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Characterization of Discriminative Stimulus Effects of the Neuroactive Steroid Pregnanolone

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ABSTRACT

Reduced pregnane neurosteroids such as allopregnanolone and pregnanolone are potent neuromodulators able to affect a number of membrane receptors, including γ-aminobutyric acid $(GABA)_A$, *N*-methyl-D-aspartate (NMDA), 5-hydroxytryptamine $(5\text{-HT})_3$, and σ_1 receptors. The present study used a drug discrimination procedure to assess further the receptor effects of pregnanolone in vivo. Rats were trained to discriminate 5 mg/kg pregnanolone from saline in a two-lever operant task maintained by food reinforcement. The opiate agonist morphine and the negative $GABA_A$ modulator dehydroepiandrosterone sulfate did not substitute for pregnanolone. All of the $GABA_A$ positive modulators tested (allopregnanolone, epipregnanolone, androsterone, pentobarbital, midazolam, and zolpidem) dose dependently substituted for pregnanolone. The direct GABA-site agonists 4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridin-3-ol and muscimol failed to substitute for pregnanolone. Ethanol and the σ_1 receptor agonist SKF 10047 fully substituted for pregnanolone, and the NMDA antagonist MK-801 partially substituted for pregnanolone. The 5-HT_3 antagonist tropisetron did not substitute at any dose tested. The 5-HT_3 agonist SR 57227A reached full substitution, whereas the other 5-HT_3 agonist tested, *m*-chlorophenylbiguanide, produced partial substitution. These results suggest that positive $GABA_A$ modulation, but not direct agonism, confers a discriminative stimulus effect similar to pregnanolone. Additionally, antagonism of NMDA receptors and activation of 5-HT_3 and σ_1 receptors modulate stimulus effects similar to the pregnanolone cue. Overall, the data suggest that pregnanolone produces discriminative stimulus effects representative of a wide-spectrum sedative hypnotic.

Neurosteroids are potent neuromodulators synthesized by the nervous system or derived from metabolites of steroid hormones such as progesterone and deoxycorticosterone (for review, see Baulieu, 1998). Two primary sites of action of neurosteroids are at $GABA_A$ and NMDA receptors (Baulieu, 1998). Binding, electrophysiological, and chloride flux data demonstrate that the reduced pregnane neurosteroids such as allopregnanolone, pregnanolone, and THDOC are positive modulators of $GABA_A$ receptors (Morrow et al., 1990; Goodnough and Hawkinson, 1995; Le Foll et al., 1997) and have sedative hypnotic effects in humans (Schultz et al., 1996). Although these neurosteroids modulate $GABA_A$ receptors in a manner that is similar to benzodiazepines and barbiturates, in vitro studies suggest that neurosteroids act at a site distinct from that of benzodiazepines (Morrow et al., 1990) and barbiturates (Gee et al., 1988). Sulfated neurosteroids such as pregnanolone sulfate, androsterone sulfate, pregnenolone sulfate, and dehydroepiandrosterone sulfate (DHEAS) negatively modulate $GABA_A$ receptors (Park-Chung et al., 1999). The glutamate system is also affected by neurosteroids. Pregnenolone sulfate positively modulates NMDA receptors, but has little effect on non-NMDA receptors (Bowlby, 1993; Park-Chung et al., 1997). Pregnanolone sulfate negatively modulates NMDA, kainate, and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (Park-Chung et al., 1994). Binding and electrophysiological data suggest separate sites of action for positive and negative neurosteroid modulators on both $GABA_A$ and NMDA receptors (Majewska et al., 1990; Park-Chung et al., 1999, 1997).

In addition to the $GABA_A$ and glutamate receptors, σ_1 sites and 5-HT_3 receptors may also be targets of neurosteroid action. Progesterone, and to a lesser extent other neurosteroids, inhibit σ_1 binding, and progesterone alters the expression of σ_1 sites (Su et al., 1988; Maurice et al., 1999). Reduced pregnane neurosteroids such as THDOC and allopregnanolone inhibit 5-HT_3 receptor function (Wetzel et al., 1998; Barann et al., 1999), acting at a site separate from that of the agonist binding site (Wetzel et al., 1998). This suggests

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ABBREVIATIONS: GABA, γ-aminobutyric acid; NMDA, *N*-methyl-D-aspartate; THDOC, allotetrahydrocorticosterone; DHEAS, dehydroepiandrosterone sulfate; THIP, 4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridin-3-ol; CPBG, chlorophenylbiguanide; FR, fixed ration; 2HPCD, 2-hydroxypropyl-β-cyclodetrin.

that neurosteroids may play a modulatory role in 5-HT_3 receptor function.

The discriminative stimulus paradigm can be used as an in vivo assay of receptor-mediated activity (Holtzman, 1990). In this paradigm, animals are trained to respond differentially depending upon the presence or absence of a particular drug effect. Depending upon the training conditions, drugs that act in a similar manner on specific receptor systems share discriminative stimulus effects. For example, positive modulators of $GABA_A$ receptors show similar generalization patterns in drug discrimination studies. Neurosteroids that are positive modulators at $GABA_A$ receptors substitute for benzodiazepines (Ator et al., 1993; Deutsch and Mastropaolo, 1993), barbiturates (Ator et al., 1993; Bowen and Grant, 1999; Rowlett et al., 1999), and ethanol (Bienkowski and Kostowski, 1997; Grant et al., 1997; Bowen et al., 1999). Another laboratory has investigated the discriminative stimulus effects of neurosteroids as a training drug, where pentobarbital and a variety of benzodiazepines completely substituted in rats trained to discriminate pregnanolone from saline (Vanover, 1997, 2000).

The focus of this study was to characterize further the discriminative stimulus properties of pregnanolone. We tested the ability of a number of $GABA_A$ positive modulators (pentobarbital, midazolam, zolpidem, allopregnanolone, epipregnanolone, androsterone, and ethanol), direct $GABA_A$ agonists [4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridin-3-ol (THIP) and muscimol], and the $GABA_A$ negative modulator (DHEAS) to substitute for pregnanolone. Additionally, we tested the ability of the uncompetitive NMDA receptor antagonist (MK-801), two different 5-HT_3 agonists [SR 57227A and *m*-chlorophenylbiguanide (CPBG)], the 5-HT_3 antagonist (tropisetron), a σ_1 agonist (SKF 10047), and the opiate agonist (morphine) to substitute for pregnanolone.

Materials and Methods

Animals. Seventeen male Long-Evans rats (Harlan Industries, Indianapolis, IN) were housed individually in a temperature-controlled vivarium on a 12-h light/dark cycle. All procedures were carried out at approximately the same time during the light phase. Rats were approximately 3 months of age weighing 314 ± 1.5 g at the start of the study. Animals were allowed to gain weight gradually until they weighed 350 g, and were then maintained at that weight ±10 g with water ad libitum. All procedures were approved by the Animal Care and Use Committee at Wake Forest University School of Medicine and in accordance with National Institutes of Health guidelines in Principles of Laboratory Animal Care (publication 85-23).

Apparatus. Rats were trained and tested in standard operant chambers (30 × 24 × 21 cm) enclosed in sound-attenuated and ventilated cubicles (Med Associates, East Fairfield, VT). A Macintosh G3 computer connected to National Instruments interface and run by Lab View instrumentation software controlled the operant sessions. One wall of the chamber consisted of an operant panel with two retractable levers and three stimulus lights above each lever. The wall opposite the levers contained a food cup equidistant from both levers, into which a 45-mg food pellet (P.J. Noyes, Lancaster, NH) could be dispensed.

Discrimination Training. A single lever was made available in the chamber and each rat was trained to press the lever under a fixed ration (FR) 1 schedule, with each response resulting in a 45-mg food pellet presentation. As response rates increased, FR requirements for pellet delivery were gradually increased from 1 to 15. Under the terminal schedule, 15 consecutive responses resulted in food pellet delivery. During this phase, the period of time between placement into the operant chamber and the start of the training session (pretreatment time) was also increased from 0 to 20 min. During the pretreatment time, the operant chamber was dark and all levers retracted.

Once rates of responding were stable, exposure to the training conditions began. After the administration of saline (0.35 ml i.p.) or 5 mg/kg pregnanolone (5 mg/ml i.p.), the rat was placed into the operant chamber. After the 20-min pretreatment period, the start of the session was indicated by illumination of the house light and extension of the condition-appropriate lever. Sessions ended after 20 pellets were delivered or 30 min had elapsed, and were conducted once a day at least 5 days/week for each rat.

After six consecutive sessions of saline administration, with only the saline-appropriate lever available, the rats received six consecutive sessions of pregnanolone administration, with only the pregnanolone-appropriate lever available. This was followed by six consecutive sessions of alternating between pregnanolone and saline injection, with only the appropriate lever presented. The right lever was designated correct after the administration of pregnanolone for half of the rats and designated correct after saline for the other half of the rats. Criteria for having acquired the discrimination were set at ≥90% of total responding and ≥10 of the first 15 responses (first FR) on the condition-appropriate lever for five consecutive sessions. Responding on the appropriate lever resulted in the delivery of a food pellet, whereas responding on the inappropriate lever resulted in resetting the ratio requirement for the correct lever to 15.

Stimulus Substitution Testing. Once the pregnanolone from water discrimination was reliably established, stimulus substitution tests were conducted approximately twice per week (usually Wednesday and Saturday), whereas training sessions occurred on the intervening days. Specifically, test sessions were conducted when the above-mentioned criteria were met for two consecutive sessions. If performance during training sessions failed to meet these criteria, discrimination training continued until criteria were met for three consecutive sessions. Test sessions were identical to training sessions except that 15 consecutive responses on any lever resulted in food delivery. All drugs and vehicles were administered (i.p.) 20 min before the start of the session. All rats were tested with 0.35 ml of saline, 0.35 ml of 20% 2-hydroxypropyl-cyclodetrin (2HPCD), pregnanolone (1–10 mg/kg), and morphine (1–9 mg/kg), to assess the basis of the discrimination. Thereafter, rats were divided into groups of 8 to 10 for the rest of the drug tests.

Drugs. Pregnanolone (3α-hydroxy-5β-pregnan-20-one) was dissolved in 20% 2HPCD (Cerestar USA, Inc., Hammond, IN). Epipregnanolone (3β-hydroxy-5β-pregnano-20-one), androsterone, and zolpidem were dissolved in 45% 2HPCD. All other drugs were dissolved in saline. The following dose ranges were tested: pregnanolone (1–10 mg/kg), morphine (1–9 mg/kg), muscimol (1–2 mg/kg), THIP (3–30 mg/kg), androsterone (5–60 mg/kg), epipregnanolone (3–10 mg/kg), and allopregnanolone (3α-hydroxy-5α-pregnan-20-one) (5 and 10 mg/kg), DHEAS (10 and 30 mg/kg), pentobarbital (1–10 mg/kg), midazolam (0.1–5 mg/kg), zolpidem (0.3–5 mg/kg), tropisetron (3-tropanyl-indole-3-carboxylate) (1–5 mg/kg), SR 57227A (3–10 mg/kg), CPBG (5–10 mg/kg), ethanol (0.5–1.5 g/kg), (+)-MK-801 (dizcilopine) (0.05–0.2 mg/kg), and SKF 10047 (+)-*N*-allylnormetazocine (5–20 mg/kg). Pregnanolone and androsterone were obtained from Steraloids (Wilton, NH). SR 57227A and CPBG were obtained from Tocris Cookson (St. Louis, MO). SKF 10047, DHEAS, MK-801, zolpidem, pentobarbital, morphine, THIP, and muscimol were obtained from Sigma/RBI (Natick, MA). Midazolam was obtained from Roche Molecular Biochemicals (Indianapolis, IN), and epipregnanolone and allopregnanolone were synthesized as described (Purdy et al., 1990).

Data Analysis. The percentage of total responses that occurred on the pregnanolone-appropriate lever (pregnanolone-appropriate responses/total session responses) and response rates (total session responses/session time in seconds) were determined for each rat

during each session. Complete substitution for the discriminative stimulus effects of a training drug was defined as 80% or greater total session responding on the pregnanolone-appropriate lever. Partial substitution was defined as responding between 50 and 80% on the drug-appropriate lever. Although response rate data from all test sessions were included in the analyses, the percentage of drug appropriate responding from each test was included only if a rat obtained at least one reinforcer during the test session. The ED_{50} values with confidence intervals (CIs) for substitution were calculated by nonlinear regression analysis (variable slope) of the dose-response curves using Prism GraphPad software, with no constraints except when there were too few or overlapping data points to define the CI and in these cases (epipregnanolone, androsterone, ethanol, SR 57227A, and SKF 10047) the bottom and top of the curve were held constant a 0 and 100, respectively. ED_{50} values and confidence intervals for allopregnanolone and CPBG were derived graphically from two doses. Significant differences in rates of responding between drug versus saline test sessions were calculated by the Bonferroni's test for multiple comparisons. All means are presented as mean ± S.E.M.

Results

All rats (n = 17) acquired the discrimination, which required between 19 and 74 sessions to meet criteria (mean = 31 ± 3) from the onset of the presentation of both levers during training. Pregnanolone showed a dose-dependent increase in pregnanolone-appropriate responding (Fig. 1) with an ED_{50} value of 1.7 mg/kg (Table 1). At the highest dose (10 mg/kg), pregnanolone also significantly depressed rates of responding ($p \leq 0.001$). The average drug-appropriate responding after saline and 20% 2HPCD was 4 ± 1.8 and 6 ± 3.3%, respectively. Rates of responding after 2HPCD were no different from saline (0.70 ± 0.02 and 0.68 ± 0.03 for saline and 20% 2HPCD, respectively).

Morphine did not substitute (Fig. 2) for pregnanolone at any dose tested and decreased response rates at 3, 5, and 10 mg/kg ($p \leq 0.007$), with only 9 of 17 rats responding at the highest dose. The $GABA_A$ agonists THIP and muscimol also did not substitute for pregnanolone at any dose tested (Fig. 2). Only one of eight rats showed drug-appropriate responding (65%) at the 10-mg/kg dose of THIP, corresponding to a

TABLE 1
ED_{50} of compounds tested for substitution in rats trained to discriminate 5 mg/kg pregnanolone

	Drug	ED_{50}	95% CI
		mg/kg	
Neurosteroids	Pregnanolone	1.7	1.3–2.2
	Allopregnanolone	5.0	0.8–6.6
	Epipregnanolone	5.5	4.4–6.8
	Androsterone	21.5	10.5–44.0
	DHEAS	NS	NS
$GABA_A$	Pentobarbital	2.9	2.2–3.8
	Midazolam	0.5	0.2–1.2
	Zolpidem	1.5	0.7–3.3
	THIP	NS	NS
	Muscimol	NS	NS
NMDA	MK-801	0.09	0.07–0.12
Alcohol	Ethanol	900	700–1100
$5\text{-}HT_3$	SR 57227A	5.5	4.4–6.8
	CPBG	8.8	7.3–10.8
	Tropisetron	NS	NS
σ_1	SKF 10047	9.7	6.0–15.6
Opiate	Morphine	NS	NS

NS, no substitution.

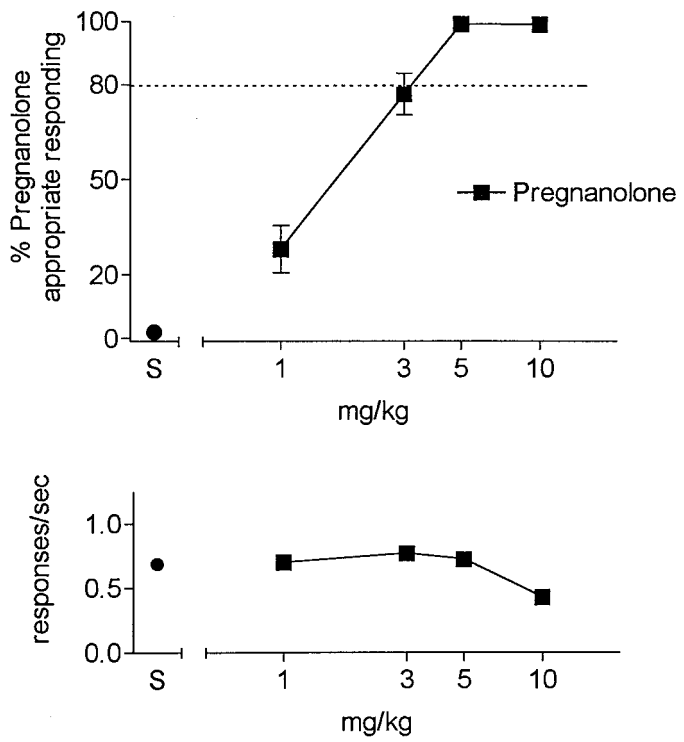

Fig. 1. Discriminative stimulus effects and response rates of pregnanolone (i.p., 20 min) in rats (n = 17) trained to discriminate 5 mg/kg pregnanolone from saline. Top, mean percentage responding on the pregnanolone-appropriate lever, and bottom, mean rates of responding during the corresponding sessions. S, saline control.

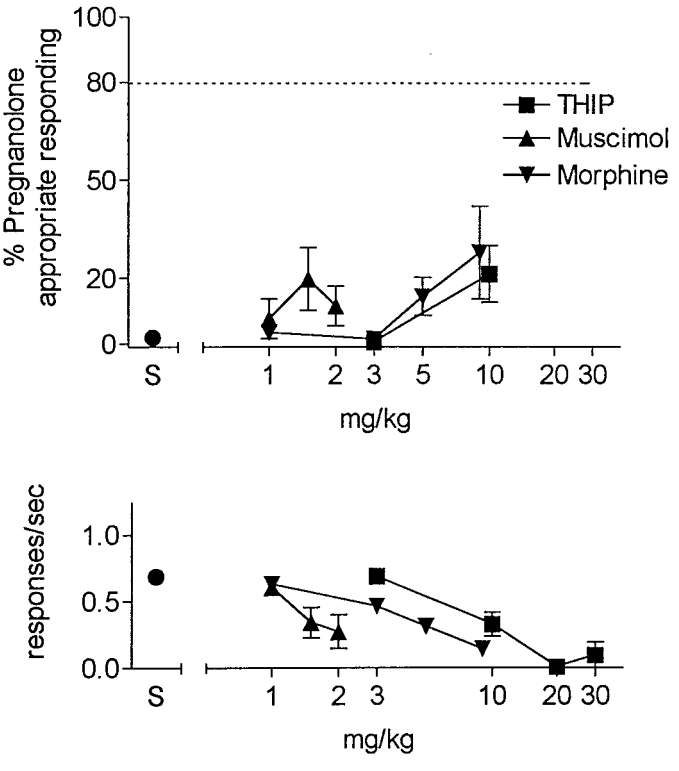

Fig. 2. Discriminative stimulus effects and response rates of THIP (n = 8–10), muscimol (n = 8–10), and morphine (n = 17) (i.p., 20 min) in rats (n = 17) trained to discriminate 5 mg/kg pregnanolone from saline. Top, mean percentage responding on the pregnanolone-appropriate lever, and bottom, mean rates of responding during the corresponding sessions. S, saline control.

significant decrease in response rates ($p \leq 0.03$). When tested with muscimol, 1 of 10 rats showed partial substitution at 1 and 1.5 mg/kg muscimol (61 and 63%, respectively), also corresponding to a decrease in response rates ($p \leq 0.05$).

The neurosteroids androsterone, epipregnanolone, and allopregnanolone produced dose-dependent substitution for pregnanolone (Fig. 3; see Table 1 for ED_{50} values). All rats ($n = 9$) tested with epipregnanolone and allopregnanolone ($n = 6$) showed complete substitution for pregnanolone. The highest dose of epipregnanolone (10 mg/kg) tested significantly decrease response rates ($p \leq 0.001$). The mean substitution of androsterone for pregnanolone was 80% at the highest dose tested (60 mg/kg), with no substitution in two of eight rats tested. Average rates of responding with androsterone were not significantly altered at any dose tested. DHEAS (10 and 30 mg/kg, $n = 8$ rats) did not substitute for pregnanolone (Fig. 2) and had no significant effect on rates of responding.

The $GABA_A$ receptor-positive modulators pentobarbital, midazolam, and zolpidem also produced complete substitution for pregnanolone, with every rat tested ($n = 9$ each) responding ≥80% on the pregnanolone-appropriate lever after at least one test dose (Fig. 4). The higher doses of zolpidem and midazolam (3 and 5 mg/kg) significantly decreased rates of responding ($p \leq 0.001$) at doses that produced full substitution. Doses of pentobarbital that produce full substitution (5 and 10 mg/kg) did not reduce response rates of responding.

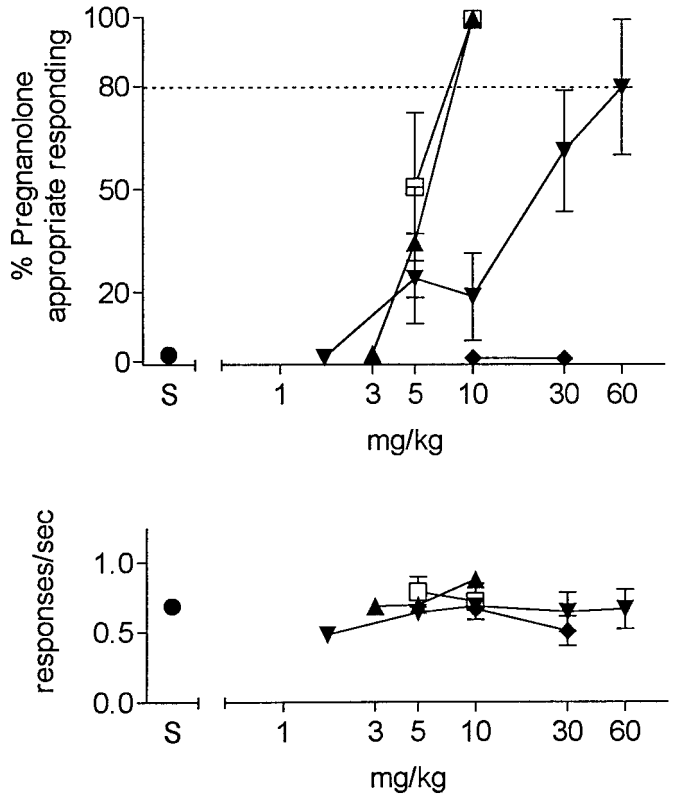

Fig. 3. Discriminative stimulus effects and response rates of epipregnanolone (▲), allopregnanolone (□), androsterone (▼), and DHEAS (♦) (i.p., 20 min; $n = 8$–10 each drug) in rats ($n = 17$) trained to discriminate 5 mg/kg pregnanolone from saline. Top, mean percentage responding on the pregnanolone-appropriate lever, and bottom, mean rates of responding during the corresponding sessions. S, saline control.

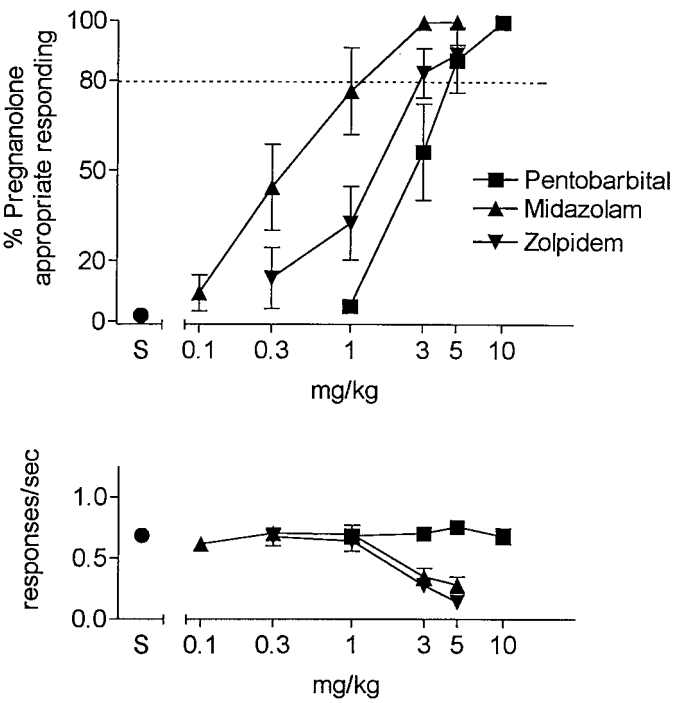

Fig. 4. Discriminative stimulus effects and response rates of pentobarbital, midazolam, and zolpidem (i.p., 20 min; $n = 8$–10 each drug) in rats trained to discriminate 5 mg/kg pregnanolone from saline. Top, mean percentage responding on the pregnanolone-appropriate lever, and bottom, mean rates of responding during the corresponding sessions. S, saline control.

The 5-HT_3 antagonist tropisetron did not substitute for pregnanolone at any dose tested (Fig. 5). The same rats ($n = 8$) were tested with the 5-HT_3 agonists SR 57227A and CPBG (Bachy et al., 1993). SR 57227A dose dependently substituted for pregnanolone, reaching complete substitution at the 10-mg/kg dose in six of eight rats tested and an $ED_{50} = 5.7$ mg/kg (Fig. 5; Table 1). SR 57227A decreased rates of responding at the 3-, 8-, and 10-mg/kg doses ($p \leq 0.002$). Partial substitution was evident with CPBG (10 mg/kg) with an average of 59% substitution at the dose of 10 mg/kg, with four of eight rats tested showing complete substitution. The highest dose of CPBG tested, 10 mg/kg, resulted in significant decrease in response rates ($p \leq 0.001$).

The NMDA antagonist (+)-MK-801 partially substituted for pregnanolone (Fig. 6) with an $ED_{50} = 0.09$ mg/kg (Table 1). The 0.2-mg/kg dose of MK-801 resulted in an average of 73% pregnanolone-appropriate responding, an effect that was accompanied by a significant decrease in response rates ($p \leq 0.002$). The σ_1 receptor agonist SKF 10047 completely substituted with an average of 93% of pregnanolone-appropriate responding at the 20-mg/kg dose with an associated decreased rate of responding ($p \leq 0.001$) (Fig. 6). Ethanol completely substituted in seven of eight rats with an average pregnanolone-appropriate responding of 98 ± 1% at 1.5 g/kg; the remaining rat showed partial substitution (52%) at this dose. Both the 1.5 and 2.0 g/kg ethanol dose resulted in significantly depressed rates of responding ($p \leq 0.001$).

Discussion

The discriminative stimulus effects of pregnanolone were mimicked by all of the $GABA_A$ positive modulators, pento-

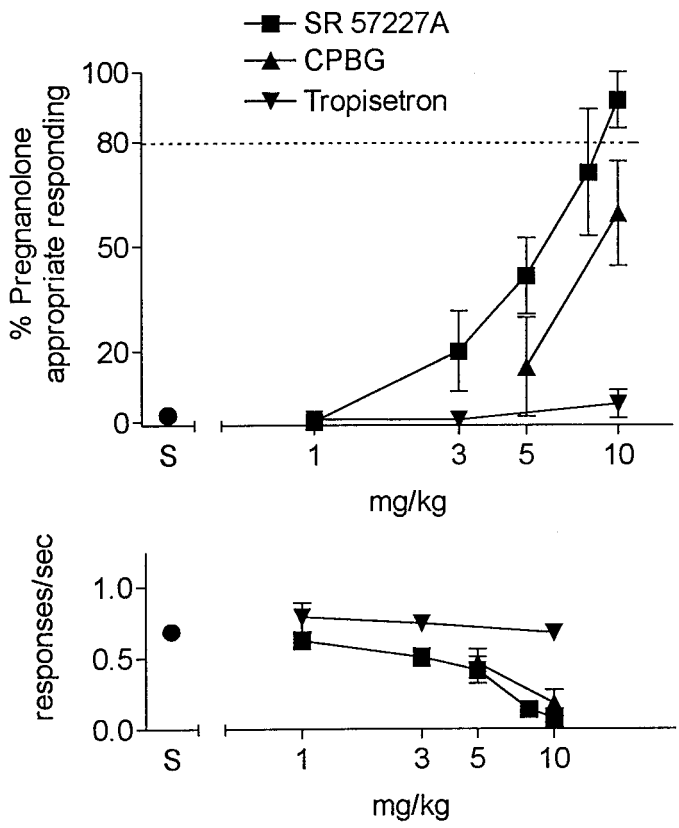

Fig. 5. Discriminative stimulus effects and response rates of SR 57727A, CPBG, and tropisetron (i.p., 20 min; n = 8–10 each drug) in rats trained to discriminate 5 mg/kg pregnanolone from saline. Top, mean percentage responding on the pregnanolone-appropriate lever, and bottom, mean rates of responding during the corresponding sessions. S, saline control.

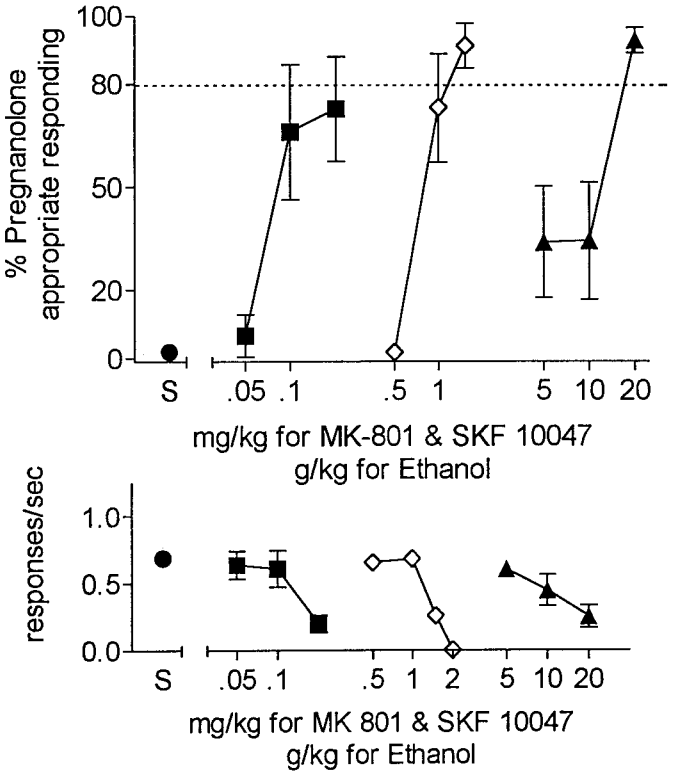

Fig. 6. Discriminative stimulus effects and response rates of MK-801 (■), ethanol (○), and SKF 10047 (▲) (i.p., 20 min; n = 8–10 each drug) in rats trained to discriminate 5 mg/kg pregnanolone from saline. Top, mean percentage responding on the pregnanolone-appropriate lever, and bottom, mean rates of responding during the corresponding sessions. S, saline control.

barbital, midazolam, zolpidem, androsterone, epipregnanolone, allopregnanolone, and ethanol. However, direct $GABA_A$ agonists did not generalize to pregnanolone. Thus, positive modulation of $GABA_A$ receptors, but not direct $GABA_A$ activation, confers a discriminative stimulus similar to that of pregnanolone. The difference in substitution patterns of the neurosteroids that are $GABA_A$ positive modulators and direct $GABA_A$ agonists is in agreement with mutagenesis studies that suggest the neurosteroid site is not the same as the GABA site on $GABA_A$ receptors (Ueno et al., 1997).

Epipregnanolone and allopregnanolone showed lower potency for pregnanolone substitution compared with pregnanolone (ED_{50} for allopregnanolone substitution was 5.0 versus 1.7 mg/kg for pregnanolone). The decreased potency of epipregnanolone may be due to its partial or low-efficacy agonist activity at $GABA_A$ receptors compared with pregnanolone (Pignataro and de Plazas, 1997). However, the similar potencies of the isomers allopregnanolone and epipregnanolone to substitute for pregnanolone in the present study suggest that the axial orientation of the hydroxyl group at C3, which is less interactive than an equatorial hydroxyl group, subtly influences the discriminative stimulus effects of these two progesterone-derived neurosteroids. To extend further the importance of steroid structure, we investigated the discriminative stimulus effects of the C 19 steroid androsterone, a positive modulator of $GABA_A$ receptors (Peters et al., 1988). In vitro data show the potency of androsterone, an axial 3-hydroxy-5-reduced androstane, to inhibit the action of competitive antagonists at $GABA_A$ receptors is about 10 times lower than that of 3-hydroxy pregnane steroids (Turner and Simmonds, 1989). Consistent with the in vitro data, a higher dose of androsterone (ED_{50} = 21.5 mg/kg) was required for generalization to pregnanolone than for pregnanolone, allopregnanolone, and epipregnanolone (Table 1). Although these $GABA_A$ positive-modulator neurosteroids differ in potency, overall their discriminative stimulus effects are similar.

The substitution of midazolam, zolpidem, and pentobarbital for pregnanolone in this study is in agreement with other studies that report similarities in the discriminative stimulus effects of benzodiazepines and barbiturates with $GABA_A$ positive-modulator neurosteroids. Specifically, THDOC substitutes for the benzodiazepines midazolam and diazepam (Ator et al., 1993; Deutsch and Mastropaolo, 1993) and allopregnanolone, pregnanolone, THDOC, and the synthetic neuroactive steroid Co 8-7071 substitute for the barbiturate pentobarbital (Ator et al., 1993; Rowlett et al., 1999). In previous neurosteroid discriminations the benzodiazepine ligands diazepam, triazolam, lorazepam, bretazenil, zalepon, and zolpidem substituted for pregnanolone (Vanover, 1997, 2000), findings that are in agreement with the present substitution of midazolam and zolpidem for pregnanolone. In addition, all three studies, where pregnanolone is used as the training stimulus, have found substitution of the barbiturate pentobarbital. Together, these data suggest that positive modulation of $GABA_A$ receptors produces similar discriminative

stimulus effects, even though neurosteroids, barbiturates, and benzodiazepines have distinct binding sites.

Many studies have looked at the effect of subunit composition on neurosteroid modulation of $GABA_A$ receptors, but no general consensus exists for the role of the σ_1-containing $GABA_A$ receptors and neurosteroid modulation (for review, see Lambert et al., 1996). The type-1 benzodiazepine receptor ligand zolpidem shows binding selectivity for $GABA_A$ receptors that contain a σ_1 subunit (Sanger, 1987). Zolpidem completely substituted for pregnanolone in the present study and in a previous study (Vanover, 2000), suggesting that the activation of the σ_1-containing $GABA_A$ receptors can produce discriminative stimulus effects similar to pregnanolone.

The present data also suggest that ligands at other neurotransmitter receptor systems can mimic the discriminative stimulus effects of pregnanolone. Among these are an uncompetitive NMDA channel antagonist, two 5-HT_3 receptor agonists, a σ_1 receptor agonist, and ethanol. However, each of these substitutions was associated with a decrease in response rates, suggesting differences in the behavioral profile between pregnanolone and the non-$GABA_A$ receptor ligands tested. Similarly, the in vitro data for pregnanolone activity at the NMDA receptor complex and the σ_1 site are not strong. For example, pregnanolone has no direct effect on NMDA responses (Park-Chung et al., 1994), however, the sulfated forms of pregnanolone and epipregnanolone do inhibit NMDA responses (Park-Chung et al., 1994, 1997). It is possible that endogenous sulfation of pregnanolone and subsequent negative modulation of NMDA responses become part of the pregnanolone training cue, although this hypothesis requires further investigation.

Stronger data exist for neurosteroid activity at σ_1 sites, where it has been shown that progesterone can displace σ_1 ligands (Su et al., 1988). Drug discrimination studies show that MK-801 and SKF 10047 share similar discriminative stimulus effects (Balster, 1989; Singh et al., 1990), an effect that has been argued to be mediated via common action at NMDA receptors (Balster, 1989). However, there is evidence that σ_1 agonists can modify NMDA responses by a site separate from that of the NMDA receptor (Yamamoto et al., 1995; Chaki et al., 1998; Maurice et al., 1999). Thus, in addition to a direct action of neurosteroids at σ_1 receptors and NMDA receptors, neurosteroids may alter NMDA function indirectly through the σ_1 site. Regardless of the exact receptor mechanism, the data in the present study are the first to clearly demonstrate that ligands with activity similar to dissociative anesthetics can produce discriminative stimulus effects similar to an endogenous neurosteroid.

In addition to possible effects of pregnanolone at NMDA and σ_1 receptors, recent evidence suggests neurosteroid activity at 5-HT_3 receptors. In general, neurosteroids have a negative modulatory effect at 5-HT_3 receptors in vitro (Wetzel et al., 1998; Barann et al., 1999). Specifically, several steroids, including progesterone, THDOC, and alphaxalone inhibit [^{14}C]guanidinium flux through 5-HT_3 receptors in N1E-115 cells (Barann et al., 1999) and progesterone and allopregnanolone inhibit 5-HT_3-mediated current in human embryonic kidney 293 cells (Wetzel et al., 1998). Paradoxically, the results from the present discrimination study show that 5-HT_3 receptor antagonist tropisetron did not substitute for pregnanolone, but the 5-HT_3 receptor agonists CPBG and SR 57227A produced partial and full substitution for pregnanolone. The results of the present study suggest that potentiation of 5-HT_3 receptors produces discriminative stimulus effects similar to pregnanolone, a finding that is opposite to predicted effects from in vitro electrophysiological and biochemical studies.

The present data confirm symmetrical generalizations between neurosteroids and benzodiazepines and barbiturates (Vanover, 2000), and it is assumed that these symmetrical generalizations are largely based on similar positive modulatory effects of benzodiazepines, barbiturates, and neurosteroids on $GABA_A$ receptors. However, the present study is one of the few to demonstrate symmetrical substitution of ethanol for another drug. Ethanol can be viewed as a heterogenous, or compound cue, with aspects of $GABA_A$ positive modulation, NMDA antagonism, and 5-HT agonism (Grant, 1999). The compound nature of the ethanol cue has been suggested as the basis for a failure of ethanol to consistently substitute in benzodiazepine and barbiturate discriminations, producing asymmetrical generalizations (Grant, 1999; Vanover, 2000). The present data show that drugs acting at several different ligand-gated receptor systems generalize to pregnanolone, and demonstrate a possible heterogenous nature to the pregnanolone cue. Thus, the symmetrical pattern of substitution between pregnanolone and ethanol emphasizes the similarities in the discriminative stimulus effects of these compounds, and suggests that pregnanolone has behavioral activity representative of a broad-spectrum sedative hypnotic.

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