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EFFECTS OF CEFTRIAXONE ON THE ACQUISITION AND MAINTENANCE OF ETHANOL DRINKING IN PERI-ADOLESCENT AND ADULT FEMALE ALCOHOL-PREFERRING (P) RATS

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Abstract—Increased glutamatergic neurotransmission appears to mediate the reinforcing properties of drugs of abuse, including ethanol (EtOH). We recently reported that the administration of ceftriaxone (CEF), a β-lactam antibiotic known to upregulate glutamate transporter 1 (GLT1) levels/activity, decreased the maintenance of EtOH intake in adult male alcohol-preferring (P) rats. In the present study, we tested whether CEF administration would reduce the acquisition and maintenance of EtOH drinking in adolescent and adult female P rats. The rats were treated with saline or 200 mg/kg ceftriaxone for 7 days (starting at 35 or 75 days old, respectively) followed by the EtOH acquisition test. Five weeks later the effects of CEF were examined regarding the maintenance of EtOH intake. For the maintenance test, half of the animals that received CEF during acquisition received CEF for 7 days and the other half received saline for 7 days. Saline-treated acquisition animals were treated similarly. The results indicated that pretreatment with ceftriaxone reduced the maintenance of EtOH intake in both animals that started as adolescents and those that started as adults. However, the beneficial effect of CEF was more pronounced in rats pretreated with CEF as adults compared with rats pretreated as adolescents. Reductions in EtOH intake by ceftriaxone were paralleled by an upregulation of GLT1 protein levels in both the nucleus accumbens (∼25% in rats starting at both ages) and prefrontal cortex (∼50% in rats starting as peri-adolescents and ∼65% in those starting as adults). These findings provide further support for GLT1-associated mechanisms in high alcohol-consuming behavior, and hold promise for the development of effective treatments targeting alcohol abuse and dependence.

Key words: ceftriaxone, EAAT2, acquisition, maintenance.

INTRODUCTION

Over half of adult Americans have a family history of alcoholism or alcohol (ethanol) abuse (Research Society for Alcoholism, 2009), and a subset of this group has this trait in multiple generations. Young men and women are initiating alcohol use earlier and experiencing more alcohol-related problems than ever before (Quine and Stephenson, 1990; Kandel et al., 1997; Nelson et al., 1998; Miller et al., 2001, 2007; Pitkanen et al., 2005; Bava and Tapert, 2010; Gore et al., 2011). This is significant as the early onset of alcohol use is a strong predictor of future alcohol dependence (Chou and Pickering, 1992; Anthony and Petronis, 1995; Grant and Dawson, 1997; Hawkins et al., 1997). Additionally, nearly half of all individuals meeting life-time diagnostic criteria for alcohol dependence do so by the age of 21, with this percentage increasing to approximately 65% by the age of 25 (Hingson et al., 2006). The danger of alcohol abuse among youth is compounded by the fact that the brain continues to mature during adolescence and young adulthood [c.f., Spear, 2010 for an overview]. Thus, it is clear that a greater understanding of alcohol abuse and its consequences among youth is needed. However, the effects of alcohol may, or may not, differ between the peri-adolescent and adult subject. Thus, when addressing this developmental question it is important to evaluate whether observed effects during peri-adolescence are also seen during adulthood.

In important reviews, Spear et al. have indicated that the boundaries of adolescence for rats often differ given the parameters (e.g., behavioral vs. neurochemical) examined (Spear and Brake, 1983; Spear, 2000, 2007). Nonetheless, neurochemical and neurobehavioral differences from postweaning through adulthood support an adolescent developmental window of postnatal days (PNDs) 28–42 (Spear and Brake, 1983; Spear, 2000, 2007). When assessing the effects of pharmacological pretreatment, during adolescence, on adult behaviors, Spear has suggested that this conservative window (PNDs 28–42) could be extended to PND 60 (Spear, 2000, 2004). This extended window allows one to

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Abbreviations: Acb, nucleus accumbens; CEF, ceftriaxone; EtOH, ethanol; GLT1, glutamate transporter 1; HRP, horseradish peroxidase; mTOR, mammalian target for rapamycin; PFC, prefrontal cortex; PNDs, postnatal days; SEM, standard error of the mean; TBST, tris-buffered saline with Tween; VEH, vehicle; VTA, ventral tegmental area.

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examine the earliest adolescent/pubertal changes in the female rat as well as the latest adolescent/pubertal changes in the male rat. These windows of development correspond with adolescent (a) changes in glutamatergic *N*-methyl-D-aspartate (NMDA) receptor binding of the prefrontal cortex (PFC) (Insel et al., 1990); (b) decreased excitatory synaptic transmission in the nucleus accumbens (Acb) relative to juveniles (Kasanetz and Manzoni, 2009); (c) greater cerebral metabolic activity relative to adults (Chugani et al., 1987; Spear, 2000, 2007); and (d) synaptic pruning/remodeling of subcortical regions, in early peri-adolescence, and cortical regions, in later peri-adolescence (Trommer et al., 1996; Casey et al., 2000; Dumas, 2004; Schochet et al., 2008).

Changes in glutamatergic neurotransmission affect many aspects of neuroplasticity associated with alcohol dependence. For example, neuroadaptations in the glutamatergic system appear to mediate ethanol tolerance, dependence, and withdrawal (Krystal et al., 2003). Additionally, the effects of ethanol withdrawal are linked to an increase in extracellular glutamate levels in rats made dependent on ethanol (Rossetti and Carboni, 1995). Ethanol-induced neuroadaptations of the glutamatergic system include alterations in *N*-methyl-D-aspartate (NMDA) receptor activity (Grant et al., 1990; Sanna et al., 1993; Snell et al., 1996; Chen et al., 1997). For instance, rapid withdrawal from chronic ethanol results in phosphorylation and re-localization of synaptic NMDA receptors (Clapp et al., 2010). In addition, chronic ethanol-induced increases in extracellular glutamate levels are associated with enhanced NMDA-receptor sensitivity in the Acb (Siggins et al., 2003).

Importantly, ethanol administration for 1 week resulted in decreased glutamate uptake in the Acb of male Sprague Dawley rats (Melendez et al., 2005). In addition, chronic ethanol consumption for 20 months down-regulates glutamate uptake in the cerebral cortex of alcohol-preferring cAA rats (Schreiber and Freund, 2000). Extracellular glutamate levels are regulated by several glutamate transporters located in neurons and glia (Gegelashvili and Schousboe, 1997; Seal and Amara, 1999; Anderson and Swanson, 2000). Glutamate transporter 1 [(GLT1), or its human homolog, the excitatory amino acid transporter 2 (EAAT2)], is the primary transporter that regulates the removal of extracellular glutamate in the central nervous system (CNS) (Ginsberg et al., 1995; Rothstein, 1995; Rothstein et al., 1995; Danbolt, 2001; Mitani and Tanaka, 2003).

The role of GLT1 in chemical dependency has been studied in drug abuse models, including those associated with excessive ethanol intake. Functional activation of GLT1 appears to reduce the rewarding effects of cocaine, morphine and methamphetamine (Nakagawa et al., 2005). In addition, upregulation of GLT1 activity by ceftriaxone, a beta-lactam antibiotic, attenuates cue-induced cocaine-seeking behavior (Sari et al., 2009; Knackstedt et al., 2010). Importantly, our laboratory has recently reported that upregulation of GLT1 level/activity by ceftriaxone resulted in a dose-dependent, long-lasting reduction in ethanol intake by adult male alcohol-preferring (P) rats (Sari et al., 2011). In the present study, we investigated the effects of up-regulating GLT1 level, by ceftriaxone, on the acquisition of ethanol intake in both peri-adolescent and adult female P rats. We also investigated the effects of ceftriaxone on the maintenance of ethanol intake in these animals five weeks after the acquisition test. Since ceftriaxone-induced reductions in ethanol intake in male P rats is associated with upregulation of GLT1 levels in the PFC and Acb, the present study also assessed whether ceftriaxone-induced reductions in ethanol intake by female P rats were associated with upregulation of GLT1 expression levels in these two brain reward regions, as well.

EXPERIMENTAL PROCEDURES

Animals

Adolescent and adult female P rats (35 and 75 days of age at the start of the experiment, respectively) were used in this study. These rats were obtained from the Indiana Alcohol Research Center breeding colonies (Indianapolis, IN, USA). No more than two rats from a litter were included in any condition or interaction between conditions. This was done to avoid litter effects and increase the generalizability of the findings (Holson and Pearce, 1992). Rats were housed in a temperature- (21 °C) and humidity- (50%) controlled vivarium which was maintained on a 12 h reverse-light/dark cycle (lights on at 2200 h). All experimental procedures were approved by the Institutional Animal Care and Use Committee of Indiana University School of Medicine and are in accordance with guidelines set by the Institutional Animal Care and Use Committee of the National Institutes of Health, and the Guide for the Care and Use of Laboratory Animals.

Adolescent female P rats

First treatment cycle/acquisition of ethanol drinking phase. Subjects were group-housed at the start of the study. Each rat received seven consecutive injections (i.p.; 1×/day) with 200 mg/kg ceftriaxone (n = 20), or an equivalent volume (2 ml/kg) of vehicle (sterile saline; n = 19). Subjects had free access to food and water throughout the experiment. The day following the first treatment cycle (i.e., 42 days of age), subjects were individually housed in hanging wire-mesh cages. Animals were given 24-h concurrent access to 15% and 30% v/v ethanol. Measures of ethanol (g/kg) and water (ml/kg) intake as well as body weights (g) were recorded 5 days/wk (Monday–Friday) for the next 5 weeks.

Second treatment cycle/maintenance of ethanol drinking phase. During Week 6, subjects were reassigned to groups for a second injection cycle (1×/day for seven consecutive days). Approximately half of the rats were exposed to the same treatment that they had received during the first treatment cycle, whereas the remaining rats were exposed to the opposite treatment, yielding four treatment groups (n = 9–10/group): vehicle–vehicle [VEH–VEH]; ceftriaxone–vehicle [CEF–VEH]; vehicle–ceftriaxone [VEH–CEF]; and ceftriaxone–ceftriaxone [CEF–CEF]. During the second treatment cycle, defined here as the maintenance test phase, ethanol (g/kg) and