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Agmatine reduces balance deficits in a rat model of third trimester binge-like ethanol exposure

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Abstract

This study examined the effects of binge-like ethanol (ETOH) exposure in neonatal rats on a cerebellar-mediated balance task, and the ability of agmatine, an *n*-methyl-*d*-aspartate receptor (NMDAR) modulator, to reverse such effects. Five neonatal treatments groups were used, including ETOH (6.0 g/kg/day), AG (20 mg/kg), ETOH plus AG (6.0 g/kg/day and 20 mg/kg), a maltose control, and a non-treated control. Ethanol was administered via oral intubation twice daily for eight days, (AG was administered with the last ETOH intubation only). Two exposure periods were used; PND 1-8 or PND 8-15. On PND 31-33, balance performance on a single dowel was tested. Treatment with AG during withdrawal in ETOH exposed animals improved performance relative to ETOH alone among the PND 1-8 exposure period. ETOH exposure during the 2nd postnatal week did not impair balance. These findings provide further support that exposure to ETOH during critical developmental periods can impair performance on a cerebellar-dependent balance task. Of perhaps greater significance, co-administration of agmatine reduced these deficits suggesting that NMDA modulation via polyamine blockade may provide a novel approach to attenuating damage associated with binge-like ETOH consumption.

Keywords: Agmatine; Alcohol; BAC; Balance; Glutamate; Ethanol; Ethanol withdrawal; Excitotoxicity; Fetal alcohol syndrome; FASD; NMDA; NR2B; Polyamines

1. Introduction

Ethanol abuse during pregnancy can cause permanent brain damage, resulting in behavioral, social, and cognitive dysfunctions (Hannigan and Armant, 2000). Such impairments can range from the more extreme Fetal Alcohol Syndrome (FAS) to more subtle fetal alcohol effects. All of these fit the umbrella term Fetal Alcohol Spectrum Disorder (FASD). The incidence of FASD has been reported to be approximately 9.1/1000 live births (Sampson et al., 1997) with an estimated annual cost of 3.6 billion dollars (Lupton et al., 2004).

Among the reports of FASD-associated impairments, deficiencies in motor skills are common, including delayed motor development, poor eye-hand coordination and fine motor dysfunction. Additionally, problems with balance in children exposed to ethanol prenatally have been noted (Kyllerman et al., 1985; Streissguth et al., 1980). Autopsy, MRI, and PET studies have associated these deficits with alterations in cerebellar volume and function (Riley et al., 2004; Riley and McGee, 2005; Roebuck et al., 1998).

Animal models have served as useful tools in the investigation of the effects of ethanol on the developing brain (Driscoll et al., 1990; Green, 2004; Tabakoff and Hoffman, 2000; Thomas and Riley, 1998; West et al., 1990; West and Goodlett 1990). In rat cerebellum, neonatal ethanol exposure produces a significant, dose-dependent loss of Purkinje cells. This neonatal exposure model is used to study a period of CNS development that overlaps the human 3rd trimester "brain growth spurt" (Dobbing and Sands, 1979). Reports of deficits in balance and other cerebellar type tasks resulting from neonatal ethanol exposure are common (Goodlett et al., 1991; Klintsova et al., 1998; Klintsova et al., 2000; Thomas et al., 1996). A particularly sensitive developmental period appears to span postnatal days (PND) 4-6 (Goodlett et al., 1998; Thomas et al., 1998). Behavioral tests of cerebellar function as well as stereological counts of cerebellar Purkinje cells have demonstrated that ethanol exposure on PND 4-5 results in more severe deficits than PND 8-9 exposure (Thomas et al.,

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1998), suggesting that the first neonatal week is a particularly sensitive period for ethanol's effects on the developing cerebellum.

A number of mechanisms have been proposed to explain how prenatal ethanol exposure affects the developing CNS (Goodlett et al., 2005; Riley et al., 2001). One mechanism that has received considerable attention and has shown potential as a clinical target for intervention involves glutamate receptor hyperactivity during withdrawal, specifically the *N*-methyl-D-aspartate receptor (NMDAR). Acute ethanol exposure inhibits NMDAR activity, and following chronic exposure a variety of compensatory responses can occur resulting in receptor function that may be up-regulated (Chandler et al., 1999; Hu and Ticku, 1995; Kalluri et al., 1998). During withdrawal, this up-regulation has been shown to result in glutamatergic hyperexcitability and cell death (Gibson et al., 2003; Hoffman et al., 1995; Iorio et al., 1993).

Thomas et al. (1997, 2002) have shown that administration of an NMDAR antagonist, MK-801, to neonatal pups during ethanol withdrawal reduced some of the deficits associated with prenatal ethanol exposure. The timing of MK-801 treatment, however, appeared critical (Thomas et al., 2001). If MK-801 was administered in the presence of ethanol, behavioral deficits were exacerbated. In contrast, when MK-801 was administered during ethanol withdrawal, behavioral deficits were reduced. NMDAR antagonists such as MK-801 have numerous limitations as potential treatments. MK-801 works via channel blockade and demonstrates little receptor subtype specificity. Thus, its wide-ranging actions can produce toxicity, may disrupt learning and memory, and can have abuse/psychotomimetic potential (Grant et al., 1991; Klein et al., 1999). Although the side effects of MK-801 preclude its use clinically, success in animal models of early ethanol exposure has generated interest in alternate NMDAR antagonists that may be more viable. At least three types of antagonists seem particularly promising; low-affinity, non-competitive NMDAR channel blockers such as memantine (Volbracht et al., 2006), which is currently used clinically for treating Alzheimer's disease, NR2B subunit antagonists such as ifenprodil or eliprodil (Nikam and Meltzer, 2002; Thomas et al., 2004), and agents that modulate rather than block NMDAR activity such as agmatine, which can act at the polyamine binding site, resulting in allosteric modulation of the receptor.

Polyamines play a variety of roles in CNS development (Slotkin and Bartolome, 1986) and can enhance NMDAR activity (Williams et al., 1991; Williams, 1994). Increased polyamine expression has also been reported in hippocampus, striatum, cortex, and cerebellum during periods of ethanol withdrawal (Davidson and Wilce 1998; Gibson et al., 2003). The concentration of polyamines is positively correlated with the severity of withdrawal-induced tremor and seizure in ethanol-dependant animals (Davidson and Wilce, 1998). Additionally, they have been shown to potentiate ethanol withdrawal-induced cell death *in vitro* (Prendergast et al., 2000; Gibson et al., 2003), and are implicated in the pathogenesis of FAS (Littleton et al., 2001; Sessa et al., 1987; Sessa and Perin, 1997). Taken together, these data suggest that inhibiting polyamine activity during ethanol withdrawal could reduce the severity of withdrawal-induced CNS damage (Littleton et al., 2001; Shibley et al., 1995), an effect that has been observed *in vitro* (Gibson et al., 2003), but not *in vivo*.

Agmatine, a polyamine precursor, is known to inhibit the NMDAR via binding at the polyamine site (Gibson et al., 2002). Exogenous administration of agmatine attenuates glutamate-induced neurotoxicity in cell cultures of rat cerebellum (Olmos et al., 1999), hippocampus (Wang et al., 2006), and cortex (Zhu et al., 2003). Additionally, agmatine reduces infarct and loss of cerebellar neurons following *in vivo* focal or global ischemia (Gilad et al., 1996; Kim et al., 2004) and brain weight loss following ischemia in neonates (Feng et al., 2002). Behaviorally, agmatine dose-dependently attenuates behaviors associated with ethanol withdrawal, including stereotypy, tremor, and wet-dog shakes, without affecting motor coordination in non-dependent animals (Uzbay et al., 2000).

In the current study, a third trimester model of chronic ethanol exposure was used to study the potential neuroprotective effects of agmatine on a cerebellar-mediated balance task. Additionally, two exposure periods (PND 1-8 and PND 8-15) were used to study temporal variables that could interact with ethanol and/or agmatine.

2. Materials and methods

2.1. Subjects

Offspring were Sprague-Dawley rats born at the University of Kentucky in the breeding colony maintained in the Psychology Department. Parent animals were obtained from Harlan Labs (Indianapolis, IN). Animals were mated nightly, and the presence of seminal plugs the following morning indicated copulation had occurred. Pregnant females were individually housed in plastic cages in a temperature controlled nursery (70°±2 F) on a 12 h light-dark cycle, with food and water provided *ad libitum*. On the day following birth (PND 1) litters were culled to 10 animals, maintaining a 1:1 ratio of males to females whenever possible.

2.2. Neonatal drug administration

Litters were randomly divided into five treatment conditions. These included 6 g/kg/day ethanol (ETOH), 20 mg/kg agmatine (AG), 6 g/kg/day ethanol plus 20 mg/kg agmatine (ETOH/AG), a maltose isocaloric control (MALT) and a non-treated control (NTC). No more than one male and one female were assigned to any treatment condition to avoid potential litter effects (Abbey and Howard, 1973). Drugs were administered via gastric intubation (.0278 ml/g bw) in a solution developed to nutritionally mimic rat milk (West et al., 1984). Animals not receiving ethanol (AG and MALT groups) instead received maltose, making the solutions for all intubated groups isocaloric. Agmatine was administered concurrently with the final ethanol exposure only, in order to have AG on board during the final withdrawal from ethanol.

Intubations were administered twice daily for eight days, at 1000 and 1400 h. Each intubation consisted of a 3 g/kg dose of