Reversal of Behavioral Effects of Pentylenetetrazol by the Neuroactive Steroid Ganaxolone

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ABSTRACT

Neuroactive steroids are naturally occurring or synthetically derived compounds many of which have anticonvulsant, anesthetic, axiolytic, anagolic or hypnotic properties. The major site of neuronal activity appears to be with a specific steroid-sensitive site on the γ-aminobutyric acid receptor/chloride ionophore complex. Ganaxolone (3α-hydroxy-3β-methyl-5α-pregn-20-one) is a synthetic neuroactive steroid protected from metabolic attack of the 3α position. Ganaxolone is an efficacious anticonvulsant agent in a variety of acute seizure models, as well as in electrical and chemical kindling models, and is currently under Phase II clinical investigation for epilepsy. A prior observation that ganaxolone appeared to reverse the marked behavioral changes induced by the convulsant pentylenetetrazol (PTZ) was systematically examined in the present study. A model to quantify PTZ-induced behaviors is described and used to evaluate ganaxolone in comparison with the anticonvulsants valproate, ethosuximide, clonazepam, diazepam and phenobarbital. All compounds were compared using dose equivalents based on their respective ED₅₀ values in preventing convulsions induced by 70 mg/kg PTZ. The ED₅₀ and lower doses of ganaxolone prevented the observed behavioral effects of PTZ as well as its depressant effects on locomotor activity and rearing of mice. In contrast, the other anticonvulsants, if effective, were much less potent. Strikingly, most of the other anticonvulsants were incapable of preventing all the behavioral effects of PTZ. Only phenobarbital prevented all the behavioral effects of PTZ and only at doses 4 to 8 times the anticonvulant ED₅₀. Rather than normalizing behavior after ganaxolone did, however, phenobarbital resulted in supranormal behavioral responses (e.g., increases in activity). Repeated administration of PTZ did not decrease the protective efficacy of ganaxolone. The results document the unique pharmacological profile of ganaxolone and suggest additional potential benefits from its use as an antiepileptic. Furthermore, because the behavioral effects of PTZ have been used to model anxiety and anxiety associated with withdrawal from drugs of abuse, ganaxolone may find additional therapeutic application in those areas.

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NAS are steroidal compounds that alter excitability in the CNS. They occur naturally as metabolites of the hormones progesterone and deoxycorticosterone or are synthetically derived compounds such as the anesthetic alphaxalone (Paul and Purdy, 1992; Lambert et al., 1995). NAS are fast-acting (Selye et al., 1941) and appear to exert their neuronal effects through a nongenomic pathway that is independent of membrane perturbation and intracellular receptors (Gee et al., 1988; Lambert et al., 1995). It is now generally accepted that many NAS work by a selective interaction with the GABA_A receptor, as was first demonstrated for alphaxalone (Harrison and Simmonds, 1984). NAS can either antagonize the Cl⁻ conductance, as for example with the sulfate ester of pregnenolone, possibly by acting on the picrotoxin site (Harrison et al., 1987; Majewska and Schwartz, 1987; Majewska, 1992; Mienville and Vicini, 1989; Wu et al., 1990), or potentiate the Cl⁻ influx (Morrow et al., 1987, 1988, 1989; Olsen et al., 1988), by increasing the probability that the Cl⁻ channel will enter an open state of long duration (Barker et al., 1987; Twyman and MacDonald, 1992) as well as increasing the frequency of channel openings (Twyman and MacDonald, 1992). The GABA-potentiating NAS are thought to act through a steroid site on the GABA_A receptor that is distinct from metabolic attack of the 3α position. Ganaxolone is an efficacious anticonvulsant agent in a variety of acute seizure models, as well as in electrical and chemical kindling models, and is currently under Phase II clinical investigation for epilepsy. A prior observation that ganaxolone appeared to reverse the marked behavioral changes induced by the convulsant pentylenetetrazol (PTZ) was systematically examined in the present study. A model to quantify PTZ-induced behaviors is described and used to evaluate ganaxolone in comparison with the anticonvulsants valproate, ethosuximide, clonazepam, diazepam and phenobarbital. All compounds were compared using dose equivalents based on their respective ED₅₀ values in preventing convulsions induced by 70 mg/kg PTZ. The ED₅₀ and lower doses of ganaxolone prevented the observed behavioral effects of PTZ as well as its depressant effects on locomotor activity and rearing of mice. In contrast, the other anticonvulsants, if effective, were much less potent. Strikingly, most of the other anticonvulsants were incapable of preventing all the behavioral effects of PTZ. Only phenobarbital prevented all the behavioral effects of PTZ and only at doses 4 to 8 times the anticonvulant ED₅₀. Rather than normalizing behavior after ganaxolone did, however, phenobarbital resulted in supranormal behavioral responses (e.g., increases in activity). Repeated administration of PTZ did not decrease the protective efficacy of ganaxolone. The results document the unique pharmacological profile of ganaxolone and suggest additional potential benefits from its use as an antiepileptic. Furthermore, because the behavioral effects of PTZ have been used to model anxiety and anxiety associated with withdrawal from drugs of abuse, ganaxolone may find additional therapeutic application in those areas.

ABBREVIATIONS: ED, effective dose; GABA, γ-aminobutyric acid; NAS, neuroactive steroids; PTZ, pentylenetetrazol; TBPS, t-butylbicyclophosphorothionate.
from both the benzodiazepine (Harrison and Simmonds, 1984; Cottrell et al., 1987b) and barbiturate sites (Cottrell et al., 1987b; Gee et al., 1987, 1988; Peters et al., 1988; Turner et al., 1989).

These GABA-potentiating NAS form an interesting class of compounds with potential clinical use as anesthetics, anticonvulsants, anxiolytics, hypnotics and analgesics (for reviews on NAS, see Simmonds, 1991; Majewska, 1992; Paul and Purdy, 1992; Gee et al., 1995; Lambertz et al., 1995). Among the most potent ligands in this class is allopregnanolone (3α-hydroxy-5α-pregnan-20-one), a major metabolite of progesterone. Allopregnanolone has been shown to protect against convulsions induced by PTZ, bicuculline, pilocarpine, nicotine, strychnine and cocaine (Belelli et al., 1989; Luntz-Leybman et al., 1990; Kokate et al., 1994; Gasior et al., 1997a). However, allopregnanolone has poor oral availability, presumably because of metabolism at the 3α-hydroxy position. To improve bioavailability, the 3α-hydroxy analog has been synthesized. This compound, ganaxolone, is a potent and efficacious anticonvulsant agent with predicted utility in the control of generalized absence and partial seizures (Carter et al., 1997) as well as for convulsions due to cocaine poisoning (Gasior et al., 1997a). Ganaxolone is now undergoing Phase II clinical trials.

In addition to preventing seizures induced by acute chemical challenge, ganaxolone blocks the development and expression of electrical and chemical kindling (Carter et al., 1997; Gasior et al., 1997a, b). Ganaxolone not only was effective in preventing seizure kindling but also appeared to block the behavioral sequella resulting from PTZ administration. Diazepam and valproate, in contrast, were not as potent or efficacious in altering seizure kindling and did not appear to affect the behavioral changes induced by PTZ challenge. In order to quantify these observations, we have developed a model to describe behavior in mice after the administration of PTZ and to compare the effects of ganaxolone on this behavior to those of other, clinically used anticonvulsants with different mechanisms of action. The results of these studies document both the quantitative and the qualitative superiority of ganaxolone in preventing the behavioral sequella engendered by high doses of PTZ given acutely or repeatedly.

Material and Methods

Subjects and treatments. Male Swiss Webster mice (Taconic Farms, Germantown, NY), weighing 30 to 50 g, resided in groups of six within a temperature-controlled vivarium on a 12-h light-dark cycle. The experiments were conducted during the light phase. All mice were naive and were utilized only once except in the repeated-dosing experiments.

Animals received a dose of one of the test compounds s.c., followed by an i.p. injection of PTZ. Ganaxolone was given 15 min before PTZ, and the other compounds were given 30 min before. Pretreatment times were based on efficacy against the acute convulsant effects of PTZ (Gasior et al., 1997a). The doses that were tested are multiples of the ED$_{50}$ values of the anticonvulsants in preventing convulsions induced by the acute administration of 70 mg/kg PTZ (Gasior et al., 1997a). Depending on the effect of 1 × ED$_{50}$, subsequent doses were either increased or decreased. To determine the effects of the compounds alone, a dose of 1 × ED$_{50}$ and the highest dose or doses tested were also given without PTZ. Eight animals participated in each treatment. Immediately after the PTZ injection, the animals were put separately into Digiscan activity monitors (Omnitech Electron-ics, Columbia, OH) with a surface area of 40 × 40 cm and with photoelectric detectors placed 2.5 cm apart along the perimeter and 10 cm (vertical activity) above the floor. Data on the total distance traveled and vertical activity were collected at 10-min intervals for 30 min.

During the recording of the locomotor activity, the animals were observed for 2-min periods at t = 5 min (immediately after all members of the group received injections), t = 15 min and t = 30 min after the administration of PTZ. Mice were observed during these periods for the presence or absence of the following postures and/or behaviors: 1) walking; 2) rearing; 3) grooming; 4) sleeping (defined as a posture in which the mice were curled up with the top of their head on the floor); 5) sitting in a corner with the tail curled around the body so that the tip of the tail was under the nose; 6) sitting or lying not necessarily in a corner, without moving at least once every 15 s; 7) lying in a way that at least one of the back limbs was clearly visible; 8) the occurrence of "hicups," or small twitching of the body; 9) the tail being in line with the rest of the body, 10) sitting or lying with the nose facing the corner and 11) sitting or lying with the tail pressed against a wall of the locomotor cage. A pilot study indicated that behaviors 1 to 5 were characteristic of saline-treated mice whereas behaviors 6 to 11 characterized PTZ-treated mice whether or not PTZ led to clonic convulsions.

In addition, we determined whether the results were influenced by the repeated administration of drugs over the time period in which seizure kindling develops. In this study, the anticonvulsant ED$_{50}$ dose of ganaxolone was compared with that of diazepam. Here, the same animals were used on days 1, 3, 5 and 8. On these days the animals were injected with ganaxolone, diazepam or vehicle s.c., 15 min (30 min in the case of diazepam) before an i.p. injection of PTZ or vehicle. The animals were immediately placed in the activity monitors, and data were obtained every 10 min for a 50-min period. At t = 5, t = 15, t = 30 and t = 45 min, the mice were observed for a 2-min period for the presence or absence of the behaviors noted above and for the occurrence of clonic convulsions (rapid clonic forelimb and hindlimb movement lasting continuously at least 5 s each with accompanying loss of righting response).

Drugs. PTZ (Sigma Chemical Co., St. Louis, MO) was dissolved in sterile saline. Ganaxolone (CCD 1-1042, CoCensys Inc. Irvine, CA) was dissolved in 40% w/v hydroxypropyl-β-cyclodextrin (Research Biochemicals International, Natick, MA) in saline. Diazepam (Hoffmann LaRoche, Nutley, NJ) and clonazepam (Sigma) were dissolved in 20% v/v propylene glycol (Sigma) in saline. Phenobarbital sodium (Ruger Chemical Co., New York, NY) was dissolved in sterile saline. Ethosuximide (Research Biochemicals) was dissolved in sterile water, with a minimal amount of Tween 80. Valproic acid (Sigma) was dissolved in sterile saline. The appropriate drug vehicles were used in control experiments for evaluation of the effects of the different drugs used in this study. Mild heating, stirring and sonication aided the dissolution of the compounds into solution. Drug doses are expressed as the drug forms noted. Injection volumes were 0.01 ml/g b.wt.

PTZ was administered in a dose of 45 mg/kg, the same dose that was used in the chemical kindling experiments in which the initial observations on the anti-PTZ-like behavioral effects of ganaxolone were made (Gasior et al., 1997b). The other drugs were used in multiples of the anticonvulsant ED$_{50}$ value, as determined by Gasior et al. (1997a) or as experimentally determined in the present study against a higher dose of PTZ. The anticonvulsant ED$_{50}$ is the dose that protected 50% of animals from clonic convulsions induced by 70 mg/kg PTZ. Using anticonvulsant potency as a basis for comparing, dose to dose, these drugs against the behavioral effects of PTZ, we uncovered a potential dissociation, as suspected, between the anticonvulsant effects and the anti-PTZ-like behavioral effects of these drugs.

Data analysis. Every animal in each observation period was characterized as either "saline-like," "PTZ-like" or "mixed" according to the following criteria. Animals that exhibited one or more of
TABLE 1
Analysis of individual behavioral components of PTZ-induced behavioral changes as a function of drug treatment

The numbers of animals (mean ± S.E.M.) that displayed specific behaviors in one of the four observational periods were summed to yield a total theoretical maximal score of 32. Behaviors 1 to 5 represent “saline-like” behaviors, and behaviors 6 to 11 represent “PTZ-like” behaviors. Ganaxolone and diazepam were given as pretreatments 15 min and 30 min, respectively, before the administration of 45 mg/kg PTZ. Drugs were administered in doses equivalent to their ED50 values for protection against convulsions engendered by 70 mg/kg PTZ.* indicates a significant difference (P < .05; Dunnett’s one-tailed test) from PTZ alone.

<table>
<thead>
<tr>
<th>“Saline-like” Features</th>
<th>Vehicle + Vehicle</th>
<th>Vehicle + PTZ</th>
<th>Ganaxolone + PTZ</th>
<th>Diazepam + PTZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Walking</td>
<td>22.75 ± 1.75*</td>
<td>1.25 ± 0.63</td>
<td>20.25 ± 1.50*</td>
<td>1.75 ± 0.25</td>
</tr>
<tr>
<td>2 Rearing</td>
<td>23.00 ± 0.41*</td>
<td>0.50 ± 0.50</td>
<td>19.75 ± 1.79*</td>
<td>0.25 ± 0.25</td>
</tr>
<tr>
<td>3 Grooming</td>
<td>21.75 ± 2.13*</td>
<td>0.50 ± 0.50</td>
<td>10.00 ± 0.71*</td>
<td>0.75 ± 0.48</td>
</tr>
<tr>
<td>4 Sleeping</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.25 ± 0.25</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>5 Tail curled</td>
<td>1.75 ± 0.86</td>
<td>0.50 ± 0.50</td>
<td>1.50 ± 0.65</td>
<td>0.00 ± 0.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>“PTZ-like” Features</th>
<th>Vehicle + Vehicle</th>
<th>Vehicle + PTZ</th>
<th>Ganaxolone + PTZ</th>
<th>Diazepam + PTZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 Motionlessness</td>
<td>0.00 ± 0.00*</td>
<td>25.75 ± 2.3</td>
<td>4.00 ± 0.82*</td>
<td>26.75 ± 1.55</td>
</tr>
<tr>
<td>7 Hindlimbs visible</td>
<td>0.00 ± 0.00*</td>
<td>12.25 ± 1.55</td>
<td>1.25 ± 0.63*</td>
<td>6.00 ± 0.41*</td>
</tr>
<tr>
<td>8 Hiccups</td>
<td>0.00 ± 0.00</td>
<td>1.75 ± 1.11</td>
<td>1.25 ± 0.48</td>
<td>1.75 ± 0.25</td>
</tr>
<tr>
<td>9 Tail straight</td>
<td>0.00 ± 0.00*</td>
<td>12.25 ± 3.12</td>
<td>0.75 ± 0.48*</td>
<td>9.25 ± 2.10*</td>
</tr>
<tr>
<td>10 Nose to corner</td>
<td>0.00 ± 0.00*</td>
<td>3.75 ± 1.03</td>
<td>0.00 ± 0.00*</td>
<td>7.25 ± 1.25</td>
</tr>
<tr>
<td>11 Tail along wall</td>
<td>0.00 ± 0.00*</td>
<td>11.50 ± 1.71</td>
<td>2.00 ± 0.92*</td>
<td>6.25 ± 1.65*</td>
</tr>
</tbody>
</table>

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cles or fell while walking when the highest dose of ethosuximide was given alone or in conjunction with PTZ. The ED$_{50}$ for reversing the behavioral effects of PTZ was 1.98 times the ED$_{50}$ (table 2).

Neither clonazepam nor diazepam resulted in significant reductions in PTZ-like signs until 8 times the ED$_{50}$. At this dose, clonazepam did not produce significant effects itself, but the animals switched between PTZ-like and saline-like behaviors. Diazepam was also tested at a dose of 16 times the ED$_{50}$, which yielded significant improvement but less than with 8 times the ED$_{50}$. In the highest dose, however, diazepam itself started to have sedating effects that manifested as non-saline-like sleeping (nonsignificant changes, table 3). The ED$_{50}$ values for the behavior-reversing effects were 3.07 times the ED$_{50}$ for clonazepam and 6.62 times the ED$_{50}$ for diazepam (table 2). Phenobarbital displayed a steep dose-effect curve with 50% effect at 1.50 times the ED$_{50}$ (table 2), in which 4 times and 8 times the ED$_{50}$ resulted in 0% PTZ-like animals. At these doses, the mice reared and ambulated frequently but hardly groomed. Phenobarbital given alone did not affect observed behavior (table 3).

Differences in the anti-PTZ effects of ganaxolone and diazepam are directly compared in table 1 for the individual behaviors that make up the PTZ behavioral rating scale. These data indicate that even for specific behaviors that are altered by PTZ, ganaxolone produced a more effective normalization of behavior toward vehicle control levels than diazepam. Again, this difference in behavioral efficacy was demonstrated by comparing doses of these two drugs that were equally efficacious in blocking the convulsant effects of PTZ (ED$_{50}$ values).

PTZ also significantly reduced locomotor activity (fig. 2). As with the direct behavioral observations (fig. 1), ganaxolone was the most potent compound tested in reversing the reductions in horizontal activity induced by PTZ. Complete reversal was achieved at 1 times the ED$_{50}$. The ED$_{50}$ for this effect was 0.38 times the ED$_{50}$.

Valproate only marginally reversed the PTZ-induced reductions in horizontal locomotor activity at 2 times the ED$_{50}$. This dose given alone did not alter the horizontal distance traveled from vehicle control values (table 3). At 4 times the ED$_{50}$, valproate itself suppressed distance traveled (table 3) and did not reverse the PTZ-effect. Ethosuximide reversed the PTZ-induced decrease in locomotion, but in higher doses than ganaxolone; this effect was seen at 4 times and 6 times the ED$_{50}$. The ED$_{50}$ of ethosuximide for this effect was 2.71 times the ED$_{50}$ (table 2). The locomotor-depressant effects of PTZ were also reversed.
by clonazepam at a high dose (8 × ED<sub>50</sub>). However, locomotion was elevated above the level of vehicle controls, as was the case when this concentration was administered alone (table 3). The ED<sub>50</sub> of clonazepam for reversing the effects of PTZ was 2.62 × ED<sub>50</sub> (table 2). Diazepam brought the distance traveled to the vehicle control level in doses of 8 × and 16 × ED<sub>50</sub>. A dose of 4.56 × ED<sub>50</sub> diazepam was predicted to be the dose that would yield 50% protection (table 2). Pheno- barbital also reversed the decreases in activity induced by PTZ, with doses of 4 × and 8 × ED<sub>50</sub> (50% effect was 1.89 × ED<sub>50</sub>), but as with clonazepam, activity at the highest dose was greater than that of vehicle controls (note the difference in scale in fig. 2).

Effects of the anticonvulsants on vertical activity are shown in figure 3. Most striking is the inability of most drugs to reverse the PTZ-induced depression of vertical activity. Only ganaxolone, clonazepam and phenobarbital reduced this behavioral effect of PTZ; with ganaxolone demonstrating the greatest potency. The effective dose of phenobarbital (8 × ED<sub>50</sub>) produced effects that were greater than those of vehicle-treated mice. ED<sub>50</sub> values for reversing the vertical activity deficit engendered by PTZ could be calculated only for ganaxolone and phenobarbital. These values were 0.56 × ED<sub>50</sub> and 2.12 × ED<sub>50</sub>, respectively (table 2). None of the compounds at a dose of 1 × ED<sub>50</sub> produced significant changes in vertical activity when given alone. Higher doses of valproate, ethosuximide, clonazepam and diazepam all significantly reduced vertical activity counts when given alone (table 3).

The effects of repeated administration of ganaxolone and diazepam are shown in figure 4. The top left panel in figure 4 shows the percentage of PTZ-like animals for controls (circles) and for diazepam (triangles) and ganaxolone (squares) treatments. Because there was little difference over the 4 days, the data were summarized and plotted as a function of time. Both control groups remained constant throughout the experiment at a level of 0 ± 0% for the vehicle control and 92.5 ± 2.1% for the PTZ control. Diazepam did not reverse the effect of PTZ within this time frame; the mean percentage of PTZ-like animals, 92.5 ± 3.9%, did not differ from PTZ controls. In contrast, ganaxolone showed a time-dependent effect; at all times the percentage of PTZ-like animals was significantly lower than without the drug, and only the level at the first observational period was significantly different.

Fig. 2. Horizontal distance traveled, in centimeters, for different doses of anticonvulsants, summed from 10 to 30 min after PTZ or vehicle injection. Error bars represent S.E.M. *indicates significant difference from vehicle + PTZ control, determined by Dunnett's one-tailed t-test, P < .05. # indicates significant difference from vehicle + vehicle control, indicated by a one-tailed t-test, P < .05. n = 8. Other details are as described in fig. 1 caption.
from saline (mean = 35.0 ± 10.1%). This difference decreased, and in the final period, ganaxolone reached maximal efficacy with a level of 0 ± 0%.

The top right panel of figure 4 shows the horizontal distance traveled for the 4 experimental days. There were no marked trends in the data (means were 2213.0 ± 249.87 for vehicle controls, 99.62 ± 60.84 for PTZ controls, 3878.82 ± 298.41 for ganaxolone-treated animals and 21.94 ± 6.94 in...
the case of diazepam treatment). There was a significant difference between the effects of ganaxolone + PTZ and PTZ alone, whereas there was no significant effect of diazepam. Activity levels were somewhat higher, compared with vehicle control, when ganaxolone was given with PTZ, and on days 2 and 4 this difference was significant.

Ganaxolone also reversed the effects of PTZ on vertical activity over successive days (fig. 4, bottom left panel), as was the case with distance traveled. The vertical activity of ganaxolone-treated mice was higher than for vehicle controls on day 1. Diazepam, in contrast, had no significant influence on vertical activity in PTZ-treated mice.

Even though behavior did not change markedly over time, the bottom right panel of figure 4 shows that the convulsant effects of PTZ increased by 33% on day 1 to 83% on day 4 (filled circles). Although both ganaxolone and diazepam completely prevented PTZ-induced convulsions on day 1, diazepam was less effective in controlling seizures over repeated tests.

**Discussion**

The present report provides quantitative observations in support of the conclusion that the neuroactive steroid ganaxolone is superior to a host of standard antiepileptic agents in controlling the behavioral disturbances that result from both acute PTZ administration and a regimen of PTZ that induces seizure kindling. These findings support previous nonsystematic visual observations of behavior and substantiate the superior potency and efficacy of ganaxolone in blocking the development of PTZ-kindled seizures (Gasior et al., 1997b). Ganaxolone was more potent than the other antiepileptic drugs studied, with potency differences that were 4-fold and greater. Ganaxolone also displayed greater efficacy than the other compounds studied. In this respect, ganaxolone was the only compound that completely prevented all of the behavioral signs of PTZ intoxication. For example, although diazepam at high enough doses reversed PTZ-like signs and the decreases in horizontal activity, diazepam was ineffective in reversing the decreases in vertical activity induced by PTZ. Phenobarbital (with reduced potency) also prevented all of the behavioral sequella of PTZ administration; however, reversal was accompanied by effects that were different from vehicle controls. Ganaxolone, in contrast, normalized behavior, returning PTZ-induced behavior to vehicle control levels. Phenobarbital, and to a lesser extent clonazepam, produced a reversal that overshot control levels.

Differences in pharmacokinetic variables cannot account for the differential effects of the drugs reported here. All of the compounds are 100% effective in preventing the clonic seizures produced by PTZ in this strain of mice (Gasior et al., 1997a and present study), and the doses used in the present study were based on their respective anticonvulsant ED₅₀ values. Because the doses of all of the drugs tested are comparable in terms of anticonvulsant efficacy, the present findings indicate that there are striking differences in the potencies and efficacies of anticonvulsants to exert anticonvulsant vs. behavioral protection against PTZ. As far as we know, this differential potency and efficacy relationship has not been previously investigated. Before different mechanisms of action are proposed to explain these relationships, additional factors must first be systematically eliminated.

The ability to block behavioral effects of PTZ may be related to the specific behavioral effects of the anticonvulsant agents alone. Potencies to block phystostigmine-induced decreases in food-maintained responding, for example, are positively correlated with drug potencies to decrease responding on their own (Genovese et al., 1990). In the present study, behavioral effects of the anticonvulsants did not appear to contribute substantially to the prevention of PTZ-induced behavioral effects. Neither ganaxolone nor phenobarbital, given alone, produced significant changes from vehicle controls under the PTZ behavioral rating scale, and all of the drugs at some doses prevented the PTZ-induced behavioral signs. Although valproate, diazepam and clonazepam all produced some observed changes in behavioral ratings when given alone at higher doses, they were all (except the highest dose of valproate) capable of preventing PTZ-induced behavioral signs from day 1. Diazepam, on the other hand, increased activity when given alone at 8× ED₅₀; this was also the only dose that reversed effects of PTZ. NAS have been reported previously to increase locomotor activity (e.g., Wieland et al., 1995), but they did not produce increases at the doses studied here (present study and Gasior et al., 1997a). For vertical activity, only ganaxolone, clonazepam and phenobarbital prevented the PTZ-induced decreases. Of these three drugs, only clonazepam decreased vertical activity when given alone. Thus the preponderance of the data do not link the behavioral effects of the anticonvulsants alone with their efficacies in reversing behavioral effects of PTZ. Nonetheless, it is possible that combinations of specific anticonvulsants with PTZ may uncover behavioral effects not observed with the drugs alone that may be incompatible with, or otherwise interfere with, the PTZ blockade.

Many anticonvulsant agents exert several of the same be-
havioral effects. Thus benzodiazepines, barbiturates, valproate and the NAS demonstrate anxiolytic efficacy in preclinical models (Vellucci and Webster, 1984; Crawley et al., 1986; Wieland et al., 1995; Britton et al., 1991). The effects of ganaxolone reported here suggest its anxiolytic activity, which should be confirmed in other animal models of anxiety. PTZ has been used to model anxiety in rodents (Lal and Sherman, 1980; Lal and Emmett-Oglesby, 1983; Lal and Fielding, 1985; Giusti et al., 1991). PTZ, in subconvulsant doses, produces anxiety in humans (Rodin and Calhoun, 1991; Kamien, 1993). NAS also appear to share discriminative stimuli effects at the low doses tested (Lal et al., 1980). NAS also appear to share discriminative stimulus effects with benzodiazepines and barbiturates, an effect that has been used to predict the subjective effects of drugs in humans (Holtzman, 1990; Preston and Bigelow, 1991; Kamien, 1993). NAS substitute for the training drug in rats trained to discriminate pentobarbital, diazepam, midazolam or ethanol from vehicle (Astor et al., 1993; Deutsch and Mastropaolo, 1993). Clearly, these common behavioral actions appear insufficient to account for the unique pharmacological effects of ganaxolone that are revealed here.

Now that pharmacokinetics and behavioral effects of the drugs alone have been ruled out as major factors in the differential potencies and efficacies observed here, the conclusion that the ability to alter convulsions and behavioral effects of PTZ may be mediated by different mechanisms becomes more tenable. What, then, is the nature of these differential mechanisms? The drugs used in this study form a heterogenous group of compounds. The drugs have different clinical profiles as well as marked differences in their pharmacology. Clinically, ethosuximide is primarily used in the treatment of generalized absence seizures, the benzodiazepines are most effective for myoclonic convulsions, phenobarbital can be used for myoclonic convulsions and in generalized tonic-clonic and partial seizures and valproate has clinical significance for generalized tonic-clonic, absence, myoclonic and partial seizures (MacDonald and Meldrum, 1989). Preclinical indications have suggested efficacy for ganaxolone in the management of generalized absence as well as simple and complex partial seizures (Carter et al., 1997; Gasior et al., 1997a, b). Thus the common clinical or suggested clinical utility of phenobarbital, valproate and ganaxolone does not predict efficacy against behavioral effects of PTZ.

The pharmacological profiles of the compounds are diverse as well. Ethosuximide acts on T calcium channels and valproate on sodium channels. Diazepam and clonazepam interact with the benzodiazepine site of the GABA\textsubscript{A} receptor as well as with sodium channels, whereas phenobarbital acts on the barbiturate site and on sodium channels (MacDonald and Meldrum, 1989). The anticonvulsant profile of ganaxolone, though different in some respects, closely resembles that of valproate (Carter et al., 1997) and clonazepam (Kokate et al., 1994). Nonetheless, neither valproate nor clonazepam demonstrated the degree of protection against behavioral effects of PTZ (present study) or against PTZ-kindled seizures (Gasior et al., 1997b) that is demonstrated by ganaxolone. Ganaxolone is unique in modulating GABA transmission by allosteric modulation of a steroid-sensitive site on the GABA\textsubscript{A} receptor complex with only weak interactions at other known anticonvulsant sites of action (Morrow et al., 1987; Lambert et al., 1995; Carter et al., 1997). The specific modulation of GABA through the steroid binding site may confer on neuroactive steroids and endogenous steroids the capacity to function as efficacious inhibitors of both epileptogenic and behavioral effects induced by PTZ with comparable potency. The novel anticonvulsant properties of NAS have previously been documented (Gasior et al., 1997a, b). In addition, ganaxolone exhibited a reduced propensity to affect cognitive function, and to produce in its motor-intoxicating interactions with ethanol, compared with valproate (R. B. Carter unpublished observations). The possibility that such


References


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