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Gene expression changes in serotonin, GABA-A receptors, neuropeptides and ion channels in the dorsal raphe nucleus of adolescent alcohol-preferring (P) rats following binge-like alcohol drinking

Jeanette N. McClintick [a,b], William J. McBride [c], Richard L. Bell [c], Zheng-Ming Ding [c], Yunlong Liu [d], Xiaoling Xuei [a,b], Howard J. Edenberg [a,b,d,*]

[a] Department of Biochemistry & Molecular Biology, Indiana University School of Medicine, Indianapolis, IN 46202, United States
[b] Center for Medical Genomics, Indiana University School of Medicine, Indianapolis, IN 46202, United States
[c] Institute of Psychiatric Research, Department of Psychiatry, Indiana University School of Medicine, Indianapolis, IN 46202, United States
[d] Department of Medical & Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN 46202, United States

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ABSTRACT

Alcohol binge-drinking during adolescence is a serious public health concern with long-term consequences. We used RNA sequencing to assess the effects of excessive adolescent ethanol binge-drinking on gene expression in the dorsal raphe nucleus (DRN) of alcohol preferring (P) rats. Repeated binges across adolescence (three 1 h sessions across the dark-cycle per day, 5 days per week for 3 weeks starting at 28 days of age; ethanol intakes of 2.5–3 g/kg/session) significantly altered the expression of approximately one-third of the detected genes. Multiple neurotransmitter systems were altered, with the largest changes in the serotonin system (21 of 23 serotonin-related genes showed decreased expression) and GABA-A receptors (8 decreased and 2 increased). Multiple neuropeptide systems were also altered, with changes in the neuropeptide Y and corticotropin-releasing hormone systems similar to those associated with increased drinking and decreased resistance to stress. There was increased expression of 21 of 32 genes for potassium channels. Expression of downstream targets of CREB signaling was increased. There were also changes in expression of genes involved in inflammatory processes, axonal guidance, growth factors, transcription factors, and several intracellular signaling pathways. These widespread changes indicate that excessive binge drinking during adolescence alters the functioning of the DRN and likely its modulation of many regions of the central nervous system, including the mesocorticolimbic system.

1. Introduction/background

A multi-national review of studies of adolescent drinking indicates that about 3/4 of adolescents have consumed alcohol by late adolescence, and 20–40% had engaged in binge drinking, the consumption of 5 or more drinks on one occasion (Marshall, 2014). In the US, adolescents (ages 12–20) drink 11% of all alcohol consumed, 90% of which is consumed during binge drinking (NIAAA, 2013).

Adolescence is a time in which brain connections mature and are remodeled, and alcohol could alter these processes. Given the legal and ethical prohibition of using adolescents in alcohol research, animal models have been used to examine the effects of ethanol on adolescents. Studies have examined the effects of chronic low dose exposure (Evrard et al., 2006) and different models of binge drinking (McBride et al., 2014; Pascual et al., 2007, 2014; Ward et al., 2014). Selectively bred alcohol preferring (P) and high-alcohol-drinking (HAD) rats engage in binge-like drinking, and adults and peri-adolescents of both sexes readily achieve blood ethanol levels ≥ 80 mg% (Bell et al., 2014). Multiple scheduled access protocols (usually three 1 h periods of access to alcohol during the dark phase) enhance this binge-like drinking, and under these conditions peri-adolescent selectively bred rats consume more alcohol than their adult counterparts (Bell et al., 2014). In general, animal studies and human observational studies during adolescence indicate that adolescents, relative to adults, are less affected by ethanol-induced sedation and motor incoordination but more affected by its rewarding and reinforcing properties (Bell et al., 2013; Spear, 2010). Greater disturbances in some cognitive functions have also been reported (Bell et al., 2013; Spear, 2010).

Alcohol dependence has two basic components: positive effects primarily attributed to ethanol stimulated release of dopamine in the reward centers of the brain and negative effects of anxiety and depression that occur after cessation of drinking (Koob, 2013). The reward

Abbreviations: Acb-sh, nucleus accumbens shell; CeA, central nucleus of the amygdala; CREB, cAMP response element-binding protein; DRN, dorsal raphe nucleus; HAD/LAD, high alcohol and low alcohol drinking rats; p-CREB, phosphorylated CREB; P/NP, alcohol preferring and non-preferring rats

* Corresponding author at: Department of Biochemistry & Molecular Biology, Indiana University School of Medicine, Indianapolis, IN 46202, United States. Tel.: +1 317 274 2353; fax: +1 317 274 4686.
E-mail address: edenberg@iu.edu (H.J. Edenberg).

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system in the brain includes the mesocorticolimbic dopamine system and extended amygdala, which intersect in the nucleus accumbens shell. Within this system, ethanol increases the release of dopamine in the shell of the nucleus accumbens, which is directly associated with the rewarding effects of ethanol consumption (McBride, 2002; McBride and Li, 1998 reviewed in Koob, 2013). Serotonin modulates the effect of ethanol on dopamine release via the 5-HT3 receptor (Campbell et al., 1996; Campbell and McBride, 1995; Engleman et al., 2008; Wozniak et al., 1990).

In rats, a genetic predisposition for excessive ethanol consumption is associated with alterations in central serotonergic activity. For example, ethanol-naïve P and HAD rats have significantly lower serotonin and/or 5HIAA5-hydroxyindoleacetic acid levels in the frontal cortex, nucleus accumbens, caudate putamen, hippocampus and hypothalamus compared with ethanol-naïve NP and LAD rats (Bell et al., 2012; Gongwer et al., 1989; Murphy et al., 1987). In addition, ethanol-naïve P, and in some cases ethanol-naïve HAD, rats have altered levels of 5HT1A, 5HT1B, 5HT2, 5HT2C and 5HT3 receptors in multiple brain regions compared with ethanol-naïve NP and LAD rats (McBride et al., 1997a,b). Thus, alterations in receptor expression may be compensating for reduced serotonin function. Selective agonists and/or antagonists for various serotonin receptors affect the alcohol-consuming behavior of rats selected for high alcohol preference (Ding et al., 2012; Lankford et al., 1996; Lankford and Myers, 1996; Long et al., 1996; Overstreet et al., 1997; Rodd-Henricks et al., 2000; Rodd et al., 2010). Other selectively bred high alcohol-consuming rat lines (Alko alcohol preferring and Sardinian alcohol preferring rats) had higher levels of serotonin in whole brain or selected regions than their low alcohol-consuming counterparts (Alko non-alcohol and Sardinian non-preferring rats) (Bell et al., 2012).

The dorsal raphe nucleus (DRN) is an origin of the central serotonergic system. About 50% of the serotonin neurons in the rat brain are in the DRN, although fewer than 50% of the neurons in the DRN are serotonergic (Waselus et al., 2011). The DRN has projections into numerous brain regions, including the mesolimbic regions important in reward and addiction (amygdala, hippocampus, caudate putamen, substantia nigra and ventral tegmental area). Projections from the raphe nuclei mediate dopamine release in the ventral tegmental area (Liu et al., 2006b; Rodd et al., 2007; Sari et al., 2011). Ethanol-naïve P rats have fewer serotonergic neurons in the DRN than ethanol-naïve NP rats and decreased serotonergic projections into terminal regions (Zhou et al., 1994). Postmortem studies of the DRN of alcoholics (Underwood et al., 2007) found less serotonin transporter in the brainstem of alcoholics.

The links between the DRN and the reward centers of the brain, its role in anxiety, stress and depression, and the changes that occur during adolescence make it an important region in which to study the effects of heavy alcohol exposure, particularly during the vulnerable period of adolescence. This study examines the effects of binge drinking by adolescent P rats on the gene expression profile of the DRN.

2. Materials and methods

2.1. Animal binge drinking protocol

Peri-adolescent male P (alcohol preferring) rats were maintained on a reverse light–dark cycle and given free-choice access to ethanol using a multiple-scheduled-access procedure, as described in Bell et al. (2011). There were 10 animals in the water control group and 11 in the ethanol binge-drinking group. Animals were given ad libitum access to food and water and those in the drinking group were given access to ethanol (15 and 30% ethanol solutions concurrently) in 3× 1-h sessions per day for 5 consecutive days/week, starting at 28 days of age. The three 1 h ethanol access sessions were scheduled so that the first 1 h session started at dark onset (1000 h), the second 1 h session began 2 h after the end of the first session (1300 h) and the third/last 1 h session began 2 h after the end of the second session (1600 h). The animals used in this study are the same ones described in McBride et al. (2014). A graph of the 15-day ethanol drinking data is given in McBride et al. (2014). In general, during week 1, ethanol intakes ranged from 3 to 4 g/kg/1 h session with a total intake of approximately 10 g/kg/day; during weeks 2 and 3, ethanol intakes ranged from 2 to 3 g/kg/1 h session with a total intake of approximately 8 g/kg/day.

The rats were sacrificed 3 h after the 1st access session on their 15th day of drinking. Brains were rapidly extracted and flash-frozen in isopentane in dry ice and stored at −80 °C until sectioning. Brains were sectioned (300 μm) and the DRN micro-punched using procedures previously described (McBride et al., 2014). The DRN was punched from coronal sections between −7.30 mm and −8.30 mm post bregma according to the rat brain atlas by Paxinos and Watson (1998). All equipment used to obtain tissue was treated with RNase Zap (Life Technologies, Carlsbad, CA). Other brain regions of these animals have been studied (McBride et al., 2014). The animals used in these experiments were maintained in facilities fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC). All research protocols were approved by the institutional animal care and use committee and are in accordance with the guidelines of the Institutional Care and Use Committee of the National Institute on Drug Abuse, National Institutes of Health, and the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council 1996).

2.2. Sample collection and quality

To extract the RNA, punches were immediately homogenized in TRIzol (Life Technologies, Carlsbad, CA) and processed according to manufacturer's protocol but with twice the suggested ratio of TRIzol to tissue (Edenberg et al., 2005). Ethanol-precipitated total RNA was further purified through Qiagen RNeasy columns (Qiagen, Hilden Germany) according to the manufacturer's protocol. The yield, concentration and purity of the RNA were measured by Nanodrop (Thermo Fisher Scientific, Waltham, MA) spectrum from 220 nm to 340 nm. Quality was also assessed by Agilent Bioanalyzer (Agilent Technologies, Santa Clara, CA); RNA integrity numbers (RIN) averaged 8.6 for the samples, showing little degradation.

2.3. RNA sequencing

Standard methods were used for RNA-seq library construction, EZBead preparation and Next-Gen sequencing using the Life Technologies SOLiD system. Briefly, total RNA (≤400 ng per sample) was first ribosome-depleted using Ribominus™ Eukaryote Kit for RNA-Seq (Ambion, CA), and whole transcriptome library was prepared and barcoded per sample using the standard protocol of SOLiD Total RNA-seq Kit (Life Technologies, Carlsbad, CA). Each barcoded library was quantified by quantitative PCR using SOLiD Library Taqman qPCR Module (Life Technologies, Carlsbad, CA), and pooled in equal molarity. EZBead preparation, bead library amplification, and bead enrichment were then conducted using Life Technologies EZ Bead™ E80 System (Life Technologies, Carlsbad, CA). And finally sequencing by ligation was carried out using standard single-read, 5′–3′ strand-specific sequencing procedure on SOLiD4™ Sequencer (50b-read), as well as on SOLiD™ 5500xl Sequencer (75 b read). The average number of mapped reads per sample was 20.8 million.

2.4. Data processing and quality assessment

We used SOLiD Instrument Control Software and SOLiD Experiment Tracking System software for the read quality recalibration. Each sequence read was scanned for low-quality regions, and if a 5-base sliding window had an average quality score less than 20, the read was truncated at that position. Any read with a length of less than 35