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Repeated ethanol treatment in adolescent rats alters cortical NMDA receptor

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Abstract

Earlier we have reported that repeated ethanol treatment during adolescence causes long-lasting impairments in spatial learning and memory. The present study was undertaken to determine the cellular mechanisms underlying the persistent ethanol-induced cognitive dysfunction in adolescent male rats. Since in adult animals ethanol is known to affect the *N*-methyl-D-aspartate (NMDA) receptor–gated ion channel, the hypothesis tested here was that adolescent ethanol exposure modulates NMDA receptor (NR) regulation in the brain. Adolescent male rats were injected daily with ethanol (2 g/kg intraperitoneally) for 5 consecutive days. Control rats received isovolumetric saline for the same number of days. Groups of control and experimental rats were sacrificed 7 days after the last ethanol/saline administration, and NR activity was measured in specific brain regions (frontal cortex, hippocampus) using the [^{3}H]MK-801 binding assay. In addition, some rats were sacrificed and their brains were used to investigate changes in NR pharmacology by measuring specific NR2 subunits immunohistochemically. Compared to saline-treated controls, ethanol-treated rats showed significant increases in [^{3}H]MK-801 maximal binding in the frontal cortex. This was associated with increased cortical NR2B subunit protein. [^{3}H]MK-801 binding in the hippocampus was minimally affected. These results indicate that ethanol exposure during the adolescent period produces brain region–specific alterations in NR activity. These changes are different from those reported in literature for ethanol administration during the perinatal period or adulthood. Together, these data suggest that adolescence represents a unique stage in brain development in its long-term sensitivity to ethanol.

Keywords: Juvenile; Alcoholism; Radioligand binding; Glutamatergic system; Brain maturation

1. Introduction

Ethanol continues to be a major substance of abuse among American adolescents. It produces cognitive dysfunction in both humans (Parsons, 1977) and rodents (Swartzwelder et al., 1995a, 1995b). At moderate to high doses, ethanol slows the speed of information processing, and impairs attention and memory recall (Maylor et al., 1988). In young adults (21–24 years), ethanol disrupts memory acquisition on semantic and figural memory tasks (Acheson et al., 1998), and in animals, ethanol has been shown to impair acquisition of spatial memory (Anisman, 1972; Devenport et al., 1983; Goodlett et al., 1987; Markwiese et al., 1998; Matthews et al., 1995; Melia et al., 1996; Sircar & Sircar, 2005).

Ethanol sensitivity varies with age. Ethanol metabolism rate is slower in young rats than in adult rats (Silveri & Spear, 2000). Adolescent rats have greater propensity to develop acute (Silveri & Spear, 1998) and chronic (Swartzwelder et al., 1998) tolerance to ethanol than adults. Ethanol-induced disruptions in long-term potentiation (LTP) are more robust in hippocampal slices from periadolescent rats than in slices from adult hippocampus (Piyapali et al., 1999; Swartzwelder et al., 1995a, 1995b). Behavioral effects of ethanol are also age specific. Adolescent rats show greater impairments in spatial memory than adult rats (Markwiese et al., 1998; Sircar & Sircar, 2005). We have earlier reported that repeated ethanol exposure in adolescent and adult male rats produces severe impairments in the acquisition of spatial memory, and unlike in adult rats, performance deficits in adolescent male rats persist for

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several weeks after the ethanol treatment period is over, indicating that the negative influence of adolescent ethanol exposure on spatial memory is relatively long lasting in adolescent rats (Sircar & Sircar, 2005).

Ethanol affects brain functions by interacting with several neurotransmitter systems including the glutamatergic system (Alele & Devaud, 2005; Heinz et al., 2005; Hoffman & Tabakoff, 1996; Larsson et al., 2005; Mehta & Ticku, 2005). Ethanol has been shown to inhibit glutamatergic neurotransmission (Hoffman et al., 1989; Lovinger et al., 1989; Spuhler-Phillips et al., 1995). Glutamatergic neurotransmission is known to play an important role in developmental plasticity, learning and memory, and neurotoxicity. The *N*-methyl-D-aspartate (NMDA) type of glutamate receptor is a ligand-gated calcium channel. The NMDA receptor (NR) is a multimeric complex consisting of several subunits—NR1, NR2 (A–D), and NR3 (A,B). The NR1 subunit is a constitutive component of the NR and is uniformly distributed in the brain. Homomeric NR1 assembly can generate a functional NMDA ion channel, while the other subunits influence the pharmacological properties of the NR. The combined expression of NR1 subunit with one or more NR2 and/or NR3 subunits yields NRs that exhibit distinctive properties. NR2 and NR3 subunits are localized more discretely in the brain than the NR1 subunit. While the NR1 subunit is an obligatory component of the native NR, the NR2 subunits confer functional and pharmacological specificity to responses elicited by the heteromeric NR (Laurie & Seeburg, 1994).

The NR undergoes ontogenic changes during brain development. In the rat brain, NR has been shown to exist in two forms, an immature form present during the neonatal period that is replaced by the mature form seen in adult rats. The temporal switching from the immature form to the mature NR configuration takes place after the second postnatal week (Sircar, 2000). The developmental switching of the NR, leading to alterations in the functional and pharmacological properties of the receptor, has been reported both in vitro and in vivo (Monyer et al., 1994; Sheng et al., 1994; Sircar, 2000; Sircar et al., 1996; Williams et al., 1993). A progressive alteration in the expression of NR2 subunits occurs during development, with more NR2B at early ages and NR2A at later ages. The developmental changes in NR subunit composition from predominance of NR2B to NR2A reflect a switch from the immature form of NR to a mature form with altered NR functional properties (Ramoa & Prusky, 1997; Sircar, 2000; Williams et al., 1993). Ramoa and Prusky (1997) reported that retinal activity alters the developmental switch from the juvenile/immature form of NR to a more mature form by affecting expression of NR2 subunits.

Several in vivo and in vitro studies in adult animals have shown that ethanol interacts with the NR, and is in fact a potent inhibitor at the receptor (Buller et al., 1993; Darstein et al., 2000; Hoffman et al., 1989; Lovinger et al., 1989; Matthews et al., 1995). In adult animals, chronic ethanol exposure has been shown to increase NR number as determined from radioligand binding studies, and increased levels of NR subunits (Follesa & Ticku, 1995; Grant et al., 1990; Snell et al., 1993, 1996). Ethanol exposure during the perinatal period has been shown to cause reductions in NR maximal binding as well as expression of NR subunits (Diaz-Granados et al., 1997; Hughes et al., 1998; Savage et al., 1991a, 1991b). In adolescent rats, acute ethanol exposure has been reported to reduce the mean amplitude of NR-mediated excitatory postsynaptic currents (EPSCs) in a dose-dependent manner (Li et al., 2002).

The long-lasting effects of ethanol treatment in adolescent rats on NR functioning remain unknown. Because adolescent ethanol exposure impairs long-term spatial memory (Sircar & Sircar, 2005), and NR has been implicated in developmental plasticity and memory formation, the hypothesis tested in this study was that NR plays an important role in long-term memory deficit following repeated ethanol exposure during adolescence. Adolescent male rats were treated with ethanol for several days, and following an ethanol-free period of 7 days, they were sacrificed and their brains were isolated and studied for NR activity. NR changes in specific brain regions were determined using radioligand NR binding; MK-801 is a potent and noncompetitive NR antagonist and acts as an NR channel blocker (Sircar et al., 1987). In addition, in a subset of ethanol-treated and saline-treated rats, changes in NR2 subunit proteins were investigated immunohistochemically.

2. Materials and methods

2.1. Animals

Adolescent male Sprague-Dawley rats (Taconic, Germantown, MD), 28 days of age at the start of experiments, were used. Animals were housed in plastic cages, three to a cage, with ad libitum access to food and water. All experimental protocols used were approved by the Institutional Animal Care and Utilization Committee, and the research was conducted according to the requirements of *NIH Guide for the Care and Use of Laboratory Animals* (revised 1996).

2.2. Ethanol treatment

Rats were randomly assigned to one of two groups—saline or 2 g/kg of ethanol, with 12 rats in each group. Starting on postnatal day (PD) 30, rats were administered ethanol by intraperitoneal (ip) injection, and the process was repeated daily for 5 consecutive days. This repeated ethanol injection protocol produces a blood ethanol level of 225 mg/dl (Little et al., 1996); blood ethanol level was not measured in this study. The dosage of ethanol used and route of administration were as previously used (Sircar & Sircar, 2005). Control rats received isovolumetric ip saline injections on the same days.