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Effects of Capsaicin Treatment on the Regulation of Ovarian Compensatory Hypertrophy and Compensatory Ovulation

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The present study investigates the effects of functional sensorial denervation, induced by administering capsaicin to hemiovariectomized adult female rats in each day of the estrus cycle, on ovulation and serum concentrations of estrogen and progesterone. The results indicate that the establishment of compensatory ovarian hypertrophy (COH) and compensatory ovulation (CO) depends on both the day of the estrous cycle when sensorial denervation was performed and on which ovary was extirpated. These results support the now accepted notion that the response of the ovaries to denervation is asymmetrical. The results seem to suggest that this asymmetric response is mediated by some specific neural information that is registered in the ovary and sent to the CNS, that such information plays a role modulating the reactivity of the ovarian compartments to gonadotropins, and that the frequency of this signal varies along the estrus cycle.

Key Words: Hemiovariectomy; steroid secretion; ovarian compensatory hypertrophy; neuroendocrinology.

Introduction

Hemiovariectomy is, by now, an experimental paradigm widely used by researchers studying neural connections between the ovaries and the central nervous system (CNS). The effects of hemiovariectomy in mammals can be analyzed in terms of compensatory hypertrophy by the *in situ* ovary, enhanced follicular activity, and an increase in both the number of ova shed and the number of corpora lutea *(1)*. The mechanisms involved in compensatory ovarian hypertrophy (COH) and compensatory ovulation (CO) in hemiovariectomized rats have been analyzed through changes in the secretion rates of gonadotropins, by the pituitary, and of steroids, by the ovaries *(1)*. There is evidence, however, that a direct neural mechanism is involved in modulating COH and CO *(2–6)*. Such modulation is achieved by at least two distinct neural pathways: the parasympathic (vagus nerve) and sympathetic (the superior ovarian nerve and ovarian plexus) systems *(3,4,7)*.

According to Chávez et al. *(3)*, bilateral abdominal vagotomy to right hemiovariectomized rat reduces COH, while sectioning the left vagus nerve induces different effects that vary according to the ovary remaining *in situ.* In right hemiovariectomized rats (left ovary *in situ*) ovulation rates, COH and the number of ova shed by ovulating animals increased after left-side vagotomy, while the same procedure to left hemiovariectomized rats (right ovary *in situ*) induces a decrease in all parameters evaluated.

In the adult rat, the participation of the ovary's noradrenergic innervation in COH depends on the day of the estrous cycle when hemiovariectomy and denervation are performed *(4)*. In the pre-pubertal rat, the participation of the innervation depends on the age of the rat at which surgery is performed *(6)*.

Several studies indicate that the information pathways connecting the CNS and the ovaries involve sensory innervation, and that from the ovary to the CNS, this information is carried by the vagus nerve *(8,9)* and the ovarian plexus *(10,11)*.

The sensory nerves innervating the ovary contain various neurotransmitters, such as substance P (SP) *(12–14)*, vasointestinal peptide (VIP), and calcitonin gene-related peptide (CGRP) *(11,15,16)*. The sensory fibbers innervating the ovary are classified as unmyelinated or C-type primary afferent nerves. In the rat, the sensory fibbers are permanently destroyed by treatment with the neurotoxin capsaicin *(17)*.

According to Nance et al. *(18)*, intrathecal capsaicin treatment had no effect on the estrous cycle, COH, or female sexual behavior of treated rats. However, these researchers found that capsaicin-treated animals showed a dramatic reduction in their fertility rates, which was found to be due to the reduced capacity of the vaginal–cervical stimulation to produce the decidual response.

Morán et al. *(19)* found that injecting capsaicin to prepubertal rats results in a decrease in the number of ova shed at first estrus. No differences in the levels of estradiol and progesterone between control and treated animals were observed. When hemiovariectomy was performed to 28-d-old rats, the group of pre-pubertal rats treated with capsaicin at

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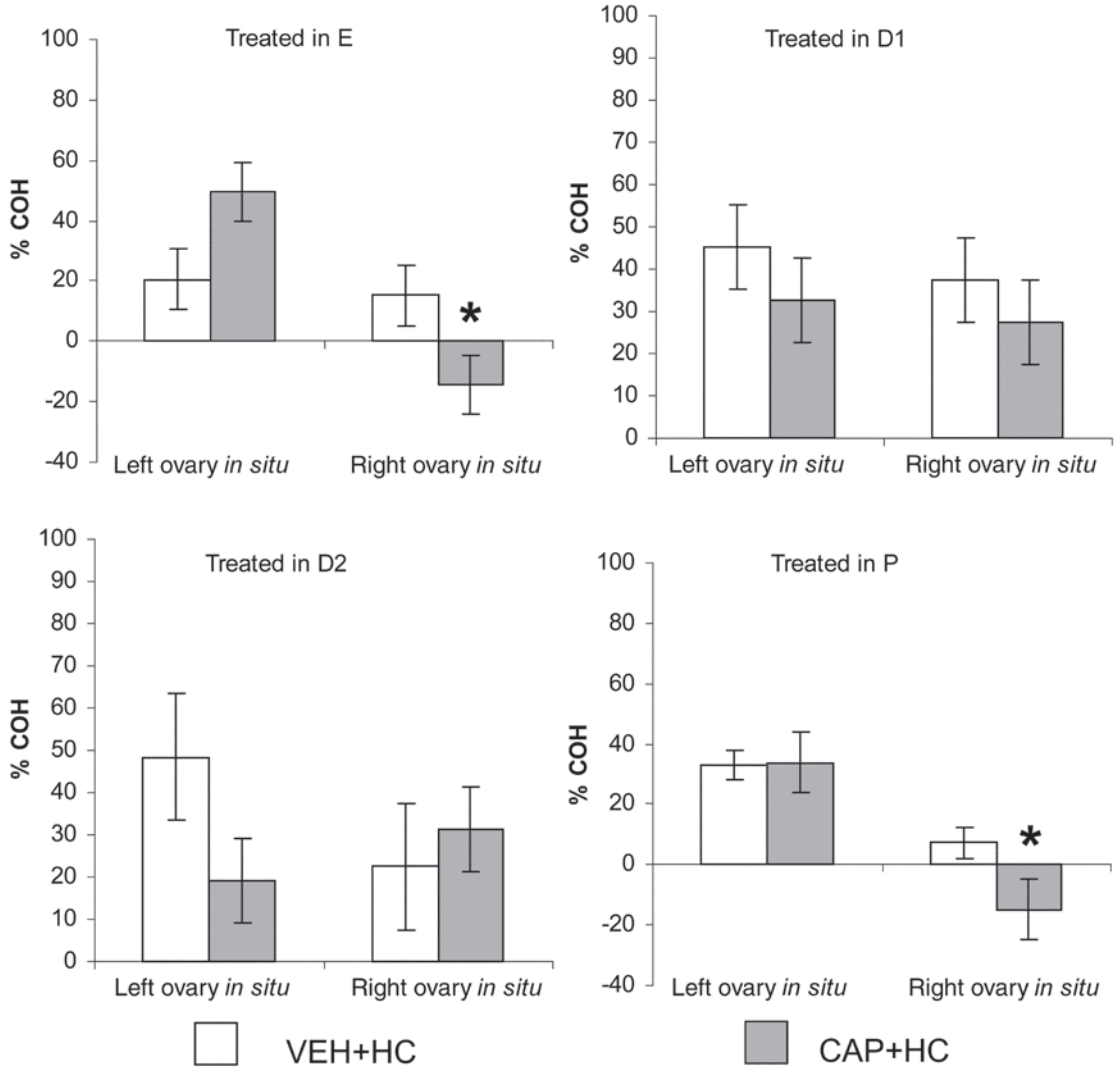


Fig. 1. Percentage (mean ± SEM) of ovarian compensatory hypertrophy (COH) by left or right ovary, in animals treated with vehicle (VEH) or capsaicin (CAP), in estrus (E), diestrus 1 (D1), diestrus 2 (D2), or proestrus (P). The animals were sacrificed the day of estrus, after presenting three consecutive 4-day cycles. *$p < 0.05$ vs VEH+HC Right ovary *in situ* (chi square test).

birth showed significantly higher COH by the left ovary than vehicle-treated animals did. Capsaicin treatment did not modify progesterone serum levels to animals with the right ovary *in situ*, but were significantly lower when the right ovary was extirpated.

The aim of the present study was to evaluate the participation of the sensorial ovarian innervation, in hemiovariectomized adult female rats, on regulating COH, CO, and the concentrations of estradiol and progesterone in serum. The evaluation was based on the effects of capsaicin treatment to adult rats. According to Trujillo et al. *(20)*, the effects of capsaicin treatment vary according to the day of the estrous cycle when the drug is injected. Consequently, we analyzed the hypothesis that neuroendocrine conditions regulating ovarian responses to hemiovariectomy vary along the estrous cycle, and that sensory denervation modifies the reactivity of the ovary *in situ*, depending also on the day of the estrous cycle when denervation is performed.

Results

Percentage of the Compensatory Ovarian Hypertrophy (COH)

In rats with the left ovary *in situ,* the COH was similar between control (vehicle-injected) animals and denervated (capsaicin-treated) groups injected with capsaicin, regardless of the day of treatment (estrus, diestrus 1, diestrus 2, or proestrus).

When the right ovary was *in situ*, the COH of rats treated with capsaicin on estrus or proestrus was lower than in rats injected with vehicle solution. Such differences, between the denervated and control rats, were not observed when capsaicin was injected on diestrus 1 or diestrus 2 (Fig. 1).

Number of Ova Shed and Percentage of Compensatory Ovulation (CO)

When left or right hemiovariectomy was performed on adult rats injected with capsaicin on diestrus 2 or proestrus,

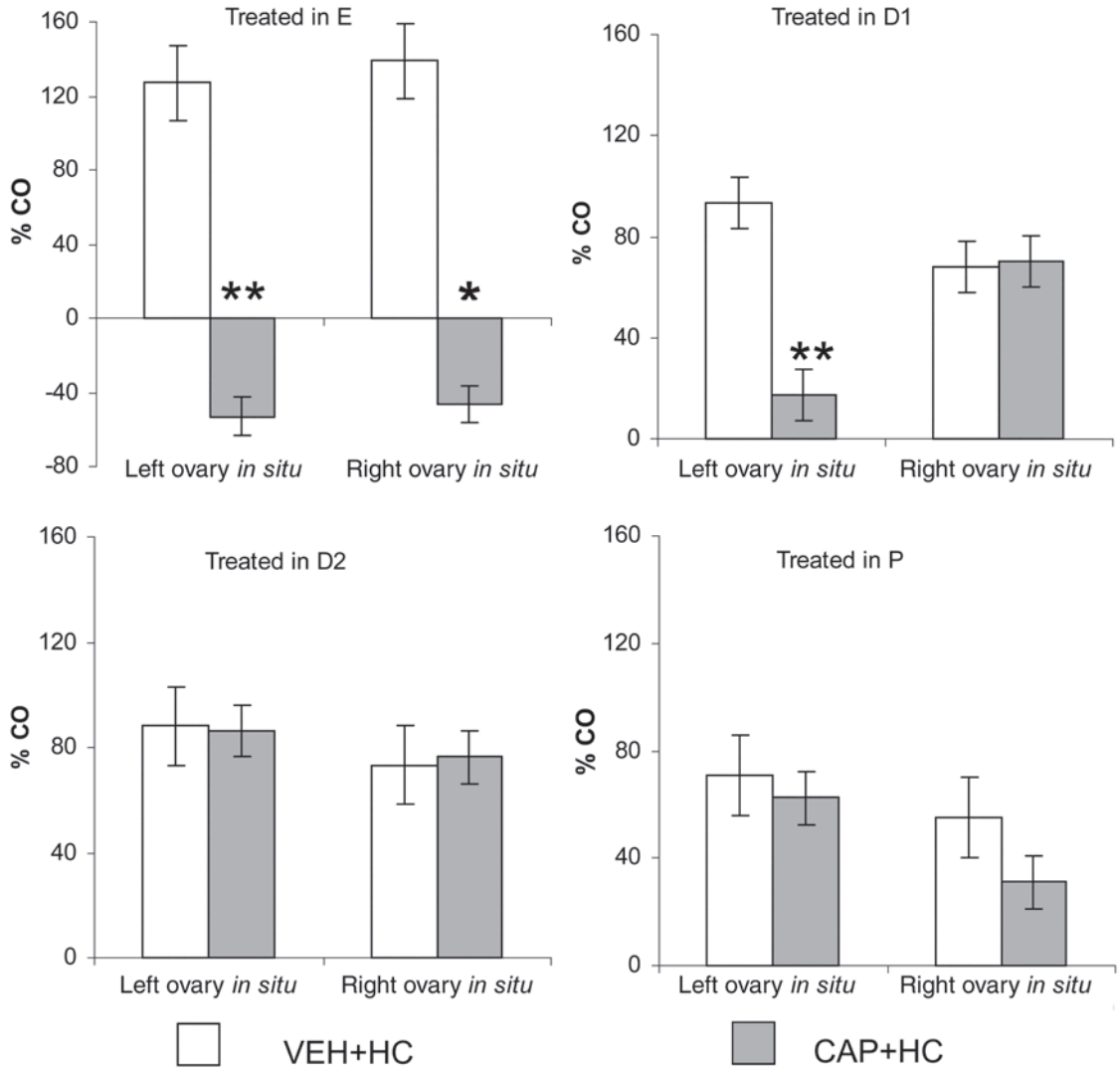


Fig. 2. Percentage (mean ± SEM) of compensatory ovulation (CO) by left or right ovary, in animals treated with vehicle (VEH) or capsaicin (CAP), in estrus (E), diestrus 1 (D1), diestrus 2 (D2), or proestrus (P). The animals were sacrificed the day of estrus, after presenting three consecutive 4-d cycles. *$p < 0.05$ vs VEH+HC Right ovary *in situ* (chi square test), **$p < 0.05$ vs VEH+HC Left ovary *in situ* (chi square test).

CO (Fig. 2) and the number of ova shed were similar to vehicle-treated rats (Table 1).

However, when the left ovary was *in situ*, the CO of rats treated with capsaicin in diestrus 1 or estrus was significantly lower than in vehicle-treated animals. Treatment with capsaicin on the day of estrus, followed by left or right hemiovariectomy, resulted in a significantly lower CO than that of vehicle-treated animals (Fig. 2).

Estradiol and Progesterone Serum Levels

The results of the estradiol and progesterone serum levels in the different experimental groups are shown on Figs. 3 and 4.

1. Rats with the left ovary *in situ*. Compared to vehicle-treated animals, estradiol serum levels were significantly higher in rats treated with capsaicin at diestrus 1. No significant differences, between vehicle and capsaicin-treated rats in diestrus 2, proestrus, or estrus were observed in estradiol serum levels (Fig. 3). No significant differences in progesterone serum levels were observed either (Fig. 4).
2. Rats with the right ovary *in situ*. Compared to vehicle-treated animals, estradiol serum levels were significantly higher in rats treated with capsaicin at estrus. No significant differences in estradiol serum levels were observed between vehicle and capsaicin-treated rats in diestrus 1, 2, or proestrus (Fig. 3). Rats treated with capsaicin in proestrus had significantly higher levels of progesterone than the vehicle-treated group. Such differences in progesterone serum levels were not observed when treatments were done on diestrus 1, diestrus 2, or estrus (Fig. 4).

Histological Observation

The histological analysis of the corpora lutea of hemiovariectomized adult rats injected with vehicle on diestrus 1 day showed healthy cells, with large nuclei, and numerous

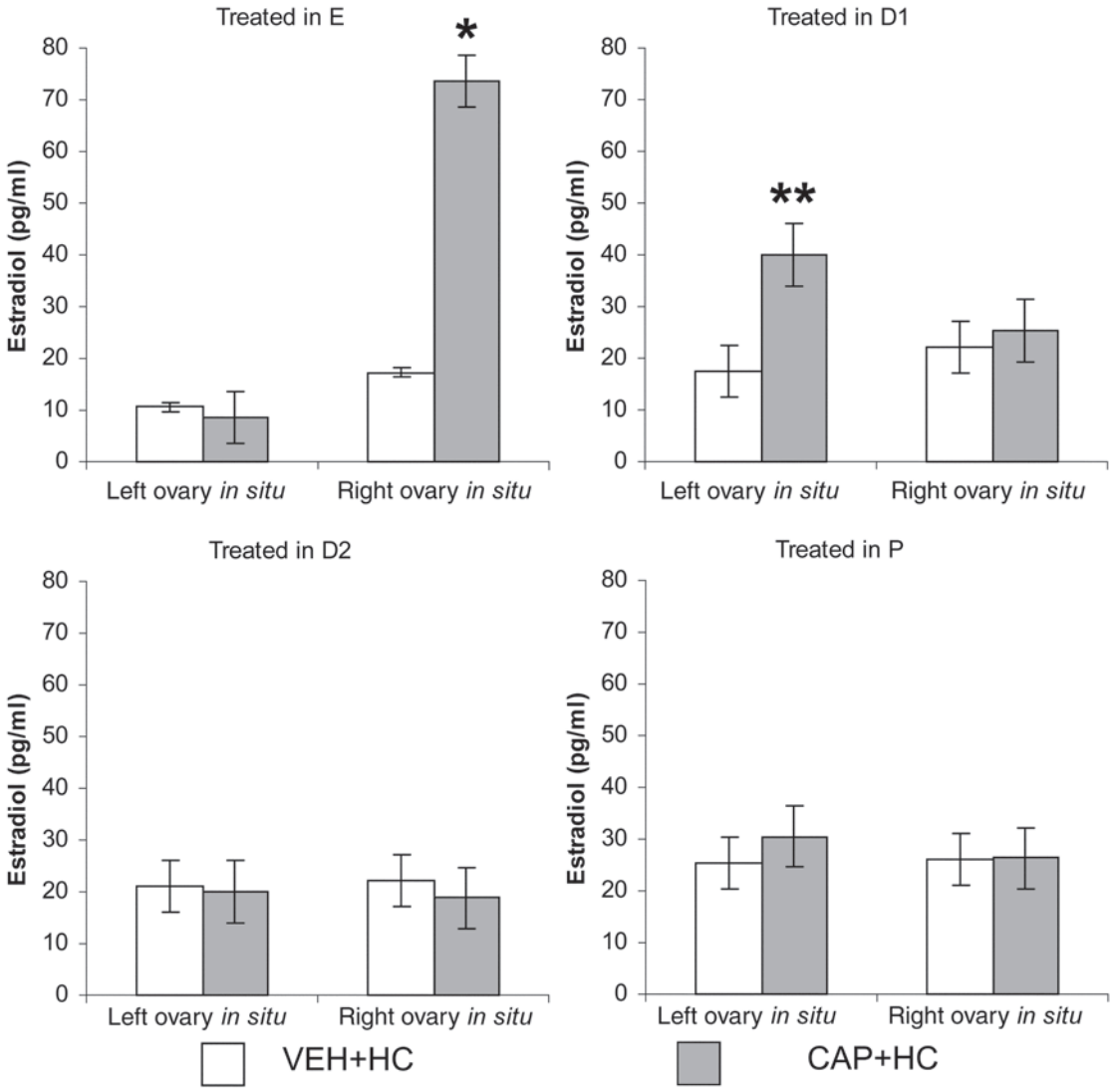
Table 1
Mean ± SEM of Number of Ova Shed
by the Left or Right Ovary, in Animals Treated with Vehicle (VEH)
or Capsaicin (CAP), in Diestrus 1 (D1), Diestrus 2 (D2), Proestrus (P) or Estrus (E)[a]

Group	Ovary *in situ*	Ova shed	Ovary *in situ*	Ova shed
Treated in E				
VEH+HC	Left	6.1 ± 1.04	Right	6.3 ± 1.85
CAP+HC	Left	4.6 ± 1*	Right	4.3 ± 1.24**
Treated in D1				
VEH+HC	Left	6.8 ± 0.5	Right	6.7 ± 0.5
CAP+HC	Left	4.5 ± 0.3*	Right	4.8 ± 0.3**
Treated in D2				
VEH+HC	Left	6 ± 0.3	Right	6 ± 0.3
CAP+HC	Left	6.2 ± 0.3	Right	6.2 ± 0.3
Treated in P				
VEH+HC	Left	6.3 ± 0.7	Right	6 ± 0.6
CAP+HC	Left	6.5 ± 0.6	Right	6.3 ± 0.6

*$p < 0.05$ vs VEH+HC Left ovary in situ. Kruskal-Wallis test followed by Mann–Whitney *U* test.

**$p < 0.05$ vs VEH+HC Right ovary *in situ*. Chi square test. Kruskal–Wallis test followed by Mann–Whitney U test.

[a]The animals were sacrificed the day of estrus, after presenting three consecutive 4-d cycles.



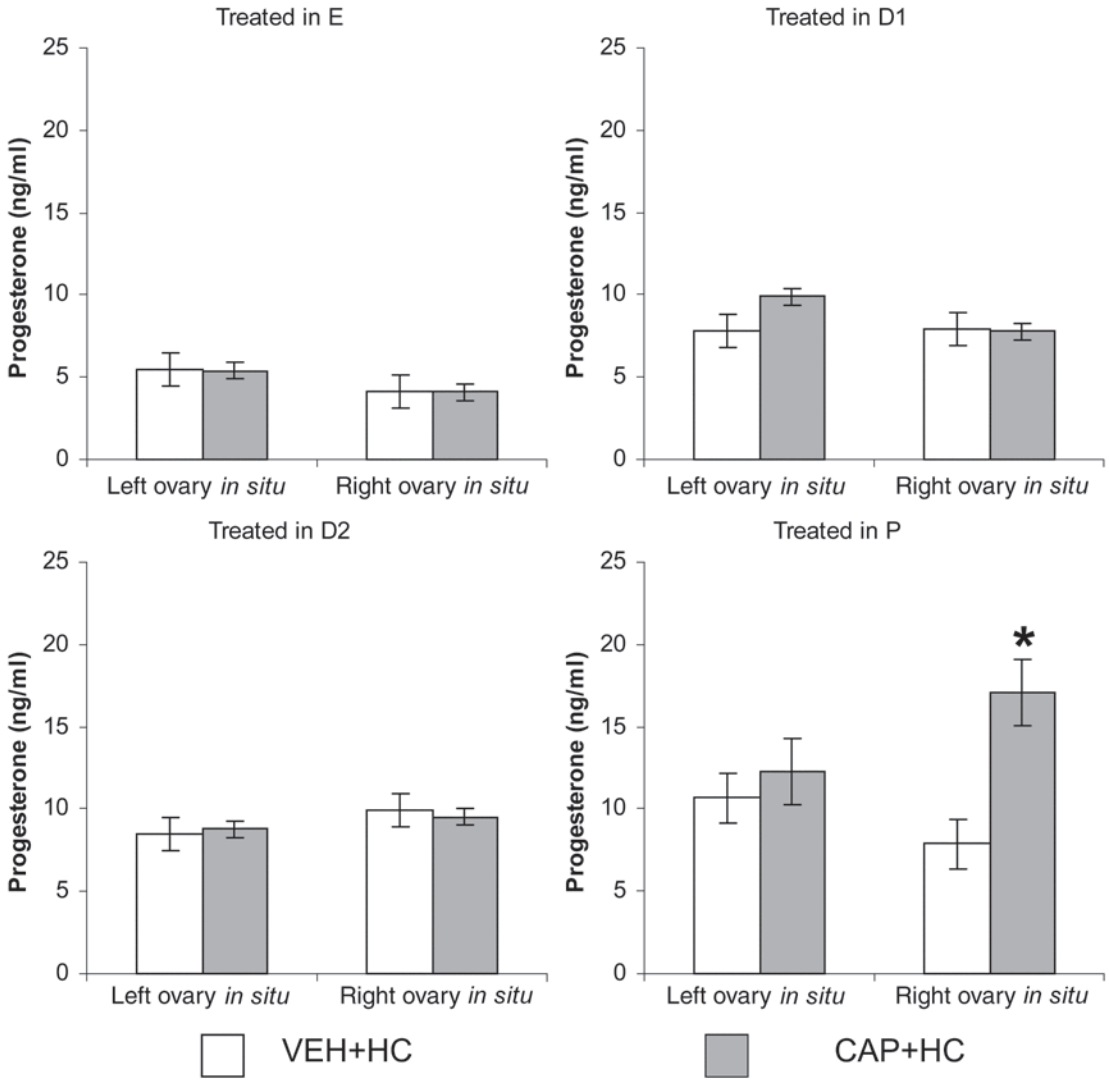


Fig. 4. Serum concentration (mean ± SEM) of progesterone (ng/mL) in animals treated with vehicle (VEH) or capsaicin (CAP), in estrus (E), diestrus 1 (D1), diestrus 2 (D2), or proestrus (P) and hemiovariectomy (HC) of the right or left ovary. The animals were sacrificed the day of estrus, after presenting three consecutive 4-d cycles. *$p < 0.01$ vs VEH+HC Right ovary *in situ*. ANOVA followed by Tukey's test.

well-vascularized mitotic cells [fresh corpora lutea *(21)*]. The corpora lutea of denervated animals had larger cytoplasmic vacuoles, and showed a drop in the number of capillaries and an increase in dense connective tissue [old corpora lutea *(21)*].

Compared to the vehicle-treated group, animals treated with capsaicin in diestrus 1 and hemiovariectomized in diestrus 1 showed a drop in the number the follicles present in the ovary. The follicles present in the ovaries of animals denervated on diestrus 1 showed the presence of pyknosis in the granulosa cell, granulosa cells in the follicular fluid, and hypertrophy of the theca cells.

Discussion

The present results suggest that the sensorial innervation of the ovary participates in ovarian reactivity to neuroendocrine regulating mechanisms, both in intact animals *(20)* and when one of the ovaries is extirpated. In both, the participation of the sensorial innervation varies along the estrus cycle.

According to Nance et al. *(18)*, injecting capsaicin intrathecal to hemiovariectomized adult rats had no apparent effect on COH. The differences in COH changes between Nance et al. and present results could be related to which ovary was removed [it was not specified by Nance et al. *(18)*],

Fig. 3. *(Opposite page)* Serum concentration (mean ± SEM) of estradiol (pg/mL) in animals treated with vehicle (VEH) or capsaicin (CAP), in estrus (E), diestrus 1 (D1), diestrus 2 (D2), or proestrus (P) and hemiovariectomy (HC) of the right or left ovary. The animals were sacrificed the day of estrus, after presenting three consecutive 4-d cycles. *$p < 0.01$ vs VEH+HC Right ovary *in situ*. ANOVA followed by Tukey's test. **$p < 0.05$ vs VEH+HC Left ovary *in situ*. ANOVA followed by Tukey's test.

the day of the estrous cycle when surgery was performed, and the via used to inject the drug (intrathecal and subcutaneous).

Several studies propose that the sensorial innervation of the ovary obtains information on ovarian functions through receptors localized around the follicles; that this information is conveyed to the hypothalamic and extra-hypothalamic centers by neural pathways, and that the afferent ovarian innervation is involved in regulating the response of the ovarian follicle to gonadotropins *(9,22–24)*. In addition, Gerendai et al. *(9)* found that the neural routes by which sensory signals arising from the ovaries reach the hypothalamus include the vagi nerves.

In the adult rat, the participation of the noradrenergic ovary innervation in COH depends on the day of the estrus cycle when hemiovariectomy and denervation are performed *(4)*, suggesting that there are significant changes in the neural mechanisms regulating the modulatory effects made by the ovarian innervation on the performance of the gonad. Present results agree with such idea.

Differences in CO and COH, between the right and left ovaries, have been described in humans *(25)*, pigs *(26)*, and monkeys *(27)*. In the rat, these differences are associated with the number of ova shed by the right and left ovaries, and such differences are related to ovarian innervation *(4, 28)*, and the age of the animal when hemiovariectomy is performed *(6)*. Present results support the idea that the ovarian sensorial innervation participates in modulating CO and COH.

Capsaicin treatment resulted in changes in CO and COH, and, as with estradiol and progesterone serum concentrations, the changes in CO and COH depend of the day of the estrous cycle when treatment was performed. Chávez et al. *(3)* showed that the CO in right or left unilaterally ovariectomized rats, with unilateral or bilateral section of the vagus nerve, was normal when compared to the control group; however, these animals showed lower COH.

Morán et al. *(19)* showed that when hemiovariectomy was performed in newborn capsaicin-treated animals, the CO by the right ovary was significantly higher than in vehicle-injected animals. When the left ovary was left *in situ,* CO from the *in situ* ovary was significantly lower than in vehicle-injected animals. COH was similar in vehicle-treated and hemiovariectomy capsaicin-treated animals.

Our results and those presented by Moran et al. *(19)*, suggest that the left and right ovaries provide different information to the central nervous system. It is possible that in unilaterally ovariectomized animals, the neuroendocrine systems controlling ovarian compensatory growth and ovulation are organized differently, depending on whether the neural information arises from the left or right ovary and arrives at the left or right hypothalamus.

There is no direct evidence showing that the sensorial innervation participates in regulating steroid biosynthetic pathways in vivo. However, Morán et al. *(19)* showed that, compared with vehicle-treated rats, capsaicin-treated rats hemiovariectomized at 20 d of age showed significantly lower progesterone serum levels when the right ovary was left *in situ*, also that the ability of the right and left ovary to secrete estradiol is different.

In capsaicin-treated animals we observed an increment in serum estrogen concentration, an increment that could result from the increased content of norepinephrine in the ovaries of these animals. In this study we did not measure ovarian norepinephrine concentration, but results from of our laboratory show that in the adult rat capsaicin administration into the bursa resulted in an increase in ovarian norepinephrine content when the treatment was in diestrus 1 (diestrus 1: 6.1 ± 1.1 vs 1.9 ± 0.3 $p < 0.05$) *(20)*. Thus, the increase in estradiol serum concentration in denervated female rats may result from the plausible increment in norepinephrine. There is evidence that norepinephrine has a stimulatory effect on the estrogen secretion by the ovaries *(28–30)*.

The results presented herein suggest that in hemiovariectomized adult rats the sensory innervation participates in the steroid biosynthetic pathways in vivo, by inhibiting the modulation of estradiol and progesterone secretion, and that such participation is different for each ovary and varies along the estrous cycle. There is evidence that capsaicin alters the sensory nerves containing SP, VIP, and CGRP *(11–16)*. These peptides play a role in the communication between primary sensory neurons and other neuronal and nonneuronal cells. Thus, it is possible that capsaicin treatment's effect on progesterone and estradiol serum levels reflect the effects on the secretion of other peptides. Based on the present results, and those available in the primary literature, we propose that capsaicin treatment affects estradiol and progesterone secretion via the sensory–sympathetic reflex.

The different ovarian response to hemiovariectomy between pre-pubertal and adult animals could be related to norepinephrine concentration at the day of estrus between rats sacrificed at the day of first vaginal estrus *(4)* and adult rats sacrificed at the day of estrus (0.67 ± 0.06 vs 1.9 ± 0.3) *(20)*.

Finally, the results presented herein support the concept of asymmetry in the response of the ovaries to denervation. These differences can be explained by the different information conveyed by the left and right ovaries to the CNS. Once in the CNS, such information participates in regulating the gonadotropin-secretion mechanism. Neuronal signals carried by the sympathetic innervation, projecting to the right and left ovaries, modulate the reactivity of the ovarian compartments to gonadotropins, and as shown, its participation varies along the estrus cycle.

Materials and Methods

All experiments were carried out in strict accordance with the Guide for Care and Use of Laboratory Animals at the National Academy of Science. The protocols were approved by the FES Zaragoza.

Table 2
Schematic Representation
of the Distribution of the Animals in the Experiments

Treatments			Hemiovariectomy left ovary *in situ*		Hemiovariectomy right ovary *in situ*	
D 1	12	VEH	D1	6	D1	6
D 2	12	VEH	D2	6	D2	6
P	12	VEH	P	6	P	6
E	12	VEH	E	6	E	6
D 1	12	CAP	D1	6	D1	6
D 2	12	CAP	D2	6	D2	6
P	12	CAP	P	6	P	6
E	12	CAP	E	6	E	6

Animals and Treatment

Adult female rats from the CIIZ-V strain (230–260 g) that had shown at least three regular estrous cycles monitored by cytological examination of daily vaginal smears, were used in this study. All animals were housed in an artificial light–dark cycle (lights on 05.00 to 19.00 h) with access to food and water *ad libitum*. All the treatments were performed between 09.00 and 11.00 h. Capsaicin (Sigma Chemical Co., St. Louis, MO, USA) was dissolved in 10% ethanol, 10% Tween 80, and 80% saline solution.

Five microliters of vehicle, or 50 mg/kg, of capsaicin was injected subcutaneously to rats in diestrus 1, diestrus 2, proestrus, or estrus.

In Table 2 we include a schematic representation of the distribution of animals per treatment.

After presenting three consecutive 4-d cycles, the rats were anesthetized with ether, laparotomized, and either the right of left ovary was extirpated on the same day of the estrous cycle when they were treated with vehicle or capsaicin. The extirpated ovary was immediately weighed in a precision balance. After hemiovariectomy, the estrous cycle was monitored by daily smears. After presenting three consecutive 4-d cycles, the animals were sacrificed on the morning of the day of estrus.

Autopsy Procedure

Animals were killed by decapitation. The blood of the trunk was collected, allowed to clot at 4°C, and centrifuged at 970.6*g*. The serum was stored at −20°C, until estradiol and progesterone were measured by specific radioimmunoassay (RIA), with kits purchased from Diagnostic Products (Los Angeles, CA, USA). The intra-and interassay coefficients of variation were 2.1% and 5.6% for progesterone, and 6.0% and 7.1% for estradiol, respectively. At autopsy, the oviducts were dissected and the number of ova counted with the aid of a dissecting microscope. The remaining ovary was dissected and weighed in a precision balance.

Compensatory ovulation (CO) and ovarian compensatory hypertrophy (COH) were calculated as previously described by Chávez et al. *(3)*, and Cruz et al. *(31)*. In brief, CO = [(number of ova shed by the *in situ* ovary – number of ova shed by the control)/number of ova shed by the control] × 100; and COH = [(weight of the ovary *in situ*– weight of the extirpated ovary)/ weight of the extirpated ovary] × 100.

Histological Analysis

For histological analysis, ovaries were fixed in Bouin's solution, embedded in paraffin wax, serially sectioned at a thickness of 10 μm, and stained with hematoxylin–eosin. All sections from three randomly chosen ovaries of rats treated with capsaicin in D1 day were examined microscopically. For comparison purposes, three ovaries from the vehicle-injected group were also examined. Follicles were identified as healthy or atretic. Follicles having one of the following characteristics were considered atretic: the presence of pyknosis in the granulosa cell, granulosa cells present in the follicular fluid, or hypertrophy of the theca cells.

Statistical Analyses

Data on the number of ova shed were analyzed by Kruskal–Wallis test, followed by Mann–Whitney *U* test. Data on the estradiol and progesterone concentrations in serum were analyzed using variance analysis (ANOVA), followed by Tukey's test. When two means were compared, a Student's "*t*" test was used. The percentage of CO and COH were analyzed by chi square test. A probability of less than 5% was considered significant.

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