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Research Report

GABAergic miniature postsynaptic currents in septal neurons show differential allosteric sensitivity after binge-like ethanol exposure

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ABSTRACT

Binge-like ethanol treatment of septal neurons blunts $GABA_AR$-mediated miniature postsynaptic currents (mPSCs), suggesting it arrests synaptic development. Ethanol may disrupt postsynaptic maturation by blunting feedback signaling through immature $GABA_ARs$. Here, the impact of ethanol on the sensitivity of mPSCs to zolpidem, zinc and 3α-hydroxy-5α-pregnan-20-one (3α-OH-DHP) was tested. The decay phase of mPSCs showed concentration-dependent potentiation by zolpidem (0.03–100 μM), which was substantially blunted after ethanol exposure. Since zolpidem potentiation exhibited a substantial age-dependent increase in untreated neurons, this finding supported the idea that ethanol arrests synaptic development. $GABA_AR$ α1 subunit protein also increased with age in untreated neurons, paralleling enhanced sensitivity to zolpidem. Surprisingly, α1 levels were not reduced by binge ethanol even though mPSCs were relatively zolpidem-insensitive. Zinc (3–30 μM) decreased mPSC parameters in a concentration- and age-related manner with older untreated cells showing less inhibition. However, there was no increase in mPSC zinc sensitivity after binge ethanol as would be expected if a general arrest of synaptic maturation had occurred. 3α-OH-DHP (3–1000 nM) induced concentration-dependent potentiation of mPSC decay. Although potentiation was age-independent, binge ethanol treatment exaggerated sensitivity to this neurosteroid. Finally, chronic picrotoxin pretreatment (100 μM) intended to mimic $GABA_AR$ inhibition from ethanol pretreatment did not significantly change mPSC modulation by zolpidem, zinc or 3α-OH-DHP. These results suggest that binge ethanol treatment selectively arrests a subset of processes important for maturation of postsynaptic $GABA_A$ Rs. However, it is unlikely that ethanol causes a broad arrest of postsynaptic development through a direct inhibition of $GABA_AR$ signaling.

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1. Introduction

Ethanol is a teratogen that can induce the fetal alcohol syndrome (FAS), diagnosed by pre- and postnatal growth retardation, facial dysmorphology and central nervous system (CNS) deficits (Sampson et al., 1997; Warren and Foudin, 2001). CNS defects associated with FAS range from structural abnormalities to cognitive dysfunction that impairs learning, memory and judgement. When gross malformations are not evident, these problems are categorized as alcohol-related neurodevelopmental disorder (ARND), fetal alcohol effects (FAE) or fetal alcohol spectrum disorder (FASD) (Sampson et al., 1997; Bookstein et al., 2002; Hoyme et al., 2005). Studies in animal models of human FAS, ARND or FAE indicate many processes in the developing brain are at risk for disruption by ethanol, including mechanisms guiding neuronal and glial precursor proliferation, differentiation and migration, as well as neuronal axon or dendrite extension, synaptogenesis and signal transduction (see for overview DuBois et al., 2004; Hsiao et al., 2004). In this regard, one form of neuronal signaling that is increasingly considered an important target for ethanol and which may play a role in alcohol's teratogenic actions is neuromodulation mediated via γ-aminobutyric acid (GABA) through type A GABA receptors ($GABA_A$Rs; see for an overview Costa et al., 2000; Olney et al., 2002; Hsiao et al., 2004). In adult brain, these ligand-gated ion channels mediate prominent synaptic inhibition, but in fetal or neonatal brain, they also provide important excitatory developmental trophic drive and participate in regulation of neurogenesis, differentiation, migration and synaptogenesis (Represa and BenAri, 2005; Vicini, 2005). Well-known CNS depressants like ethanol, benzodiazepines and barbiturates which evoke neuroadaptative tolerance and dependence-related changes in adult brain from repeated interaction with $GABA_A$Rs also appear capable of triggering brain injury by distorting the trophic role of immature $GABA_A$Rs in developing brain (Costa et al., 2000; Olney et al., 2002; DuBois et al., 2004; Hsiao et al., 2004 for an overview).

Recently, we have identified a developmental distortion of $GABA_A$R function in septal neurons following ethanol intoxication in rat pups on postnatal days 4–9 (Hsiao et al., 1998, 2001, 2004), using a well-established model of binge-like drinking-related fetal brain injury during the human 3rd trimester (West et al., 1994). Rats receiving this type of ethanol exposure exhibit spatial learning and memory deficits in Morris water maze (Goodlett and Johnson, 1997) that parallel cognitive impairments in a virtual water maze-like task in children diagnosed with FAS (Hamilton et al., 2003). Because local injection of drugs that distort $GABA_A$R activity within the medial septal area of normal rats can reversibly impair Morris water maze spatial learning and memory performance (Brioni et al., 1990), it seems likely that ethanol-induced $GABA_A$R dysfunction in septal neurons could contribute to cognitive deficits associated with FAS, ARND and FAE. In hippocampal neurons in vitro, drugs that block GABA tone delay the developmental switch of $GABA_A$Rs from excitatory to inhibitory transducers (Ganguly et al., 2001). Since ethanol can inhibit $GABA_A$Rs on immature septal and cerebellar neurons (Hsiao et al., 1998, 1999), intoxication may diminish the local GABAergic tone septal neurons require for synaptic maturation (Hsiao et al., 1998, 2001, 2004). In this regard, we recently examined the impact of binge-like ethanol exposure on development of postsynaptic $GABA_A$Rs in newly forming synapses (DuBois et al., 2004). After 6 days in vitro (DIV 6), embryonic day 20 septal neurons begin to show robust miniature postsynaptic currents (mPSCs) due to asynchronous release of single vesicles of GABA. These events rapidly increase in frequency and show essentially complete bicuculline sensitivity, consistent with synapse formation involving postsynaptic $GABA_A$Rs. Analysis of GABAergic mPSC decay kinetics, which can provide a useful index of receptor maturation (Hollrigel and Soltesz, 1997; Dunning et al., 1999; Verderio et al., 1999; Cohen et al., 2000; Vicini et al., 2001), suggested that binge ethanol exposure arrested development of synaptic $GABA_A$Rs (DuBois et al., 2004). We proposed that impaired development of postsynaptic signaling results from a direct inhibition by ethanol of endogenous $GABA_A$R-mediated tone at newly formed synapses that broadly halts mechanisms driving synaptic maturation.

In the present study, we further characterized the impact of ethanol treatment on the development of postsynaptic $GABA_A$R by determining changes in sensitivity of GABA mPSCs to several allosteric modulators with distinct actions on $GABA_A$R function. Sensitivity to the positive modulators zolpidem and 3α-OH-DHP was examined since these agents potentiate $GABA_A$Rs through actions on distinct allosteric sites and were differentially influenced by developmental age (Dunning et al., 1999 and unpublished observations). Inhibitory actions of Zn^{2+} were examined since septal neurons are known to become less sensitive to the negative modulatory actions of this metal during early stages of development (see for an overview Hsiao et al., 1998, 2001). Results suggest that binge ethanol treatment does not simply arrest postsynaptic maturation but more likely acts on several distinct mechanisms that can regulate signal transduction by postsynaptic $GABA_A$Rs.

2. Results

2.1. *Ethanol levels in cultures during binge-like exposure*

Media samples taken immediately after the 3-h ethanol vapor treatment on DIV 6–11 contained 310 ± 8 mg/dl ethanol (n = 113), and decreased to 140 ± 4 mg/dl (n = 103) by 21 h after treatment. Across the 6 daily treatments (Fig. 1), peak ethanol concentrations ranged from 251 ± 9 to 364 ± 22 mg/dl and troughs 21 h later from 112 ± 5 to 162 ± 9 mg/dl. These results were similar to those in earlier studies in DIV 6–11 cultured septal neurons where peak ethanol levels averaged ~325 mg/dl media and declined to ~125 mg/dl by 21 h (DuBois et al., 2004; Hsiao et al., 2004). We have shown previously that this repeated ethanol or control exposure paradigm does not decrease the survival of septal neurons relative to that of untreated cultured cells through DIV 15 (Hsiao et al., 2004). It is important to note that peak ethanol levels in culture media (Fig. 1) are comparable to the daily blood ethanol peak that rat pups experience 90 min after treatment over postnatal days