Available online at www.sciencedirect.com
SCIENCE DIRECT®
Pharmacology, Biochemistry and Behavior 76 (2003) 451–462
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Neurosteroid consumption has anxiolytic effects in mice

Deborah A. Finn[a,b,*], Amanda J. Roberts[b,1], Season Long[b], Michelle Tanchuck[b], Tamara J. Phillips[a,b]

[a]Department of Veterans Affairs Medical Center, Portland Alcohol Research Center, Portland, OR 97239, USA
[b]Department of Behavioral Neuroscience, Oregon Health & Science University, Portland, OR 97239, USA

Received 23 March 2003; received in revised form 24 August 2003; accepted 3 September 2003

Abstract

The neurosteroids allopregnanolone (ALLOP) and pregnanolone (PREG), like ethanol, potentiate γ-aminobutyric acid$_A$ receptor function. PREG-hemisuccinate (PREG-HS) is a negative modulator of *N*-methyl-D-aspartate (NMDA) receptors. Because C57BL/6J (B6) and DBA/2J (D2) mice differ in ethanol preference, voluntary consumption of ALLOP and PREG-HS (50 μg/ml solution) versus tap water was measured in B6 and D2 mice for a minimum of 8 days. Mice were acclimated to a reverse light–dark cycle prior to the initiation of experiments. In the first study, both B6 and D2 mice exhibited preference for the PREG-HS solution. In the second study, neither strain exhibited significant preference for the ALLOP solution versus water. However, the ALLOP-consuming B6 and D2 mice exhibited significant anxiolysis when they were tested on the elevated plus maze following 8 days of ALLOP consumption, compared to separate animals that consumed only water. A subsequent study determined that systemic administration of PREG-HS had significant anxiolytic effects in both B6 and D2 mice, when assessed on the elevated plus maze. Plasma ALLOP levels in the steroid-consuming mice from both studies were significantly increased versus basal levels only in the D2 strain. While the pattern of steroid intake or strain differences in steroid conversion may have influenced the differential change in plasma ALLOP levels, it is noteworthy that both strains consumed doses of ALLOP, and presumably doses of PREG-HS, that were anxiolytic.
© 2003 Elsevier Inc. All rights reserved.

Keywords: Neuroactive steroid; Preference drinking; Inbred strains; C57BL/6J; DBA/2J; Allopregnanolone; Pregnanolone-hemisuccinate; Elevated plus maze

1. Introduction

The reduced A-ring metabolites of progesterone [i.e., 3α-hydroxy-5α-pregnan-20-one or allopregnanolone (ALLOP) and 3α-hydroxy-5β-pregnan-20-one or pregnanolone (PREG)] and deoxycorticosterone (i.e., 3α,21-dihydroxy-5α-pregnan-20-one or 5α-THDOC) are potent positive modulators of γ-aminobutyric acid$_A$ (GABA$_A$) receptors (see reviews by Gasior et al., 1999; Lambert et al., 1995; Paul and Purdy, 1992). Nanomolar concentrations of ALLOP and 5α-THDOC potentiate the action of GABA (Gee et al., 1988; Morrow et al., 1987) and interact with sites on GABA$_A$ receptors in a noncompetitive manner (see review by Belelli et al., 1990). Administration of ALLOP and PREG produce anxiolytic, locomotor stimulant, ataxic, hypnotic and anticonvulsant effects (e.g., Finn et al., 1997b; Gasior et al., 1999; Palmer et al., 2002; Weiland et al., 1995). Taken in conjunction with the demonstration that stress, estrous cycle and pregnancy can increase endogenous ALLOP to levels that are pharmacologically relevant (see Barbaccia et al., 1994, 1996; Concas et al., 1998; Paul and Purdy, 1992), the available evidence suggests that GABAergic steroids modify the functioning of central GABA$_A$ receptors in vivo.

Drug discrimination studies demonstrated that ALLOP, PREG and 5α-THDOC share discriminative stimulus effects with pentobarbital, EtOH and diazepam (e.g., Ator et al., 1993; Bowen et al., 1999; Grant et al., 1997; Rowlett et al., 1999) and that PREG can function as a discriminative stimulus (Vanover, 1997, 2000). Intravenous self-administration of PREG maintained operant responding above saline levels in rhesus monkeys, indicating that this steroid can function as a positive reinforcer (Rowlett et al., 1999).

* Corresponding author. VAMC Research (R&D-49), Department of Veterans Affairs Medical Center, 3710 SW U.S. Veterans Hospital Road, Portland, OR 97239, USA. Tel.: +1-503-721-7984; fax: +1-503-273-5351.
E-mail address: finnd@ohsu.edu (D.A. Finn).
[1] Present address: Department of Neuropharmacology, SP-315, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA.

0091-3057/$ – see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2003.09.004

Another study demonstrated a dose-dependent conditioned place preference in ALLOP-treated DBA/2J (D2) mice, which was evident by the greater amount of time spent on a distinctive floor paired with ALLOP (Finn et al., 1997a). These data suggest that ALLOP has the ability to enhance the motivational value of environmental cues. Recent work from our laboratory also indicated that rats preferred to consume an ALLOP/tap water solution versus plain tap water (Sinnott et al., 2002a). Collectively, these results suggest that PREG and ALLOP possess positive motivational effects as well as similar subjective effects to drugs with abuse liability that are $GABA_A$ receptor modulators. Combined with the demonstration that ALLOP increases voluntary EtOH consumption (Sinnott et al., 2002b) and EtOH-reinforced operant responding (Janak et al., 1998) in male rodents, these findings also raise the possibility that endogenous steroids with $GABA_A$ receptor-agonist properties may play a role in modulating the rewarding effects of some drugs of abuse, including EtOH.

In contrast to the pharmacological profile for PREG, the sulfated form of the neurosteroid PREG (i.e., PREG-S), is a negative modulator of *N*-methyl-D-aspartate (NMDA) receptors (Irwin et al., 1994; Park-Chung et al., 1994). Additionally, sulfation of PREG at C-3 also reverses the modulation of $GABA_A$ receptors from positive to negative (Park-Chung et al., 1999). Inhibitory activity at $GABA_A$ receptors is retained when a hemisuccinate group is substituted for the sulfate at C-3, suggesting that it is the negative charge, rather than the sulfate group, that confers inhibitory activity (Park-Chung et al., 1999). While synthetic steroids bearing a hemisuccinate group may be more stable than sulfated steroids, due to their resistance to sulfatases, it was recently demonstrated that hemisuccinated steroids are able to penetrate the blood–brain barrier (Weaver et al., 1997). Specifically, systemic administration of PREG-hemisuccinate (PREG-HS) produced peak levels in brain at 10 min postinjection (Sadri-Vakili et al., 2003) and exhibited sedative, anticonvulsant, and analgesic properties (Weaver et al., 1997). Thus, this synthetic steroid may represent a new class of potentially useful therapeutic agents.

Several inbred mouse strain comparisons of free-choice EtOH consumption have demonstrated that C57BL/6J (B6) mice are extreme EtOH preferrers, whereas D2 mice are EtOH avoiders (e.g., Belknap et al., 1978, 1993; McClearn and Rodgers, 1959; Phillips et al., 1994). Although neurochemical differences between these strains have been identified, there has not been definitive evidence for an association between activity of a specific enzyme or neurotransmitter system and the genetic differences in EtOH preference (Phillips and Crabbe, 1991). B6 and D2 mice also are well known for their difference in sensitivity to EtOH's locomotor stimulant effects, with D2 mice exhibiting a larger stimulant response across a range of doses (e.g., Dudek et al., 1991). Recent work comparing the discriminative stimulus effects of EtOH in B6 and D2 mice suggested that, while the initial stimulus effects of 1.5 g/kg EtOH might be more salient in the D2 strain, the substitution profiles of several $GABA_A$ receptor-positive modulators, including PREG, were similar in the two strains (Shelton and Grant, 2002). This result would suggest that B6 and D2 mice do not differ in sensitivity to PREG's stimulus effects. However, these strains have been found to differ in behavioral sensitivity to ALLOP (Finn et al., 1997b; Palmer et al., 2002). B6 mice were more sensitive than D2 mice to the anxiolytic and anticonvulsant effects of ALLOP, in that the behavioral effects occurred at lower doses in B6 versus D2 mice (Finn et al., 1997b). B6 mice also exhibited locomotor stimulation following administration of a lower dose of ALLOP, but peak stimulant response was greater in D2 than in B6 mice (Palmer et al., 2002). Thus, the pattern of differences in sensitivity to $GABA_A$ receptor-agonist neurosteroids in these two strains appears to differ, depending on the behavioral trait examined.

Since rats have recently been shown to exhibit preference for an ALLOP solution (Sinnott et al., 2002a) and B6 and D2 mice differ in EtOH preference (e.g., Belknap et al., 1978, 1993; McClearn and Rodgers, 1959; Phillips et al., 1994), we hypothesized that B6 and D2 mice might also differ in their consumption of neurosteroid solutions. Due to the different pharmacological profile between ALLOP and PREG-HS in their action at $GABA_A$ receptors, the present studies measured voluntary consumption of ALLOP and PREG-HS in B6 and D2 mice. We also determined whether the animals consumed pharmacologically active (i.e., anxiolytic) doses of ALLOP by assessing their behavior on the elevated plus maze at the end of the drinking study. In addition, a separate study assessed whether injection of PREG-HS had anxiolytic properties in B6 and D2 mice, measured by elevated plus maze behavior.

2. Materials and methods

2.1. Subjects

Drug naïve, male B6 and D2 mice were used in all experiments. The animals were purchased from The Jackson Laboratory (Bar Harbor, ME) at 5 weeks of age and were initially housed four per cage during 2-week acclimation to a reverse 12:12-h light–dark cycle (lights off at 0900 h). During this time all animals had free access to food and water. The reverse light–dark cycle permitted the investigators to collect drinking data during the animals' dark phase, the time when mice would be expected to engage in the most consummatory behavior.

2.2. Preference testing: general procedures

Mice were individually housed in standard shoebox cages with ad libitum access to food and two tubes (inverted 25-ml graduated cylinders) containing tap water for 2 days.

For the subsequent 8–10 days, all mice were given a choice between a bottle containing tap water and a neurosteroid-containing bottle (50 μg/ml suspension in tap water). This concentration was chosen because it was at the limit of solubility and had the potential for mice to consume pharmacologically active doses, once factors such as hepatic metabolism and bioavailability were taken into account (Dr. R. Purdy, personal communication). Every 2 days (PREG-HS study) or every 4 days (ALLOP study), the position of the bottles was switched to control for side preferences in drinking. This is our common practice in drinking studies (e.g., Phillips et al., 1994) because it gives the mice adequate time to discern the position of the steroid-containing bottle and stabilize consumption from both sides of the cage. Tube position alternation occurred less often in the ALLOP study to improve the probability of higher intake, assuming that intake was associated with detection of the ALLOP-containing tube. Food was distributed in association with both tubes to disrupt food-associated drinking. Daily fluid consumption was recorded at 6 and 24 h following lights off. Pairs of tubes containing the same solutions as on experimental cages were monitored on two empty cages to control for evaporation and leakage. Daily consumption was corrected by subtracting the average depletion from these control tubes. Corrected consumption (ml) was converted to dose of neurosteroid (mg/kg), based on the animal's body weight (body weight was measured every 4 days). Preference ratios were calculated as the volume of steroid consumed (ml) divided by the total volume consumed from both tubes (ml). All procedures adhered to the United States Public Health Service—National Institutes of Health Guidelines for the care and use of laboratory animals and were approved by the local Institutional Animal Care and Use Committee.

2.3. Experiment 1: PREG-HS preference test

The initial study examined two-bottle choice voluntary consumption of PREG-HS, because this compound was reported to be water soluble and could readily cross the blood–brain barrier (Sadri-Vakili et al., 2003; Weaver et al., 1997). A 50 μg/ml suspension of PREG-HS (Steraloids, Wilton, NH) was prepared in tap water and sonicated for 45 min prior to use. Suspensions were stored in the refrigerator and stirred daily prior to use (i.e., filling of the drinking tubes). Fresh suspensions were prepared every 2–3 days. Neurosteroid drinking was monitored for eight consecutive days ($n = 10$/strain), as described above.

2.4. Experiment 2: ALLOP preference test

Once we had determined that mice would consume a neurosteroid solution, we desired to ascertain whether they would consume an anxiolytic dose under free-choice conditions. Since finding preference for two neurosteroid solutions would strengthen conclusions about neurosteroid preference and consumption, this second study measured voluntary ALLOP consumption.

Pilot studies determined that ALLOP (purchased from Dr. R. Purdy, custom synthesis) could be prepared as a 50 μg/ml suspension in 0.5% v/v EtOH in tap water and that B6 and D2 mice did not differ in consumption from a 0.5% v/v EtOH/tap water bottle versus a bottle containing tap water. The mean ± S.E.M. 24-h preference ratio for the 0.5% EtOH solution was 0.44 ± 0.09 for B6 mice and 0.54 ± 0.07 for D2 mice. These values were not significantly different. Therefore, tap water was used in the alternate bottle.

ALLOP suspensions (50 μg/ml) were stored in the refrigerator and stirred daily prior to use (i.e., filling of the drinking tubes). Fresh suspensions were prepared every 2–3 days. Steroid preference drinking was monitored for 9–10 consecutive days, as a span of 2 days was required for subsequent elevated plus maze testing. One group of mice was given unlimited access to tap water and the 50 μg/ml ALLOP solution in a two bottle choice paradigm ($n = 12$/strain). A separate group of animals ($n = 12$/strain) was individually housed with two water bottles from which to drink. This group of animals served as the control, non-ALLOP exposed group for the elevated plus maze testing upon completion of the drinking portion of the study.

2.5. Elevated plus maze testing

Because we could test only 24 mice on a single day for anxiety-like behavior on the elevated plus maze, it was necessary to complete testing on two consecutive days. Half of the neurosteroid-consuming and half of the water-consuming mice were tested on each day. Thus, mice were given access to their appropriate drinking tubes for 1 or 2 days following our evaluation of preference to maintain neurosteroid access until plus maze testing was completed. Tube positions were not switched from those of the four preceding days. Half of the animals were tested after the 6-h consumption measurement on Day 9 and the other half at the same time on Day 10. Mice were individually removed to an adjacent room and tested on the elevated plus maze for a 5-min trial. Plus maze testing occurred between 1500 and 1700 h. The elevated plus maze was used, based on its documented ability to detect both anxiolytic- and anxiogenic-like drug effects in mice (Lister, 1987).

Briefly, the elevated plus maze was constructed of clear Plexiglas and consisted of two open (0.5 cm lip) and two walled (15.5 cm high) horizontal perpendicular arms extending from a central platform (5 × 5 cm), 50 cm above the floor. Each mouse was placed on the central platform and allowed to explore the entire apparatus freely for 5 min. The number of entries into the open and closed arms as well as the amount of time spent in each arm was recorded. An arm entry was recorded when all four paws entered an arm. With this apparatus and the moderate lighting conditions utilized in the present study, mice prefer the closed arms of

the plus maze. Anxiolytic drugs typically increase the proportion of open arm entries and the time spent on the open arm. In the present study, the percent of open arm entries and open arm time were taken as indices of anxiety, whereas the total number of entries and number of closed arm entries were taken as estimates of locomotor activity (e.g., Rodgers and Johnson, 1995). Choice of these variables also was based on earlier work in which 16 inbred mouse strains, including B6 and D2, were assessed for their performance on the elevated plus maze (Trullas and Skolnick, 1993).

2.6. Experiment 3: Assessment of PREG-HS on the elevated plus maze

In this study, male B6 and D2 mice ($n = 40$/strain) were housed in a colony room on a regular light–dark cycle (lights on at 0600) for a minimum of 1 week before behavioral testing. Testing on the elevated plus maze occurred over 2 days, between 0900 and 1200 h. On the morning of behavioral testing, mice were moved to the procedure room 1 h preceding the testing and weighed. Then, B6 and D2 mice ($n = 10$/dose) were injected intraperitoneally with vehicle (20% w/v 2-hydroxypropyl β-cyclodextrin; β-cyclodextrin; Sigma, St. Louis, MO) or PREG-HS (5, 10, or 20 mg/kg in β-cyclodextrin). At 20 min postinjection, the mouse was placed on the plus maze in the center area for a 5-min test, as described above. Total number of entries into the open and closed arms, as well as time spent in open arms, closed arms and center area were recorded.

2.7. Radioimmunoassay (RIA)

Upon completion of the 8-day drinking study (Experiment 1) or 5-min test on the elevated plus maze (Experiment 2), each mouse was euthanized and trunk blood collected for subsequent analysis of plasma for ALLOP concentration by RIA. A separate group of naive B6 and D2 mice was euthanized to serve as the control group for the PREG-HS-consuming animals in Experiment 1, since there was no separate group of animals that consumed only water as in Experiment 2. The RIA for ALLOP was adapted from the method of Purdy et al. (1990) and is described in detail elsewhere (Finn and Gee, 1994). The RIA utilized a polyclonal antiserum, which was kindly provided by CoCensys (Irvine, CA) and [^{3}H]allopregnanolone (54 Ci/mmol; New England Nuclear, Boston, MA). Counts per min were normalized and fit to a least-squares regression equation produced by log-logit transformation of the standards. Mass of samples was calculated by interpolation of the standards and correction for recovery. The minimum detectable limit in the present assay was 25 pg. The intraassay coefficient of variation averaged 14%, and the interassay coefficient of variation in seven assays averaged 15%. While the RIA for ALLOP is not as quantitative or as sensitive as more recently published methods such as gas chromatography–mass spectrometry (GC-MS; Alomary et al., 2001; Uzunov et al., 1996), at the time that these assays were conducted, the sensitivity of the antibody was high ($IC_{50} = 0.185$ ng) and the minimum detectable limit in the present assays (amount of ALLOP causing a significant displacement of binding relative to total binding) was 25 pg. Additionally, cross-reactivity of the antibody with progesterone (5%) and the majority of other endogenous steroids (<2%) was low (Finn and Gee, 1994). However, cross reactivity with 3α-hydroxypregn-4-ene-20-one, another $GABA_A$-receptor active neurosteroid (Morrow et al., 1990), was 84%. While significant levels of the major cross-reactant steroid are not reported in circulation, this steroid could be formed by alternate pathways when the major 5α-reductase pathway is inhibited (Morrow et al., 2001). Thus, it is not known whether low levels of 3α-hydroxypregn-4-ene-20-one contributed to the detection of ALLOP by RIA in the present studies.

2.8. Data analysis

Data are expressed as mean ± S.E.M.. Separate analyses were conducted on the 6 h and 24 h consumption determinations (i.e., volume, dose and preference ratio). For the PREG-HS and ALLOP drinking studies, the average dose, preference ratio, and fluid consumption for each animal were calculated from the values obtained on each day of steroid drinking.

One-way analysis of variance (ANOVA) was used to assess strain effects on the drinking variables average volume, average dose and average preference ratio for PREG-HS and ALLOP consumption. Two-way ANOVA assessed strain and treatment (Exp. 2) or dose (Exp. 3) effects on the plus maze variables total arm entries, closed arm entries, percent of open arm entries, and percent of open arm time. Plasma ALLOP concentration also was analyzed for strain differences. Two-way ANOVA was used to compare average water versus neurosteroid intake (ml) between the inbred strains. Significant interactions were followed up with Simple Main Effects analysis. Since we had *a priori* reasons for determining whether neurosteroid consumption would differentially increase endogenous ALLOP levels, the effect of treatment on plasma ALLOP concentration was compared within each strain. The requirement for statistical significance was set at $P \leq .05$.

3. Results

3.1. Experiment 1: PREG-HS preference test

At the beginning of this drinking study, B6 mice weighed 25.9 ± 0.49 g and D2 mice weighed 24.6 ± 0.61 g. These values did not differ significantly.

There was no strain difference in 24-h fluid consumption. However, a strain difference was found for the amount consumed from the water versus PREG-HS tubes [$F(1,36) = 6.31$, $P < .02$]; the average intake from the PREG-HS-containing bottle was 2 × that of water intake in B6 mice, but only 1.4 × that of water intake in D2 mice (Fig. 1A). Simple Main Effects analysis indicated that both strains consumed more fluid from the PREG-HS- versus water-containing tube [$Ps < .01$]. However, B6 mice consumed more of the PREG-HS suspension than did the D2 mice [$P < .02$], whereas tap water consumption was similar in the two strains.

During the first 6 h of the dark phase, the strains also differed in the amount that they consumed from the water versus PREG-HS bottles [$F(1,36) = 10.91$, $P < .003$] (Fig. 1B). Simple Main Effects analysis indicated that only the B6 mice consumed more fluid from the PREG-HS- versus water-containing tube ($P < .0001$). Comparisons between the two strains indicated that tap water consumption did not differ, and that B6 mice consumed more of the PREG-HS suspension than did the D2 mice ($P < .0003$). These data suggest that B6 mice had a greater avidity for the PREG-HS suspension than D2 mice.

The average dose (mg/kg) of PREG-HS consumed during the first 6 and 24 h also was significantly greater in B6 versus D2 mice [$Fs(1,18) \geq 4.87$ $Ps < .05$] (Fig. 1C). Preference ratios for 24-h intake were 0.68 ± 0.04 for the B6 and 0.60 ± 0.03 for the D2 mice and did not differ significantly. However, preference ratios were 0.67 ± 0.03 for B6 and 0.57 ± 0.04 for D2 mice during the first 6 h of intake and were significantly different [$F(1,18) = 4.29$, $P = .05$]. Overall, both strains exhibited preference for the PREG-HS-containing suspension, although B6 mice exhibited a greater preference than did D2 mice during the early segment of the dark phase.

Plasma ALLOP concentration was measured in the neurosteroid-consuming and naive B6 and D2 mice upon completion of the 8 days of steroid drinking (Fig. 1D). Whereas there was no overall effect of strain or treatment on ALLOP levels, there was a trend for a significant interaction between strain and treatment [$F(1,32) = 2.80$, $P = .10$]. Subsequent analyses indicated that consumption of PREG-HS significantly increased plasma ALLOP levels in D2 mice, when compared with values in naive mice [$P < .05$], whereas this increase in plasma ALLOP levels was not seen in the B6 mice consuming PREG-HS.

3.2. Experiment 2: ALLOP preference test

At the beginning of this drinking study, B6 mice weighed 23.06 ± 0.41 g and D2 mice weighed 22.64 ± 0.53 g. These values did not differ significantly.

While there was no strain difference in overall fluid consumption for the 24 h measures, the strains did differ

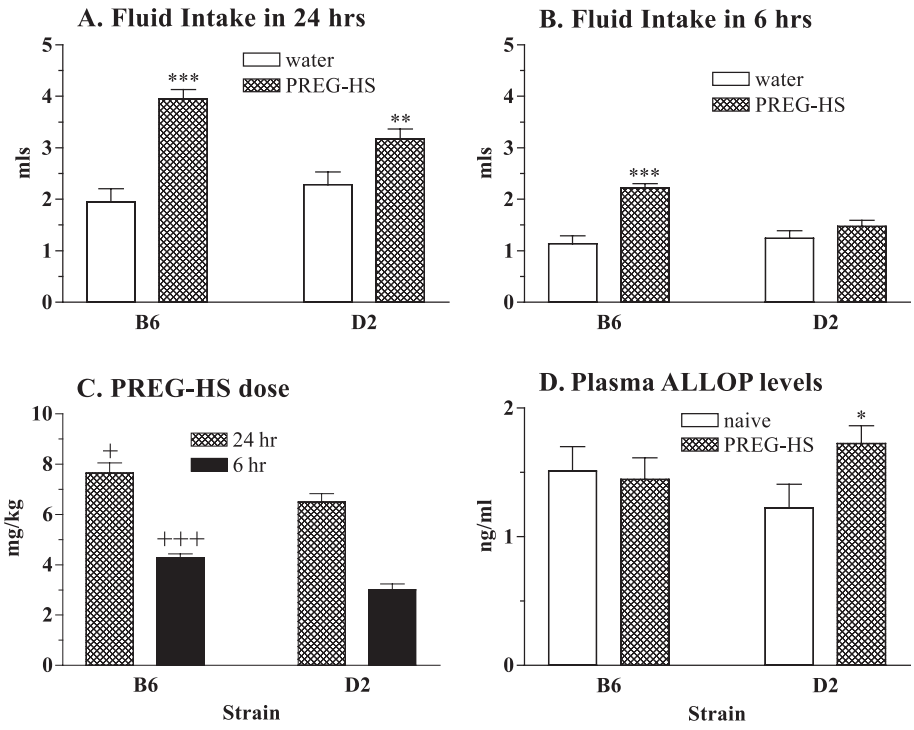

Fig. 1. PREG-HS consumption in B6 and D2 mice. Depicted is the average fluid consumption from the tap water- and PREG-HS-containing bottles across 24 h (A) or the first 6 h of the dark cycle (B), the corresponding dose of PREG consumed (C), and plasma ALLOP levels upon completion of the 8-day drinking study (D). Values represent the mean ± S.E.M. for $n = 10$ mice per strain, except for the naive mice depicted in panel D, where the $n = 8$ per strain. $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$ versus respective water-containing bottle (panels A and B) or naive group (panel D), Simple Main Effects analysis. $^{+}P < .05$, $^{+++}P < .001$ versus D2 mice, ANOVA.

in the amount consumed from the water versus ALLOP bottles [$F(1,44) = 4.69$, $P < .05$] (Fig. 2A). Average neurosteroid intake was 0.8 × that of water intake in the B6 mice, whereas neurosteroid intake was 1.3 × that of water intake in the D2 mice. D2 mice tended to consume more from the bottle containing the ALLOP suspension versus tap water [$P < .07$]. Simple Main Effects analysis also indicated that D2 mice consumed more of the ALLOP suspension than did the B6 mice [$P < .02$], whereas tap water consumption was similar in the two strains.

Similar results were found from analysis of data from the initial 6 h of the dark phase (Fig. 2B); there was a trend for the strains to differ in fluid consumption from the tap water- and ALLOP-containing bottles [$F(1,44) = 3.50$, $P < .07$]. D2 mice tended to consume more ALLOP suspension than water [$P = .09$]. D2 mice drank more ALLOP than B6 mice [$P < .03$], whereas the water consumption of the two strains was similar.

The dose (mg/kg) of ALLOP consumed in 24 h also was significantly greater in D2 versus B6 mice [$F(1,22) = 10.78$, $P < .005$] (Fig. 2C). There was a significant difference in preference ratios between the two strains [$F(1,22) = 4.56$, $P < .05$]; preference ratios were 0.58 ± 0.06 for the D2 and 0.45 ± 0.02 for the B6 mice. Similar results were found from the analysis of the 6 h consumption data. Dose of ALLOP consumed was significantly greater in D2 versus B6 mice [$F(1,22) = 9.10$, $P < .01$] (Fig. 2C), and there was a trend for a strain difference in preference ratio [$F(1,22) = 2.92$, $P = .10$]. Six-hour preference ratios were 0.54 ± 0.05 for the D2 mice and 0.45 ± 0.02 for the B6 mice. Overall, neither strain showed evidence of a preference for the ALLOP suspension.

Assessment of ALLOP dose and preference ratio in the mice prior to plus maze testing yielded comparable results to those described above following the 8 days of voluntary consumption (see also Fig. 2C). The dose of ALLOP consumed during the first 6 h of the dark phase on Day 9 or 10, immediately prior to plus maze testing, was significantly higher in D2 versus B6 mice [$F(1,22) = 4.78$, $P < .04$]; the ALLOP dose consumed was 3.69 ± 0.67 mg/kg for D2 and 2.06 ± 0.31 mg/kg for B6 mice. There was no significant strain difference in preference ratio; ratios were 0.61 ± 0.10 for D2 and 0.49 ± 0.07 for B6 mice.

Since the ALLOP was prepared as a suspension in a 0.5% EtOH solution, we also calculated the EtOH dose (g/kg) that was consumed in the ALLOP suspension. Both the 6 and 24 h EtOH doses consumed were significantly greater in D2 versus B6 mice [Fs(1,22)>9.1, Ps < .01] (Fig. 2D), consistent with the strain difference in consumption of the ALLOP suspension at these times. Additionally, the dose of EtOH consumed during the first 6 h of the dark phase immediately prior to testing on the elevated plus maze was significantly greater in D2 versus B6 mice [$F(1,22) = 4.78$, $P < 05$]; the EtOH dose consumed was 0.16 ± 0.025 g/kg for B6 and 0.29 ± 0.05 g/kg for D2 mice.

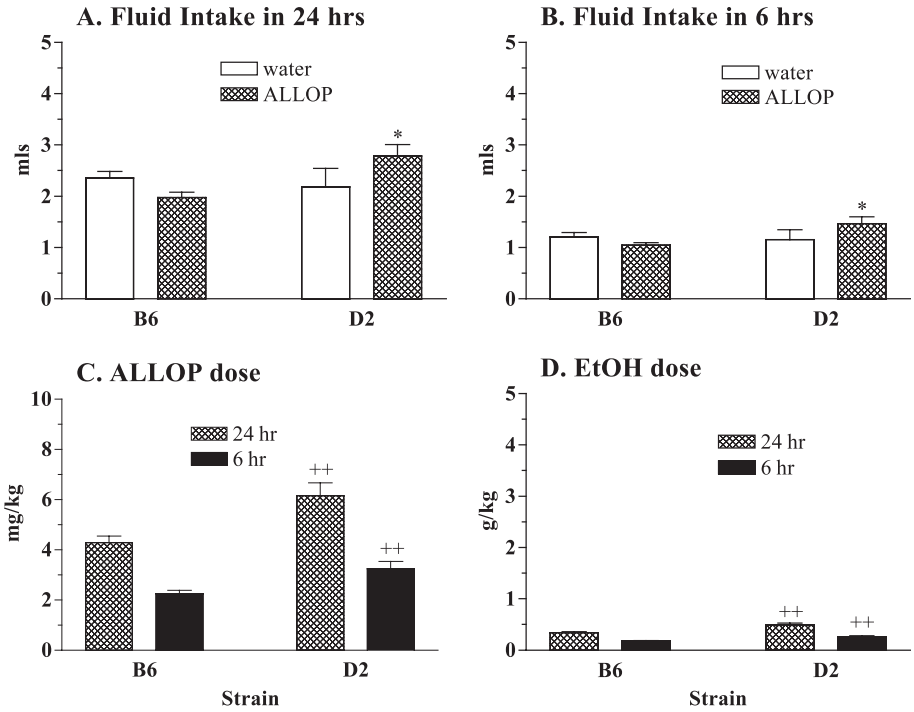

Fig. 2. ALLOP consumption in B6 and D2 mice. Depicted is the average fluid consumption from the tap water- and ALLOP-containing bottles across 24 h (A) or the first 6 h of the dark cycle (B), and the corresponding dose of ALLOP (C) and EtOH (D) that was consumed from the ALLOP suspension. Values represent the mean ± S.E.M. for $n = 12$ mice per strain. * $P < .05$ versus B6 ALLOP-containing bottle, Simple Main Effects analysis. $^{++}P < .01$ versus B6 mice, ANOVA.

3.3. Elevated plus maze

There was a significant increase in percent of open arm entries in the ALLOP-consuming mice versus mice that consumed only water [$F(1,44) = 7.09$, $P < .02$] (Fig. 3A).

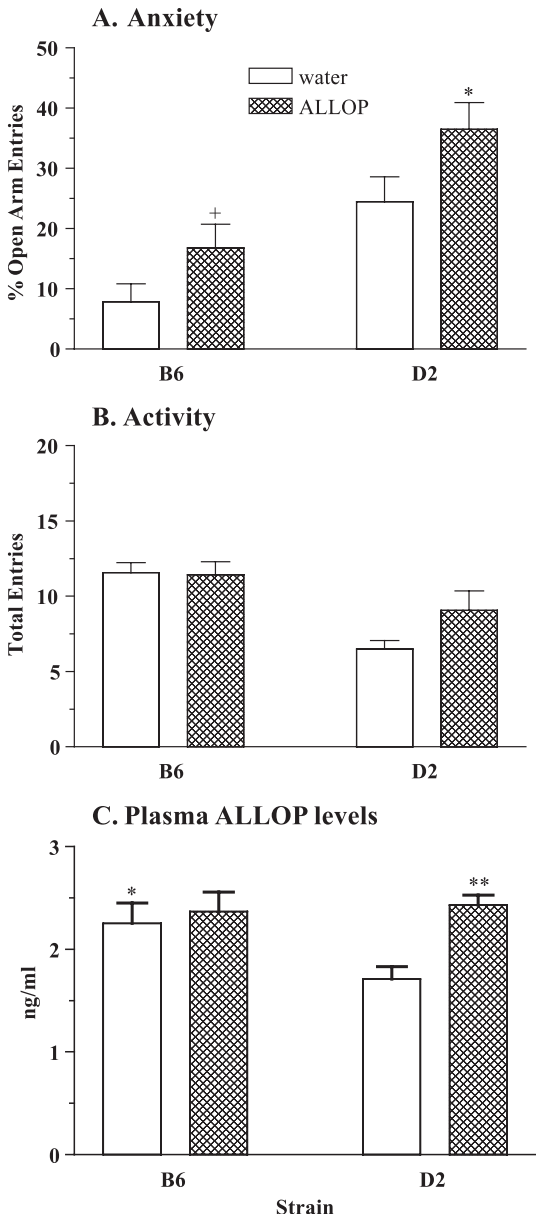

Fig. 3. ALLOP consumption produces anxiolytic effects on the elevated plus maze in B6 and D2 mice. Depicted are percent of open arm entries (A) and total entries (B) during a 5-min test on the elevated plus maze following 9–10 days of neurosteroid preference drinking and the corresponding plasma ALLOP levels (C). Water bars refer to separate groups of animals that consumed only tap water during the preference-drinking phase of the study. ALLOP consumption had significant anxiolytic-like effects, regardless of strain. Values represent the mean ± S.E.M. for $n = 12$ mice per strain and treatment for the behavioral data and $n = 9–12$ per strain and treatment for the plasma ALLOP data. * $P < .05$, * * $P < .01$ versus water-consuming D2 mice, ANOVA and Simple Main Effects analysis. $^{+}P < .05$ versus water-consuming B6 mice, ANOVA.

The percent of open arm entries was greater in D2 versus B6 mice [$F(1,44) = 21.31$, $P < .0001$], but there was no interaction between strain and treatment. Similar results were found for the analysis of percent of open arm time (data not shown); consumption of the ALLOP solution significantly increased percent of open arm time [$F(1,44) = 4.11$, $P < .05$], and the percent of time spent in the open arms was significantly greater in D2 versus B6 mice [$F(1,44) = 3.89$, $P = .05$]. These findings indicate that neurosteroid-consuming mice drank doses of ALLOP that had anxiolytic effects, when compared with animals that drank only water.

Analysis of total entries, an index of locomotor activity, indicated that there was no significant effect of treatment (Fig. 3B). However, total entries in B6 mice were significantly greater than in D2 mice [$F(1,44) = 17.22$, $P < .0003$]. The interaction between strain and treatment was not significant, even though total entries in neurosteroid-consuming D2 mice were somewhat greater than in their respective water-consuming controls. Closed arm entries, another index of activity, also were significantly influenced by strain (i.e., B6>D2) [$F(1,44) = 68.39$, $P < .0001$], but not by treatment (data not shown). These results suggest that consumption of the ALLOP suspension did not significantly alter activity levels on the elevated plus maze.

Plasma ALLOP concentration was measured in the neurosteroid- and water-consuming B6 and D2 mice upon completion of the plus maze testing (Fig. 3C). Consumption of ALLOP significantly increased plasma ALLOP levels [$F(1,38) = 7.72$, $P < .01$]. There was a trend for a strain difference in plasma ALLOP concentration (i.e., B6>D2) [$F(1,38) = 3.20$, $P = .08$], and a trend for a significant interaction between strain and treatment [$F(1,38) = 3.63$, $P = .06$]. Subsequent analyses indicated that plasma ALLOP levels were significantly higher in water-consuming B6 versus D2 mice [$P < .03$]. Consumption of ALLOP significantly increased plasma ALLOP levels only in D2 mice, when compared with values in the respective water-consuming mice [$P < .003$].

3.4. Experiment 3: Assessment of PREG-HS on the elevated plus maze

There was no significant difference in body weight of the B6 and D2 mice prior to testing on the elevated plus maze. B6 mice weighed 23.8 ± 0.3 g and D2 mice weighed 23.7 ± 0.3 g.

Systemic injection of PREG-HS significantly increased percent of open arm entries in both B6 and D2 mice [$F(3,72) = 8.54$, $P < .001$] (Fig. 4A). The 10 and 20 mg/kg doses of PREG-HS significantly increased percent of open arm entries in D2 mice, while only the highest dose of PREG-HS produced a significant increase in percent of open arm entries in B6 mice. Similar results were found for the analysis of percent of open arm time (data not

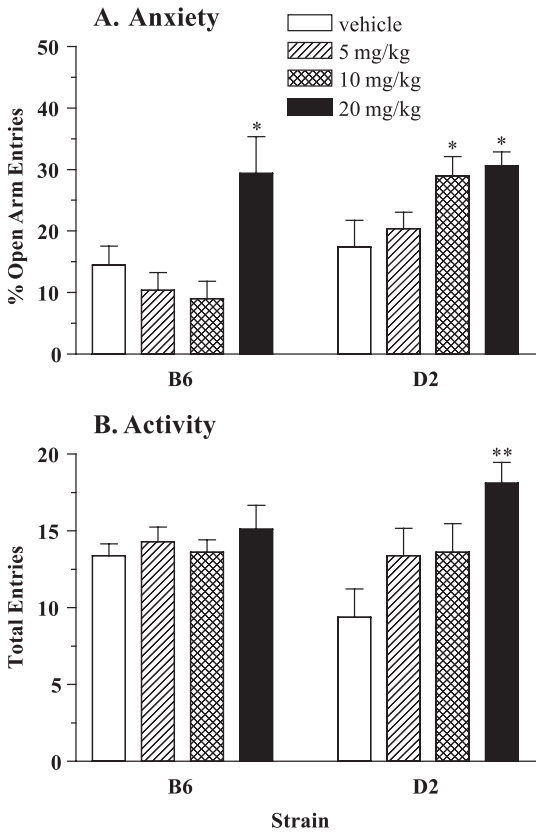

Fig. 4. Systemic administration of PREG-HS produces anxiolytic effects on the elevated plus maze in B6 and D2 mice. Depicted are percent of open arm entries (A) and total entries (B) during a 5-min test on the elevated plus maze at 20 min postinjection of the designated dose of PREG-HS or vehicle. Values represent the mean ± S.E.M. for $n = 10$ mice per strain and dose of PREG-HS. * $P < .05$, * * $P < .01$ versus respective vehicle-injected mice, Dunnett's multiple comparison test.

shown); injection of PREG-HS significantly increased percent of open arm time [$F(3,72) = 8.74$, $P < .001$], and the percent of time spent in the open arms was significantly greater in D2 versus B6 mice [$F(1,72) = 11.34$, $P < .001$]. These findings indicate that administration of PREG-HS produced anxiolytic effects in both B6 and D2 mice, when compared with respective vehicle-injected animals.

Analysis of total entries, an index of locomotor activity, was significantly influenced by PREG-HS dose [$F(3,72) = 4.40$, $P < .01$] (Fig. 4B). While there was not a significant strain difference in total arm entries in this study, administration of the 20 mg/kg dose of PREG-HS significantly increased total entries in D2, but not B6 mice. Closed arm entries, another index of activity, was significantly influenced by treatment [$F(3,72) = 3.40$, $P < .03$] and tended to be influenced by strain (i.e., B6>D2) [$F(1,72) = 3.07$, $P = .08$] (data not shown). These results suggest that systemic administration of PREG-HS significantly altered activity levels on the elevated plus maze only in D2 mice.

4. Discussion

4.1. Neurosteroid preference

The present findings showed that B6 and D2 male mice preferentially consumed PREG-HS in an unlimited access two-bottle choice paradigm. Both inbred mouse strains exhibited preference for the PREG-HS solution; however, preference was significantly greater in B6 than in D2 mice during the first 6 h of access. Thus, a greater EtOH preference in B6 versus D2 mice corresponds with greater PREG-HS preference in the B6 strain. In contrast, neither strain exhibited preference for the ALLOP solution. The basis for the differential consumption of the neurosteroid suspensions in D2 and B6 mice is unclear, but could be related to their pharmacological properties (i.e., ALLOP is a positive modulator of $GABA_A$ receptors, while PREG-HS is a negative modulator of $GABA_A$ and NMDA receptors). Additionally, when one considers that mice may regulate their neurosteroid dose as well as total fluid intake, preference may not be seen even when a solution has motivational or reinforcing effects.

Overall preference ratios for the neurosteroid solutions in the present study were lower than recent findings in which male rats displayed a strong preference for an equivalent ALLOP suspension (i.e., 50 μg/ml), when tested in an unlimited access two-bottle choice paradigm (Sinnott et al., 2002a). In that study, rats consumed approximately 80% of their total fluid from the ALLOP-containing bottle over the last 3 days of the 10-day drinking study, and exhibited significant preference for the ALLOP suspension beginning on Day 6 of the study. In the present studies, neurosteroid consumption had stabilized by Days 4–5 of the 8-day drinking studies. The species difference in preference ratio for ALLOP could be due to differences in body weight and the fact that in both studies, the rats and mice were drinking the same concentration of ALLOP (i.e., 50 μg/ml). In other words, it would be necessary for rats to consume a greater volume of the ALLOP solution than mice to achieve a comparable dose, and presumably pharmacological effect, of ALLOP. Consistent with this idea, the average dose of ALLOP consumed by rats during the 24-h access period ranged from 2.44 to 3.75 mg/kg (Sinnott et al., 2002a). This dose range of ALLOP is similar to that consumed by the B6 and D2 mice during the first 6 h of access to the neurosteroid suspension in the present study, and which was subsequently shown to be anxiolytic in both inbred strains.

It is possible that differences in the pharmacological properties of the two neurosteroid suspensions were interacting with assessments of neurosteroid preference and thus, contributing to the difference in preference ratios for the two neurosteroids that were found in the present study. While the anxiolytic properties of ALLOP are well documented (e.g., Finn et al., 1997b; Gasior et al., 1999; Weiland et al., 1995), the results from the present study also demonstrated that PREG-HS has anxiolytic properties. It is likely that the

anxiolytic properties of these two neurosteroids are due to their distinct pharmacological profile (i.e., positive modulation of $GABA_A$ receptors by ALLOP and negative modulation of NMDA and $GABA_A$ receptors by PREG-HS). Since ALLOP is at least 100 times more potent than PREG-HS (Gee et al., 1988; Morrow et al., 1987; Park-Chung et al., 1999; Weaver et al., 2000), it is possible that consumption of a lower ALLOP versus PREG-HS dose was required to produce a pharmacological effect in the present drinking studies, and hence, contributed to the differences in preference for the two neurosteroid suspensions.

EtOH drinking studies indicate that the B6 and D2 strains are among the extremes in voluntary EtOH consumption (e.g., Belknap et al., 1978, 1993; McClearn and Rodgers, 1959; Phillips et al., 1994), with B6 mice voluntarily consuming >10 g/kg of EtOH per day and D2 mice consuming <2 g/kg EtOH per day. When trying to compare results from neurosteroid drinking studies with EtOH preference studies, it should be noted that a number of factors could influence EtOH preference, such as central nervous system sensitivity to alcohol, taste, smell, caloric value, polydipsia, and acetaldehyde metabolism (discussed in McBride and Li, 1998; Phillips and Crabbe, 1991). D2 mice are much more sensitive to the aversive taste and odor properties of EtOH, when compared to B6 mice (Belknap et al., 1978, 1993), two factors that contribute to the EtOH aversion exhibited by the D2 strain in EtOH drinking studies. Yet, other animal models of EtOH reinforcement suggest that EtOH has positive motivational effects in D2 mice, such as conditioned place preference (e.g., Cunningham et al., 1992), intravenous EtOH self-administration (Grahame and Cunningham, 1997), and EtOH-stimulated locomotor activity (e.g., Dudek et al., 1991). Recent drug discrimination studies indicate that both B6 and D2 mice will acquire a 1.5 g/kg EtOH and saline discrimination, but that D2 mice may have an enhanced sensitivity to the initial discriminative stimulus effects of EtOH (Shelton and Grant, 2002). Because the D2 strain is very sensitive to the aversive taste and odor properties of EtOH, we ensured that neither the D2 nor B6 mice exhibited preference or aversion for a 0.5% EtOH solution versus tap water prior to initiating the ALLOP drinking study (see Materials and methods). Thus, it is unlikely that the presence of EtOH in the ALLOP suspension significantly influenced preference for this neurosteroid, particularly since neither strain exhibited preference or aversion for the 0.5% concentration of EtOH. We attempted to minimize potential aversive taste and odor properties in the present drinking studies so these factors would not confound our assessment of preference for the two neurosteroid suspensions.

4.2. Elevated plus maze

With regard to the ALLOP drinking study, both strains of mice consumed doses of ALLOP that were anxiolytic, when the animals were subsequently tested on the elevated plus maze. There was a significant increase in percent of open arm entries and time in the ALLOP-consuming animals versus animals that consumed tap water. Thus, consumption of ALLOP produced a significant decrease in the natural tendency of rodents to avoid the open arms of the elevated plus maze, which is considered to represent an alteration in anxiety or emotionality. Treatment did not significantly alter total entries or closed arm entries, suggesting that the anxiolytic effect of ALLOP was not associated with a concomitant change in general activity level. These findings are consistent with previous work in which injection of ALLOP produced anxiolytic effects at doses that did not affect activity in B6 and D2 mice, when they were tested on the elevated plus maze (Finn et al., 1997b; Rodgers and Johnson, 1998). Importantly, the dose of EtOH that was consumed in the ALLOP suspension was 10-fold lower than the doses that produce anxiolytic effects in mice (i.e., 1.5–2.5 g/kg; Boehm et al., 2002; D. Finn, unpublished) or the doses that can enhance the ataxic effect of GABAergic neurosteroids (Vanover et al., 1999). Thus, it is unlikely that the dose of EtOH consumed contributed to the anxiolytic effect of ALLOP that was observed in the present study.

Systemic administration of PREG-HS also had anxiolytic effects in both B6 and D2 mice, which was evident by the significant increase in percentage of open arm entries and time on the elevated plus maze. But, in contrast to the results with consumption of ALLOP, injection of PREG-HS significantly altered total entries and closed arm entries. While the highest dose of PREG-HS significantly increased total entries only in D2 mice, the 10 mg/kg dose of PREG-HS was anxiolytic without altering activity in this strain. Thus, these findings suggest that PREG-HS can produce anxiolytic effects at doses that do not affect activity in both B6 and D2 mice. Since it has been hypothesized that the anxiolytic properties of a drug may contribute to its reinforcing effects (discussed in Grant, 1995), it is possible that ALLOP (and PREG-HS) may have been consumed voluntarily and in some instances preferentially, due to its reinforcing properties.

The present findings also are consistent with earlier work indicating that B6 and D2 mice differ in basal levels of anxiety and activity, measured on the elevated plus maze (Trullas and Skolnick, 1993). In both studies, closed arm entries and total entries were significantly greater in B6 versus D2 mice, suggesting that general activity level is greater in B6 mice. A similar strain difference in basal level of activity has been reported by others using automated activity monitors (e.g., Phillips et al., 1995; Wenger, 1989). For the basal level of anxiety measures, the percent of open arm entries and time were significantly decreased in B6 versus D2 mice in the ALLOP drinking study, suggesting that anxiety level was greater in B6 mice. However, the strain difference in basal level of anxiety was not as pronounced in the study with PREG-HS. It is not known

if these differences reflect the time of day at which the animals were tested (i.e., during dark phase in ALLOP study and during light phase in PREG-HS study).

4.3. Plasma ALLOP levels

Plasma ALLOP concentrations in the animals consuming water indicate that basal ALLOP levels were significantly lower in D2 versus B6 mice. Plasma ALLOP levels also were lower in naive D2 versus B6 animals from the PREG-HS drinking study, but the difference was not significant. Overall, this finding for a strain difference in basal ALLOP concentration is consistent with recent results from our laboratory (Finn et al., 1997b). Basal performance on the plus maze appeared to be inversely related to endogenous ALLOP levels (compare values in water-consuming animals in Fig. 3). This finding would suggest that there is no correlation between endogenous ALLOP levels and basal levels of anxiety or that additional factors unrelated to endogenous ALLOP level are contributing to basal performance on the elevated plus maze.

There was no strain difference in plasma ALLOP levels in the neurosteroid-consuming B6 and D2 mice from both drinking studies. The significant increase in plasma ALLOP levels in the D2 mice consuming the PREG-HS suspension was surprising in light of the fact that PREG-HS consumption was significantly lower in this strain (i.e., 24-h dose was 6.5 mg/kg for D2 and 7.65 mg/kg for B6). Thus, we cannot rule out at the present time whether or not strain differences in neurosteroid conversion contributed to the significant increase in plasma ALLOP levels in the D2 mice consuming the PREG-HS suspension. Since brain ALLOP levels were not determined in the present study, it is also possible that plasma ALLOP concentration does not adequately reflect brain ALLOP levels following consumption of neurosteroid suspensions.

However, the significant increase in plasma ALLOP levels in the ALLOP-consuming D2 mice most likely reflects the greater neurosteroid consumption of this strain (i.e., 3.7 mg/kg for D2 and 2.1 mg/kg for B6 prior to plus maze testing), rather than a strain difference in metabolism of ALLOP or in stability of the neurosteroid solution. Previous work has demonstrated that B6 and D2 mice did not differ in plasma ALLOP levels following injection of ALLOP at doses ranging from 1 to 32 mg/kg (Finn et al., 1997b), suggesting that the strains do not differ in ALLOP metabolism. With regard to stability of the neurosteroid solution, the same solution was used for both inbred strains and was verified daily to be in suspension. Additionally, solutions were prepared fresh every 2–3 days, stored in the refrigerator and stirred daily prior to use (i.e., filling of the drinking tubes). Even though aqueous steroid solutions can be subject to some oxidation (Dr. R. Purdy, personal communication), a pilot study determined that the concentration of ALLOP in the solution did not change significantly over 7 days of measurement. There was an 8% decrease in the concentration of ALLOP on the first day after the solution was prepared, which decreased to 15% when the solution was tested 1 week later (D. Finn, unpublished), confirming the stability of the neurosteroid solution during the time frame that it was utilized in the present study.

Even though the neurosteroid-drinking B6 and D2 mice consumed different doses of ALLOP and had similar plasma ALLOP levels, ALLOP consumption had anxiolytic effects in B6 and D2 mice. This suggests that either a threshold concentration of endogenous ALLOP was necessary for anxiolysis, the pattern of ALLOP intake differed between B6 and D2 mice in the 6-h period prior to plus maze testing, or that B6 mice could consume a lower dose of ALLOP to achieve an anxiolytic effect. This third possibility is consistent with the recent finding that B6 mice were more sensitive than D2 to the anxiolytic effect of exogenously administered ALLOP (Finn et al., 1997b). Additionally, since we do not know the pattern of intake of the ALLOP solution in the 6 h prior to plus maze testing, it is also possible that the plasma ALLOP levels do not reflect the true endogenous concentration of ALLOP as accurately as the values in the water-consuming animals, since brain ALLOP levels were not measured in this study.

5. Conclusion

The present results are consistent with a growing body of evidence suggesting that neurosteroids possess mildly reinforcing properties and discriminative stimulus effects that are similar to drugs with abuse liability (Ator et al., 1993; Bowen et al., 1999; Finn et al., 1997a; Grant et al., 1997; Rowlett et al., 1999; Sinnott et al., 2002a; Vanover, 1997, 2000). The fact that two inbred strains of mice, which differ markedly in EtOH preference, exhibited preference for a PREG-HS suspension and voluntarily consumed anxiolytic doses of ALLOP (without exhibiting preference for the ALLOP suspension) is noteworthy. The difference in preference for these two neurosteroids most likely reflects their different actions at $GABA_A$ and NMDA receptors, even though both compounds have anxiolytic properties. These findings indicate that further investigation of the interaction of neurosteroids with alcohol consumption is warranted.

Acknowledgements

The research was supported by NIAAA grants P50 AA10760, RO1 AA12439 and the Department of Veterans Affairs. We thank Charlotte Wenger for expert technical assistance and Dr. Rachna Sinnott for critical commentary on a draft of this manuscript. ALLOP was synthesized by, and purchased from, Dr. Robert H. Purdy. The antibody to ALLOP was a generous gift from Co-Censys (Irvine, CA).

References

Alomary AA, Fitzgerald RL, Purdy RH. Neurosteroid analysis. Int Rev Neurobiol 2001;46:97–115.

Ator NA, Grant KA, Purdy RH, Paul SM, Griffiths RR. Drug discrimination analysis of endogenous neuroactive steroids in rats. Eur J Pharmacol 1993;241:237–43.

Barbaccia ML, Roscetti G, Trabucchi M, Cuccheddu T, Concas A, Biggio G. Neurosteroids in the brain of handling-habituated and naïve rats: effect of CO_2 inhalation. Eur J Pharmacol 1994;261:317–20.

Barbaccia ML, Roscetti G, Trabucchi M, Mostallino MC, Concas A, Purdy RH, et al. Time-dependent changes in rat brain neuroactive steroid concentrations and $GABA_A$ receptor function after acute stress. Neuroendocrinology 1996;63:166–72.

Belelli D, Lan NC, Gee KW. Anticonvulsant steroids and the GABA/benzodiazepine receptor-chloride ionophore complex. Neurosci Biobehav Rev 1990;14:315–22.

Belknap JK, Coleman RR, Foster K. Alcohol consumption and sensory threshold differences between C57BL/6J and DBA/2J mice. Physiol Psychol 1978;6:71–4.

Belknap JK, Crabbe JC, Young ER. Voluntary consumption of ethanol in 15 inbred mouse strains. Psychopharmacology 1993;112:503–10.

Boehm II SL, Reed CL, McKinnon CS, Phillips TJ. Shared genes influence sensitivity to the effects of ethanol on locomotor activity and anxiety-like behaviors, and the stress axis. Psychopharmacology 2002; 161:54–63.

Bowen CA, Purdy RH, Grant KA. Ethanol-like discriminative stimulus effects of endogenous neuroactive steroids: effect of ethanol training dose and dosing procedure. J Pharmacol Exp Ther 1999;289:405–11.

Concas A, Mostallino MC, Porcu P, Follesa P, Barbaccia ML, Trabucchi M, et al. Role of brain allopregnanolone in the plasticity of gamma-aminobutyric acid type A receptor in rat brain during pregnancy and after delivery. Proc Natl Acad Sci 1998;95:13284–9.

Cunningham CL, Niehus DR, Malott DH, Prather LK. Genetic differences in the rewarding and activating effects of morphine and ethanol. Psychopharmacology 1992;107:385–93.

Dudek BC, Phillips TJ, Hahn ME. Genetic analysis of the biphasic nature of the alcohol dose–response curve. Alcohol Clin Exp Res 1991;15: 262–9.

Finn DA, Gee KW. The estrus cycle, sensitivity to convulsants and the anticonvulsant effect of a neuroactive steroid. J Pharmacol Exp Ther 1994;271:164–70.

Finn DA, Phillips TJ, Okorn DM, Chester JA, Cunningham CL. Rewarding effect of the neuroactive steroid 3α-hydroxy-5α-pregnan-20-one in mice. Pharmacol Biochem Behav 1997a;56:261–4.

Finn DA, Roberts AJ, Lotrich F, Gallaher EJ. Genetic differences in behavioral sensitivity to a neuroactive steroid. J Pharmacol Exp Ther 1997b;280:820–8.

Gasior M, Carter RB, Witkin JM. Neuroactive steroids: potential therapeutic use in neurological and psychiatric disorders. Trends Pharmacol Sci 1999;20:107–12.

Gee KW, Bolger MB, Brinton RE, Coirini H, McEwen BS. Steroid modulation of the chloride ionophore in rat brain: structure–activity requirements, regional dependence and mechanism of action. J Pharmacol Exp Ther 1988;246:803–12.

Grahame NJ, Cunningham CL. Intravenous ethanol self-administration in C57BL/6J and DBA/2J mice. Alcohol Clin Exp Res 1997;21:56–62.

Grant KA. Animal models of the addiction process. In: Kranzler HR, editor. The pharmacology of alcohol abuse. Handbook of Experimental Pharmacology, vol. 114. Berlin: Springer-Verlag, 1995. pp. 185–229.

Grant KA, Azarov A, Shively CA, Purdy RH. Discriminative stimulus effects of ethanol and 3α-hydroxy-5α-pregnan-20-one in relation to menstrual cycle phase in cynomolgus monkeys (*Macaca fascicularis*). Psychopharmacology 1997;130:59–68.

Irwin RP, Lin SZ, Rogawski MA, Purdy RH, Paul SM. Steroid potentiation and inhibition of *N*-methyl-D-aspartate receptor-mediated intracellular Ca^{2+} responses: structure activity studies. J Pharmacol Exp Ther 1994; 271:677–82.

Janak PH, Redfern JEM, Samson HH. The reinforcing effects of ethanol are altered by the endogenous neurosteroid, allopregnanolone. Alcohol Clin Exp Res 1998;22:1106–12.

Lambert JJ, Belelli D, Hill-Venning C, Peters JA. Neurosteroids and $GABA_A$ receptor function. Trends Pharmacol Sci 1995;16:295–303.

Lister RG. The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology 1987;92:180–5.

McBride WJ, Li T-K. Animal models of alcoholism: neurobiology of high alcohol-drinking behavior in rodents. Crit Rev Neurobiol 1998;12: 339–69.

McClearn GE, Rodgers DA. Differences in alcohol preference among inbred strains of mice. Q J Stud Alcohol 1959;20:691–5.

Morrow AL, Suzdak PD, Paul SM. Steroid hormone metabolites potentiate GABA receptor-mediated chloride ion flux with nanomolar potency. Eur J Pharmacol 1987;142:483–5.

Morrow AL, Pace JR, Purdy RH, Paul SM. Characterization of steroid interactions with γ-aminobutyric acid receptor-gated chloride ion channels: evidence for multiple recognition sites. Mol Pharmacol 1990;37: 263–70.

Morrow AL, VanDoren MJ, Fleming R, Penland S. Ethanol and neurosteroid interactions in the brain. Int Rev Neurobiol 2001;46:349–77.

Palmer AA, Miller MN, McKinnon CS, Phillips TJ. Sensitivity to the locomotor stimulant effects of ethanol and allopregnanolone shares common genetic influence. Behav Neurosci 2002;116:126–37.

Park-Chung M, Wu FS, Farb DH. 3α-hydroxy-5β-pregnan-20-one sulfate: a negative modulator at the NMDA receptor. Mol Pharmacol 1994;46: 146–50.

Park-Chung M, Malayev A, Purdy RH, Gibbs TT, Farb DH. Sulfated and unsulfated steroids modulate γ-aminobutyric $acid_A$ receptor function through distinct sites. Brain Res 1999;830:72–87.

Paul SM, Purdy RH. Neuroactive steroids. FASEB J 1992;6:2311–22.

Phillips TJ, Crabbe JC. Behavioral studies of genetic differences in alcohol action. In: Crabbe JC, Harris RA, editors. The genetic basis of alcohol and drug actions. New York: Plenum, 1991. pp. 25–104.

Phillips TJ, Crabbe JC, Metten P, Belknap JK. Localization of genes affecting alcohol drinking in mice. Alcohol Clin Exp Res 1994;18:931–41.

Phillips TJ, Huson M, Gwiazdon C, Burkhart-Kasch S, Shen EH. Effects of acute and repeated ethanol exposures on the locomotor activity of BXD recombinant inbred mice. Alcohol Clin Exp Res 1995;19:269–78.

Purdy RH, Moore Jr PH, Rao N, Hagino N, Yamaguchi T, Schmidt P, et al. Radioimmunoassay of 3α-hydroxy-5α-pregnan-20-one in rat and human plasma. Steroids 1990;55:290–6.

Rodgers RJ, Johnson NJT. Factor analysis of spatiotemporal and ethological measures in the murine elevated plus-maze test of anxiety. Pharmacol Biochem Behav 1995;52:297–303.

Rodgers RJ, Johnson NJT. Behaviorally selective effects of neuroactive steroids on plus-maze anxiety in mice. Pharmacol Biochem Behav 1998;59:221–32.

Rowlett JK, Winger G, Carter RB, Wood PL, Woods JH, Woolverton WL. Reinforcing and discriminative stimulus effects of the neuroactive steroids pregnanolone and Co 8-7071 in rhesus monkeys. Psychopharmacology 1999;145:205–12.

Sadri-Vakili G, Johnson DW, Janis GC, Gibbs TT, Pierce RC, Farb DH. Inhibition of NMDA-induced striatal dopamine release and behavioral activation by the neuroactive steroid 3α-hydroxy-5β-pregnan-20-one hemisuccinate. J Neurochem 2003;86:92–101.

Shelton KL, Grant KA. Discriminative stimulus effects of ethanol in C57BL/6J and DBA/2J inbred mice. Alcohol Clin Exp Res 2002;26:747–57.

Sinnott RS, Mark GP, Finn DA. Reinforcing effects of the neurosteroid allopregnanolone in rats. Pharmacol Biochem Behav 2002a;72:923–9.

Sinnott RS, Phillips TJ, Finn DA. Alteration of voluntary ethanol and saccharin consumption by the neurosteroid allopregnanolone in mice. Psychopharmacology 2002b;162:438–47.

Trullas R, Skolnick P. Differences in fear motivated behaviors among inbred mouse strains. Psychopharmacology 1993;111:323–31.

Uzunov DP, Cooper TB, Costa E, Guidotti A. Fluoxetine-elicited changes in brain neurosteroid content measured by negative ion mass fragmentography. Proc Natl Acad Sci 1996;93:12599–604.

Vanover KE. Discriminative stimulus effects of the endogenous neuroactive steroid pregnanolone. Eur J Pharmacol 1997;327:97–101.

Vanover KE. Effects of benzodiazepine receptor ligands and ethanol in rats trained to discriminate pregnanolone. Pharmacol Biochem Behav 2000; 67:483–7.

Vanover KE, Suruki M, Robledo S, Huber M, Wieland S, Lan NC, et al. Positive allosteric modulators of the $GABA_A$ receptor: differential interaction of benzodiazepines and neuroactive steroids with ethanol. Psychopharmacology 1999;141:77–82.

Weaver Jr CE, Marek P, Park-Chung M, Tam SW, Farb DH. Neuroprotective activity of a new class of steroidal inhibitors of the *N*-methyl-D-aspartate receptor. Proc Natl Acad Sci 1997;94:10450–4.

Weaver CE, Land MB, Purdy RH, Richards KG, Gibbs TT, Farb DH. Geometry and charge determine pharmacological effects of steroids on *N*-methyl-D-aspartate receptor-induced Ca^{2+} accumulation and cell death. J Pharmacol Exp Ther 2000;293:747–54.

Weiland S, Beluzzi JD, Stein L, Lan NC. Comparative behavioral characterization of the neuroactive steroids 3α-OH, 5α-pregnan-20-one and 3α-OH, 5β-pregnan-20-one in rodents. Psychopharmacology 1995;118: 65–71.

Wenger GR. The role of control activity levels in the reported strain differences to the behavioral effects of drugs in mice. Pharmacol Biochem Behav 1989;32:241–7.