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Review

# Manipulation of GABAergic steroids: Sex differences in the effects on alcohol drinking- and withdrawal-related behaviors

Deborah A. Finn [a,b,*], Ethan H. Beckley [b], Katherine R. Kaufman [b], Matthew M. Ford [b]

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

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ABSTRACT

Alcoholism is a complex disorder that represents an important contributor to health problems worldwide and that is difficult to encompass with a single preclinical model. Additionally, alcohol (ethanol) influences the function of many neurotransmitter systems, with the interaction at γ-aminobutyric acid$_A$ (GABA$_A$) receptors being integral for ethanol's reinforcing and several withdrawal-related effects. Given that some steroid derivatives exert rapid membrane actions as potent positive modulators of GABA$_A$ receptors and exhibit a similar pharmacological profile to that of ethanol, studies in the laboratory manipulated GABAergic steroid levels and determined the impact on ethanol's rewarding- and withdrawal-related effects. Manipulations focused on the progesterone metabolite allopregnanolone (ALLO), since it is the most potent endogenous GABAergic steroid identified. The underlying hypothesis is that fluctuations in GABAergic steroid levels (and the resultant change in GABAergic inhibitory tone) alter sensitivity to ethanol, leading to changes in the positive motivational or withdrawal-related effects of ethanol. This review describes results that emphasize sex differences in the effects of ALLO and the manipulation of its biosynthesis on alcohol reward- versus withdrawal-related behaviors, with females being less sensitive to the modulatory effects of ALLO on ethanol-drinking behaviors but more sensitive to some steroid manipulations on withdrawal-related behaviors. These findings imply the existence of sex differences in the sensitivity of GABA$_A$ receptors to GABAergic steroids within circuits relevant to alcohol reward versus withdrawal. Thus, sex differences in the modulation of GABAergic neurosteroids may be an important consideration in understanding and developing therapeutic interventions in alcoholics.



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## Introduction

The present review describes results that were presented at a recent symposium entitled, "Sex-Specific Therapeutic Strategies Based on Neuroactive Steroids: In Search for Innovative Tools for Neuroprotection". Certainly, the importance of sex and the impact of gonadal steroids on brain function and behavior are being increasingly recognized (e.g., Young and Becker, 2009). Given the sex differences in human disease, it is desirable to consider sex differences in preclinical models. Sex differences in ethanol intake are well-documented in rodents, with intake in females higher than that in males (e.g., Belknap et al., 1993; Chester et al., 2008; Finn et al., 2004b; Lancaster et al., 1996; Yoneyama et al., 2008). Sex-dependent sensitivity to other drugs also occurs, with females generally being more sensitive to the rewarding effects of drug than males (see review by Carroll et al. (2004)). For example, female rats acquire intravenous self-administration of cocaine, methamphetamine and nicotine faster than males. Female rats also self-administer more cocaine with longer duration of "binges" and greater loss of circadian control over drug intake in an escalation model, and exhibit greater extinction

* Corresponding author. VAMC Research (R&D-49), 3710 SW U.S. Veterans Hospital Road, Portland, OR 97239, USA. Fax: +1 503 273 5351.
*E-mail address:* finnd@ohsu.edu (D.A. Finn).

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responding on the drug associated lever after drug removal and greater reinstatement after drug priming than males (see Carroll et al. (2004) and references therein). Thus, research that incorporates male and female subjects can identify sex-specific mechanisms as well as general mechanisms underlying aspects of addiction.

Steroid hormones exert genomic effects via binding to their receptors and altering the transcription of genes, but rapid membrane effects of steroids provide another level of diversity in the mechanisms by which steroids can influence brain function and behavior (e.g., Brann et al., 1995; Lösel and Wehling, 2003; McEwen, 1991; Rupprecht and Holsboer, 1999). For instance, a vast variety of brain functions are affected by sex steroids (e.g., testosterone, Hajszan et al., 2008; estrogen, Tokuyama et al., 2008; progesterone, Quesada and Micevych, 2008) and adrenal steroids (e.g., corticosterone, Uchoa et al., 2009; aldosterone, Shen et al., 2009). Genomic or rapid membrane effects have been observed via steroid interaction at androgen receptors (e.g., Claessens et al., 2008), glucocorticoid and mineralocorticoid receptors (e.g., Kawata et al., 2008), liver X receptors (e.g., Matsumoto et al., 2009), estrogen receptors (e.g., Kelly and Rønnekleiv, 2008) progesterone receptors (e.g., Brinton et al., 2008) and vitamin D receptors (e.g., Garcion et al., 2002). Besides membrane effects of steroid hormones at their "classic" receptor, some steroids and their derivatives have rapid membrane actions via an interaction with ligand-gated ion channels (e.g., Belelli and Lambert, 2005; Finn et al., 2004a; Paul and Purdy, 1992; Rupprecht and Holsboer, 1999). This evidence for rapid non-genomic effects of steroids gave rise to the term "neuroactive steroids."

The term "neurosteroid" was introduced by Baulieu (1981) to designate a steroid hormone derivative found in brain at concentrations that were independent of its plasma concentration, and the story of their discovery and function has been reviewed recently (Baulieu et al., 2001). Given that the enzymes identified in classic steroidogenic tissues are found in the brain (Mellon and Vaudry, 2001), brain "neuroactive steroid" levels most likely reflect a combination of neuroactive compounds produced *de novo* as well as peripherally-derived steroids that are metabolized to neuroactive compounds in the brain. Thus, it has been suggested that the term "neurosteroid" be broadened to include both sources of "neuroactive steroids" (Mellon and Griffin, 2002). Consistent with this idea, we will use the term neurosteroids to refer to both sources of neuroactive steroids in this review and will focus on the effects of neurosteroids that potentiate the action of γ-aminobutyric acid (GABA) at $GABA_A$ receptors (Fig. 1).

The progesterone derivative allopregnanolone (ALLO) is the most potent endogenous modulator of $GABA_A$ receptors identified (reviewed in Belelli and Lambert (2005); Belelli et al. (1990); Finn et al. (2004a); Purdy et al. (1990); Rupprecht and Holsboer (1999); Veleiro and Burton (2009)). Endogenous ALLO levels fluctuate within a range of concentrations previously shown to potentiate the action of GABA at $GABA_A$ receptors. Exposure to various stressors (including injection or consumption of alcohol) increased brain ALLO levels to the equivalent of 10–30 nM in male rats (e.g., Barbaccia et al., 2001; Finn et al., 2004b; Paul and Purdy, 1992; VanDoren et al., 2000). Similar concentrations are achieved in female rats at estrus, and increase to approximately 100 nM during pregnancy (Paul and Purdy, 1992). These findings suggest that fluctuations in endogenous ALLO levels could modify the functioning of central $GABA_A$ receptors *in vivo* — an idea that is supported by the finding that increasing endogenous ALLO levels in the dentate gyrus revealed a physiologically relevant neurosteroid tone that was sufficient to modulate $GABA_A$ receptors (Belelli and Herd, 2003).

Alcohol (ethanol) has many pharmacodynamic properties (Spanagel, 2009; Vengeliene et al., 2008;), but its ability to potentiate the action of GABA at $GABA_A$ receptors appears to be integral for its reinforcing and discriminative stimulus effects (Chester and Cunningham, 2002; Grant, 1999) as well as its effects on withdrawal-related hyperexcitability (Devaud et al., 2006; Grobin et al., 1998). Given that ethanol injection and consumption can increase cortical ALLO to pharmacologically active levels in male rodents (Barbaccia et al., 1999; Finn et al., 2004b; VanDoren et al., 2000), the ability of ethanol to increase endogenous ALLO levels may potentiate or prolong ethanol's effect via dual (i.e., ethanol + ALLO) actions at $GABA_A$ receptors. Likewise, a reduction in ethanol's steroidogenic effect (e.g., use of finasteride (FIN), a 5α-reductase inhibitor that blocks the metabolism of progesterone to ALLO) may reduce the action of ethanol at $GABA_A$ receptors. Consistent with this idea, ethanol was found to have a direct and indirect effect on $GABA_A$ receptor function, with the indirect effect being due to steroidogenesis in that it was blocked by FIN (Sanna et al., 2004). Thus, the underlying hypothesis of the studies summarized here is that fluctuations in neurosteroid levels (and the resultant change in GABAergic inhibitory tone) alter sensitivity to ethanol, due

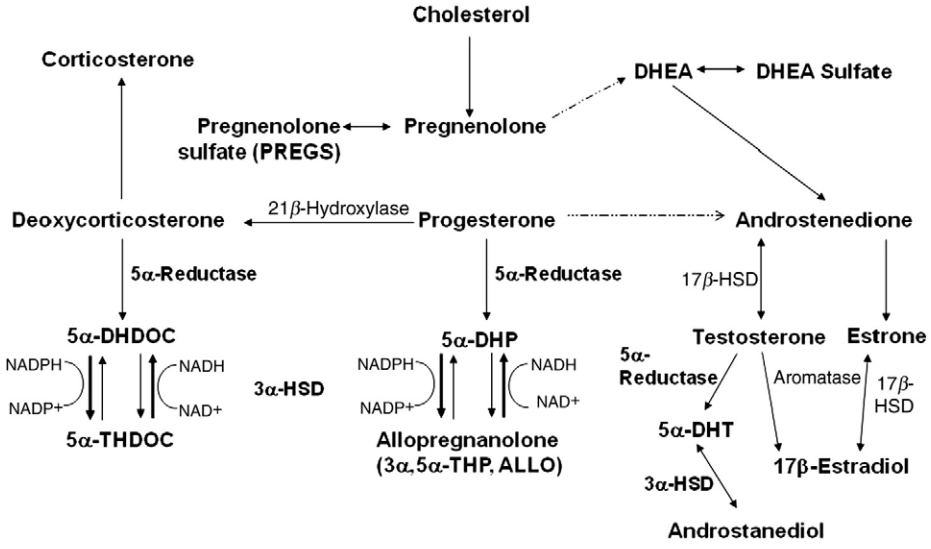


**Fig. 1.** The biosynthesis of select steroids with genomic and non-genomic effects. Depicted is the biosynthetic pathway for ALLO, 5α-THDOC, and androstanediol, which are potent positive modulators of $GABA_A$ receptors and are formed from the two step reduction of the parent steroids, progesterone, deoxycorticosterone, and testosterone, respectively. The rate-limiting enzyme in GABAergic steroid biosynthesis is 5α-reductase. Also depicted are the sulfated derivatives of pregnenolone and DHEA, which are negative modulators of $GABA_A$ receptors, and corticosterone, which has been shown to have excitatory effects. The broken lines indicate that 17-OH pregnenolone and 17-OH progesterone are omitted from the figure in the formation of DHEA and androstenedione, respectively. *Abbreviations*: DHEA, dehydroepiandrosterone; DHDOC, dihydrodeoxycorticosterone; THDOC, tetrahydrodeoxycorticosterone; DHP, dihydroprogesterone; DHT, dihydrotestosterone; 3α-HSD, 3α-hydroxysteroid dehydrogenase. (Adapted from Finn et al., 2006a).