

# Endocrine and Metabolic Responses to Long-Term Monotherapy with the Antiepileptic Drug Valproate in the Normally Cycling Rhesus Monkey

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An association between epilepsy and reproductive disturbances with an apparent increase in a polycystic ovarian syndrome (PCOS) has been reported. Whether this association can be attributed to epilepsy itself or is related to antiepileptic drug therapy, in particular valproate (VPA), remains controversial. We studied effects of a long-term VPA treatment on cycling monkeys, postulating that, if VPA monotherapy were to promote abnormal endocrine and metabolic parameters that are characteristic of PCOS, changes in cyclicality would be readily demonstrated.

After a 2-month control, a 12- to 15-month VPA monotherapy was initiated in 7 regularly cycling rhesus monkeys. Overall mean levels of VPA were  $88.7 \pm 4.0$  (SE)  $\mu\text{g/ml}$ . Mean body weight increased progressively during VPA treatment from  $8.5 \pm 0.5$  kg before treatment to  $9.6 \pm 0.7$  kg in the last week of treatment ( $P < 0.05$ ). Monkeys continued to have regular ovulatory menstrual cycles throughout VPA monotherapy. Length of the cycles was  $28 \pm 0.58$  d in control and

$28.4 \pm 1.18$  d in the last 3 months of VPA treatment. Follicular and luteal lengths and peak preovulatory estradiol and integrated luteal progesterone levels did not differ between control and treatment. Ovaries from VPA-treated monkeys showed histological evidence of ovulation, and none had characteristic features of PCOS. Endocrine PCOS markers, such as increased early follicular LH/FSH ratio and androgen levels were not different in control and VPA treatment cycles. LH and 17-hydroxyprogesterone responses to GnRH agonist challenges and the insulin response to glucose tolerance tests were similar in control and VPA groups. Lipid profiles were not affected by VPA treatment. The data indicate that a 12- to 15-month therapeutic exposure to VPA does not induce cyclic hormonal or morphological ovarian abnormalities or characteristics of the PCOS when administered to nonepileptic normally cycling nonhuman primates. (*J Clin Endocrinol Metab* 88: 2908–2915, 2003)

**M**ENSTRUAL AND REPRODUCTIVE disturbances have been reported in women with epilepsy (1–4) and bipolar disease (5), with an apparent increased finding of polycystic appearing ovaries and to a lesser degree of a polycystic ovarian-like syndrome (PCOS; Refs. 1 and 6). One group of investigators has suggested that these cyclic abnormalities are related to a specific antiepileptic drug, valproate (VPA), rather than to the brain disorder itself (7, 8). Some authors have suggested that VPA therapy in women with epilepsy induces hyperandrogenism (8–10) and a metabolic syndrome with centripetal obesity, hyperinsulinemia, lipid abnormalities, and polycystic appearing ovaries, and that, therefore, VPA treatment in young women is relatively contraindicated (11). Other investigators have concluded that the prevalence of PCOS is increased in epilepsy independently of antiepileptic drug treatment, or they contest the association between epilepsy and PCOS (1, 12, 13). The persisting controversy may reflect the difficulty in separating menstrual and metabolic disturbances in women with epilepsy from effects of the therapy itself in small clinical series. It may also mirror the observation that the finding of polycystic ovaries in young women is not unusual, encompassing

as much as 20% of the normal population in some studies (14).

VPA is a branched fatty acid that has a broad spectrum of antiepileptic efficacy in the prevention of both partial and generalized seizures (15). Because this drug is an FDA-approved treatment for epilepsy, migraine, and bipolar disease, it is important to determine its potential for inducing reproductive disturbances. For this reason, we have studied the effect of a 12- to 15-month VPA treatment in normally cycling nonhuman primates. We have postulated that if VPA monotherapy were to promote abnormal endocrine and metabolic parameters characteristic of PCOS in these normally cycling rhesus monkeys, changes in cyclic patterns could be readily demonstrated. Alternatively, maintenance of normal reproductive parameters despite this long-term therapy would substantiate the observation that VPA itself does not affect reproductive endocrinology in the normal individual.

## Materials and Methods

### Animals

The experimental protocol was approved by the Animal Care and Use Committee of Columbia University and was performed in accordance with the NIH guide for the care and use of laboratory animals. Ten adult female rhesus monkeys (*Macaca mulatta*), 11–16 yr of age, were selected for this experiment. The animals were housed in individual cages in temperature- and light-controlled (lights on from 0730–1930 h) rooms. They were fed Purina monkey chow (Ralston-Purina, St. Louis, MO)

Abbreviations: CV, Coefficient(s) of variation; DCL, degenerating corpora lutea; DHEAS, dehydroepiandrosterone sulfate; NS, not significant; PCOS, polycystic ovarian syndrome; RCL, recent corpora lutea; VPA, valproate.

twice a day and fresh fruit or vegetables. Water was available at all times. The experiments were conducted between September 2000 and February 2002.

In a preliminary step, we investigated whether each animal had normal menstrual cycles. Blood samples were obtained daily by venipuncture (a process to which the animals had previously been habituated) for estradiol and progesterone measurements over a period of two menstrual cycles. Menstruation was determined by daily vaginal swabbing. Seven monkeys that showed two normal menstrual cycles, according to criteria established previously in our laboratory (see *Experimental protocol*), were then immediately included in the study. Before the study, these animals had a mean ( $\pm$ SE) body weight of  $8.5 \pm 0.5$  kg, excluding one animal (no. 7) who weighed 14.7 kg.

### Experimental protocol

The study was designed to investigate potential effects of long-term VPA treatment on cyclic ovarian function. The content of four sprinkle capsules (Depakote Sprinkle capsules, Abbott Laboratories, North Chicago, IL; 125 mg/capsule), mixed with a variety of jams, jellies, and syrups (changed weekly) and coated on various cookies or fruits, was given orally twice a day at 0900 and 1600 h; each animal was closely observed for ingestion. The individual doses as well as total daily dosing of VPA in this monkey study exceeded on a milligram per kilogram basis the maximum daily dose recommended in the human (60 mg/kg). However, because the conversion factor for equieffective individual doses of VPA in the monkey compared with the human has been reported to be three (16), the daily human therapeutic dose (20 mg/kg) was multiplied by a factor of three for an equieffective monkey dose of 60 mg/kg. This dose was administered twice daily for a total dose of 120 mg/kg·d.

VPA blood levels were measured biweekly throughout the experiment at 1100 h, 2 h after morning drug administration. If VPA blood levels were less than 60  $\mu$ g/ml or if the animal failed to ingest the mixture completely, the mix into which the drug was administered was immediately changed to make it more palatable to that individual animal. The overall course of VPA treatment extended from 12.7–15.7 months.

To determine menstrual cyclicity, menstruation was monitored, and blood samples were obtained daily for the duration of the treatment. Hematocrits were verified at frequent intervals; no animals required supplemental iron therapy. Midcycle peak estradiol levels and daily levels of progesterone during the entire luteal phase were measured in each control and treatment cycle. Follicular and luteal phase lengths were recorded, and the integrated daily progesterone luteal concentrations were calculated. The rise of progesterone above 0.4 ng/ml (coupled to the preceding decrease in estradiol) was used to mark the beginning of the luteal phase. To determine whether endpoints relevant to the development of PCOS were influenced by the experimental therapy, LH/FSH ratios were determined at monthly intervals on d 1 of each control and treatment cycle. Ovarian androgens (androstenedione, total testosterone) were measured in each cycle at their cyclic peak, which paralleled the time of the estradiol peak, whereas the adrenal androgen dehydroepiandrosterone sulfate (DHEAS) was measured on d 2 of the cycle.

At the end of VPA treatment, two tests were performed. For the GnRH agonist stimulation test, Lupron (Abbott Laboratories, 1 mg) was injected sc on d 1–4 of the cycle. Blood samples were obtained at 30-min intervals for the first 4 h, then at 6 h and 22 h after injection. The LH and 17-hydroxyprogesterone responses were measured. The glucose tolerance test was initiated after a 16-h fasting period. Dextrose (Abbott Laboratories; 0.1 g/kg) was given iv on d 2–5 of the follicular phase, and

blood samples were taken at +5, 10, 20, and 30 min for glucose and insulin level measurements. For comparison, seven normally cycling monkeys ( $9.5 \pm 0.5$  kg) from our colony, tested at the same time of the cycle, served as controls. The lipid profile of each animal was compared in the control period and in the last month of treatment.

After completion of treatment, all animals were ovariectomized. Both ovaries were placed in saline, examined grossly to identify corpora lutea and cystic changes, and photographed. Each ovary was then bisected. Half of the ovary was fixed in a 10% formaldehyde solution. The tissue was then dehydrated in alcohol, embedded in paraffin, sectioned at 5  $\mu$ m, and mounted serially. The histological sections were stained with hematoxylin-eosin and examined under the microscope. The other half of the ovary was frozen and stored at  $-80$  C for potential future analysis.

### Assays and statistical analysis

Serum estradiol, progesterone, testosterone, insulin, and VPA were measured by commercial chemiluminescent immunoassays using the Immulite system (Diagnostic Products Corp., Los Angeles, CA). Intra- and interassay coefficients of variation (CV) were 9.3% and 10.5% for estradiol, 6.6% and 7.9% for progesterone, 7.4% and 9.8% for testosterone, 4.7% and 8.2% for insulin, and 3.8% and 9.5% for VPA, respectively. LH and FSH levels were measured by in-house recombinant homologous RIA (17, 18). Intra- and interassay CV were 7.9% and 13.1% for LH and 5.0% and 6.1% for FSH, respectively. Androstenedione and 17-hydroxyprogesterone were measured by ELISA (Diagnostic Systems Laboratories, Inc., Webster, TX). Intra- and interassay CV were 2.4% and 8.0% for androstenedione and 2.1% and 8.8% for 17-hydroxyprogesterone, respectively. DHEAS was measured by a commercial RIA (Coat-A-Count, Diagnostic Products Corp.), with an intra- and interassay CV of 5.9% and 8.2%, respectively. Glucose levels were measured by a blood glucose testing system (Precision G, Abbott Diagnostic Laboratories, Bedford, MA). Enzymatic tests (Roche Diagnostics, Indianapolis, IN) were used to measure serum total cholesterol, high-density lipoprotein cholesterol after precipitation, low-density lipoprotein cholesterol and triglycerides, the latter after lipase hydrolysis.

Cycle parameters, such as the length of the follicular and luteal phases, hormone concentrations, as well as luteal function as reflected by integrated progesterone values in the luteal phase, were compared in the control, first and last trimester of treatment. For luteal progesterone evaluation, the areas under the luteal phase progesterone curves (from the day of LH surge +1 to the day of menstruation -1) were calculated by trapezoidal analysis. Insulin sensitivity after long-term VPA treatment was evaluated using fasting glucose and insulin levels by the quantitative insulin sensitivity check index method (19) as follows:  $1/[\log(\text{insulin } \mu\text{U/ml}) + \log(\text{glucose mg/dl})]$ .

Comparisons were made by multiple ANOVA followed by the Tukey test. Paired Student's *t* tests were used for the comparison of body weights before and after VPA treatment. The level of significance was established at *P* less than 0.05.

## Results

Overall mean levels of VPA were  $88.7 \pm 4.0$   $\mu$ g/ml, as measured 2 h after morning drug administration, with the mean ( $\pm$ SE) for the seven individual monkeys as follows: 97.6 ( $\pm$ 5.1), 97.7 ( $\pm$ 6.7), 102.2 ( $\pm$ 4.4), 75.4 ( $\pm$ 3.1), 87.8 ( $\pm$ 5.7), 83.5 ( $\pm$ 3.7), and 77.8 ( $\pm$ 6.0)  $\mu$ g/ml. [According to the manufacturer's description of the drug, as outlined in the Physicians Desk Reference, the therapeutic range in epilepsy is com-

**TABLE 1.** Cyclic parameters in VPA-treated monkeys (mean  $\pm$  SE)

	Mean of 2 control cycles	Mean of first 3 treatment cycles	Mean of last 3 treatment cycles	Mean of overall treatment cycles
Follicular length (d)	12.4 $\pm$ 0.59	12.8 $\pm$ 0.46	12.2 $\pm$ 0.46	12.8 $\pm$ 0.28
Estradiol peak (pg/ml)	217.1 $\pm$ 22.3	241.8 $\pm$ 22.89	161.8 $\pm$ 16.7	246.9 $\pm$ 7.25
Luteal length (d)	15.3 $\pm$ 0.24	15.4 $\pm$ 0.20	14.8 $\pm$ 0.70	15.5 $\pm$ 0.13
Integrated progesterone (ng/ml)	46.2 $\pm$ 0.88	46.4 $\pm$ 1.63	46.7 $\pm$ 2.51	48.5 $\pm$ 4.26
LH/FSH ratio in early follicular phase	0.59 $\pm$ 0.05	0.58 $\pm$ 0.03	0.66 $\pm$ 0.04	0.64 $\pm$ 0.03

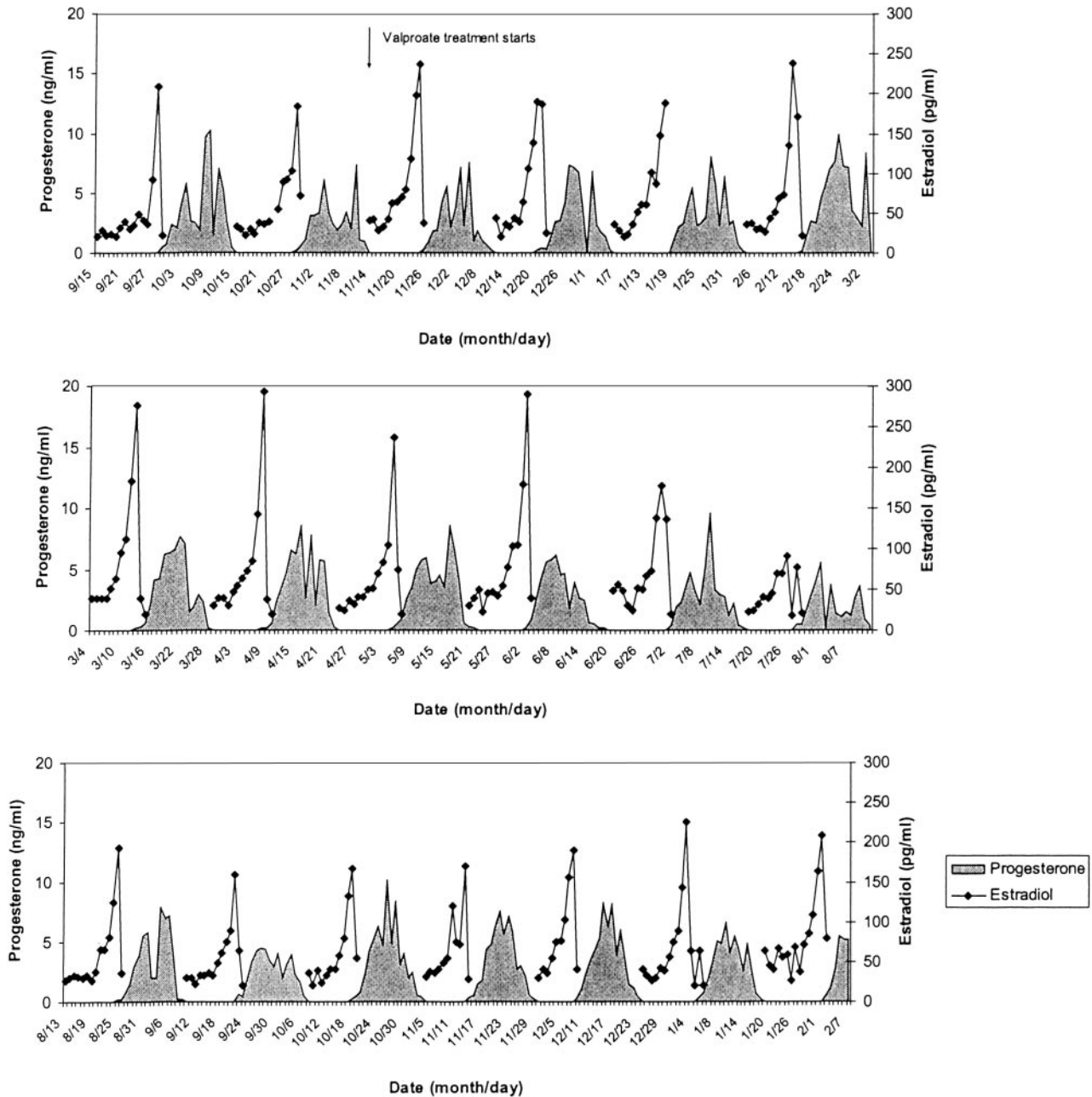


FIG. 1. Effects of VPA treatment on the menstrual cycle, as illustrated by daily estradiol levels in the follicular phase (*lines*) and by daily progesterone levels in the luteal phase (*shaded areas*) in an individual monkey (no. 89N111). Two control cycles were first documented, starting on 9/15/00. VPA treatment was started at the *arrow* and continued until 2/7/02. Data are continuous from levels 1–3. The data indicate that long-term VPA therapy does not interfere with the normal menstrual cycle.

monly considered to be 50–100  $\mu\text{g}/\text{ml}$ ]. Because peak VPA concentrations are reported to occur at 1.2 h after oral administration of 100 mg/kg in the monkey (20), our peak levels were most likely higher.

Mean ( $\pm\text{SE}$ ) body weight in six of the seven animals increased progressively during treatment with VPA from  $8.5 \pm 0.5$  kg before treatment began to  $8.5 \pm 1.0$  after 3 months of treatment,  $9.2 \pm 0.6$  after 9 months, and  $9.6 \pm 0.7$  kg in the last week of treatment ( $P < 0.05$  vs. pretreatment). Body weight in monkey 7 was 14.2 kg in the last week of treatment vs. 14.7 kg before treatment.

#### Menstrual cyclicity

All monkeys, except one, continued to have regular ovulatory menstrual cycles throughout VPA monotherapy. One exception to that pattern was monkey 4JU who, after three regular menstrual cycles during the first 3 months of treatment, had a 102-d amenorrheic period that terminated in a normal ovulatory cycle. After this period, she resumed normal-length cycles. Overall mean ( $\pm\text{SE}$ ) length of menstrual cycles was  $28 \pm 0.58$  d in control cycles,  $28.1 \pm 0.54$  d in the first 3 months of treatment, and  $28.4 \pm 1.18$  d in the last 3

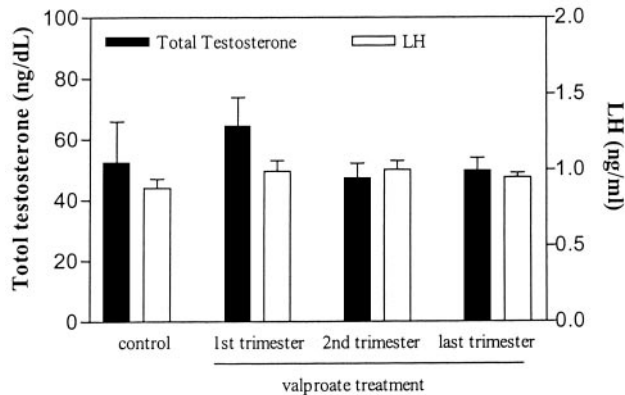


FIG. 2. Mean ( $\pm$ SE) total testosterone and LH levels before treatment and during the first to last trimesters of VPA treatment. There were no significant differences between pretreatment controls and treated groups.

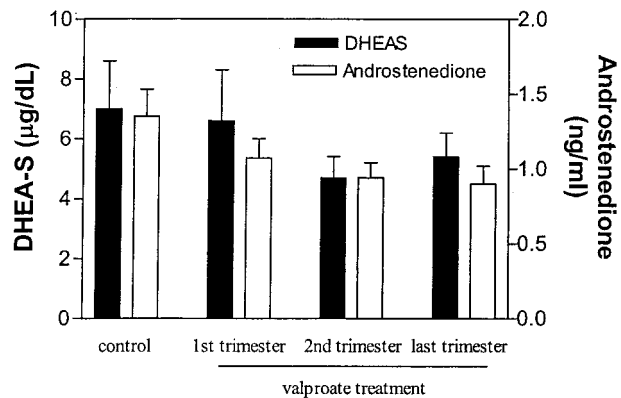


FIG. 3. Mean ( $\pm$ SE) DHEAS and androstenedione levels before treatment and during the first to last trimesters of VPA treatment. No significant differences were observed between pretreatment controls and treated groups.

months of treatment. Mean follicular and luteal length did not differ between the control period and the first and last trimesters of the treatment (Table 1). Similarly, peak pre-ovulatory estradiol levels and integrated luteal progesterone were similar in all groups (Table 1). One monkey (no. 7) had a significant decrease in integrated luteal progesterone levels during the months of May to September only ( $33.3 \pm 1.5$  vs.  $48.3 \pm 2.3$  ng/ml), a phenomenon that occurs spontaneously in about 10–15% of the animals in our colony during the summer months. Early follicular phase LH/FSH ratios were similar in control and treatment cycles (Table 1). Figure 1 illustrates daily follicular estradiol and luteal progesterone levels in successive control (September and October 2000) and treatment cycles (November 2000 to February 2002) in an individual monkey.

#### Baseline and stimulated hormone levels

Figure 2 compares mean LH levels on d 2 of the cycle and mean testosterone levels measured on the day of the pre-ovulatory estradiol peak in control cycles and in the first, second, and last trimesters of VPA treatment. No significant difference was observed. Figure 3 compares mean DHEAS and androstenedione levels before and during VPA treat-

ment. Although there was a tendency for the levels to decrease with treatment, the decrease was not significant. Figure 4 compares the mean LH (*top left panel*) and 17-hydroxyprogesterone (*top right panel*) responses to a GnRH agonist challenge test in control and VPA-treated monkeys. Although LH was significantly increased 22 h after the agonist administration, there was no difference between the control and VPA-treated groups. No significant differences were observed in regard to 17-hydroxyprogesterone. Figure 4 (*bottom*) illustrates glucose levels (*left panel*) and the insulin response (*right panel*) to a glucose tolerance test. No significant differences were observed between VPA-treated and control groups. Quantitative insulin sensitivity check index values were  $0.3057 \pm 0.0037$  and  $0.3050 \pm 0.004$  in control and VPA-treated animals, respectively, and were not significantly different. Mean ( $\pm$  SE) total cholesterol was  $204.9 \pm 9.4$  and  $188.4 \pm 10.3$  mg/dl before and at the end of VPA treatment [not significant (NS)], respectively; high-density lipoprotein cholesterol was  $88.0 \pm 6.3$  and  $88.2 \pm 4.3$  mg/dl (NS); low-density lipoprotein cholesterol was  $100.6 \pm 6.2$  and  $88.1 \pm 9.5$  mg/dl (NS); and triglycerides were  $81.0 \pm 22.6$  and  $93.3 \pm 16.5$  ng/dl (NS).

#### Ovarian histology

All 14 ovaries from the 7 VPA-treated monkeys were reviewed on gross and microscopic examinations. Ovaries measured from  $1.0 \times 0.7 \times 0.5$  cm to  $2.2 \times 0.8 \times 0.7$  cm. The characteristic histological features of PCOS in humans (multiple superficial cortical cysts and a region of subcortical stromal fibrosis resulting in the appearance of a white capsule) were not identified in any of the ovaries (Fig. 5). In addition, in humans with PCOS, the central portion of the ovarian cortex usually consists of a homogenous stroma that lacks stigmata of ovulation, such as corpora lutea or corpora albicantia. All 14 ovaries from the VPA-treated monkeys showed histological evidence of ovulation. Table 2 illustrates histological landmarks in each ovary as well as the hormonal background at that time. Recent corpora lutea (RCL) were identified in five of seven monkeys, whereas degenerating corpora lutea (DCL) and/or corpora albicantia were seen in all ovaries. Numerous primordial follicles were present in all ovaries. Developing follicles, including preantral and antral follicles, were identified in all ovaries. Figure 6 shows a section of the whole ovary in monkey 53-124 treated with VPA for 477 d. Note the recently matured corpus luteum and developing follicles at different stages present in the background. No hyperthecosis was observed.

#### Discussion

PCOS is a multisymptomatic disease that includes ovulatory dysfunction, hyperandrogenemia, insulin resistance, and the presence of polycystic ovaries (21, 22). Several studies have described an association between epilepsy and a PCOS in women under treatment with various antiepileptic medications, although no study has been specifically designed to diagnose the syndrome (1–3). One possibility explaining this association is that the seizure disorder itself increases the risk of PCOS (4, 12). This hypothesis is supported by observations of an increased incidence of poly-

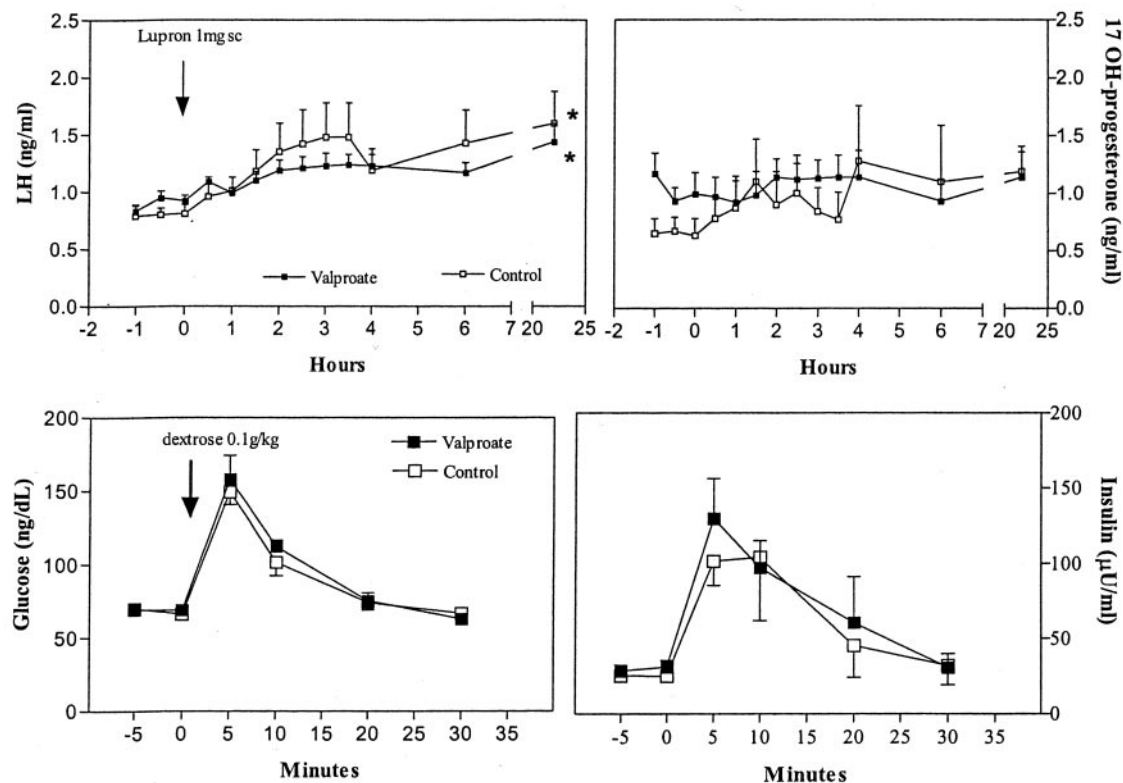


FIG. 4. *Top*, GnRH challenge test: mean ( $\pm$ SE) LH (*left panel*) and 17-hydroxyprogesterone (*right panel*) responses to sc administration of the GnRH antagonist Lupron (1 mg) on d 2–4 of the cycle in control (*open squares*) and in VPA-treated monkeys (*closed squares*). \*,  $P < 0.05$  vs. baseline. There was no significant difference in the overall response between the control and VPA-treated groups. *Bottom*, Glucose tolerance test: mean ( $\pm$ SE) glucose (*left panel*) and insulin (*right panel*) responses to a glucose tolerance test in control (*open squares*) and VPA-treated (*closed squares*) monkeys. After a 16-h fasting, dextrose (0.1 g/kg) was given iv on d 2–5 of the cycle. Responses in the control and VPA-treated groups were not significantly different.

cystic appearing ovaries in unmedicated epileptic women (1) or in treated women with epilepsy regardless of the anti-epileptic therapy (6). Another possibility is that the anti-epileptic treatment induces PCOS, and that this is most likely to arise with VPA. One hypothesis is that certain brain disorders, such as epilepsy and bipolar disease, are associated with disruption of the hypothalamic pituitary axis and with menstrual disturbance and anovulatory cycles, whereas VPA causes hyperandrogenism and carbohydrate intolerance (4, 10, 23). Women with specific brain disorders on VPA may present with a syndrome very closely resembling PCOS (8, 11). Other groups argue that there is no association between VPA and any reproductive or metabolic health disorder (1, 12, 24), and it has been suggested that other possible causative factors may be implicated in the genesis of PCOS (13). The purpose of our study was to investigate prospectively on a day-to-day basis whether any of the symptoms associated with PCOS can be elicited in normal monkeys by a prolonged VPA treatment.

Disorders of the menstrual cycle, including menstrual cycle irregularities, ovulatory dysfunction, and amenorrhea, are the most common finding associated with PCOS (21). These have also been described in women with epilepsy, with the highest frequency of anovulatory cycles in women with primary (idiopathic) generalized epilepsy receiving VPA. Hyperandrogenemia has also been consistently de-

scribed in women with epilepsy receiving VPA for epilepsy as well as bipolar disease (5, 8, 9, 11, 25). However, our data clearly demonstrate that prolonged VPA treatment in normal monkeys with regular menstrual cycles neither induces cyclic irregularities nor results in anovulation or amenorrhea. Regular cycles and appropriate hormone levels were monitored on a daily basis over the entire control and treatment periods, which lasted from 12.7–15.7 months. The estradiol profiles characteristic of normal follicular development, as well as progesterone secretion representative of the normal corpus luteum, remained cyclic and within normal limits.

PCOS is also characterized by major hormonal dysfunction. In a majority of patients, PCOS is accompanied by an increase in GnRH pulse frequency (26, 27), and, because the increased GnRH pulse generator frequency enhances the synthesis of LH over that of FSH (28), PCOS women preferentially secrete LH over FSH and show a typical increased LH/FSH ratio (29). Although experiments have shown that an acceleration of GnRH pulse frequency readily increases the LH/FSH ratio in the monkey (30), no significant increase in this ratio was observed in our animals treated with VPA. Furthermore, the increase in LH after GnRH agonist stimulation was similar in the control and VPA-treated groups, in contrast to the response in women with PCOS where it is enhanced (31). A prime characteristic of PCOS is hyperandrogenism (22). Measurements of testosterone were per-

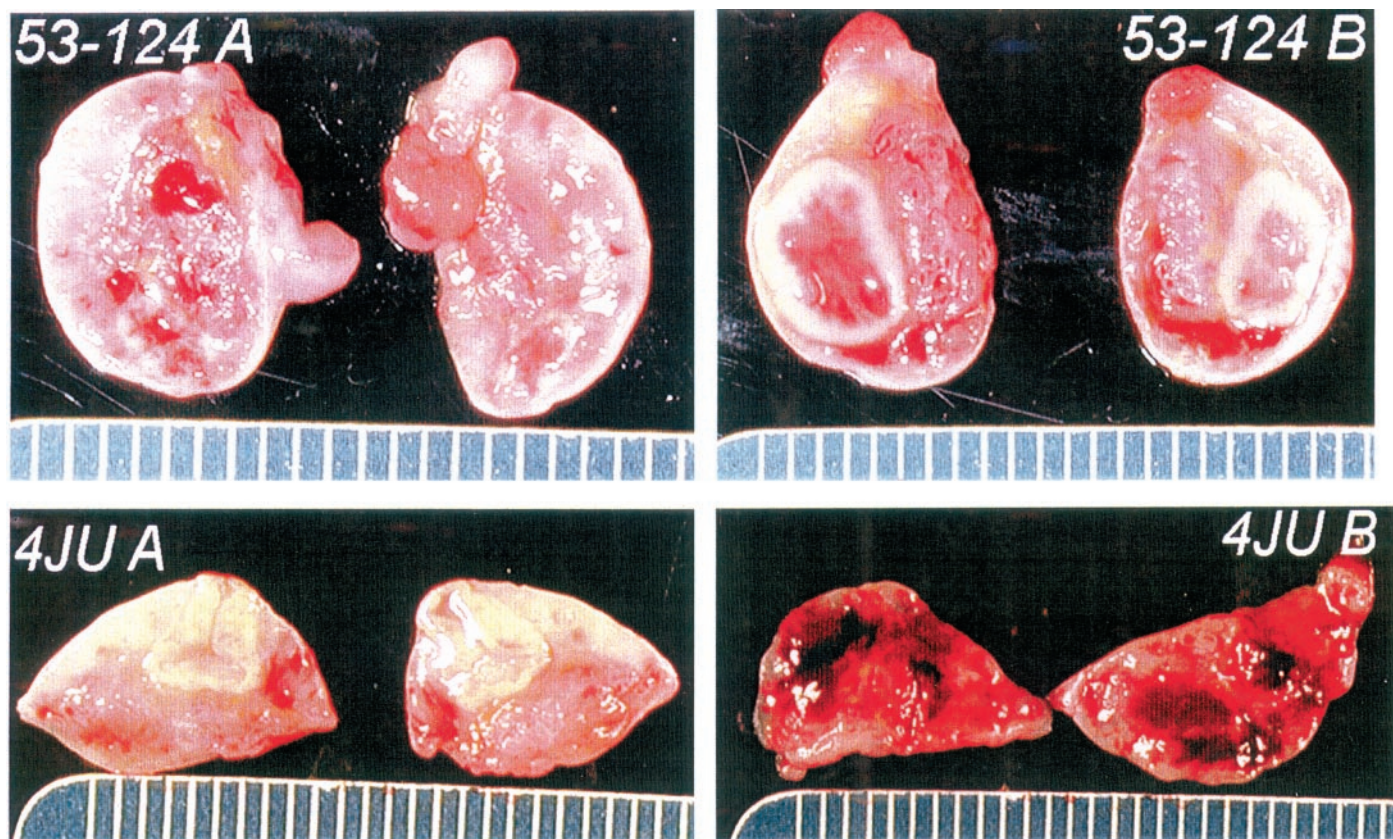


FIG. 5. Gross view of two pairs of ovaries obtained from monkeys that had been treated for 477 and 438 d with VPA. One pair (no. 53-124) shows a RCL and the other a DCL (no. 4JU). Both pairs lack a dense fibrous capsule and multiple subcortical ovarian cysts (the typical features of PCOS).

TABLE 2. Cyclic hormonal status of each ovary at the end of VPA treatment

Monkey	Cycle day	E <sub>2</sub> (pg/ml)	Prog (ng/ml)	Days of treatment	Corpus luteum dating	Follicle dating
88N182	21	76	1.7	436	RCL (POD 10–12)	–
					–	–
4JU	7	49	<0.2	438	DCL	DF
					DCL	–
626	17	37	0.7	386	DCL	–
					RCL (POD 2–3)	–
7	12	75	<0.2	480	DCL	DF
					DCL	–
89N111	20	<20	5.2	421	RCL (POD 10–12)	–
					–	–
89D361	22	<20	0.5	416	RCL (POD 13–14)	–
					–	–
53-124	20	<20	2.5	477	RCL (POD 10–12)	–
					–	–

E<sub>2</sub>, Estradiol; Prog, progesterone; POD, post ovulation day; DF, dominant follicle.

formed at the time of the preovulatory estradiol peak in each control and treatment cycle, but there were no significant changes between pretreatment and early or late treatment samples. Functional ovarian hyperandrogenism in PCOS women is also characterized by 17-hydroxyprogesterone and androstenedione hyperresponsiveness to GnRH stimulation (32, 33). However, the response in the VPA-treated animals was not different from that in controls. Although not significant, there was a tendency for DHEAS, an androgen produced by the adrenals, to decrease over time during treat-

ment. A similar tendency was seen for androstenedione. Previous reports have indicated a significant decrease in DHEAS within 3 months of VPA treatment in female but, not in male, epileptic patients (34, 35).

Ovarian morphology in PCOS is characterized by cortical thickening, subcapsular follicular cysts, hyperplastic theca interna, and stromal hyperplasia (21). In our animals, the ovaries were removed at the end of the 12.7- to 15.7-month VPA therapy, and no such changes were observed in any of the 14 ovaries that were removed. In fact, all ovaries dem-

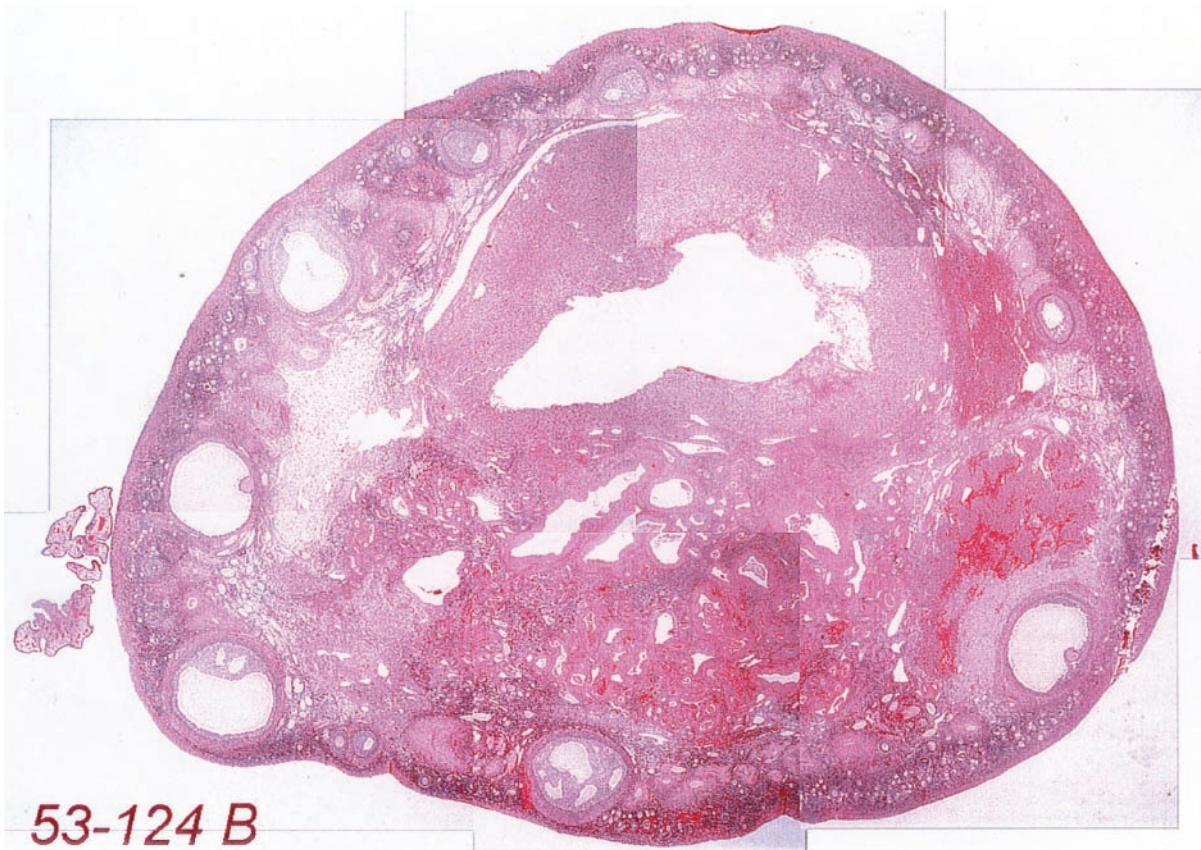


FIG. 6. A whole-mount section of an ovary from monkey 53-124. This ovary was obtained during the luteal phase and contains a recent (mature) corpus luteum and developing follicles at different stages. No subcortical fibrosis or superficial cortical cysts are seen.

onstrated normal cyclic histological features, including antral and mature follicles and active corpora lutea, in accord with the stage of the cycle when they were removed (36). This is notwithstanding previous observations that polycystic ovarian characteristics can readily be induced in the rhesus monkey experimentally, for instance after prenatal androgen treatment (37) or after active immunization to estradiol (38). In contrast to the nonhuman primate, a 3-month VPA treatment in the rat was reported to result in a dose-dependent increase in the number of ovarian follicular cysts and reduction in the number of corpora lutea (39). However, the absence of concomitant increases in LH, androgens, and insulin in these animals suggests a different mechanism of action than that responsible for PCOS and a possible direct effect of VPA at higher doses on the ovary in this species (7, 40). Such a direct effect on the ovary was not observed in our animals.

A side effect of VPA therapy observed in six of our seven animals was a significant increase in body weight, in the presence of normal glucose tolerance tests. This increase became significant after 9 months of VPA therapy. This change in body weight may not be surprising because the use of VPA has been previously reported to induce weight gain (41, 42). The mechanisms leading to weight gain during VPA medication are, however, still unknown. In women with epilepsy, this VPA-related weight gain has been associated with hyperinsulinemia (11).

Although the metabolic fate of VPA is similar in monkeys compared with humans (43), there are several pharmacoki-

netic differences that need to be taken into account in this study. VPA is eliminated more rapidly in the monkey, by a 10-fold higher metabolic clearance rate (19, 44). However, monkeys have less drug-binding activity (less protein bound) and higher free (active) drug levels compared with the human at similar total plasma concentrations (10–15% in humans *vs.* 46% in monkeys; Ref. 20). Peak plasma levels were repeatedly measured at 2 h post dose and determined to reach the therapeutic range. However, additional time points throughout the day were not examined. A pharmacokinetic simulation suggested that levels were not likely maintained within the therapeutic range throughout the 24-h period. However, due to the use of equieffective dosing (16) and decreased drug-binding capacity, it is likely that daily exposure to free VPA concentrations in our monkeys was comparable to human daily exposure from therapeutic doses.

In conclusion, our data suggest that, although effects on body weight were observed, a long period of therapeutic exposure to VPA did not induce cyclic hormonal or morphological abnormalities or hormonal characteristics of the PCOS when administered to nonepileptic normally cycling nonhuman primates. These results do not support the hypothesis that VPA treatment *per se*, at least of this duration, is entirely responsible for the induction of PCOS in some women with epilepsy and bipolar disease. This conclusion is evidently limited by the 12- to 15-month length of treatment with VPA, compared with chronic treatment in humans with epilepsy, and the fact that although VPA levels reached the therapeutic range, these levels were not

likely maintained within the therapeutic range throughout each 24-h period. Overall, the data suggest that VPA treatment by itself may not be associated with the development of PCOS, but rather the increased incidence of this syndrome in women with epilepsy and bipolar disease may represent either a result of the disorder or an interaction between a brain disorder and VPA, which predisposes susceptible individuals to this syndrome.

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