SEX DIFFERENCES IN THE EFFECT OF FINASTERIDE ON ACUTE ETHANOL WITHDRAWAL SEVERITY IN MICE

By

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Abstract

The neurosteroid allopregnanolone (ALLO) is a 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 ALLO from progesterone and can reduce certain effects of EtOH. Treatment with finasteride during chronic EtOH exposure decreased EtOH withdrawal severity and blood EtOH concentrations (BECs) upon the initiation of withdrawal, suggesting an effect of finasteride on EtOH pharmacokinetics. Thus, the purpose of the present study was to determine the effect of finasteride on acute EtOH withdrawal severity, using a procedure that would minimize any interaction between finasteride and EtOH metabolism. Male and female C57BL/6J and DBA/2J mice received a pretreatment of finasteride (50 mg/kg i.p.) or vehicle 24 hours prior to an injection of EtOH (4 g/kg i.p.) or saline. Handling-induced convulsions (HICs) were scored prior to the finasteride or vehicle injection, and then over a 24 hr period after EtOH or saline injection. In another experiment, mice were treated identically to that described above, but were killed at selected time points to assess BEC (2 hr) or plasma estradiol and corticosterone levels (0, 2, 8, and 24 hrs). In a final study, retro-orbital blood samples were collected at 30, 60, 120, and 240 minutes post-EtOH administration to access finasteride's effects on EtOH clearance parameters. Pretreatment with finasteride increased acute EtOH withdrawal severity in female C57BL/6J and DBA/2J mice but decreased acute EtOH withdrawal severity in male mice of both strains. Finasteride did not alter BECs, EtOH clearance, estradiol, or corticosterone concentrations in a manner that appeared to contribute to the sex difference in finasteride's effect on acute EtOH withdrawal-related HICs. Collectively, these findings suggest that male and female C57BL/6J and DBA/2J mice may differ in their sensitivity to changes in ALLO or other GABAergic steroid metabolite levels

during acute EtOH withdrawal. These findings indicate that sex differences in the modulation of ALLO and other 5α-reduced steroids may be an important consideration in understanding and developing therapeutic interventions for male and female alcoholics.

Introduction

Drug abuse is a complex disease that affects both the individual and society at multiple levels (Rice, 1999; Volpicelli, 2000). Specifically, alcohol (ethanol) abuse is often a chronic, progressive disease that is the source of major social, economic, and public health problems. According to the National Council on Alcoholism and Drug Dependence, approximately 18 million Americans abuse alcohol, while more than 100,000 die each year as a result of alcohol-related causes (Ericson, 2001; Grant, 2000; Harwood, 2000). As a result, experimental research has focused on the factors leading to the development and maintenance of alcohol abuse, in an effort to develop effective prevention and treatment strategies.

Although human research has been essential in understanding alcoholism, animal models have allowed more experimental control and more invasive techniques to better elucidate some of the neurochemical mechanisms underlying alcohol abuse (Stewart and Li, 1997). Due to the multidimensional and complex factors involved in alcoholism, most research models focus on a particular facet of the disease. For example, research has examined the effects of short-term or acute alcohol exposure, while other research has investigated changes that occur with long-term or chronic alcohol intake. Within these models of alcohol exposure, different effects of alcohol have been examined, such as sensitization, tolerance and withdrawal (Crabbe, 2002; Lovinger and Crabbe, 2005). Neurochemical, genetic, and behavioral approaches have also been used to elucidate how different effects of alcohol contribute to the development, maintenance, and relapse of alcohol abuse (Lewis, 1990; Miller and Gold, 1993).

Several animal models have been used to study genetic differences in alcohol withdrawal (discussed in Grahame, 2000; Crabbe, 2002). Selective breeding has been used to create lines of mice that demonstrate extremes in alcohol-related behavior. For example, the selectively bred

Withdrawal-Seizure Prone (WSP) and Withdrawal-Seizure Resistant (WSR) lines of mice have been bred to have severe (WSP) or mild (WSR) handling-induced convulsions (HICs) during withdrawal after exposure to chronic alcohol (Kosobud and Crabbe, 1986). The WSP and WSR selected lines can be used to determine whether there is a genetic correlation between the selection phenotype and other traits of interest. Inbred strains of mice are another tool that is commonly used to examine differences in behavior or neurobiology during alcohol withdrawal (discussed in Grahame, 2000; Crabbe, 2002). Inbred strains of mice are generated by more than 20 generations of brother-sister matings. Since mice within a given strain are essentially clones of one another, differences in behavior or neurobiology within the inbred strain are attributed to environmental factors or allelic differences due to chance. Differences observed between different inbred strains are due to genetic contributions. For example, the inbred strains C57BL/6J and DBA/2J mice differ in a number of alcohol-related behaviors and underlying neurobiological substrates (Crabbe, 2002). Thus, these inbred strains of mice are frequently used to examine mechanisms involved in an aspect of alcohol-related phenotypes.

Much research has focused on the interaction of alcohol and various neurotransmitter systems. Alcohol affects several different neurotransmitter systems including dopamine, opioid, serotonin, cannabinoids, neuropeptides, glutamate, and γ-aminobutyric acid (GABA) (Buonopane and Petrakis, 2005). GABA has been of particular interest due to its involvement in a number of alcohol-related behaviors, such as sedation, anesthesia, and anxiolysis (Boehm et al., 2004), as well as the rewarding and conditioning effects of alcohol (Buonopane and Petrakis, 2005). For example, alcohol potentiates GABA function while GABAergic antagonists block some alcohol-related symptoms (Davies, 2003). Certain endogenous neurochemicals, like neuroactive steroids, alter GABAergic transmission and have been examined for their

involvement in the effects of alcohol (Finn et al., 2004a; Morrow et al., 2001b). Specifically, neurosteroids predominately refer to steroids that are synthesized in the brain from cholesterol or other steroid hormone precursors (Reddy, 2003).

One mechanism where by steroid hormones can alter behavior is via their "classical" genomic action, which involves binding to a hormone response element and changing gene transcription. These effects develop over hours to days and remain once the steroid has left the brain (McEwen, 1991). However, recent studies have demonstrated that neurosteroids, including the progesterone derivative allopregnanolone (ALLO; 3α-hydroxy-5α-pregnan-20-one), can also alter neuronal $GABA_A$ receptor excitability by exerting rapid effects through membrane-bound receptors (Belelli and Lambert, 2005; Reddy, 2003). These non-genomic effects of neurosteroids can produce immediate changes within seconds or minutes. For example, early patch-clamp studies demonstrated that these steroids promoted the open-state of the GABA-gated ion channel but did not alter the single channel conductance of the receptor (Lambert et al., 1987). Thus, ALLO's rapid effects on GABAergic transmission may have important implications for its ability to influence brain changes and behavior during alcohol exposure (Finn et al., 2004a).

Physiological Relevance of Allopregnanolone

Action at $GABA_A$ Receptors

ALLO is a potent positive modulator of $GABA_A$ receptors that increases GABA-stimulated chloride flux at low nanomolar concentrations (Morrow et al., 1987). Neurosteroids like ALLO interact with known modulatory sites on $GABA_A$ receptors in a non-competitive manner (Belelli et al., 1990; Gee et al., 1988). ALLO interacts with $GABA_A$ receptors in a stereospecific manner. Specifically, two features necessary for activity are a 5α or 5β reduced

steroid A-ring and a 3α-OH group. The interaction with $GABA_A$ receptors is relatively specific, in that ALLO does not interact with other neurotransmitter systems in the nanomolar and low micromolar range. However, pregnane neurosteroids, such as ALLO and pregnanolone, do interact with neuronal nicotinic acetylcholine (nnAch), serotonin type 3 ($5\text{-}HT_3$), and *N*-methyl-$_D$-aspartate (NMDA) receptors within the 10-100 μM range (Finn et al., 2004a; Stoffel-Wagner, 2001). Whether the pregnane steroid is a positive or negative modulator of these neurotransmitters, in part, depends on whether it is sulfated or unsulfated (Finn et al., 2004a; Stoffel-Wagner, 2001).

Neurosteroids that are positive modulators of $GABA_A$ receptors, like ALLO, exert anesthetic, hypnotic, anticonvulsant, anxiolytic, and motor stimulant effects in animals (Gasior et al., 1999). These behavioral observations suggest that ALLO may modify central $GABA_A$ receptors *in vivo*. Thus, fluctuations in endogenous ALLO levels may be important for $GABA_A$ receptor modulation. Supporting this notion is the finding that low concentrations of ALLO (applied via perfusion) enhance inhibitory transmission in the CA1 region of the hippocampus (Belelli and Herd, 2003). Similarly, blocking the oxidation of ALLO to 5α-dihydroprogesterone with indomethacin (a 3α –hydroxysteroid dehydrogenase or 3α-HSD inhibitor) unmasked a GABAergic inhibitory tone (Belelli and Herd, 2003). These findings suggest that local steroid metabolism maybe important in shaping $GABA_A$ receptor inhibition in the central nervous system (CNS).

Endogenous Allopregnanolone Levels

Endogenous concentrations of ALLO have been detected in brain, plasma, adrenal glands, and gonads in rodents (Finn et al., 2004b; Paul and Purdy, 1992; Purdy et al., 1990, 1991). Although ALLO can be synthesized *de novo* in the brain by neurons and glia, peripheral

progesterone and ALLO can also contribute to brain ALLO levels (Mellon and Vaudry, 2001; Rupprecht and Holsboer, 1999). Therefore, ALLO levels in the brain represent a combination of ALLO synthesized *de novo* as well as conversion from circulating progesterone.

Basal brain and plasma ALLO levels are higher in females than in males (Finn et al., 2004b; Paul and Purdy, 1992), since ALLO is synthesized from progesterone. Specifically, endogenous levels in females can fluctuate from 10 to 30 nM during the estrous cycle and can increase to approximately 100 nM during pregnancy (Concas et al., 1998; Finn et al., 2004a; Finn and Gee, 1994; Paul and Purdy, 1992). However, ALLO concentrations in males can also increase to 10-30 nM following exposure to various stressors such as ambient temperature swim, footshock, restraint stress, or CO_2 inhalation (Barbaccia et al., 2001; Purdy et al., 1991). These concentrations observed *in vivo* are physiologically relevant, since *in vitro* studies have demonstrated that these low nM concentrations potentiate the action of GABA at $GABA_A$ receptors (Belelli et al., 1990; Gee et al., 1988; Lambert et al., 1995; Morrow et al., 1987).

Fluctuations in ALLO levels in humans have been implicated in several behaviors. For example, ALLO may play an important role in the expression of premenstrual syndrome (PMS), a chronic, cyclical disorder, associated with symptoms of depression, anxiety, and mood swings. In women who do not have PMS, ALLO levels mirror that of progesterone, with the higher levels occurring during the luteal phase rather than the follicular phase (Genazzani et al., 1998). However, in women with PMS, serum ALLO levels are lower during the luteal phase than normal controls (for review see Reddy, 2003). Similarly, plasma and cerebrospinal fluid ALLO levels are decreased in patients with depression, an effect that is reversed by a clinically effective treatment with the antidepressant, fluoxetine (Strohle et al., 1999; Uzunova et al., 1998). That is, there is an inverse relationship between endogenous ALLO levels and symptoms of depression

in depressed patients (Uzunova et al., 1998). Improvement in depressed symptoms is positively correlated with an increase in ALLO following treatment with antidepressants (Uzunova et al., 1998). Interestingly, fluctuations in ALLO levels are also reported in human alcoholics. Specifically, there is a significant decrease in plasma ALLO levels in alcoholics during withdrawal from alcohol (Romeo et al., 1996). In alcoholic patients, there is an inverse relationship between endogenous plasma ALLO levels and symptoms of depression and anxiety (Romeo et al., 1995). Collectively, these findings suggest that fluctuations and changes in endogenous ALLO levels may be important in a number of behaviors, including PMS, depression, and alcohol withdrawal.

Allopregnanolone and Ethanol Interactions

One research interest over the past 10 years has been to examine the interaction of ethanol (EtOH) with the neuroactive steroid ALLO for a number of reasons. First, ALLO is a very potent positive modulator of $GABA_A$ receptors that exerts anxiolytic, sedative, anticonvulsant, and locomotor stimulant effects in animals (Gasior et al., 1999; Lambert et al., 1995; Paul and Purdy, 1992; Rupprecht and Holsboer, 1999). EtOH has a pharmacological profile similar to ALLO, with some of these behavioral effects believed to be due to the ability of EtOH to potentiate $GABA_A$ receptor function (see review by Grobin et al., 1998). Second, fluctuations in endogenous ALLO levels are correlated with some of EtOH's pharmacological effects (Dazzi et al., 2002; Morrow et al., 2001a, 2001b; VanDoren et al., 2000). Third, since EtOH withdrawal can increase seizure susceptibility and anxiety, ALLO's anticonvulsant and anxiolytic effects may modulate the severity of EtOH withdrawal (Finn et al., 2004a; Gasior et al., 1999).

Although ALLO is an anticonvulsant and acts to increase GABAergic inhibition, it is unclear how ALLO and EtOH interact at the $GABA_A$ receptor. Since ALLO and EtOH both can alter GABAergic transmission, endogenous levels of ALLO may be particularly important in determining the net behavioral outcome during withdrawal. That is, since ALLO levels are higher in female mice relative to male mice, this sex difference in ALLO levels may be important in how ALLO's anticonvulsant effects alter EtOH withdrawal severity in male and female mice. Thus, one aim of the current studies was to determine potential sex differences in the pharmacological manipulation of ALLO during EtOH withdrawal, thereby examining the indirect effects of ALLO on EtOH withdrawal severity, measured by HICs.

Acute Ethanol Administration and Withdrawal

Acute administration of EtOH in doses ranging from 1.35 to 4.0 g/kg significantly increased cortical ALLO levels to pharmacologically active concentrations at 40 to 80 minutes following injection in male rats (Barbaccia et al., 1999; VanDoren et al., 2000). Studies comparing male and female C57BL/6J mice show that an acute injection of EtOH produced a 1.7-fold increase in brain ALLO levels as well as a significant increase in plasma corticosterone levels in male but not female mice (Finn et al., 2004b). Systemic EtOH administration increased plasma and cerebral cortical levels of ALLO in male and female rats (Morrow et al., 1998). Interestingly, consumption of EtOH significantly increased brain ALLO levels in male but not female mice (Finn et al., 2004b). Studies in humans demonstrate that during intoxication, ALLO levels increase in both male and female adolescents (Torres and Ortega, 2003; Torres and Ortega, 2004), an increase that is not observed in male or female adults (Holdstock et al., 2005). Although acute EtOH administration altered ALLO levels in male and female rats and adolescent humans, a sex difference in EtOH's effects occurred only in mice. Despite the fact that these

observed sex differences are not fully understood, the sex differences in the effect of EtOH administration on endogenous ALLO levels in mice suggest that the hormonal milieu may impact EtOH's effects on GABAergic neurosteroids.

The ability of acute administration of EtOH to increase plasma and brain levels of ALLO in rodents recently was shown to be primarily of adrenal and gonadal origin (Khisti et al., 2003; O'Dell et al., 2004). Specifically, following EtOH administration, brain and plasma ALLO levels were lower in adrenalectomized animals relative to sham controls (Khisti et al., 2003). Thus, stress from systemic administration of EtOH may activate the hypothalamic-pituitary-adrenal (HPA) axis, causing synthesis of systemic ALLO, which in turn produces an increase in brain ALLO.

Fluctuations in endogenous ALLO levels might modulate some of EtOH's pharmacological effects (Dazzi et al., 2002; Morrow et al., 2001a, 2001b; VanDoren et al., 2000). The 5α-reductase inhibitor finasteride blocks the formation of ALLO from progesterone and was recently found to reduce some effects of EtOH (see Table 1). For example, pretreatment with finasteride reversed the anticonvulsant effect induced from an acute EtOH injection and reduced the EtOH-induced increase in cortical ALLO levels (VanDoren et al., 2000). Finasteride pretreatment also antagonized the antidepressant-like effect of EtOH in the forced swim test procedure (Hirani et al., 2002) and the anxiolytic effect of EtOH in an elevated plus maze test in male rodents (Hirani et al., 2005). In contrast, progesterone administration (5 mg/kg i.p. for 5 days), which increased cortical content of ALLO in male rats, 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

Table 1.

Summary of Finasteride's Effects on Measures of EtOH Sensitivity and Withdrawal

Model	Finasteride Dose	Strain/ Species	Sex	Finding	Reference
Chronic EtOH (72 hrs vapor exposure)	50 mg/kg a day for 4 days; i.p.	WSP and WSR Mice	Males	↓ HICs, ↓ anxiety-like behavior, and ↓ BECs in WSP mice only	Gorin et al., 2005
Chronic EtOH (72 hrs vapor exposure)	50 mg/kg a day for 4 days; i.p.	C57BL/6J and DBA/2J Mice	Males and Females	↓ HICs in female DBA/2J mice; nonselective ↓ HIC in males of both strains; ↓ BEC	Finn et al., 2004c
Acute EtOH (4 g/kg; i.p.)	50 mg/kg; i.p.	WSP and WSR Mice	Males	↑ HICs in WSPs only	Gorin et al., 2005
Acute EtOH (4 g/kg; i.p.)	50 mg/kg; i.p.	C57BL/6J and DBA/2J Mice	Males and Females	↑ HICs in females ↓ HICs in males	Present study
Acute EtOH (1 or 2 g/kg; i.p.)	0, 50, or 100 mg/kg; i.p.	DBA/2J Mice	Males	↓ EtOH-stimulated activity; did not modulate EtOH-induced place conditioning	Gabriel et al., 2004
Acute EtOH (2.5 g/kg; i.p.)	50 mg/kg; sc	Swiss mice	Males	↓ antidepressant effect of EtOH (i.e. immobility time in forced swim test)	Hirani et al., 2002
Acute EtOH (2 g/kg; i.p.)	2 x 50 mg/kg; sc	Sprague-Dawley Rats	Males	↓ EtOH-induced anxiety-like behavior (i.e. time spent and # of entries into open arms of plus maze)	Hirani et al., 2005
Acute EtOH (2 g/kg; i.p.)	2 x 50 mg/kg; sc	Sprague-Dawley Rats	Males	No change in the motor incoordinating effects of EtOH (i.e., Majchrowicz test, rotarod, and righting reflex)	Khisti et al., 2004
Acute EtOH (2 g/kg; i.p.)	2 x 25 or 50 mg/kg; sc	Sprague-Dawley Rats	Males	↓ EtOH's anticonvulsant effect; ↓ EtOH-induced increase in ALLO	VanDoren et al., 2000
Acute EtOH (0.25-1.0 g/kg; i.p.)	25 mg/kg a day for 6 days; sc	Sprague-Dawley Rats	Males	Co-administration with progesterone ↓ cortical ALLO levels and ↓ EtOH-induced modulation of dopamine content	Dazzi et al., 2002
Acute EtOH (1.5 g/kg; i.p.)	50 mg/kg; sc	Sprague-Dawley Rats	Males	↓ inhibitory effect of EtOH on spontaneous firing of hippocampal pyramidal neurons	Tokunaga et al., 2003
Acute EtOH (3 alcoholic drinks- 0.8 g/kg for men and 0.7 g/kg for women)	100 mg; orally	Humans	Males and Females	Among A-allele homozygotes, finasteride ↓ several subjective effects of EtOH	Pierucci-Lagha et al., 2005

progesterone on cortical levels of ALLO and on modulation of dopamine content by EtOH. Since finasteride pretreatment did not alter EtOH-induced ataxia (Khisti et al., 2004) or conditioned place preference (Gabriel et al., 2004), it is not known by what mechanism finasteride may decrease certain behavioral and physiological effects of EtOH.

Previous findings from our laboratory demonstrated that a 50 mg/kg dose of finasteride produced an 80% decrease in brain ALLO levels in mice at 24 hr post injection, similar to the 75% decrease reported in rats following this dose (Van Doren et al., 2000). Briefly, male DBA/2J mice were injected with saline or finasteride (50 or 100 mg/kg) and killed at selected time points following injection. ALLO levels were measured in extracted whole brain and plasma samples using radioimmunoassay. The 50 mg/kg dose of finasteride produced a 60% or 80% decrease in plasma and brain ALLO levels at 8 and 24 hours post injection, respectively (Finn, unpublished). However, the 100-mg/kg dose of finasteride produced only a 26 to 69% decrease in plasma and brain ALLO levels at 8-24 hours post injection, respectively. Thus, we chose the 50 mg/kg dose for use in the present studies based on the aforementioned findings and the fact that a 100 mg/kg dose was not used in any other study.

Since the terminal elimination half-life of circulating finasteride in humans is 4.7 to 7.1 hr (Steiner et al., 1996), the use of an acute EtOH withdrawal paradigm should allow the clearance of finasteride from serum prior to the EtOH injection. In this model of EtOH withdrawal, a single, acute injection of a sedative dose of EtOH initially produces a depressant effect that is followed by rebound hyperexcitability as the EtOH is metabolized (i.e. at approximately 4-8 hours post-injection). Thus, circulating finasteride should be eliminated before EtOH exerts its effects, eliminating a potential interaction with EtOH pharmacokinetics as observed in our chronic EtOH withdrawal paradigm (described below).

Chronic Ethanol Withdrawal

Recent studies have demonstrated that C57BL/6J and DBA/2J mice differ in behavioral sensitivity to ALLO (Finn et al., 1997) as well as in EtOH withdrawal severity (Crabbe, 1998). DBA/2J mice exhibit more severe HICs than C57BL/6J mice during both chronic (Crabbe, 1998; Crabbe et al., 1983) and acute (Roberts et al., 1992) EtOH withdrawal. Therefore, these two inbred strains can be used to examine genetic differences in sensitivity to ALLO or change in endogenous ALLO levels during EtOH withdrawal.

Initial studies from our laboratory examined the chronic EtOH exposure paradigm, in which C57BL/6J and DBA/2J mice received finasteride injections (50 mg/kg, i.p.) during exposure to 72 hr EtOH vapor. In our chronic EtOH administration experiment, we found that treatment with finasteride produced a significant reduction in withdrawal severity in DBA/2J versus C57BL/6J mice (Finn et al., 2004c). Withdrawal severity was indexed by HIC, which is a sensitive measure of CNS excitability. Treatment with finasteride significantly decreased HICs in female DBA/2J mice, produced a nonselective suppressive effect on HIC in male mice of both strains, but did not alter withdrawal severity in female C57BL/6J mice. That is, finasteride produced a slight decrease in both the air- and EtOH-treated groups of male mice, although the magnitude of finasteride's effect was greater in the EtOH-treated animals. Thus, the effect of finasteride was not selective for EtOH treatment in the male mice. This result was opposite to our prediction, as we had hypothesized that decreasing endogenous ALLO levels would decrease GABAergic inhibition and increase withdrawal severity. However, an unanticipated finding was that finasteride treatment significantly decreased BEC upon the initiation of withdrawal, suggesting that finasteride might affect EtOH withdrawal severity via an alteration in EtOH pharmacokinetics (Finn et al., 2004c). In other words, the decrease in BEC may be altering

withdrawal severity by decreasing the effective alcohol dose and perhaps, the development of physical dependence, rather than altering sensitivity to alcohol.

Interestingly, another study from our laboratory found that finasteride altered BECs during the chronic EtOH paradigm. Using the WSP and the WSR selected lines of mice, we found that pretreatment with finasteride decreased HICs in the WSP line but also produced a decrease in BECs upon the initiation of withdrawal in both WSP and WSR mice (Gorin et al., 2005). These findings were consistent with our previous study in male and female C57BL/6J and DBA/2J mice and provided further evidence that finasteride might affect EtOH pharmacokinetics.

Finasteride's Effects on Other Systems

Effect on Blood Ethanol Concentrations

As discussed above, in our chronic EtOH administration paradigm, treatment with finasteride significantly altered BECs following 72 hrs of EtOH vapor exposure. To determine EtOH concentrations during this chronic EtOH paradigm, BECs were assessed at several time points over the course of vapor exposure. At 24 hrs following chronic EtOH vapor exposure, treatment with finasteride did not affect BECs in male and female C57BL/6J and male DBA/2J mice (Finn et al., 2004c). However, there was a significant 34% decrease in BEC in the finasteride-treated DBA/2J females. Relative to saline-treated controls, finasteride treatment produced a slight, but nonsignificant decrease in BEC in all strains and sexes after 48 hrs of exposure to EtOH vapor. Following 72 hrs of EtOH vapor exposure, treatment with finasteride produced a significant decrease in BECs in both strains and sexes. Similarly in WSP and WSR mice, pretreatment with finasteride did not affect BECs at the 24 hr time point, but produced a 5-18% decrease in BECs following 48 hrs of EtOH vapor exposure. At the 72 hr time point,

BECs in the finasteride-treated WSP mice were decreased by 14%, whereas BECs were decreased by 23% in finasteride-treated WSR mice. It is not known whether a 14-35% decrease in BEC is effective in decreasing the EtOH dose enough to alter withdrawal severity (i.e. HICs). To our knowledge, no research has been done to look at the effect of finasteride on EtOH pharmacokinetics following an acute EtOH injection. Thus, one aim of the current studies was to examine the effect of finasteride on BECs during acute EtOH withdrawal.

Absorbed alcohol is rapidly carried throughout the body and blood and is metabolized at a steady state (Goldstein, 1983). Specifically, alcohol is metabolized first to acetaldehyde and then to acetic acid, a process that primarily is mediated by hepatic enzymes (Goldstein, 1983; Grisel et al., 2002). Alcohol is oxidized in the liver, catalyzed by the cytosolic enzyme alcohol dehydrogenase (ADH), thereby producing acetaldehyde, a highly toxic substance (Goldstein, 1983). Acetaldehyde dehydrogenase (ALDH) is involved in the conversion of acetaldehyde to acetic acid, a normal metabolite in humans. Although the liver is responsible for approximately 90% of alcohol metabolism, the remainder is eliminated through breath, saliva, sweat, urine, or feces (Goldstein, 1983).

There are important sex differences that can affect how alcohol is metabolized and eliminated. For example, previous studies have suggested that women have higher BECs than men (see Desroches et al., 1995). This sex difference in BECs was originally attributed to differences in the volume of distribution of alcohol. However, later research has demonstrated that liver ADH activity differs in males and females, with female mice having higher enzymatic activity than male mice (see Rao et al., 1997). Although the exact mechanisms of sex differences in alcohol metabolism are not fully understood, these differences may play an important role when examining how different drugs, like finasteride, differentially affect EtOH

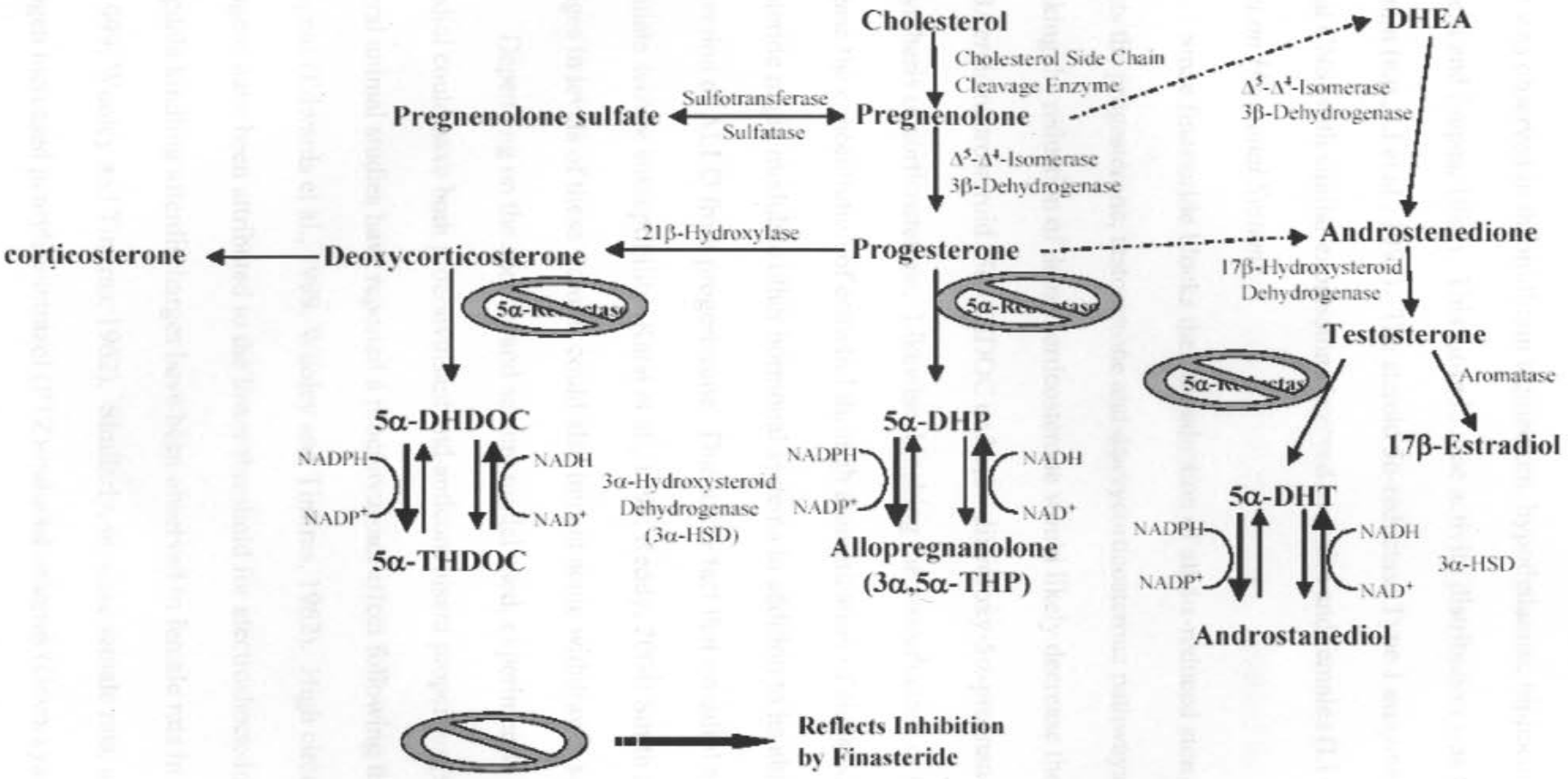
withdrawal. Thus, one aim of the present studies was to determine whether finasteride differentially affected EtOH clearance in male and female mice.

Distribution and Biological Role of 5α-Reductase

As discussed above, finasteride is a 5α-reductase inhibitor that blocks the reduction of all 5α-reduced steroids. Although the general profile of the biosynthesis of 5α-reduced steroids is presented in Figure 1, the distributions of the biosynthetic enzymes vary depending on the tissue, part of the body, or developmental stage. In primates and rodents, there are two distinct 5α-reductase isozymes, Type I and Type II. In humans, Type I is found in sebaceous glands of the skin, liver, muscle, and brain, while low levels are found in the prostate (Thigpen et al., 1993). Type II 5α-reductase isozyme is found in the prostate, seminal vesicle, epididymis, hair follicles, and liver (Thigpen et al., 1993). Based on the reduction of testosterone to dihydrotestosterone (DHT), the Type I enzyme is responsible for approximately 1/3 of circulating DHT levels, whereas Type II 5α-reductase is responsible for the remaining 2/3 of DHT levels (Gisleskog et al., 1998). In humans, finasteride preferentially inhibits the Type II 5α-reductase isozyme (Andersson et al., 1991). Thus, the most common therapeutic use of finasteride (typically 1 to 5 mg/day) is to decrease DHT levels at the androgen receptor in the prostate and scalp of men, for the treatment of benign prostatic hyperplasia and androgenetic alopecia (male pattern hair loss).

In rodents, both Type I and Type II isozymes demonstrate comparable inhibition following finasteride administration (Azzolina et al., 1997). For this reason, finasteride has a number of experimental applications, including the ability to inhibit the synthesis of ALLO and other GABAergic neurosteroids. Based on the conversion of progesterone to 5α – dihydroprogesterone (5α-DHP), the highest distribution of 5α-reductase activity in the mouse

Figure 1.



Presented is a general profile for the biosynthesis of the GABAergic neuroactive steroid ALLO (3α,5α-THP) as well as the inhibitory effect of finasteride on other 5α-reduced steroids (adapted from Finn et al., in press). The distribution and activity of the various steroidogenic enzymes in target tissues, including gonads, adrenals, and brain, will impact the levels of steroid hormones and their neuroactive metabolites.

brain was observed in the midbrain tegmentum, hypothalamus, hippocampus, and cerebral cortex (Roselli and Snipes, 1984). This 5α-reductase activity distribution was found to be similar in the rat brain (e.g., Li et al., 1997). The steroid 5α-reductase Type 1 enzyme is widely expressed in the rat CNS, with similar expression observed in males and females (Li et al., 1997).

Effect on Hormonal Systems

Since finasteride blocks the 5α-reduction of all 5α-reduced steroids, this inhibitor also affects the progesterone, testosterone and deoxycorticosterone pathways (see Figure 1). Blocking the reduction of deoxycorticosterone would likely decrease the production of another GABAergic neurosteroid (5α-THDOC or 3α,21-dihydroxy-5α-pregnan-20-one) and favor the biosynthesis of corticosterone. Likewise, blocking the 5α-reduction of testosterone could increase the concentration of estradiol through aromatization of elevated testosterone. Thus, finasteride might modulate other hormonal systems in addition to its ability to inhibit the conversion of ALLO from progesterone. Due to the fact that estradiol and corticosterone can modulate seizure susceptibility (Karst et al., 1999; Reddy, 2004; Smith and Woolley, 2004), changes in levels of these steroids could also impact acute withdrawal severity.

Depending on the species and seizure model used, experimental studies suggest that estradiol could have both proconvulsant and anticonvulsant properties (see Veliskova, 2006). Several animal studies have reported a proconvulsant effect following the acute administration of estrogens (Edwards et al., 1999; Wooley and Timiras, 1962). High circulating levels of estrogens have been attributed to the lower threshold for electroshock-induced seizures, and amygdala kindling afterdischarges have been observed in female rats in proestrous (Edward et al., 1999; Wooley and Timiras, 1962). Similarly, in naïve female rats, acute administration of estrogen increased pentylenetetrazol (PTZ)-induced seizures (Gevorkyan et al., 1989). In

contrast, anticonvulsant effects of estrogen have been observed in ovariectomized (OVX) female rats. Specifically, 17β-estradiol administration had an anticonvulsant effect on seizures induced by picrotoxin (Schwartz-Giblin et al., 1989) and kainic acid (Veliskova et al., 2000) in OVX female rats, when compared with OVX rats with no estrogen replacement. In humans with tonic-clonic seizures, an improvement in seizures has been reported following estrogen treatment (Whitehead and McNeil, 1952). Thus, the effect of estrogens on seizures is affected by multiple factors, including the animal and seizure model used, the dose of estrogen administered, and the hormonal levels of the subject.

Testosterone also exhibits both proconvulsant and anticonvulsant properties (see Reddy, 2004; Frye and Reed, 1998; Pesce et al., 2000; Thomas and McLean, 1991), perhaps due to how testosterone was metabolized. In a study using intact male mice and rats, testosterone was protective against PTZ-induced seizures when the formation of estradiol was inhibited (Reddy, 2004). Systemic administration of 3α-androstanediol was protective against PTZ-induced seizures and picrotoxin-induced seizures (Reddy, 2004b). Conversely, the aromatization of testosterone to estrogen facilitated the proconvulsant properties of testosterone (Reddy, 2004). Thus, alterations in how testosterone was metabolized could potentially affect seizure susceptibility.

Similarly, deoxycorticosterone is converted to 5α-dihydrodeoxycorticosterone via 5α-reductase, another likely site of action of finasteride, or to corticosterone. By blocking 5α-reductase, one might expect to observe an increase in deoxycorticosterone and hence corticosterone levels. Corticosterone can demonstrate proconvulsant and anticonvulsant properties depending upon the animal model employed. However, most of the data seems to support the proconvulsant effects of corticosterone. A corticosterone-releasing pellet (100

mg/day) increased tonic-clonic seizures induced by kindling epileptogenesis in male rats (Karst et al., 1999). In naïve male C57BL/6J and DBA/2J mice, administering corticosterone (to produce levels comparable to that observed during acute EtOH withdrawal) significantly increased HICs (Roberts et al., 1992). Administration of corticosterone also increased the severity of acute EtOH withdrawal (Roberts et al., 1994) as well as the susceptibility to chemically-induced convulsions (Roberts and Keith, 1995) in male mice. Paradoxically, a single injection of corticosterone or other adrenal steroid precursors to 15 day old rats produced an anticonvulsant effect against PTZ-induced seizures (Edwards et al., 2002). Interestingly, deoxycorticosterone, a corticosterone precursor that increases during stress, protected mice against PTZ, methyl-6,7-dimethoxy-4-ethyl-beta-carboline-3-carboxylate, picrotoxin, and amygdala-kindled seizures (Reddy and Rogawski, 2002). However, the authors suggest that this anticonvulsant effect may be due to changes in 5α -THDOC levels, a 5α,3α-reduced metabolite of deoxycorticosterone (Reddy and Rogawski, 2002). Overall, corticosterone appears to exert predominantly proconvulsant effects, although downstream metabolites of deoxycorticosterone may have anticonvulsant properties under certain experimental conditions.

In summary, finasteride's differential alteration of the steroid biosynthetic pathway in male and female mice may be another potential mechanism by which withdrawal severity is affected. Thus, another aim of the current studies was to examine the effect of finasteride on estradiol and corticosterone levels during acute EtOH withdrawal, since an increase in levels of these steroids with proconvulsant properties might contribute to withdrawal severity.

Summary of Experiments

While several studies have investigated sex differences in alcohol-related behaviors, few studies have examined sex differences in the modulatory effect of finasteride (and presumably,

ALLO or other GABAergic steroid metabolites) during EtOH withdrawal. Due to the fact that male and female mice differ in endogenous ALLO levels (females > males) and in alcohol withdrawal severity (females < males) ,and that studies from our laboratory found a sex difference in the effect of EtOH injection and exposure on ALLO levels, it seems important to determine whether there is a sex difference in finasteride's effect on EtOH withdrawal. To our knowledge, there are no studies that have examined this relationship.

Although a previous study from our laboratory explored sex differences in the pharmacological manipulation of ALLO during withdrawal from chronic EtOH, these findings were complicated by finasteride's effects on BECs. The current set of experiments sought to reduce finasteride's effect on BECs by examining the role of pharmacological manipulation of ALLO (or other steroid metabolites) in the acute EtOH administration paradigm. Thus, the purpose of the present studies was to characterize the effect of finasteride on acute EtOH withdrawal severity measured by HICs, in male and female C57BL/6J and DBA/2J mice. We predicted that pretreatment with finasteride would increase acute withdrawal severity in both strains and sexes. Specifically, since finasteride acts to decrease ALLO levels and thus GABAergic inhibition, pretreatment with finasteride may act to increase withdrawal-related seizures. However, based on the fact that there was a sex difference on finasteride's effect in the chronic administration study, it is possible that there will be a sex difference in finasteride's effect on HICs in the acute administration paradigm. Although pretreatment with finasteride may increase withdrawal severity in male and female mice, the magnitude of this increase may be larger in female mice (i.e. since female mice have higher endogenous ALLO levels, finasteride may be producing a larger, more physiological relevant decrease in brain inhibition).

Due to finasteride's effects on BECs in our previous chronic study, another aim of the current studies was to determine whether finasteride altered EtOH clearance in this acute withdrawal model. We examined EtOH elimination by measuring BECs at several time points following pretreatment with finasteride. It was hypothesized that the acute EtOH administration paradigm would eliminate finasteride's effect on BECs, an effect that was expected at all time points during withdrawal and in both strains and sexes.

Since finasteride treatment likely alters other 5α-reduced steroids, another study sought to determine whether finasteride increased estradiol or corticosterone levels in a manner that would be consistent with an increase in acute withdrawal severity. That is, we examined hormone levels over several time points to see if their change mirrored the time course of HICs. It was predicted that pretreatment with finasteride would not alter plasma estradiol or corticosterone levels at any time point, in both strains and sexes. Overall, the findings of these studies will help elucidate sex differences in the effect of pharmacological inhibition of ALLO or other steroid metabolites during EtOH withdrawal, which may help in the development of therapeutic interventions for male and female alcoholics.

Methods

Subjects

Naïve male and female C57BL/6J and DBA/2J mice aged 8-10 weeks were used in all experiments (Jackson Laboratory, Bar Harbor, ME). Animals were separated by strain and sex and maintained in groups of four in individually ventilated cages (Thorens) with *ad libitum* food and water under a 12:12 hour light/dark cycle at 26 ± 1°C. All procedures were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* as adopted and promulgated by the U.S. National Institutes of Health and were approved by the local Institutional Animal Care and Use Committee.

Drugs

Finasteride was solubilized in 20% w/v 2-hydroxypropyl-β-cyclodextrin (β-cyclodextrin; Cerestar, USA, Hammond, IN) and prepared as a 5-mg/ml solution to be injected as 0.01 ml/g of body weight. In all experiments, mice received a pretreatment of finasteride (50 mg/kg, i.p.; Steraloids, Newport, RI) or an equivalent volume of saline (i.p.) depending upon group assignment. The following day, mice received an injection of EtOH (4 g/kg, i.p.; Pharmco Products, Brookfield, CT) or an equivalent volume of saline (i.p.) depending upon group assignment.

Experiment 1: Withdrawal Severity

Prior to the injection of finasteride or vehicle, baseline HICs were measured. Male and female mice received a pretreatment of finasteride (50 mg/kg, i.p.) or saline 24 hrs prior to an injection of EtOH (4 g/kg, i.p.; Pharmco Products, Brookfield, CT) or saline, depending upon group assignment. Following the EtOH or saline injection, mice were scored every hour for 12 hrs and again at 24 and 25 hrs for HIC severity.

Handling-Induced Convulsions

Scoring for HICs was done according to a previously published scale (Table 2; Finn and Crabbe 1999). This procedure involved lifting the animal by the tail, gently spinning it 180° if necessary, and observing a single, rapid convulsion. 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.

Experiment 2: Hormone Assessment

In a second experiment, mice received a pretreatment of finasteride (50 mg/kg, i.p.) or saline 24 hours prior to an injection of EtOH (4 g/kg, i.p.) or saline. Separate groups of mice were killed at selected time points to assess BECs, estradiol or corticosterone concentrations. Mice were killed at 0, 2, 8, or 24 hrs to assess hormone concentrations over the time course of withdrawal. Specifically, the 0 hr time point was chosen in order to access hormone levels prior to an injection of EtOH, but following the pretreatment with finasteride or saline. The 2 hr time point reflected the point prior to initiation of EtOH withdrawal hyperexcitability when animals would have high BECs. Thus, this time point was used to assess the effect of an acute injection of EtOH on hormone levels, in order to compare it to hormone levels during peak withdrawal and BECs. In our experience, peak withdrawal typically occurred around the 8 hr time point. The 24 hr time point accessed hormone concentrations following the termination of withdrawal. BEC was measured in animals that were killed at 2 hrs to estimate whether finasteride altered EtOH concentrations at this single time point.

BEC Determination

A modification of the method originally described by Roach and Creaven (1968) was utilized. Briefly, a 20-µl sample of blood from the tip of the tail was added to 50-µl of chilled

Table 2.

HIC Scoring Scale (from Finn and Crabbe, 1999)

Symptom	*HIC Score*
Severe, tonic-clonic convulsion elicited before lifting by the tail, with quick onset and long duration, often continuing for several seconds after the mouse is released	7
Severe, tonic-clonic convulsion when lifted by the tail, with quick onset and long duration, often continuing for several seconds after the moue is released	6
Tonic-clonic convulsion when lifted by the tail, often with onset delayed by as much as 1 to 2s	5
Tonic convulsion when lifted by the tail	4
Tonic-clonic convulsions after gentle 180°spin	3
No convulsion when lifted by the tail, but tonic convulsion elicited by gentle 180° spin	2
Only facial grimace after gentle 180° spin	1
No convulsion	0

5% $ZnSO_4$ and stored on ice. Distilled water (300-µl) and 0.3N $Ba(OH)_2$ (50-µl) were added to each sample. Each sample was shaken for 5 sec and centrifuged for 5 min at 12,000 rpm. The supernatant was transferred to a crimp top glass vial and analyzed for EtOH concentrations by gas chromatography (Model 6890N, Agilent Technologies, Palo Alto, CA, USA) with flame ionization detection. Seven pairs of EtOH standards (0.25-4.0 mg/ml) were used to establish a standard curve.

Radioimmunoassay (RIA)

At selected time points, separate groups of mice were killed and trunk blood collected for subsequent analysis of plasma 17β-estradiol and corticosterone concentrations by RIA. Due to decreased sensitivity of the antibody used in the ALLO RIA after long-term storage, we were unable to analyze ALLO levels in the present study. RIAs were conducted across several days due to the number of samples to be analyzed. In order to assess the reliability of measures and the consistency of the assay across days, intra- and inter-assay variation was determined. Intra-assay variation was determined by interpolating the counts per minute (cpm) obtained from the standard curve and comparing these concentrations with the mass of the standards. Inter-assay variability was determined by comparing the concentration of steroid that produced 90% and 50% inhibition of isotope across assays.

Estradiol determination

Using 50-µl of plasma, estradiol concentrations were obtained by following the manufacturer's instructions on the ImmuChem Double Antibody 17β-Estradiol [^{125}I] RIA kit from ICN Pharmaceuticals (Costa Mesa, CA). Counts per minute were normalized and fit to a least-squares regression equation produced by log-logit transformation of the standards (10-3,000 pg/ml) using Prism Version 4 Software (GraphPad Software Inc, San Diego, CA). Mass

of samples was calculated by interpolation of the standards. The minimal detectable limit of the assay was 0.34-1.25 pg/ml, based on actual values from individual assays. Intra- and inter-assay coefficients of variation were 1% and less than 11%, respectively, when calculated across 4 assays. The specificity of the assay was fairly high, with 20% cross-reactivity to estrone, 1.5% cross-reactivity to estriol, and less than 1% cross-reactivity to other endogenous steroids (based on the manufacturer's information).

Corticosterone determination

Plasma (5-µl) was diluted with 100-µl sterile water and stored at 4°C until assayed. Samples were immersed in boiling water for 5 minutes to denature corticosterone-binding globulin. The RIA was adapted from a previously reported procedure (Keith et al., 1978) and employed [^{125}I] corticosterone from ICN Pharmaceuticals and antiserum from Ventrex (Portland, ME). Counts per minute were normalized and fit to a least-squares regression equation produced by log-logit transformation of the standards (10-10,000 pg). Mass of samples was calculated by interpolation of the standards. The minimal detectable limit of the assay was 0.04-0.07 µg/dl, based on actual values from individual assays. Intra- and inter-assay coefficients of variation were 2% and 17%, respectively, when calculated across 4 assays. The specificity of the assay was very high, with only 4% cross-reactivity to deoxycorticosterone, 1% cross-reactivity to 5β-pregnanedione, and <0.6% cross-reactivity to other endogenous steroids (Keith et al., 1978).

Experiment 3: EtOH Clearance

A separate time course study examined the effects of finasteride on EtOH clearance parameters. Separate groups of male and female C57BL/6J and DBA/2J received a pretreatment of finasteride (50 mg/kg, i.p.) or saline 24 hours prior to an injection of EtOH (4 g/kg, i.p.). Animals were tested for ethanol clearance rate using previously published methods (Shen et al.,

1995). Specifically, retro-orbital blood samples were collected at 30, 60, 120, and 240 minutes post EtOH injection for BEC determination. Mice were briefly restrained while a 20 µl sample of blood was collected, eyes were alternated for each time point to minimize trauma. The blood sample was drawn from the orbital sinus in less than one minute, the eye was carefully blotted to stop the flow of blood, and the mouse was then released back to its home cage. Recent work suggests that multiple retro-orbital blood sampling did not significantly alter BECs relative to animals that had received a single blood sampling (Kamens et al, submitted). BECs were determined as described in experiment 2, above.

Data Analysis

Analysis of variance (ANOVA) was used to assess strain (C57BL/6J versus DBA/2J), sex (male versus female), treatment (EtOH versus saline), and drug (finasteride versus saline) effects on the dependent variables hourly HICs, area under the withdrawal curve (AUC), peak withdrawal, BEC, and hormone concentrations. A repeated measure ANOVA was used to assess the effect of the independent variables on hourly HICs. AUC was calculated over the 25 hr period (AUC25) following the single injection of a 4 g/kg dose of EtOH from the hourly HIC scores. Peak withdrawal was calculated by taking the average of the highest HIC score, the previous hourly score, and the following hourly score.

Linear regression analysis was performed on the retro-orbital BEC time course data for each repeatedly sampled animal. Based on the slope of the regression line, an estimate of clearance rate (mg/ml/hr) was obtained. A crude estimate of volume of distribution (ml) and volume of distribution accounting for body weight (ml/g) was determined by dividing the amount of EtOH (mg) by the estimated BEC at time = 0 (based on the regression slope and the y-intercept of the regression line). An estimate for total clearance time (min) was determined by

the x-intercept of the regression line. A repeated measures ANOVA was used to assess the effects of strain, sex, drug, and time on BECs, while a three-way ANOVA was used to assess the effects of strain, sex, and drug on several of the clearance parameters.

All statistical analyses were conducted using Systat (version 10; Point Richmond, CA). Based on the findings of our chronic study (Finn et al., 2004c), our *a priori* hypothesis was that finasteride would differentially alter withdrawal in C57BL/6J and DBA/2J male and female mice (as discussed in the Introduction). Thus, each group was analyzed separately in the absence of significant interactions. Post-hoc analyses were conducted using Tukey's post-hoc test. Data are expressed as the mean ± SEM. Significance was set at $p \leq 0.05$.

Results

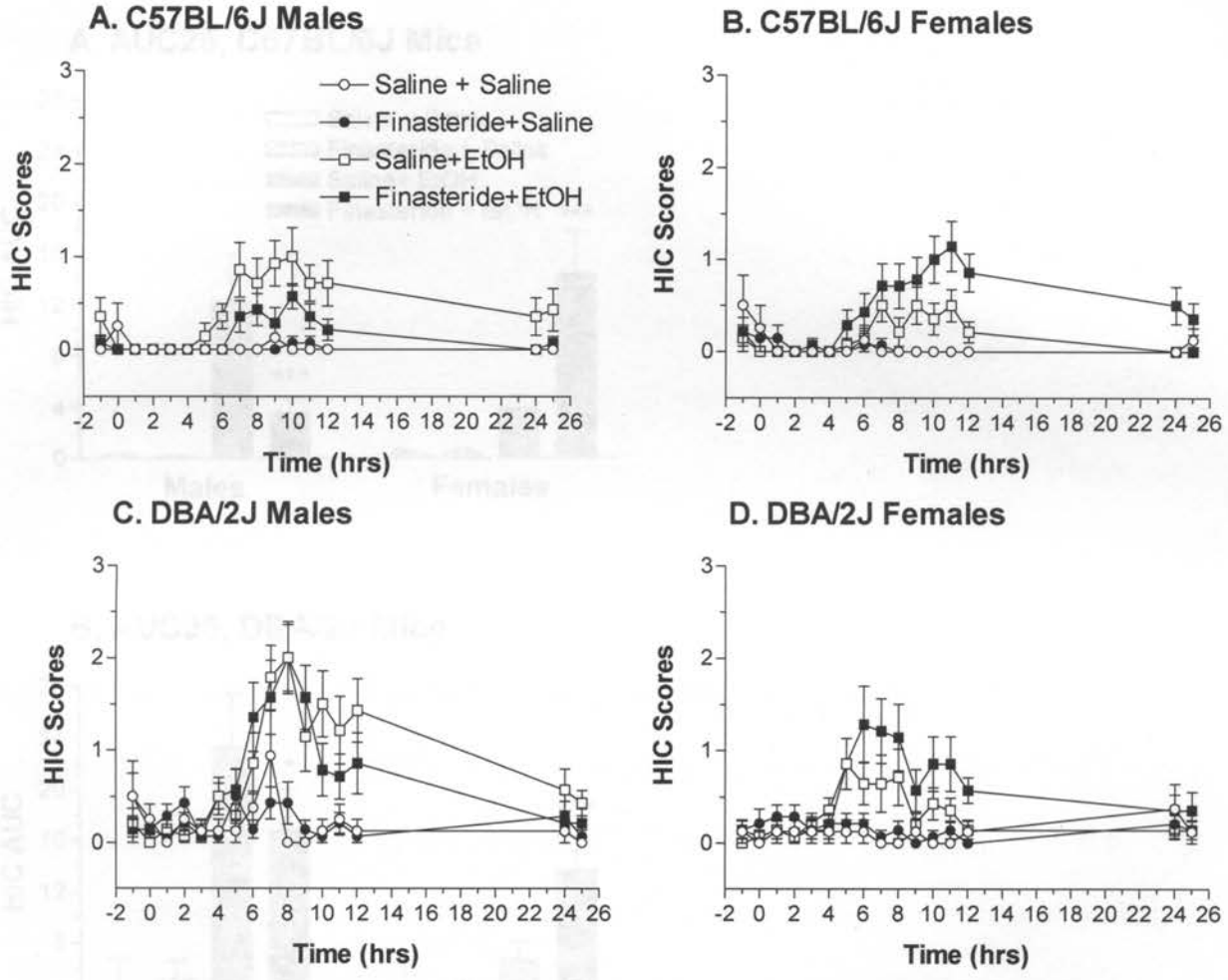
Experiment 1: Withdrawal Severity

HIC Scores and AUC25

Hourly HIC scores, collapsed across time, were significantly higher in DBA/2J versus C57BL/6J mice, in male than in female mice, and in mice treated with EtOH versus saline [$Fs(1,184) > 4.13$, $ps < 0.05$] (Figure 2). Thus, EtOH injection produced an increase in HICs relative to saline treated animals, and acute EtOH withdrawal was greater in DBA/2J mice than in C57BL/6J mice and was greater in male than female mice. There were several significant interactions involving strain, sex, treatment, and drug [$Fs(1,184) > 6.70$, $ps < 0.05$], indicating that treatment with finasteride and EtOH differentially altered HIC scores in male and female C57BL/6J and DBA/2J mice. Repeated measures ANOVA also indicated that hourly HIC scores changed significantly across time [$F(15,2760) = 17.46$, $p < 0.001$]. The significant interactions involving time, strain, sex, treatment, and drug [$Fs(15,2760) > 2.027$, $ps < 0.05$] and post-hoc analyses (not shown) provide support for the conclusion that pretreatment with finasteride decreased acute EtOH withdrawal severity in male C57BL/6J and DBA/2J mice and increased acute EtOH withdrawal severity in female mice of both strains. The results of the hourly HIC scores mimicked that of AUC25, another index of EtOH withdrawal severity, which will be discussed in detail.

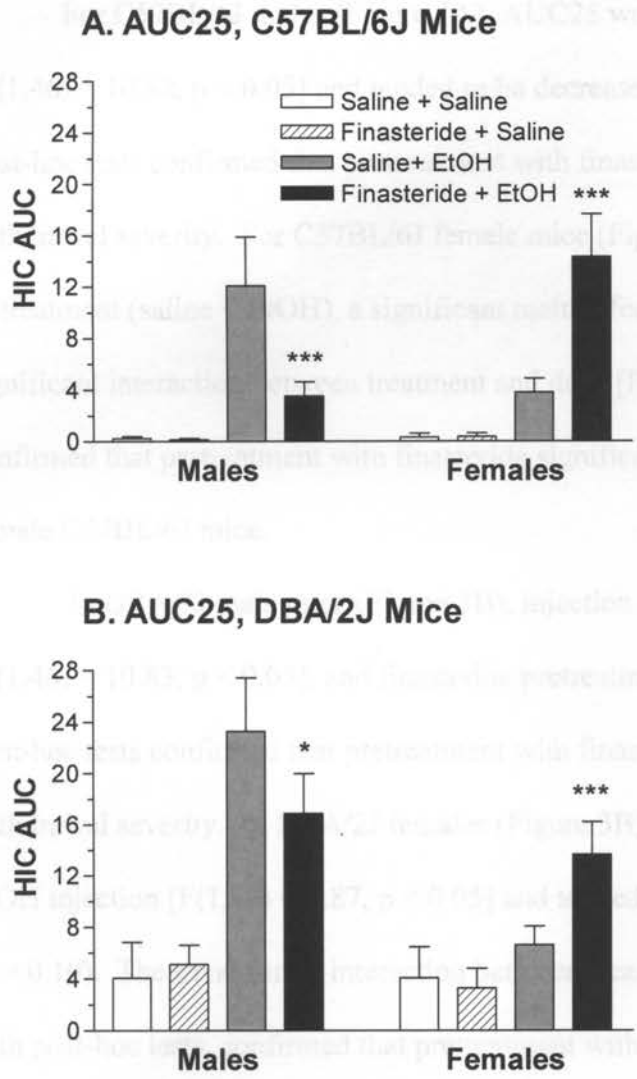
AUC25 (Figure 3) was significantly higher in DBA/2J than in C57BL/6J mice and following injection of EtOH [$Fs(1,184) > 18.34$, $ps < 0.05$]. There was a trend for AUC25 to be higher in male than in female mice ($p = 0.055$). The significant three-way interactions between strain, sex, and treatment as well as between sex, treatment, and drug [$Fs(1,184) > 4.26$, $ps < 0.05$], suggested that finasteride pretreatment altered acute EtOH withdrawal differently in the

Figure 2.



Hourly HIC scores in intact C57BL/6J male (A), C57BL/6J female (B), DBA/2J male (C), and DBA/2J female (D) mice following a 4 g/kg injection of EtOH or saline and pretreatment with finasteride (50 mg/kg) or saline. Values represent the mean ± S.E.M. for n=8-14/group.

Figure 3.



The effect of pretreatment with finasteride on EtOH withdrawal severity, measured by area under the withdrawal curve (AUC25), in C57BL/6J (A) and DBA/2J (B) intact male and female mice. Values represent the mean ± S.E.M. for the animals depicted in Figure 2. *$p < 0.05$, ***$p < 0.001$, versus respective saline + EtOH group

male and female mice. Based on these multiple significant interactions, each strain and sex was analyzed separately.

For C57BL/6J males (Figure 3A), AUC25 was significantly increased by EtOH injection [$F(1,46) = 10.82$, $p < 0.05$] and tended to be decreased by finasteride pretreatment ($p = 0.07$). Post-hoc tests confirmed that pretreatment with finasteride significantly decreased EtOH withdrawal severity. For C57BL/6J female mice (Figure 3A), there was a significant main effect of treatment (saline < EtOH), a significant main effect of drug (saline < finasteride) and a significant interaction between treatment and drug [$Fs(1,46) > 3.32$, $ps < 0.05$]. Post-hoc tests confirmed that pretreatment with finasteride significantly increased EtOH withdrawal severity in female C57BL/6J mice.

In DBA/2J male mice (Figure 3B), injection of EtOH significantly increased AUC25 [$F(1,46) = 10.83$, $p < 0.05$], and finasteride pretreatment tended to decrease AUC25 ($p = 0.06$). Post-hoc tests confirmed that pretreatment with finasteride significantly decreased EtOH withdrawal severity. In DBA/2J females (Figure 3B), AUC25 was significantly increased by EtOH injection [$F(1,46) = 9.87$, $p < 0.05$] and tended to be increased by finasteride pretreatment ($p = 0.10$). The trend for an interaction between treatment and drug ($p = 0.07$), in conjunction with post-hoc tests, confirmed that pretreatment with finasteride significantly increased EtOH withdrawal severity in DBA/2J female mice.

In EtOH-treated animals, peak withdrawal severity (Table 3) was significantly greater in DBA/2J mice than C57BL/6J mice and was significantly greater in male mice relative to female mice [$Fs(1,104) > 4.08$, $ps < 0.05$]. There were multiple significant interactions involving strain, sex, and drug [$Fs(1,104) > 6.42$, $ps < 0.05$], suggesting that peak withdrawal was differentially altered by finasteride in the male and female C57BL/6J and DBA/2J mice. In C57BL/6J male

Table 3.

Peak Withdrawal Scores and Peak Withdrawal Hour in EtOH-Injected Mice

Strain and Sex	Drug	Peak Withdrawal Score (HIC Score)	Peak Withdrawal Hour
C57BL/6J Males	Saline	1.12 ± 0.29 (n = 14)	7.36 ± 1.11 (n = 14)
	Finasteride	0.48 ± 0.08 (n = 14)+	7.43 ± 0.89 (n = 14)
C57BL/6J Females	Saline	0.61 ± 0.14 (n = 14)	7.71 ± 0.78 (n = 14)
	Finasteride	1.17 ± 0.21 (n = 14)*	8.43 ± 0.95 (n = 14)
DBA/2J Males	Saline	2.02 ± 0.29 (n = 14)	8.43 ± 0.59 (n = 14)
	Finasteride	1.93 ± 0.29 (n = 14)	6.36 ± 0.59 (n = 14)
DBA/2J Females	Saline	1.05 ± 0.20 (n = 14)	6.07 ± 0.68 (n = 14)
	Finasteride	1.41 ± 0.58 (n = 14)	6.93 ± 0.58 (n = 14)

Peak withdrawal scores (i.e. the average of the highest HIC score, the previous score, and the following score) and the hour that peak withdrawal occurred were calculated for C57BL/6J and DBA/2J male and female mice. Values represent mean ± S.E.M. for the EtOH-injected animals depicted in Figure 1. + $p < 0.10$, * $p < 0.05$ versus saline pretreatment

mice, pretreatment with finasteride tended to decrease peak withdrawal severity ($p = 0.07$). In C57BL/6J female mice, pretreatment with finasteride significantly increased peak withdrawal severity [$F(1,46) = 4.13$, $p < 0.05$]. There was no significant effect of pretreatment with finasteride on peak withdrawal severity in male or female DBA/2J mice. In EtOH-treated animals, strain, sex, and drug did not significantly affect the hour at which peak withdrawal occurred.

Experiment 2: Hormone Assessment

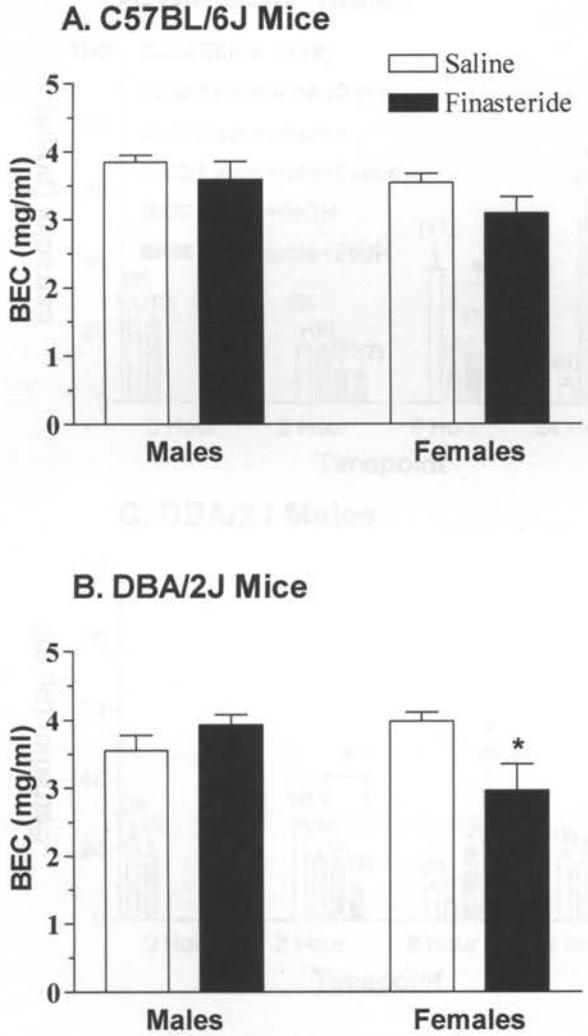
BEC

BEC was measured at 2 hours following EtOH administration in mice that had been pretreated with either saline or finasteride (Figure 4). BEC was significantly lower in finasteride pretreated mice and in female versus male mice [$Fs(1,70) > 4.72$, $ps < 0.05$]. There was a significant interaction between drug and sex [$F(1,70) = 6.54$, $p < 0.05$] and a trend for a three-way interaction between treatment, sex, and strain ($p = 0.055$), suggesting that finasteride differentially altered BEC in the male and female mice. Specifically, pretreatment with finasteride did not alter BECs in male and female C57BL/6J mice or in male DBA/2J mice. However, finasteride pre-treatment significantly decreased BECs in female DBA/2J mice by 26% [$F(1,16) = 6.18$, $p < 0.05$]. Thus, pretreatment with finasteride significantly reduced BECs following acute administration of a high EtOH dose only in DBA/2J female mice.

Estradiol Concentrations

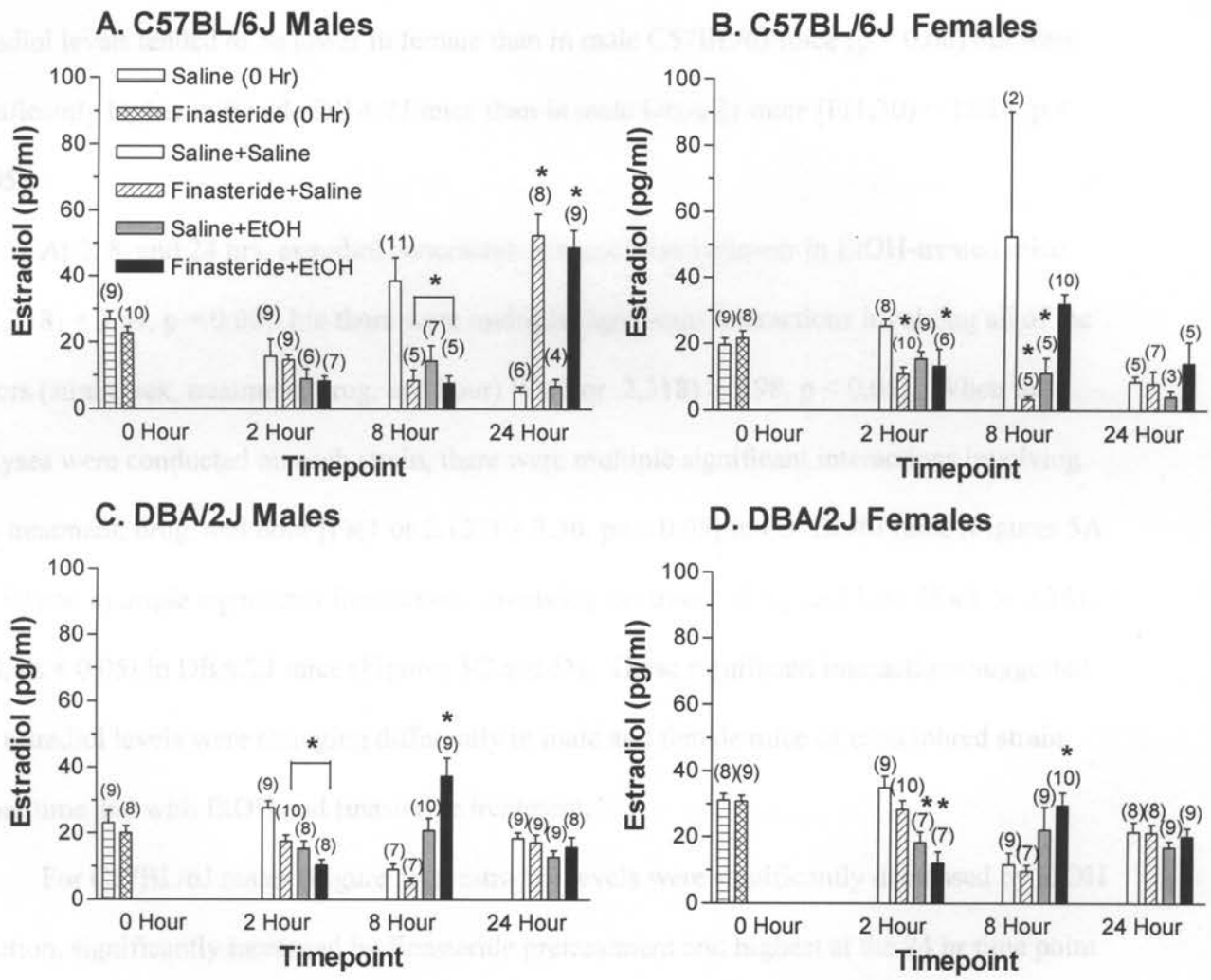
Due to the fact that not all groups were equally represented in the 0 hr condition (i.e., since these animals did not receive injections of either EtOH or saline), this time point was analyzed separately from 2, 8, and 24 hrs (Figure 5). At 0 hr, there was a significant main effect

Figure 4.



Tail blood BEC was measured in C57BL/6J (A) and DBA/2J (B) mice at 2 hours following EtOH administration. Values represent the mean ± S.E.M. for n=10/group. *$p < 0.05$ versus respective saline pretreatment group

Figure 5.



Plasma estradiol concentrations in C57BL/6J male (A), C57BL/6J female (B), DBA/2J male (C), and DBA/2J female (D) mice following a 4 g/kg injection of EtOH or saline that was given 24 hrs after pretreatment with finasteride or saline. Values represent the mean ± S.E.M. for the number of animals in parentheses. * at least $p < 0.05$, versus respective Saline+Saline group

of strain (C57BL/6J < DBA/2J) on estradiol concentrations as well as a significant interaction between strain and sex [Fs(1,62) > 4.27, ps < 0.05]. The interaction was due to the fact that estradiol levels tended to be lower in female than in male C57BL/6J mice ($p = 0.06$) but were significantly higher in female DBA/2J mice than in male DBA/2J mice [$F(1,30) = 12.16$, $p < 0.005$].

At 2, 8, and 24 hrs, estradiol concentrations tended to be lower in EtOH-treated mice [$F(1,318) = 3.09$, $p = 0.08$], but there were multiple significant interactions involving all of the factors (strain, sex, treatment, drug, and hour) [Fs(1 or 2,318) > 3.98, $p < 0.05$]. When the analyses were conducted on each strain, there were multiple significant interactions involving sex, treatment, drug, and hour [Fs(1 or 2,137) > 8.36, ps < 0.05] in C57BL/6J mice (Figures 5A and B) and multiple significant interactions involving treatment, drug, and hour [Fs(1 or 2,181) > 6.65, ps < 0.05] in DBA/2J mice (Figures 5C and D). These significant interactions suggested that estradiol levels were changing differently in male and female mice of each inbred strain across time and with EtOH and finasteride treatment.

For C57BL/6J males (Figure 5A), estradiol levels were significantly decreased by EtOH injection, significantly increased by finasteride pretreatment and highest at the 24 hr time point (2 hr < 8 hr < 24 hr) [Fs (1 or 2,74) > 4.74, ps < 0.05]. The significant interaction between drug and hour [$F(2,74) = 35.190$, $p < 0.001$] suggested that the effect of finasteride on estradiol levels differed at the various time points. At the 2 hr time point, there was a trend for estradiol levels to be lower in EtOH-treated mice ($p = 0.066$). At 8 hrs, estradiol levels were significantly lower following finasteride pretreatment [$F(1,24) = 7.585$, $p < 0.05$] and tended to be lower following EtOH injection ($p = 0.07$). The trend for an interaction between treatment and drug ($p = 0.09$), in conjunction with post-hoc tests, confirmed that finasteride-treated and EtOH-treated animals had

lower plasma estradiol concentrations relative to the saline-treated control animals ($p < 0.05$). At the 24 hr time point, finasteride pretreatment significantly increased estradiol levels [$F(1,23) = 59.30$, $p < 0.001$], an effect that was confirmed with post-hoc tests.

For C57BL/6J female mice (Figure 5B), estradiol concentrations were significantly decreased by finasteride pretreatment and were lowest at the 24 hr time point (24 hr < 2 hr < 8 hr) [Fs(1 or 2,63) > 4.60, ps < 0.05]. The multiple interactions between treatment, drug, and hour [Fs (1 or 2,63) > 3.49, ps < 0.05] suggested that the effect of EtOH and finasteride on estradiol levels differed at the various time points. At the 2 hr time point, there was a significant main effect of drug (finasteride < saline) and a significant interaction between treatment and drug [Fs(1,29) > 4.76, ps < 0.05]. Confirmation with post-hoc tests showed that both finasteride-treated groups had lower plasma estradiol levels relative to saline-treated control animals ($p < 0.05$). Similarly, at 8 hrs, finasteride pretreatment significantly decreased plasma estradiol levels, with a significant interaction between treatment and drug [Fs(1,18) > 2.53, ps < 0.05]. Post-hoc tests revealed that relative to the saline-treated control animals, finasteride + saline-treated and EtOH + saline-treated animals had lower plasma estradiol concentrations ($p < 0.005$). There were no significant main effects or interactions at 24 hrs.

For DBA/2J males (Figure 5C), there were multiple significant interactions between treatment, drug, and hour [Fs(1 or 2,91) > 6.26, ps < 0.05] on estradiol concentrations, suggesting that they were differentially altered across time by finasteride and EtOH. At the 2 hr time point EtOH injection and finasteride pretreatment significantly decreased estradiol levels [Fs(1,31) > 15.11, ps ≤ 0.001]. Post-hoc tests confirmed that relative to saline-treated control animals, finasteride and EtOH produced a decrease in estradiol concentrations ($p < 0.005$). At 8 hrs, estradiol levels were significantly increased by EtOH injection, with a significant interaction

between treatment and drug [$Fs(1,29) > 6.53$, $ps < 0.05$]. Post-hoc tests confirmed that the combination of finasteride and EtOH produced the greatest increase in plasma estradiol concentrations relative to the saline-treated control group ($p < 0.001$). There were no significant main effects or interactions at the 24 hr time point.

For DBA/2J female mice (Figure 5D), there was a significant interaction between treatment and hour [$F(2,90) = 19.12$, $p < 0.001$] on plasma estradiol levels, suggesting that EtOH injection differentially altered estradiol concentration across time. At 2 hrs, estradiol levels were significantly lower in the EtOH-treated mice [$F(1,29) = 26.01$, $p < 0.001$], and they tended to be lower in mice pretreated with finasteride ($p = 0.06$). Post-hoc tests confirmed that treatment with EtOH significantly decreased estradiol concentrations relative to saline-treated counterparts ($p < 0.01$). However, at the 8 hr time point, EtOH treatment significantly increased plasma estradiol levels [$F(1,31) = 10.376$, $p < 0.05$]. This main effect of EtOH was confirmed with post-hoc tests ($p < 0.05$). There were no significant main effects or interactions at 24 hrs.

Corticosterone Concentrations

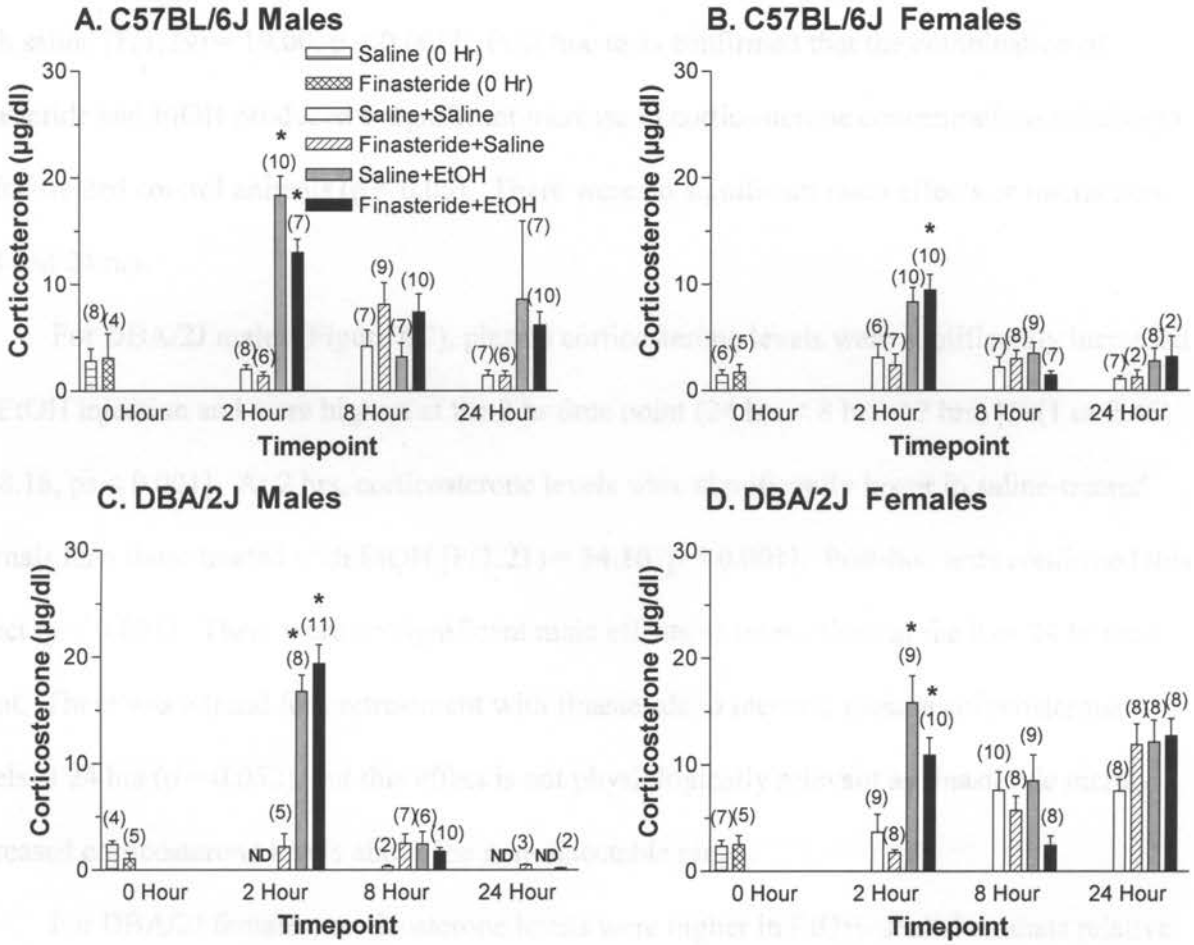
Due to the fact that several groups in the DBA/2J male mice fell below the minimum detectable limit (saline+saline at 2 hrs and 24 hrs, as well as the saline+EtOH group at 24 hrs), it was necessary enter the minimal detectable limit of 0.04 μg/dl (see methods) for those animals in order to analyze the data. It should be noted that corticosterone values ≤ 0.04 μg/dl would be consistent with low "non-stressed" basal levels. Since corticosterone levels were measurable in all other DBA/2J male groups as well as in the DBA/2J females and the C57BL/6J males and females, we do not believe that the undetectable levels reflect insensitivity of the assay.

Baseline corticosterone levels were not significantly affected by strain or pretreatment condition (Figure 6). At 2, 8, and 24 hrs, corticosterone levels were significantly higher in EtOH versus saline-injected mice and were highest at the 2 hr time point [Fs(1 or 2,289) > 3.33, ps < 0.001]. There was a trend for corticosterone levels to be higher in DBA/2J mice relative to C57BL/6J mice ($p = 0.07$). There were multiple significant interactions involving all of the factors (strain, sex, treatment, drug, and time point) [Fs(1 or 2,289) > 4.18, ps < 0.05], suggesting that corticosterone levels were changing across time differently in the male and female mice treated with finasteride and/or EtOH.

For C57BL/6J males (Figure 6A), corticosterone levels were significantly higher in the EtOH-treated group relative to the saline-treated group and were highest at the 2 hr time point (24 hrs < 8 hrs < 2 hrs) [Fs(1 or 2,82) > 3.33, ps < 0.05]. The significant interaction between the treatment and hour [$F(2,82) = 9.96$, $p < 0.001$] suggested that EtOH treatment differentially altered plasma corticosterone at various time points. At the 2 hr time point, plasma corticosterone levels were significantly increased by EtOH injection and significantly decreased by finasteride pretreatment [Fs(1,27) > 4.78, ps < 0.05]. The trend for an interaction between the two factors ($p = 0.097$), in conjunction with post-hoc tests, confirmed that treatment with EtOH increased corticosterone concentrations at 2 hrs ($p < 0.001$) relative to saline-treated-control groups. At 8 hrs, corticosterone levels were significantly elevated in the mice pretreated with finasteride [$F(1,29) = 5.33$, $p < 0.05$]. There were no significant main effects or interactions at the 24 hr time point. However, there was a trend for treatment with EtOH to increase corticosterone levels relative to saline-treated animals ($p = 0.10$).

For C57BL/6J females (Figure 6B), corticosterone levels were significantly increased by EtOH injection and were highest at the 2 hr time point (24 hrs $\leq$ 8 hrs < 2 hrs), with a significant

Figure 6.



Plasma corticosterone levels in C57BL/6J male (A), C57BL/6J female (B), DBA/2J male (C), and DBA/2J female (D) mice following a 4 g/kg injection of EtOH or saline that was given 24 hrs after pretreatment with finasteride or saline. Values represent mean ± S.E.M. for the number of animals in parentheses. N.D. = not detectable * at least $p < 0.05$, versus respective Saline+Saline group

interaction between treatment and time [Fs(1 or 2,71) > 7.98, ps < 0.05]. At 2 hrs, corticosterone levels were significantly higher in EtOH-treated animals relative to those treated with saline [F(1,29) = 19.00, p < 0.001]. Post-hoc tests confirmed that the combination of finasteride and EtOH produced a significant increase in corticosterone concentrations relative to saline-treated control animals (p < 0.05). There were no significant main effects or interactions at 8 and 24 hrs.

For DBA/2J males (Figure 6C), plasma corticosterone levels were significantly increased by EtOH injection and were highest at the 2 hr time point (24 hrs < 8 hrs < 2 hrs) [Fs(1 or 2,45) > 18.18, ps < 0.001]. At 2 hrs, corticosterone levels were significantly lower in saline-treated animals than those treated with EtOH [F(1,21) = 34.16, p < 0.001]. Post-hoc tests confirmed this effect (p < 0.001). There were no significant main effects or interactions at the 8 or 24 hr time point. There was a trend for pretreatment with finasteride to increase plasma corticosterone levels at 24 hrs (p = 0.052), but this effect is not physiologically relevant as finasteride merely increased corticosterone levels above the non-detectable range.

For DBA/2J females, corticosterone levels were higher in EtOH-treated animals relative to those treated with saline and varied significantly across time (8 hrs < 2 hrs < 24 hrs) [Fs(1 or 2,91) > 7.89, ps < 0.05]. The significant interactions between treatment and time point as well as between drug and time point [Fs(2,91) > 3.91, ps < 0.05] suggested that EtOH and/or finasteride altered corticosterone levels differently at the various time points. There was a trend for an interaction between treatment and drug (p < 0.08). At 2 hrs, corticosterone levels were significantly increased by EtOH injection [F(1,32) = 34.77, p < 0.001] and tended to be decreased by finasteride pretreatment (p = 0.07). Post-hoc tests confirmed that relative to the saline-treated control animals, EtOH produced an increase in plasma corticosterone levels (p <

0.05). Finasteride pretreatment also significantly reduced corticosterone levels at 8 hrs [$F(1,31) = 4.99$, $p < 0.05$]. Post-hoc tests revealed that there was a trend for finasteride-treatment to decrease corticosterone concentrations ($p = 0.10$) relative to saline-treated controls. There were no significant main effects or interactions at the 24 hr time point.

Experiment 3: EtOH Clearance

BEC Timecourse

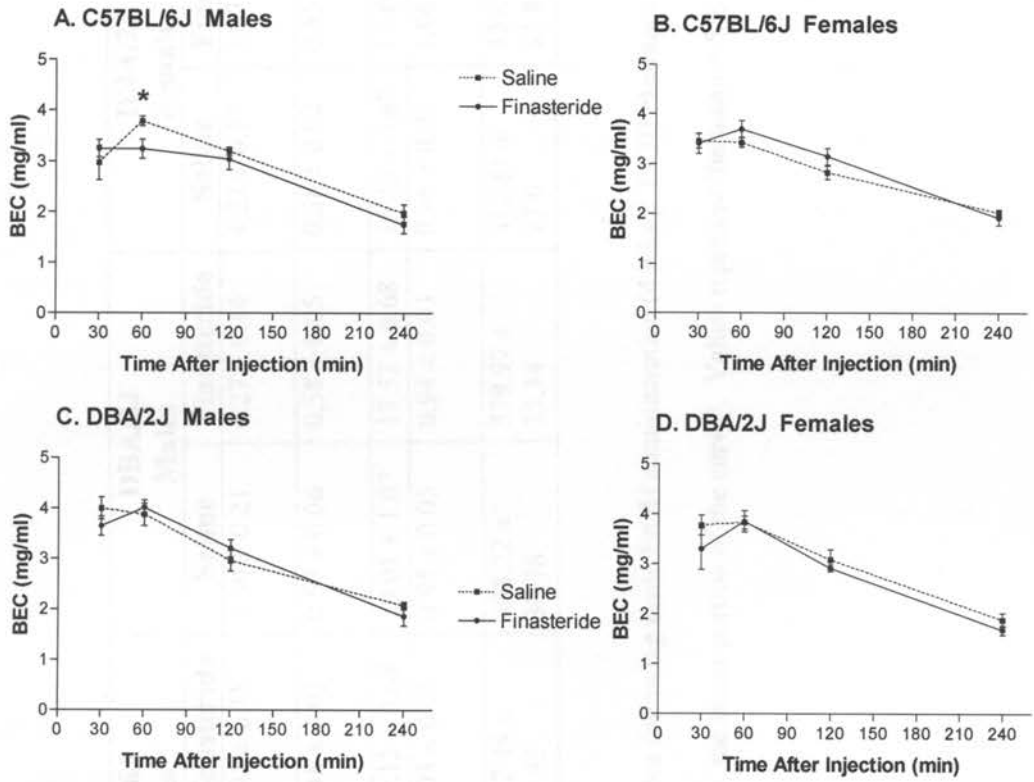
Retro-orbital BECs were measured at 30, 60, 120, and 240 minutes post EtOH injection (Figure 7). Repeated measures ANOVA indicated that that BECs changed significantly across time [$F(3,210) = 229.95$, $p < 0.001$]. There was a significant interaction between time and strain [$F(3,210) = 4.98$, $p < 0.005$] and a trend for a significant interaction between time, strain, and drug ($p = 0.10$). Due to our *a priori* hypothesis and significant interactions, each strain and sex was analyzed separately to determine the effect of finasteride on BECs at each time point.

In C57BL/6J male mice (Figure 7A), pretreatment with finasteride did not significantly alter BECs relative to saline-treated controls at the 30, 120, and 240 minute time point. However, at 60 minutes post EtOH injection, pretreatment with finasteride significantly reduced BECs relative to saline-treated animals [$F(1,18) = 6.61$, $p < 0.02$]. In C57BL/6J females, DBA/2J males, and DBA/2J females (Figure 7B-D, respectively), there were no significant differences in BECs at any time point between finasteride-treated and saline-treated animals.

Clearance Parameters

Although BECs were collected at 30, 60, 120, and 240 minutes post-injection, EtOH clearance rates for individual animals were based on the 60 – 240 minute time points (the linear portion of the curve). For differences in clearance rates (Table 4), ANOVA revealed that C57BL/6J mice had slower clearance rates than DBA/2J mice [$F(1,71) = 14.99$, $p < 0.001$]. Due

Figure 7.



Retro-orbital BECs at 30, 60, 120, and 240 minutes following a 4 g/kg injection of EtOH that was given 24 hrs after pretreatment with finasteride or saline in C57BL/6J male (A), C57BL/6J female (B), DBA/2J male (C), and DBA/2J female (D) mice. Values represent mean ± SEM for n=9-11/group. * $p < 0.05$ versus saline-treated controls

Table 4.

Ethanol Clearance Parameters

Clearance Parameters	C57BL/6J Males		C57BL/6J Females		DBA/2J Males		DBA/2J Females	
	Saline	Finasteride	Saline	Finasteride	Saline	Finasteride	Saline	Finasteride
BEC at time = 0 (mg/ml)	3.71 ± 0.16	3.65 ± 0.17	3.75 ± 0.1	3.95 ± 0.18	4.30 ± 0.21	4.27 ± 0.14	4.22 ± 0.17	3.97 ± 0.29
Clearance Rate (mg/ml/h)	0.39 ± 0.05	0.45 ± 0.03	0.43 ± 0.02	0.48 ± 0.03	0.57 ± 0.06	0.58 ± 0.05	0.58 ± 0.02	0.55 ± 0.09
V_d (ml)	26.15 ± 1.67	26.74 ± 1.62	19.76 ± 0.97	18.12 ± 0.68	19.01 ± 1.07	17.52 ± 0.68	14.43 ± 0.67	15.86 ± 1.3
V_d/body weight (ml/g)	1.1 ± 0.06	1.12 ± 0.05	1.08 ± 0.03	1.03 ± 0.05	0.95 ± 0.05	0.94 ± 0.03	0.96 ± 0.03	1.06 ± 0.09
Total Clearance Time (min)	394.97 ± 48.26	431.14 ± 21.27	470.05 ± 22.93	432.19 ± 27.55	408.22 ± 39.78	379.97 ± 25.34	412.81 ± 17.6	329.85 ± 33.8 *

Retro-orbital blood BECs were collected at 30, 60, 120, and 240 minutes following acute EtOH administration (4 g/kg). EtOH clearance parameters were calculated based on the 60-240 minute time points (i.e. the linear portion of the curve). Values represent the mean ± S.E.M. for n = 9-11/group. * $p < 0.05$ versus respective saline-treated group

to our *a priori* hypothesis, each strain and sex were analyzed separately. For C57BL/6J male and female mice, pretreatment with finasteride did not significantly affect EtOH clearance rates, when compared to saline-treated controls (i.e., pretreatment with finasteride produced only a 12-15% increase versus saline treatment). Similarly, in DBA/2J males, pretreatment with finasteride did not significantly affect EtOH clearance rates (i.e., produced only a 2% increase relative to saline-treated controls). Although it was not significant, pretreatment with finasteride produced a 5% decrease in EtOH clearance rates relative to saline-treated controls in DBA/2J female mice.

For many of the other clearance parameters, there was a significant main effect of strain. Specifically, the estimate of BEC at time 0 (an extrapolation based on the slope) was significantly lower in C57BL/6J mice versus DBA/2J mice [$Fs(1,71) < 14.99$, $ps < 0.002$]. Volume of distribution accounting for body weight (ml/g) was significantly lower and total clearance time (minutes) was significantly faster in DBA/2J mice than in C57BL/6J mice [$Fs(1,71) < 7.16$, $ps < 0.03$]. For C57BL/6J males and females and DBA/2J males, finasteride pretreatment did not significantly alter any of the clearance parameters. However, for DBA/2J female mice, pretreatment with finasteride significantly decreased total clearance time relative to saline-treated controls [$F(1,17) = 4.43$, $p < 0.05$].

Since we had collected tail blood samples (experiment 2) and retro-orbital eye blood samples (experiment 3) at 120 minutes post-EtOH injection, we compared the values from these two studies (Table 5). Overall, in all strains and sexes, BECs from retro-orbital samples were lower than from tail blood samples. However, the direction of finasteride's effect (i.e., lower or higher relative to saline-treated controls) on BECs was the same in all strains and sexes, except C57BL/6J female mice. Since the effect of finasteride in C57BL/6J female mice was not

Table 5.

Comparison of Tail and Retro-orbital Blood BECs at 120 Minutes

Animals	Treatment	Tail Blood BEC (mg/ml)	Retro-orbital BEC (mg/ml)
C57BL/6J Males	Saline	3.85 ± 0.10 (n = 10)	3.19 ± 0.08 (n = 10)
	Finasteride	3.59 ± 0.27 (n = 10)	3.04 ± 0.21 (n = 9)
C57BL/6J Females	Saline	3.54 ± 0.13 (n = 10)	2.83 ± 0.14 (n = 10)
	Finasteride	3.09 ± 0.24 (n = 10)	3.15 ± 0.17 (n = 10)
DBA/2J Males	Saline	3.55 ± 0.23 (n = 9)	2.96 ± 0.20 (n = 10)
	Finasteride	3.93 ± 0.15 (n = 11)	3.21 ± 0.17 (n = 10)
DBA/2J Females	Saline	3.98 ± 0.13 (n = 9)	3.08 ± 0.20 (n = 9)
	Finasteride	2.96 ± 0.39 (n = 9)	2.92 ± 0.07 (n = 10)

Tail blood (experiment 2) and retro-orbital blood (experiment 3) BECs at 2 hours following acute EtOH administration (4 g/kg). Values represent the mean ± S.E.M. for n = 9-11/group.

significant for either tail or eye blood BECs, the discrepancy between the two methods may reflect subtle experimental differences.

Discussion

The present experiments were designed to assess whether male and female C57BL/6J and DBA/2J mice would differ in response to pharmacological inhibition of the neurosteroid biosynthetic enzyme 5α-reductase on acute EtOH withdrawal severity. Pretreatment with finasteride decreased acute EtOH withdrawal severity, measured by hourly HIC and AUC25, in male C57BL/6J and DBA/2J mice. However, pretreatment with finasteride increased acute EtOH withdrawal severity in female mice of both strains. This sex difference in finasteride's effect on acute EtOH withdrawal was not due to changes in BECs at the 2 hr time point or to changes in EtOH clearance parameters. Similarly, the finasteride- and EtOH-induced alterations in plasma estradiol and corticosterone levels did not appear to change in a manner that could explain the sex difference in acute EtOH withdrawal severity, measured by HICs. Collectively, these findings suggest that male and female C57BL/6J and DBA/2J mice may differ in their sensitivity to manipulation of ALLO levels or other GABAergic steroid metabolites during acute EtOH withdrawal.

HIC Data

Acute EtOH withdrawal severity, measured by hourly HIC scores and AUC, was significantly greater in DBA/2J versus the C57BL/6J strain of mice, consistent with previously observed strain differences (Crabbe, 1998; Crabbe et al., 1983; Roberts et al., 1992). Similarly, peak withdrawal severity was significantly greater in DBA/2J mice compared to C57BL/6J mice. Another interesting finding was that acute EtOH withdrawal severity was significantly higher in male mice relative to female mice. Specifically, hourly HIC scores, AUC, and peak withdrawal severity were higher in saline + EtOH treated male mice than female mice. This sex difference

in acute withdrawal severity is consistent with reports that chronic EtOH withdrawal severity is decreased by approximately 25% in female rodents (e.g. Devaud et al., 2003).

Finasteride pretreatment decreased EtOH withdrawal severity in male mice, a finding that is consistent with a chronic withdrawal study from our laboratory. Treatment with finasteride during chronic EtOH exposure reduced the severity of EtOH withdrawal, measured by HICs in male C57BL/6J and DBA/2J mice (Finn et al., 2004c). Although finasteride decreased BECs upon the initiation of withdrawal in the chronic study, there was no comparable effect of finasteride on BEC in the male mice in the present study. Thus, the fact that finasteride decreased acute withdrawal severity in male mice does not appear to be due to an interaction between finasteride and BECs.

In contrast, finasteride increased acute EtOH withdrawal severity in female mice of both strains. However, pretreatment with finasteride had the opposite effect on chronic EtOH withdrawal severity in that HICs were significantly decreased in female DBA/2J mice and were not significantly altered in female C57BL/6J mice. Consistent with the chronic EtOH study in male mice, the finasteride-treated female mice had a significant decrease in BECs upon the initiation of chronic EtOH withdrawal. Whereas an indirect effect of finasteride on EtOH pharmacokinetics could have decreased chronic EtOH withdrawal severity in female DBA/2J mice (and male C57BL/6J and DBA/2J mice) by decreasing the "effective" EtOH dose during the development of physical dependence, it cannot explain the results in female C57BL/6J mice in the chronic study. In these animals, BEC was decreased 45% but chronic withdrawal was not affected. These data suggest that the opposite effect of finasteride on acute versus chronic withdrawal severity in female mice was not solely due to alterations in BECs. Although speculative, other mechanisms (e.g. ion channel adaptations) may be recruited during chronic

exposure that do not occur from a single injection of EtOH, thereby altering sensitivity to finasteride during withdrawal.

Peak withdrawal was also examined to determine the manner in which finasteride might be altering withdrawal severity. As described above, peak withdrawal scores were greater in DBA/2J mice relative to C57BL/6J mice and in male versus female mice, a finding that is consistent with previous studies and our AUC data. Interestingly, the hour at which peak withdrawal occurred was significantly different between the two strains. In EtOH-treated animals, the highest HIC scores appear to occur at later time points in C57BL/6J mice relative to DBA/2J mice, indicating that withdrawal was occurring approximately 1 hr earlier in the DBA/2J mice. In the current study, pretreatment with finasteride did not affect the time at which peak withdrawal occurred. However, there was trend for pretreatment with finasteride to decrease peak withdrawal scores in C57BL/6J male mice, while pretreatment with finasteride significantly increased peak withdrawal scores in female C57BL/6J mice. This finding is consistent with the hourly HIC and AUC data. Thus, finasteride may be acting to increase or decrease the severity of withdrawal, rather than precipitating withdrawal (i.e., causing withdrawal to occur sooner) or elevating HIC scores for longer duration.

BEC and Clearance Data

Notably, the current findings indicate that we can rule out a finasteride-induced alteration in EtOH's pharmacokinetics as the cause of the observed increase in acute EtOH withdrawal severity in female mice or the decrease in acute EtOH withdrawal in male. Likewise, there were no sex differences in EtOH clearance parameters that could explain the differences in hourly HIC and AUC between the male and female mice.

There were no systematic changes in retro-orbital or tail BECs in the male or female C57BL/6J or DBA/2J mice following finasteride pretreatment. Specifically, the only significant difference observed at any time point was a finasteride-induced decrease in retro-orbital BEC at 60 minutes in C57BL/6J male mice. This decrease in BEC occurred prior to the initiation of withdrawal and was not reflected in later time points. Similarly, there was no significant difference in any EtOH clearance parameter between finasteride and saline-treated C57BL/6J males. Thus, finasteride's effect on retro-orbital BEC at 60 minutes post-EtOH injection did not appear to be representative of finasteride's effects on EtOH clearance parameters in these animals.

The finding that finasteride significantly decreased tail BECs at 2 hrs post EtOH injection in female DBA/2J mice was not consistent with an increase in withdrawal severity and was not observed when assessed by retro-orbital sampling. In fact, one would expect the opposite; if the concentration of EtOH was reduced, withdrawal should be reduced as well. However, it should be noted that due to the fact that all groups were injected with the same dose of EtOH, the lower BECs observed in the DBA/2J female group pre-injected with finasteride might reflect a more rapid rate of EtOH elimination due to finasteride. Although there was no difference in clearance rates between saline-treated and finasteride-treated DBA/2J female mice, pretreatment with finasteride decreased total clearance time relative to saline-treated DBA/2J females (an effect that was not observed in C57BL/6J animals or DBA/2J male mice). This could explain the significant finasteride-induced decrease observed in tail BECs at 2 hrs post-EtOH injection, but it is unclear why a comparable difference in BEC at 2 hrs was not observed with the orbital samples. Nonetheless, if DBA/2J female mice were demonstrating a more rapid rate of clearance due to finasteride, it might also be predicted that withdrawal signs would begin

sooner in the finasteride-treated DBA/2J females relative to saline-treated animals, which did not occur based on our HIC data (Figure 2) and the hour at which peak withdrawal occurred (Table 3).

Although there were no overall sex and drug effects on any of the EtOH clearance parameters, there were a number of strain differences in the elimination of EtOH. Notably, clearance rates were lower in C57BL/6J mice relative to DBA/2J mice. This finding is consistent with the previous literature, which suggests that C57BL/6J mice have approximately 10-15% slower elimination rates than DBA/2J mice (Grisel et al., 2002; Faulkner et al., 1990). Interestingly, an estimate for the volume of distribution accounting for body weight and total clearance time were lower in DBA/2J mice relative to C57BL/6J mice. Although one would expect that a slower rate of elimination would correspond to a longer total clearance time and vice versa, it is possible that there are other factors involved. For example, we did not examine differences in water distribution, thermoregulation, or metabolic enzymes that can affect EtOH metabolism and elimination. Similarly, our values of volume of distribution and total clearance time represent crude estimates that were based on the slope of the regression line on the BEC data.

It should also be noted that in experiment 2, tail blood samples were examined for BEC, while experiment 3 utilized retro-orbital blood samples. Retro-orbital bloods were used in the EtOH clearance study instead of tail nick or saphenous vein sampling due to the fact that retro-orbital eye blood samples are highly correlated with, and representative of, brain EtOH concentrations during the first 30-60 minutes following EtOH administration (Smolen and Smolen, 1989; Gentry et al., 1983). Tail blood sampling may not provide an accurate representation of brain EtOH during the early time points post-EtOH injection. Similarly, since

EtOH produces hypothermia and rodents thermoregulate with their tail, hypothermia-induced constriction of the tail veins may confound BECs obtained from this region (Lomax et al., 1981).

Upon comparing tail blood BECs and retro-orbital BECs at 120 minutes post-EtOH injection, there appeared to be sampling method differences, with retro-orbital BECs being lower than tail derived BECs. However, it should be noted that this effect was uniform in both strains and sexes. Importantly, the direction of drug effects (i.e. finasteride versus saline) remained the same regardless of whether tail or eye bloods were used to obtain BECs. Thus, our interpretation of any drug effects on BECs was not altered, based on which blood collection method was utilized. Any differences in BECs between tail blood and retro-orbital blood sampling may reflect subtle experimental differences or may be due to the fact that while tail bloods were collected one time, retro-orbital blood samples were collected repeatedly from the same animal.

Although there were interesting findings in the BEC time point data and EtOH clearance parameters, there were no systematic changes following finasteride pretreatment that were consistent with the observed sex difference in acute withdrawal severity. Overall, finasteride-induced alterations in BECs did not contribute to differences in HICs during acute EtOH withdrawal.

Hormone Data

Work from other laboratories also suggests that estradiol and corticosterone may influence convulsions (as described in the Introduction; Edwards et al., 2002; Karst et al., 1999; Reddy; 2004). If finasteride was altering these hormone levels, then the sex difference in acute EtOH withdrawal severity might be a result from a finasteride-induced change in the levels of these hormones.

In general, finasteride pretreatment did not appear to have a major impact on estradiol or corticosterone concentrations. By blocking 5α-reductase, an accumulation of testosterone might occur, which could be converted to estradiol via aromatase (since it could not be reduced to DHT). The fact that aromatase expression in rodents appears to be reduced relative to primates (see Syed and Khosla, 2005) suggests that finasteride might not favor an increase in estradiol levels via the aromatization pathway. Consistent with this idea, we did not observe a persistent increase in estradiol levels in the finasteride-treated groups. Although estradiol concentrations were higher in female than in male DBA/2J mice as one would expect, it was surprising that there was no sex difference in estradiol concentrations between male and female C57BL/6J mice. This may be due to the fact that all animals remained intact and that the female mice were not synchronized with regard to hormonal cycling. Further studies examining estradiol concentrations in animals that are synchronized with regard to cycling are necessary to further characterize these observed effects.

At the 8 hr time point, when peak withdrawal typically occurs, pretreatment with finasteride produced an increase in plasma estradiol levels relative to saline-treated animals in EtOH-treated male and female DBA/2J mice. However, it is unlikely that this finasteride-induced increase in plasma estradiol at 8 hrs produced an opposite effect on HICs in male and female mice (i.e. a decrease in HICs in males and an increase in HICs in females). Although it is possible that male and female mice have different sensitivities to an increase in estradiol levels, other factors (such as 5α-reduced steroids) may be mediating the observed sex difference in acute withdrawal severity.

Consistent with previous findings from our laboratory, treatment with EtOH increased corticosterone concentrations relative to saline treated groups, particularly at the 2 hr time point

(Finn et al., 2004c). This increase at the 2 hr time point appeared to be due to activation of the hypothalamic-pituitary-adrenal (HPA) axis in response to the injection of EtOH, an effect that decreased over time. In agreement with this suggestion, early work in male C57BL/6J and DBA/2J mice found that the EtOH-induced activation of the HPA axis, measured by higher plasma concentrations of adrenocorticotropic hormone and corticosterone, was greater in DBA/2J than in C57BL/6J mice (Roberts et al., 1992). This activation of the HPA axis in response to the administration of EtOH has been previously demonstrated in other laboratories (e.g., Rivier, 1993) and was found to vary in female rats, depending on the estrous cycle phase. At 8 hrs, when peak withdrawal typically occurs, treatment with finasteride had no effect on plasma corticosterone levels, suggesting that changes in corticosterone were not contributing to the sex difference in acute withdrawal severity.

The mechanism by which finasteride differentially altered acute EtOH withdrawal severity in male and female mice is not known. Another potential site of action of finasteride is deoxycorticosterone, which can be converted to 5α-dihydrodeoxycorticosterone via 5α-reductase, or to corticosterone. By blocking 5α-reductase, one might expect to observe an increase in deoxycorticosterone and hence corticosterone levels. Corticosterone can demonstrate proconvulsant and anticonvulsant properties depending upon the animal model employed (see Introduction). Thus, finasteride's differential alteration of this pathway in male and female mice is another potential explanation for the differences in acute withdrawal severity. However, it is unlikely that this is the case in the present studies, since corticosterone concentrations did not change in a manner that could explain the HIC and AUC25 data. That is, the EtOH-induced increase in corticosterone levels at 2 hrs in both strains and sexes was not sustained throughout the time course of withdrawal and was not potentiated by pretreatment with finasteride.

Since finasteride can block the conversion of progesterone, deoxycorticosterone, and testosterone to GABAergic neurosteroids, there may be a complex interaction between finasteride, EtOH, and their subsequent interaction with $GABA_A$ receptors. Specifically, a finasteride-induced decrease in ALLO, 5α-THDOC, or androstanediol levels (Figure 1) would thereby decrease GABAergic inhibition, making EtOH withdrawal seizures more pronounced. Fluctuations in endogenous GABAergic neurosteroid levels may modulate $GABA_A$ receptors, thereby changing inhibitory tone and seizure severity. For example, if finasteride was differentially altering ALLO levels in male and female mice, this could explain the differences in acute EtOH withdrawal severity. Since basal ALLO levels are higher in females than in males, finasteride may be producing a larger, more physiologically relevant decrease in brain inhibition in female mice, thereby increasing brain excitability. Conversely, finasteride's effects on the lower basal brain ALLO levels in the male mice may not produce enough of a change in neuronal inhibition to alter seizure susceptibility. Thus, the sex difference in EtOH withdrawal severity observed in the current study may be due to differences in ALLO levels. Although we were unable to measure brain ALLO levels in the present studies, the dose of finasteride was chosen based on our pilot data and previous work to decrease endogenous ALLO levels by 60-80% (Finn et al., unpublished). Additional studies are necessary to characterize the efficacy of finasteride on brain ALLO levels across strains and sex.

Another possibility is that a finasteride-induced decrease in GABAergic inhibition altered EtOH sensitivity in the male mice, since decreasing endogenous ALLO levels has been shown to decrease sensitivity to certain effects of EtOH in male rodents (e.g., Dazzi et al., 2002; Van Doren et al., 2000). As summarized in Table 1, finasteride pretreatment alters sensitivity to some, but not all, effects of EtOH. For example, finasteride modulates EtOH-induced HICs,

depression-like behavior, anxiety-like behavior and changes in ALLO levels, but does not modulate EtOH-induced place conditioning or motor incoordinating effects. While this may seem curious, it could be that finasteride affects alcohol-related behavioral or physiological responses with a strong GABAergic component. Thus, there may be important sex differences in sensitivity to finasteride's effects and the interaction with EtOH sensitivity. Further studies are necessary to characterize finasteride's effects on additional alcohol-related behaviors in male and female mice.

Future Directions

Although the use of finasteride is an indirect tool to examine how ALLO may contribute to withdrawal severity, the current study did not directly measure changes in ALLO levels. Thus, it would be of great interest to directly measure ALLO levels during the course of withdrawal to determine whether the inhibition of this neurosteroid is in fact modulating finasteride's effect on EtOH withdrawal severity.

Due to finasteride's alteration of other 5α-reduced steroids, it would also be interesting to manipulate and control for hormonal cycling to investigate the contribution of other steroid hormones in finasteride's effect on EtOH withdrawal severity. Specifically, it may be informative to see whether gonadectomy would eliminate finasteride's effect on HICs, and whether steroid replacement would restore the effect observed in intact animals.

Although we presumed in our studies that plasma hormone levels would correspond to brain hormone levels, further studies are needed to determine whether changes in brain ALLO levels, brain estradiol levels, and brain corticosterone levels are mediating the observed sex difference in acute withdrawal severity.

Recent work from our laboratory has shown that basal 5α-reductase type 1 (*Srd5a1*) enzyme activity from cortical and hippocampal microsomes was significantly greater in C57BL/6J mice than in DBA/2J mice, while basal *Srd5a1* activity from liver microsomes was significantly lower in C57BL/6J mice versus DBA/2J mice (Finn and Roselli, unpublished). Chronic EtOH exposure slightly increased cortical *Srd5a1* activity in female DBA/2J mice and differentially altered hippocampal *Srd5a1* activity in C57BL/6J and DBA/2J mice (i.e. decreased activity in DBA/2J mice and did not significantly affect activity in C57BL/6J mice). These findings suggest that there are brain regional differences in the responsivity of the *Srd5a1* enzyme during chronic EtOH exposure. Further studies are needed to elucidate *Srd5a1* activity during acute EtOH administration and withdrawal. Perhaps there are sex differences in *Srd5a1* activity following an acute EtOH injection that correspond to differences in acute EtOH withdrawal severity.

Based on the putative complex interaction between finasteride, EtOH, and their interaction with $GABA_A$ receptors, it may be important to look at changes in GABAergic function. That is, it would be interesting to see whether finasteride alters $GABA_A$ receptor function (e.g., chloride flux) in a manner that is predicted, based on the changes in excitability that we observed. For example, perhaps finasteride is causing a decrease in GABAergic function in female mice, thereby explaining the observed increase in HICs. In male mice, finasteride may increase GABA function or make EtOH less effective at GABA receptors. Thus, the aforementioned studies may be important for elucidating the role of ALLO in alcohol-related behaviors.

It is also important to note that finasteride's decrease in acute EtOH withdrawal severity may have important implications in human men taking finasteride. That is, in men already

taking finasteride for the treatment of benign prostatic hyperplasia and androgenetic alopecia, there appears to be no increased risk with regard to acute alcohol-related effects. Specifically, since pretreatment with finasteride produced a decrease in acute EtOH withdrawal-related seizures in male mice, finasteride does not appear to exacerbate withdrawal symptoms in males. Based on the findings of the current set of experiments, if human men taking finasteride concurrently use alcohol, there does not appear to be an increased risk in the severity of withdrawal-related symptoms. This may be particularly important for a cohort of men who have also been diagnosed with alcoholism. Further studies are necessary to examine the effect of finasteride on withdrawal-related symptoms in humans.

In conclusion, pretreatment with finasteride increased acute EtOH withdrawal severity in female C57BL/6J and DBA/2J mice but decreased acute EtOH withdrawal severity in male mice of both strains. However, finasteride did not alter BECs, estradiol or corticosterone concentrations in manner that appeared to contribute to the HIC results. Collectively, these findings suggest that male and female C57BL/6J and DBA/2J mice may differ in their sensitivity to changes in levels of ALLO or other GABAergic steroid metabolites during acute EtOH withdrawal. Additional studies are necessary to characterize this sex difference.

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