Ethanol-Like Discriminative Stimulus Effects of Endogenous Neuroactive Steroids: Effect of Ethanol Training Dose and Dosing Procedure

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ABSTRACT

A number of endogenous steroids exhibit rapid, nongenomic effects on the central nervous system and are called neuroactive steroids. The rapid mechanisms of action include modulation of γ-aminobutyric acid type A (GABA_A) and N-methyl-D-aspartate (NMDA) receptors, which are two receptors implicated in the behavioral effects of ethanol. It was hypothesized that neuroactive steroids that positively modulate GABA_A receptors or negatively modulate NMDA receptors, analogous to the actions of ethanol, would produce discriminative stimulus effects similar to ethanol. Two groups of male Long-Evans rats (n = 6–8/group) were trained to discriminate between 1.0 or 2.0 g/kg ethanol (i.g.) and water (i.g.). The neuroactive steroids allopregnanolone, pregnanolone, epipregnanolone, allotetrahydrodeoxycorticosterone, pregnanolone sulfate, epipregnanolone sulfate, dehydroepiandrosterone, dehydroepiandrosterone sulfate, pregnenolone, and pregnenolone sulfate (PS), all administered i.p., were tested for substitution with acute and cumulative dosing procedures (n = 4–8/steroid). The GABA_A-positive modulatory steroids allopregnanolone, pregnanolone, and allotetrahydrodeoxycorticosterone substituted for ethanol, as did the low-efficacy steroid 3β,5β-P. GABA_A-negative modulators, such as dehydroepiandrosterone sulfate and PS, and all of the NMDA modulators tested, including PS, pregnanolone sulfate, and epipregnanolone sulfate, did not substitute for ethanol. These results show that certain endogenously occurring neuroactive steroids produce discriminative stimulus effects similar to those of ethanol.

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ABBREVIATIONS: CNS, central nervous system; 3α,5α-P, 3α-hydroxy-5α-pregnan-20-one (allopregnanolone); 3α,5β-P, 3α-hydroxy-5β-pregnan-20-one (pregnanolone); 3α,5α-THDOC, 3α,21-dihydroxy-5α-pregnan-20-one (allotetrahydrodeoxycorticosterone); 3β,5β-P, 3β-hydroxy-5β-pregnan-20-one (epipregnanolone); GABA, γ-aminobutyric acid; NMDA, N-methyl-D-aspartate; 3α,5β-PS, 3α-hydroxy-5β-pregnan-20-one sulfate (pregnanolone sulfate); 3β,5β-PS, 3β-hydroxy-5β-pregnan-20-one sulfate (epipregnanolone sulfate); PS, 3β-hydroxy-pregnan-5-en-20-one sulfate (pregnenolone sulfate); DHEA, 3β-hydroxy-androst-5-en-17-one sulfate (dehydroepiandrosterone sulfate); pregnenolone, 3β-hydroxy-pregnan-5-en-20-one; DHEAS, 3β-hydroxy-androst-5-en-17-one (dehydroepiandrosterone); FR20, Fixed ratio 20.
this may result from CNS conversion of progesterone to its metabolites. Indeed, the progesterone metabolites 3α-hydroxy-5β-pregn-20-one sulfate (pregnenolone sulfate, or 3α,5β-PS) and 3β-hydroxy-5β-pregn-20-one sulfate (epipregnenolone sulfate, or 3β,5β-PS) were found to inhibit NMDA-mediated calcium responses (Irwin et al., 1994; Park-Chung et al., 1994). Although the behavioral profiles of these naturally occurring steroids have yet to be investigated, 3α,5β-P hemisuccinate, a synthetic analog of 3α,5β-PS, inhibits NMDA receptor function and exhibits sedative, anti-convulsant, and analgesic properties (Weaver et al., 1997). Attenuation of NMDA-mediated activity also is implicated in the behavioral effects of ethanol (see Grant, 1994).

Some endogenous neuroactive steroids modulate GABA<sub>α</sub> and/or NMDA receptors in a manner opposite to that of ethanol. 3β-Hydroxyprogren-5β-20-ketone sulfate (pregnenolone sulfate, or PS) and 3β-hydroxyandrosten-5β-17-ketone sulfate (dehydroepiandrosterone sulfate, or DHEAS) inhibit GABA<sub>α</sub> receptor function (Carette and Poulain, 1984; Majewska, 1992). Furthermore, PS positively modulates NMDA receptors, although somewhat less potently than it inhibits GABA<sub>α</sub> receptor function (Wu et al., 1991). The behavioral effects of PS and DHEAS are consistent with inhibition of GABA<sub>α</sub> receptor function and exhibits sedative, anti-convulsant, and analgesic properties (Weaver et al., 1997). Attenuation of NMDA-mediated activity also is implicated in the behavioral effects of ethanol (see Grant, 1994)....

Materials and Methods

Subjects. Adult male Long-Evans rats (n = 6–8/group; Harlan Industries, Indianapolis, IN) were maintained at 350 g (± 10 g) for the duration of the study. Daily rations were comprised of food obtained during operant sessions, followed by 10 to 14 g/day of rat chow (Prolab 3000; Agway Inc., Syracuse, NY) at least 1 h after the session. Animals were individually housed in standard clear plastic cages (23.0 × 45.0 × 20.0 cm) within a temperature- and humidity-controlled vivarium. A 12-h light/dark cycle was in effect, with lights on at 0600 h. Water always was available in the home cage. All rats were experimentally naive at the start of the investigation. The protocol for this study was reviewed and approved by the Wake Forest University Animal Care and Use Committee, in compliance with North Carolina state and federal regulations.

Apparatus. Rats were trained and tested in operant chambers (28 cm × 22 cm × 21 cm; Coulbourn Instruments, Allentown, PA) enclosed in ventilated, sound-attenuating cubicles. The operant panel was located on the right-hand wall of each chamber. Each panel contained a centrally located house light positioned above two sets of stimulus lights, which were located above the two retractable levers (4.5 cm wide, protruding 3 cm from the wall and 7 cm above the grid floor). A food cup, into which 45 mg of food pellets (P. J. Noyes Company Inc., Lancaster, NH) was dispensed, was positioned equidistant between the two levers. Chamber operation and data acquisition were conducted by a computer system (Dell System 310, Austin, TX; Med Associates Inc., East Fairfield, VT).

Discrimination Training. Each rat was trained to press the levers with food reinforcement, as described previously (Grant et al., 1997). The terminal schedule of reinforcement was a fixed ratio of 20 (FR20). The period between placement of the rat into the darkened chamber and the start of the training session (i.e., pretreatment time) was 30 min. The session began with the illumination of the house light and extension of the available lever(s). Training sessions ended after 25 pellet presentations or 30 min. Experimental sessions were conducted once per day, typically 6 days/week, for each rat.

Once response rates on the two levers were stable (i.e., five consecutive sessions in which response rates were within 2 S.D. of the mean), exposure to the training conditions began. For 5 days each, water (2.3 or 4.7 ml i.g.) and ethanol (1.0 or 2.0 g/kg i.g.; 15%) were administered with only the condition-appropriate lever available. The ethanol-appropriate lever was counterbalanced within each group. Under the FR20, 20 consecutive responses on the lever resulted in food pellet delivery. Sessions ended after 25 pellet presentations or 30 min.

After vehicle or drug administration only the condition-appropriate lever available, discrimination training began. Ethanol or water was administered i.g. before placement of the rat in the operant chamber. After the 30-min pretreatment time, both levers were presented and food delivery was contingent upon condition-appropriate lever available. The ethanol-appropriate lever was counterbalanced within each group. Under the FR20, 20 consecutive responses on the lever resulted in food pellet delivery. Sessions ended after 25 pellet presentations or 30 min.

Stimulus Substitution Testing. Once the discriminations were reliably established, stimulus substitution tests were conducted approximately twice per week (usually Wednesday and Saturday). Training sessions occurred on the intervening days. Test sessions were conducted when the criteria mentioned above were met for two consecutive training sessions. If performance during training sessions failed to meet these criteria, discrimination training continued until the criteria were met for three consecutive training sessions. For all test sessions, drugs were administered i.p. and 20 consecutive responses on either lever resulted in food delivery.
Ethanol and neuroactive steroids were tested for substitution with an acute dosing procedure in the 1.0 and 2.0 g/kg ethanol training groups. Using this dosing regimen, one dose of a drug was examined during each test session. For each animal, stimulus substitution testing started with the administration of an intermediate drug dose. The lowest dose tested was that which resulted in at least 50% below the vehicle rate obtained from the preceding ethanol or water session, whichever was observed first.

In addition, ethanol and neuroactive steroid dose-response functions were determined in the 2.0 g/kg ethanol training group in single test sessions with a cumulative dosing procedure. A cumulative dosing test session was comprised of up to eight trials, each starting after an injection of vehicle or a dose of the test drug. At any time point, the dose of drug injected was such that when added to preceding doses, it yielded the desired cumulative dose of the drug. Specifically, after i.p. injection of vehicle, the rat was placed into the operant chamber. The first trial began after a 5-min pretreatment period and terminated after five pellet presentations or 5 min. Then the rat was removed from the operant chamber briefly to administer the first dose of drug 10 min after the preceding injection. The second trial began after another 5-min pretreatment period (i.e., 10 min after the beginning of the previous trial) and terminated after five pellet presentations or 5 min. A cumulative dosing test session was completed after administration of a predetermined cumulative dose (i.e., 56 mg/kg for steroids) or after a trial in which ≥80% ethanol-appropriate responding occurred or the response rate was at least 50% below the vehicle rate obtained during the initial vehicle trial, whichever was observed first.

Under acute and/or cumulative test conditions, the effects of selected i.p. doses of ethanol (0.25–2.0 g/kg), 3α,5α-P (0.03–56.0 mg/kg), 3α,5β-P (0.03–30.0 mg/kg), 3β,5α-THDOC (3.0–56.0 mg/kg), 3α,5β-PS (3.0–56.0 mg/kg), 3β,5β-PS (10–56 mg/kg), PS (3.0–56.0 mg/kg), 3β-hydroxyandrost-5-en-17-one (DHEA; 5.6–56.0 mg/kg), and DHEAS (10–56 mg/kg) were tested. Doses were calculated as the base, typically varied by quarter- or eighth-log units, and were tested once per rat. Neuroactive steroids were examined in a minimum of four rats under 1.0 and 2.0 g/kg ethanol acute dosing and 2.0 g/kg ethanol cumulative dosing test conditions. The rats tested were randomized across steroids and across test conditions.

**Drugs.** Ethanol (95%; The Warner-Graham Company, Cockeysville, MD) was diluted with tap water to a concentration of 15% (w/v). Neuroactive steroids synthesized by the procedure of Purdy et al. (1998) included 3α,5α-P, 3α,5β-P, 3α,5α-THDOC, 3α,5β-PS, and 3β,5β-PS. Other steroids were obtained from Sigma Chemical Co., St. Louis, MO (3β,5β-P, pregnenolone, DHEA, and DHEAS) and Research Biochemicals International, Natick, MA (PS). 3α,5α-P, 3α,5β-P, 3β,5α-THDOC, and 3β,5β-PS were suspended in sterile Intralipid emulsion (20%; Kabi Pharmacia, Clayton, NC). 3α,5β-PS, pregnenolone and DHEA were suspended in 45% (w/v) 2-hydroxypropyl-γ-cyclodextrin (Research Biochemicals International in sterile water. PS was suspended in 1% (v/v) Tween 80 in saline. DHEAS was dissolved in saline.

**Data Analysis.** The percentage of total responses occurring on the ethanol-appropriate lever and the response rate was determined for each rat during each test session or test trial. Complete substitution of a test drug for the discriminative stimulus effects of 1.0 or 2.0 g/kg ethanol was defined as ≥80% total session or trial responding on the ethanol-appropriate lever. Substitution and rate suppression ED response values were determined for animals responding ≥80% on the ethanol-appropriate lever and exhibiting ≥50% reduction in response rates compared with control values, respectively. Individual substitution and rate suppression ED values were calculated by regression analysis of the linear portion of the dose-effect curve with log-transformed data. If only two points comprised the linear portion of the dose-effect curve, ED values were estimated (SigmaPlot 4.16; Abacus Concepts, Inc., Berkeley, CA). Substitution ED values, response rates, and the percentage of rats tested that showed complete substitution of a drug for ethanol were compared by factorial (between-group) and repeated measures (within-group) ANOVA (p < .05; StatView 4.5; Jandel Scientific, San Rafael, CA). Between training groups, differences in the rank-order potency of neuroactive steroids to substitute for ethanol were examined with the Mann-Whitney U test (p < .05; StatView 4.5). Although response rate data from all test sessions were included in the analyses, ethanol-appropriate responding data were included only if a rat obtained at least one reinforcer during the test session or trial.

**Results.** Of the 16 rats that began discrimination training, 14 rats successfully acquired the 1.0 g/kg (n = 8) and 2.0 g/kg (n = 6) ethanol discriminations. Two rats became ill and were removed from the experiment. An average (± S.D.) of 68 (± 28) and 43 (± 11) training sessions were required for acquisition of the 1.0 and 2.0 g/kg ethanol discriminations, respectively. Ethanol administration (tested acutely or cumulatively) resulted in complete substitution for the 1.0 or 2.0 g/kg ethanol cue in all rats. The threshold dose for acute injections of ethanol to completely substitute differed slightly between the 1.0 g/kg ethanol-trained rats (0.75 g/kg) and the 2.0 g/kg ethanol-trained animals (1.0 g/kg; Fig. 1). However, the threshold substitution dose was 1.0 g/kg ethanol for either test dosing procedure in the 2.0 g/kg ethanol-trained group (Fig. 1). The potency of ethanol to substitute was similar across training groups and test dosing procedures (Table 1). Across the ethanol doses tested under acute conditions (0.5–
TABLE 1
Mean (±S.D.) and range of ED_{50} values of test compounds that completely substituted for ethanol under different test conditions

<table>
<thead>
<tr>
<th>Test Compounds</th>
<th>1.0 g/kg Ethanol versus H_{2}O—Acute Test Dosing</th>
<th>2.0 g/kg Ethanol versus H_{2}O—Acute Test Dosing</th>
<th>2.0 g/kg Ethanol versus H_{2}O—Cumulative Test Dosing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>0.53 (±0.20) (0.18–0.71 g/kg)</td>
<td>0.67 (±0.19) (0.38–0.88)</td>
<td>0.74 (±0.34) (0.38–1.25)</td>
</tr>
<tr>
<td>3α,5α-P</td>
<td>5.6 (±5.7) (0.56–13.0 mg/kg)</td>
<td>NA^b</td>
<td>17.7 (±6.6) (7.8–21.0 mg/kg)</td>
</tr>
<tr>
<td>3α,5β-P</td>
<td>3.7 (±3.0) (0.053–7.6 mg/kg)</td>
<td>10.7 (±9.1) (0.56–22.0 mg/kg)</td>
<td>9.2 (±3.3) (7.2–13.0 mg/kg)</td>
</tr>
<tr>
<td>3α,5α-THDOC</td>
<td>10.1 (±3.4) (7.0–13.0 mg/kg)</td>
<td>11.5 (±10.1) (4.1–23.0 mg/kg)</td>
<td>9.4 (±4.4) (4.1–13.0 mg/kg)</td>
</tr>
<tr>
<td>3β,5β-P</td>
<td>6.4 (±2.3) (1.7–7.8 mg/kg)</td>
<td>7.5 (±6.4) (0.54–13.0 mg/kg)</td>
<td>31.0 (±11.6) (21.0–41.0 mg/kg)</td>
</tr>
</tbody>
</table>

Values represent mean ED_{50} values (±S.D.) and the range of ED_{50} values from 3 to 8 rats per test compound.

^a NA indicates that a test compound did not substitute completely for ethanol in at least three of the rats tested. All test compounds were administered i.p.

1.0 g/kg), average response rates were not different from average control rates (range of ethanol versus control rates: 0.40–1.81 versus 0.66–2.07 responses/s). Higher ethanol doses were administered under cumulative dosing test conditions, resulting in reduced response rates [mean (± S.D.) rates of 1.33 (± .46) versus 0.01 (± .01) under control versus cumulative 2.0 g/kg ethanol administration], and an average rate-suppression ED_{50} value [95% confidence interval (CI)] of 1.49 g/kg (1.23–1.75). Acute and cumulative vehicle injections resulted in less than 5% average ethanol-appropriate responding (range: 0–9%; Fig. 1) and did not alter average response rates compared with control values (range of acute vehicle rates versus control rates, 0.70–1.75 versus 0.67–1.94 r/s; range of cumulative vehicle rates versus control rates, 0.47–2.33 versus 0.57–1.99 responses/s).

Acutely administered GABA_{A}-positive modulatory steroids 3α,5α-P, 3α,5β-P, and 3α,5α-THDOC, as well as the low efficacy steroid 3β,5β-P, substituted for ethanol in rats trained to discriminate 1.0 g/kg ethanol (Fig. 2). Individual steroids completely substituted for 1.0 g/kg ethanol in 80 to 100% of the animals tested (Fig. 2). Substitution ED_{50} values varied between rats, indicating interanimal differences in sensitivity to the ethanol-like discriminative stimulus effects of these steroids, particularly 3α,5β-P (Table 1). Although orderly dose-effect relationships were prominent, a number of inverted U-shaped dose-response curves indicated that 3α,5β-P and 3α,5α-THDOC, as well as 3β,5β-P, produced ethanol-like effects across narrow dose ranges in some animals (Fig. 2). Across the steroid doses tested, average response rates were not different from average control rates (range of steroid versus control rates, 0.0–2.14 versus 0.72–2.07 r/s).

Acutely administered 3α,5α-P, 3α,5β-P and 3α,5α-THDOC, as well as the low efficacy GABAergic steroid 3β,5β-P, substituted for ethanol in rats trained to discriminate 2.0 g/kg ethanol (Fig. 3). Individual steroids completely substituted for 2.0 g/kg ethanol in 75 to 80% of the animals tested (Fig. 3). Similar to the findings in the 1.0 g/kg ethanol group, sensitivities to the ethanol-like discriminative stimulus effects of these steroids varied between rats (Table 1). The average and rank-order potencies of each steroid did not differ between 1.0 and 2.0 g/kg ethanol training groups. In contrast, the percentage of rats that showed complete substitution of these steroids for ethanol was significantly different between groups (F(1, 6) = 16.3; p < .05). Across the acute steroid doses tested, average response rates were not different from average control rates (range of steroid versus control rates: 0.0–1.75 versus 0.52–2.07 r/s).

Cumulatively administered GABA_{A}-positive modulatory steroids 3α,5α-P, 3α,5β-P, and 3α,5α-THDOC, as well as the low-efficacy steroid 3β,5β-P, substituted for ethanol in rats trained to discriminate 2.0 g/kg ethanol (Fig. 4). Individual steroids completely substituted for 2.0 g/kg ethanol in 75 to 80% of the animals tested (Fig. 4). The differences in indi-
individual substitution ED$_{50}$ values were modest (Table 1). In rats tested with both acute and cumulative administration, only the low-efficacy steroid 3β,5β-P exhibited altered potency to substitute for 2.0 g/kg ethanol between dosing conditions ($F_{1,12} = 21.4; p < .05$). The percentage of rats tested that showed complete substitution of these steroids for 2.0 g/kg ethanol was significantly different between acute and cumulative dosing procedures ($F_{1,13} = 11.0; p < .05$). Across the doses tested (5.6–56.0 mg/kg), only 3α,5α-P resulted in average response rates that were different from average control rates (mean ± S.D.) rates of 1.20 (± 0.64) versus 0.04 (± 0.05) under control versus steroid conditions. The average ED$_{50}$ values (95% CI) of 3α,5α-P, 3α,5α-THDOC, and 3α,5α-P to suppress response rates were 13.7 mg/kg (6.6–20.8; $n = 5$), 16 mg/kg (11–21; $n = 3$) and 38 mg/kg (32–44; $n = 3$), respectively. 3β,5β-P, up to 56 mg/kg, decreased response rates in only one of five rats tested, and thus the average ED$_{50}$ value for rate suppression was not determined.

Neuroactive steroids which modulate NMDA receptors and negatively modulate GABA$_A$ receptors did not substitute for 1.0 or 2.0 g/kg ethanol in the majority of subjects. Across the three test conditions, average ethanol-appropriate responding was no greater than 33% after any dose of these neuroactive steroids, reflecting complete substitution in a maximum of two of the rats tested with a steroid. Table 2 shows the maximal average ethanol-appropriate responding of each steroid and the dose at which it occurred. When tested up to 56 mg/kg per molar dose of steroid, only cumulative 3β,5β-PS resulted in average response rates that were different from average control rates (mean ± S.D.) rates of 1.39 (± 0.45) versus 0.33 (± 0.47) under control versus steroid conditions.

![Fig. 3. Ethanol-like discriminative stimulus effects of acutely administered (i.p.) GABA$_A$-active steroids in rats (n = 6) trained to discriminate 2.0 g/kg ethanol from water. Each panel depicts ethanol-appropriate responding (%) of an individual animal after 3α,5α-P, 3α,5β-P, 3α,5α-THDOC, and 3β,5β-P administration. Each animal was tested with at least two of the four steroids. Some data points have been offset slightly for clarity between neuroactive steroid dose-response curves.](image)

![Fig. 4. Ethanol-like discriminative stimulus effects of cumulatively administered (i.p.) GABA$_A$-active steroids in rats (n = 6) trained to discriminate 2.0 g/kg ethanol from water. Each panel depicts ethanol-appropriate responding (%) of an individual animal after 3α,5α-P, 3α,5β-P, 3α,5α-THDOC, and 3β,5β-P administration. Each animal was tested with at least two of the four steroids. Some data points have been offset slightly for clarity between neuroactive steroid dose-response curves.](image)

**Table 2**

<table>
<thead>
<tr>
<th>Steroid</th>
<th>1.0 g/kg Ethanol vs H$_2$O—Acute Test</th>
<th>2.0 g/kg Ethanol vs H$_2$O—Acute Test</th>
<th>2.0 g/kg Ethanol vs H$_2$O—Cumulative Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/kg; i.p.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3α,5α-PS</td>
<td>(5.6–56.0) 3 (± 5)</td>
<td>1 (± 1)</td>
<td>1 (± 2)</td>
</tr>
<tr>
<td>3β,5β-PS</td>
<td>(5.6–56.0) 1 (± 1)</td>
<td>12 (± 26)</td>
<td>25 (± 50)</td>
</tr>
<tr>
<td>Pregnenolone</td>
<td>(10–56) 18 (± 29)</td>
<td>3 (± 5)</td>
<td>not tested</td>
</tr>
<tr>
<td>PS</td>
<td>(10–56) 7 (± 16)</td>
<td>3 (± 4)</td>
<td>1 (± 2)</td>
</tr>
<tr>
<td>DHEA</td>
<td>(10–56) 7 (± 10)</td>
<td>1 (± 1)</td>
<td>25 (± 42)</td>
</tr>
<tr>
<td>DHEAS</td>
<td>(10–56) 4 (± 10)</td>
<td>1 (± 1)</td>
<td>33 (± 53)</td>
</tr>
<tr>
<td>DHEAS</td>
<td>(10–56) 17 mg/kg</td>
<td>56 mg/kg</td>
<td>17 mg/kg</td>
</tr>
</tbody>
</table>

*Values represent maximal mean percentage of ethanol-appropriate responding (the dose at which maximal responding occurred). Means are based on data from three to six rats per dose.

The average ED$_{50}$ values (95% CI) of 3β,5β-PS and PS to suppress response rates were 31 mg/kg (25–37; $n = 4$) and 8.1 mg/kg (4.7–11.4; $n = 4$), respectively.

**Discussion**

The present study investigated the ethanol-like discriminative stimulus effects of a number of endogenously occurring neuroactive steroids which exhibit activity at GABA$_A$ and NMDA receptors. The results replicate and extend previous neuroactive steroid findings in ethanol discriminations.
As reported in earlier discriminations using rats (Ator et al., 1993; Bienkowski and Kostowski, 1997; Bowen and Grant, 1998) and monkeys (Grant et al., 1996, 1997), the GABA_A-positive modulators 3α,5α-P and 3α,5α-THDOC produced ethanol-like discriminative stimulus effects. Another GABA_A-positive modulatory steroid, 3α,5β-P, and the low-efficacy steroid 3β,5β-P also produced discriminative stimulus effects similar to ethanol. The present results also confirm a report that i.p. administration of DHEAS, a neuroactive steroid with GABA_A antagonist activity, does not substitute for the discriminative stimulus effects of ethanol (Bienkowski and Kostowski, 1997). Similarly, pregnenolone, PS, and DHEA were devoid of ethanol-like discriminative stimulus effects after i.p. administration. Together, these findings suggest that GABAergic neuroactive steroids produce interoceptive effects similar to ethanol and strengthen the data for positive modulation of GABA_A receptors as a basis for the discriminative stimulus effects of ethanol.

Substitution of GABA_A-active steroids for ethanol varied significantly as a function of the ethanol training dose and test dosing procedure. Acute administration of 3α,5α-P, 3α,5β-P, 3α,5α-THDOC, and the low-efficacy steroid 3β,5β-P resulted in complete substitution for ethanol in 80 to 100% and 40 to 67% of the animals tested in the 1.0 and 2.0 g/kg ethanol discriminations, respectively. This finding is consistent with reports that GABA_A-mediated activity is a more prominent component of the discriminative stimulus effects of 1.0 versus 2.0 g/kg ethanol (Grant and Colombo, 1993; Green and Grant, 1998). It is possible that reduced substitution of acute steroid administration for ethanol may reflect increased liver metabolic activity in rats trained to discriminate the higher, 2.0 g/kg ethanol dose. However, this explanation is challenged by the finding that cumulative steroid administration resulted in a greater percentage of rats tested that showed complete substitution (i.e., 75–80%) in the 2.0 g/kg ethanol discrimination. A more likely possibility is that the incremental rise in neuroactive steroid concentrations associated with cumulative dosing may have allowed the rate-imparing effects of these steroids to be separated from their 2.0 g/kg ethanol-like discriminative stimulus effects. It also is plausible that an acute increase in neuroactive steroid concentrations is functionally similar to a chronic increase, resulting in GABA_A receptor desensitization (Yu and Ticku, 1995; Friedman et al., 1996). In that case, the gradual increase in steroid levels accomplished with cumulative dosing may result in less perturbation of GABA_A receptor function.

In contrast to the differences in the percentage of animals exhibiting complete substitution, the potencies of GABA_A-active steroids to substitute for ethanol were not altered as a function of ethanol training dose or test dosing procedure. The only significant difference was observed with cumulative administration of the low-efficacy steroid 3β,5β-P, which exhibited decreased potency to substitute for 2.0 g/kg ethanol compared with acute 3β,5β-P substitution for 1.0 or 2.0 g/kg ethanol. This result was ascribed to the cumulative dosing procedure itself, which involved increasing neuroactive steroid concentrations at 10-min intervals but did not account for ongoing steroid metabolism. That is, the first steroid dose was the most precise, and all subsequent “cumulative” steroid doses were subject to overestimation due to metabolism of a portion of the previously injected steroid dose. Thus, the substitution ED_{50} values obtained with this procedure were approximations and would be expected to be higher than the actual values.

In other behavioral assays, efficacy and/or potency differences between GABA_A-positive modulatory steroids have been reported. Bitran et al. (1991) reported that 3α,5α-P exhibited more potent, but less efficacious, anxiolytic activity than 3α,5β-P in the elevated plus-maze. In another study, 3α,5α-P and 3α,5β-P showed similar anticonvulsant activities, and 3α,5β-P demonstrated enhanced potency and/or efficacy in multiple tests of anxiolytic activity (Wieland et al., 1995). Consistent with the latter finding, 3α,5β-P was more potent in producing ethanol-like discriminative stimulus effects under each of the three test conditions as compared with 3α,5α-P. Acutely, 3α,5β-P also substituted in a greater percentage of animals trained to discriminate 1.0 and 2.0 g/kg ethanol compared with 3α,5α-P.

We observed a lack of ethanol substitution after i.p. administration of the NMDA antagonist steroids 3α,5β-PS and 3β,5β-PS. Unlike other NMDA antagonists that substitute for ethanol (Grant and Colombo, 1992; Sanger, 1993; Shelton and Balster, 1994), these steroid sulfates did not substitute for the discriminative stimulus effects of 1.0 or 2.0 g/kg ethanol. The results may be due to an inability of i.p. administered sulfate esters of neuroactive steroids to penetrate the CNS because i.p. 3β,5β-P hemisuccinate, a synthetic analog of 3β,5β-PS that crosses the blood-brain barrier, produces ethanol-like discriminative stimulus effects (K.A. Grant, unpublished observations). Assuming this hypothesis, the data suggest that a 30-min pretreatment is not enough time for sufficient hydrolysis of these steroid sulfates to the unesterified, GABA_A-active, ethanol-like steroids 3α,5β-P and 3β,5β-P.

As hypothesized, NMDA-agonist and GABA_A-antagonist steroids did not produce robust ethanol-like discriminative stimulus effects under any test conditions. After administration of pregnenolone, PS, DHEA, and DHEAS, the average ethanol-appropriate response was consistently low and complete substitution for ethanol was observed in few rats. Of these neuroactive steroids, only DHEA was tested in an earlier ethanol discrimination, and the present results are consistent with those obtained in the previous study (Bienkowski and Kostowski, 1997). However, it should be noted that acute administration of pregnenolone produced some 1.0 g/kg ethanol-like activity and cumulative administration of DHEA and DHEAS resulted in some 2.0 g/kg ethanol-like activity. One possible explanation for these results is endogenous conversion of pregnenolone, DHEA, and DHEAS into GABA_A-positive modulatory steroids such as 3α,5α-P and androsterone (3α-hydroxy-5α-androstan-17-one).

Brain and plasma concentrations of the neuroactive steroids 3α,5α-P and 3α,5α-THDOC are relatively low in male rats under most conditions. After exposure to an acute stressor, endogenous levels of these steroids rapidly increase to concentrations that have been reported to modulate GABA_A receptor function in vitro (Purdy et al., 1991; Paul and Purdy, 1992; Barbaccia et al., 1996, 1997). Because our experiments were conducted in nonstressed male rats, it is likely that endogenous neuroactive steroid levels were inconsequential and that the data reflect the effects of exogenous steroid administration.

In summary, the present results replicated and extended earlier findings with endogenously occurring neuroactive ste-
roids in ethanol discriminations. The ethanol-like discriminative stimulus effects of 3α,5α-P and 3α,5α-TDZOC were confirmed and the GABA<sub>A</sub>-positive modulatory steroid 3α,5β-P and the low-efficacy steroid 3β,5β-P also substituted for ethanol. Although the potencies of these steroids were relatively consistent, the percentage of animals tested that showed complete substitution of GABA<sub>A</sub>-active steroids for ethanol differed as a function of ethanol training dose and test dosing procedure. The present results confirmed an earlier report that i.p. DHEAS did not substitute for ethanol. DHEA, pregnenolone, and PS also did not produce ethanol-like interoceptive effects. Because the negative results with 3α,5β-PS and 3β,5β-PS may reflect a lack of CNS penetration, future in vivo studies should use novel neuroactive steroid derivatives that easily cross the blood-brain barrier to investigate NMDA receptor involvement in the interactions between ethanol and neuroactive steroids. Overall, the present findings strengthen the data for positive modulation of GABA<sub>A</sub> receptors as a basis for the discriminative stimulus effects of ethanol and indicate that a number of endogenously occurring neuroactive steroids can produce interoceptive effects similar to those of ethanol.

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References


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