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In rats with the polycystic ovary syndrome, the monoaminergic activity in the celiac superior mesenteric ganglion depends on the vagal innervation

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RESEARCH

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ABSTRACT

Background
One of the mechanisms involved in the polycystic ovarian syndrome (PCOS) development is the hyperactivity of the sympathetic nervous system, which reaches the ovaries pathway the ovarian superior nerve (SON). The somas of the neurons originating the SON are located mainly in the celiac superior mesenteric ganglia (CSMG), which in turn receives innervation by the vagus nerve, suggesting that the neural information arriving to the CSMG through the vagus nerve may modulate the role played by the SON in the persistence of PCOS.

Aims
The aim of the present study was to assess the participation of the vagus nerve in the regulation of the monoaminergic activity in the CSMG of rats with PCOS induced by estradiol valerate (EV-induced PCOS).

Methods
Ten-day old rats injected with EV dissolved in corn oil, at 24-days of age were submitted to unilateral or bilateral vagotomy. The animals were sacrificed at 90–92 days of age, after presenting vaginal oestrous smear preceded by a proestrus.

Results
In rats with EV-induced PCOS, unilateral or bilateral vagotomy resulted in lower noradrenaline (NA) levels; bilateral vagotomy yielded lower serotonine (5-HT) levels in the CSMG, and the dopamine (DA) levels were not modified by the vagotomy.

Conclusion
Present result suggests that in rats with EV-induced PCOS the CSMG serves as communication channel between the vagus nerve and sympathetic innervation and this communication is related to the persistence of EV-induced PCOS.

Key Words
Polycystic ovarian syndrome (PCOS), noradrenaline, vagotomy, celiac superior mesenteric ganglia (CSMG)

What this study adds:

1. What is known about this subject?
One of the mechanisms involved in PCOS development is the hyperactivity of the sympathetic nervous system.

2. What new information is offered in this study?
In rats with EV-induced PCOS, sympathetic hyperactivity is regulated by vagal information.

3. What are the implications for research, policy, or practice?

It is important to know the mechanisms by which the PCOS develops, in order to have therapeutic alternatives to infertility in patients with PCOS.

Background

In humans and other mammal, the exposure to high concentration of androgens or oestrogens during prenatal and early postnatal life disrupts normal endocrine functions and decreases fertility.[1]

The polycystic ovary syndrome (PCOS) is the most common cause of infertility in woman, characterized by a complex pathophysiology, including anovulation, oligomenorrhea, follicular cysts, hyperandrogenism, hyperestrogenism, and variable levels of gonadotropins in blood, glucose metabolic disorders, cardiovascular diseases, dyslipidaemia and cancer.[2] The experimental models used in the study of the PCOS includes injecting long lasting estradiol or testosterone esters;[3-5] as well as exposure of adults rats to constant light, or chronic cold stress.[6] Injecting of estradiol valerate (EV) to infantile or adult rats results in acyclicity, anovulation, polycystic ovaries, hyperandrogenism, characteristics similar to those observed in women with PCOS.[7,8]

The aetiology of PCOS is multifactorial and is attributed to genetic, nutritional, and environmental factors; as well as primary defects in the reproductive axis, including alterations in the patterns of gonadotropins secretion.[2,9] The onset of PCOS is associated with increased activity of the ovarian sympathetic nerves.[1,4,10-12]

In adult rats with EV-induced PCOS, the bilateral section of the superior ovarian nerve (SON) resulted in spontaneous ovulation by both ovaries.[10] In juvenile rats with EV-induced PCOS, the unilateral section of the SON results in spontaneous ovulation and higher levels of noradrenaline (NA) in the innervated ovary. These results suggest that aside from an increase in ovarian noradrenergic tone in the ovaries, in the pathogenesis of the PCOS participate other neural mechanisms.[4]

According to Gerendai et al.,[13] a multi-synaptic neural pathway between the ovary and the central nervous system is involved in regulating ovarian functions. In the adult rat, bilateral vagotomy altered the oestrous cycle,[14] blocked pseudo-pregnancy induction,[15] increased the number of ova shed by ovulating adult and pre-pubertal rats,[16,17] and in pregnant rats resulted in lower luteinizing hormone (LH) basal levels, causing foetal resorption.[18] Taken together, these results suggest that the information arriving to the ovaries through the vagus nerve participates in regulating ovarian functions. The effect of the unilateral or bilateral vagotomy on ovulation suggest that the sensory fibres in the vagus nerve carry information from the periphery to the hypothalamus, which in turn participates in the regulation of gonadotropin releasing hormone (GnRH) and gonadotropin secretion.[13,18] Another possibility is that the ovarian vagal innervation modulates the effects of gonadotropins on the ovarian follicles.[16]

According to Berthoud and Powley,[19] communication between the sympathetic and parasympathetic fibres is apparent at the celiac superior mesenteric ganglion (CSMG) level. The neurons originating the SON and ovarian plexus nerve (OPN) are located in the CSMG.[20] The vagus nerve may modulate the postganglionic outflow directly or indirectly some or all of the potential modulatory inputs to these postganglionic neurons, allowing the vagal system to exert a more selective influence on sympathetic outflow. The vagal projections form varicose terminal-like structures suggest the presence of synaptic contacts surrounding each individual ganglion cell.[19]

Delgado et al.;[21] used the *ex vivo* celiac ganglion-SON-ovary (CG–SON–O) system of the rat provide evidence that the celiac ganglion has a direct neural effect on ovarian physiology. In a previous study we showed that unilateral or bilateral vagotomy to 24 day old rats with PCOS induced by EV injection, results in spontaneous ovulation in both ovaries (number of ova shed in rats with EV-left vagus nerve section: 11.5±1.9, EV-right vagus nerve section: 7.2±2.0 and EV-bilateral vagus nerve section: 7.0±1.0) with ovarian morphology similar to control animals, suggesting that the vagus nerve is a neural pathway participating in maintaining PCOS.[22] Since the vagus nerves innervate the ovaries directly and indirectly through its synapsis in the CSMG, where the somas of neurons originating in the SON are located, we presume that in the CSMG the vagus nerve modulates the activity of those neurons originating the SON and the OPN. Then, the aim of the present study was to analyse if the ovulation observed in rats with EV-induced PCOS by the section of the vagus nerves was accompanied by changes in the monoaminergic activity in the CSMG. For this purpose, we measured the amounts of noradrenaline (NA), dopamine (DA), serotonin (5-HT) and their metabolites in the CSMG of those rats where the unilateral or bilateral section of the vagus nerves restored the ovulation.

Method

This study was performed using pre-pubertal or adult female rats of the CIIZ-V strain from our own breeding stock. Animals were maintained under controlled lighting conditions (lights on from 05:00 to 19:00 h); with free access to food (Purina S.A, México) and tap water.

Animal treatment

Ten-day old rats were injected with either a single dose of 0.1ml corn oil (vehicle Vh) or 2mg EV (Sigma Chem. Co., St. Luis, Mo. USA) dissolved in 0.1ml corn oil. When the rats injected with Vh or EV reached 24 days of age, groups of seven animals were randomly allotted to one of the following groups: 1) sham surgery; 2) sectioning of the left vagus nerve; 3) sectioning of the right vagus nerve, and 4) bilateral sectioning of the of vagus nerve.

Sectioning the vagus nerve and sham surgery procedures were performed between 10:00 and 12:00 h, following previously described methodology.[17,22] In brief, the rats were anesthetized with ether and a ventral incision, including skin, muscle and peritoneum was performed. Subsequently, the stomach and liver were manipulated to expose the oesophagus. The left, right, or both vagal trunks were cut with fine forceps. Sham-surgery involved the same procedures except that the vagus trunks were not touched. After surgery, the abdominal wall was sutured and the animals returned to their cage. All animals were sacrificed when they presented vaginal oestrous preceded by a proestrus between 90–92 days of age, In brief, estrus cyclicity was monitored by examining vaginal lavage during the two weeks after the presence of vaginal canalization and during two weeks before the animals were euthanized. Following Marcondes et al.[23] methodology, every morning between 9 and 10am, vaginal smears were obtained with a sterile inoculating loop, 3mm loop, soaked in normal saline, placed on a standard slide, stained with hematoxylin-eosin and observed under a light microscope.

Autopsy procedures

The animals were sacrificed by decapitation between 10.00 AM and noon. At autopsy, the CSMG was removed and stored at -70ºC until monoamines and their metabolites were measured using high performance liquid chromatography (HPLC).

Monoamines Levels

The concentration of monoamines (NA, DA, 5-HT) and their metabolites (4-hydroxy-3-methoxyphenyl glycol (MHPG), 3,4-dihydroxyphenilacetic acid (DOPAC) and 5-hydroxyindole-3-acetic acid (5-HIAA)) in the CSMG were measured following methodologies previously described.[23] In brief, the CSMG was weighed in a precision balance, homogenized in 300µl of 0.1N perchloric acid, and centrifuged at 12,000×g, at 4°C for 30 min. The supernatant was filtered using 0.2µm regenerated cellulose filters. Twenty microliters of this extract were injected into a chromatography column via a Rheodyne injection valve. The HPLC system consisted of an isocratic pump (L-250 model; Perkin Elmer Co., Norwalk, CT, USA), a Rheodyne injection valve (7125 model; Perkin Elmer Co), an ultrasphere ODS preanalytical column (5cm 3 4.6mm) and a Biophase ODS C-18 analytical (25cm 3 4.6mm, 5mm particle size; Bionalitical Systems Inc., West Lafayette, IN, USA) column. The content of monoamines and their metabolites were detected electrochemically using a LC-4A amperometric detector and a LC-5A glassy carbon traducer cell at a potential of 850mV (Bionalitical Systems Inc.). The mobile phase consisted of 0.1M citrate buffer (Merck-México, SA.) at pH 3.0, with 175 mg of 1-octane-sulfonic acid (Sigma Chemical Co., St. Louis, MO, USA), filtered and degassed under vacuum. Immediately after degassing, 20ml of acetonitrile and 21.5ml of tetrahydrofurane for chromatography (Merck, Darmastadt, Germany) were added until a total volume of 500 ml was reached. The mobile phase was pumped at a flow rate of 1.2ml/min. Stock standards (Sigma Chemical Co.) were prepared and diluted with 0.1M perchloric acid the day of the experiment.

The system was calibrated by producing a 0.1–2ng/ml standard range curve. Monoamines and their metabolites were identified by the relative retention times compared to standards. Using a 1020 Perkin- Elmer Nelson integrator, the concentrations of monoamines and their metabolites were determined by comparing standards with the highest peaks obtained from the samples. Results are expressed as pg of neurotransmitter/mg wet tissue. The sensitivity for all neurotransmitters was 0.01ng.

Neural activity was estimated as previously described, following Kerdelhué et al.[24] Neural activity = [Neurotransmitter Metabolite] / [Neurotransmitter]. Increases in this ratio is considered an indication of greater neurotransmitter turnover and therefore increased neuronal activity.[24]

Statistical analyses

Results are expressed as mean ± standard error of the mean (SEM). Comparisons among the concentrations of monoamines, their metabolites, and the monoaminergic activity in the groups different were analysed using a two-way analysis of variance (ANOVA), p values less than 0.05

were considered to be statistically significant.

Results

The NA, DA and 5-HT levels in rats injected with Vh or EV were not modifying by sham surgery (Table 1). The group with sham surgery was used as a comparison group to evaluate the effect of vagotomies.

Noradrenergic system in the Celiac Superior Mesenteric Ganglia

Sham surgery to EV-injected rats resulted in higher NA levels than in the Vh-injected sham surgery group. In EV-injected animals, unilateral or bilateral vagotomy yielded lower NA levels than in EV-injected sham surgery group (Figure 1A). Sham surgery to EV-injected rats yielded in higher MHPG levels than in the Vh-injected sham surgery group. MHPG levels were higher in animals Vh-injected with left vagotomy than in the Vh-injected sham surgery group. In turn, compared to the EV-injected sham surgery group, MHPG levels were lower in rats EV-injected with unilateral or bilateral vagotomy (Figure 1B). Compared to Vh-injected sham surgery animals, the EV-injected sham surgery group showed higher noradrenergic activity in the CSMG. In Vh-injected rats, the noradrenergic activity was higher in rats with left vagotomy and lower in rats with right or bilateral vagotomy in comparison with Vh-injected sham surgery group. Left vagotomy in EV-injected rats resulted in lower noradrenergic activity than Vh-injected group submitted to same surgery. Compared to the EV-injected sham surgery group, animals with right vagotomy showed lower noradrenergic activity, while bilateral vagotomy group the noradrenergic activity was higher (Figure 1C).

Dopaminergic system in the Celiac Superior Mesenteric Ganglia

In comparison with the sham surgery animals, the DA levels in the CSMG were not modified by unilateral or bilateral vagotomy (Figure 2A). Bilateral vagotomy to Vh-injected rats yielded lower DOPAC levels than Vh-injected sham surgery group. In EV-injected rats, DOPAC levels were higher in rats with left vagotomy and lower in rats with right vagotomy in comparison with Vh-injected submitted to same surgery animals (Figure 2B). Dopaminergic activity in EV-injected rats submitted to sham surgery were lower than Vh- injected animals with same surgery. Compared to the Vh-injected sham surgery group, dopaminergic activity was lower in Vh-injected with left or bilateral vagotomy rats. In EV-injected rats, the left or bilateral vagotomy resulted higher dopaminergic activity, while right vagotomy yielded lower dopaminergic activity, in comparison to their respective Vh-injected groups (Figure 2C).

Serotonergic system in the Celiac Superior Mesenteric Ganglia

Compared to their respective Vh-injected or sham surgery group, 5-HT levels were lower in the EV-injected bilateral vagotomy rats (Figure 3A). 5-HIAA levels in EV-injected rats submitted to sham surgery were higher than Vh- injected animals with same surgery. In Vh-injected right vagotomy animals resulted in lower 5-HIAA levels than Vh-injected sham surgery rats. In EV-injected groups, unilateral or bilateral vagotomy yielded lower 5-HIAA levels than the EV-injected sham surgery group (Figure 3B). Serotonergic activity was lower in Vh-injected rats and unilateral vagotomy respect to the Vh-injected sham surgery group. Serotonergic activity in EV-injected rats submitted to sham surgery were higher than Vh- injected animals with same surgery. Compared to the EV-injected sham surgery group, serotonergic activity was lower in EV-injected with unilateral vagotomy groups and higher in the EV-injected bilateral vagotomy animals (Figure 3C).

Discussion

The results obtained in the present study show that injecting EV to pre-pubertal female rats increases NA levels in the CSMG, without changes in DA and 5-HT levels, while unilateral or bilateral vagotomy results in lower NA and 5-HT levels suggesting that the vagus nerve has a stimulating role in the regulation of the CSMG monoaminergic tone.

Experimentally, both the increase of the testosterone concentration by the injection of testosterone propionate (TP) or estradiol by the injection of estradiol benzoate[26] (EB) or EV27 results in the development of ovarian polycystic, and in some cases with neuroendocrine and metabolic alterations similar to those of PCOS.[27]

According to Matthews,[28] the small intense fluorescence cells (SIF) present in the CSMG contain DA and 5-HT granules. SIF cells receive and send synapses, but their physiological role is obscure.[29] To our knowledge, there is not information on the role of DA and 5-HT neurons in the regulatory mechanisms exerted by the CSMG on ovarian functions. While prolonged androgenization results in decreased NA, DA, and 5-HT,[30] EV injection results in increased 5-HT, DA and NA levels.5 The effects of TP injection on the NA, DA and 5-HT levels in the hypothalamus[30] and the ventromedial-arcuate nucleus of the hypothalamus[5] vary according to the age and the amount of hormone injected.

In adult rats, the EV injection increases ovarian NA content, enhanced NA uptake and release from ovarian nerve terminals,[7] increase intraovarian synthesis of neural growth factor and its low affinity neurotrophin receptor p-75, on the other hand in the CSMG increase the tyrosine hydroxylase mRNA level.[31] In adult female rats, combining cold and restraint stress procedures results in higher NA levels in CSMG than control.[32] According to Sotomayor-Zárate et al.,[1] injection of 0.1mg EV to newborn rats results in a higher concentration of NA in the ovaries and no change the celiac ganglion. In our study, we observed that injection of 2.0mg EV to 10-day-old rats resulted in an increase in NA concentration in both ovaries[4] and CSMG (current study), supporting the idea that an increase in the noradrenergic system activity is part of the mechanisms elicited by the experimental PCOS inductors. Likewise, the differences in the NA levels by the EV injection can be attributed to the dose of EV used and the age of the treated animals.

According to Anesetti et al.,[33] 15-days old rat injected with estradiol cypionate (a long lasting oestrogen) significantly increased the ovaries' catecholaminergic innervations, the sympathetic celiac neuronal size, and the expression of the neurotrophin receptor p-75, seven days after treatment. Injecting adult gilts with estradiol 17β during 38 days resulted in lower number of neurons present in the ganglia innervating the ovaries.[34,35] In the present study, higher estradiol levels resulting from EV treatment lead to NA levels increases in the CSMG 70 days after treatment. Higher NA levels could be explained by noradrenergic neurons size increases described by Anesetti et al.[33]

Ovarian sympathetic innervation regulates to steroidogenesis, folliculogenesis and corpus luteum development and regression in various species.[36,37] This innervation not only includes the neural components that enter the ovary, as is the case of the SON and OPN, but also intermediate structures such as CSMG, which was capable of receiving and integrating signals coming from the central nervous system and organizing responses that influence ovarian physiology.[38,39] According to Berthoud and Powley,[19] the vagal pre-ganglionic efferent innervates the ganglion cells of CSMG. These vagal contacts may either play a role by directly modulating the post-ganglionic outflow or by accessing the potential modulatory inputs to these post-ganglionic neurons, thus allowing the vagal system to exert a more selective influence on sympathetic outflow.

Aguado[39] and Gerendai et al.,[40] proposed that the CSMG is part of a multi-synaptic neural network connecting the central nervous system and the ovary. According to Follesa et al.,[41] the acute stimulation of the vagus nerve increased the brain-derived neurotrophic factor and fibroblast growth factor mRNAs in the hippocampus and cerebral cortex, as well in the concentration of NA in the prefrontal cortex of the rat. In the present study changes in the concentration of neurotransmitters and their metabolites in rats with unilateral or bilateral vagotomy may be explained by differences in the neural information carried by the left and right vagus nerve. This interpretation is supported by the effects of unilateral or bilateral vagotomy on ovulation.[16,17]

Several hypothesis have been proposed to explain the etiology of the PCOS, including failings in the pulsatile secretion of GnRH, resulting in chronic anovulation9 and the hyperactivity of the sympathetic ovarian innervation.[7,12] Chaudhari and Nampoothiri[30] suggest that neurotransmitters, independently or in combination with one another, regulate GnRH release. Then, neurotransmitter alteration could be one of the reasons for disturbed GnRH release, consequently directing the ovarian dysfunction in PCOS.

Mravec[42] proposed that vagal sensory fibers activated directly by epinephrine and norepinephrine represent the afferent extremity of a negative feedback loop that adjusts the activity of the system sympatho-adrenal according to the actual levels of plasma catecholamines and tissues. Then, it is possible that the vagus nerve also adjust the activity of the sympatho-ovarian system.

There is evidence that the vagus nerve stimulation increase the firing rate in neurons in the locus coeruleus (LC)[43,44] and c-fos expression,[45] suggesting that vagus nerve modulates of ovarian function via the regulation of the monoaminergic activity.

There is experimental evidence showing that the left and right vagus nerve plays different role in the regulation of rat's spontaneous ovulation,[16] and compensatory ovarian hypertrophy.[46] Also, the section of the left or right vagus nerve affect in a different way the DNA/protein relationship and acetylcholinesterase activity in the cerebellum, hypothalamus, striatum and medulla.[47] In our study, compared to the EV-injected sham surgery group, in the animals with PCOS EV induced, left vagotomy result in lower serotonergic activity without change in noradrenergic and dopaminergic activity. The right vagotomy yielded lower noradrenergic, dopaminergic and serotonergic activity, while bilateral vagotomy produced higher noradrenergic and serotonergic activity without change in dopaminergic

activity. The differential effects of right, left and bilateral vagus nerve section on the CSMG activity can be relate to the differences in the ovarian neural connections between the ovaries and the central nervous system (CNS).[13] Tóth et al.[48] showed that neural connections between the left ovary and several brain structures, such as the nucleus of the solitary tract, the dorsal nucleus of the vagus, the A5 noradrenergic cell group, are more abundant than between these cell groups and the right gonad. A similar relationship was observed for adrenal glands.[49]

Present results suggest that during the PCOS the vagus nerve may regulate the transmission of sensory information from the ovary to the spinal cord. The density of sympathetic nerve fibres is higher in the cystic ovaries of women,[50] in the ovaries of rats injected with EV[32] and in gilts with cystic ovaries induced by injecting dexamethasone.[51,52] Cholinergic[53] and sensory[54] ovarian innervations are also modified in gilts with cystic ovaries induced by injecting dexamethasone. Gerendai et al.[55] showed that there was almost no viral labeling in the CNS from the ovaries with precystic morphology, suggesting that the lack of viral labeling is connected with the alterations in the ovary.

Conclusion

In EV-induced PCOS rats, unilateral or bilateral vagotomy restores ovulation and decreases norepinephrine concentration in CSMG. Since PCOS is characterized by the increase in the noradrenergic tenor of the ovary and some regions of the hypothalamus, from our results we suggest that the restoration of ovulation in the animals with PCOS would imply the decrease of noradrenergic tenor in the ganglion by effect of the vagotomy. Since the neuronal bodies that give rise to the SON and the OPN are located in the CSMG, vagotomy could modify the synthesis of NA by these neurons (Figure 4).

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PEER REVIEW

Not commissioned. Externally peer reviewed.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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ETHICS COMMITTEE APPROVAL

All experiments were carried out in strict accordance with the Mexican Law of Animal Treatment and Protection Guidelines; and followed the Mexican Official Standard NOM-062-ZOO-1999 specifications. The Institutional Committee for the Care and Use of Animals of the Facultad de Estudios Superiores Zaragoza approved all experimental protocols. All possible efforts were made to minimize the number of animals used and their suffering.

Table 1: Mean ± SEM of Noradrenaline (NA), Dopamine (DA) and Serotonin (5-HT) levels in the Celiac Superior Mesenteric Ganglia (CSMG) of rats injected with vehicle (Vh) or estradiol valerate (EV) at day 10 of life, untouched or sham surgery at day 24 of life, sacrificed at day 90-92 of life

Group	NA	DA	5-HT
Vh	3729±771	390±41	2641±888
Vh-Sham	6116.2±461.4	377.7±37	2181.6± 162.1
EV	7331±1523	425±50	3519±467
EV-Sham	10646.9±1500	458.5±45	2408.9±247.7

Figure 1: Noradrenergic system. Mean ± SEM of (A) NA, (B) MHPG and (C) MHPG/NA levels in rats injected with vehicle (Vh) or estradiol valerate (EV) at day 10 of life, followed by sham surgery or with unilateral (LSVN or RSVN) or bilateral vagotomy (BSVN) at day 24 of life, sacrificed at day 90-92 of life. a p<0.05 *vs.* paired Vh group, b p<0.05 *vs.* their sham group (two-ways ANOVA)

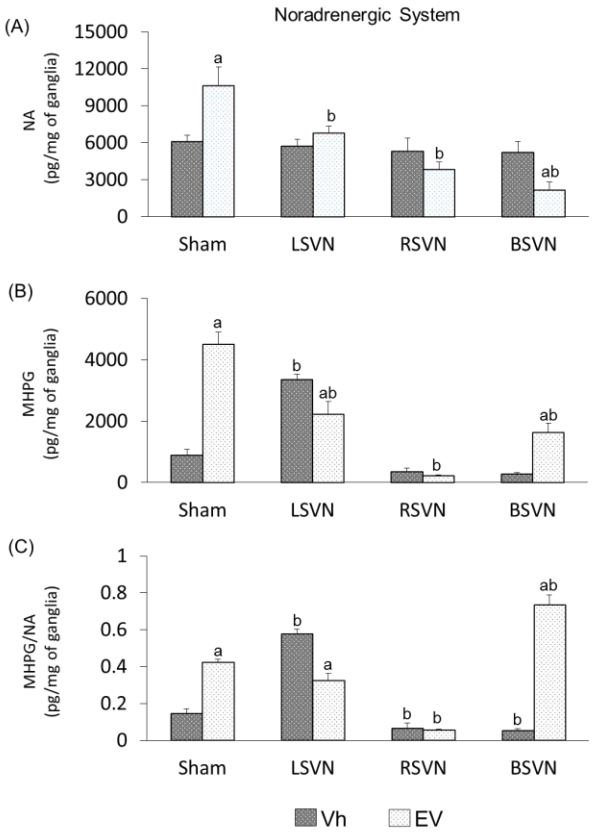

Figure 2: Dopaminergic system. Mean ± SEM of (A) DA, (B) DOPAC and (C) DOPAC/DA levels in rats injected with vehicle (Vh) or estradiol valerate (EV) at day 10 of life, sham surgery or with unilateral (LSVN or RSVN) or bilateral vagotomy (BSVN) at day 24 of life, sacrificed at day 90-92 of life. a p<0.05 *vs.* paired Vh group, b p<0.05 *vs.* their sham group (two-ways ANOVA)

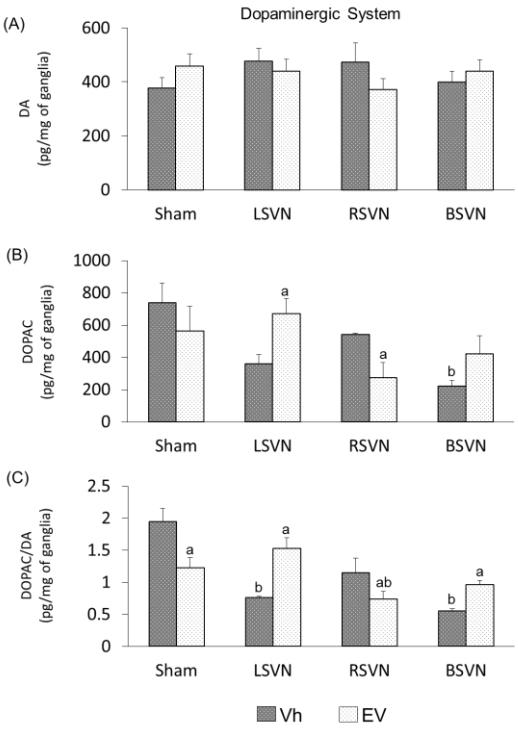

Figure 3: Serotonergic system. Mean ± SEM of (A) 5-HT, (B) 5-HIAA and (C) 5-HIAA/5-HT levels in rats injected with vehicle (Vh) or estradiol valerate (EV) at day 10 of life, sham surgery or with unilateral (LSVN or RSVN) or bilateral vagotomy (BSVN) at day 24 of life, sacrificed at day 90-92 of life. a p<0.05 *vs.* paired Vh group, b p<0.05 *vs.* their sham group (two-ways ANOVA)

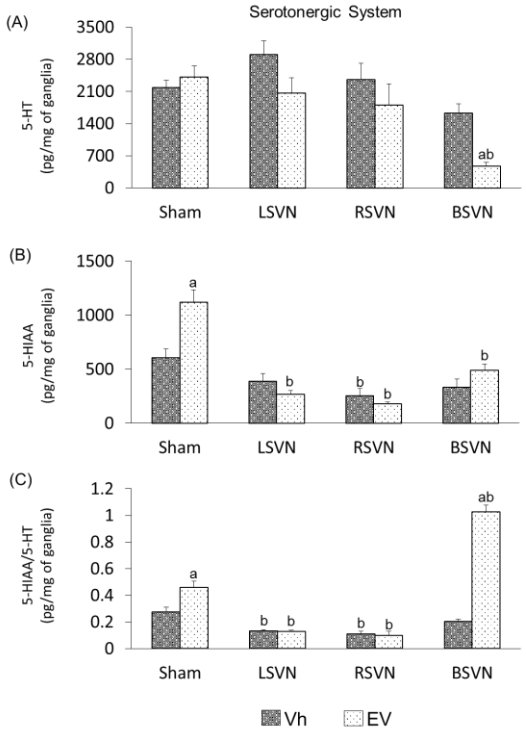

Figure 4: Mechanism the action to vagus nerve in rats with EV-induced PCOS. The schematic shows that in animals with PCOS induced by administration of EV, the vagus nerve stimulates (+) increased secretion of NA in the CSMG, which is the origin of sympathetic innervation and therefore the site of interaction between the vagal and sympathetic innervation. From the increase in concentration of NA in the CSMG, the ovary also is under hiper-noradrenergic tone (+++) which will come via the SON and in response the development and persistence of PCOS is presented. PCOS: polycystic ovarian syndrome, EV: estradiol valerate, NA: noradrenaline, CSMG: celiac superior mesenteric ganglia, SON: superior ovarian nerve