Endocrinology & Metabolic Syndrome
ISSN: 2161-1017

Domínguez et al. Endocrinol Metabol Syndrome 2011, S:4
DOI: 10.4172/2161-1017.S4-001

Review Article Open Access

The Ovarian Innervation Participates in the Regulation of Ovarian Functions

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Abstract

The release of gonadotropins is the main endocrine signal regulating ovulation and the release of hormones by the ovaries. Several types of growth factors modulate the effects of gonadotropins on the follicular, luteal and interstitial compartments of the ovaries. During the last 30 years, numerous studies have indicated that the ovarian innervations play a role in modulating the effects that gonadotropin have on the ovaries' ability to ovulate and secrete steroid hormones. This literature review presents a summary of the experimental results obtained by analyzing the effects of stimulating or blocking the well-known neural pathways participating in the regulation of ovulation and secretion of steroid hormones. Together, the results suggest that various neurotransmitter systems modulate the effects of gonadotropins on ovulation and the ovaries capacity to secrete steroid hormones. In addition, the ovaries asymmetric capacity for ovulation and hormone secretion could be explained by the asymmetries in their innervations.

Introduction

Ovarian functions, such as hormone secretion and the release of cells (oocytes) able to be fertilized are regulated by hormonal signals originating in the pituitary, adrenals, thyroid, pancreas, thymus and the ovary itself. Gonadotropins (follicle stimulating hormone (FSH) and luteinizing hormone (LH) are the main hormones regulating both ovulation and hormone secretion. FSH and LH secretion is regulated by the gonadotropin releasing hormone (GnRH) secreted by neurons located in the hypothalamus. Hormonal signals (estradiol, progesterone) and neural signals arising from intra and extra-hypothalamic regions regulate GnRH secretion. GnRH secretion is pulsating, and the stimulation of FSH and LH secretion depends on the GnRH pulse frequency. In the ovaries FSH acting on the follicular granulosa cells, stimulates follicular growth and maturation, aromatase synthesis resulting in estrogen secretion, and LH receptors in granulosa cells. LH stimulates progesterone and androgens secretion by the theca-interstitial cells. Androgens are the precursor for estrogen secretion. LH acting on mature follicles stimulates aromatase and estrogen secretion, and ovulation [1,2].

The ovarian innervations arriving to the ovary via the superior ovarian nerve (SON), the ovarian plexus nerve (OPN), and the vagus nerve modulates the reactivity of the three ovarian compartments (follicular, luteal and interstitial) to hormonal signals. The regulation of ovarian functions by neural signals implies the sequential and ordered release of one or more neurotransmitters at a particular frequency and amplitude.

Evidence that the ovaries send neural information to several regions of the central nervous system (CNS) [3,4], and that this information participates in regulating gonadotropin secretion [3,4,5] has been documented. The ovaries share neural information [3,4], and the celiac-superior mesenteric ganglia (CG-MG) facilitate the communication between the ovaries [6,7].

The main idea on how the endocrine system is regulated is based on the notion that the CNS controls the functions of the endocrine glands by releasing neuropeptides through the hypothalamus and that the pituitary amplifies such hormonal signals. Endocrine signals from the hypothalamus that stimulate the release of follicle stimulating hormone (FSH) and luteinizing hormone (LH) are the main regulators for growth, differentiation and maturation of the ovarian follicles, the release of oocyte II (ovulation), and the secretion of steroid and peptidergic hormones by the ovaries. Since the growth and maturation of follicles is continuous, the effects that FSH and LH have during the estrous cycle can be explained by the oscillatory number of hormone receptors in the follicles and interstitial gland cells through the cycle. The response of the ovaries to neuroendocrine signals changes according to the day of the ovarian cycle, and even perhaps to the time of day.

The participation of neural signals regulating ovarian functions can be explained as modulatory, since the release of neurotransmitters such as acetylcholine (ACh), catecholamines and neuropeptides is time specific and amplitudes and frequencies vary during the estrous cycle, influencing the response of the follicles and interstitial gland to gonadotropins in different ways. Furthermore, neurotransmitters may also affect the expression of growth factors and/or other molecules participating in regulating the complex processes of ovulation and hormone secretion.

The effects of pituitary hormones are modulated by neural signals arriving to the endocrine glands, indicating that the CNS regulates the functions of endocrine glands through hormonal and neural signals. The existence of neural pathways carrying information from the endocrine glands to the CNS [5,6] suggests that such neural information works to inform the CNS of the hormonal and/or neural signals requirements of the endocrine gland. Then, the endocrine glands receive specific neuroendocrine signals for its function, instead of having the CNS sending neuroendocrine signals without having precise information of the peripheral requirements [7].

Ovarian innervations

Neural signals arrive to the ovaries via two noradrenergic-peptidergic nerves that originate in the CG-MG, and through neurons

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Received August 29, 2011; **Accepted** October 14, 2011; **Published** October 19, 2011

Citation: Domínguez R, Cruz-Morales SE (2011) The Ovarian Innervation Participates in the Regulation of Ovarian Functions. Endocrinol Metabol Syndrome S4:001. doi:10.4172/2161-1017.S4-001

located at the paravertebral ganglia [8]. By injecting True blue, a fluorescent retrograde tracer, into the SON or the OPN, afferent perikarya were observed in the lower thoracic-upper lumbar dorsal root ganglia and projected towards the ovary via both nerve routes. Postganglionic sympathetic efferent perikarya were located in both, the pre-vertebral and thoracolumbar paravertebral ganglia, and were observed to use the SON and the OPN to reach the ovary. When True blue was applied to the SON, 17% of labeled cells in the paravertebral ganglia were immunoreactive for vasoactive intestinal polypeptide (VIP); while treatment to the OPN resulted in only 1% of labeled cells immunoreactive to VIP [8].

Some VIP positive cells have axons that are not part of the SON or the OPN. In the rat, substance P (SP) and VIP positive fibers are present in the para- and pre-vertebral ganglia projecting to the ovary and the dorsal root ganglia at segmental levels T12-L1 [9]. Many sensory neurons projecting towards the ovary contain nitric oxide (NO) synthase and presumably release NO [10], a gaseous molecule that modulates ovarian functions. The participation of the vagus nerve in ovarian innervations has been shown by injecting horseradish peroxidase into the left or right ovaries [11], or by using the transneuronal viral tracing technique [3,4,6]. Studies using the viral transneuronal tracing technique have shown the existence of neurons in several nuclei of the brain stem and the hypothalamus, indicating they receive and/or send neural signals to the ovaries. These neurons are located at the ventro-lateral medulla, the gigantocellular nucleus, the area postrema, the vagal nuclei nucleus of the solitary tract, the dorsal nucleus of the vagus, A1 and A5 noradrenergic cell groups, the caudal raphe nuclei, the locus coeruleus, the hypothalamic para-ventricular nucleus, and the lateral hypothalamus [3, 4].

Some neurons in the nucleus of the solitary tract, the dorsal nucleus of the vagus, A5 noradrenergic cell group, the raphe magnus, and the hypothalamic para-ventricular nucleus are connected to both ovaries, and these regions receive more information from the left ovary than from the right [3,4]. Additionally, some neurons are simultaneously connected to both ovaries and adrenals, suggesting that the adrenals also participate in regulating ovarian functions through hormonal and neural signals [4].

Several neurotransmitters and the enzymes participating in their synthesis or degradation (such as noradrenaline (NA), dopamine (DA), serotonin, acetyl-cholinesterase, gamma aminobutiric acid (GABA), VIP, SP, neuropeptide Y (NPY), cholecystokinin-8, the calcitonin gene-related peptide (CGRP), and corticotrophin releasing hormone (CRH)) are present in the fibbers innervating the internal theca of the follicles, the interstitial gland and the ovarian vasculature [14-20].

In the ovaries of cows, VIP innervations occur in all ovarian regions (the ovarian hilus, the cortex and the medulla), as well as in the innervating blood vessels and ovarian follicles, especially in primordial and primary follicles. SP immuno-reactive fibers occur in solitary intra-ovarian nerve fibers of all ovarian regions, but mainly in those innervating the blood vessels of the ovarian medulla that is in close vicinity to primordial and primary follicles. Some VIP nerve fibers supplying intra-ovarian blood vessels were simultaneously positive for tyrosine hydroxilase (TH) and NPY, and fibers simultaneously positive to VIP and SP were also observed [21].

In the ovaries of the rat, CGRP is present in nerves that form dense plexuses surrounding the ovarian vasculature, in the interstitial tissue, and near the ovarian follicles. Surgical sectioning of the OPN eliminates CGRP immuno-reactivity, while sectioning the SON or the abdominal vagus trunk does not. CGRP and SP co-exist in several axons [16]. Peripheral CRH is secreted by post-ganglionic sympathetic and unmyelinated sensory afferent neurons. In the human ovary CRH receptors are present in thecal and stromal cells, and in the follicular fluid. Ovarian CRH regulates ovarian steroidogenesis and is involved in follicular maturation, ovulation, and luteolysis [20].

In mammals, the mature ovary synthesize several neurotrophins, including nerve growth factor (NGF), neurotrophin 3 (NT-3), and neurotrophin 4/5 (NT-4/5), and express neurotrophin receptors, including p75 NGFR, trkA (the NGF receptor) and trkB (the receptor for NT-4/5 and brain-derived neurotropic factor [22].

In adult gilts, long-term estradiol treatment down regulates the population of ovary supplying noradrenergic neurons and estrogen receptors expressed in the sympathetic chain ganglia [23].

According to Harrison and Weir [24], the ovaries receive innervations from the cervico-vaginal plexus, a paired structure situated laterally to the uterine cervix, where a pool of small fluorescent short adrenergic neurons is located.

Neurons in the ovary

The ovary of primates has a network of neurons, some of which are catecholaminergic [25]. In the rat, from the juvenile period (10 to 20 days of age) onwards, neurons are present in the ovarian hilum, the medulla, and in the ovarian cortex. The size of the neuronal soma increases during the pre-pubertal period, reaching its maximum values before puberty. Catecholaminergic neurons are found in both the ovarian cortex and the medulla, and are either isolated or clustered in ganglion-like structures. Some neurons show NPY immunoreactivity [26]. Four types of ganglia have been identified in post-pubertal and young adult rats: the mesovarial, the hilar, the medullary, and the cortical ganglia. Isolated neurons were also found dispersed in blood vessels reaching the ovary medulla and near the follicles [27].

Origin of ovarian innervating nerves

In pig fetuses, neurons expressing low affinity for neurotrophin receptors migrate from the neural crest to form the para-aortic autonomic ganglia. Some neurons migrate ventrally from the ganglia into the genital ridge, and later into the developing ovary. A catecholaminergic subset of neurons is associated with pubertal maturation of the ovary and subsequent reproductive functions [28]. Electron microscopy and immuno-histochemical studies showed the existence of neural fibers containing catecholamines in the ovary of rats before birth. These fibers innervate blood vessels, and other catecholaminergic fibers are associated with primordial ovarian cells [29].

In the rat, as early as fetal day 22, NPY nerves are present in the connective tissue immediately peripheral to the ovary. On postnatal day 2, NPY nerves are observed in the connective tissue septa of the developing ovary; and at day 12, NPY nerves surround developing follicles and blood vessels of the ovarian cortex [30]. During ovarian development follicle cells lack of FSH receptors; the neuroendocrine signals coming from the innervations on the developing ovary and from other cells stimulate FSH receptor synthesis [31-33].

Ovarian innervations reach the ovaries before the onset of folliculogenesis and before follicles acquire FSH receptors. The *in-vitro* stimulation of the ovaries of two-day-old rats with isoproterenol (agonist of beta-adrenoreceptors), VIP or forskolin increases the follicular levels of the mRNA encoding for cytochrome P-450 aromatase (P-450arom) and FSH receptors [34].

In humans, from week 24 of gestation to 10-months of postnatal age, neurons containing neuro-filaments are found in the hilum and medulla of the ovary. Most of these neurons co-express the low affinity neurotrophin receptor (p75NTR), and some are catecholaminergic [35].

In human embryos, primordial germ cells preferentially migrate along autonomic nerve fibers from the dorsal mesentery to the developing gonad, where they arrive via a fine nerve plexus [36].

In the ovaries of rodents, sheep, cows, and primates (including humans), neurotrophins participate in recruiting the ovarian innervation and the early regulation of follicular development and ovulation. Neurotrophins promote the initial differentiation of primordial follicles by maintaining the proliferation of both mesenchymal and granulosa cells, and by inducing the synthesis of FSH receptors. Neurotrophins participate in the ovulatory cascade by increasing the release of prostaglandin E2, reducing gap junction communication, and inducing cell proliferation within the thecal compartment of pre-ovulatory follicles [31].

In humans and rodents, neurotrophin expression begins during fetal life, before the formation of primordial follicles, and acts as a signal required for follicular assembly, early follicular growth, and oocyte survival [32]. In embryonic ovaries of rats, neurotrophin receptor p75 NGFR is predominantly expressed in mesenchymal cells. According to Dissen et al. [22], p75 NGFR mRNA content increases after birth and remains elevated at the time of follicular assembly.

The participation of the ovarian innervations in regulating spontaneous and induced ovulation

The participation of different neurotransmitters and neural signals arriving to the ovaries through its innervations have been analyzed by studying the effects of injecting neurotransmitters or blocking agents peripherally or intra-ovarian, and/or sectioning or stimulating the neural pathways arriving to the ovaries or the innervation region.

Extirpating one ovary produces an acute neural stimulation of all the innervations arriving to the *in-situ* ovary and modifies the responsiveness of endocrine cells to hormonal signals. Sectioning the nerves innervating one ovary causes an acute neural stimulation of the denervated organ and the CNS areas with neural connections to the ovaries. The physiological response resulting from sectioning one of the pathways innervating the ovary are different than the response produced by extirpating one ovary because the partially denervated ovary still has two neural pathways regulating its functions (Figure 1).

The function of ovaries after denervation is dependent upon the time elapsed between denervation and function´s assessment [12]. Then, the effect resulting from ovarian denervation or neural stimulation on ovarian functions (ovulation and hormone secretion) will depend on the lapse between surgery or stimulation treatment and the time their effects are evaluated. In chronic and sub-chronic denervation studies, we observe the adaptation of the ovarian regulatory systems to the lack of one or several neuro-endocrine signals [37-41].

Peripheral pharmacological denervation or stimulation

The chronic blockade of noradrenergic peripheral terminals, induced by injecting guanethidine to cyclic rats during one month, resulted in a lower number of ova shed by ovulating animals [42,43], while the noradrenergic denervation of newborn rats resulted in higher numbers of ova shed [43]. These results suggest that the noradrenergic ovarian innervations play different roles in regulating follicular growth and maturation (stimulating in adult rats, and inhibitory in newborns). Such interpretation is supported by the results obtained by inducing ovulation through the sequential injection of gonadotropins to newborn rats previously denervated with guanethidine, and the non-ovulatory response with the same treatment in control rats [44].

Chronic noradrenergic denervation affects follicular responses to exogenous gonadotropin stimulation in different ways: when gonadotropins are injected immediately after the end of the guanethidine treatment, the number of ova shed was similar to control animals, and lower when the stimulation was done 90 days after the end of denervation treatment [42]. In 12- and 18-month-old rats, the acute noradrenergic denervation with guanethidine increases the percent of healthy follicles; while the combined treatment of guanethidine denervation followed by stimulation with gonadotropins partially reestablished ovulation in 12-month-old rats. The same treatment doubled the number of ova shed in young animals [45]. Taken together these results suggest that during the pre-pubertal and aging phases the peripheral noradrenergic system inhibits the modulating effects of gonadotropins, while in adult animals the noradrenergic system stimulates the growth and maturation of ovarian follicles.

The ovarian noradrenergic innervation seems to be associated with the gonadotropic regulation of folliculogenesis. In newborn and 10-days old female guinea pigs, the peripheral noradenergic denervation with guanethidine injection results in the earlier onset of puberty and ovulation, and in follicles with larger mean-diameters [46]. In adult guinea-pig, guanethidine injection depletes the content of ovarian norepinephrine, with more pronounced effects on the left ovary than on the right, and increases the percent of healthy follicles. In addition, the corpora lutea in the ovaries of these animals were old, suggesting that the animals did not ovulate in the cycle that they were sacrificed. Estrogen concentrations in serum were higher in denervated animals treated at the follicular phase, while progesterone levels were higher in animals denervated at the luteal phase [47]. These results suggest that in the adult normal female guinea-pig the noradrenergic ovarian innervation regulates follicular development and the secretion

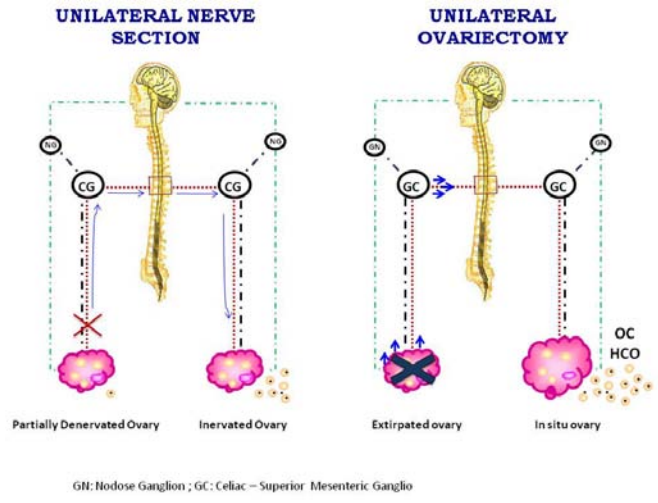

Figure 1: The diagram shows the differential effects of unilateral sectioning of one of the neural pathways innervating the ovaries and the extirpation of one ovary. The ovaries receive at least three neural pathways. The sectioning of one of them still leaves two pathways that will "compensate" the lack of one of them. The extirpation of one ovary, as in unilateral ovariectomy, results in the lack of the neural pathways arising from the extirpated ovary. Then, the inervated ovary does not have information on the functions of the other ovary.

of estrogen and progesterone in an inhibitory way, and that the participation of the noradrenergic ovarian innervation varies along the estrous cycle.

According to Farrar et al. [48] the noradrenergic nerves supplying the ovary normally exert an inhibitory tonic that influences the selection of follicles for maturation and/or for their rupture and subsequent luteinization. Chávez et al. [38,39] showed that the inhibitory effects of noradrenergic nerves are on FSH follicles responses, and depends on the integrity of the vagus nerve. Studies [49-51] suggest that the sympathetic innervation may play a permissive role in these events. In adult hens, the chronic injection of adrenaline or NA increases ovulation rate and estradiol levels [52].

In newborn guinea-pig, the peripheral sympathetic denervation induced by guanethidine treatment resulted in earlier vaginal opening and ovulation, and triggering the response to exogenous gonadotropins [46,53]. In adult guinea-pig the peripheral noradrenergic denervation induced by guanethidine injection inhibited ovulation. Compared to control animals denervated adult animals injected with guanethidine for three weeks showed no differences in the number of corpora lutea. However, all the corpora lutea from the treated group were old, and a significant decrease in ovarian norepinephrine content was observed. This suggests that the sympathetic denervation affected the luteolytic process but not ovulation. Based on these results the authors concluded that the ovarian innervation participates in regulating follicular development in an inhibitory way [47].

The superior ovarian nerve and ovarian functions

The role played by the SON on ovarian functions has been studied by analyzing the effects of unilaterally or bilaterally sectioning the SON on ovulation and steroid hormone secretion. Bilateral sectioning the SON of neonatal rats decreases the number of follicles in their ovaries at 30 and 90-days of age. The ovulatory response induced by the sequential injection of gonadotropin on day 30 was lower than normal, but normal in animals treated at day 90 of age [54]. These results suggest that during the post-surgery period the system adapts and is able to compensate the lost information arriving from the SON innervation, and that this compensation becomes apparent sometime after day 30 post-surgery.

In prepubertal rat, sectioning the suspensory ligament or the SON reduces the content of NA and adrenergic nerves in the ovaries [55]. In prepubertal rats the acute unilateral sectioning of the left SON reduces the concentration of NA in the denervated ovary, while the concentration of NA in the innervated ovary increases. In turn, sectioning the right SON reduces NA concentration in the denervated ovary to levels below the analytical method's sensitivity, without apparent changes in the left ovary. A slow recovery of NA levels in the denervated ovary occurs during puberty [56].

In prepubertal rats, unilaterally sectioning the SON results in an acute decrease of ovarian NA concentration in the denervated ovary, though sectioning the left SON resulted in a significant increase of NA concentration in the right ovary. In turn, NA concentration in the ovaries of bilaterally denervated rats was below the analytical method's sensitivity. In bilaterally denervated animals, amine concentrations recovered to normal levels after several days, and the recovery time depended on the age the animals were treated at [56]. This suggests that the noradrenergic innervation of the OPN increases and normalize the NA content in the innervated ovary, although normal ovarian functions are not recovered. These and other results support the idea that the neural regulation of ovarian functions requires the modulated (specific frequency and amplitude) release of neurotransmitters.

Bilaterally sectioning the SON briefly affects ovarian blood flow when measured on the morning of the day of proestrus, without apparent effects in the afternoon [55]. In pre-pubertal rats, unilaterally sectioning the SON results in lower numbers of ova shed by the denervated ovary and increases the number of ova shed by the innervated ovary [58].

Unilaterally stimulation of the ovarian pedicle at 07.00 hours of diestrus 1 (D1) blocks spontaneous ovulation without affecting LH release. Unilateral ovariectomy on estrus, D1 or diestrus 2 (D2) also decreased ovulation rates (number of ovulating animals over number of treated animals) [59]. Stimulating the right ovarian pedicle on the day of estrus, as well as stimulating the right or left pedicle on D2, decreased the noradrenergic activity of the pre-optic anterior (POA) area, suggesting the existence of a neural pathway between the ovaries and the CNS [60].

In pre-pubertal rats, the bilateral mechanical stimulation of the ovarian pedicle resulted in a higher number of ova shed when treated rats reached puberty (vaginal opening). The subsequent super-stimulation with pregnant mare gonadotropin (PMSG) did not modify the number of ova shed. Injecting human chorionic gonadotropin (hCG) decreased the number of ova shed; while injecting PMSG+hCG further increased the number of ova shed [61]. These results suggest that stimulating the ovarian pedicle elicits changes in the follicular sensitivity to FSH and LH during puberty.

In adult rats, unilaterally sectioning the right ovary's SON decreases the number of ova shed by the denervated ovary, while sectioning the left ovary's SON resulted in lower numbers of ova shed by both ovaries. The bilateral sectioning of the SON resulted in lower number of ova shed by the right ovary, without affecting the number of ova shed by the left ovary (Figure 2) [37]. Injecting hCG to rats with unilateral sectioning of the SON resulted in lower ovulation rates by the denervated ovary and normal ovulation rates by the innervated one [37].

In the pre-pubertal rat, the unilateral or bilateral sectioning of the SON increases the number of follicular atresia and decreases the number of growing follicles in the denervated ovary, and the degree of these effects depends on the time elapsed between surgery and autopsy. Estradiol and progesterone levels also depend on the integrity of the SON's ovarian innervation and the time elapsed between surgery and

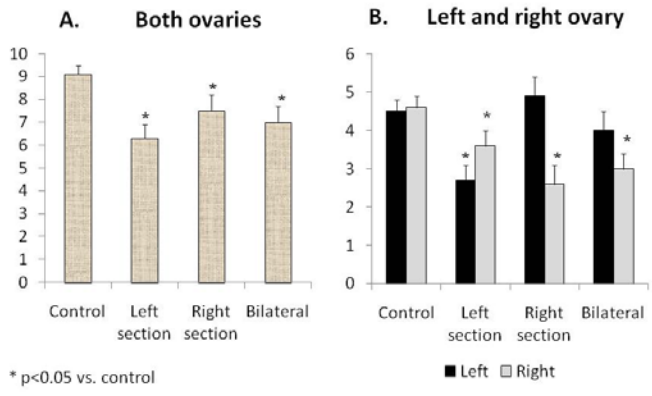

Figure 2: Number of ova shed by both ovaries (panel A) or by left or right ovary of rats with unilateral or bilateral section of the superior ovarian nerve, sacrificed at the day of vaginal estrous after 20 days of surgery. *p<0.05 vs. control (Duncan's test after ANOVA). Based on data published by Chávez et al.[37].

autopsy. Progesterone levels decrease as a function of time elapsed between surgery and autopsy [62].

In female guinea-pig, unilaterally or bilaterally sectioning the SON on day 5 of the estrous cycle (early luteal phase) increased the number of fresh corpora lutea. Similar effects were observed when the left SON was sectioned during the late follicular phase (day 1) or the medium luteal phase (day 8) [63]. These results suggest that in the guinea-pig the SON modulates ovulation in an inhibitory and asymmetric way, that this modulation varies along the estrous cycle, and that the influence of the SONs on ovulation is strongest during the early luteal phase, decreasing thereafter.

The estrous cycles of the rat and the guinea-pig are quite different, both in length and in the capacity of their corpora lutea to secrete progesterone; which in the guinea-pig lasts 8 to 10 days [64] and only a few hours in the rat [65]. Another difference is that the ovary of the guinea-pig receives a richer sympathetic innervation than the rat's ovaries [66].

The vagus nerve

The acute, sub-chronic, and chronic effects (19 to 40 days after surgery) of bilateral sectioning the abdominal vagus nerve of adult and pre-pubertal rat results in higher number of ova shed by ovulating animals [41,67]. These results suggest that in the rat, the ovaries adapt to the lack of direct or indirect ovarian vagal innervation by stimulating follicular growth and maturation. The results are different in cyclic hamsters, where the abdominal sectioning of the vagus nerve resulted in a lower number of ova shed and a higher number of atretic follicles [68].

In adult rats the participation of vagal information regulating ovulation is asymmetric and varies during the estrous cycle and the time of day [41,67-71]. The effects of sectioning unilaterally the sub-diafragmatic vagus nerve depend on the day of the estrous cycle in which the treatment was performed, and were found to be asymmetric. For instance, compared to the sham-surgery group, ovulation rates were lower in rats with the left vagus nerve sectioned on estrus. In the left ovary, the number of ova shed was lower in sham-surgery treated rats and in animals with bilateral (right and left) sectioning of the vagus nerve; whereas the number of ova shed by the right ovary was not modified by these treatments. No changes in FSH plasma levels were observed in animals with the right or left vagus nerve sectioned at estrus. The results indicate that vagal manipulations performed at the beginning of the estrous cycle (day of estrus and D1) induce stronger changes on ovulation than manipulations during the second half of the cycle (D2 and pro-oestrus). In addition, the left ovary is more sensitive to changes in ovarian innervation than the right one [69].

Sensory innervations

Injecting the anesthetic lidocaine-adrenaline (Xylocaine) into one or both ovaries at 13:00 h of proestrus did not affect ovulation. However, injecting a saline solution into the right ovary and xylocaine into the left ovary lowered the number of ova shed by both ovaries. These results support the hypothesis of neural communication between the ovaries. Xylocaine treatment on the right ovary of rats treated with saline solution in the left ovary did not modify the number of ova shed by either ovary. In ULO rats, xylocaine treatment on the left ovary blocked ovulation. Ovulation was not reestablished by injecting hCG at 14.00 h; however, all animals treated with xylocaine at 18:00 h ovulated [72]. These results support the idea that ULO modifies the neuroendocrine system regulating ovulation, and that the signals arising from the left ovary are crucial for spontaneous and induced ovulation.

In newborn rats the sensory denervation induced by injecting capsaicin decreased the number of ovarian follicles, the number of ova shed by ovulating animals, and the ovulatory response to the sequential injection of gonadotropins. The observed increase in atretic follicles may explain the decrease in the number of ova shed. Compared to non-denervated rats, the right ovary of ULO rats treated on day 20 of life showed higher compensatory ovulation, while the compensatory ovulation of the left ovary was below normal (non-treated animals). Such differences were not observed when ULO was performed at day 28 of age [73].

In capsaicin treated animals, the compensatory ovulation and number of ova shed after ULO treatment depends on the day of the cycle when ULO was performed (Figure 3) [74]. Capsaicin denervated animals copulate normally but their fertility rates were low [74,75]. Such results were explained by the reduced capacity of the vaginal–cervical stimulation to produce the decidual response in denervated animals [75].

The cervico-vaginal plexus

Experimental and clinical results indicate that neural signals originating in the cervico-vaginal plexus participate, via multisynaptic pathways, in regulating gonadotropin release inducing ovulation, and perhaps acting directly at the ovarian level.

Experimental studies show that the innervation originating in the cervico-vaginal plexus may participate in regulating spontaneous ovulation. For instance, in newborn guinea-pigs, sectioning and cauterizing the utero-vaginal junction delays normal ovulation to a period similar to pregnancy [76]. Compared to the control group, the unilateral thermo cautery-lesion of the cervico-vaginal plexus to 4 or 5 day old cyclic rats resulted in lower numbers of ova shed and medium sized follicles, and in higher numbers of small follicles; luteinized follicles trapped ova were observed in some ovaries of treated animals [77].

Additional evidence of the innervations' participation in regulating ovulation

In ULO rats, the lack of ovulation by the auto-grafted ovary was interpreted as an indication that the ovarian innervation participates in regulating ovulation [78]. Such idea is supported by the lack of ovulation by the auto-grafted ovary of ULO rats sequentially injected

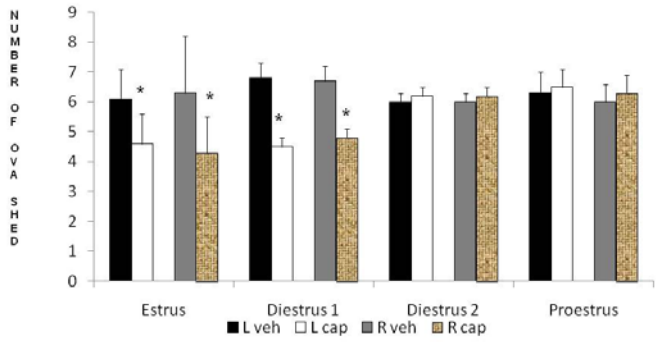

Figure 3: Number of ova shed in rats with ULO performed on each day of the estrous cycle, injected with vehicle (veh) or capsaicin (cap) in the in situ ovary, sacrificed in estrus day after 3 consecutive 4 day cycles. *p<0.05 vs. veh (Kruskal-Wallis test followed by Mann–Whitney U test). Based on data published by Trujillo et al.[74].

with gonadotropins, and by the ovulation induced by injecting gonadotropin to animals denervated with guanethidine injection treatment [79].

The bilateral or unilateral sectioning of the SON restores ovulation in rats with blocked ovulation resulting from polycystic ovarian syndrome (PCOS), induced with estradiol valerate [80,81], or in rats with blocked ovulation resulting from a lesion to the dorsal raphe nucleus [82]. Uchida et al. [83] showed the existence of neural reflexes from the abdominal skin to the ovaries that affect ovarian blood flow and SON activity. Stimulating the left abdomen produced a stronger effect on the left ovarian sympathetic nerve activity than stimulating the right abdomen.

The participation of the ovarian innervations in regulating hormones secretion

The effects of unilateral or bilateral laparotomy on progesterone, testosterone and estradiol serum levels depend on the day of the cycle and the surgical approach (dorsal or ventral) used [84-89].

1. Using sophisticated in vitro methodologies, Aguado's group analyzed the coeliac ganglion-SON-ovary system, as well as the effects of stimulating or blocking noradrenergic, cholinergic and peptidergic synapses on ovarian steroid hormones release.Taken together the results show thatThe cholinergic stimulation increases the basal release of NO by the ovaries and inhibits the release of steroid hormones [90].

2. Adding ACh into the ganglion compartment on the first proestrous day inhibited progesterone and estradiol release, and increased androstenedione secretion. The same treatment on the first estrous day lowered progesterone release and increased androstenedione, estradiol and NO secretion. ACh addition on D1 resulted in lower progesterone secretion, which was later followed by its increase. The secretion of androstenedione and estradiol also increased with cholinergic stimulation, while NO secretion decreased [91].

3. Adding NA to the ganglion isolated on the first pro-estrous day acutely decreased the release of progesterone and estradiol. Progesterone and androstenedione secretion occurred after NA stimulation. Addition of NA to ganglions obtained from rats on their first estrous day increased the ovarian release of progesterone, androstenedione and NO. In turn, the same experiment performed on ganglions obtained from rats in D1 resulted in the initial decrease of progesterone release followed by its increase; higher androstenedione and estradiol release, and lower NO levels [91].

4. Adding NA to the ganglion obtained from rats on the first proestrus day increased aromatase mRNA expression, while ganglionic cholinergic stimulation on the first estrous or the D1 also increased aromatase mRNA expression [91].

5. NPY, VIP or SP applied directly on the ovaries obtained from cyclic rats on D1 decrease progesterone secretion, while the same treatment on ovaries obtained from rats on D2 stimulates it. Adding NPY or VIP to the ganglions obtained from rats in D1 increased progesterone release, while adding SP decreased it. The three neuropeptides increased progesterone release in ganglions obtained from rats in D2 [92].

6. In ganglions obtained from prepubertal rats, NA inhibits the ovarian secretion of progesterone and increases the secretion of androstenedione, estradiol and NO. Adding alpha (α) and beta (β) adrenergic antagonists also reduced progesterone secretion. Blocking α-receptors stimulates androstenedione secretion and later diminished it, while blocking β receptors reduced androstenedione release. Blocking α or β receptors reduced estradiol and NO secretion [93].

7. The neurons originating at the OPN are located in the superior mesenteric ganglion (SMG). Stimulating with KCl the SMG obtained from rats on estrus resulted in lower progesterone secretion by the left and right ovaries. The acute release of estradiol by the left ovary was initially lower and increased with time, whereas the release of estradiol by the right ovary increased. ACh ganglion treatment increased the liberation of NO in the left ovary and decreased it in the right one; progesterone secretion decreased in both ovaries, estradiol increased in the left ovary compartment with no apparent changes in the right ovary; and androstenedione secretion was lower in the left ovary and similar to the control group in the right ovary [94].

8. In SMGs obtained from rats on D1 or D2, adding ACh to the ganglions increased the release of progesterone, androstenedione and NO, as well as the activity of 3beta-HSD; while 20alpha-HSD activity decreased. The gene expression of 3beta-HSD increased while the 20alpha-HSD was lower only on ganglions obtained from animals on D2 [95].

9. In the celiac ganglion obtained from rats in D2, NA addition lowers the release of ovarian progesterone by acting on its beta receptors. NA effects were blocked by the addition of a GABA-A receptor antagonist to the ganglia. Adding VIP or NPY to the celiac ganglions increased progesterone release. Adding VIP decreased ovarian nitrite levels, and the expression of NGF/trkA receptor mRNA. Androstenedione levels were not modified with neuropeptides or NE ganglionic stimulation [96].

10. Adding ACh to the celiac ganglions obtained from rats in proestrus did not modify the pre-ovulatory progesterone peak. However, adding ACh to ganglions obtained from rats on D1 inhibited the progesterone peak occurring in the afternoon and decreased ovarian NA release [97].

In the adult rat, the ovarian denervation induced by bilateral sectioning the SON modifies estrogen and progesterone secretion depending on the day and hour of the estrous cycle treatment is performed [55]. Similar results were obtained with adult guinea-pigs where denervation was induced by guanethidine injection. Denervation at the follicular phase resulted in higher concentrations of estrogen in serum than in the control group, while such effects were not observed in animals treated on the luteal phase. The opposite effect occurred with progesterone levels, suggesting that the participation of the noradrenergic peripheral innervation in hormone secretion varies during the estrous cycle [47].

The acute effects of abdominal laparotomy on progesterone, testosterone and estradiol serum levels varies according the day of the cycle when treatment is performed and also on the surgical approach used. Ventral laparotomy results in greater hormone level changes than the unilateral or bilateral dorso-lateral approach; suggesting that different neural pathways arising from the abdominal wall participate and play different roles in regulating the release of steroid hormones by the ovaries [88]. The acute effects of ventral laparotomy (1 hour after treatment) on progesterone, testosterone and estradiol are different for each hormone, vary along the estrous cycle and depend on the hour of the day when laparotomy is performed [89]. Compared to dorso-lateral left-sham surgery rats, left ULO (right ovary in situ) did not modify progesterone levels, while testosterone levels increased and estradiol

levels decreased. Right ULO did not modify hormone levels, suggesting that the lack of neural information arising from the left ovary affect the release of enzymes participating in hormone synthesis by the right ovary [84].

Blocking muscarine receptors with atropine sulphate on the day of estrus increased progesterone, testosterone and estradiol levels, suggesting that the role of ACh in regulating ovarian hormones secretion is mediated by its binding to muscarinic receptors [84]. When muscarine receptors are blocked on D1 or proestrus, estradiol levels were lower than in control [87]. Progesterone levels in rats treated on D2 or proestrus were higher than control [85]. Testosterone levels in rats with muscarinic receptors blocked on D1 were higher than in control animals**.** In rats with bilateral dorso-lateral sham surgery, injecting atropine sulphate increased testosterone levels. Unilateral left dorso-lateral sham surgery on proestrus also increased testosterone levels, while right sham surgery did not. Compared to animals with a unilateral left peritoneal perforation, testosterone serum levels decreased significantly when left hemiovariectomy (right ovary in-situ) was performed on proestrus [86].

Left dorso-lateral sham surgery performed on D1, D2, or proestrus resulted in an acute decrease of estradiol serum levels. Right dorso-lateral sham surgery on D2 increased or proestrus resulted in lower estradiol serum levels. Bilateral sham surgery on proestrus decreased hormone levels (Figure 4) [87].

Removing the right ovary (left ovary in situ) on D1 or proestrus resulted in higher estradiol serum levels, while the same procedure on D2 resulted in lower levels of estradiol in serum. In animals treated on proestrus hormone levels were lower than in the control group. The effects of blocking muscarinic receptors to ULO rats were different than in the sham surgery group depending on the in situ ovary and the day of the cycle analyzed [87]. Taken together, the effects of blocking muscarinic receptors on the release of steroid hormones in ULO rats depends on the ovary remaining *in situ* and the hormone studied. These data suggest that the neuroendocrine mechanisms regulating the functions of the enzymes participating in the regulation of steroid hormones synthesis are regulated by the cholinergic system and that it is different for each enzymes group and ovary.

Dorso-lateral sham surgery on D1, D2, or proestrus did not modify progesterone serum levels. Right ULO (left ovary in situ) on D2 resulted in higher progesterone levels than in the sham surgery group [83].

Unilaterally sectioning the SON has acute effects on progesterone, testosterone and estradiol levels; and the degree of these effects depend on the day of the cycle when surgery is performed and the hormone studied.

The results of unilaterally sectioning the SON are different in rats with both ovaries and in ULO rats. The effects depend on the day of the cycle in which treatment is performed, which SON is sectioned (left or right), the hormone studied, and in ULO rats the order in which surgeries are performed. For instance, ULO followed by sectioning the ipsilateral SON of the in situ ovary, or sectioning one SON followed by the extirpation of the innervated ovary [89]. The results support the idea that ULO modifies the neuroendocrine system regulating the release of ovarian steroid hormones and that the SON is a neural pathway carrying and sending neural information from/to the CNS. The results also suggest that the neural information arriving to the ovaries modulates the activity of the enzymes participating in the hormones synthesis in different way, which seems to be asymmetric.

Ovarian asymmetries in ovulation and hormone secretion

It is commonly accepted that paired organs are physiologically and pathologically similar. However, experimental and pathological evidence indicate that endocrine-paired organs are not identical in their function and control. According to Bernstein [96], endocrine asymmetry is part of a broader phenomenon he coined as topoendocrinology [topological endocrinology].

At present, there is not a clear understanding on the existence of endocrine-paired organs that have the same functions, secrete the same hormones, and are regulated by the same endocrine signals. As stated by diZerega et al. [99] "Despite similar exposure to pituitary gonadotropins by perfusion of both ovaries with the same peripheral blood, only 1 of the 2 ovaries sponsors the single dominant follicle in the typical menstrual cycle." The information reviewed suggests that the main difference between the right and left ovaries is related to their ability to regulate the signals from the neuroendocrine system participating in the regulation of ovarian functions. These regulatory differences are related to the innervations received by each ovary and

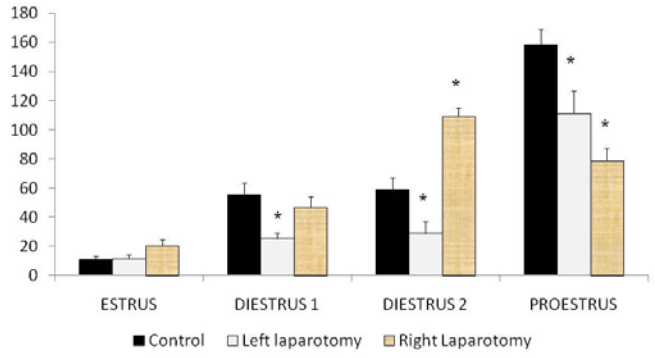

Figure 4: Acute effects of dorso-lateral laparotomy performed at 13.00 h of each day of the estrous cycle and sacrificed 1 h later, on estradiol serum levels. *p<0.05 vs. control (ANOVA followed by Tukey's test). Based on data published by Cruz et al. [87].

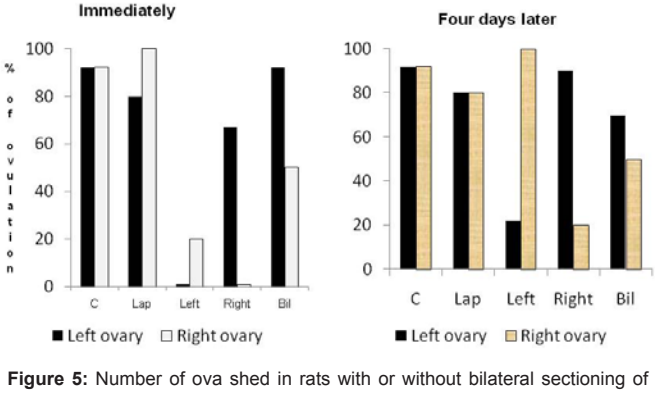

Figure 5: Number of ova shed in rats with or without bilateral sectioning of the vagus nerve followed 20 days later by the monoaminergic depletion by injecting reserpine 3 h before ovine FSH injection followed 53 h later by LH administration, sacrificed 20 h later. In the other group reserpine was injected 53 h later than reserpine injection and LH was injected 3 h later. The animals were sacrificed 20 h after LH injection of Effects o bilateral vagus nerve sectioning on the number of ova shed by rats with monoaminergic depletion performed by reserpine injection before FSH or LH injection. *p<0.05 vs. no sectioning (ANOVA followed by Tukey's test). Based on data published by Chávez et al. [37].

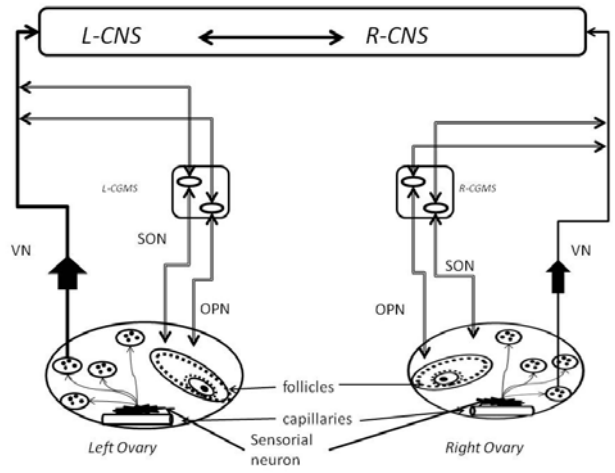

Figure 6: The diagram shows the neural pathways connecting in a dual way, the vagus nerve with the right and left celiac-superior mesenteric ganglia (CSMG). The CSMG connects also in a dual way with the ovaries through the right and left superior ovarian nerve (SON) and the ovarian plexus nerve (OPN). Their endings arrive to the blood vessels, the interstitial gland and near the follicles. On the wall of the capillaries, there are located sensorial neurons, which act as chemical sensors registering changes in the amounts of chemicals signals arising from the growing and maturating follicles. The vagus nerve carries neural information originated in the ovarian ganglia to the left and right central nervous system (CNS), which in turn have neural communication between them. The left ovary sends a higher amount of neural information to the CNS than the right ovary.

their communication with the CNS. The different ovulatory ability by the right or left ovary with SON sectioning after gonadotropin injection, support such idea (Figure 5) [100]. Other results supporting the interpretation are: the neural information sent by the left and right ovaries, reaches different regions of the CNS [3,4]. During the estrous cycle, the communication with the CGMS through the SON innervating the right and left ovaries changes in a mirror way [4]. ULO in rats with unilateral sectioning of the SON have different effects on ovulatory and hormones release abilities than unilateral sectioning of the SON in ULO rats [89,101] or in animals with unilateral sectioning of the vagus nerve [40].

Therefore, aside from the role played by hormonal signals and the participation of the ovarian innervation on regulating its actions, to better understand the neuroendocrine mechanisms regulating ovarian functions we must consider the neural signals arising from each ovary to the other ovary and the CNS. Because there is a neuronal component in each ovary, we presume that these neurons participate directly, and in asymmetric way, in the translation of neural information arriving to the ovaries and in the neural information sending for each ovary. In Figure 6, we present a proposed mechanism explaining these interactions.

Acknowledgments

We thank MSc Alvaro Dominguez-González for assistance in the English revisión, and MSc Rosa Linares and Dr. Alejandro D. Domínguez-González for helping with the figures. DGAPA-UNAM Grants IN 200405, IN209508 and IN218911-3 supported this review.

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This article was originally published in a special issue, **Hormonomics** handled by Editor(s). Dr. Hanrui Zhang, University of Missouri-Columbia, USA