Differential Change in Neuroactive Steroid Sensitivity during Ethanol Withdrawal

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ABSTRACT
The progesterone metabolite 3α-hydroxy-5α-pregn-20-one (3α,5α-P or allopregnanolone) is a potent positive modulator of γ-aminobutyric acid (GABA) receptors. Although it is well documented that chronic ethanol (EtOH) administration produces cross-tolerance to the positive modulatory effect of benzodiazepines and GABA at GABA_A receptors, recent findings suggest that sensitivity to 3α,5α-P is enhanced during EtOH withdrawal. In addition, EtOH-naive inbred strains of mice, which differ in EtOH withdrawal severity (DBA/2 > C57BL/6), had marked differences in behavioral sensitivity to 3α,5α-P. Therefore, the present study was conducted to determine whether C57BL/6 (B6) and DBA/2 (D2) mice would be differentially sensitive to several of the pharmacological effects of 3α,5α-P during EtOH withdrawal. Male mice were exposed to EtOH vapor or air for 72 h. During withdrawal from EtOH, animals were injected with 3α,5α-P (0, 3.2, 10, or 17 mg/kg i.p.) and tested for activity and anxiolysis on the elevated plus maze, muscle relaxation, ataxia, and seizure protection following pentylenetetrazol. Sensitivity to the anticonvulsant effect of 3α,5α-P was enhanced during EtOH withdrawal in B6, but not D2 mice. In contrast, sensitivity to the muscle relaxant effects of 3α,5α-P was reduced in EtOH-withdrawing B6 and D2 mice, with a suggestion of decreased sensitivity to the anxiolytic effect of 3α,5α-P during EtOH withdrawal in B6. These results suggest that sensitization to the anticonvulsant effect of 3α,5α-P during EtOH withdrawal does not generalize across all genotypes nor does it generalize to all of the pharmacological effects of 3α,5α-P.

Chronic administration of ethanol (EtOH) leads to the development of tolerance, or reduced sensitivity, to most of EtOH’s pharmacological effects (Kalant et al., 1971). The efficacy of γ-aminobutyric acid (GABA), benzodiazepines, and barbiturates also is reduced in animals following exposure to chronic EtOH. These findings suggest that chronic EtOH administration produces tolerance to EtOH and cross-tolerance to other positive modulators of GABA_A receptors (Buck and Harris, 1990; Morrow, 1995; Khanna et al., 1997).

The progesterone metabolite 3α-hydroxy-5α-pregn-20-one (3α,5α-P or allopregnanolone) is a recently identified potent positive modulator of GABA_A receptors (for review, see Paul and Purdy, 1992; Lambert et al., 1995; Gasior et al., 1999). Consistent with the cross-tolerance to benzodiazepine and barbiturate effects just described, recent work found that chronic EtOH exposure produced reduced sensitivity to the anticonvulsant effect of diazepam in male and female rats during EtOH withdrawal (Devaud et al., 1996, 1998). However, in similarly treated rats, enhanced sensitivity to the anticonvulsant effect of 3α,5α-P was observed during EtOH withdrawal (Devaud et al., 1996, 1998). Progressive increases in response to chronically administered drugs are termed sensitization, and characterize certain effects of EtOH, such as low-dose locomotor activation (Masur and Boerngen, 1980; Phillips et al., 1998). Because chronic administration of EtOH can produce divergent changes in sensitivity to a subsequent dose of EtOH (i.e., sensitization or tolerance, depending on the pharmacological effect; Khanna et al., 1997; Phillips et al., 1998), the observed sensitization to the anticonvulsant effect of 3α,5α-P during EtOH withdrawal may not generalize to other pharmacological effects of 3α,5α-P.

Recently, we have shown that C57BL/6J (B6) and DBA/2J (D2) mice differ in behavioral sensitivity to 3α,5α-P (Finn et al., 1997). B6 mice were more sensitive than D2 animals to the anxiolytic, locomotor stimulant, and anticonvulsant effects of 3α,5α-P. In contrast, D2 were more sensitive than B6

ABBREVIATIONS: EtOH, ethanol; GABA, γ-aminobutyric acid; 3α,5α-P, 3α-hydroxy-5α-pregn-20-one; B6, C57BL/6; D2, DBA/2; WSP, Withdrawal Seizure-Prone; WSR, Withdrawal Seizure-Resistant; BEC, blood EtOH concentration; PTZ, pentylenetetrazol; MC twitch, myoclonic twitch; FF clonus, face and forelimb clonus; RB clonus, running bouncing clonus; THE, tonic hindlimb extension; RIA, radioimmunoassay; CORT, corticosterone.
to the muscle relaxation and ataxia produced by 3α,5α-P. The B6 and D2 inbred strains differ markedly in basal seizure susceptibility (D2 > B6) as well as in a number of EtOH-related behaviors (Phillips and Crabbe, 1991). For example, D2 mice exhibit more severe handling-induced convulsions than B6 after withdrawal from both acute (Roberts et al., 1992) and chronic (Crabbe et al., 1983; Crabbe, 1998) EtOH administration. Therefore, the strain difference in sensitivity to the anticonvulsant effect of 3α,5α-P (i.e., B6 > D2) is consistent with their differences in basal seizure susceptibility and EtOH withdrawal severity (i.e., B6 < D2). That is, the greater sensitivity to 3α,5α-P in the B6 inbred strain might confer some protection against basal seizure susceptibility as well as EtOH withdrawal severity because 3α,5α-P is a potential positive modulator of GABA_A receptors.

Genetic differences in EtOH withdrawal hyperexcitability may be due in part to modulatory effects of endogenous GABA agonist steroids, such as 3α,5α-P. Such genetic differences in the modulatory effects of endogenous 3α,5α-P at GABA_A receptors could result from differences in sensitivity to 3α,5α-P, or from altered biosynthesis, following exposure to chronic EtOH; these differences could lead to changes in neural excitability during EtOH withdrawal. Recent findings in our laboratory in mice selectively bred for sensitivity (Withdrawal Seizure-Prone, WSP) and resistance (Withdrawal Seizure-Resistant, WSR) to handling-induced convulsions following exposure to chronic EtOH inhalation are consistent with this hypothesis. Briefly, WSP mice were cross-tolerant to the anticonvulsant effect of a single dose of 3α,5α-P, whereas sensitivity was unchanged in similarly treated WSR animals (D.A.F., unpublished data). If the change in sensitivity to the anticonvulsant effect following exogenous administration of 3α,5α-P is indicative of a change in sensitivity to endogenous 3α,5α-P concentration, then exposure to chronic EtOH may render an EtOH withdrawal sensitive strain (i.e., WSP or D2) cross-tolerant to 3α,5α-P, which would produce less of a positive modulatory effect at GABA_A receptors and therefore, would increase neuronal excitability.

Because B6 and D2 differ in behavioral sensitivity to 3α,5α-P (Finn et al., 1997) and chronic EtOH exposure produced enhanced sensitivity to the anticonvulsant effect of 3α,5α-P in rats (Devaud et al., 1996), we wanted to determine (1) whether chronic EtOH exposure would produce enhanced sensitivity to other pharmacological effects of 3α,5α-P and 2) whether the two genotypes would differ in the change in sensitivity to 3α,5α-P during EtOH withdrawal. Because B6 have mild, and D2 have severe EtOH withdrawal, we hypothesized that B6 would become more sensitive to the anticonvulsant effect of 3α,5α-P, whereas D2 would not. If this were the case (i.e., sensitization in B6 and cross-tolerance in D2), these differential changes in sensitivity to the anticonvulsant effect of 3α,5α-P between B6 and D2 would be consistent with their differences in EtOH withdrawal severity.

Materials and Methods

Subjects

Drug-naive male B6 and D2 mice were used in all experiments. The animals were purchased from the Jackson Laboratory (Bar Harbor, ME) at 6 weeks of age, housed four per cage with ad libitum access to food and water, and acclimated to a 12:12 h light/dark cycle for a minimum of 2 weeks before experimentation. All procedures adhere to the U.S. Public Health Service-National Institutes of Health Guidelines for the care and use of laboratory animals and were approved by two local Institutional Animal Care and Use Committees.

Chronic EtOH Administration

Animals were exposed to EtOH vapor or air for 72 h using our standardized method for inducing EtOH dependence (for details, see Terdal and Crabbe, 1994). During the experiment, the animals were housed in stainless steel ¼-in. hardware cloth cages inside a large Plexiglas chamber. Three such cages, measuring 48 × 35 × 18 cm, could be suspended by a flat metal guide rail inside a 71 × 66.5 × 114-cm chamber made of ½-in. Plexiglas, with a separate door allowing access to each wire mesh cage. Each wire mesh cage was subdivided into six compartments, so that each large Plexiglas chamber could house a maximum of 18 individual cages of mice. Ten centimeters below each wire mesh cage was a stainless steel drop pan with absorbent underpads, which were changed daily. A closed food hopper and water bottle were freely available. Chamber temperatures ranged from 28 to 30°C, depending on the total number of mice in the chamber. Airflow rate in the large Plexiglas chambers was 55 l/min. Because the volume of each large chamber was 538 liters, the air or EtOH vapor in each chamber was replaced approximately every 10 min. The presence of three large Plexiglas chambers allowed the simultaneous treatment of EtOH- and air-exposed animals.

The present studies used the alcohol dehydrogenase inhibitor pyrazole hydrochloride (pyrazole; Sigma Chemical Co., St. Louis, MO) to stabilize blood EtOH concentration (BEC). On day 1, mice in the EtOH groups were weighed, injected i.p. with a priming dose of EtOH (1.5 g/kg) and pyrazole (1.0 mmol/kg), and exposed to EtOH vapor (6 mg EtOH/l air for D2 and 9 mg EtOH/l air for B6) inside inhalation chambers. Different vapor doses were used to achieve equal BECs for the genotypes so that any genetic differences in behavioral responses could not be ascribed to differences in EtOH pharmacokinetics. At 24 and 48 h, the animals were briefly removed from the chambers, weighed, injected i.p. with pyrazole, and placed back into the chamber. Tail blood samples were taken from a subset of the animals each day to monitor BECs. Air-exposed animals also received daily pyrazole injections, but were injected with saline on day 1 and were exposed to air inside the inhalation chamber. At 72 h, all animals were removed from the chambers and tail blood samples were taken for subsequent analysis of BEC. Tails were nicked for the air-exposed groups, but no blood sample was taken. The mice were housed in polypropylene cages with cob bedding, taking to a procedure room for behavioral testing, and weighed. At peak withdrawal (i.e., 5.5–9.5 h postremoval from the inhalation chamber) the EtOH- and air-exposed animals were tested for behavioral sensitivity to 3α,5α-P. Subsequent 3α,5α-P dose groups for each treatment and genotype were counterbalanced across the 4-h time frame for behavioral testing.

BEC Determination

A 20-μl sample of blood from the tip of the tail was added to 50 μl of chilled 5% ZnSO_4 and stored on ice. Fifty microliters of 0.3 N Ba(OH)_2 and 300 μl of distilled water were added to each sample. The samples were shaken for 5 s and centrifuged for 5 min at 12,000 rpm. The supernatant was transferred to crimp-top glass vials and analyzed for EtOH concentration by gas chromatography. Four pairs of external standards of known EtOH concentration (0.5–4.0 mg/ml) were used to establish a standard curve.

Behavioral Sensitivity to 3α,5α-P

Behavioral testing occurred between 1:00 PM and 5:00 PM (i.e., during peak EtOH withdrawal). Each mouse was tested at 10-min intervals on several behavioral tasks (Table 1). The procedures have
TABLE 1
Sequence of behavioral tests

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Behavioral Task</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Injection with 3α,5α-P i.p. (0, 3, 2, 10, or 17 mg/kg in 20% β-cyclodextrin*)</td>
</tr>
<tr>
<td>10</td>
<td>Elevated plus maze, 5-min trial</td>
</tr>
<tr>
<td>20</td>
<td>Forelimb grip strength (three trials, compared with baseline)</td>
</tr>
<tr>
<td>30</td>
<td>Accelerating Rotarod performance (three trials, compared with baseline)</td>
</tr>
<tr>
<td>40</td>
<td>Timed tail vein infusion of PTZ (5 mg/ml in saline, 0.5-ml/min infusion rate)</td>
</tr>
</tbody>
</table>

* 3α,5α-P was synthesized by, and purchased from, Robert H. Purdy, Ph.D. (Veterans Affairs Medical Center, San Diego, CA).
* 20% β-cyclodextrin (2-hydroxypropyl β-cyclodextrin; Research Biochemicals International, Natick, MA).
* PTZ (Sigma Chemical Co., St. Louis, MO).

been described recently (Finn et al., 1997) and will be outlined briefly. 1) Muscle relaxation. Mice are placed on a tray, allowed to grasp a horizontal bar connected to a strain gauge, and then pulled gently until they lose their grip. This procedure allows each mouse to be tested for baseline ability and ability following 3α,5α-P injection. The steroid effect was expressed as a change from baseline. 2) Ataxia. Each mouse was placed on a stationary Rotarod that began to accelerate linearly (20 rpm/min) in its rate of rotation until the mouse fell off. The latency to fall was then used to calculate the speed (rpm) at which the mouse could no longer remain on the Rotarod. This procedure allowed each mouse to be tested for Rotarod performance at baseline and following 3α,5α-P injection. The steroid effect was expressed as a change from baseline rpm. 3) Anxiolysis. The elevated plus maze consists of two open and two enclosed horizontal perpendicular arms extending from a central platform (5 × 5 cm), 50 cm above the floor. Each mouse was placed on the central platform facing an intersection between open and closed arms and allowed to explore freely for 5 min. During the 5-min test period, the number of entries into the open and closed arms as well as the amount of time spent in the open and closed arms was measured. For an arm entry to be measured, all four paws had to be within the arm. The percentages of open-arm time and open-arm entries were used as indices of anxiety. To demonstrate the predicted anxiogenic effect of EtOH withdrawal, we wanted to use conditions under which naïve animals spent equal time in both open and closed arms. Pilot testing showed that we could achieve this condition if we adjusted the sides of the open arm upward (from the initial configuration of 0.5 cm to 1.2-cm height; data not shown). 4) Activity. The information obtained from elevated plus maze testing (i.e., total number of arm entries) was used as an estimate of locomotor activity. 5) Seizure protection. Mice were administered the convulsant pentyleneetrazol (PTZ, 5 mg/ml in saline) via timed tail vein infusion into a lateral vein (0.5-ml/min infusion rate). The apparatus and procedure for tail vein infusion have been described in detail (Kosobud and Crabbe, 1990). Briefly, an animal was placed into a Plexiglas container (5-cm diameter) that allowed it to be hand held by the tail and visualized during the infusion. A 27-gauge butterfly needle, connected to the PTZ-containing syringe on an infusion pump (Sage Instruments, model 355; Orion, Boston, MA), was inserted into a lateral vein. The needle was held in place with one hand while the latencies for onset to myoclonic twitch (MC twitch), face and forelimb clonus (FF clonus), running bounding clonus (RB clonus), and tonic hindlimb extension (THE) were recorded in seconds. Subsequently, the latencies were converted to threshold convulsant dosage of PTZ to elicit each convulsion endpoint (i.e., milligram of drug per kilogram body weight). Therefore, this method allowed for observation and qualitative analysis of several different endpoints that characterize PTZ-induced convulsions (i.e., MC twitch, FF clonus, RB clonus, and THE).

Radioimmunoassay (RIA)

On completion of behavioral testing, the mice were euthanized and trunk blood collected for subsequent analysis of plasma 3α,5α-P and corticosterone (CORT) concentration by radioimmunoassay.

Radioimmunoassay (RIA)

Data Analysis

Data are expressed as the means ± S.E. ANOVA was used to assess strain and dose effects on BEC, to ensure that the two genotypes were matched for EtOH exposure. Then ANOVA was used to determine the influence of strain, treatment, and dose on the dependent variables muscle relaxation (percentage of baseline forelimb grip strength), ataxia (percentage of baseline Rotarod performance), activity (total arm entries on elevated plus maze), anxiolysis (percentage open-arm time and open-arm entries on plus maze), seizure protection (threshold dose for onset to MC twitch, FF clonus, RB clonus, and THE), plasma 3α,5α-P concentration, and plasma CORT concentration. Due to differences in susceptibility to PTZ in the EtOH- versus air-exposed mice, the seizure protection data were transformed to percentage of change in PTZ threshold dose of the mean for the respective vehicle-injected group. If three-way interactions were obtained, the data for each strain were then analyzed separately. When appropriate, simple main effects analyses followed by Tukey post hoc comparisons were then used to examine significant treatment and dose effects within each strain. Mice with less than two total entries on the elevated plus maze were eliminated from the plus maze analysis (n = 6 EtOH-exposed B6, n = 5 EtOH-exposed D2, and n = 1 air-exposed B6). In addition, data from four air-exposed B6 animals with incorrect injections were eliminated from the analyses. Significance was set at P < .05.

Results

Exposure to EtOH vapor produced stable BEC at the time of removal from the inhalation chamber. Mean ± S.E. BEC was 1.05 ± 0.06 mg/ml for B6 and 1.21 ± 0.05 mg/ml for D2. There was a trend for a difference between the strains in BEC (F = 3.83; df = 1, 79; P = .054). However, there was no
effect of \(3\alpha,5\alpha\)-P dose or interaction between strain and dose (both \(F < .8\)). These results indicate that the chronic EtOH exposure was similar in the two genotypes when they subsequently were divided into different \(3\alpha,5\alpha\)-P dose groups.

**Elevated Plus Maze.** Total number of entries on the elevated plus maze, which was used as an index of activity, was significantly decreased during EtOH withdrawal in both B6 and D2 mice (Fig. 1). This conclusion is supported by the significant main effect of treatment (\(F = 115.86; df = 1, 140; P = .0001\)). Although there was no overall main effect of strain on total entries, there was a significant effect of dose of \(3\alpha,5\alpha\)-P (\(F = 5.04; df = 3, 140; P < .005\)) on the total number of entries on the elevated plus maze. Interactions between strain and treatment (\(F = 9.93; df = 1, 140; P < .003\)) and between strain and dose (\(F = 12.31; df = 3, 140; P < .0001\)) were significant, but the three-way interaction was not significant.

Simple main effects analysis for the significant strain by treatment interaction indicated that total entries differed in the two strains when they were exposed to chronic EtOH (\(F = 5.45; df = 1, 140; P < .03\)) (i.e., B6 > D2), but not to air. In addition, chronic EtOH exposure significantly decreased total entries in both B6 (\(F = 18.61; df = 1, 140; P < .0001\)) (Fig. 1A) and D2 (\(F = 73.76; df = 1, 140; P < .0001\)) mice (Fig. 1B). Analyses subsequent to the strain by dose interaction indicated that the overall effect of dose of \(3\alpha,5\alpha\)-P on total entries was significant in B6 (\(F = 5.28; df = 3, 140; P < .002\)), with a trend for significance in D2 (\(F = 2.58; df = 3, 140; P = .056\)) mice. The two genotypes differed significantly in total entries following injection of 3.2 mg/kg \(3\alpha,5\alpha\)-P (\(F = 6.19; df = 1, 140; P < .02\)) (i.e., B6 > D2) and 10 mg/kg \(3\alpha,5\alpha\)-P (\(F = 6.74; df = 1, 140; P = .01\)) (i.e., B6 < D2), with a trend for a difference in total entries following injection of the 17-mg/kg dose of \(3\alpha,5\alpha\)-P (\(F = 3.11; df = 1, 140; P = .08\)) (i.e., B6 < D2). Overall, these findings indicate that EtOH withdrawal decreased total entries on the elevated plus maze in both genotypes, whereas the effect of dose of \(3\alpha,5\alpha\)-P on total entries was significant only in B6 mice.

The percentage of time spent in the open arms of the elevated plus maze, which was used as an index of anxiety, was significantly influenced by strain (\(F = 5.26; df = 1, 140; P < .03\)) and dose of \(3\alpha,5\alpha\)-P (\(F = 5.01; df = 3, 140; P < .003\)) (Fig. 2). Surprisingly, there was no main effect of treatment on percentage of open-arm time, suggesting that we could not detect an increase in anxiety during EtOH withdrawal with our experimental paradigm. However, the interaction between strain, treatment and dose of \(3\alpha,5\alpha\)-P was significant (\(F = 3.05; df = 3, 140; P < .04\)).

To follow-up the three-way interaction for percentage of open-arm time, data from B6 and D2 mice were analyzed separately. Neither treatment, dose of \(3\alpha,5\alpha\)-P, nor their interaction significantly affected percentage of open-arm time in D2 mice (Fig. 2B). In B6 mice, there was a main effect of dose of \(3\alpha,5\alpha\)-P (\(F = 6.33; df = 3, 66; P < .001\)), with a trend for an interaction between treatment and dose of \(3\alpha,5\alpha\)-P (\(F = 2.34; df = 3, 66; P = .08\)) (Fig. 2A). Simple main effects analysis indicated that treatment significantly influenced percentage of open-arm time in animals injected with the 10-mg/kg dose of \(3\alpha,5\alpha\)-P (\(F = 5.39; df = 1, 66; P < .03\)) (i.e., EtOH < air). In addition, dose of \(3\alpha,5\alpha\)-P significantly influenced percentage of open-arm time in the EtOH-exposed (\(F = 5.86; df = 3, 66; P < .002\)) and air-exposed (\(F = 2.74; df = 3, 66; P = .05\)) mice. In the air-exposed B6 mice, injection of the 17-mg/kg dose of \(3\alpha,5\alpha\)-P significantly increased percentage of open-arm time versus mice injected with vehicle. In the B6 mice undergoing EtOH withdrawal, injection of the 17-mg/kg dose of \(3\alpha,5\alpha\)-P significantly increased percentage of open-arm time versus mice injected with the 3.2 mg/kg and 10 mg/kg doses of \(3\alpha,5\alpha\)-P. Therefore, injection of the 17 mg/kg dose of \(3\alpha,5\alpha\)-P significantly increased percentage of open arm time versus vehicle, only in air-exposed B6 mice.

Similar results were found when percentage of open-arm entries were analyzed (Fig. 3). Both strain (\(F = 5.74; df = 1, 140; P < .02\)) and dose of \(3\alpha,5\alpha\)-P (\(F = 3.78; df = 3, 140; P = .01\)) significantly influenced percentage of open-arm entries, whereas there was no significant effect of treatment. In contrast to the results with percentage of open-arm time, the interaction between main effects did not reach statistical significance.

**Forelimb Grip Strength.** A decrease in percentage of baseline forelimb grip strength following injection was used as an indication of muscle relaxation. The percentage of baseline grip strength was significantly influenced by treatment (\(F = 13.78; df = 1, 152; P < .0005\)) and dose of \(3\alpha,5\alpha\)-P (\(F = 33.33; df = 3, 152; P < .0001\)), with a trend for a main effect of strain (\(F = 3.64; df = 1, 152; P = .058\)) (Fig. 4). Interactions between strain and dose (\(F = 3.15; df = 3, 152; P < .03\)) and between treatment and dose (\(F = 2.99; df = 3, .001\)) were significant.

**Fig. 1.** The effect of \(3\alpha,5\alpha\)-P on total entries, an index of activity, in air- and EtOH-exposed B6 (A) and D2 (B) mice. Animals were tested on the elevated plus maze at 10-min postinjection of \(3\alpha,5\alpha\)-P or vehicle at a time corresponding to peak withdrawal in the EtOH-exposed mice. Values represent means ± S.E. The n/dose of \(3\alpha,5\alpha\)-P for each treatment and genotype was as follows. For B6, n = 10/dose (vehicle and 3.2 mg/kg) or 7/dose (10 and 17 mg/kg) for EtOH-exposed mice and n = 9/dose (vehicle and 3.2 mg/kg), n = 8 (10 mg/kg) or n = 7 (17 mg/kg) for air-exposed mice. For D2, n = 10/dose (3.2 and 10 mg/kg) or n = 8 (vehicle) or n = 6 (17 mg/kg) for the EtOH-exposed D2 and n = 10/dose for the air-exposed D2.
152; \( P < .04 \) were significant, with a trend for a three-way interaction between main effects \( (F = 2.49; df = 3, 152; P = .06) \). When the analysis was limited to the animals injected with vehicle, there was no effect of treatment on percentage of baseline grip strength, suggesting that EtOH withdrawal did not influence baseline grip strength in either B6 or D2 mice.

Subsequent analyses conducted in B6 mice (Fig. 4A) confirmed the main effect of treatment \( (F = 9.05; df = 3, 72; P < .004) \) and dose of 3a,5a-P \( (F = 7.43; df = 3, 72; P < .0003) \) on percentage of baseline grip strength, with a significant interaction between main effects \( (F = 2.67; df = 3, 72; P = .05) \).

Simple main effects analyses indicated that 3a,5a-P significantly decreased percentage of baseline grip strength only in the air-exposed B6 mice \( (F = 8.16; df = 3, 72; P < .0002) \). Injection of the 17-mg/kg dose of 3a,5a-P significantly decreased percentage of baseline grip strength versus values in similarly treated animals injected with vehicle or with the 3.2-mg/kg dose of 3a,5a-P. Simple main effects analyses also indicated that percentage of baseline grip strength was significantly decreased in the air-versus EtOH-exposed B6 mice following injection of the 10-mg/kg \( (F = 5.14; df = 1, 72; P < .05) \).
analyses conducted in D2 mice (Fig. 4B) found that percentage of baseline grip strength was significantly influenced by both treatment (F = 4.67; df = 1, 79; P < .04) and dose of 3α,5α-P (F = 33.06; df = 3, 79; P < .0001), with a significant interaction between main effects (F = 2.70; df = 3, 79; P = .05). Simple main effects analysis indicated that dose of 3α,5α-P significantly influenced percentage of baseline grip strength in both the EtOH- (F = 15.49; df = 3, 79; P < .0001) and air-exposed mice (F = 20.26; df = 3, 79; P < .0001). In the air-exposed D2 mice, injection of any dose of 3α,5α-P significantly decreased percentage of baseline grip strength versus values for similarly treated animals injected with the 3.2-g/kg dose of 3α,5α-P. Post hoc tests in the EtOH-exposed mice indicated that injection of the 10- and 17-mg/kg doses of 3α,5α-P also significantly decreased percentage of baseline grip strength versus vehicle. Injection of the 10- and 17-mg/kg doses of 3α,5α-P also significantly decreased percentage of baseline grip strength versus vehicle. The decrease in percentage of baseline grip strength following injection of 17-mg/kg 3α,5α-P also was significantly greater than that in animals injected with the 3.2- and 10-mg/kg doses of 3α,5α-P. Therefore, the muscle relaxant effect of 3α,5α-P was evident following all three doses of 3α,5α-P in the air-exposed D2 mice, but only was evident following the two highest doses of 3α,5α-P in the EtOH-exposed animals. In addition, simple main effects analyses indicated that percentage of baseline grip strength was significantly decreased in the air- versus EtOH-exposed D2 mice, with a trend for a difference between the two genotypes (F = 3.41; df = 1, 150; P = .067) (Fig. 5). Collectively, these results suggest that sensitivity to 3α,5α-P-induced muscle relaxation is reduced in EtOH- versus air-exposed D2 mice.

**Rotarod Performance.** A decrease in percentage of baseline Rotarod performance was used as an indication of ataxia. The percentage of baseline Rotarod performance was significantly influenced by dose of 3α,5α-P (F = 8.96; df = 3, 150; P < .0001), with a trend for a difference between the two genotypes (F = 3.41; df = 1, 150; P = .067) (Fig. 5). Although there was no significant effect of treatment on percentage of baseline Rotarod performance, there was a trend for an interaction between strain and treatment (F = 3.09; df = 1, 150; P = .08) and between treatment and dose of 3α,5α-P (F = 2.50; df = 3, 150; P = .06). Simple main effects analysis indicated that B6 and D2 mice differed in percentage of baseline Rotarod performance following exposure to chronic EtOH (F = 5.26; df = 1, 150; P < .03) (i.e., B6 > D2), but not following exposure to air. Analyses subsequent to the treatment by dose interaction indicated that percentage of baseline Rotarod performance was significantly greater in the EtOH- versus air-exposed mice injected with the 17-mg/kg dose of 3α,5α-P (F = 5.33; df = 1, 150; P < .03), an effect that appeared to be due primarily to the results in B6 mice. In addition, the effect of 3α,5α-P dose on percentage of baseline Rotarod performance was significant in the air-exposed mice (F = 7.45; df = 3, 150; P = .0001), with a trend for significance in the EtOH-exposed animals (F = 2.63; df = 3, 150; P = .052). Although these results are not conclusive, they are suggestive that the ataxic effect of 3α,5α-P, measured by a significant decrease in percentage of baseline Rotarod performance, was more pronounced in air- versus EtOH-exposed B6 mice.

**Seizure Susceptibility.** Drugs that alter seizure threshold can be tested for pro- or anticonvulsant activity by pretreating mice and observing the effect on PTZ seizure threshold. A decreased PTZ seizure threshold indicates proconvulsant activity, whereas an increased PTZ threshold suggests anticonvulsant activity. By administering PTZ via timed tail vein infusion, distinct convulsive responses were produced as a function of dose. Although these seizure manifestations might appear to be on a continuum, there are two qualitatively distinct seizure components that are mediated by separable and independent anatomical circuits (Gale, 1988). MC twitch and FF clonus appear to be associated with forebrain neural circuits, whereas RB clonus and THE depend on hindbrain circuitry. Genetic susceptibility to these two seizure types also may be distinct (Kosobud and Crabbe, 1990). Therefore, results for MC twitch and FF clonus were analyzed as similar types of convulsions as were results for RB clonus and THE.

When the analysis was limited to vehicle-injected animals, basal seizure susceptibility to PTZ, measured by the threshold dose for onset to MC twig (F = 19.04; df = 1, 38; P < .0003) and FF clonus (F = 17.17; df = 1, 38; P < .0004) was significantly influenced by genotype (i.e., D2 < B6 for PTZ threshold dose) (Fig. 6). The threshold dose for onset to FF twitch and FF clonus appeared to be associated with forebrain neural circuits, whereas RB clonus and THE depend on hindbrain circuitry. Genetic susceptibility to these two seizure types also may be distinct (Kosobud and Crabbe, 1990). Therefore, results for MC twitch and FF clonus were analyzed as similar types of convulsions as were results for RB clonus and THE.
Due to the differences in basal seizure susceptibility to PTZ in EtOH- and air-exposed mice, sensitivity to the anticonvulsant effect of 3α,5α-P was determined as the percentage of change in PTZ threshold dose (Figs. 7 and 8). An increase in percentage of change in threshold dose would be interpreted as an anticonvulsant effect. The percentage of change in threshold dose for onset to RB clonus was significantly influenced by dose of 3α,5α-P (F = 32.97; df = 3, 152; P < .0001), whereas there was no significant effect of treatment or genotype. However, the interaction between strain and treatment (F = 17.19; df = 1, 152; P = .0002) and between strain, treatment, and dose of 3α,5α-P (F = 3.575; df = 3, 152; P < .02) was significant. Similar results were found when the percentage of change in threshold dose for onset to FF clonus was analyzed (data not shown). Therefore, subsequent analyses for the percentage of change in threshold dose for onset to MC twitch were conducted on the two strains separately.

In B6 mice the percentage of change in MC twitch threshold dose was influenced significantly by treatment (F = 5.63; df = 1, 73; P = .02) (i.e., EtOH > air) and was increased significantly by dose of 3α,5α-P (F = 19.63; df = 3, 73; P < .0001) (Fig. 7A). The interaction between main effects also was significant (F = 3.05; df = 3, 73; P < .04). Simple main effects analysis indicated that dose of 3α,5α-P significantly increased the percentage of change in MC twitch threshold dose in both the air- (F = 4.25; df = 3, 73; P < .01) and EtOH-exposed mice (F = 20.725; df = 3, 73; P < .0001). Post
hoc analyses in the air-exposed B6 mice indicated that the percentage of change in MC twitch threshold dose was significantly greater in animals injected with 10- or 17-mg/kg 3α,5α-P versus animals injected with vehicle. In the B6 mice undergoing EtOH withdrawal, the percentage of change in MC twitch threshold dose was significantly greater in mice injected with 10- or 17-mg/kg 3α,5α-P versus vehicle. The percentage of change in MC twitch threshold dose in animals injected with 17-mg/kg 3α,5α-P also was significantly greater than values in animals injected with either 3.2- or 10-mg/kg 3α,5α-P. Simple main effects analysis also indicated that treatment significantly influenced the percentage of change in MC twitch threshold dose following injection of 17-mg/kg 3α,5α-P ($F = 13.55; df = 1, 73; P = .0005$) (i.e., EtOH > air). These findings suggest that the 17-mg/kg dose of 3α,5α-P was more efficacious as an anticonvulsant in the EtOH- versus air-exposed B6 mice.

In D2 mice (Fig. 7B), both treatment ($F = 12.19; df = 1, 78; P < .001$) (i.e., EtOH < air) and dose of 3α,5α-P ($F = 13.865; df = 3, 78; P < .0001$) significantly influenced the percentage of change in threshold dose for onset to MC twitch. However, the interaction between main effects was not significant. Nonetheless, it is noteworthy that the overall percentage of change in MC twitch threshold dose with treatment, when collapsed across dose of 3α,5α-P, was opposite in D2 (i.e., EtOH < air) versus B6 (i.e., EtOH > air) mice. Because the data were analyzed as percentage of change in threshold dose to correct for baseline differences due to treatment, the significant effect of treatment in the present results suggests that dose of 3α,5α-P was more efficacious as an anticonvulsant in EtOH-exposed B6 and less efficacious in EtOH-exposed D2, versus their respective air-exposed animals.

Analyses conducted on the two later convulsion endpoints (i.e., RB clonus and THE) were similar. Therefore, only the results for percentage of change in THE threshold dose will be reported (Fig. 8). Dose of 3α,5α-P significantly increased the percentage of change in threshold dose for onset to THE ($F = 88.38; df = 3, 152; P < .0001$). Although there was no significant effect of strain or treatment on percentage of change in THE threshold dose, the interaction between strain and treatment ($F = 10.08; df = 1, 152; P < .002$) and between strain, treatment and dose of 3α,5α-P ($F = 3.405 df = 3, 152; P < .02$) was significant.

In B6 mice (Fig. 8A), both treatment ($F = 7.17; df = 1, 73; P < .01$) (i.e., EtOH > air) and dose of 3α,5α-P ($F = 29.98; df = 3, 73; P < .0001$) significantly influenced the percentage of change in THE threshold dose. The interaction between main effects also was significant ($F = 2.60; df = 3, 73; P = .05$). Subsequent analyses indicated that dose of 3α,5α-P significantly increased the percentage of change in THE threshold dose in the air- ($F = 6.88; df = 3, 73; P = .0004$) and EtOH-exposed mice ($F = 28.72; df = 3, 73; P < .0001$). For the air-exposed B6 mice, the increase in percentage of change in THE threshold dose was significantly greater in mice injected with the 17-mg/kg dose of 3α,5α-P versus vehicle or versus mice injected with 3.2-mg/kg 3α,5α-P. In the mice undergoing EtOH withdrawal, the percentage of change in THE threshold dose was significantly greater in animals injected with the 10- and 17-mg/kg doses of 3α,5α-P versus vehicle. Values in the mice injected with 17-mg/kg 3α,5α-P also were significantly greater than that in mice injected with the 3.2- and 10-mg/kg doses of 3α,5α-P. Simple main effects analysis also indicated that the percentage of change in threshold dose for onset to THE was significantly greater in EtOH- versus air-exposed B6 mice. Nonetheless, it is noteworthy that the overall percentage of change in THE threshold dose was significantly greater in EtOH- versus air-exposed B6 mice injected with the 17-mg/kg dose of 3α,5α-P ($F = 12.68; df = 1, 73; P = .0007$). Collectively, these results suggest that 3α,5α-P was more efficacious as an anticonvulsant in EtOH- versus air-exposed B6 mice.

In D2 mice (Fig. 8B), dose of 3α,5α-P significantly increased the percentage of change in threshold dose for onset to THE ($F = 70.65; df = 3, 78; P < .0001$). However, there was no effect of treatment on the percentage of increase in THE threshold dose, nor was the interaction between main effects significant. These results suggest that sensitivity to the anticonvulsant effect of 3α,5α-P was similar in the EtOH- and air-exposed D2 mice.

**RIA**. Plasma 3α,5α-P concentration in the EtOH- and air-exposed B6 and D2 mice following completion of the behavioral testing (i.e., ~50 min postinjection of 3α,5α-P or vehicle) is depicted in Fig. 9. Dose of 3α,5α-P ($F = 77.505; df = 3, 149; P < .0001$) significantly influenced plasma 3α,5α-P concentration, producing a dose-dependent increase in plasma 3α,5α-P levels in both genotypes. There was a trend for an effect of strain on plasma 3α,5α-P concentration ($F = 2.81; df = 1, 149; P < .10$), whereas the effect of treatment was not significant. In addition, there was no significant interaction between strain, treatment and dose of 3α,5α-P, suggesting that any change in sensitivity to 3α,5α-P during EtOH withdrawal was not due to treatment differences in plasma 3α,5α-P concentration among genotypes.

When the analysis was limited to the vehicle-injected an-

Fig. 8. Sensitivity to the anticonvulsant effect of 3α,5α-P, measured by the percentage of change in threshold dose for onset to THE in B6 (A) and D2 (B) mice. Shown are the means ± S.E. for the mice depicted in Fig. 7. **P < .01, ***P < .001 versus respective vehicle-treated group; +++P < .001 versus respective EtOH-exposed dose group of 3α,5α-P.
imals, basal 3α,5α-P concentration (Fig. 9C) was decreased significantly during EtOH withdrawal ($F = 4.54; df = 1, 37; P = .04$), with a trend for a difference between the two strains ($F = 2.54; df = 1, 37; P = .12$) (i.e., B6 > D2). Although the interaction between main effects was not significant, basal 3α,5α-P concentration was decreased by 15% in B6 and 50% in D2 during EtOH withdrawal compared with similarly treated air-exposed mice.

Plasma CORT concentration on completion of the behavioral testing (Fig. 10) was significantly influenced by strain ($F = 9.93; df = 1, 150; P < .003$) (i.e., B6 < D2) and treatment ($F = 15.39; df = 1, 150; P = .0002$) (i.e., EtOH < air). In addition, dose of 3α,5α-P significantly decreased plasma CORT concentration ($F = 15.33; df = 3, 150; P < .0001$), no doubt due to the anxiolytic effect of this steroid. The interaction between strain and treatment also was significant ($F = 6.52; df = 1, 150; P < .02$). Simple main effects analysis indicated that the two strains differed in plasma CORT levels when exposed to air ($F = 10.72; df = 1, 150; P < .002$), but not when exposed to EtOH. In addition, the overall effect of EtOH withdrawal to increase plasma CORT concentration was significant only in B6 mice ($F = 14.685; df = 1, 150; P = .0002$). The lack of a significant effect of treatment in D2 mice may be due to the elevated basal CORT concentrations in the air-exposed animals.

When the analysis was limited to the vehicle-injected animals (Fig. 10C), basal CORT concentration was significantly influenced by strain ($F = 6.33; df = 1, 38; P < .02$), but not by treatment. In addition, the interaction between main effects was not significant. Therefore, the lack of a significant effect of treatment on basal CORT concentrations suggests that the vehicle-injected animals were moderately stressed.

### Discussion

Chronic EtOH exposure produced changes in sensitivity to exogenous administration of a GABA agonist steroid, which varied, depending on the genotype and pharmacological effect. Recent findings that chronic EtOH exposure enhanced neuroactive steroid stimulation of $[^3H]$muscimol binding (Negro et al., 1993) as well as potentiation of GABA$_A$ receptor-mediated chloride influx (Devaud et al., 1996) suggested that the sensitization to the anticonvulsant effect of 3α,5α-P that was demonstrated in rats (Devaud et al., 1996, 1998) might generalize to all of the pharmacological effects of 3α,5α-P. Consistent with the results in rats, the present findings indicate that sensitization to the anticonvulsant effect of 3α,5α-P was observed in B6 mice during EtOH withdrawal. In contrast, EtOH-exposed D2 mice were either cross-tolerant or exhibited no change in sensitivity to the anticonvulsant effect of 3α,5α-P, depending on the convulsion endpoint. The only other clear change in sensitivity to 3α,5α-P with chronic EtOH exposure occurred with muscle relaxation, where sensitivity to the muscle relaxant effect of 3α,5α-P was reduced during EtOH withdrawal in both genotypes. Importantly, these differences in behavioral sensitivity to 3α,5α-P in EtOH- versus air-exposed B6 and D2 mice were not due to differences between groups in plasma 3α,5α-P concentration. Therefore, in conjunction with the recent report that rats were sensitized to the anticonvulsant effect of 3α,5α-P during EtOH withdrawal (Devaud et al., 1996, 1998), the present findings suggest that sensitization to the anticonvulsant effect of 3α,5α-P during EtOH withdrawal does not generalize across all genotypes nor does it generalize to all of the pharmacological effects of 3α,5α-P.
The differential change in sensitivity to the anticonvulsant effect of 3α,5α-P during EtOH withdrawal in B6 versus D2 mice was not due to strain differences in EtOH exposure because there were no differences in the BEC among the groups of animals that were injected with vehicle or 3α,5α-P before behavioral testing during EtOH withdrawal. In the present study, BEC was determined immediately on initiation of withdrawal (i.e., on removal from inhalation chambers) and was not determined immediately before the behavioral testing. However, separate studies (D.A.F., unpublished data) have determined that BEC at 6-h postremoval from the inhalation chambers was negligible (mean ± S.E., 0.06 ± 0.03 mg/ml; n = 13) in animals with an initial BEC similar to that in the present study (1.16 ± 0.05 mg/ml). Therefore, it is unlikely that strain differences in EtOH pharmacokinetics contributed to the present findings as blood EtOH should have been nearly eliminated at the time of behavioral testing.

Chronic EtOH exposure produces well documented bidirectional changes in GABA A receptor subunit gene expression (Buck et al., 1991; Montpied et al., 1991; Mhatre et al., 1993; Mhatre and Ticku, 1994; Devaud et al., 1995, 1996; Mahmoudi et al., 1997). Overall, α1- and α2-subunit mRNA levels significantly decrease, whereas α4-, β1–3-, γ1-, and γ2S-subunit mRNA levels were significantly increased following various chronic EtOH exposure paradigms. Comparison between the EtOH-induced changes in GABA A receptor subunit mRNA levels versus peptide levels suggests that they are highly correlated during EtOH dependence (Devaud et al., 1997). Because studies using recombinantly expressed receptors have demonstrated that the GABA A receptor subunit composition can determine the pharmacological properties of these receptors (Sieghart, 1995), it is possible that chronic EtOH-induced changes in GABA A receptor subunit mRNA and peptide levels would lead to alterations in assembly of receptors and in sensitivity of these receptors to positive modulators.

With regard to neuroactive steroids, potentiation of GABA A receptor function does not exhibit an absolute requirement for any specific subunit (Lambert et al., 1995). However, recent work has found that recombinantly expressed receptors incorporating the γ1- (Puia et al., 1993) or α6- (Im et al., 1994; Hauser et al., 1995; Lambert et al., 1996) subunits had enhanced sensitivity to neuroactive steroids, whereas inclusion of the δ-subunit (Zhu et al., 1996) or recently identified ε-subunit (Davies et al., 1997) reduced sensitivity of these receptors to neuroactive steroids. Therefore, it is possible that strain and species differences in the brain regional distribution of GABA A receptors as well as in the chronic EtOH-induced alterations in subunit composition of GABA A receptors or post-translational modifications underlie the genotypic differences in neuroactive steroid sensitivity during EtOH withdrawal. The effects of chronic EtOH exposure on gene expression of specific GABA A receptor subunits are currently unknown in B6 and D2 mice. These studies are underway (K. Buck, unpublished data) and hopefully will provide information on the potential role of different GABA A receptor isoforms in the differential changes in sensitivity to 3α,5α-P during EtOH withdrawal.

Recent findings indicate that intra-amygdala administra-
tion of benzodiazepines produces anxiolysis without locomotor stimulation, muscle relaxation, ataxia, or seizure protection (Lotrich and Gallaher, 1998). Therefore, the bidirectional changes in sensitivity to the various pharmacological effects of 3α,5α-P in the present study suggest that chronic EtOH exposure is producing differential changes in GABA<sub>A</sub> receptors in the various brain regions that may underlie the distinct pharmacological properties of 3α,5α-P. Importantly, the enhanced sensitivity to the anticonvulsant effect and reduced sensitivity to the muscle relaxant effect of 3α,5α-P in B6 mice emphasizes the therapeutic potential of neuroactive steroids or their analogs in the treatment of alcohol dependence.

Even though the B6 and D2 strains were matched for chronic EtOH exposure, the two genotypes differ markedly in withdrawal severity (i.e., D2 > B6), when it is indexed by an increase in handling-induced convulsions following removal from the inhalation chambers (Crabbe, 1998). It is noteworthy that the enhanced sensitivity to the anticonvulsant effect of 3α,5α-P was evident only in B6 mice following exposure to chronic EtOH, an inbred strain that has modest withdrawal compared with a panel of inbred strains (Crabbe et al., 1983; Crabbe, 1998). This finding is consistent with our prediction that the change in sensitivity to the anticonvulsant effect of 3α,5α-P would be inversely related to withdrawal severity (i.e., ↑ sensitivity, ↓ withdrawal).

Withdrawal from chronic EtOH exposure also can produce changes in sensitivity to convulsant drugs (Watson and Little, 1995; Finn and Crabbe, 1999; Mhatre and Gonzalez, 1999). In the present study, basal seizure susceptibility to PTZ was increased in EtOH-withdrawing B6, but not D2, mice injected with vehicle. Although this finding might suggest that withdrawal severity in B6 was greater due to the significant increase in sensitivity to PTZ in the EtOH-versus air-exposed mice, EtOH naive D2 were much more sensitive to PTZ than B6 naive mice (i.e., compare air-exposed B6 versus D2 in Fig. 6). Therefore, the lack of a significant change in sensitivity to PTZ in EtOH-versus air-exposed D2 mice may be due to a “floor effect” in that it would be difficult to detect a further decrease in PTZ threshold dose with the current experimental paradigm because this genotype is extremely sensitive to PTZ in the absence of EtOH (Kosobud and Crabbe, 1990). It is also possible that independent mechanisms contribute to PTZ-induced versus EtOH withdrawal-induced convulsions. Nonetheless, when EtOH withdrawal is indexed by convulsions elicited by handling, withdrawal severity is greater in the D2 inbred strain.

The predicted anxiogenic effect of EtOH withdrawal was not apparent in the present study. An elevated plus maze was used to measure anxiety because this animal model (Pellow et al., 1985; Lister, 1987) is able to detect both anxiolytic and anxiogenic drug effects in mice (Lister, 1987). Because naïve animals typically spend ~25% of the test time in the open arms, the sides of the open arms were adjusted upward so that naïve animals would spend equal time in both open and closed arms, to facilitate detection of an anxiogenic effect of EtOH withdrawal. Therefore, it was surprising that the vehicle-injected, air-exposed mice spent less than the predicted 50% of time in the open arm. In a separate study in which B6 and D2 mice were tested only on the elevated plus maze during peak withdrawal from exposure to 72 h of EtOH vapor or air, there was a significant decrease in open-arm entries in the EtOH-exposed D2, but not B6, mice versus their respective air-exposed controls (D.A.F., unpublished data). Therefore, we are able to detect an anxiogenic effect of EtOH withdrawal following exposure to 72 h of EtOH vapor, with a greater increase in anxiety in D2 versus B6 mice, when the animals are not tested on several behavioral tasks. Consequently, it is unclear if the pyrazole injections in the air-exposed animals or the baseline grip strength and Rotarod testing of the animals before injection in the present study produced an increased level of anxiety in the air-exposed mice, which then made it difficult to detect an anxiogenic effect of withdrawal in the EtOH-exposed animals. Consistent with this notion, plasma CORT concentrations were elevated in the air-exposed animals (i.e., > 20 µg/dl) compared with a typical basal plasma CORT concentration of 2 to 5 µg/dl in naive animals or plasma CORT levels of 6 to 8 µg/dl in air-exposed B6 and D2 mice that were not behaviorally tested (D.A.F., unpublished data). This suggests that the air-exposed mice were moderately stressed.

It is unlikely that stress associated with repeated testing of an animal contributed to the change in sensitivity to 3α,5α-P that was observed following chronic EtOH exposure. First, we have previously validated the methodology for repeated testing of an animal and demonstrated that seizure susceptibility to PTZ, as well as sensitivity to the anticonvulsant effect of 3α,5α-P, was not different in B6 or D2 mice that were tested once versus repeatedly tested before the seizure susceptibility determination (Finn et al., 1997). Second, comparison of plasma 3α,5α-P and CORT concentrations in vehicle-injected animals in the present study with values from similarly treated B6 and D2 mice that were not behaviorally tested (D.A.F., unpublished data) suggests that plasma 3α,5α-P levels did not differ in tested versus untested mice (i.e., values in EtOH- versus air-exposed B6 and D2 mice were similar to that in the present study). However, plasma CORT concentrations did differ in that values in air-exposed mice were significantly lower than those in the present study (as discussed above) and were significantly increased during EtOH withdrawal in both genotypes. Therefore, the lack of significant differences in basal 3α,5α-P concentrations in tested versus untested mice, coupled with the similar innate sensitivity to the anticonvulsant effect of 3α,5α-P in repeatedly tested versus singly tested EtOH naïve mice, suggests that stress associated with activation of the hypothalamic-pituitary-adrenal axis did not influence sensitivity to the pharmacological effects of 3α,5α-P in the present study.

In conclusion, the present results indicate that two inbred strains that differ markedly in chronic EtOH withdrawal severity also differ in the change in sensitivity to the anticonvulsant effect of 3α,5α-P during EtOH withdrawal. If the present findings with exogenous administration of 3α,5α-P are indicative of a change in sensitivity to endogenous 3α,5α-P concentration, then exposure to chronic EtOH may render an EtOH-withdrawal-resistant strain more sensitive to 3α,5α-P than an EtOH-withdrawal-sensitive strain. Importantly, this relationship between genetic differences in EtOH withdrawal severity and 3α,5α-P sensitivity only may be relevant to the anticonvulsant effect because the sensitization to the anticonvulsant effect of 3α,5α-P during EtOH withdrawal in B6 mice did not generalize to all pharmacological effects of 3α,5α-P.
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