced by adrenal medullar secretion while regulating testicular blood flow *(14)*. According to Klein et al. *(15)*, blood flow to the ovaries is not modified after stimulation of the superior ovarian nerve.

We think that these neural pathways, reaching the ovary and/or the adrenals, play significant roles in steroidogenesis regulation; thus, differences in progesterone serum concentration between left- and right-hemiovariectomized rats may be explained on the basis of differences in ovarian innervation *(5)*. Consequently, we propose that the right ovary sends inhibitory regulatory stimuli through the secretion of testosterone to the left ovary.

Because bilateral adrenalectomy significantly reduced the concentration of testosterone in serum, it is possible that the concentration of testosterone, measured in unilaterally ovariectomized rats (left ovary *in situ*), was influenced by the secretion of testosterone from the adrenals.

According to D'Albora et al. *(16)*, some of the neurons in the rat's ovary may be catecholaminergic and others neuropeptide Y immune-reactive. Some of the neurotransmitters (norepinephrine and vasoactive intestinal peptide) delivered to the ovary, via the extrinsic innervation of the gland, are involved in the regulation of ovarian steroidogenesis *(17)*. In addition, evidence that the ovaries receive cholinergic innervation has been reported *(12,18)*, as well as the existence of muscarinic receptors in the ovaries of primates, and the synthesis of acetilcholine by granulose cells *(19)*. Therefore, the observed effects on progesterone concentrations in plasma, caused by injecting atropine sulfate before hemiovariectomy (extirpation of the right or left ovary), support the idea of differences in the participation of the cholinergic innervation regulating hormonal secretion by the ovaries and/or adrenals.

The existence of two cholinergic pathways (one stimulatory, another inhibitory) to the adrenals and/or ovaries is suggested by both: the increase in testosterone concentration in plasma, observed in rats with an induced cholinergic blockade by atropine sulfate injection before unilateral-sham operation; and, by the "mirror" differences in testosterone concentration between left and right sham-operated rats treated with atropine sulfate before sham-surgery procedures.

Cholinergic innervation of the right ovary seems to play a stimulatory role in hormonal regulation and, because cholinergic blockade by atropine sulfate resulted in a significant increase in testosterone and estradiol serum concentration, cholinergic innervation of the left ovary seems to play an inhibitory regulatory role.

Compared to control rats, the increase of testosterone concentration observed in adrenalectomized rats suggests that the ovaries are the origin of such testosterone secretion. Our results suggest that under normal conditions, the ovarian cholinergic innervation regulates the secretion of both hormones in an inhibitory way. This conclusion is based on the increases in testosterone and estradiol concentration in plasma observed in rats subjected to cholinergic blockade by atropine sulfate injection. The idea of two cholinergic regulatory effects on each ovary is supported by the decrease in testosterone and estradiol serum concentration in rats with the right ovary *in situ*, and sustains the hypothesis of the asymmetric ability to secrete estrogens by the ovaries. The increase of estradiol concentration in plasma, observed in rats with bilateral adrenalectomy, agrees with the findings and conclusions of Cardenas *(20)* and Shors et al. *(21)*. Both groups of researchers reported that exposure to relatively acute stressful events enhances estra-diol concentration in an immediate and persistent fashion, and that the effects of stress on hormone concentration vary according to the type of stressor used. Because in our study exposure to ether stress did not modify estradiol concentration in serum, we presume that the peritoneum perforation elicited the necessary signals for such "stressful" response.

According to Puder et al. *(22)*, in the presence of sufficient estrogens, activation of the hypothalamic-pituitary-adrenal axis leads to the stimulation of LH release. Since left side sham surgery did not cause apparent changes in progesterone and testosterone serum concentration, we suggest the existence of an asymmetric neural component linking the central nervous system with the ovaries. It seems that at least two different neural pathways related to the regulation of gonadotropin release exist, one stimulatory and the other inhibitory.

Hemiovariectomy blocked the effects of sham-operation on LH concentration in serum, observed 1 h after surgery. Such results suggest that the left ovary is the origin of the stimulatory signal, while the right ovary is influenced by a clue that inhibits LH secretion. LH concentration decreases in adrenalectomized rats could be explained through an inhibitory action of corticotrophin-releasing factor on gonadotropin-releasing hormone (GnRH) *(23)*, because 24 h after adrenalectomy this effect was still observed (unpublished results).

According to Gerendai et al. *(24)*, there is a multisynaptic neuronal pathway between the ovary and the central nervous system. The vagus nerve is the most probable neural link, because the peritoneum is a vagal-innervated structure. According to Chen et al. *(25)*, neurons containing neurokinin B receptor in the nucleus tractus solitarius are involved in the integration of noxious afferent information from the peritoneum in the rat. Evidence that vagotomy decreases steroid secretion *(26)*, reduces the number of ova shed *(27)*, and inhibits the development of compensatory ovarian hypertrophy *(28)* has been reported. Based on morphological studies, Gerendai et al. *(29)* suggest that several brain structures are involved in the control of ovarian function, and act via the vagus or sympathetic nerves. Therefore, the differential effects on steroid and LH secretion observed on unilaterally sham-operated rats could be based on asymmetric cues carried by parasympathetic fibbers originating in the central nervous system, as proposed by Frankel et al. *(30)*.

Taken together, the results presented in this study support the idea that left and right ovaries play different roles in the regulation of hormone secretion, and that such differences are related to ovarian innervation *(9)*. Furthermore, the idea of a possible relation between the hormones of the hypothalamic–pituitary–adrenal axis, and those of the hypothalamic–pituitary–gonadal axis *(23)*, is supported by the results presented herein.

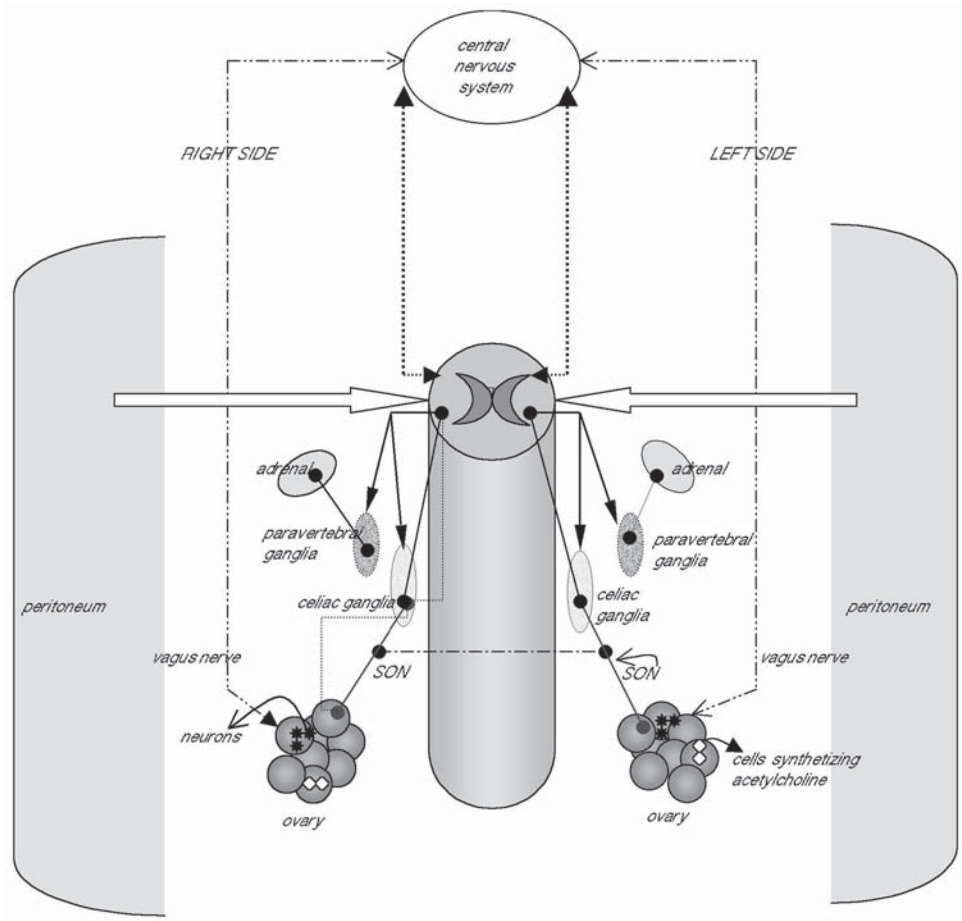

Fig. 3. A possible explanation for the participation of the innervation in the regulation of ovarian hormone secretion. The central nervous system sends different neural signals to the right and left ovaries. The final common pathway for each ovary is the superior ovarian nerve (SON). It also sends and receives neural asymmetric information from each adrenal and the peritoneum. In the ovary such innervation modulates the response of the follicles to the gonadotropins via the release of neurotransmitters, mainly norepinephrine, and by acting on the neurons of the ovary; it is also possible that it affects the non-neuronal cholinergic system of the ovary. To close the loop, some of the neurons on the ovary register changes in several parameters of the ovarian function and such changes are sending to the central nervous system via the vagus nerve, the paravertebral ganglia, and the intermediolateral column, and the contralateral ovary via sympathetic nerves (ovarian plexus?).

Fig. 3 represents a possible explanation on the interplay between the ovaries and adrenals via their innervation.

Materials and Methods

The study was conducted with virgin adult female rats of the CIIZ-V strain from our own stock, with 195–225 g body weight. 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 the parameters of the NIH Guide. The experiments were approved by the Committee of the Facultad de Estudios Superiores Zaragoza. Estrus cycles were monitored by daily vaginal smears. Only rats showing at least two consecutive 4-d cycles were used in the experiment. All surgeries were performed under ether anesthesia, between 13:00 and 13.15 h on the day of estrus.

Rats were randomly allotted to each of the experimental groups (8–10 rats by group). Rats of different groups were treated simultaneously and sacrificed 1 hour after surgery.

Experimental Groups

Control Group

Nontreated cyclic rats sacrificed at 14:00 h on the day of estrus.

Sham-Operated Group

Rats were submitted to a unilateral incision in the back, 2 cm below the last rib. The incision affected skin, muscle, and peritoneum, but the ovary was not touched. After this procedure the wound was sealed.

Hemiovariectomy

The right or left ovary was extirpated and the wound sealed afterward.

Ether Anesthesia

Rats were anesthetized during 10 min and sacrificed 1 h later.

Bilateral Adrenalectomy

A bilateral incision below the last rib, including skin and muscle, was performed, the adrenals were subsequently re-moved, and the wound sealed.

Bilateral Adrenalectomy in Hemiovariectomized Rats

Groups of rats were hemiovariectomized (right or left ovary), the adrenals were extirpated, and the wounds sealed.

Atropine Sulfate-Treated Groups

Sham-operated, bilateral adrenalectomized and hemi-ovariectomized rats were subcutaneously injected with 300 mg/kg of atropine sulfate (Sigma Chemical Co., St. Louis, MO, USA) 1 h before surgery, and were sacrificed 1 h after surgery. For control purposes, a group of untouched rats was injected with the same amount of atropine sulfate at 13.00 h and sacrificed 1 h later.

Autopsy Procedure

All rats were sacrificed by decapitation. The blood of the trunk was collected, allowed to clot at room temperature for 30 min, and centrifuged at 3000 rpm during 15 min. The serum was stored at –20°C, until estradiol-17β, testosterone, progesterone, and LH concentration were measured.

Hormone Assay

LH concentration was measured by specific radioimmunoanalysis (RIA), using the double-antibody technique, with reagents and protocol supplied by the National Hormone and Pituitary Program (Baltimore, MD). Intra- and interassay variation coefficients were 5.1% and 6.5%, respectively. Results are expressed in ng/mL, based on LH-RP-3. The concentration of estradiol, testosterone and progesterone in serum were measured by RIA, using a kit purchased from Diagnostic Products (Los Angeles, CA). The Intra- and interassay variation coefficients were 6.9% and 10.8% for estradiol, 5.3% and 9.87% for progesterone, and 5.6% and 8.7 for testosterone, respectively. Results are expressed in ng/mL (progesterone concentration) or pg/mL (testosterone and estradiol concentration).

Statistics

Data on hormonal concentrations in serum were analyzed using multivariate analysis of variance (MANOVA) followed by Tukey's test. The differences in hormone serum concentration between the control group and the ether-anesthesia-treated group were analyzed by Student's *t* test. A probability value of less than 5% was considered significant.

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References

1. Greenwald, G. S. and Roy, S. K. (1994). In: *The physiology of reproduction.* Knobil, E. and Neild, J. D. (eds.). Raven Press: New York.
2. Otani, T. and Sasamoto, S. (1982). *J. Reprod. Fertil.* **65,** 347–353.
3. Chávez, R., Cruz, M. E., and Domínguez, R. (1987). *J. Endocrinol.* **113,** 397–401.
4. Burden, H. W., Leonard, M., Smith, C. O., and Lawrence, I. E. Jr. (1983). *Anat. Rec.* **207,** 623-627.
5. Klein, C. M. and Burden, H. W. (1988). *Neurosc. Lett.* **85,** 217–222.
6. Malishevskaia, V. A. and Brindak, O. I. (1980). *Arkhiv Anatomii, Gistologii I Embriologii* **79,** 95–99.
7. Bleier, W. J. and Ehteshami, M. (1981). *J. Reprod. Fertil.* **63,** 181–183.
8. Rao, Ch. V. and Edgerton, L. A. (1984). *J. Reprod. Fertil.* **70,** 61–66.
9. Domínguez, R., Cruz, M. E., and Chávez, R. (1988). In: *Growth factors and the ovary.* Hirshfield, A. N. (ed.). Plenum Press: New York.
10. Mittwoch, V. (1975). *Lancet* **1,** 401–402.
11. Marut, E. L. and Hodgen, G. D. (1982). *Steroids* **39,** 461–469.
12. Kornya, L., Bodis, J., Koppan, M., Tinneberg, H. R., and Torok, A. (2001). *Gynecol. Obstet. Invest.* **52,** 104–107.
13. Engeland, W. C. and Gann, D. S. (1989). *Neuroendocrinology* **50,** 124–131.
14. Cross, B. A. and Silver, I. A. (1962). *J. Reproduc. Fertil.* **3,** 377–395.
15. Klein, C. M., Ray, R. H., and Burden, H. W. (1989). *Brain Res.* **479,** 194–200.
16. D'Albora, H., Lombide, P., and Ojeda, S. R. (2000). *Cell Tissue Res.* **300,** 47–56.
17. Ojeda, S. and Urbanski, H. F. (1994). In: *The physiology of reproduction.* Knobil, E. and Neill, J. D. (eds.). Raven Press: New York.
18. Burden, H. W. (1978). The vertebrate ovary. In: *Comparative biology.* Jones, R. E. (ed.). Plenum Press: New York.
19. Fritz, S., Wessler, I., Breitling, R., et al. (2001). *J. Clin. Endocrin. Metab.* **86,** 349–354.
20. Cardenas, H. (1992). *Biol. Res.* **25,** 15–20.
21. Shors, T. J., Pickett, J., Wood, G., and Paczynski, M. (1999). *Stress* **3,** 163–171.
22. Pudder, J. J., Freda, P. U., Goland, R. S., Ferin, M., and Wardlaw, S. I. (2000). *J. Clin. Endocrin. Metab.* **85,** 2184–2188.
23. Rivier, C. and Rivest, S. (1991). *Biol. Reprod.* **45,** 523–532.
24. Gerendai, I., Tóth, I. E., Boldogköi, Z., Medveczky, I., and Halász, B. (1998). *Neuroendocrinology* **68,** 244–256.
25. Chen, L. W., Guan, Z. L., and Ding, Y. Q. (1997). *J. Hirnforschung.* **38,** 363–367.
26. Lawrence, J. E., Burden, H. W., and Louis, T. M. (1978). *J. Reprod. Fertil.* **53,** 131–136.
27. Nakamura, Y., Kato, H., and Terranova, P. F. (1992). *Endocrinol. Jap.* **39,** 141–145.
28. Burden, H. W. and Lawrence, I. E. Jr. (1977). *Neuroendocrinology* **23,** 368–378.
29. Gerendai, I., Tóth, I. E., Boldogköi, Z., Medveczky, I., and Halász, B. (2000). *J. Auton. Nervous Syst.* **80,** 40–45.
30. Frankel, A. I., Chapman, J. C., and Cook, B. (1989). *J. Endocrinol.* **122,** 485–488.