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Research Report

# The neurosteroid environment in the hippocampus exerts bi-directional effects on seizure susceptibility in mice

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ABSTRACT

The progesterone derivative allopregnanolone (ALLO) rapidly potentiates γ-aminobutyric $acid_A$ ($GABA_A$) receptor mediated inhibition. The present studies determined whether specific manipulation of neurosteroid levels in the hippocampus would alter seizure susceptibility in an animal model genetically susceptible to severe ethanol (EtOH) withdrawal, Withdrawal Seizure-Prone (WSP) mice. Male WSP mice were surgically implanted with bilateral guide cannulae aimed at the CA1 region of the hippocampus one week prior to measuring seizure susceptibility to the convulsant pentylenetetrazol (PTZ), given via timed tail vein infusion. Bilateral intra-hippocampal infusion of ALLO (0.1 μg/side) was anticonvulsant, increasing the threshold dose of PTZ for onset to myoclonic twitch and face and forelimb clonus by 2- to 3-fold. In contrast, infusion of the 5α-reductase inhibitor finasteride (FIN; 2 μg/side), which decreases endogenous ALLO levels, exhibited a proconvulsant effect. During withdrawal from chronic EtOH exposure, WSP mice were tolerant to the anticonvulsant effect of intra-hippocampal ALLO infusion, consistent with published results following systemic injection. Finally, administration of intra-hippocampal FIN given only during the development of physical dependence significantly increased EtOH withdrawal severity, measured by handling-induced convulsions. These findings are the first demonstration that bi-directional manipulation of hippocampal ALLO levels produces opposite behavioral consequences that are consistent with alterations in GABAergic inhibitory tone in drug-naive mice. Importantly, EtOH withdrawal rendered WSP mice less sensitive to ALLO's anticonvulsant effect and more sensitive to FIN's proconvulsant effect, suggesting an alteration in the sensitivity of hippocampal $GABA_A$ receptors in response to fluctuations in GABAergic neurosteroids during ethanol withdrawal.



## 1. Introduction

One well-documented nongenomic effect of steroid hormones and their derivatives is the rapid potentiation of γ-aminobutyric $acid_A$ ($GABA_A$) receptor function. The progesterone derivative allopregnanolone (ALLO) is the most potent endogenous positive modulator of $GABA_A$ receptors yet identified and fluctuations in endogenous levels *in vivo* occur within the

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range of concentrations that potentiate GABAergic inhibition *in vitro* (Belelli and Lambert, 2005). Clinical and preclinical research shows that ALLO fluctuations can contribute to diverse disorders such as premenstrual and postpartum dysphoric disorder (Follesa et al., 2004), catamenial epilepsy (Herzog and Frye, 2003), depression (Uzunova et al., 1998) and ethanol (EtOH) withdrawal (Romeo et al., 1996). The inverse relationship between endogenous ALLO levels and anxiety, depression, and seizure susceptibility are consistent with ALLO's pharmacological profile, since exogenous administration produces anxiolytic, anticonvulsant and antidepressant effects (Gasior et al., 1999; Hirani et al., 2002). This inverse relationship was reported in cohorts of male subjects with depression (Uzunova et al., 1998) or in the early phase of alcoholic withdrawal (Romeo et al., 1996). Treatment with drugs which restored ALLO levels to those found in control subjects significantly reduced the symptoms of anxiety and depression in both the depressed and alcoholic subjects (Romeo et al., 2000; Ströhle et al., 1999). Thus, endogenous ALLO levels may be important in maintaining normal GABAergic brain function.

Recent studies have demonstrated that withdrawal from chronic EtOH exposure significantly decreased plasma ALLO levels in ethanol withdrawal seizure-prone mouse genotypes (Finn et al., 2004a) and hippocampal ALLO levels in rats (Cagetti et al., 2004). These decreases in endogenous ALLO levels corresponded to increased anxiety and seizure susceptibility and decreased expression of the biosynthetic enzyme 5α-reductase (Cagetti et al., 2003; Cagetti et al., 2004; Finn et al., 2004a), suggesting that there might be an inverse relationship between GABAergic neurosteroid levels and behavioral changes in excitability during EtOH withdrawal. Given this evidence, it was hypothesized that a sustained reduction in ALLO to levels below the physiologically relevant range during EtOH withdrawal would decrease GABAergic inhibition *in vivo* and contribute to the manifestation of a more severe withdrawal syndrome.

Results in one genetic animal model of EtOH withdrawal severity, namely the Withdrawal Seizure-Prone (WSP) and -Resistant (WSR) selected lines, are consistent with the idea that genetic differences in EtOH withdrawal severity are due, in part, to differences in the modulatory effects of GABAergic neurosteroids. The WSP and WSR selected lines were bred in duplicate for their severity of (WSP) or resistance to (WSR) handling-induced convulsions (HICs) following chronic EtOH exposure (Crabbe et al., 1985). EtOH withdrawal convulsions are at least 10-fold higher in the WSP than in the WSR line following an equivalent exposure to 72 h EtOH vapor (Crabbe et al., 1985). Notably, there is a persistent decrease in endogenous ALLO levels as well as decreased sensitivity to ALLO during EtOH withdrawal in WSP, but not in WSR mice (Beckley et al., 2008; Finn et al., 2006; Finn et al., 2004a). Tolerance to the anticonvulsant effect of ALLO during EtOH withdrawal in WSP mice corresponded to a decrease in functional sensitivity of $GABA_A$ receptors to ALLO. Thus, the WSP line is an ideal animal model to examine critical brain regions important for ALLO's modulatory effects on brain function during EtOH-naive and EtOH withdrawal conditions.

Investigations into the neuroanatomical substrates influenced by withdrawal from chronic EtOH exposure determined that *c-fos* expression was significantly increased in the hippocampus during withdrawal from chronic EtOH exposure (Dave et al., 1990; Morgan et al., 1992), and that co-administration of the convulsant pentylenetetrazol (PTZ) during EtOH withdrawal produced a further increase in *c-fos* expression (Putzke et al., 1996). Relevant to GABAergic neurosteroids, several studies documented that the brain sites where ALLO shows the highest potency for modulation of $GABA_A$ receptor function across brain regions are: hippocampus>cortex=amygdala (Finn and Gee, 1993; Gee et al., 1988). However, there is heterogeneity within the hippocampus since electrophysiological studies determined that $GABA_A$ receptors in the CA1 region of the hippocampus were much more sensitive to the positive modulatory effect of neurosteroids than receptors in the dentate gyrus (Belelli and Herd, 2003; Harney et al., 2003). Additionally, chronic EtOH withdrawal produced significant decreases in the expression and activity of hippocampal 5α-reductase in rats (Cagetti et al., 2004) and in WSP mice (Finn et al., 2004a). Thus, the hippocampus is a brain region relevant for seizure susceptibility during EtOH withdrawal, $GABA_A$ receptor sensitivity to ALLO, and for changes in the expression and activity of the 5α-reductase during EtOH withdrawal.

The goal of the present set of experiments was to examine the physiological significance of manipulation of GABAergic neurosteroid levels in the hippocampus of WSP mice that were either EtOH naive or undergoing EtOH withdrawal. Separate studies determined whether: 1) manipulating ALLO levels in the hippocampus would produce bi-directional effects on seizure susceptibility, measured by the change in sensitivity to PTZ-induced convulsions (i.e., an increase in hippocampal ALLO with ALLO infusions would be anticonvulsant and conversely, a decrease in hippocampal ALLO with finasteride (FIN) infusion would be proconvulsant), 2) withdrawal from chronic EtOH exposure would produce tolerance to the anticonvulsant effect of hippocampal ALLO infusion similar to results following systemic ALLO administration, measured by the change in sensitivity to PTZ-induced convulsions (Finn et al., 2006), and 3) intra-hippocampal infusions of FIN during the development of physical dependence would increase chronic EtOH withdrawal severity, measured by HICs.

## 2. Results

### 2.1. *Intra-hippocampal infusion revealed an anticonvulsant effect of ALLO and a proconvulsant effect of FIN*

Since the hippocampus is an integral component of the limbic seizure circuitry (Gale, 1988), the overall hypothesis of these studies was that enhancing the positive modulation of hippocampal $GABA_A$ receptors via ALLO infusion would be anticonvulsant and that reducing the positive modulation of hippocampal $GABA_A$ receptors via FIN injection and infusion would be proconvulsant. The CA1 region of the hippocampus was targeted for drug delivery, based on the enhanced sensitivity of $GABA_A$ receptors in this region versus the dentate gyrus (Belelli and Herd, 2003; Harney et al., 2003). Seizure