0013-7227/93/1336-2696$03.00/0
Endocrinology

Vol. 133, No. 6
Printed in U.S.A.

Ovarian Steroidal Response to Gonadotropins and β-Adrenergic Stimulation Is Enhanced in Polycystic Ovary Syndrome: Role of Sympathetic Innervation*

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ABSTRACT

Experimental induction of a polycystic ovarian syndrome (PCOS) in rodents by the administration of a single dose of estradiol valerate (EV) results in activation of the peripheral sympathetic neurons that innervate the ovary. This activation is evidenced by an increased capacity of ovarian nerve terminals to incorporate and release norepinephrine (NE), an increase in ovarian NE content, and a decrease in ovarian β-adrenergic receptor number in the ovarian compartments receiving catecholaminergic innervation. The present experiments were undertaken to examine the functional consequences of this enhanced sympathetic outflow to the ovary. The steroidal responses of the gland to β-adrenergic receptor stimulation and hCG were examined *in vitro* 60 days after EV administration, *i.e.* at the time when follicular cysts are well established. EV-treated rats exhibited a remarkable increase in ovarian progesterone and androgen responses to isoproterenol, a β-adrenergic receptor agonist, with no changes in estradiol responsiveness. Basal estradiol release was, however, 50-fold higher than the highest levels released from normal ovaries at any phase of the estrous cycle. The ovarian progesterone and androgen responses to hCG were enhanced in EV-treated rats, as were the responses to a combination of isoproterenol and hCG. Transection of the superior ovarian nerve (SON), which carries most of the catecholaminergic fibers innervating endocrine ovarian cells, dramatically reduced the exaggerated responses of all three steroids to both β-adrenergic and gonadotropin stimulation. SON transection also reduced the elevated levels of ovarian NE resulting from EV treatment and caused up-regulation of β-adrenoreceptors. Most importantly, SON transection restored estrous cyclicity and ovulatory capacity. The results indicate that the increased output of ovarian steroids in PCOS is at least in part due to an enhanced responsiveness of the gland to both catecholaminergic and gonadotropin stimulation. The ability of SON transection to restore a normal response indicates that the alteration in steroid output results from a deranged activation of selective components of the noradrenergic innervation to the ovary. These findings support the concept that an alteration in the neurogenic control of the ovary contributes to the etiology of PCOS. (*Endocrinology* **133:** 2696–2703, 1993)

POLYCYSTIC ovarian syndrome (PCOS) is a complex pathophysiology characterized by ovulatory failure, amenorrhea, hyperandrogenemia, and variable levels of circulating gonadotropins (1–3). Frequently, PCOS is accompanied by obesity, hirsutism, and, in the vast majority of cases, infertility. A hallmark of PCOS is the elevation in circulating levels of androgenic hormones and their precursors (1); this excess is accompanied by moderately elevated levels of estrogen, which mainly derive from peripheral aromatization of the heightened androgen (A) levels.

In spite of the progress made toward characterizing the different components of the syndrome and devising therapeutical strategies for its treatment, little is known about the primary factors that initiate the dysfunction. Although ovulatory surges of gonadotropins fail to occur in PCOS, and the pattern of basal gonadotropin secretion is disturbed (1, 4, 5), it is unlikely that PCOS results from a primary hypothalamic defect. On the one hand, the patterns of pulsatile LH release and LH/FSH ratios of secretion are inconsistent among PCOS patients (5). On the other, the hypothalamic-pituitary axis retains its responsiveness to both negative and positive feedback of estradiol (E_2) (4) and can reinitiate cyclic function after appropriate therapeutic manipulations (1). Both of these observations suggest that the alterations in neuroendocrine hypothalamic function in PCOS are secondary to a defect located elsewhere.

It is now clear that a major factor responsible for the acyclicity of PCOS is a tonic inhibition of gonadotropin secretion effected by the elevated circulating estrogen levels that result from the peripheral aromatization of A (for reviews, see Refs. 1 and 4). The validity of this view, which derived from clinical observations, is supported by experimental data in laboratory rodents showing the ability of estradiol valerate (EV), a long-acting estrogen, to cause acyclicity, anovulation, and formation of ovarian cysts (6, 7). These and other considerations have suggested that the primary defect underlying the etiology of PCOS may be intraovarian, a possibility that has recently redirected investigative efforts toward the study of intraovarian molecules involved in paracrine/autocrine regulation, such as insulin-like growth factor-I (for reviews, see Refs. 1 and 8). Never-

Received March 30, 1993.

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* This work was supported by grants from the Departamenyo Técnico de Investigaciones, Universidad de Chile B 3344–9213; Fondecyt 90–1001 and 1931049, and the Rockefeller Foundation (to H.E.L.), and NIH Grants HD-24870, HD-18185, and RR-00163 (SRO). This is publication 1886 of the Oregon Regional Primate Research Center.

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theless, the ability of LHRH to induce ovulation in humans (1) and rats (9) and the restoration of ovarian cyclic function by administration of the antiestrogen clomiphene citrate in humans (1, 4) or hemiovariectomy in rats (10) suggest that, as in the hypothalamus, an ovarian defect is not the primary cause of PCOS.

The extraordinary complexity of the syndrome dictates the need to search for alternative sources of dysfunction that may contribute to or be primarily responsible for the initiation of abnormalities leading to the development of PCOS. The nervous system is one such source. That alterations in the neurogenic control of the ovary may play a role in the initiation and/or maintenance of PCOS has not been addressed previously, but is inferentially suggested by the finding of an increased density of catecholaminergic nerves in the ovaries of PCOS patients (11), and the marked effectiveness of ovarian wedge resection to initiate ovulatory cycles (1, 3, 12) in the face of only transient decreases in circulating steroid levels (1). Interestingly, PCOS symptoms are most commonly initiated around the time of puberty (13), a time during which the adult density of ovarian sympathetic innervation is acquired in primates (14). In a companion paper (15), we present evidence that the activity of sympathetic nerves is enhanced in PCOS induced in rats by the administration of EV and have postulated that a derangement of sympathetic inputs to the ovary contributes to the maintenance of PCOS. In the present study, we demonstrate that the steroidal response of the ovary to both β-adrenergic and gonadotropin stimulation is markedly enhanced in PCOS, and that ablation of the sympathetic innervation to endocrine cells of the ovary restores a normal steroidal response and results in initiation of estrous cyclicity and ovulation. A preliminary report of these findings has appeared (16).

Materials and Methods

Animals

Virgin adult cycling rats, weighing 200–220 g were obtained from a stock of Sprague-Dawley animals maintained at the University of Chile. The animals were kept on a 12-h light, 12-h dark photoperiod (lights on from 0700–1900 h) and allowed free access to pelleted rat chow and tap water. Animals showing regular 4-day cycles were used for the experiments. A PCOS condition was induced by the administration of EV (Sigma Chemicals, St. Louis, MO; single im injection, 2 mg/rat diluted in 0.2 ml corn oil), as described by Brawer *et al.* (6). Control rats were injected with oil. All experiments were performed 60 days after the injection, when follicular cysts are first detected (7).

Transection of the superior ovarian nerve (SON)

The SON was selected for transection because it carries sympathetic fibers that predominantly innervate the endocrine component of the ovary, in contrast to the plexus nerve, which mainly innervates the ovarian vasculature (17). Control and EV-treated rats were lightly anaesthetized with ether, the ovaries were exposed through a dorsal incision, and the SON was sectioned with a microcautery, as previously described (18). All experiments were carried out 10 days after the surgical procedure. Estrous cyclicity was daily monitored by vaginal lavages obtained between 1000–1200 h.

Steroid response to β-adrenergic and/or gonadotropin stimulation

The ovaries from control and EV-treated rats were halved and incubated *in vitro* in 2 ml Krebs-Ringer bicarbonate buffer, pH 7.4, for 3 h at 37 C, as previously reported (19–21), in the presence of D,L-isoproterenol-HCl (10^{-5} M; Sigma), hCG (2.5 IU; Sigma), or a combination of the two. The experimental design was such that all four ovarian halves were simultaneously used; one served as a control, and the other three were subjected to different stimulatory treatments. Progesterone (P), E_2, and A released into the incubation medium were measured by RIA, as previously described (22). In the case of testosterone measurements, the values obtained are reported as A because the antiserum used cross-reacts with 5α-dihydrotestosterone (22).

Measurement of β-adrenergic receptors

The assay was performed in membranes isolated from whole ovaries. The membranes were prepared by differential centrifugation, and the binding reaction was performed using [^{3}H]dihydroalprenolol (SA, 92.0 Ci/mmol; DuPont-New England Nuclear, Boston, MA) as the ligand, following a procedure described in detail previously (19, 20). The concentration of [^{3}H]dihydroalprenolol used was 10 nM; nonspecific binding was assessed by incubating membranes in the presence of 1 mM D,L-propranolol (Sigma). Results are expressed as femtomoles of dihydroalprenolol bound per mg protein/30 min.

Measurement of norepinephrine (NE) and proteins

To measure NE content, the ovaries were first homogenized in 0.2 M perchloric acid, the homogenates were then centrifuged at 15,000 × *g* for 10 min, and the supernatants were collected for radioenzymatic assay of NE (23), as previously described (24). Proteins were measured by the method of Lowry *et al.* (25), and the acid-insoluble material was dissolved in 1 M NaOH, using BSA (fraction V) as the standard.

Histology

The ovaries from control, EV-treated, and EV-treated/denervated rats were cleaned of adherent fat tissue, fixed in Zamboni's fixative, embedded in paraffin, sectioned at 8 μm, and stained with hematoxylin-eosin, as previously reported (21).

Statistics

Differences between two groups were analyzed with Student's *t* test. Comparisons between several groups were performed using a one-way analysis of variance, followed by the Student-Newman-Keuls multiple comparison test for unequal replications (26).

Results

Ovarian P release in EV-induced PCOS and after SON denervation

In vitro basal P release changed little during the estrous cycle and was not significantly affected by EV treatment (Fig. 1A). Activation of β-adrenoreceptors with isoproterenol significantly increased P release at all phases of the estrous cycle (Fig. 1B); in agreement with previous observations (19), the greatest response was observed at estrus ($P < 0.01$). The ovaries of EV-treated rats responded significantly more ($P < 0.05$) than those at any phase of the cycle, including estrus (Fig. 1B). SON denervation, which by itself caused a marginal ($P < 0.05$) increase in basal P release (perhaps due to the appearance of new corpora lutea), blunted the P response of EV-treated rats ($P < 0.01$).

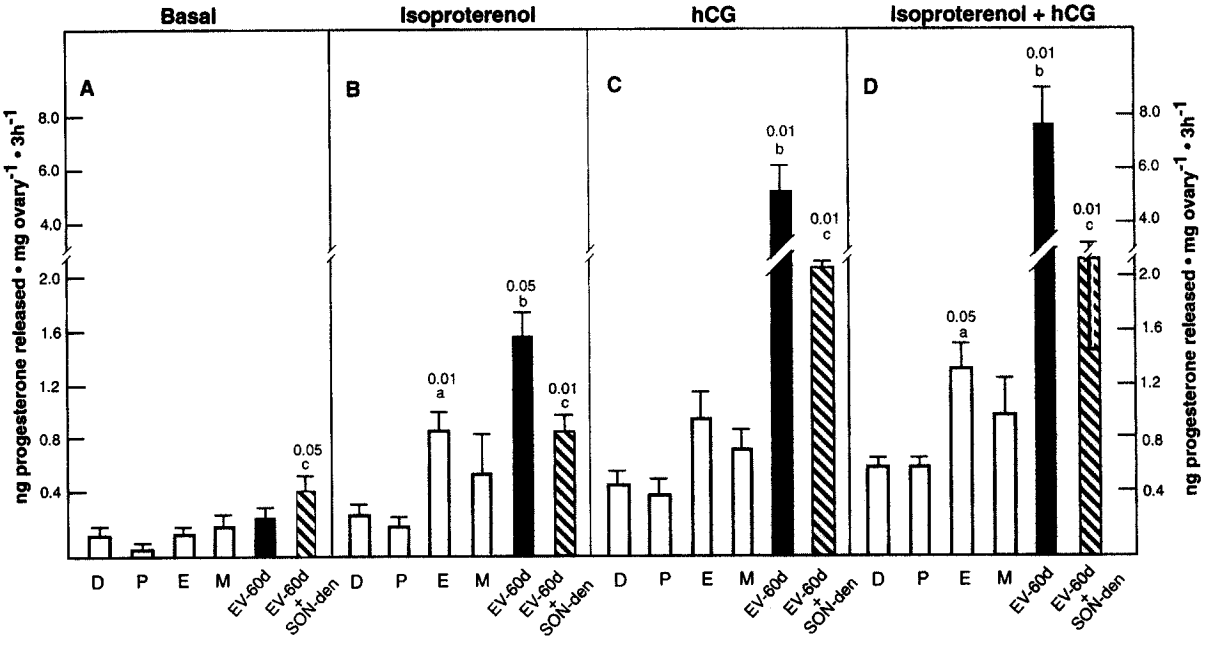

FIG. 1. Neural-dependent increase in ovarian P response to β-adrenergic and gonadotropin stimulation in PCOS induced by EV. The ovaries of cycling, EV-treated, and EV-treated animals subjected to transection of the SON were halved and incubated for 3 h in Krebs-Ringer bicarbonate buffer alone (A), isoproterenol (10 μM), or hCG (2.5 IU). The amount of P released into the incubation medium was measured by RIA. D, Diestrus; P, proestrus; E, estrus; M, metestrus; EV-60d, EV-treated rats 60 days after a single injection of the steroid; EV-60d + SON Den, EV-treated rats 10 days after SON transection. a, Statistically different at the indicated level from the other phases of the estrous cycle; b, significantly higher than the response of normal cycling animals; c, significantly different than the response of EV-treated rats with intact innervation. In this and subsequent figures, each *bar* represents the mean ± SEM of four or five independent observations per group.

Like isoproterenol, hCG stimulated P release at all phases of the estrous cycle, with a tendency to a greater response at estrus (Fig. 1C). The P response to hCG was markedly enhanced ($P < 0.01$) in EV-treated rats and, as in the case of isoproterenol, was significantly ($P < 0.01$) reduced by SON denervation (Fig. 1C). Incubation of the ovaries with both isoproterenol and hCG resulted in an additive effect at all phases of the estrous cycle and in EV-treated animals (Fig. 1D). As before, SON transection decreased the response.

Ovarian A release in EV-induced PCOS and after SON denervation

Basal A release was highest ($P < 0.01$) during the proestrous phase of the estrous cycle (Fig. 2A). The ovaries from EV-treated rats had a basal A output as high as that of proestrous rats; SON transection reduced this basal release by about 40%, but the levels attained were not significantly lower than those seen at proestrus.

Only proestrous ovaries responded to isoproterenol with a significant ($P < 0.01$) increase in A release (Fig. 2B). The response of EV-treated animals was even greater ($P < 0.05$) and was reduced by SON transection to levels lower than those in control proestrous animals (Fig. 2B). Paralleling the effect of isoproterenol, hCG stimulated A output only in proestrous ovaries and elicited an even greater ($P < 0.01$) release from the ovaries of EV-treated rats (Fig. 2C). As before, this effect was abolished by SON transection (Fig. 2C). A similar profile of responses was observed when the ovaries were exposed to a combination of isoproterenol and hCG (Fig. 2D).

Ovarian E_2 release in EV-induced PCOS and after SON denervation

Basal E_2 release was highest ($P < 0.05$) during the proestrous phase of the cycle and was dramatically increased ($P < 0.001$) in EV-treated rats (Fig. 3A). SON transection decreased the response, which returned to levels seen in normal cycling animals (Fig. 3A). Isoproterenol did not stimulate E_2 release in cycling rats, and failed to further increase the already elevated basal output of the steroid in EV-treated animals (Fig. 3B). As in the case of A, only proestrous ovaries responded to hCG or to the combination of hCG plus isoproterenol with E_2 release (Fig. 3, C and D). Neither treatment was able to further increase the already elevated basal E_2 levels observed in EV-treated rats. As seen with ovaries incubated without stimulatory agents, SON transection markedly reduced E_2 release from ovaries treated with isoproterenol, hCG, or a combination of the two (Fig. 3, B–D).

Effect of SON denervation on estrous cyclicity and ovarian morphology

Ten days after transection of the SON of normal cycling rats, 76% (7 of 9) of the animals continued to display normal 4-day estrous cycles. Representative profiles illustrating responses are depicted in Fig. 4. After denervation of

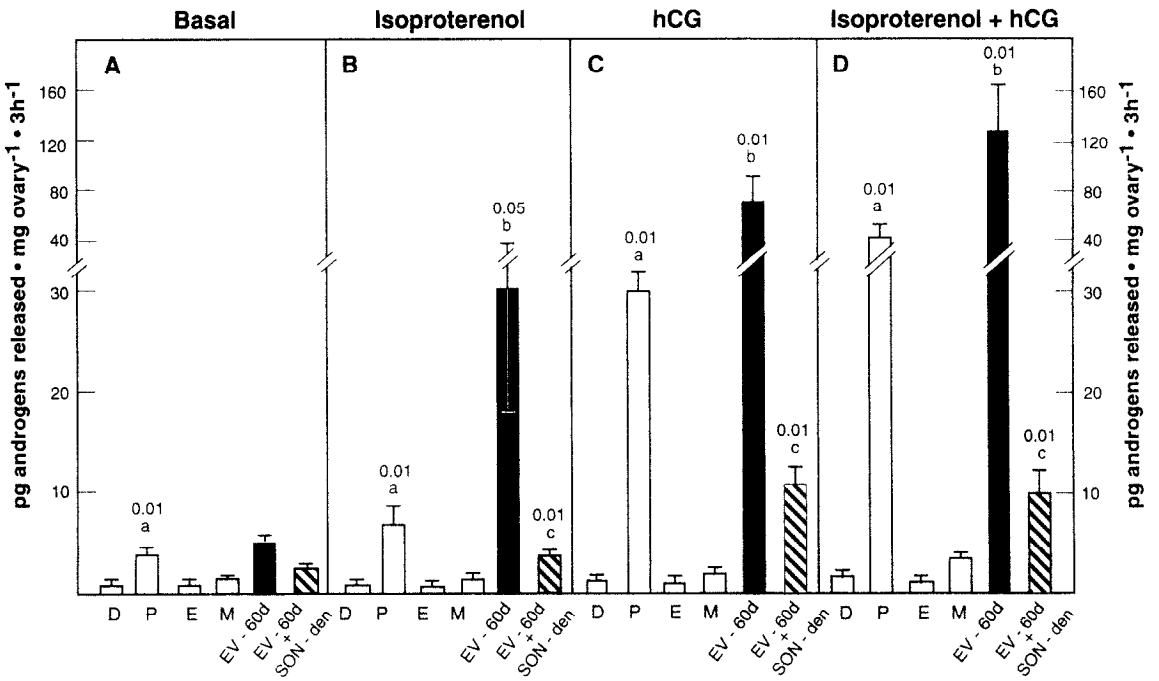

FIG. 2. Neural-dependent increase in ovarian A response to β-adrenergic and gonadotropin stimulation in PCOS induced by EV. For abbreviations and key to statistical significance, see Fig. 1.

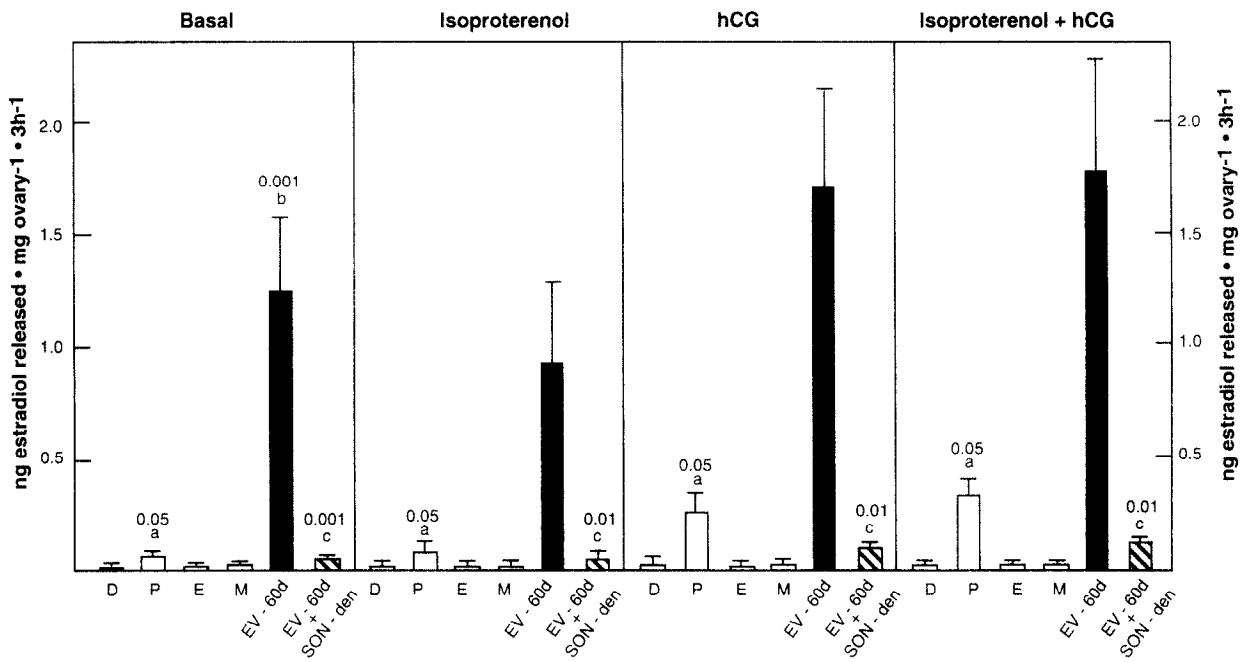

FIG. 3. Neural-dependent increase in basal ovarian E secretion without a concomitant increase in response to either β-adrenergic or gonadotropin stimulation in PCOS induced by EV. For abbreviations and key to statistical significance, see Fig. 1.

EV-treated rats that were in constant estrus, 70% of them (7 of 10) reinitiated regular estrous cycles. The remaining 30% either failed to cycle or exhibited an abnormal pattern of cyclicity. Figure 4 illustrates these responses.

Histological examination of the ovary revealed that transection of the SON in EV-treated rats had indeed resulted in ovulation and formation of corpora lutea. Figure 5A shows an ovary of an intact cycling rat killed in the metestrous phase of the cycle. Numerous corpora lutea and several small antral follicles characteristic of this phase of the cycle were found. The ovaries of EV-treated rats displayed the characteristic features of PCOS (Fig. 5B), including severely atretic large antral follicles, follicular cysts with a well developed thecal cell layer and a diminished granulosa cell compartment, and luteinized cysts (6, 7). Transection of the SON of normal cycling controls did not affect the ability of the ovary

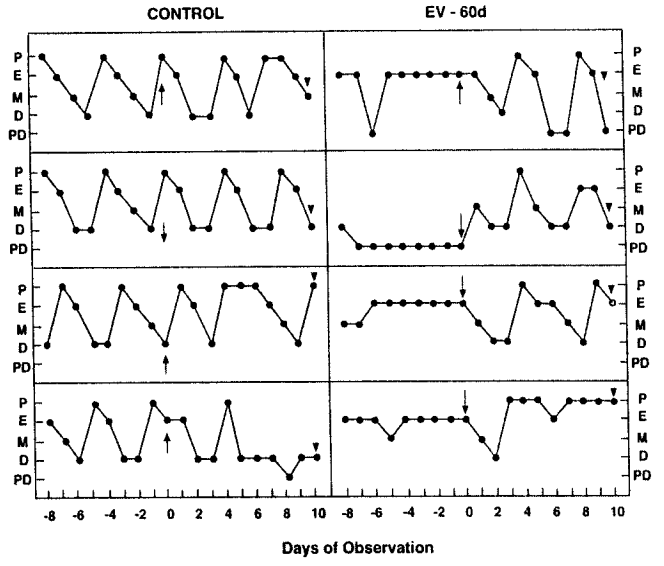

FIG. 4. Estrous cyclicity 10 days after transecting the SON of cycling control rats (*left panels*) and animals in which a polycystic ovary/anovulatory condition had been induced by EV administration (*right panels*). Four representative profiles per group are shown. P, Proestrus; E, estrus; M, metestrus; D, diestrus; PD, pseudodiestrus. Day 0 is the day of SON transection (*arrow*); all rats were killed 10 days after SON transection (*arrowhead*). 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.

to ovulate, as evidenced by the presence of corpora lutea. Figure 5C depicts the ovary of one such control which was killed in the diestrous phase of the estrous cycle. Several corpora lutea can be observed, as well as some large antral follicles, as would be expected in this phase of the cycle. SON transection, on the other hand, resulted in striking changes in the morphological aspect of ovaries from EV-treated rats. Numerous corpora lutea were readily apparent as well as a marked attenuation of the cystic condition (Fig. 5D), indicating that the resumption of estrous cyclicity caused by SON transection was a consequence of ovulation and formation of functionally competent corpora lutea.

Effect of SON denervation on ovarian NE and β-adrenoreceptor contents

As shown in the companion paper (15), EV-induced PCOS is accompanied by an increase in ovarian NE content and a decrease in β-adrenergic receptor number (Table 1). The receptor number in polycystic ovaries was as low as that in normal ovaries in the estrous phase of the cycle. SON denervation depleted ovarian NE content in both cycling and EV-treated animals and resulted in up-regulation of β-adrenoreceptors. In EV-treated rats, this up-regulation was apparent only in animals that were in the diestrous phase of the cycle after reinitiating cyclicity (Table 1).

Discussion

In both women suffering from PCOS and laboratory rodents in which the syndrome is induced experimentally, the ovary produces excessive amounts of certain steroids. The nature of the steroid inappropriately produced appears to depend on the species. In women, ovarian A secretion, but not that of estrogen, is markedly enhanced (1, 4). In spite of the absence of changes in ovarian estrogen output, circulating estrogen levels, in particular those of estrone, are elevated due to peripheral aromatization of A (1, 4). In rats, one study reported no changes in plasma levels of aromatizable A (9), a surprising finding because the cystic ovaries of rats with PCOS exhibit a degree of thecal hyperplasia similar to that of humans affected with the syndrome (6, 7). However, as in humans, circulating E_2 levels are elevated in rats with PCOS (6, 27). Perhaps circulating levels of aromatizable A in rats are not elevated because they are extensively metabolized to estrogens within the ovary.

The persistent estrogen negative feedback resulting from the chronic elevation of plasma estrogen levels is thought to be a key factor in maintaining an inappropriate pattern of gonadotropin secretion and perpetuating the acyclicity of PCOS (1). Experimental evidence for this concept was provided by the ability of E_2 to induce acyclicity and maintain a persistent anovulatory condition in rats (6, 28). These and other observations (1–4, 7, 27) have raised the question of which is the primary defect responsible for the initiation of inappropriate secretion of ovarian steroids in PCOS. The defect does not appear to reside within the neuroendocrine hypothalamus or anterior pituitary gland, because the hypothalamic-pituitary unit is able to reinitiate menstrual cyclicity in response to blockade of estrogen negative feedback with the antiestrogen clomiphene citrate (1, 12, 13). Moreover, in both humans and rats with PCOS, the pattern of LH secretion varies considerably both among individuals (5) and with progression of the pathology (27), indicating that maintenance of the cystic condition is independent of the prevailing pattern of gonadotropin release. A primary ovarian defect, though currently under experimental scrutiny, may not be responsible either, because polycystic ovaries can ovulate and reinitiate cyclic function under appropriate hormonal treatment (1, 9) or after hemiovariectomy (10). The current state of affairs has been recently summarized by Yen (1), who states that anovulation of patients with PCOS is not due to an inherent defect of the hypothalamic-pituitary-ovarian axis, but, rather, to "a variety of factors and sites outside the reproductive axis."

The present findings demonstrate that the ovarian steroidal responsiveness to both β-adrenergic stimulation and gonadotropins is strikingly enhanced in animals with EV-induced PCOS, and this abnormal response can be reversed by selectively ablating the neural input to endocrine cells of the ovary. The activation of noradrenergic outflow to the ovary observed in animals with PCOS (15) suggests that an abnormally heightened sympathetic tone to the gland underlies the steroidal hyperresponsiveness of polycystic ovaries.

Previous observations have shown that catecholamines acting via β-adrenergic receptors of the β_2-subtype stimulate P (19, 29, 30) and A secretion (19, 31) on their own and facilitate the stimulatory effect of gonadotropins on the se-

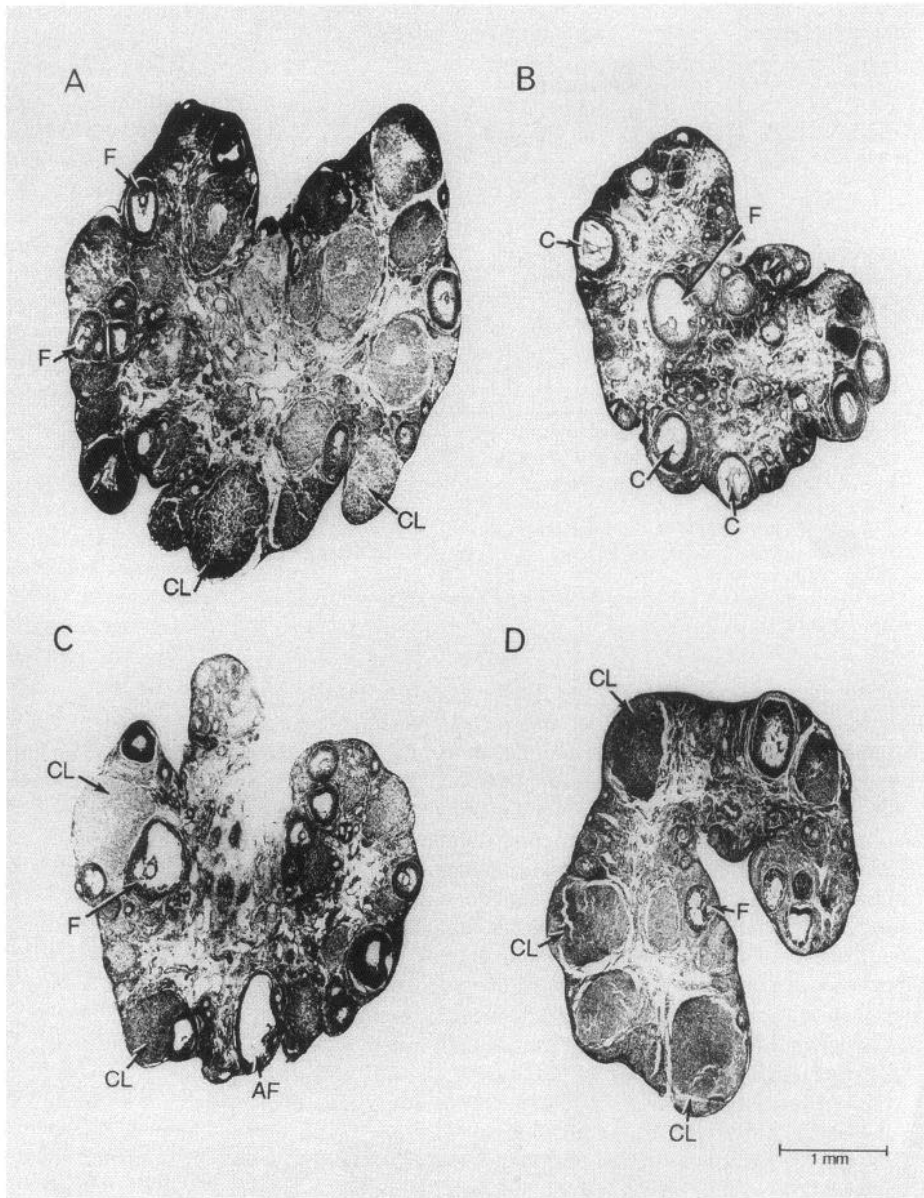

FIG. 5. Restoration of normal ovarian histology after transection of the SON in rats suffering from PCOS induced by EV. A, Ovary from a rat in the metestrous phase of the cycle; B, polycystic ovary; C, ovary from a rat in the diestrous phase of the cycle, subjected to SON transection 10 days previously; D, ovary from an EV-treated rat 10 days after SON transection. Notice the development of corpora lutea and regression of cystic follicles after SON transection. F, Normal follicle; CL, corpus luteum; C, follicular cyst; AF, atretic follicle. The sections are 8 μm thick and stained with hematoxylin-eosin (magnification, ×24).

cretion of these steroids in an additive manner (31, 32). These studies also demonstrated the inability of β-adrenergic receptor stimulation to affect E_2 release (19, 29, 32). The present results are in keeping with these earlier observations, as they show that both the steroid selectivity of β-adrenergic stimulation and the additive effect with hCG are maintained in PCOS, despite the striking increase in responsiveness shown by the cystic ovaries.

It is noteworthy that the P and A 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 P response to Zinterol, a β_2-adrenergic agonist, during the first estrus at puberty (19), and the P response to isoproterenol during the adult estrus (this paper). In both cases, ovarian β-adrenergic receptor content was reduced (15, 19), but activation of the remaining receptors resulted in a much greater stimulation of P secretion than at the other phases of the cycle. Surprisingly, polycystic ovaries also show an enhanced steroid responsiveness to hCG in spite of having a low hCG receptor content (33). Evidence exists that in the ovary, gonadotropins and β-adrenoreceptors are coupled to the same adenylyl cyclase pool, so that desensitization of the cAMP response to one type of receptor leads to similar alterations in response to the other (34). Thus, it is plausible that the steroid response to both β-adrenergic and gonadotropin stimulation increases in PCOS because of enhanced coupling of the receptors to adenylyl cyclase. Two observations support this possibility. One of them showed that coupling of β-adrenoreceptors to adenylyl cyclase can be increased by E_2 in rat mammary tissue (35). The other demonstrated the existence of a constitutive activation of adenylyl cyclase in *McCune-Albright*

TABLE 1. Effect of SON denervation on the NE content and β-adrenergic receptor number of ovaries from EV-treated rats

	NE content (ng/ovary)	β-Adrenergic receptor no. (fmol/mg protein)
Diestrus (n = 5)		
Control	13.6 ± 0.8	265.6 ± 6.1[a]
Denervated	3.6 ± 0.2[b]	365.7 ± 12.4[b]
Estrus (n = 5)		
Control	11.3 ± 0.9	108 ± 25
Denervated	3.8 ± 0.1[b]	195 ± 15[b]
EV-treated (n = 4)		
Control	24.3 ± 2.2[c]	96.5 ± 8.4
Denervated		
E[d]	4.2 ± 0.1[b]	114.5 ± 5.5
D_2[e]	7.2 ± 1.3[b]	267.7 ± 12.4[b]

Transection of the SON was performed 10 days before collection of the ovaries. Results are expressed as the mean ± SEM.

[a] $P < 0.01$ *vs.* estrus and EV-treated.

[b] $P < 0.01$ *vs.* control.

[c] $P < 0.01$ *vs.* control estrus and diestrus.

[d] Ovaries collected on the day of estrus, 10 days after reinitiation of estrous cyclicity.

[e] Ovaries collected on the day of diestrus, 10 days after reinitiation of estrous cyclicity.

syndrome and suggested that the multiple abnormalities of this disease, which include an increased ovarian steroid output and unilateral formation of follicular cysts, are caused by persistent activation of cAMP formation (36).

The ability of SON transection to reduce the steroid response and increase β-adrenoreceptor content toward normalcy suggests that both abnormalities are at least in part due to an exaggerated sympathetic outflow to the ovary. It is noteworthy that ovarian NE release is highest during both the estrous phase of the normal cycle and in PCOS (15), two conditions during which steroidal hyperresponsiveness occurs in the face of decreased β-adrenoreceptor content. Further experimentation will be required to determine whether cAMP formation in response to β-adrenergic/hCG stimulation is enhanced in PCOS and to define the molecular mechanisms underlying the abnormality.

The restoration of estrous cyclicity and ovulation resulting from ablation of the SON, which carries the bulk of the sympathetic innervation to ovarian endocrine cells (17), further implies a neural abnormality in the maintenance of PCOS. Although the effect of SON transection may be attributed to a compensatory increase in circulating gonadotropin levels, as shown after hemiovariectomy (10), steroid secretion drops for only a few minutes after SON transection (37), making it unlikely that a sustained increase in gonadotropin levels would be responsible for the restoration of ovarian function that follows nervotomy. Moreover, SON transection does not affect the timing of puberty (18) and, as observed here, only causes irregularity of estrous cyclicity in some animals without affecting ovulatory capacity in normal cycling adult rats. In a recent study, Desjardins and Brawer (38) showed that polycystic ovaries transplanted under the kidney capsule remain polycystic despite being severed from their innervation. As the cystic condition was evident in ovaries first transplanted and then injected with EV and in ovaries removed from animals with PCOS, it was concluded that the innervation was not important for either the development or maintenance of PCOS. These findings are difficult to reconcile with the present observations and with the report by the same group that hemiovariectomy of rats with PCOS, which leads to much less pronounced changes in circulating gonadotropins than autotransplantation of both ovaries (39), results in ovulation and rapid restoration of estrous cyclicity (10). Based on the ability of hemiovariectomy to restore normal ovarian function in rats with PCOS, one would predict a similar course of events after autotransplantation of both polycystic ovaries, unless the recovery is prevented by other factors related to the transplantation paradigm. On a short term basis, the sensitivity of cystic ovaries transplanted under the kidney capsule to circulating catecholamines (40) may be heightened by the compensatory increase in β-adrenoreceptor function that follows interruption of the ovarian innervation (18). Three weeks after transplantation, reinnervation of the ovary is complete (39), so a sympathetic tone would be expected to be operative in transplanted ovaries at the time of EV administration.

Further evidence in support of neural involvement in the maintenance of PCOS was recently provided by the finding that the expression of the genes encoding nerve growth factor (NGF) and one of its receptors, the so-called low affinity NGF receptor, increased dramatically in the ovary within 30 days after EV administration, *i.e.* at a time preceding the formation of cysts (Lara, H. E., G. A. Dissen, S. R. Ojeda, unpublished data). NGF produced by tissues innervated by the peripheral nervous system is required for the survival and function of the innervating sympathetic neurons (41). It is thought that one of the functions of the low affinity NGF receptor may be to transfer NGF from its sites of synthesis to the innervating fibers. The simultaneous increases in the synthesis of both NGF and its receptor before the formation of cysts suggest that after EV administration, the neurons innervating the ovary become subjected to an enhanced neurotrophic influence that contributes to their hyperactivation and to maintaining an abnormally heightened catecholaminergic tone on ovarian steroid-secreting cells.

We believe that the results of this study and those reported in the companion paper (15) provide strong evidence for an involvement of the nervous system in the maintenance of PCOS. They do not, however, demonstrate that the primary defect that initiates the syndrome resides within the nervous system, a possibility that requires careful consideration.

Acknowledgments

We thank Ms. Janie Gliessman and Diane Hill for editorial assistance, and Mr. Gabriel Aravena for animal care.

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