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Interaction of chronic ethanol exposure and finasteride: sex and strain differences

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Abstract

The neurosteroid allopregnanolone (ALLOP) is a very potent positive modulator of γ-aminobutyric acid$_A$ (GABA$_A$) receptors that can modulate ethanol (EtOH) withdrawal. The 5α-reductase inhibitor finasteride blocks the formation of ALLOP from progesterone and was recently found to reduce some effects of EtOH. Thus, the present studies were conducted to determine the effect of finasteride on chronic EtOH withdrawal severity in male and female C57BL/6 (B6) and DBA/2 (D2) mice. The animals were exposed to 72 h EtOH vapor or air and received four injections of finasteride (50 mg/kg ip) 24 h prior to, and each day of, the EtOH vapor exposure. Upon removal from the inhalation chambers, handling-induced convulsions (HICs) were measured hourly for the first 12 h and then again at 24 h. EtOH withdrawal severity was significantly greater in D2 than in B6 mice. Pretreatment with finasteride significantly decreased EtOH withdrawal severity only in the female D2 mice, produced a nonselective suppressive effect on HIC in male B6 and D2 mice, and did not significantly alter HIC in female B6 mice. Finasteride pretreatment significantly decreased blood EtOH concentration (BEC) upon initiation of withdrawal, suggesting that finasteride may affect withdrawal severity via an alteration in EtOH pharmacokinetics.

Keywords: C57BL/6; DBA/2; Allopregnanolone; 5α-reductase; Handling-induced convulsions; Withdrawal; Neurosteroid

1. Introduction

The progesterone derivative allopregnanolone (ALLOP) is both a neuroactive steroid, based on its rapid membrane actions via an interaction with ligand-gated ion channels (see Paul and Purdy, 1992; Lambert et al., 1995; Rupprecht and Holsboer, 1999), and a neurosteroid, based on evidence that its levels in the brain were independent from plasma concentrations (e.g., Paul and Purdy, 1992; Corpechot et al., 1993; Mellon, 1994). Results over the past 20 years have demonstrated that ALLOP is a very potent positive modulator of γ-aminobutyric acid$_A$ (GABA$_A$) receptors (e.g., Morrow et al., 1987; Gee et al., 1988; Belelli et al., 1990; Lambert et al., 1995). Importantly, the positive modulatory effect of neurosteroids at GABA$_A$ receptors is relatively specific, in that these steroids do not interact with other neurotransmitter receptors in the nanomolar to low micromolar concentration range (e.g., Rupprecht and Holsboer, 1999).

Since ALLOP is a derivative of progesterone, endogenous levels in females can range from 10 to 30 nM and can increase to approximately 100 nM during pregnancy (Paul and Purdy, 1992; Finn and Gee, 1994; Concas et al., 1998; Finn et al., 2004b). However, ALLOP concentration in males also can increase to the equivalent of 10–30 nM following exposure to various stressors (e.g., Purdy et al., 1991; Barbaccia et al., 2001). These concentrations achieved in vivo are comparable to those that have been shown to potentiate the action of GABA at GABA$_A$ receptors in vitro (e.g., Morrow et al., 1987; Gee et al., 1988; Belelli et al., 1990; Lambert et al., 1995), suggesting that fluctuations in endogenous ALLOP levels may modulate GABA$_A$ receptors. Support for this idea is provided by the recent report in which manipulation of endogenous ALLOP levels in the dentate gyrus revealed an endogenous neurosteroid tone that

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was sufficient to modulate $GABA_A$-receptor-mediated synaptic inhibition (Belelli and Herd, 2003).

The pharmacological profile of ALLOP is similar to that of ethanol (EtOH). While the effects of ALLOP appear to be primarily mediated via its interaction with $GABA_A$ receptors, EtOH interacts with multiple receptor systems, with one effect being to potentiate $GABA_A$ receptor function (see Grobin et al., 1998; Morrow et al., 2001). Taken in conjunction with the putative neuromodulatory effects of neuroactive steroids in vivo, work from several laboratories has examined the potential influence of neurosteroids on EtOH's acute and chronic effects.

Acute administration of EtOH increased plasma and brain levels of ALLOP (Barbaccia et al., 1999; VanDoren et al., 2000), an effect that was recently shown to be of adrenal and gonadal origin (Khisti et al., 2003; O'Dell et al., 2004). Additionally, blockade of progesterone's conversion to ALLOP with the 5α-reductase inhibitor finasteride partially reduced the EtOH-induced increase in cortical ALLOP levels and blocked the anticonvulsant effect of EtOH and the inhibitory effect of EtOH on spontaneous neural activity (VanDoren et al., 2000). These data suggest that an EtOH-induced increase in endogenous ALLOP levels may potentiate or prolong certain behavioral effects of EtOH.

Recent findings indicate that chronic EtOH administration alters ALLOP concentration in rodents and in human alcoholic patients. Cerebral cortical ALLOP levels were significantly reduced in EtOH-dependent male but not in female rats (Janis et al., 1998), suggesting that endogenous ALLOP concentrations may be differentially altered by chronic EtOH exposure in male and female rats. In male mice of two genetic animal models of EtOH withdrawal severity, chronic EtOH exposure and withdrawal differentially altered plasma ALLOP levels in the Withdrawal Seizure-Prone (WSP) and Withdrawal Seizure-Resistant (WSR) selected lines as well as in the C57BL/6 (B6) and DBA/2 (D2) inbred mouse strains (discussed in Finn et al., 2004a). Plasma ALLOP concentrations decreased in all EtOH-dependent genotypes, but the decrease persisted during EtOH withdrawal only in the genotypes with varying degrees of withdrawal severity (i.e., WSP, D2 and B6). In a small cohort of alcoholic patients, an inverse relationship between endogenous ALLOP levels and symptoms of alcohol withdrawal (i.e., increased subjective ratings of anxiety and depression) was demonstrated, when compared to control subjects (Romeo et al., 1996). Overall, these findings are suggestive of a relationship between endogenous ALLOP levels and behavioral changes in excitability during EtOH withdrawal.

The B6 and D2 inbred strains differ in withdrawal from both acute (Roberts et al., 1992) and chronic (Crabbe et al., 1983; Crabbe, 1998) EtOH administration, as indexed by an increase in handling-induced convulsions (HICs). Notably, chronic EtOH exposure produced a less severe EtOH withdrawal in female than in male B6D2F2 mice (i.e., the F2 cross of B6 and D2 progenitors; Buck et al., 2002) and produced enhanced sensitivity to the anticonvulsant effect of ALLOP in male B6 but not in male D2 mice (Finn et al., 2000), consistent with data in male and female rats (e.g., Devaud et al., 1996; reviewed in Devaud et al., 2003). Taken in conjunction with the finding that ALLOP levels were significantly reduced in EtOH-dependent male but not in female rats (Janis et al., 1998), the purpose of the present study was to examine the physiological consequence of pharmacologically decreasing ALLOP levels on EtOH withdrawal severity in male and female B6 and D2 mice. We pharmacologically manipulated ALLOP levels by administering the 5α-reductase inhibitor finasteride (Stoner, 1990; Freeman et al., 1993), which blocks the conversion of progesterone to ALLOP. Thus, we hypothesized that male and female B6 and D2 mice would be differentially sensitive to the effect of inhibiting the enzyme 5α-reductase (and concomitant decrease in endogenous ALLOP levels) on chronic EtOH withdrawal severity. Because HIC is a sensitive measure of central nervous system excitability, we assessed the effect of finasteride on central nervous system excitability, measured by HIC.

2. Methods

2.1. Subjects

Drug naive male and female B6 and D2 mice were purchased from the Jackson Laboratory (Bar Harbor, ME) at 6 weeks of age, separated by strain and sex, housed four per cage with free access to food and water, and acclimated to a 12:12 light/dark cycle (lights on 0600 h) for a minimum of 2 weeks prior to experimentation. All procedures adhered to the U.S. 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.

2.2. Procedure

The effect of finasteride (50 mg/kg ip; Steraloids, Newport, RI) on chronic EtOH withdrawal severity was assessed. Briefly, mice were exposed to 72 h EtOH vapor or air using our standard method for inducing EtOH dependence (e.g., Finn et al., 2000), which utilized the alcohol dehydrogenase inhibitor pyrazole hydrochloride (pyrazole; Sigma, St. Louis, MO) to stabilize blood EtOH concentration (BEC). The dose of finasteride was chosen, based on pilot studies that found that administration of 50 or 100 mg/kg significantly decreased plasma and brain ALLOP at 8 and 24 h postinjection, but not at 4 h postinjection. At 24 h postinjection, ALLOP concentration was decreased 66% and 62% in plasma and 80% and 69% in brain, by the 50- and 100-mg/kg doses of finasteride, respectively (Finn, unpublished). Thus, animals received a total of four injections of finasteride, 24 h prior to, and each

day of, the exposure to EtOH vapor or air. There were a total of four treatment groups: EtOH + finasteride, EtOH + vehicle, air + finasteride, and air + vehicle. The EtOH groups had 10–14 animals per strain and sex, while the air groups had 6–10 animals per strain and sex.

HICs were measured at baseline (i.e., prior to the first finasteride injection and the exposure to EtOH vapor or air), and then during a 24-h period of EtOH withdrawal. Immediately following removal from the inhalation chambers at 72 h, mice were scored hourly for HIC for the first 12 h, and then again at 24 h.

2.3. Chronic EtOH vapor and air exposure

During the experiment, mice were housed in stainless steel 0.25-in. hardware cloth cages inside a large Plexiglas chamber (see Finn et al., 2000 for details on the inhalation chambers). On Day 1, mice in the EtOH groups were weighed, injected with a priming dose of EtOH (1.5 g/kg ip) and pyrazole (68.1 mg/kg ip), followed by an injection of vehicle (20% w/v 2-hydroxypropyl-β-cyclodextrin; β-cyclodextrin ip; Cerestar USA, Hammond, IN) or finasteride (50 mg/kg in β-cyclodextrin ip), and exposure to EtOH vapor (7.2 mg EtOH/l air for B6 males and females, 5.0 mg EtOH/l air for D2 males, and 4.9 mg EtOH/l air for D2 females) inside the inhalation chambers. Different vapor concentrations were used to achieve equivalent BECs so that any sex or strain difference in HIC could not be ascribed to differences in EtOH pharmacokinetics. At 24 and 48 h, the mice were briefly removed from the chambers, weighed, injected with pyrazole and finasteride or vehicle, and placed back into the chamber. Tail blood samples were taken from a subset of the animals each day to monitor BEC. At 72 h, the EtOH groups were removed from the inhalation chambers and tail blood samples were taken from all animals for subsequent analysis of BEC (except for one B6 male and one D2 female mouse). The mice were housed in polypropylene cages with cob bedding and taken to a procedure room for HIC assessments.

Air-exposed animals also received daily pyrazole plus finasteride or vehicle injections, but were injected with saline on Day 1 and were exposed to air inside the inhalation chamber. At 72 h, the air groups were removed from the inhalation chamber, and tails were nicked but no blood was taken. The mice were housed in polypropylene cages with cob bedding and taken to a procedure room for HIC assessments.

2.4. 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. Distilled water (300 μl) and 0.3 N $Ba(OH)_2$ (50 μl) were added to each sample. The samples were shaken for 5 s and centrifuged at 12,000 rpm. The supernatant was transferred to a crimp top glass vial and analyzed for EtOH concentration by gas chromatography. Four pairs of EtOH standards (0.5–4.0 mg/ml) were used to establish a standard curve.

2.5. HIC assessment

Scoring for HIC was done according to a previously published scale (Finn and Crabbe, 1999). This procedure involved lifting the animal by the tail, gently spinning it 180° if necessary, and observing convulsions. HIC scores ranging from 1 to 3 required the gentle spin to elicit a tonic or clonic convulsion, whereas convulsions elicited by merely lifting the mouse by the tail were scored as 4 to 6.

2.6. Data analysis

Withdrawal severity was assessed by examining the hourly HIC scores and by calculating area under the withdrawal curve (AUC). AUC24 was calculated over the 24-h period following termination of EtOH exposure after correcting for HIC in air-exposed animals at each time point. An HIC difference score was calculated for each animal by subtracting the mean HIC score for the respective air-exposed vehicle-injected mice at each hour from the individual animal's HIC at that hour. Analysis of variance (ANOVA) was used to assess strain (B6 versus D2), sex (male versus female), treatment (EtOH versus air), and drug (finasteride versus vehicle) effects on the dependent variables BEC, hourly HIC scores, and AUC24. When significant interactions were obtained, post hoc tests were conducted. Correlation and regression analyses were conducted on the individual BEC versus AUC24 data for each strain and sex to examine this relationship in the vehicle-injected versus finasteride-injected mice. Data are expressed as the mean ± S.E.M. Significance was set at $P \leq .05$.

3. Results

At the beginning of the experiment, male B6 mice weighed 25.5 ± 0.45 g ($n = 42$), female B6 mice weighed 18.7 ± 0.16 g ($n = 34$), male D2 mice weighed 25.3 ± 0.33 g ($n = 40$), and female D2 mice weighed 19.6 ± 0.22 g ($n = 40$). Body weight in the female mice was significantly lower than that in the male mice [$F(1,152) = 10.60$, $P < .002$], whereas body weights in the two strains did not differ.

Exposure to 72 h EtOH vapor produced stable BEC at the time of removal from the inhalation chamber. When collapsed across drug treatment condition, mean ± S.E.M. BEC was 1.28 ± 0.11 mg/ml for B6 males ($n = 21$), 1.20 ± 0.12 mg/ml for B6 females ($n = 22$), 1.03 ± 0.07 mg/ml for D2 males ($n = 28$), and 1.12 ± 0.10 mg/ml for D2 females ($n = 19$). There were no sex differences in BEC, although values were significantly higher in B6 than in D2 mice [$F(1,82) = 5.24$, $P < .05$]. Interestingly, BEC was significantly lower in the finasteride- versus vehicle-injected mice, although the EtOH exposure was identical in these

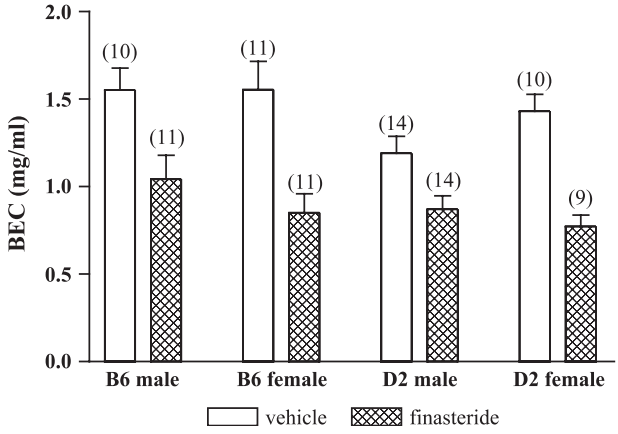

Fig. 1. The effect of pre-treatment with finasteride or vehicle during the induction of physical dependence upon BEC upon the initiation of withdrawal. Male and female B6 and D2 mice were exposed to 72 h EtOH vapor or air and received four injections of finasteride (50 mg/kg ip) or vehicle 24 h prior to, and each day of, the EtOH vapor exposure. BEC was assessed in mice upon removal from the inhalation chambers at 72 h. Values represent the mean ± S.E.M. for the number of animals in parentheses.

two groups [$F(1,82) = 46.83$, $P < .001$; Fig. 1]. That is, for each strain and sex, finasteride- and vehicle-treated mice were exposed to EtOH vapor in the same inhalation chamber. However, there was no significant interaction between main effects, suggesting that the effect of finasteride to lower BECs was similar across the strains and sexes. BEC after 72 h of EtOH vapor exposure was decreased in the finasteride-pretreated animals by 33% in male B6 mice, 45% in female B6 mice, 27% in male D2 mice, and 46% in female D2 mice, when compared to values in the respective vehicle-treated mice.

BEC was assessed in a subset of the animals following 24 and 48 h of EtOH vapor exposure, primarily to ensure that the EtOH exposure produced comparable BEC across the strains and sexes. After 24 h of EtOH vapor exposure, BECs did not differ significantly in vehicle- or finasteride-treated male B6, female B6, or male D2 mice. However, BECs in finasteride-treated female D2 mice were 34% lower than in vehicle-treated mice, a difference that reached significance ($t = 4.03$, $df = 10$, $P < .005$). Examination of the BECs after 48 h of EtOH vapor exposure revealed that finasteride pretreatment produced a slight but nonsignificant decrease in BEC in all the strains and sexes, when compared with respective vehicle-treated mice.

Hourly HIC scores were significantly influenced by strain (D2>B6), treatment (EtOH>air), and drug [vehicle>finasteride; Fs(1,140)>5.64, Ps < .02], but not by sex (Fig. 2). The interactions between strain and sex, sex and treatment, and strain and treatment were significant [Fs(1,140)>4.47, Ps < .05]. Notably, there was a trend for an interaction between sex, strain, treatment, and drug [$F(1,140) = 2.31$, P=.13], suggesting that the change in HIC scores by treatment and drug differed in male and female B6 and D2 mice. Repeated-measures ANOVA also indicated that hourly HIC scores were significantly influenced by time [$F(13,1820) = 32.10$, $P < .001$] and that there were numerous interactions between time and strain, condition and drug

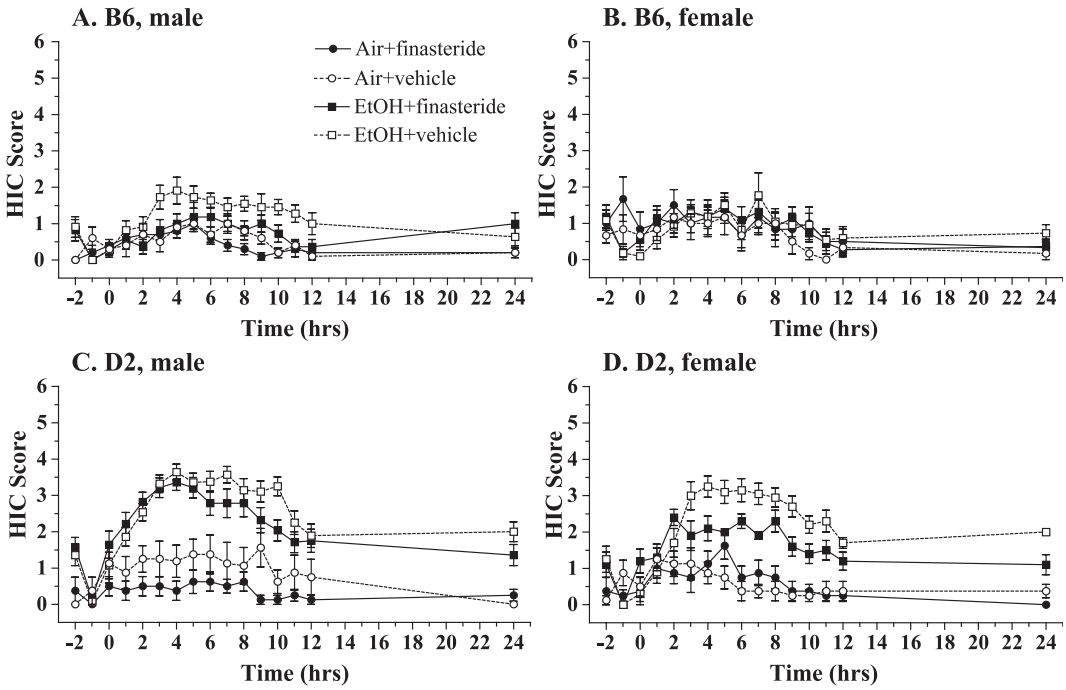

Fig. 2. Sex and strain differences in the effect of finasteride pre-treatment on hourly HIC scores in air- and EtOH-exposed male B6 (A), female B6 (B), male D2 (C), and female D2 (D) mice. HIC was scored at baseline ($t = -2, -1$), immediately upon removal from the inhalation chambers after exposure to 72 h of EtOH vapor or air ($t = 0$), hourly for 12 h following removal from the inhalation chambers and then again at 24 h. Values represent the mean ± S.E.M; n/group is shown in Fig. 3.

[Fs(13,1820) ≥ 1.71, Ps ≤ .05]. These results provide support for the conclusion that EtOH withdrawal and drug treatment differentially altered hourly HIC scores in male and female B6 and D2 mice. Therefore, subsequent analyses were conducted on each strain and sex separately.

In male B6 mice (Fig. 2A), hourly HIC scores were significantly increased during EtOH withdrawal versus air-exposed mice [$F(1,38) = 12.18$, $P < .005$] and were significantly reduced in finasteride- versus vehicle-injected mice [$F(1,38) = 4.37$, $P < .05$]. HIC scores changed significantly across time [$F(13,494) = 8.69$, $P < .001$] and were differentially altered in the EtOH- versus air-exposed mice [$F(13,494) = 2.61$, $P < .005$], consistent with the fact that the EtOH-exposed mice were undergoing withdrawal. The trend for an interaction between time and drug [$F(13,494) = 1.53$, $P = .10$] suggests that there were subtle differences in the suppressive effect of finasteride on HIC scores in the EtOH- versus air-exposed mice.

In contrast, hourly HIC scores in the female B6 mice (Fig. 2B) were only significantly influenced by time [$F(13,390) = 5.99$, $P < .001$]. Notably, HIC scores were not increased significantly during EtOH withdrawal, nor were they significantly altered by finasteride.

Results in the male D2 mice (Fig. 2C) were comparable to those in the male B6 mice. Hourly HIC scores were significantly increased during EtOH withdrawal [$F(1,40) = 63.59$, $P < .001$] and significantly decreased by finasteride pretreatment during the development of physical dependence [$F(1,40) = 4.09$, $P < .05$]. HIC scores changed significantly across time [$F(13,494) = 8.69$, $P < .001$] and were differentially altered in the EtOH- versus air-exposed mice [$F(13,494) = 2.61$, $P < .005$], consistent with the fact that the EtOH-exposed mice were undergoing withdrawal. However, the lack of significant interaction between time and drug suggests that the change in HIC scores across time with finasteride pretreatment was similar in the EtOH- and air-exposed D2 male mice.

A slightly different pattern of results was found in the female D2 mice (Fig. 2D). While hourly HIC scores were significantly increased during EtOH withdrawal [$F(1,32) = 86.81$, $P < .001$], there was a trend for finasteride to decrease HIC scores [$F(1,32) = 2.91$, $P < .10$]. However, the significant interaction between treatment and drug [$F(1,32) = 4.79$, $P < .04$] indicates that finasteride differentially altered HIC scores in the EtOH- versus air-exposed female D2 mice. As in the male B6 and D2 mice, HIC scores changed significantly across time [$F(13,416) = 9.59$, $P < .001$] and were differentially altered in the EtOH- versus air-exposed mice [$F(13,416) = 5.72$, $P < .001$], consistent with the fact that the EtOH-exposed mice were undergoing withdrawal. However, in contrast to the results in male mice, the interaction between hour, treatment, and drug was significant [$F(13,416) = 3.28$, $P < .001$], providing additional support for the conclusion that finasteride pretreatment significantly suppressed HIC scores only in female D2 mice undergoing EtOH withdrawal.

Another index of EtOH withdrawal severity, AUC24 (Fig. 3), was analyzed and the results were similar to that described above for the hourly HIC scores. AUC24 was significantly increased during EtOH withdrawal in male, but not female B6 mice [$F(1,38) = 11.44$, $P < .002$; Fig. 3A]. In male D2 mice, AUC24 was significantly increased during EtOH withdrawal [$F(1,40) = 44.80$, $P < .001$], with a trend for finasteride pretreatment to decrease AUC24 [$F(1,40) = 2.24$, $P = .14$; Fig. 3B]. In the female D2 mice, AUC24 was significantly increased during EtOH withdrawal [$F(1,32) = 81.23$, $P < .001$] and decreased by finasteride [$F(1,32) = 7.58$, $P < .01$]. A trend for an interaction between treatment and drug [$F(1,32) = 3.50$, $P = .07$] and post hoc tests confirmed that finasteride pretreatment significantly decreased AUC24 only during EtOH withdrawal in the female D2 mice.

Correlation and regression analyses were conducted to determine whether the relationship between BEC and AUC24 was comparable between finasteride- and vehicle-injected mice. Based on the strain and sex differences described above, each strain and sex was analyzed separately. In male B6 mice, BEC was not significantly correlated with AUC24 in either EtOH-exposed treatment group (rs = .09, $n = 10$/treatment; Fig. 4A). While the slope of the regression lines did not differ, there was a downward shift in the regression line for the finasteride- versus vehicle-injected

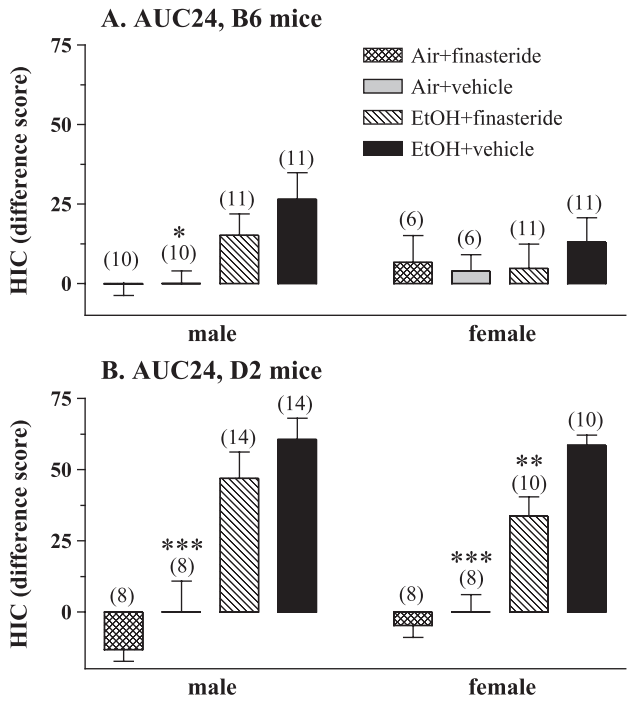

Fig. 3. The effect of finasteride on EtOH withdrawal severity, measured by AUC24, in B6 (A) and D2 (B) mice. AUC24 was calculated over the 24-h period following termination of EtOH or air exposure, after correcting for HIC in the air-exposed animals at each time point (see Methods for details). Values represent the mean ± S.E.M. for the number of animals in parentheses. * $P < .05$, ** $P < .01$, *** $P < .001$, versus respective EtOH + vehicle-treated mice; Tukey's post hoc test.

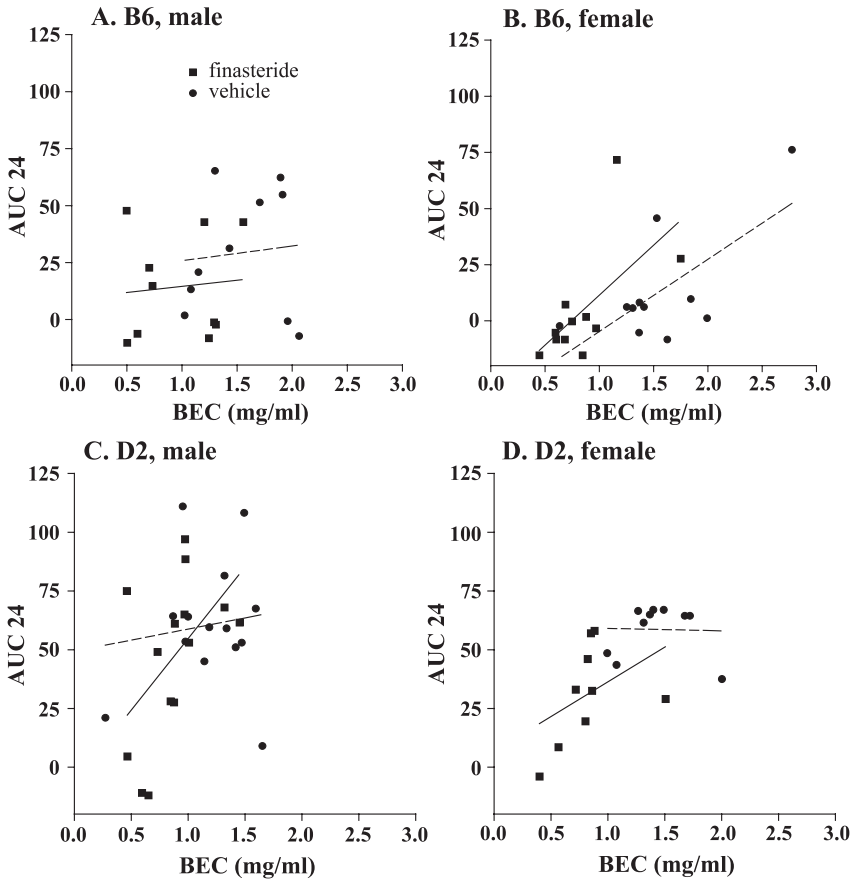

Fig. 4. The relationship between BEC and AUC24 in EtOH-exposed male B6 (A), female B6 (B), male D2 (C), and female D2 (D) mice. Depicted is a scatterplot of the values in individual mice with corresponding regression lines for finasteride-injected (solid line) and vehicle-injected (dashed line) mice.

B6 male mice, consistent with the conclusion that finasteride pretreatment decreased EtOH withdrawal severity. In contrast, BEC was significantly positively correlated with AUC24 in female B6 mice (rs $\geq .63$, Ps $< .05$, $n = 11$/treatment; Fig. 4B). The slopes of the regression lines did not differ, but the regression line for the finasteride-injected mice was shifted to the left of that for the vehicle-injected mice. Although there was no significant effect of finasteride on EtOH withdrawal severity in female B6 mice, the leftward shift with finasteride pretreatment would be consistent with a pharmacokinetic explanation for the effect of finasteride on EtOH withdrawal severity (i.e., ↓ BEC and EtOH dose would be consistent with a ↓ EtOH withdrawal severity).

The correlation and regression analyses in the D2 mice were different from that described for the B6 mice. In D2 male mice, there was a trend for a significant positive correlation between BEC and AUC24 only in the finasteride-injected mice (r=.50, $P < .07$, $n = 14$), whereas the data in the vehicle-injected mice were not correlated (r=.12, $n = 14$; Fig. 4C). Likewise, the positive correlation between BEC and AUC24 in female D2 mice did not reach significance (r=.43, $n = 9$) in the finasteride-injected mice, while the data in the vehicle-injected mice were not correlated ($r = -.03$, $n = 10$; Fig. 4D). In both male and female D2 mice, the considerable difference in the slope of the regression lines for the finasteride- versus vehicle-injected mice did not reach significance. Nonetheless, the relatively flat slope for the regression line in the vehicle-injected D2 mice suggests that this inbred strain exhibits high EtOH withdrawal across a range of BEC.

4. Discussion

The present studies examined whether there were sex and strain differences in the effect of finasteride on chronic EtOH withdrawal in a genetic animal model of EtOH withdrawal severity. The decision to use the 5α-reductase inhibitor finasteride was based on the following: First, finasteride reversed progesterone's anticonvulsant effect in male mice (Kokate et al., 1999) and produced anxiogenic "withdrawal-like" effects in pseudopregnant female rats (Smith et al., 1998). Second, 5α-reductase enzyme activity in the frontal cortex was found to be inversely correlated with behavioral measures of anxiety in male rats (Steimer et al., 1997), and basal cortical and hippocampal 5α-reductase enzyme activity was significantly higher in male and female B6 mice versus male and female D2 mice (Finn et al., 2004a). Because we also found that chronic EtOH exposure produced a persistent decrease in hippocampal ALLOP levels in

D2 mice (↓ 38% for males and 50% for females) during withdrawal, while hippocampal ALLOP levels were increased in similarly treated B6 mice (↑ 30% for males and ↑ 36% for females; Finn et al., 2004a), we reasoned that a chronic EtOH-induced inhibition of 5α-reductase, and the concomitant decrease in endogenous ALLOP concentration, might contribute to the increased anxiety and seizure susceptibility in D2 mice during EtOH withdrawal. Therefore, the present studies attempted to directly test the involvement of 5α-reductase activity in EtOH withdrawal severity.

Consistent with published findings (e.g., Crabbe et al., 1983; Crabbe, 1998), chronic EtOH withdrawal severity was significantly greater in D2 than in B6 mice, measured by AUC24 and hourly HIC scores. Pretreatment with finasteride during the induction of physical dependence produced an overall decrease in EtOH withdrawal severity, concomitant with a decrease in BEC upon removal from the inhalation chamber. Notably, there were sex and strain differences in the selectivity of finasteride to suppress HIC during EtOH withdrawal. That is, finasteride significantly decreased EtOH withdrawal only in female D2 mice, produced a nonselective significant decrease in HIC in male D2 and B6 mice, and had no effect on HIC in female B6 mice. While these findings were the opposite of what we initially predicted, they suggest that the differential effect of finasteride on EtOH withdrawal severity was dependent on the sex and genotype of the animal.

Early work demonstrated that BEC at withdrawal was highly correlated with BEC examined at several earlier points during the 72-h period (Crabbe et al., 1983). Therefore, the sex and genotype differences in withdrawal are not likely due to differential EtOH exposure. If anything, the small but significant difference in BEC between B6 and D2 mice is biased against the finding that D2 mice had greater withdrawal AUC. Nonetheless, two additional points regarding the BEC during the induction of physical dependence and effects of finasteride are noteworthy. First, withdrawal was minimal in female B6 mice, making it difficult to ascertain whether the lack of effect of finasteride in this group was due to insensitivity to finasteride or to a "floor" effect. Second, examination of the limited BEC data ($n = 4–8$/group) during the period of EtOH vapor exposure suggests that finasteride pretreatment produced a slight but nonsignificant decrease in BEC at the 48-h time point in both sexes and genotypes and that the female D2 mice might be more sensitive to the effect of finasteride to decrease BEC. In the finasteride-pretreated female D2 mice, BEC was decreased 34% at 24 h and 24% at 48 h, when compared to the vehicle-treated group. For comparative purposes, finasteride pretreatment decreased BEC at 48 h by 19% in B6 males, 15% in B6 females, and 8% in D2 males, when compared to the respective vehicle-treated mice. Thus, with the exception of female D2 mice, the effect of finasteride pretreatment on BEC did not emerge initially during the period of EtOH exposure, suggesting that finasteride may indirectly alter EtOH pharmacokinetics.

The dose of finasteride administered in the present study decreased endogenous ALLOP levels by 66% in plasma and 80% in brain, when assessed at 24 h after injection. The 50-mg/kg dose of finasteride appeared to be maximally effective, because administration of a 100-mg/kg dose did not produce a further decrease in endogenous ALLOP levels (Finn, unpublished). This finding suggests that finasteride does not completely deplete endogenous ALLOP concentrations in mice, consistent with results in rats (VanDoren et al., 2000). The effect of repeated finasteride administration on endogenous ALLOP is not known. Nonetheless, the major route of progesterone metabolism in the rodent brain is via 5α-reduction, which is an irreversible reaction in mammalian cells (see Celotti et al., 1997).

Recent findings from studies examining the interaction of ALLOP with EtOH and other GABAergic compounds have found that manipulation of endogenous ALLOP levels can alter certain behavioral and physiological effects of EtOH. For example, manipulation of endogenous ALLOP levels altered the ability of an acute EtOH injection to increase cortical ALLOP levels (Biggio et al., 2003; Serra et al., 2003). Increasing endogenous ALLOP levels with progesterone injections abolished the ability of an EtOH injection to increase cortical ALLOP levels, whereas decreasing endogenous ALLOP levels with social isolation increased the ALLOP response to an EtOH injection in male rats. Progesterone administration (5 mg/kg ip for 5 days) also increased the cortical content of ALLOP in male rats and potentiated the biphasic effect of varying doses of EtOH on dopamine content (i.e., shifted the inverted U-shaped dose–response curve to the left; Dazzi et al., 2002). Coadministration of finasteride prevented the effect of progesterone on cortical levels of ALLOP and on modulation of dopamine content by EtOH. Earlier work also demonstrated that finasteride partially reduced the EtOH-induced increase in cortical ALLOP levels and blocked the anticonvulsant effect of EtOH and the inhibitory effect of EtOH on spontaneous neural activity (VanDoren et al., 2000). Collectively, these data suggest that finasteride decreases certain behavioral and physiological effects of EtOH. However, it is not known whether finasteride altered EtOH pharmacokinetics in these studies, because BEC was not measured.

While the mechanism by which finasteride pretreatment reduced chronic EtOH withdrawal severity is not known, several possibilities come to mind. First, if activation of the neurosteroid site on $GABA_A$ receptors was important for EtOH-induced enhancement of GABA-mediated responses, then finasteride pretreatment, and the concomitant decrease in endogenous ALLOP concentrations, might decrease the GABAergic adaptive responses that were relevant to the development of physical dependence during EtOH vapor exposure. Second, because finasteride pretreatment significantly decreased BEC upon the initiation of withdrawal, although the EtOH exposure was identical to that of the vehicle-injected animals, finasteride might reduce the development of physical dependence by altering EtOH phar-

macokinetics. That is, lowering the effective EtOH dose might explain finasteride's effect on AUC24 to some degree. However, this explanation seems most parsimonious for the results in female D2 mice, because the BEC data at 24 and 48 h suggest that BECs might have been consistently suppressed by finasteride throughout the induction of physical dependence only in these animals. Additionally, the correlational data tentatively suggest that there were strain and sex differences in the ability of finasteride to produce a parallel shift in the slope of the regression line for the relationship between BEC and AUC24 when compared to respective vehicle-injected mice, which implies that multiple factors might contribute to the effect of finasteride on EtOH withdrawal. Third, finasteride pretreatment produced a slight but nonsignificant decrease in AUC24 and hourly HIC scores in the air-exposed animals that was most pronounced in male D2 mice, suggesting that finasteride also might alter HIC via a nonspecific effect on motor activity. Recent findings document a locomotor suppressive effect of finasteride within the first 120 min postinjection in male D2 mice (Gabriel et al., 2004). However, this effect of finasteride of motor activity did not persist at later time points, which argues against a nonspecific effect of finasteride on HIC in the present study. Clearly, additional studies are necessary to discern the mechanism by which finasteride pretreatment is altering chronic EtOH withdrawal severity.

In conclusion, use of a genetic animal model of EtOH withdrawal severity demonstrated that male and female B6 and D2 mice are differentially sensitive to the effect of finasteride pretreatment on chronic EtOH withdrawal severity. Finasteride pretreatment and the concomitant decrease in endogenous ALLOP levels decreased EtOH withdrawal severity in three of the four strains and sexes that were tested, in contrast to our original prediction for an inverse relationship between endogenous ALLOP levels and EtOH withdrawal severity. Notably, the reduction in EtOH withdrawal was much more pronounced in D2 versus B6 mice, and significant only in the D2 females. Because finasteride pretreatment can block some effects of EtOH, it is possible that pretreatment with finasteride may reduce the development of physical dependence via an alteration in EtOH pharmacokinetics. However, additional studies will be necessary to determine whether there are genetic differences in the effect of 5α-reductase inhibition on additional measures of EtOH sensitivity and EtOH dependence and whether finasteride would produce a similar effect when administered upon the initiation of withdrawal.

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