

Changes in Sympathetic Nerve Activity of the Mammalian Ovary During a Normal Estrous Cycle and in Polycystic Ovary Syndrome: Studies on Norepinephrine Release

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ABSTRACT Although it has been known for many years that the ovary is innervated by catecholaminergic nerve fibers and much experimental evidence has strengthened the notion that catecholamines are physiologically involved in the control of ovarian function, scarce evidence has been presented as to the role of sympathetic activity in ovarian pathologies that affect reproductive function. The purpose of this article is to provide a succinct overview of the findings in this area and discuss them relative to the pathology of polycystic ovary syndrome, the most common ovarian pathology in women during their reproductive years. *Microsc. Res. Tech.* 59:495–502, 2002. © 2002 Wiley-Liss, Inc.

ORIGIN AND INTRAOVARIAN DISTRIBUTION OF SYMPATHETIC NERVES

Postganglionic sympathetic fibers innervating the ovary derive from neuronal cell bodies of the ovarian ganglion, which is located at the origin of the ovarian artery, and from cell bodies of the celiac and renal plexuses (Baljet and Drukker, 1979; reviewed by Burden, 1985). In the rat, the ovary receives its sympathetic innervation from two sources: (1) the ovarian plexus nerve (PN), which travels along the ovarian artery, and (2) the superior ovarian nerve (SON), which is associated with the suspensory ligament (Lawrence and Burden, 1980). In general, the SON fibers innervate predominately the secretory components of the ovary, i.e., interstitial glands and follicles, whereas the PN fibers are mostly perivascular (Lawrence and Burden, 1980).

The intraovarian distribution of sympathetic fibers is similar in all species, but the density of the network varies considerably among species (Burden, 1985). Importantly, the fibers are associated with the vasculature, travel along the interstitial tissue, and surround developing follicles, but penetrate neither the corpus luteum nor the granulosa cell layer of follicles. The ovary's sympathetic innervation rapidly recovers after the organ's transplantation to an ectopic site (Lara et al., 1991). It takes 28 days for the nerve fibers to penetrate the ovary and reinnervate the gland. More recently, we found that the rat ovarian penetration of nerve fibers is accompanied by a complete biochemical biosynthetic system as ovarian norepinephrine (NE) returns to control values 28 days after surgical denervation of the SON (Fig. 1). This capacity of the ovary is probably due to the trophic support exerted by abundant amounts of nerve growth factor (NGF) and its receptors present within the gland. Immunoblockade of NGF actions during the early postnatal period not only

deprives the ovary from sympathetic nerves, but also renders it incapable of reinnervation on during adulthood (Lara et al., 1991).

Regarding the intraovarian origin for catecholamines, some studies have appeared to show that the ovary of primate and other species also present neuronal-like cell bodies that presumably synthesize catecholamines based on the presence of tyrosine hydroxylase (TH), either the protein or the gene for the enzyme (D'Albora et al., 2000; Dees et al., 1995). Because TH is the limiting enzyme for the synthesis of NE, its presence in the ovary has been used to describe the basic capacity to produce its own neurotransmitter.

The plasticity of ovarian sympathetic innervation is supported not only by the ability of the ovary to be easily reinnervated after autotransplantation (Lara et al., 1991) or SON section of the ovary (Fig. 1), but also as a consequence of estradiol administration to previously denervated rats by guanethidine administration with a procedure (Lara et al., 1990) that induces a chronic degeneration of sympathetic nerves after treatment of neonatal rats (Fig. 2). Because guanethidine permanently destroys postganglionic sympathetic nerves (Johnson and Manning, 1984), the ability of estradiol to reestablish sympathetic nerves could be the result of neuronal differentiation from neuronal-like cells already present in the ovary, stimulated to grow by increased NGF levels induced by estradiol

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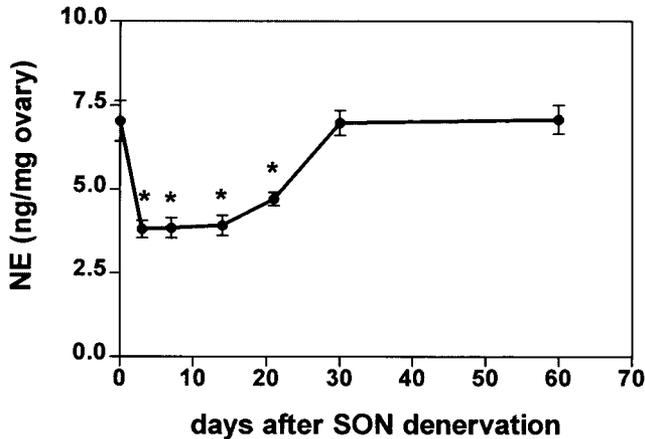


Fig. 1. Changes in ovarian norepinephrine (NE) concentration after surgical section of superior ovarian nerve (SON). NE was measured by a radio enzymatic assay as described in Ferruz et al. (1991). * $P < 0.05$ vs. control (0 days).

action on NGF gene expression (Lara et al., 2000; unpublished data).

NATURE OF THE CATECHOLAMINERGIC NEUROTRANSMITTERS WITHIN OVARIAN SYMPATHETIC FIBERS

Morphological studies, in which ovarian sympathetic nerves were visualized by histofluorescence methods, suggested that the fibers were mainly noradrenergic (Jacobowitz and Wallach, 1967; Owman et al., 1967).

This notion was later confirmed by selective biochemical measurement of ovarian catecholamines (Bahr and Ben-Jonathan 1981), a study that also showed that epinephrine and dopamine, though detectable, constitute a minor fraction of ovarian catecholamines. However, some differences exist between species; there are high levels of dopamine found in the human ovary (Lara et al., 2001). A possible physiological role of ovarian dopamine needs to be clarified because of the presence of D_1 -dopaminergic receptors in human granulosa cells, and its coupling to the intracellular third messenger, dopamine and cAMP-related phosphoprotein, M_r 32,000 (DARPP-32) phosphorylation (Mayerhofer et al., 1999, 2000).

CONTENT AND RELEASE OF NOREPINEPHRINE AS AN INDEX OF NEURONAL ACTIVITY

A variety of evidence suggests changes in sympathetic nerve activity through an estrous cycle. Ovarian NE content has been found to be lower in large than in small antral follicles, but to increase markedly in preovulatory follicles (Veldhuis et al. 1980). That these changes in ovarian NE may be related to an increased preovulatory release of NE from the nerve terminals is suggested by several observations, including an increase in NE content in porcine follicular fluid before ovulation (Bahr and Ben-Jonathan 1985), and an enhancement of NE levels in ovarian perfusates at the time of the preovulatory luteinizing hormone (LH) surge (Wolf et al., 1986). Lara and Belmar (1991) used the isolated cat ovary to study the biochemical organization of noradrenergic nerve terminals, and to deter-

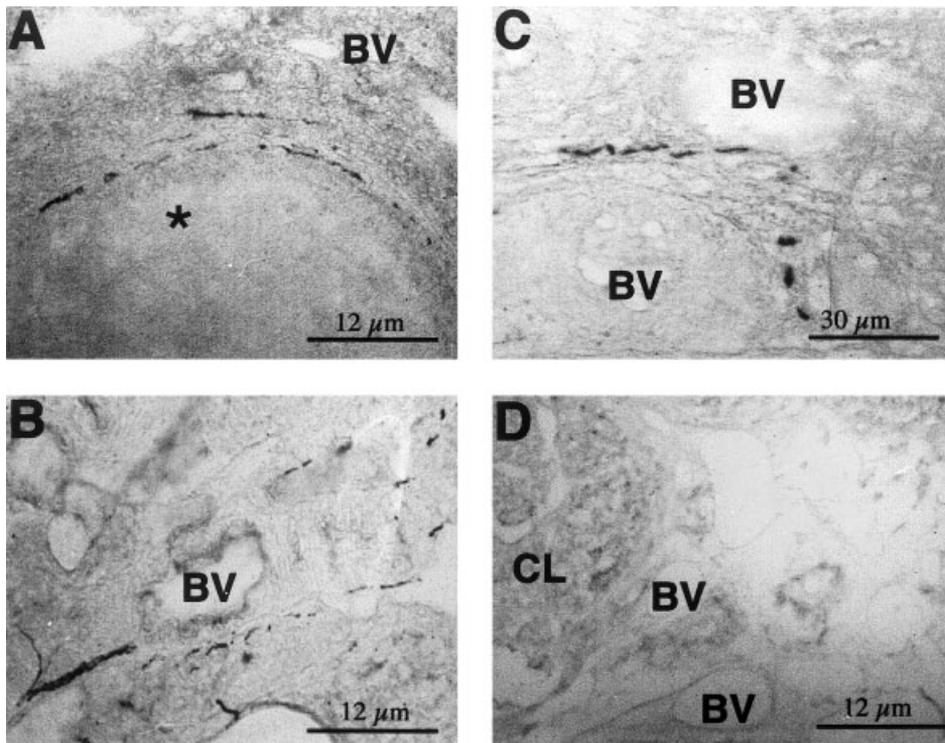


Fig. 2. The effect of estradiol administration on the appearance of tyrosine hydroxylase positive fibers in the ovary after guanethidine-induced sympathetic denervation. The presence of TH positive fibers in the ovary was analyzed according to Mayerhofer et al. (1999). **A:** Ovary of a control rat, positive for the detection of tyrosine hydroxylase. **D:** The appearance of an ovary of a denervated rat. **B,C:** Ovaries of two different denervated rats, treated with estradiol to develop cysts. No TH-immunoreactive fibers appeared in denervated rat ovaries but notice that denervated rats treated with estradiol are almost indistinguishable from the control rats. BV = blood vessel; CL = corpus luteum; * = large antral follicle.

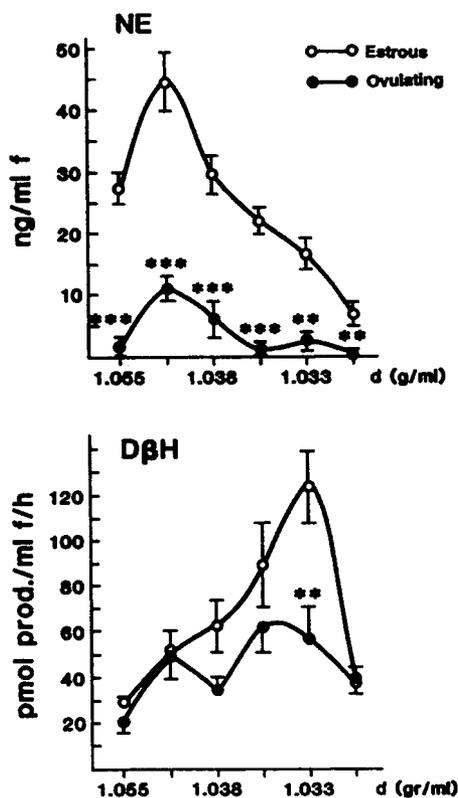


Fig. 3. NE (ng/ml of gradient fraction) and D β H (pmol product/ml of gradient fraction/h) distribution obtained after centrifugation of the vesicular fraction (P₃) in an isosmotic Percoll gradient. Values are expressed as mean \pm SEM of 6 experiments in each experimental group (estrous cat: open circles) and ovulating cats (closed circles). The abscissa represent density of the fractions. *** $P < 0.001$ vs. estrus. ** $P < 0.02$ vs. estrus. Reproduced from Lara and Belmar (1991) with permission from the publisher.

mine the occurrence of changes in the storage and releasable pool of NE during the ovulatory period.

They found a postovulatory decrease in NE stored in intracellular vesicles of the sympathetic nerve terminals, and a partial reduction in the activity of dopamine- β -hydroxylase, the enzyme that catalyzes the final step in NE biosynthesis and is located in soluble and membrane-bound form in the membrane of the vesicles releasing NE (Fig. 3). Since these changes are characteristically seen after a sympathetic discharge, their occurrence in postovulatory ovaries further strengthens the view that the activity of ovarian noradrenergic nerves increases during the hours encompassing the preovulatory surge of gonadotropins.

Depending on the experimental animal model studied, the changes occurring in NE content as a consequence of changes in sympathetic activity are not always of a sufficient magnitude to be detected. Ovarian NE content did not change significantly throughout the estrous cycle in the rat (Ferruz et al., 1991). However, the clear changes found in the concentration of β -adrenergic receptors of the ovary in the different stages of the rat estrous cycle and the intimate ligand-receptor relationship previously found in the ovary (Aguado and Ojeda 1984), strongly suggest that changes in nerve

activity exist in relationship with postsynaptic cells involved with ovarian secretory activity. The finding that the total amount of the neurotransmitter released from the nerve terminals is only a small fraction of total NE (Hughes and Roth, 1974) and that its release from nerve terminals is rapidly incorporated into the post-synaptic neurons, strengthens the notion that changes in NE release that are physiologically coupled with ovarian function are normally not accompanied by changes in NE content.

Ferruz et al. (1991) explored the changes in NE release from nerve terminals of the ovary throughout the estrous cycle as an index of neuronal activity of the sympathetic nerves instead of simply neurotransmitter content (Fig. 4). They found an increased release of NE from the ovarian sympathetic nerves of the rat during proestrus and estrus; diestrus had the lowest release activity. In addition, these results correlated well with a ligand-induced, down-regulation in the number of β -adrenergic receptors (Ferruz et al., 1991). Therefore, determination of any substance in ovarian homogenates provides only an estimate of the total content but does not differentiate between the storage vs. the releasable (and physiologically active) pool of NE.

NEUROCHEMICAL CHARACTERIZATION OF NE RELEASE FROM THE OVARY

Two different techniques to study norepinephrine release from the ovary have been published. Wolf et al. (1986) presented one study using a push-pull tubing method to study NE release from the ovary of freely moving rats. They found an increase in NE levels (measured with a radioenzymatic assay), in the ovary perfusates, during the preovulatory LH surge. In our laboratory, we studied the *in vitro* release of NE from ovaries preloaded with tracer amounts of ³H-NE. Radioactive NE is rapidly incorporated into sympathetic nerve terminals via a catecholamine-specific neuronal transmembrane carrier (Lara and Belmar, 1991). Once ³H-NE is incorporated into nerve terminals, it is distributed within the releasable pool of NE (Lara and Belmar, 1991) and can be used as a marker to follow the activity of this pool when trains of electrical pulses are applied transmurally to the preparation, mimicking the frequency of discharge occurring at the neurons and controlling its own release.

Because NE released from ovarian nerve terminals appears to be a major determinant of receptor activation in the gland (Ojeda and Aguado, 1985), the regulation of the release is a predominant factor in the control of NE availability for receptor activation. Using the above method, Ferruz et al. (1992) demonstrated that NE release in the ovary is regulated at the prejunctional level by NE and Neuropeptide Y (NPY) via the activation of specific receptors.

The release of NE from nerve terminals requires extracellular calcium. This dependency is a basic requirement to define the release of ³H-NE as neuronal when the procedure is used in whole tissues. The fact that NE release from the ovary is strictly dependent on extracellular calcium strongly suggests a neuronal origin for ³H-NE released under stimulation. A similar pattern of release was recently demonstrated to occur in the human ovary (Lara et al., 2001) permitting the

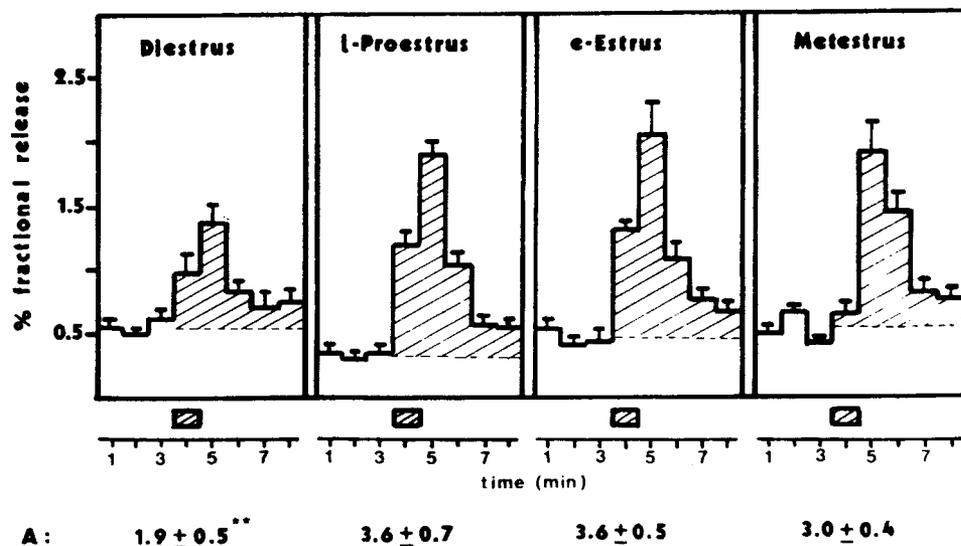


Fig. 4. Induced release of $^3\text{H-NE}$. Ovaries from rats in different stages of the estrous cycle were incubated with $^3\text{H-NE}$, and the preparation was stimulated (hatched boxes) for 1 minute at 80V, 2 msec length each stimulus, at a frequency of 10 Hz. Hatched areas correspond to the total amount of NE released under stimulation; the value is presented in A. Results are means \pm SEM from 4–6 individual experiments for each stage of the estrous cycle. L-proestrus represents rats sacrificed at 17:00 hours of proestrus and e-estrus represent rats sacrificed at 09:00 hours of the day of estrus. $**P < 0.01$ vs. proestrus, estrus and metestrus. Reproduced from Ferruz et al. (1991) with permission of the publisher.

use of this technique to study changes in sympathetic nerve activity during normal and pathological states.

While intraovarian push-pull and microdialysis-canulla techniques (see below) well represent what is happening in the internal milieu of the ovary (with a delay of 10 minutes), the tubing used is difficult to keep in position in intraperitoneal organs for long-lasting experiments (e.g., in our experiments only 20% of the microdialysis probes remained in position after 30 days of estradiol administration to rats). Additionally, these experiments also need to be followed by HPLC with electrochemical detection to measure the exact amount of NE being released from the ovary. While studying the release of recently taken up $^3\text{H-NE}$ does have the drawback of using a radioactive tracer, it is much more simple and sensitive than the other methods. Also the release of $^3\text{H-NE}$ recently taken up accepted as an index of neuronal activity presents several advantages as compared with other techniques. One difference from a push-pull technique, as used in the rat ovary by Wolf et al. (1986), is that it does not need the whole animal, instead it uses only a small (10–20 mg) piece of tissue that is incubated in vitro, making it extremely useful for studies in human biopsies (Lara et al., 2001) and for monitoring the activity of ovarian nerves during pathological states in a non-invasive, in vitro procedure and—as we did in the rat ovary through the estrous cycle (Fig. 4)—to obtain results similar to the ones obtained previously with the push-pull tubing method. Incorporation of the radioactive tracer in the presence of inhibitors of the enzymes that destroy NE (monoamineoxidase and catechol-O-methyl transferase) permits the incorporation of the intact neurotransmitter into the nerves. If inhibitors of the neuronal uptake are added, the amount of NE that overflows from the tissue (measured by scintillation counting) properly represents the release of NE from nerve terminals. A disadvantage of this method is that due to the deafferentiation of tissue from the central control of neuronal activity, there is no way to evaluate time-related events and compare the result with the status

of the whole animal, as the push-pull or microdialysis techniques do.

Probably, the local application of mini probes to monitor the release of catecholamines by fast-scan cyclic

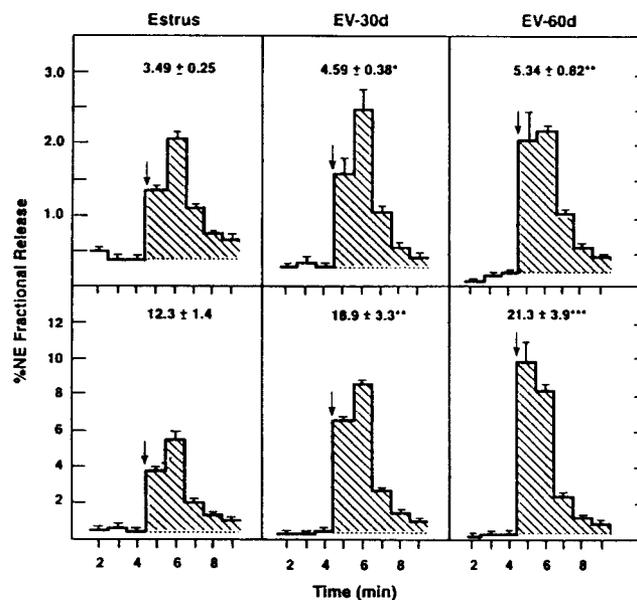


Fig. 5. Increased release of newly incorporated $^3\text{H-NE}$ by transmural stimulation of the ovary in rats with PCOS induced by estradiol valerate administration. Rats in estrus served as control animals. Treated rats were studied 30 (EV-30d) and 60 (EV-60d) days after EV administration. **Top:** NE release when stimulating (80V, 10 Hz, 2 msec, 1 min) pulses (arrows) were delivered in the absence of tetraethylammonium (TEA). **Bottom:** NE release in the presence of TEA. Numbers above profiles correspond to the total amount of NE released during stimulation (hatched area). Results are expressed as the percent fractional release and represent the mean \pm SEM of four individual experiments per group. $*P < 0.05$; $**P < 0.02$; $***P < 0.001$ (vs. estrus). Reproduced from Lara et al. (1993) with permission from the publisher.

voltametry as has been used in the central nervous system (Kume-Kick and Rice, 1998), will certainly improve the study of sympathetic nerve activity *in vivo*, with almost no delay between the response of the nerve and the detection of the neurotransmitter-dependent electrochemical reaction (i.e., "real-time" experiments). Because the probes are extremely small, they can be located between cells, easily determining the neurotransmitter locally released at the moment when nerve depolarization occurs at the nerve terminal.

CHANGES IN SYMPATHETIC NERVE ACTIVITY IN PCOS AND THE STEROIDOGENIC RESPONSE

An understanding of the role of sympathetic nerves in ovarian physiology has been clarified by the studies on the release of norepinephrine within the organ. The relationship between neuronal activity, β -adrenergic receptors, and the stimulatory effect of NE on steroid secretion found in the ovary (Ferruz et al., 1991), gives further support for a regulatory and complementary role between gonadotropins and sympathetic nerves.

Studies on the regulation of the release of NE as a mechanism to regulate ovarian nerve activity strongly suggest that it is not only regulated by the firing rate of the neurons supplying the ovary but also locally at the nerve terminals by follicle stimulating hormone (FSH) and LH. The capacity of LH to increase the induced release of NE from the ovary during late proestrus and of FSH to stimulate NE release during estrus, gives strong support that NE release is under a fine control by its own neurotransmitters and peptides, and by hormonal signals of gonadotropic origin. The increase in the release of NE during proestrus and its potentiation by gonadotropins reinforce the idea of a participation on NE in the preovulatory increase of steroids secretion (Aguado and Ojeda, 1984) but in coordination with LH and FSH acting on presynaptic receptors to regulate NE release. Thus, participation of the sympathetic nervous system in ovarian pathologies that affect steroid secretion and follicular development could be a natural consequence of autonomic dysfunction, like many others where sympathetic nerves participate.

We have obtained evidence that hyperactivation of the sympathetic innervation of the ovary participates in the development and maintenance of polycystic ovary in the rat. Polycystic ovary (PCO) syndrome is a complex disease characterized by ovulatory failure and the presence of ovarian cysts, amenorrhea, hyperandrogenemia, and variable levels of circulating gonadotropins (Yen, 1999). PCO syndrome is widely recognized as the most common cause of infertility in women. Lara et al. (1993) found that PCO induced by the administration of a single dose of estradiol valerate to rats results in profound changes in ovarian catecholamine homeostasis, which were initiated before the development of cysts and persist after the cysts were formed. These changes include an increased ovarian NE content, enhanced NE release from ovarian nerve terminals and down-regulation of β -adrenergic receptors in theca-interstitial cells, the ovarian compartment directly innervated by sympathetic nerves. The initial neural process affected in PCO appears to be the release of NE from a small, easily releasable pool

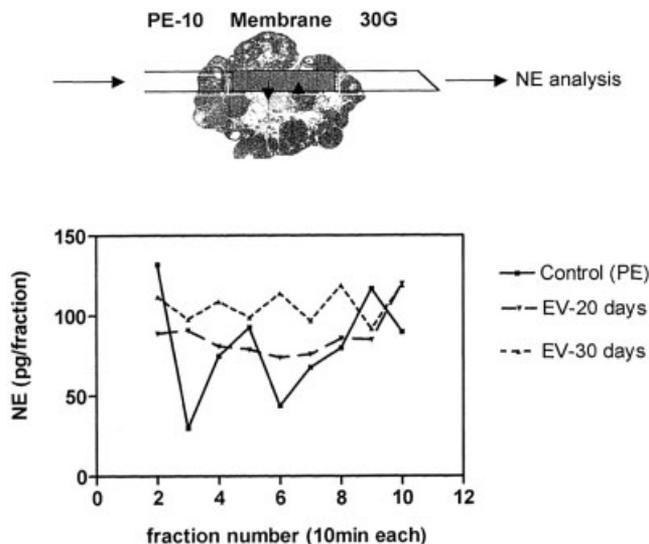


Fig. 6. Release of norepinephrine from the rat ovary as determined by a microdialysis probe chronically implanted in the ovary. **Top:** Microdialysis probe built with PE-10 tubing connected to the dialysis membrane (cut-off 5 kDa) from one side and with a small piece of a 30G needle to the other side. A connection between the membrane and PE-10 or the needle was made with silica-fuse tubing. Saline at a flow of 3 μ l/min was delivered by a peristaltic pump and samples collected in microtubes on ice. Ten-microliter samples were used for NE determination by radio enzymatic analysis (Ferruz et al., 1991). **Bottom:** Profile of NE for control rats and rats after 20 or 30 days of estradiol valerate administration.

(Hughes and Roth, 1974). This is suggested by the finding of the increase in NE content and by the ability of tetraethylammonium, a drug that increases evoked NE efflux by releasing NE from the storage pool (Wakade 1980), to further accentuate the difference in NE release between PCO and control ovaries (Fig. 5). NE release in PCO rats is clearly increased within 30 days of EV administration and is even more pronounced after 60 days (Fig. 5). The increase in NE-induced release found 30 days after estradiol administration could be the result of an increase in the size of the NE pool to be released and/or a change in the amount released under basal condition. We recently obtained some evidence in support of this last possibility, after developing a microdialysis system to monitor the *in vivo* progression of NE release from the ovary after PCO is induced. We found that NE release to the microdialysis probe is pulsatile in nature as demonstrated by the changing levels of NE in the superfusion medium (Fig. 6). It is interesting to note that the pulsatile pattern of release of NE in control animals is very similar to the pattern of pulsatile release of LH and FSH. Probably the direct effects we found for gonadotropins throughout the estrous cycle (Ferruz et al., 1991) could be responsible for the pulsatile levels of NE released from the ovary. The microdialysis pattern of NE secretion showed changes after 20 and 30 days of estradiol administration to induce PCO in rats; the pulsatile secretion found in controls was eliminated after 20 and 30 days of EV administration. This change in the secretion pattern could be associated with the decrease in LH plasma levels previously described to

occur in rats after 30 days of EV administration (Brawer et al., 1986). Probably, the decreased levels of LH are responsible for losing the pulsatile pattern of NE secretion we found after 30 days of estradiol administration. But, in addition to the change in the pattern of NE secretion after 20 and 30 days of EV, the total amount of NE released per unit of time increased (calculated as the area under the curve) and confirmed previous observations of NE release measured as the induced release of recently taken up $^3\text{H-NE}$ (Lara et al., 1993).

The increased activity of sympathetic nerves during development of PCO in rats is accompanied with a strikingly enhanced ovarian steroidal responsiveness to both β -adrenergic stimulation and gonadotropins and this abnormal response can be reversed by selectively ablating the neural input to endocrine cells of the ovary (Barria et al., 1993). It is noteworthy that the progesterone and androgen secretory responses of cystic ovaries to isoproterenol were enhanced in the face of a reduced β -adrenergic receptor content. A similar paradox was noted when studying the progesterone response to zinterol, a β_2 -adrenergic agonist during the first proestrus at puberty (Ojeda et al., 1985) and the progesterone response to isoproterenol during adult rats in estrus (Barria et al., 1993). In both cases, the β -adrenergic receptor content was reduced, but the activation of the remaining receptors resulted in a much greater stimulation of progesterone secretion than in other phases of the cycle. Probably, coupling of β -adrenoceptors to adenylyl cyclase is increased during PCO. The constitutive activation of adenylyl cyclase in McCune-Albright syndrome also includes an increased ovarian steroid output and unilateral formation of follicular cysts (Weinstein et al., 1991). The activation of noradrenergic outflow to the ovary observed in animals with PCO suggests that an abnormally heightened sympathetic tone to the gland underlies the steroidal hyperresponsiveness of polycystic ovaries. The restoration of estrous cyclicity and ovulation resulting from ablation of the SON (Fig. 7), which carries the bulk of the sympathetic innervation to ovarian endocrine cells (Lawrence and Burden, 1980), further implies a neural abnormality in the maintenance of PCO condition.

Although these data give strong evidence for an involvement of the nervous system in the maintenance of PCO, the primary stimulus is given by an overdose of estradiol; thus they do not demonstrate that the primary defect that initiates the syndrome resides within the nervous system.

EFFECT OF STRESS AND β -ADRENERGIC STIMULATION OF THE FORMATION OF OVARIAN CYSTS

To discriminate from a direct effect of estradiol as a primary etiologic factor in cyst formation, Paredes et al. (1998) tested the capacity of stress to activate sympathetic nerves and to induce changes in follicular development and cyst formation. Because kinetic studies using the mouse, hamster, and rat have shown that the large preovulatory follicles ovulating in response to LH surge actually enter the growing pool of follicles around 20 days earlier (Greenwald and Roy, 1994), it was used a chronic intermittent cold and restraint stress throughout 3 and 11 weeks (3 hours each day

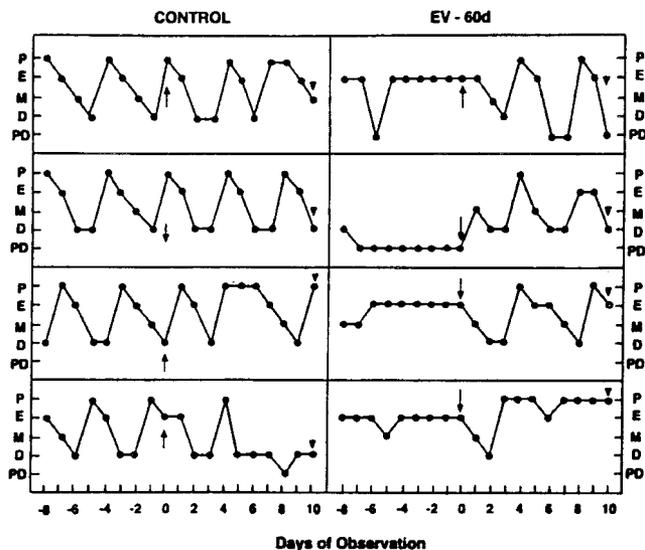


Fig. 7. Estrous cyclicity 10 days after transecting the superior ovarian nerve (SON) of cycling control rats (Left) and animals in which a polycystic ovary/anovulatory condition had been induced by EV administration (Right). Four representative profiles per group are shown. P, proestrus; E, estrus; M, metestrus; D, diestrus; PD, pseudodiestrus. Day 0 is the day of SON transection (arrow). Notice that some control animals exhibit irregular estrous cycles after SON transection, and that most of the EV-treated rats reinitiate regular cyclicity after ablation of the SON. Reproduced from Barria et al. (1993) with permission from the publisher.

from Monday to Friday). This combined-stress procedure induced an increase in sympathetic nerve activity of the ovary, in corticosterone plasma levels and catecholamines from the adrenal gland. Although the increase in NE found in the celiac ganglion represents a general effect of stress on the sympathetic nerves similar to that described at the superior cervical ganglion after restraint stress (Nankova et al., 1996), the local effect of stress in the ovary was demonstrated by the increase in the release in $^3\text{H-NE}$ recently taken up by the ovary. The increase in the activity of the autonomic nerves was also correlated with the appearance of precystic follicles in the ovary. Longer periods of stress did not develop more changes in follicular development. Instead, after 11 weeks of stress, there was no cyst formation and even a recovery in sympathetic nerve activity to control values was found. The participation of corticosterone of adrenal origin could be responsible for the reversion of the effect of stress in ovarian physiology. Supporting this, an inhibitory effect of corticosterone on NE synthesis has been demonstrated (Axelrod and Reisine, 1984). In addition, Galvez et al (1999) found that adrenalectomy alone, increases $^3\text{H-NE}$ release and up-regulated β -adrenergic receptors of the ovary. A further increase in the release on NE occurred when the same stress procedure was applied to adrenalectomized rats. These data strongly suggest that chronic stress is able to increase sympathetic nerve activity and that the increase in nerve activity is associated with changes in follicular development and the appearance of precystic structures in the ovary. Because the stress response is a multifactor event that

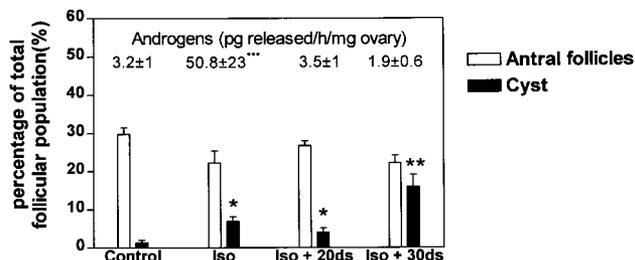


Fig. 8. The effect of in vivo isoproterenol administration on follicular development in rats. Iso: rats treated with isoproterenol for 10 days and sacrificed the next days after finishing the treatment. Iso + 20ds, rats treated for 10 days with isoproterenol and sacrificed 20 days after finishing the isoproterenol treatment. Iso + 30ds, rats treated with isoproterenol for 10 days and sacrificed 30 days after finishing the isoproterenol treatment. The ovary was fixed Zamboni's fixative, embedded in paraffin, cut in 8- μ m slices, stained with hematoxylin-eosin; antral and cystic follicles were counted in each slice. The right ovary was incubated for 3 hours in Krebs-Ringer phosphate buffer and androgens released to the medium were measured by radioimmunoassay technique (Barria et al, 1993). Numbers above bars represent the mean value \pm SEM of 5 animals for each experimental group. * $P < 0.05$; ** $P < 0.01$ vs. control rats.

involves complex neuroendocrine responses, we recently applied a method to directly stimulated β -adrenergic receptors by in vivo administration of the β -adrenergic agonist isoproterenol. We administered isoproterenol (125 μ g/k/day) for 10 days, a procedure previously described to induce cardiac hypertrophy in the rat (Benjamin et al., 1989), to study the changes that β -adrenergic receptor overstimulation might provoke in ovarian follicular development. Because of the time needed to complete the follicular development studies (Greenwald and Roy, 1994), we chose three different times to study: immediately after finishing the treatment, then 20 and 30 days after (Fig. 8). At the end of the isoproterenol treatment, the ovary had already developed follicular cysts. While an increased number of cysts were present 20 days after treatment, a more clear increase in the number of cysts was found after 30 days. Probably the mechanism involved in this process is associated with a hyperandrogenism provoked by the chronic β -adrenergic stimulation induced by isoproterenol as the ovaries of these rats presented an increased capacity to secrete androgens when incubated in vitro (Fig. 8, numbers). It is interesting to note that the increased secretory capacity of androgens of the ovary is only presented while the β -agonist is present, because androgen production returned to control levels 20 and 30 days after the end of treatment. Thus, a chronic β -agonist-induced increase in androgen production could lead to aberrant follicular development, finally causing the ovary to develop cysts in a process that is complete 30 days after of the end of isoproterenol administration.

CONCLUSIONS

The variety of evidence presented in this chapter strongly suggests that a chronic increase in ovarian sympathetic nerve activity is related to changes in follicular development, producing a non-cyclic anovulatory ovary that develops cysts. The process seems to

be reversible if the sympathetic activity is attenuated. This data could offer a new alternative to treat the polycystic ovarian syndrome using β -adrenergic antagonists in patients resistant to the standard procedure of clomiphene citrate treatment and provides a non-expensive means to reinstate ovulatory cycles in PCOS-affected women.

REFERENCES

- Aguado LI, Ojeda SR. 1984. Ovarian adrenergic nerves play a role in maintaining preovulatory steroid secretion. *Endocrinology* 114: 1944–1946.
- Axelrod J, Reisine TD. 1984. Stress hormones: their interactions and regulation. *Science* 224:452–458.
- Bahr JM, Ben-Jonathan N. 1985. Elevated catecholamines in porcine follicular fluid before ovulation. *Endocrinology* 117:620–623.
- Baljet B, Drukker J. 1979. Extrinsic innervation of the abdominal organs in the female rat. *Acta Anat* 104:243–267.
- Barria A, Leyton V, Ojeda SR, Lara HE. 1993. Ovarian steroid response to gonadotropins and β -adrenergic stimulation is enhanced in polycystic ovary syndrome: Role of sympathetic innervation. *Endocrinology* 133:2696–2703.
- Benjamin IJ, Jilil JE, Tan LB, Cho K, Weber KT, Clark WA. 1989. Isoproterenol-induced myocardial fibrosis in relation to myocyte necrosis. *Circ Res* 65:657–670.
- Braver JR, Muñoz M, Farooki R. 1986. Development of polycystic ovarian condition (PCO) in the estradiol valerate-treated rat. *Biol Reprod* 35:647–655.
- Burden HW. 1985. The adrenergic innervation of mammalian ovaries. In: Ben-Jonathan N, Bahr JM, Weiner RI, editors. *Catecholamines as Hormone Regulators*. New York: Serono Symposia Publications from Raven Press; p 261–278.
- D'Albora H, Lombide P, Ojeda SR. 2000. Intrinsic neurons in the rat ovary: an immunohistochemical study. *Cell Tissue Res* 300:47–56.
- Dees WL, Hiney JK, Schulte TD, Mayerhofer A, Danilchick M, Dissen GA, Ojeda SR. 1995. The primate ovary contains a population of catecholaminergic neuron-like cells expressing nerve growth factor receptors. *Endocrinology* 136:5760–5768.
- Ferruz J, Barria A, Galleguillos X, Lara HE. 1991. Release of norepinephrine from the rat ovary: Local modulation by gonadotropins. *Biol Reprod* 45:592–597.
- Ferruz J, Ahmed CE, Ojeda SR, Lara HE. 1992. Norepinephrine release in the immature ovary is regulated by autoreceptors and neuropeptide Y. *Endocrinology* 130:1345–1351.
- Galvez A, Paredes A, Fiedler JL, Venegas M, Lara HE. 1999. Effects of adrenalectomy on the stress-induced changes in ovarian sympathetic tone in the rat. *Endocrine* 10:131–135.
- Greenwald GS, Roy SK. 1994. Follicular development and its control. In: Knobil E, Neill JD, editors. *The physiology of reproduction*. New York: Raven Press. p 629–724
- Hughes J, Roth RH. 1974. Variation in noradrenaline output with changes in stimulus frequency and train length. Role of different noradrenaline pools. *Br J Pharmacol* 51:373–381.
- Jacobowitz D, Wallach EE. 1967. Histochemical and chemical studies of the autonomic innervation of the ovary. *Endocrinology* 81:1132–1139.
- Johnson EM, Manning PT. 1984. Guanethidine-induced destruction of sympathetic neurons. *Int Rev Neurobiol* 25:1–37.
- Kume-Kick J, Rice ME. 1998. Dependence of dopamine calibration factors on media Ca^{2+} and Mg^{2+} at carbon-fiber microelectrodes used with fast-scan cyclic voltammetry. *J Neurosci Methods* 84:55–62.
- Lara HE, Belmar J. 1991. Release of norepinephrine from the rat ovary: Changes after ovulation. *Biol Reprod* 44:752–759.
- Lara HE, McDonald JK, Ojeda SR. 1990. Guanethidine-mediated destruction of ovarian sympathetic nerves disrupts ovarian development and function. *Endocrinology* 127:2199–2209.
- Lara HE, Dees WL, Hiney JK, Dissen GA, Rivier C, Ojeda SR. 1991. Functional recovery of the developing rat ovary after transplantation: contribution of the extrinsic innervation. *Endocrinology* 129:1849–1860.
- Lara HE, Ferruz JL, Luza S, Bustamante D, Borges Y, Ojeda SR. 1993. Activation of ovarian sympathetic nerves in polycystic ovary syndrome. *Endocrinology* 133:2690–2695.
- Lara HE, Dissen GA, Leyton V, Paredes A, Fuenzalida H, Fiedler JL, Ojeda SR. 2000. An increased intraovarian synthesis of nerve growth factor and its low-affinity receptor is a principal component of steroid-induced polycystic ovary in the rat. *Endocrinology* 141: 1059–1071.

- Lara HE, Porcile A, Espinoza J, Romero C, Luza SM, Fuhrer J, Miranda C, Roblero J. 2001. Release of norepinephrine from human ovary. Coupling to steroidogenic response. *Endocrine* 15:187–192.
- Lawrence IE, Burden HW. 1980. The origin of the extrinsic adrenergic innervation to the rat ovary. *Anat Rec* 196:51–59.
- Mayerhofer A, Hemmings HC, Snyder GL, Greengard P, Boddien S, Berg U, Brucker C. 1999. Functional dopamine-1 receptors and DARPP-32 are expressed in human ovary and granulosa cells in vitro. *J Clin Endocrinol Metab* 84:257–264.
- Mayerhofer A, Fritz S, Grünert R, Sanders S, Duffy DM, Ojeda SR, Stouffer RL. 2000. D1-receptor, DARPP-32 and PP-1 in the primate corpus luteum and luteinized granulosa cells: Evidence for phosphorylation of DARPP-32 by dopamine and human chorionic gonadotropin. *J Clin Endocrinol Metab* 85:4750–4757.
- Nankova B, Kvetnansky, Hiremagalur B, Sabban B, Rusnak M, Sabban EL. 1996. Immobilization stress elevates expression for catecholamine biosynthetic enzymes and some neuropeptides in rat sympathetic ganglia: effects of adrenocorticotropin and glucocorticoids. *Endocrinology* 137:5597–5604.
- Ojeda SR, Aguado LI. 1985. Adrenergic control of the prepubertal ovary: Involvement of local innervation and circulating catecholamines. In: Ben-Jonathan N, Bahr JM, Weiner RI, editors. *Catecholamines as hormones regulators*. New York: Serono Symposia Publications from Raven Press; p 293–310.
- Owman CH, Rosengreen E, Sjöberg NO. 1967. Adrenergic innervation of the human female reproductive organs: a histochemical and chemical investigation. *Obstet Gynecol* 30:763–773.
- Paredes A, Gálvez A, Leyton V, Aravena G, Fiedler JL, Bustamante D, Lara HE. 1998. Stress promotes development of ovarian cyst in rats. The possible role of sympathetic nerve activation. *Endocrine* 8:309–315.
- Veldhuis JD, Harrison TS, Hammond JM. 1980. β_2 -adrenergic stimulation of ornithine decarboxylase activity in porcine granulosa cells in vitro. *Biochim Biophys Acta* 627:123–130.
- Wakade AR. 1980. A maximum contraction and substantial quantities of tritium can be obtained from tetraethylammonium treated ^3H -noradrenaline preloaded, rat vas deferens in response to a single electrical shock. *Br J Pharmacol* 68:425–436.
- Weinstein LS, Shenker A, Gejman PV, Merino MJ, Friedman R, Spiegel AM. 1991. Activating mutations of the stimulatory G protein in the McCune-Albright syndrome. *N Engl J Med* 325:1688–1695.
- Wolf R, Meier-Freitmam A, Duker EM, Wuttke W. 1986. Intraovarian secretion of catecholamines, oxytocin, beta-endorphin and gamma-amino butyric acid in freely moving rats: development of a push-pull tubing method. *Biol Reprod* 35:599–607.
- Yen SSC. 1999. Polycystic ovary syndrome (hyperandrogenic chronic anovulation). In: Yen SSC, Jaffe RB, editors. *Reproductive endocrinology*. Philadelphia: WB Saunders Co. 4th ed. p 436–476.