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Discriminative stimulus effects of pregnanolone in rats: role of training dose in determining mechanism of action

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Abstract

Rationale—Positive GABA_A modulators acting at different binding sites often produce similar behavioral effects; however, their effects are not identical. Actions of neuroactive steroids at other receptors, in addition to GABA_A receptors, might account for some differences between neuroactive steroids and other positive modulators, like benzodiazepines.

Objective—Multiple mechanisms of other drugs (e.g., ethanol) have been elucidated by comparing their discriminative stimulus effects across different training doses; the current study used that approach to examine the mechanisms of action of the neuroactive steroid pregnanolone.

Methods—Separate groups of rats (n=6-8/group) discriminated pregnanolone from vehicle while responding under a fixed-ratio 10 schedule of food presentation. Two groups initially discriminated 3.2 mg/kg; once stimulus control was established, the training dose was systematically decreased to 1.33 mg/kg in one group and increased to 7.5 mg/kg in the other group. Other rats discriminated either 1.33 or 7.5 mg/kg without training at another dose.

Results—Stimulus control was established in 24-28 sessions in all groups. Positive GABA_A modulators produced ~80% pregnanolone-lever responding, regardless of training dose; rank-order potency was flunitrazepam>midazolam>pregnanolone=pentobarbital. Ethanol produced some drug-lever responding (42%) only in rats discriminating 1.33 mg/kg whereas the N-methyl-D-aspartate (NMDA) receptor antagonist ketamine and the serotonin (5-HT₃) receptor agonist 1-(m-chlorophenyl)-biguanide (CPBG) occasioned predominantly vehicle-lever responding in all rats.

Conclusions—There was little difference in discriminative stimulus effects of pregnanolone across different training conditions, confirming a predominant, if not exclusive, role of GABA_A receptors in these effects of pregnanolone.

Keywords

pregnanolone; discriminative stimulus effects; rats

Neuroactive steroids act at distinct modulatory sites on γ -aminobutyric acid_A (GABA_A) receptors (Hosie et al. 2006). Their behavioral effects are similar to those produced by positive modulators acting at benzodiazepine sites (e.g., anxiolytic, sedative and anticonvulsant effects; Wieland et al. 1997; Vanover et al. 1999; Reddy and Rogawski 2001). Nevertheless, actions at distinct sites on GABA_A receptors confer some differences, particularly during chronic treatment when, for example, tolerance developed to the rate-decreasing (McMahon and France 2002b) and anticonvulsant effects of benzodiazepines (Gonsalves and Gallagher 1987; Löscher et al. 1996) and not to those of neuroactive steroids

(McMahon and France 2002a; Kokate et al. 1998). Pharmacologically selective procedures like drug discrimination can reveal differences among positive modulators after acute administration. These procedures are often used to determine mechanism of action (Shannon and Holtzman 1976; Winger and Herling 1982), and the conditions under which drugs are studied can impact results. For example, neuroactive steroids and the benzodiazepine lorazepam produced drug-lever responding in rats discriminating the barbiturate pentobarbital from vehicle, and neither neuroactive steroids nor pentobarbital produced drug-lever responding in rats discriminating lorazepam (Ator and Griffiths 1983; Ator et al. 1993). Although the reason for asymmetric substitution is not clear, these studies indicate the need to examine positive modulators under different conditions.

Besides training drug, other factors, such as training dose, can impact discrimination results. Training dose influences many aspects of the discrimination, including length of training to establish or maintain stimulus control as well as pharmacological selectivity; generally, smaller training doses increase the number of training sessions and decrease selectivity (Colpaert et al. 1980a; Colpaert and Janssen 1982). An exception to this rule occurs with training drugs that have multiple mechanisms of action. When the training drug acts through more than one receptor (e.g., ethanol), drugs that share at least one mechanism can mimic its effects, and drugs from different pharmacological classes produce drug-lever responding (Grant and Colombo 1993a; Shelton and Balster 1994). For example, in rats discriminating 1 g/kg of ethanol from vehicle, pentobarbital occasioned 98% responding on the ethanol lever and the N-methyl-D-aspartate (NMDA) receptor antagonist dizocilpine occasioned 67% ethanol-lever responding; in other rats discriminating a larger dose (2 g/kg), pentobarbital and dizocilpine produced 64 and 99% ethanol-lever responding, respectively (Grant and Colombo 1993b). Thus, the role of different receptors in the discriminative stimulus effects of ethanol varies with training dose.

In the current study, stimulus control was established with different training doses to determine whether receptors other than GABA_A receptors contribute to the discriminative stimulus effects of the neuroactive steroid pregnanolone. Other receptors that have been implicated in its effects include NMDA, serotonin (5-HT₃), and sigma₁ receptors (Maurice et al. 2001; Rupprecht et al. 2001; Dubrovsky 2005). In rats discriminating 5 mg/kg of pregnanolone from vehicle, dizocilpine and the 5-HT₃ receptor agonists CPBG and SR 57227A produced drug-lever responding (Engel et al. 2001); however, in other rats (Bai and Gerak 2011), only positive GABA_A modulators produced pregnanolone-lever responding, indicating that the discriminative stimulus effects of pregnanolone were mediated by GABA_A receptors. There were a number of procedural differences between studies, including training dose (Engel et al. 2001; Bai and Gerak 2011); the current study addressed the discrepancy by establishing stimulus control with different training doses in separate groups. The smallest and largest discriminable doses were determined by initially training two groups to discriminate 3.2 mg/kg of pregnanolone (Bai and Gerak 2011) and then systematically decreasing the training dose to 1.33 mg/kg in one group and increasing it to 7.5 mg/kg in a second group. This approach identified doses of pregnanolone that might be trained directly, and two additional groups were trained initially to discriminate doses that were determined to be the smallest and largest discriminable doses (1.33 and 7.5 mg/kg, respectively). In this study, the only procedural difference among groups was training dose, thereby eliminating the possibility that other factors contributed to differences in pharmacological selectivity across groups. By studying the widest possible range of training doses under otherwise identical conditions, this study was designed to determine the importance of receptors other than GABA_A receptors in the discriminative stimulus effects of pregnanolone.

Methods

Subjects

Twenty-nine male Sprague-Dawley rats (Harlan, Indianapolis, IN, USA) weighed 250-300 g on arrival and were housed individually on a 14 hr light/10 hr dark cycle. Weights were increased and then maintained at 320-330 g for the duration of the experiment with grain-based pellets during sessions (BioServ, Inc., Frenchtown, NJ, USA) and rodent chow (Harlan Teklad, Madison, WI, USA) in the home cage where free access to water was also provided. Rats were maintained in accordance with the Institutional Animal Care and Use Committee at The University of Texas Health Science Center at San Antonio, and with the 2011 *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources 2011).

Apparatus

Eight chambers were located in ventilated, sound-attenuating cabinets, and contained a response panel with 2 levers, 2 stimulus lights, a pellet dispenser and a food cup. An interface connected chambers to a computer that controlled experimental events and recorded data using MED-PC IV software (MED Associates, Inc., St. Albans, VT). White noise was present in the experimental room.

Procedure

Rats discriminated pregnanolone from vehicle while responding under a fixed-ratio 10 schedule of food presentation. Sessions consisted of a 10-min timeout, when responses had no programmed consequence, and a 30-min response period. Rats received vehicle or pregnanolone immediately before sessions. During response periods, stimulus lights were illuminated and 10 consecutive responses on the lever designated correct by the injection resulted in the delivery of food; responses on the other lever reset the response requirement on the correct lever. Lever designation was counterbalanced across rats. Sessions ended after 40 min or delivery of 100 food pellets, whichever occurred first. For each training dose, stimulus control was considered adequate when the following criteria were satisfied for five consecutive or six of seven training sessions: 80% of the total responses emitted on the correct lever and <10 responses on the incorrect lever prior to delivery of the first food pellet.

Sixteen rats were initially trained to discriminate 3.2 mg/kg of pregnanolone from vehicle (Bai and Gerak 2011). As rats satisfied the above criteria, they were alternately assigned to a group, and the training dose was progressively (0.25 log unit) decreased in one group and increased in the other. Rats were trained at each dose until they satisfied the criteria, and then the dose was again decreased or increased. Training doses were systematically changed in this manner until either the criteria were not satisfied by all rats within 70 sessions or response rates were decreased to <20% of the control rate for 10 sessions during which pregnanolone was administered; thereafter, the training dose was increased in rats discriminating the small dose or decreased in rats discriminating the large dose by 0.125 log unit. Training continued with these final training doses (1.33 and 7.5 mg/kg) until rats satisfied the criteria at which point testing began. This systematic approach established the smallest and largest training doses. A history of discriminating one training dose might impact results with a subsequent training dose, and this possibility was examined by directly training two additional groups to discriminate vehicle from either 1.33 or 7.5 mg/kg of pregnanolone; no other training doses were used in these two groups.

For all 4 groups, test sessions were identical to training sessions except that rats received vehicle or a dose of pregnanolone (0.24-7.5 mg/kg) before sessions and completing the

response requirement on either lever resulted in the delivery of food. Pregnanolone dose-effect curves were determined once in 6 (training dose: 7.5 mg/kg), 7 (training dose: 1.33 mg/kg and 3.2→7.5 mg/kg), and 8 (training dose: 3.2→1.33 mg/kg) rats. Test sessions were separated by at least 2 training sessions; if necessary, additional training sessions were conducted between tests until the criteria were satisfied for 2 of the 3 sessions immediately preceding a test session. After determination of the pregnanolone dose-effect curve, the procedure was changed from a single cycle to multiple cycles, thereby allowing a cumulative-dosing procedure to be used to generate dose-effect curves. Multiple-cycle sessions were divided into 15-min cycles. Each cycle began with a 10-min timeout followed by a 5-min response period. Stimulus lights were extinguished and the response period ended after 5 min or the delivery of 10 food pellets, whichever occurred first; any time remaining between the end of the response period and the end of the cycle was a timeout. Under the multiple-cycle procedure, some training sessions began with administration of the training dose of pregnanolone followed by sham cycles, with responding on the drug-appropriate lever resulting in the delivery of food for each cycle. For other sessions, 1-3 cycles during which vehicle or sham injections were given preceded pregnanolone administration. For still other sessions, vehicle and sham injections were given on each of up to 8 cycles. Training continued until the above criteria were satisfied during each cycle for 5 consecutive or 6 of 7 sessions. After the procedure was changed, adequate stimulus control could not be maintained in rats trained initially to discriminate 1.33 mg/kg and they were removed from the study.

Dose-effect curves were determined under the multiple-cycle procedure by giving vehicle during the first cycle followed by increasing doses of drug at the beginning of each subsequent cycle such that the cumulative dose increased up to a dose that resulted in either 80% responding on the pregnanolone-appropriate lever or decreased the rate of lever pressing to 20% of the control rate for individual rats. Injections were administered during the first minute of each cycle. Dose-effect curves were generated for 7 different drugs in each of the 3 remaining groups: pregnanolone (3.2→1.33 mg/kg: determined twice in 7 rats; 3.2→7.5 mg/kg: determined twice in 8 rats; 7.5 mg/kg: determined twice in 6 rats), the benzodiazepine midazolam (3.2→1.33 mg/kg: determined twice in 4 rats and once in 3 additional rats; 3.2→7.5 mg/kg: determined twice in 7 rats and once in 1 additional rat; 7.5 mg/kg: determined twice in 6 rats), the benzodiazepine flunitrazepam (3.2→1.33 mg/kg: determined once in 6 rats; 3.2→7.5 mg/kg: determined once in 7 rats; 7.5 mg/kg: determined once in 6 rats), pentobarbital (3.2→1.33 mg/kg: determined twice in 4 rats and once in 2 additional rats; 3.2→7.5 mg/kg: determined twice in 8 rats; 7.5 mg/kg: determined twice 6 rats), ethanol (3.2→1.33 mg/kg: determined twice in 5 rats and once in 2 additional rats; 3.2→7.5 mg/kg: determined twice in 5 rats and once in 2 additional rats; 7.5 mg/kg: determined twice in 5 rats and once in 1 additional rat), the NMDA receptor antagonist ketamine (3.2→1.33 mg/kg: determined twice in 4 rats and once in 2 additional rats; 3.2→7.5 mg/kg: determined twice in 4 rats and once in 4 additional rats; 7.5 mg/kg: determined twice in 6 rats), and the 5-HT₃ receptor agonist 1-(m-chlorophenyl)-biguanide (CPBG; 3.2→1.33 mg/kg: determined twice in 4 rats and once in 3 additional rats; 3.2→7.5 mg/kg: determined twice in 6 rats and once in 2 additional rats; 7.5 mg/kg: determined twice in 6 rats). During this period of testing, rate-decreasing effects were evident following administration of the training dose in each rat discriminating 7.5 mg/kg from vehicle. This apparent increased sensitivity to pregnanolone has been observed previously when large training doses of pregnanolone were used (rate-decreasing effects: Gerak et al. 2008; seizures: Shannon et al. 2005). When these rate-decreasing effects were observed, the training dose was temporarily decreased to 5.6 mg/kg and the frequency of vehicle training sessions was increased. Once rats received all 10 food pellets following administration of 5.6 mg/kg, the training dose was increased to 7.5 mg/kg and the criteria satisfied for 2 consecutive training sessions before testing resumed. Also during this period, some rats

discriminating 1.33 mg/kg from vehicle were not satisfying the criteria under the multiple-cycle procedure; consequently, their training sessions were changed to a single, 15-min cycle, although test sessions continued to be conducted using the multiple-cycle procedure.

Drugs

Initially, pregnanolone (5 β -pregnan-3 α -ol-20-one; Steraloids, Inc., Newport, RI, USA) was dissolved in 45% (w/v) 2-hydroxypropyl- γ -cyclodextrin (Sigma-Aldrich Co., St. Louis, MO, USA). During this study, 2-hydroxypropyl- γ -cyclodextrin was not available, so the vehicle was changed to 45% (w/v) of 2-hydroxypropyl- β -cyclodextrin (Sigma-Aldrich Co., St. Louis, MO, USA). Dose-effect curves determined before and after the change in vehicle were similar; consequently, 2-hydroxypropyl- β -cyclodextrin was used for the remainder of the study. Midazolam hydrochloride (Bedford Laboratories, Bedford, OH), ketamine hydrochloride (racemate; Fort Dodge Laboratories, Fort Dodge, IA), and ethanol were purchased as commercially prepared solutions and diluted with sterile 0.9% saline. Flunitrazepam (Sigma-Aldrich Co., St. Louis, MO) was dissolved in a vehicle containing 20% emulphor, 10% ethanol, and 70% sterile 0.9% saline. Pentobarbital sodium and CPBG (Sigma-Aldrich Co., St. Louis, MO) were dissolved in sterile 0.9% saline. Drugs were administered i.p. typically in a volume of 1 ml/kg body weight with the exception of ethanol for which a 15% v/v solution was used for all injections. Doses are expressed in the form listed above in mg/kg or g/kg body weight.

Data analyses

The median number of sessions required for rats to satisfy the criteria under their first training dose was compared among groups using a Kruskal-Wallis test followed by a Dunn's Multiple Comparison test. Stimulus control was further compared among groups by examining the percentage of training sessions during which the criteria were satisfied at different times during the study. Under the single-cycle procedure, the percentage was determined for the 20 training sessions that immediately preceded the change to multiple cycles unless fewer than 20 sessions were conducted with the final training dose in which case all training sessions were included. Under the multiple-cycle procedure, the 20 training sessions used to calculate the percentage occurred immediately after the first test except for two rats trained initially to discriminate 1.33 mg/kg. In one rat, no test sessions were conducted under the multiple-cycle procedure and all 16 training sessions were used; in a second rat, only 17 training sessions occurred after the first test session and those 17 sessions along with 3 sessions that preceded the test were used. Percentages were compared among groups using one-way analysis of variance followed by Tukey's Multiple Comparison Test and within groups using a paired t-test.

Control response rates (mean $\pm$ 1 SEM), obtained during training sessions in which vehicle or sham injections were given and rats satisfied the criteria, were compared to rates obtained during training sessions in which the final training dose of pregnanolone was given. For individual rats, rates were averaged across 5 training sessions; when the multiple-cycle procedure was used, rates were first averaged across cycles within a session and then averaged across sessions. Rates were compared among groups using a one-way analysis of variance, whereas rates obtained under different training conditions within a group were compared using a one-way repeated measures analysis of variance; in both cases, analysis of variance was followed by Tukey's Multiple Comparisons Test. Discrimination data were not included in the analyses when response rate was <20% of control for an individual rat, and rates had to be >20% of control for at least half of the rats in order to plot discrimination data for that particular dose.

The percentages of responses on the drug-appropriate lever were plotted as a function of dose. For drugs that produced 80% drug-lever responding, slopes of dose-effect curves were compared among groups by fitting straight lines to the linear portion of individual dose-effect curves using GraphPad Prism version 5.01 for Windows (GraphPad Software, San Diego, CA). The linear portion included one data point below 25%, one point above 75%, and all points in between. Slopes were compared among groups using an F-ratio test, and when slopes were not different, a simpler model was used by selecting a common slope. The dose of each drug needed to produce 50% drug-appropriate responding (ED_{50}) was estimated for individual rats using linear regression, and ED_{50} values were then compared across groups using a one-way analysis of variance followed by Tukey's Multiple Comparisons Test. Significance was set at $P < 0.05$.

Results

Stimulus control was readily established with pregnanolone regardless of training dose, and the number of training sessions required to satisfy criteria (24-28 sessions) did not vary significantly among the 4 groups. Rats that were initially trained to discriminate 3.2 mg/kg of pregnanolone did not differ in the number of training sessions needed to satisfy the criteria with the final training dose of pregnanolone, regardless of whether the training dose was decreased to 1.33 mg/kg or increased to 7.5 mg/kg. Although acquisition of stimulus control was similar across groups, maintenance of stimulus control was not. Rats that initially discriminated either 3.2 or 7.5 mg/kg satisfied the criteria during 82% of training sessions whereas rats trained initially to discriminate 1.33 mg/kg satisfied the criteria during significantly fewer training sessions (i.e., 73%; $F(3,28)=11.15$, $P < 0.0001$). The percentage of training sessions during which rats satisfied the criteria was further decreased in both groups discriminating 1.33 mg/kg when the procedure was changed from single to multiple cycles (i.e., 56% in 1.33 mg/kg only: $t(6)=5.07$, $P=0.0023$; 71% in 3.2 mg/kg $\rightarrow$ 1.33 mg/kg: $t(6)=4.22$, $P=0.0056$); the percentage did not change in either group discriminating 7.5 mg/kg. Under the multiple-cycle procedure, the percentage of training sessions during which the criteria were satisfied was smaller in rats trained initially to discriminate 1.33 mg/kg, as compared to all other groups ($F(3,27)=17.18$, $P < 0.0001$).

In vehicle training sessions, response rates were not significantly different across groups (Table 1). For rats discriminating 7.5 mg/kg, rates following vehicle administration were not significantly changed when the procedure was switched from single to multiple cycles. In rats whose training dose was decreased to 1.33 mg/kg, pregnanolone did not alter response rates, as compared to vehicle, although rates were decreased during both drug and vehicle sessions when the procedure was changed to multiple cycles ($F(3,27)=7.91$, $P=0.0014$). Finally, in rats whose training dose was increased to 7.5 mg/kg, pregnanolone decreased response rates, as compared to vehicle ($F(3,31)=4.63$, $P=0.012$); reduced rates were evident in rats responding under the single-cycle procedure although pregnanolone did not decrease rates when the procedure was changed to multiple cycles. Repeated administration of 7.5 mg/kg of pregnanolone decreased responding in all rats, although this effect occurred at different points in the experiment. For some rats, the training dose decreased rates 2 months after their first training session with 7.5 mg/kg, whereas for other rats, rate-decreasing effects were not evident for more than a year later.

In rats responding under the single-cycle procedure, pregnanolone dose dependently increased drug-lever responding. A dose of 0.75 mg/kg of pregnanolone produced, on average, 80% drug-lever responding in rats whose training dose was decreased to 1.33 mg/kg (circles, upper left panel, Figure 1) whereas 1.33 mg/kg was needed to produce 80% drug-lever responding in rats trained initially to 1.33 mg/kg (diamonds, upper left panel, Figure 1). An even larger dose (4.2 mg/kg) produced 80% drug-lever responding in all rats

discriminating 7.5 mg/kg from vehicle (triangles and squares, upper left panel, Figure 1). Slopes of straight lines fitted to the four curves were not different. ED_{50} values for pregnanolone (± 1 SEM) were not different in rats whose training dose was decreased to 1.33 mg/kg ($ED_{50} = 0.51 \pm 0.09$ mg/kg) and those trained initially to 1.33 mg/kg ($ED_{50} = 0.84 \pm 0.11$ mg/kg); likewise, ED_{50} values were not different in rats whose training dose was increased to 7.5 mg/kg ($ED_{50} = 2.32 \pm 0.60$ mg/kg) and those trained initially to 7.5 mg/kg ($ED_{50} = 2.83 \pm 0.37$ mg/kg). Differences in ED_{50} values were detected between rats discriminating 1.33 mg/kg and those discriminating 7.5 mg/kg ($F(3,25) = 8.79$, $P = 0.0004$). When the procedure was changed to cumulative dosing, the potency of pregnanolone decreased with 3.2 mg/kg needed to produce 80% drug-lever responding in rats discriminating 1.33 mg/kg and 10 mg/kg needed in rats discriminating 7.5 mg/kg (upper right panel, Figure 1). A common slope best fit the three lines; while ED_{50} values were not different between the two groups discriminating 7.5 mg/kg, ED_{50} values were significantly different between rats discriminating 1.33 mg/kg and those discriminating 7.5 mg/kg ($F(2,18) = 13.98$, $P = 0.0002$). Comparing dose-effect curves within each group revealed that neither the slopes nor the potency of pregnanolone, as determined by ED_{50} values, were changed when the procedure was switched from single to multiple cycles. These doses of pregnanolone did not markedly alter response rates under these conditions (lower panels, Figure 1).

Drugs acting at other sites on $GABA_A$ receptors also produced pregnanolone-lever responding in all rats (upper panels, Figure 2). Slopes of dose-effect curves for each positive modulator were not different across groups, regardless of training dose. ED_{50} values for each positive modulator were smaller in rats whose training dose was decreased to 1.33 mg/kg, as compared to ED_{50} values in rats whose training dose was increased to 7.5 mg/kg; ED_{50} values in rats initially trained to 7.5 mg/kg were not different from either of the other two groups (midazolam: $F(2,18) = 6.12$, $P = 0.0094$; flunitrazepam: $F(2,16) = 4.87$, $P = 0.022$; pentobarbital: $F(2,15) = 8.27$, $P = 0.0038$). The benzodiazepines did not markedly alter response rates (lower left and center panels, Figure 2). While 5.6 mg/kg of pentobarbital had little effect on rates in any group, 10 mg/kg of pentobarbital, which was not studied in rats discriminating 1.33 mg/kg, decreased rates to $<60\%$ of control in rats discriminating 7.5 mg/kg (lower right panel, Figure 2).

Other drugs did not produce 80% pregnanolone-lever responding (upper panels, Figure 3). Ethanol (1 g/kg) produced, on average, 42% pregnanolone-lever responding in rats discriminating 1.33 mg/kg and 20% pregnanolone-lever responding in other rats. That dose of ethanol decreased mean rates to 0.68 ± 0.19 responses/sec and larger doses eliminated responding (lower left panel, Figure 3). Up to doses that eliminated responding, rats responded on the vehicle lever after administration of ketamine and CPBG (center and right panels, Figure 3).

Discussion

Many effects of neuroactive steroids are mediated by $GABA_A$ receptors, although these drugs appear to have actions at other receptors (Maurice et al. 2001; Rupprecht et al. 2001; Dubrovsky 2005). One procedure in which receptors other than $GABA_A$ receptors have been implicated is drug discrimination. Specifically, while the discriminative stimulus effects of the neuroactive steroid pregnanolone seem to be mediated predominantly by $GABA_A$ receptors, one study suggested that several receptor types might contribute to these effects, including NMDA and 5-HT₃ receptors (Engel et al. 2001). Other studies have shown that the discriminative stimulus effects of pregnanolone are mediated only by $GABA_A$ receptors (Bai and Gerak 2011); however, procedural differences between studies, particularly differences in training dose, might account for the discrepancy. To determine

whether multiple mechanisms contribute to the discriminative stimulus effects of pregnanolone, the smallest and largest discriminable doses were determined by initially training rats to discriminate 3.2 mg/kg, a dose used successfully in previous experiments (Bai and Gerak 2011), and then systematically decreasing or increasing the training dose in separate groups. The smallest and largest discriminable doses differed by 6-fold. Importantly, the only procedural difference among groups was training dose, thereby eliminating the possibility that differences other than training dose could account for discrepancies.

The discriminative stimulus effects of pregnanolone were pharmacologically selective in rats, regardless of training dose. Positive modulators produced 80% drug-lever responding, and their potencies were predicted by training dose, with smaller doses needed in rats discriminating 1.33 mg/kg, as compared to rats discriminating 7.5 mg/kg. Thus, increasing the training dose of pregnanolone resulted in quantitative and not qualitative differences. In contrast, when the training dose of ethanol was increased, there was a decrease in ethanol-lever responding produced by positive modulators, indicating a qualitative change in the ethanol discriminative stimulus and supporting the involvement of multiple receptors (Grant and Colombo 1993b). That the pregnanolone discriminative stimulus does not change qualitatively with training dose suggests a predominant, and possibly exclusive, contribution of GABA_A receptors to the discriminative stimulus effects of pregnanolone. Moreover, while receptors other than GABA_A receptors have been implicated in mediating effects of pregnanolone, drugs acting at two of those receptors, NMDA and 5-HT₃, did not produce pregnanolone-lever responding. Thus, in rats discriminating pregnanolone from vehicle, the discriminative stimulus effects of pregnanolone are pharmacologically selective for GABA_A receptors, regardless of training dose, suggesting that receptors other than GABA_A receptors contribute little or not at all to these effects.

The discrepancy between studies in substitution profile of pregnanolone (current study; Engel et al. 2001) could be due to several factors. First, drugs other than positive modulators produced pregnanolone-lever responding only when rates were low; discrimination data were included in the analyses even when responding was markedly decreased (at least one reinforcer delivered under a fixed ratio 15 schedule during a 30-min response period; Engel et al. 2001), which could disrupt stimulus control. When discrimination data obtained under very low rates were excluded, as in the current study, only positive modulators produced pregnanolone-lever responding. Second, NMDA receptor antagonists produce intermediate effects in rats discriminating pharmacologically different drugs; this effect appears to be due to a general disruption in stimulus control and occurs when rates are decreased (Koek et al. 1995). This effect might have contributed to pregnanolone-lever responding produced by some drugs in Engel et al. (2001), including dizocilpine and SKF 10047, which has actions at NMDA and sigma₁ receptors (Mori et al. 2001), although it did not alter results obtained with ketamine in the current study.

While reduced pharmacological selectivity can indicate that multiple mechanisms contribute to discriminative stimulus effects, it can also be indicative of other things. For example, decreasing the training dose of any drug can reduce pharmacological selectivity (Colpaert et al. 1980a). Small training doses can impact drug discrimination procedures in other ways, such as increasing the number of sessions required to establish or maintain stimulus control (Colpaert et al. 1980a, b; Colpaert and Janssen 1982). In the current study, while pharmacological selectivity was not markedly different in rats discriminating small and large doses of pregnanolone, stimulus control was decreased in rats discriminating the small dose, as compared to rats discriminating the large dose. Establishing stimulus control was not different among groups, with the number of sessions needed to satisfy the testing criteria initially similar; however, maintenance of stimulus control was significantly decreased in

rats trained initially to discriminate 1.33 mg/kg, as indicated by the percentage of training sessions during which criteria were satisfied. In addition, this percentage decreased over time in both groups discriminating 1.33 mg/kg and did not change in either group discriminating 7.5 mg/kg. Although little is known about the consequences of acquiring a discrimination at one training dose and then establishing another discrimination in the same animal with a different dose, the difficulty in maintaining stimulus control suggests that the pregnanolone discriminative stimulus is weaker when the smaller training dose was used. In addition, ethanol produced slightly more pregnanolone-lever responding in rats discriminating 1.33 mg/kg, as compared to rats discriminating 7.5 mg/kg. While any drug-lever responding is likely due to actions of ethanol at GABA_A receptors, this subtle difference in pharmacological selectivity among the groups, along with changes in stimulus control across the experiment, suggests that 1.33 mg/kg of pregnanolone produces a weaker stimulus than that produced by either 3.2 or 7.5 mg/kg.

In summary, across different training doses, the pregnanolone discriminative stimulus is mediated predominantly, if not exclusively, by GABA_A receptors; other receptors, such as NMDA and 5-HT₃, do not play a substantial role. Actions of neuroactive steroids at receptors other than GABA_A receptors do not account for differences between neuroactive steroids and benzodiazepines. Other possible explanations for these differences include the presence of two binding sites for neuroactive steroids on GABA_A receptors with only one benzodiazepine site and the presence of neuroactive steroid sites on a larger number of GABA_A receptors, as compared to the number of receptors containing benzodiazepine sites (Doble and Martin 1992; Hosie et al. 2006). Thus, differences in actions at GABA_A receptors might be entirely responsible for differences in the behavioral effects of neuroactive steroids and benzodiazepines.

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Figure 1.

Pregnanolone dose-effect curves determined in rats when a single dose was administered during each session (left panels) or when a cumulative-dosing procedure was used (right panels). Each symbol represents the effects of pregnanolone in rats discriminating different doses of pregnanolone from vehicle. Ordinates, upper panels: percentage of total responses emitted on the pregnanolone lever; lower panels: response rates (responses/sec). Abscissa: dose in mg/kg. Points above V represent the effects of vehicle.

Figure 2.

Dose-effect curves for the positive GABA_A modulators midazolam, flunitrazepam and pentobarbital determined in rats responding under a cumulative-dosing procedure. Different symbols represent effects in rats discriminating different doses of pregnanolone from vehicle. Ordinates, upper panels: percentage of total responses emitted on the pregnanolone lever; lower panels: response rates (responses/sec). Abscissae: dose in mg/kg. Points above V represent the effects of vehicle.

Figure 3.

Dose-effect curves for ethanol, ketamine and CPBG determined in rats responding under a cumulative-dosing procedure. Different symbols represent the effects in rats discriminating different doses of pregnanolone from vehicle. Ordinates, upper panels: percentage of total responses emitted on the pregnanolone lever; lower panels: response rates (responses/sec). Abscissae: dose in mg/kg. Points above V represent the effects of vehicle.

Table 1

Mean response rates (± 1 SEM) obtained in the absence of drug and following administration of the training dose of pregnanolone. Values represent the mean rates obtained across 5 training sessions during which the criteria were satisfied.

Responses/sec (± 1 SEM)				
Training dose (mg/kg)	Vehicle (single cycles)	Pregnanolone (single cycles)	Vehicle (multiple cycles)	Pregnanolone (multiple cycles)
3.2 $\rightarrow$ 1.33	1.31 (± 0.14)	1.45 (± 0.10)	1.08 (± 0.08) [*]	1.17 (± 0.08) [#]
3.2 $\rightarrow$ 7.5	1.28 (± 0.09)	0.85 (± 0.08) [*]	1.09 (± 0.11)	0.86 (± 0.13)
1.33	1.53 (± 0.09)	1.45 (± 0.08)	n.s.	n.s.
7.5	1.27 (± 0.09)	1.24 (± 0.17)	1.03 (± 0.10)	1.15 (± 0.14)

n.s. not studied

^{*} significantly different from rates obtained after administration of vehicle in rats responding under the single-cycle procedure.

[#] significantly different from rates obtained after administration of pregnanolone in rats responding under the single-cycle procedure.