Enhanced Anticonvulsant Activity of Ganaxolone after Neurosteroid Withdrawal in a Rat Model of Catamenial Epilepsy

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ABSTRACT
Perimenstrual catamenial epilepsy, the exacerbation of seizures in association with menstruation, may in part be due to withdrawal of the progesterone metabolite allopregnanolone (3α-hydroxy-5α-pregn-20-one), an endogenous anticonvulsant neurosteroid that is a positive allosteric modulator of γ-aminobutyric acidA receptors. Neurosteroid replacement is a potential approach to therapy, but natural neurosteroids have poor bioavailability and may be converted to metabolites with undesired progestational activity. The synthetic neuroactive steroid ganaxolone (3α-hydroxy-3β-methyl-5α-pregnane-20-one) is an orally active analog of allopregnanolone that is not converted to the hormonally active 3-keto form. To assess the potential of ganaxolone in the treatment of catamenial seizure exacerbations, a state of persistently high serum progesterone (pseudopregnancy) was induced in 26-day-old female rats with gonadotropins, and neurosteroids were withdrawn on postnatal day 39 with finasteride, a 5α-reductase inhibitor that blocks the conversion of progesterone to allopregnanolone. Finasteride treatment during pseudopregnancy results in a reduction in the threshold for pentylenetetrazol seizures. During this state of enhanced seizure susceptibility, there was a 3-fold increase in the anticonvulsant potency of ganaxolone (control ED50 = 3.5 mg/kg; withdrawn = 1.2 mg/kg) without a change in the potency for induction of motor toxicity in the rotarod test. The plasma concentrations of ganaxolone did not differ significantly in control and withdrawn animals; the estimated plasma concentrations of ganaxolone producing 50% seizure protection were ~500 and ~225 ng/ml in control and withdrawn rats, respectively. Unlike neurosteroid, neurosteroid withdrawal was associated with a decrease in the anticonvulsant potency of diazepam (control ED50 = 1.9 mg/kg; withdrawn = 4.1 mg/kg) and valproate (control ED50 = 279 mg/kg; withdrawn = 460 mg/kg). The enhanced anticonvulsant potency of ganaxolone after neurosteroid withdrawal supports the use of ganaxolone as a specific treatment for perimenstrual catamenial epilepsy.

Catamenial epilepsy, the recurrent exacerbation of seizures at specific times during the menstrual cycle, affects from 10 to 72% of women with epilepsy (Ansell and Clarke, 1956; Laidlaw, 1956; Rosciszewska, 1986; Tauboll et al., 1991; Duncan et al., 1993). In many women with catamenial epilepsy, catamenial seizure clustering occurs just before or during menstruation, in association with a fall in serum progesterone levels (Newmark and Penry, 1980; Herzog et al., 1994). Progesterone has anticonvulsant properties in large part due to its conversion to the neuroactive steroid allopregnanolone, a potent positive modulator of γ-aminobutyric acidA receptors (Laidlaw, 1956; Kokate et al., 1994, 1999). Thus, perimenstrual seizure exacerbations in women with catamenial epilepsy could be related to neurosteroid withdrawal. Although natural progesterone therapy benefits some women with catamenial epilepsy (Herzog, 1986, 1995), it may be associated with undesired hormonal side effects. GABA A receptor-modulating neurosteroids, which are devoid of such hormonal actions, may provide a rational alternative approach to therapy (Reddy and Kulkarni, 2000). However, certain obstacles prevent the clinical use of endogenously occurring neurosteroids. Importantly, natural neurosteroids such as allopregnanolone have low bioavailability because they are rapidly inactivated and eliminated by glucuronide or sulfate conjugation at the 3α-hydroxy group. In addition, the 3α-hydroxy group of allopregnanolone may undergo oxidation to the ketone, restoring activity at steroid hormone receptors (Rupprecht et al., 1993). Ganaxolone (CCD 1042; 3α-hydroxy-3β-methyl-5α-pregnane-20-one), the synthetic 3β-methyl analog of allopregnanolone, overcomes these limitations (Carter et al., 1997). Like allopregnanolone, ganaxolone is a positive allosteric modulator of GABA A receptors and is an effective anticonvulsant in the pentylenetetrazol (PTZ) seizure test as

ABBREVIATIONS: GABA, γ-aminobutyric acid; PTZ, pentylenetetrazol; CL, confidence limits.
well as in other anticonvulsant screening models (Carter et al., 1997; Gasior et al., 1997). However, ganaxolone is orally active, and adequate blood levels can be maintained in human subjects with two or three times daily dosing (Montghan et al., 1997). In addition, although ganaxolone is extensively metabolized, the potentially hormonally active 3-keto derivative is not formed.

To evaluate the potential of ganaxolone in the treatment of perimenstrual seizure exacerbations, we developed a rat model of catamenial epilepsy in which female pseudopregnant rats were abruptly withdrawn from neurosteroids to simulate the drop occurring in women before the menses. Pseudopregnancy, a state in which progesterone and allopregnanolone are chronically elevated, was produced by gonadotropin treatment. Neurosteroid withdrawal was induced by the administration of finasteride, a 5α-reductase inhibitor that blocks the conversion of progesterone to allopregnanolone (Azzolina et al., 1997). After neurosteroid withdrawal, animals exhibit a marked enhancement in seizure susceptibility when challenged with PTZ. Unexpectedly, we found that the anticonvulsant potency of ganaxolone was enhanced in the period after neurosteroid withdrawal, whereas the potencies of two reference anticonvulsants diazepam and valproate were reduced.

Materials and Methods

Animals. Female 26-day-old (70–80 g) and 40- to 45-day-old (200–250 g) Sprague-Dawley rats (Taconic) were housed in groups of four under a 12-h light/dark cycle in an environmentally controlled animal facility. Animals were allowed to acclimate with free access to food and water for a 24-h period before use. All procedures were performed in strict compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals under a protocol approved by the National Institutes of Health Animal Use Committee.

Pseudopregnancy Model of Catamenial Epilepsy. Rats were injected with pregnant mare serum gonadotropin (20 IU/rat.s.c.) at 10:00 AM on postnatal day 27 followed 48 h later by human chorionic gonadotropin (10 IU/rat.s.c.). The day of human chorionic gonadotropin treatment (day 29) was considered day 0 of pseudopregnancy. At 11:00 AM on day 11 of pseudopregnancy, neurosteroid withdrawal was induced with finasteride (100 mg/kg in 50% 5α-cyclodextrin,i.p.), which blocks the conversion of progesterone to allopregnanolone via inhibition of 5α-reductase isoenzymes (Kokate et al., 1999). In some experiments, animals were injected with vehicle alone. At 24 h after finasteride treatment, plasma allopregnanolone levels were reduced from 44.5 to 6.4 ng/ml; there was no effect on serum progesterone levels (D.S. Reddy, H.-Y. Kim, and M.A. Rogawski, unpublished observations).

PTZ Seizure Test. Protective activity against PTZ-induced clonic seizures was evaluated according to the procedure described by White et al. (1995). Testing was carried out on day 12 of pseudopregnancy (24 h after vehicle or finasteride administration) for pseudopregnant control or pseudopregnant withdrawn animals or in naive cycling (nonpseudopregnant) control animals. Rats were injected s.c. with ganaxolone or i.p. with diazepam and valproate and 15 min (ganaxolone) or 30 min (diazepam and valproate) later received an s.c. injection of PTZ at a dose of 90 mg/kg. The pretreatment times were based on the time to peak effect (White et al., 1995; see Carter et al., 1997). The 50% convulsant dose (CD_{50}) for PTZ is 60 mg/kg in naive (dietrous) female rats, 73 mg/kg in pseudopregnant animals, and 46 mg/kg in pseudopregnant withdrawn (D.S. Reddy, H.-Y. Kim, and M.A. Rogawski, unpublished observations). With the dose of PTZ used in this study, all animals in each of the three groups experienced seizures in the absence of anticonvulsant pretreatment. Animals were observed for a 30-min period after PTZ administration. Rats failing to show clonic spasms lasting longer than 5 s were scored as protected.

Rotarod Motor Toxicity Test. Ganaxolone and other test drugs were evaluated for motor toxicity in an accelerating rotarod test (initial speed 5 rpm, increasing 5 rpm/30 s; Jones and Roberts, 1968). Rats were acclimatized to the rotarod (Ugo Basile, Milan, Italy) 30 min before the start of the experiment. Rats that successfully remained on the rotarod for more than 2 min were selected for drug testing. After administration of the test drug, rats were given three successive opportunities to remain on the rotarod continuously for 2 min. An animal was considered to have motor toxicity if it fell from the rotarod more than twice in the 2-min period.

Ganaxolone Plasma Level Determinations. Animals were anesthetized with CO₂ gas, and –2 ml carotid blood was collected in heparinized tubes. The plasma was separated by centrifugation at 12,000g for 10 min and stored at −20°C in 10-ml glass tubes containing 7.5% EDTA solution (68 μl). The concentration of ganaxolone was analyzed by liquid chromatography-mass spectroscopy using a Hewlett-Packard liquid chromatograph (analytical column: Genesis C18; 4 μm, 3 × 30 mm, Jones Chromatography) and Micromass Quattro II mass spectrometer. Briefly, a 0.2-ml plasma sample was added to a tube containing evaporated internal standard (epiallopregnanolone). The steroid and internal standard were extracted with 4 ml of hexane. Each sample was analyzed using the atmospheric pressure chemical ionization technique under acidic conditions. A standard curve was plotted using pure ganaxolone in methanol mixed with 0.2 ml of blank rat plasma.

Drugs and Hormones. Pregnant mare serum gonadotropin and human chorionic gonadotropin were administered in sterile saline. Finasteride and ganaxolone were made fresh each day in aqueous 50% 2-hydroxypropyl-β-cyclodextrin (β-cyclodextrin; Research Biochemicals International, Natick, MA). By itself, β-cyclodextrin at concentrations as high as 50% failed to affect PTZ seizures. Diazepam and sodium valproate were dissolved in sterile isotonic saline. The diazepam solution contained a maximum of 20% propylene glycol and 5% ethyl alcohol. Drug solutions were administered s.c. or i.p. in a volume equaling 1% of the animal’s body weight. Ganaxolone was a gift of CoCensys (Irvine, CA). Epiallopregnanolone (3β-hydroxy-5α-pregnan-20-one) was obtained from Steraloids (Newport, RI). Diazepam injection was obtained from Elkins-Sinn (Cherry Hill, NJ). All other drugs and hormones were obtained from Sigma Chemical Co. (St. Louis, MO).

Data Analysis. To construct dose-effect curves, drugs were tested at several doses spanning the dose producing seizures in 50% of animals (CD_{50}), or resulting in 50% seizure protection (ED_{50}) or motor toxicity (TD_{50}). Each group consisted of six to eight rats. CD_{50}, ED_{50}, and TD_{50} values with 95% confidence limits (CL) were determined by log-probit analysis using the Litchfield and Wilcoxon procedure (PHARM/PCS Version 4.2; Microcomputer Specialists, Philadelphia, PA). Dose-response data were fit to the logistic function 100/[1 + (D_{0}/x^{n})^{H}] where x is the dose administered; D_{0} is either the CD_{50}, ED_{50}, or TD_{50}; and n_{H} is an empirical parameter describing the steepness of fit. When appropriate, the n_{H} values were determined simultaneously using ALLFIT 2.7 (abs.cit.nih.gov/dl/allfit/; DeLean et al., 1978). The significance of differences between the dose-response curves was determined using the Litchfield-Wilcoxon χ² test, where the criterion for statistical significance was P < .05. Protective index, a quantitative measure of the margin between doses producing anticonvulsant protection and motor toxicity, was calculated by dividing the TD_{50} value by the ED_{50} value. Statistical differences among mean plasma ganaxolone levels were analyzed by one-way ANOVA followed by Student’s t test.

Results

Lack of Effect of Finasteride on Seizure Susceptibility. To confirm that finasteride treatment as in the pseudo-
pregnancy model of catamenial epilepsy does not itself alter the convulsant activity of PTZ, dose-response relationships for PTZ (30–80 mg/kg s.c.) induction of clonic seizures were determined in naive control rats 24 h after i.p. injection of vehicle or 100 mg/kg finasteride. The PTZ CD50 values in the vehicle and finasteride-pretreated groups were 55 mg/kg (95% CL, 45–67; n = 45) and 57 mg/kg (95% CL, 46–69; n = 36), indicating that finasteride does not affect seizure susceptibility.

**Anticonvulsant Activity of Ganaxolone after Neurosteroid Withdrawal.** In naive female control rats, ganaxolone (0.625–15 mg/kg s.c.) protected against PTZ-induced seizures in a dose-dependent fashion (Fig. 1). Ganaxolone also protected against PTZ-induced seizures in pseudopregnant control rats and in rats that had been withdrawn from neurosteroid by finasteride treatment. In neurosteroid-withdrawn rats, the dose-response curve for anticonvulsant activity of ganaxolone was significantly (P < .05) shifted in a parallel fashion to the left from that of control rats. A marginal enhancement in the potency of ganaxolone was observed in pseudopregnant animals at dose above 1.25 mg/kg. The ED50 values derived from these data are given in Table 1. There was a significant decrease (64%) in the ED50 value of ganaxolone for seizure protection in neurosteroid-withdrawn rats compared with naive control animals, indicating an enhancement in the anticonvulsant potency of ganaxolone after neurosteroid withdrawal. To confirm that the enhanced potency of ganaxolone is not due to an action of finasteride unrelated to neurosteroid withdrawal, the anticonvulsant ED50 value for ganaxolone was determined in naive control rats that had not been pretreated with finasteride (Table 1).

**Motor Toxicity of Ganaxolone.** The motor toxicity of ganaxolone (1.25–20 mg/kg s.c.) was assessed with the rotarod test. In control animals, ganaxolone induced motor impairment at doses within the same range as those that were protective in the PTZ seizure test (Fig. 2). Ganaxolone was slightly less potent in pseudopregnant control and neurosteroid-withdrawn animals, but the TD50 values derived from the dose-response data (Table 1) were not significantly different. Because the anticonvulsant ED50 value for ganax-

### Table 1

<table>
<thead>
<tr>
<th>Agent</th>
<th>PTZ Test (ED50)</th>
<th>Motor Impairment (TD50)</th>
<th>Protective Index (TD50/ED50)</th>
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<tr>
<td>Ganaxolone</td>
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<td></td>
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<td>Control</td>
<td>3.46</td>
<td>5.61</td>
<td>1.62</td>
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<td></td>
<td>(2.06–5.82)</td>
<td>(3.52–8.94)</td>
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<td>7.42</td>
<td>3.12</td>
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<td></td>
<td>(1.35–4.26)</td>
<td>(5.23–10.53)</td>
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<td>7.89</td>
<td>6.36</td>
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<tr>
<td></td>
<td>(0.65–2.36)</td>
<td>(5.67–10.97)</td>
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<tr>
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<td>0.68</td>
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<td>(1.67–3.63)</td>
<td>(1.19–2.37)</td>
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<td>1.56</td>
<td>0.38</td>
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<td>(3.11–5.42)</td>
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<td>0.88</td>
</tr>
<tr>
<td></td>
<td>(421–503)</td>
<td>(333–490)</td>
<td></td>
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</table>

* P < .05 versus control (χ² test).
* P < .05 versus pseudopregnant (χ² test).

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**Fig. 1.** Protection against pentylenetetrazol-induced seizures by ganaxolone in naive control female rats, finasteride-treated (neurosteroid-withdrawn) pseudopregnant rats, and pseudopregnant control rats. Animals were injected with pentylenetetrazol 15 min after the indicated dose of ganaxolone. Each point represents data from six to eight animals. The curves indicate logistic fits to the data points; ED50 values are given in Table 1.

**Fig. 2.** Effects of ganaxolone in the accelerating rotarod test in control female rats, finasteride-treated (neurosteroid-withdrawn) pseudopregnant rats, and pseudopregnant control female rats. Animals were tested 15 min after ganaxolone administration. Each point represents data from six to eight animals.
Ganaxolone plasma concentrations were determined 15 min after the administration of various doses of ganaxolone (0.625–7.5 mg/kg s.c.) in control and pseudopregnant neurosteroid-withdrawn rats. Plasma ganaxolone concentrations in both control and neurosteroid-withdrawn animals increased monotonically in a dose-dependent fashion (Fig. 3). Ganaxolone levels in withdrawn animals were slightly lower than those in controls with 5 and 7.5 mg/kg ganaxolone, but there were no significant differences among the data at any of the doses (P > .05). These results indicate that the enhanced anticonvulsant potency of ganaxolone in neurosteroid-withdrawn rats is not due to pharmacokinetic factors leading to increased plasma ganaxolone levels.

Anticonvulsant Activity and Motor Toxicity of Diazepam and Valproate. For comparison, the anticonvulsant activity and motor toxicity of diazepam and valproate were examined in control, pseudopregnant, and pseudopregnant neurosteroid-withdrawn rats. In control and pseudopregnant animals, diazepam (0.4–7.5 mg/kg i.p.) was highly effective in protecting against PTZ-induced seizures (Fig. 4 and Table 1). However, in contrast to the situation with ganaxolone, where neurosteroid withdrawal was associated with enhanced anticonvulsant activity, the anticonvulsant potency of diazepam was markedly reduced in pseudopregnant animals that had undergone neurosteroid withdrawal. As shown in Fig. 5, the motor toxicity of diazepam was similar in all three groups. Thus, the protective index for diazepam was reduced in animals that experienced neurosteroid withdrawal (Table 1).

Valproate (100–800 mg/kg i.p.) was also effective in protecting against PTZ-induced seizures in control and pseudopregnant animals (Fig. 6 and Table 1). As with diazepam, there was a reduction in the potency of valproate after neurosteroid withdrawal, and there appeared to be a steepening of the dose-response curve. Moreover, like diazepam, neurosteroid withdrawal was not associated with a significant change in the motor toxicity of valproate (Fig. 7), so the protective index of valproate was also reduced in withdrawn animals (Table 1).

Discussion

The principal observation in this study is that the anticonvulsant potency of ganaxolone is enhanced in a model of
perimenstrual (progesterone withdrawal type) catamenial epilepsy. There was no corresponding increase in the motor toxicity of ganaxolone. This suggests that the potentiated anticonvulsant activity of ganaxolone results from specific alterations in the brain mechanisms responsible for seizures and is not due to pharmacokinetic factors. Although the protective index of ganaxolone compares unfavorably with that of many conventional anticonvulsant agents, diazepam and valproate, were substantially reduced after neurosteroid withdrawal. Thus, neurosteroid-derived anticonvulsants such as ganaxolone may be particularly suited for the treatment of catamenial seizure exacerbations, which are often resistant to therapy with conventional anticonvulsants (Newmark and Penry, 1980).

The measurements of plasma ganaxolone levels allow us to estimate the plasma concentrations associated with seizure protection and motor toxicity. In control and neurosteroid-withdrawn animals, the threshold plasma concentrations for seizure protection were 200 to 250 and 100 ng/ml, respectively, and the estimated plasma concentrations producing 50% seizure protection were in the range of 450 to 550 and 200 to 250 ng/ml. Thus, ganaxolone protects against the PTZ-induced seizures in neurosteroid-withdrawn rats at plasma concentrations that are not anticonvulsant in control animals.

Perimenstrual (progesterone withdrawal type) catamenial epilepsy. There was no corresponding increase in the motor toxicity of ganaxolone. This suggests that the potentiated anticonvulsant activity of ganaxolone results from specific alterations in the brain mechanisms responsible for seizures and is not due to pharmacokinetic factors. Although the protective index of ganaxolone compares unfavorably with that of many conventional anticonvulsant agents (White et al., 1995), withdrawal was associated with increased separation between the doses producing seizure protection and motor side effects, suggesting that the drug may be better tolerated during the perimenstrual period of increased seizure frequency. However, it remains to be determined whether the enhanced potency of ganaxolone generalizes to other behavioral effects of neurosteroids, including their sedative-hypnotic, anxiolytic, and cognitive impairing effects, which may be important determinants of side effects in clinical use. Measurements of plasma ganaxolone levels revealed no increase in ganaxolone levels after withdrawal, confirming that the enhanced anticonvulsant potency was a pharmacodynamic effect and not related to pharmacokinetic factors. Interestingly, the protective activities of two other anticonvulsant agents, diazepam and valproate, were substantially reduced after neurosteroid withdrawal. Thus, neurosteroid-derived anticonvulsants such as ganaxolone may be particularly suited for the treatment of catamenial seizure exacerbations, which are often resistant to therapy with conventional anticonvulsants (Newmark and Penry, 1980).

The measurements of plasma ganaxolone levels allow us to estimate the plasma concentrations associated with seizure protection and motor toxicity. In control and neurosteroid-withdrawn animals, the threshold plasma concentrations for seizure protection were 200 to 250 and <100 ng/ml, respectively, and the estimated plasma concentrations producing 50% seizure protection were in the range of 450 to 550 and 200 to 250 ng/ml. Thus, ganaxolone protects against the PTZ-induced seizures in neurosteroid-withdrawn rats at plasma concentrations that are not anticonvulsant in control animals.

The animal model of catamenial epilepsy used in this study was designed to simulate the most common form of catamenial epilepsy in which the frequency of seizures increases in the perimenstrual period. Pseudopregnancy was induced by treatment with gonadotropins, resulting in increased ovarian production of progesterone and its 5α and 3α A-ring-reduced metabolite allopregnanolone. Diestrous plasma allopregnanolone levels in the rat are 9.3 ng/ml, and on day 12 of pseudopregnancy, the plasma levels of allopregnanolone are elevated 5-fold to 44.5 ng/ml (D. S. Reddy, H.-Y. Kim, and M. A. Rogawski, unpublished observations). The administration of the 5α-reductase inhibitor finasteride causes a fall in plasma allopregnanolone to a level of 6.4 ng/ml (24 h after dosing), which is modestly below that in the diestrous period. This fall in allopregnanolone is associated with a marked enhancement in the susceptibility to PTZ seizures (Frye and Bayon, 1998; Moran and Smith, 1998). It is attractive to speculate that a similar increase in seizure susceptibility accounts for the exacerbation of seizures in women with perimenstrual catamenial epilepsy. Although the cause of the enhanced seizure susceptibility is not well understood, there is some evidence that allopregnanolone withdrawal is associated with changes in the kinetic properties of GABA_A receptors that predispose to heightened brain excitability (Smith et al., 1998). While finasteride may reduce serum allopregnanolone below control diestrous levels, Smith et al. (1998) have reported that brain levels do not fall below control levels at 24 h. Thus, the enhanced seizure susceptibility after finasteride treatment is not due to an absolute neurosteroid deficiency, and this supports the view that the heightened susceptibility is due to changes in GABA_A receptor properties. In nonpseudopregnant animals, finasteride pretreatment did not affect the convulsant activity of PTZ, indicating that it does not have proconvulsant properties in this.
model. This observation confirms our previous report that 
finasteride, at a dose much higher than used here to induce 
neurosteroid withdrawal, failed to affect the convulsant 
threshold of PTZ in mice (Kokate et al., 1999). The lack of 
effect of finasteride on seizure threshold in control animals 
suggests that basal neurosteroid levels do not have a 
substantial influence on seizure susceptibility. However, alter-
ations in neurosteroid levels during the estrous cycle may 
affect seizure susceptibility in some models (Finn and Gee, 
1994; Frye et al., 1998).

A variety of considerations indicate that the enhanced 
activity of ganaxolone in our model is specifically related to 
neurosteroid withdrawal and is not due to pseudopregnancy 
per se, to the effects of the hormone treatments used to 
induce pseudopregnancy, or to finasteride. Thus, pseudo-
 pregant rats not undergoing withdrawal did not exhibit an 
overall enhanced sensitivity to ganaxolone. However, at high 
doses, ganaxolone appeared to have slightly greater potency 
in pseudopregnant control animals, although this effect did 
not reach statistical significance (Fig. 1). This enhanced ac-
tivity may be due to the synergism between the high level of 
endogenous neurosteroid (present only in this group of ani-
mals) and the exogenously administered ganaxolone. In addi-
tion, finasteride pretreatment failed to alter the anticon-
vulsant potency of ganaxolone in control (not pseudopregnant) animals. Taken together, these observa-
tions permit the conclusion that allotropregnolone with-
drawal and not the pseudopregnant state or finasteride treat-
ment, is responsible for the enhanced sensitivity to gan-
axolone. Indeed, neuroactive steroids have previously been 
shown to have enhanced anticonvulsant activity after diaze-
pam (Tsuda et al., 1997) and ethanol withdrawal (Devaud 
et al., 1996, 1998), which, like ganaxolone, act as positive al-
losteric modulators of GABA\textsubscript{A} receptors. Moreover, there is a 
suggestion that during diestrous, when progesterone levels 
fallen, allopropregnolone has increased potency as a 
GABA\textsubscript{A} receptor modulator (Finn and Gee, 1993) and as an 
anticonvulsant against PTZ-induced seizures (Finn and Gee, 
1994). The mechanisms accounting for the enhanced anticonvul-
sant potency of ganaxolone after neurosteroid withdrawal 
were not addressed in this study. One attractive possible 
mechanism is that the neurosteroid sensitivity of GABA\textsubscript{A} 
receptors relevant to the anticonvulsant activity of ganax-
olone is enhanced after neurosteroid withdrawal. Recently, 
however, Smith et al. (1998) have reported that hippocam-
pal GABA\textsubscript{A} receptors show reduced sensitivity to alloprop-
regnolone 24 h after withdrawal from allopropregnolone. 
Therefore, if changes in GABA\textsubscript{A} receptor sensitivity do ac-
count for the present results, a distinct population must be 
involved.

In contrast to the enhanced sensitivity to ganaxolone, we 
observed a marked decrease in the anticonvulsant potency of 
diazepam and valproate. Our results are consistent with a 
recent study demonstrating a reduction in the sedative ef-
ects of the benzodiazepine lorazepam after progesterone 
withdrawal (Moran et al., 1998). This benzodiazepine insen-
sitvity has been attributed to a specific increase in the ex-
pression of the \(\alpha 4\) GABA\textsubscript{A} receptor subunit (Smith et al., 
1998). A similar benzodiazepine insensitivity has been noted 
after withdrawal from barbiturates and ethanol (Buck and 
Harris, 1990; Roca et al., 1990). In addition, attenuated ben-
zodiazepine sensitivity has been observed clinically in pa-
patients with premenstrual syndrome (Sundström et al., 
1997a,b), a condition that may be related to catamenial epi-
lepsy. However, it remains to be determined whether women 
with catamenial epilepsy have reduced sensitivity to benzo-
diazepines. Valproate also had markedly attenuated anticon-
vulsant potency after neurosteroid withdrawal. Although the 
basis for the anticonvulsant activity of valproate is not well 
understood, it may act in part through effects on GABA-
mediated inhibition (Rogawski and Porter, 1990). In fact, 
there is evidence of cross-tolerance between benzodiazepines 
and valproate (Gent et al., 1986). Thus, the reduced activity 
of diazepam and valproate may have a similar underlying 
basis.

In summary, the results of this study demonstrate that 
there is enhanced anticonvulsant sensitivity to ganaxolone 
during neurosteroid withdrawal, whereas the anticonvulsant 
activities of diazepam and valproate are reduced. There is 
no corresponding enhancement of the motor toxicity of gan-
axolone, so its protective index is greater after neurosteroid 
withdrawal than under ordinary circumstances. Thus, gan-
axolone could be of use in the treatment of perimenstrual 
catamenial epilepsy, a condition that is often resistant to 
other anticonvulsant therapies.

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