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Ovarian Asymmetry

Contents

Abstract

Ovarian asymmetry reaches its maximum expression in birds, where only the left ovary develops. In mammals, differences in ovarian performance can be seen in the bat *Taphozous melanopogon melanopogon*, where ovulation occurs only in the right ovary; or in the white-toothed shrews, *Crocidura russula monacha*, where the left ovary plays a dominant role over the right one. In humans, the right ovary receives more innervation than the left. This review evaluates established concepts on ovarian asymmetry, and presents evidence that ovarian innervation plays a role in the differentiation between right and left ovaries. Analyses of the effects of surgical denervation, and on the effects of systemic or local injection of neurotoxins, or drugs, affecting neuronal transmission, have shown that the ovarian innervation arriving through the superior ovarian nerve plays a direct role in regulating ovarian follicle responses to the effects of gonadotropins. The ovary sends neural information of its activities to the central nervous system via the vagus nerve. The response to the neural signal arising from the ovary can be ipsilateral or contralateral. Therefore, the regulation of ovarian functions depends not only on the effects of gonadotropins, but also, on the modulating effects of the ovarian innervation on gonadotropin actions and the specific ovary under study.

Key words: ovary, asymmetry, ovarian regulation, ovulation.

Invited Mini-review

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Introduction

There is evidence that most of the paired endocrine organs present asymmetry. Such differences, between left and right organs, can be observed in humans and in wild animals; and are also evidenced as consequence of pathologic conditions or when animals are submitted to certain experimental procedures.

In all common mono-ovulating animals (such as women, monkeys, cows, ewes, etc.), during the typical menstrual, or estrous cycle, only one of the ovaries grows the single dominant follicle, despite exposure to similar pituitary gonadotropin concentrations by perfusion of both ovaries with the same peripheral blood. In the present overview, we will try to show the most conspicuous examples on this subject, and we will present experimental evidence suggesting that such asymmetry is linked to ovarian innervation.

Ovarian Asymmetry in Birds

In most birds, only the left ovary ovulates and secretes estradiol; extirpation of the left ovary results in the development of the right one which, up to that point, has the aspect of a testicle and secretes testosterone. Steroid hormones appear to play an important role in gonadal sex differentiation. The onset of P450 aromatase mRNA and its protein activity were simultaneously detected in the left and right ovaries after 147 h of incubation. The asymmetric function of P450 aromatase gene expression was observed at 156 h after incubation, when morphological gonadal differentiation is first recognized (Villalpando *et al.*, 2000).

Ovarian Asymmetry in Mammals

According to Vercellini *et al.* (1998), the incidence of endometriotic ovarian cysts in humans is significantly higher in the left than in the right ovary, while the incidence of rhabdomyosarcoma is significantly higher in the right than in the left ovary (Nielsen et al.,1998).

In true hermaphroditism, individuals with both testicular and ovarian tissue have been observed. These two types of tissue are asymmetrically distributed: ovaries being more common on the left side and testes and ovo-testes on the right. Hermaphrodite mice also exhibit bilateral asymmetry of gonad differentiation, but in the opposite direction: ovaries on the right, testes and ovotestes on the left (Mittwoch,2000).

In poli-ovulant mammals, differences in ovulatory ability by the left and right ovary are also observed. In bats, anatomical and/or functional asymmetries of the female reproductive organs are far more frequent and profound than those observed in any other mammalian order. Dextral (right) dominance is most frequent, but cases of sinistral (left) dominance have been described (Wimsatt,1979). For instance, in the bat *Taphozous melanopogon melanopogon*, folliculogenesis occurs in both ovaries; but only in the right ovary, a single Graafian follicle attains maturity and ovulates (Jaroli & Lall, 1987). In the white-toothed shrews (*Crocidura russula monacha*) the left ovary is dominant (Hellwing & Funkenstein, 1977).

According to O and Chew (1987), on day 9 of pregnancy of the golden hamster, the number of corpora lutea in the right ovary was greater than that in the left. Although the percentages of ova fertilized on the left and right side were not different, the percentage of ova wastage was higher on the left side than on the right. After mating, the number of sperm was higher on the right horn than in the left one.

In mice, the right ovary produces more eggs than the left one, and this characteristic is reversed in iv/iv *situs inversus* animals (Brown *et al.*,1992). In the rat, we have observed that in cyclic rats, during the day of estrus, the left ovary releases an average of six ova, while the right one releases only four. In mares, the right ovary ovulates significantly more in two months, while the left ovary does so in two other months. In lactating and dry mares however, the ovulation rate by the left and right ovaries was equal, although a significant increase in the number of pregnancies in the right uterine horn of dry mares, and in the left uterine horn of lactating mares was observed (Butterfield & Matthews, 1979).

Mongolian gerbil dams have a tendency to gestate more male fetuses in their right uterine horn and more females in their left uterine horn. Female gerbils that had both ovaries removed and portions of their right ovary placed in both ovarian capsules gestated significantly more male fetuses than females that had both ovaries removed and portions of their left ovaries placed in both ovarian capsules. On the other hand, female gerbils that had both ovaries removed and returned to their original location gestated more males in the right uterine horns than in the left. Females that had the position of their ovaries exchanged gestated more male fetuses in the left uterine horn than in the right. These data support the hypothesis that lateral asymmetries are present in gerbil ovaries rather than in gerbil uterine horns (Clark *et al.*, 1994). At present, no real explanation for these differences is available, but it is possible that differences in ovarian innervation play a role on such event.

Ovarian Innervation

Ovaries receive their innervation through the superior ovarian nerve, the ovarian plexus and the vagus nerves. The participation of the hypogastric nerves in ovarian innervation is not clear. A slight asymmetry in the population of efferent perikaria from the superior ovarian nerve and the ovarian pedicle nerve exists, where the right ovary receives motor input from a greater number of cells in the celiac-superior mesenteric ganglia than the left ovary does (Klein & Burden, 1988). In the hen, although only the left ovary is functional, after the injection of horseradish peroxidase into the ovary, labeled neurons were found bilaterally in the paravertebral ganglia T2-LS2, prevertebral ganglia, dorsal motor nucleus of the vagus, and the dorsal root ganglia T2-LS2, but not in the nodose ganglia (Ohmori *et al.*,1994). In human fetuses of 40-42 mm parietal-coccygeal length, the formations of extra- and intra-organic ovarian nerves grow into the ovarian anlagen. The nerves reach the ovarian anlagen, the ovarian artery and its branches. During

the whole period of intrauterine ontogenesis the nerves concentrate in the mediastinum, the albugineous membrane, and in the ovarian interstitial connective tissue. As the gonads descend, the number of extra-organic nerves increases, and so does the asymmetry in the innervation between the right and left ovary. The number of neural fibbers and trunklets in the plexus around the left ovarian artery are greater than around the right one (Malishevskaia & Brindak, 1980). The connections between the central nervous system and the ovaries have been studied by intra-ovaric injection of the trans-neuronal viral infection with a seudorabies virus. Labeled neurons have been detected in the spinal cord (intermediolateral cell column), the brain stem (vagal nuclei, area postrema, parapyramidal nucleus, caudal raphe nuclei, A1, A5, A7 noradrenergic cell groups, locus coeruleus, Barrington's nucleus and periaqueductal gray), the hypothalamus (paraventricular nucleus, anterior hypothalamus, arcuate nucleus, *zona incerta*), and in some tele-encephalic structures (amygdala, bed nucleus of the stria terminalis) (Gerendai & Halasz, 1997; Gerendai *et al.*, 2002).

The presence of ganglia (mesovarial, hilar, medullary and cortical) and isolated neurons in the ovaries of some strains of rats, primates and humans, has been described. Isolated neurons are dispersed along blood vessels in the ovary, the medulla and near the follicles. Some neurons were catecholaminergic, as indicated by their content of immunoreactive tyrosine hydroxylase (TH), while others showed neuropeptide Y (NPY) immuno-reactivity. In both, the ovarian cortex and the medulla, TH-positive neurons were observed, either in isolation or clustered in ganglion-like structures (D'Albora *et al.*, 2002).

Experimental Approaches

Ovarian Autograph

The participation of ovarian innervation in regulating ovulation was proposed on the basis of the lack of ovulation observed in auto-grafted ovaries of hemispayed adult rats (Domínguez & Riboni, 1971). The fact that the grafted ovary does not respond to exogenous gonadotropins, but does so when the peripheral noradrenergic innervation is blocked by guanethidine administration, supported such interpretation (Ayala & Domínguez, 1988). When the same experiment was performed in pre-pubertal hemiovariectomized rats, and the left ovary was autografted, the graft showed corpora lutea. In turn, when the right ovary was grafted, no signs of ovulation were observed (D'Albora *et al.*, 1992).

The Role of the Ovarian Innervation

The role of the superior ovarian nerve in the mechanisms regulating ovarian functions has received the attention of several researchers. Analysis on the effects of unilateral and bilateral sectioning of the superior ovarian nerve has elucidated that, through the modulation of the ovary's response to gonadotropins, neural information plays a

significant role regulating ovarian hormone secretion and ovulation. In the adult rat, sectioning one of the superior ovarian nerves results in a decrease, or total lack of ovulation by the denervated ovary; and the effects on ovulation vary according to the day of the estrous cycle when sectioning is performed (Chávez & Domínguez, 1994). Similar results were observed when sectioning of the superior ovarian nerves was performed on pre-pubertal animals (Morales *et al.*,1993). In pre-pubertal animals with the left superior ovarian nerve sectioned, ovulation did not occur after the injection of PMSG. The same treatment to rats with the right superior ovarian nerve sectioned resulted in ovulation by the left ovary, and not the right. In rats with bilateral sectioning of the superior ovarian nerve, the ovulation rate by the right ovary was 50% lower than by the left (Morales *et al.*,1998).

When the superior ovarian nerve of 4 day-old rats was sectioned, a delay of vaginal opening, a notable disruption on estrous cyclicity, and a large number of corpora lutea in the ovaries, probably due to an attenuation of the luteolytic process, was observed. The circulating levels of FSH, prolactin, and growth hormone were lower in rats with the superior ovarian nerve sectioned than in controls animals. LH levels did not vary. Serum progesterone levels did not differ between control and treated animals, but serum estradiol concentrations were higher in denervated rats, and there was a rise in the ovarian content of norepinephrine (Forneris & Aguado, 2002). Sectioning the superior ovarian nerve blocked the changes (decrease or increase) in progesterone levels induced by the intra-cerebro-ventricular injection of epinephrine or propranolol in rats on diestrus day 1 or 2 (Aguado, 2002). In adult rats with bilateral sectioning of the superior ovarian nerve, performed on the morning of proestrus, progesterone and estradiol secretion dropped within 4 min, and remained at about 50-60% of pre-section values thereafter. At 16:00 h of proestrus, progesterone levels were markedly increased, while sectioning the superior ovarian nerve produced a transient (8-min) decrease in progesterone levels, though estradiol concentrations remained low. Sectioning of the superior ovarian nerve during estrus failed to alter the output of either steroid, and did not alter blood flow (Aguado & Ojeda, 1884).

Hemiovariectomy as a Tool

Hemiovariectomy is one of the experimental tools frequently used to analyze the existence of asymmetry between the ovaries. When the hemiovariectomy was performed to 20 day-old rats, the percentage of compensatory ovulation by the left ovary was higher than by the right ovary ($279 \pm 40\%$ vs. 60 ± 13, $p < 0.01$ Student's t test). The destruction of sensory innervation by capsaicin administration resulted in an increase of the compensatory ovulation by the right ovary and a decrease by the left. These results suggest that the compensatory response of the ovary depends on which ovary remains *in situ*. In addition, the results on the lack of sensory innervation show that the performances of the right and left ovary are affected in different ways (Apolonio *et al.*, 2000).

In the prepubertal hemiovariectomized guinea pigs, where at puberty, compensatory ovarian hypertrophy by the left ovary was significantly higher than by the

right one (Riboni, 2002). In hemiovariectomized adult rats, when the right ovary is *in situ*, 27/32 animals ovulated on the expected day of estrous, while when the left ovary was left *in situ* only 16/38 did (p<0.01, Chi-Square Test). Unilateral and contra-lateral sectioning of the vagus nerve restores ovulation by the left ovary (Chávez *et al.*, 1987).

Left hemiovariectomy (rats with the right ovary left *in situ*), performed at 13:00 h of the day of estrus caused no apparent changes in progesterone concentration. In turn, testosterone serum concentration increased and estradiol concentrations in plasma diminished significantly. On the other hand, right hemiovariectomy (rats with the left ovary *in situ*) did not result in apparent changes in progesterone, testosterone, and estradiol serum concentrations. Injecting atropine sulphate to left-hemiovariectomized rats (right ovary *in situ*) resulted in higher progesterone concentrations, a significant reduction of serum testosterone concentrations, and a non-significant reduction in estradiol levels. Rats with right-hemiovariectomy, or with cholinergic blockade, showed no changes in progesterone concentration in serum, and a significant increase in testosterone and estradiol serum concentrations. Bilateral adrenalectomy resulted in a decrease in progesterone serum concentration and a significant increase in testosterone and estradiol serum concentrations. Adrenalectomy to rats with the left ovary extirpated (right ovary *in situ*) showed a decrease in progesterone and testosterone concentration in serum, without significant changes in estradiol serum concentration. Adrenalectomy to rats with the right ovary extirpated (left ovary *in situ*), had a decrease in progesterone serum concentration, a significant increase in testosterone serum concentration and no apparent changes in estradiol serum concentration. Twenty-four hours after surgery, the adrenalectomized animals with left ovary *in situ* had a higher concentration of estradiol than those rats with right ovary *in situ* (Barco *et al.*, 2003).

Effects of Drugs Blocking Peripheral Innervation

Peripheral noradrenergic denervation, induced by the administration of guanethidine sulphate, resulted in a significant decrease in ovarian norepinephrine content, which was higher for the left ovary than for the right one. The levels of estrogen and progesterone in serum, as well as the mean number of follicles and their diameters were different, depending on the time of the estral cycle when treatments were performed (Trujillo & Riboni, 2002).

In the rat, injecting lidocaine-epinephrine into one ovary affects ovulation in different ways, depending on the ovary injected. In animals with the left ovary left in-situ, and lidocaine-epinephrine treatment performed immediately before the extirpation of the contralateral ovary, ovulation did not occur. In turn, animals with the right ovary in situ, subjected to the same treatment, ovulated. Injecting human chorionic gonadotropin (hCG) did not restore ovulation (Domínguez *et al.*, 1998).

In the prepubertal guinea pig the sympathetic denervation induced by guanethidine administration resulted in a significant increase of compensatory ovarian

hypertrophy by each ovary. All hemiovariectomized animals with the left ovary in situ ovulated, while only two out of five animals with the right ovary in situ did. Denervated animals did not ovulate (Riboni, 2002).

Taken together, the results obtained by different experimental approaches on the regulation of ovarian function by the central neural system-pituitary-ovary axis could be summarized as proposed in Figure 1.

Based on the results summarized in the present review, but primarily on the results and interpretations by Gerendai and Halasz (1997), Gerendai et al. (2001, 2002), Aguado (2002), and the results from our own laboratory, we propose the following explanation for the regulation of ovarian functions.
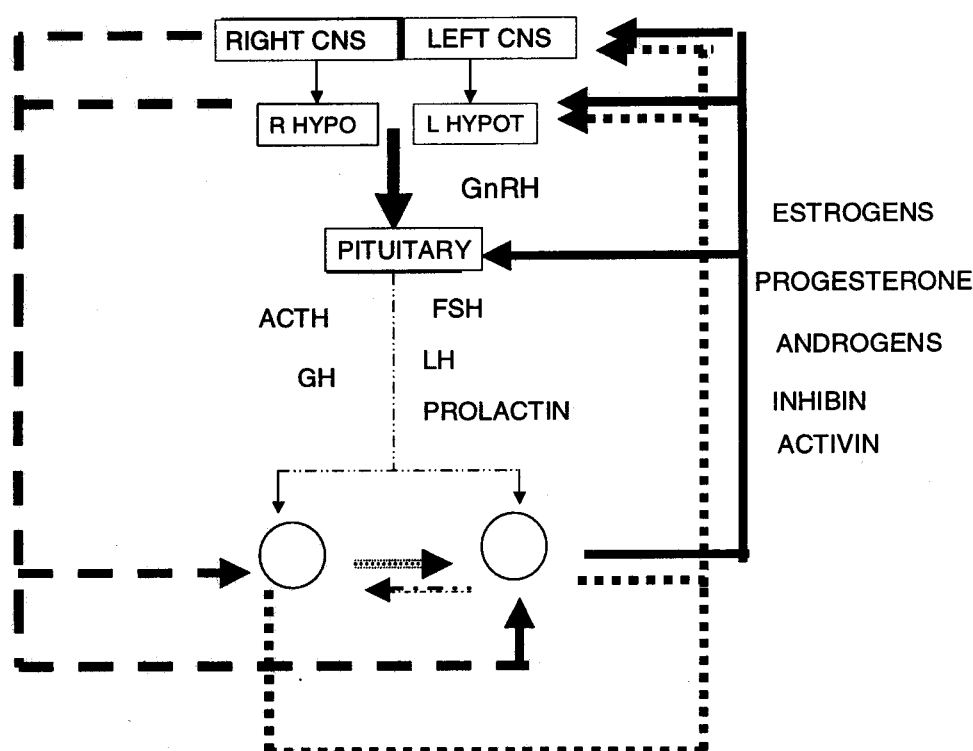

Figure 1. Theoretical explanation for the asymmetric control of the hypothalamic-pituitary–ovarian axis. See text for explanation.

The central nervous system (CNS) (raphe, vagi nuclei, amygdala, locus coeruleus, etc.) modulates the functions of the hypothalamus in different ways, depending on the side (right or left) where the signal originates. In turn, the hypothalamus, which is also lateralized and asymmetric, sends a signal to the pituitary; indicated by the variations of GnRH pulsatility.

The pituitary gland releases gonadotropins and other endocrine signals acting on both ovaries, which in turn, show different levels of response. The central nervous system (hypothalamic and extra-hypothalamic areas) sends neural signals to the ovaries through the superior ovarian nerve. The innervation arriving through the superior ovarian

nerve modulates the response of the follicles and the intersticial gland through the release of neurotransmitters, mainly norepinephrine, and by acting on the ovary's neurons. The signals and responses of the left and right ovary are different. To close the loop, some of the neurons on the ovary register changes in several ovarian function parameters (follicular growth and differentiation, pulsatile release of hormones, etc.), by modifications in the release of local molecules.

In summary, one ovary sends information to the other through innervations arising in the neurons present in the ovary; the signal goes to the intermediolateral column via sympathetic nerves (ovarian plexus?), and that signal arrives to the ovary via the superior ovarian nerve. The ovary also sends neural signals to the central nervous system via the vagus nerve, which works as an efferent pathway from the ovary to the central nervous system.

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