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Gene expression changes in the nucleus accumbens of alcohol-preferring rats following chronic ethanol consumption

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ABSTRACT

The objective of this study was to determine the effects of binge-like alcohol drinking on gene expression changes in the nucleus accumbens (ACB) of alcohol-preferring (P) rats. Adult male P rats were given ethanol under multiple scheduled access (MSA; three 1-h dark cycle sessions/day) conditions for 8 weeks. For comparison purposes, a second ethanol drinking group was given continuous/daily alcohol access (CA; 24 h/day). A third group was ethanol-naïve (W group). Average ethanol intakes for the CA and MSA groups were approximately 9.5 and 6.5 g/kg/day, respectively. Fifteen hours after the last drinking episode, rats were euthanized, the brains extracted, and the ACB dissected. RNA was extracted and purified for microarray analysis. The only significant differences were between the CA and W groups ($p<0.01$; Storey false discovery rate = 0.15); there were 374 differences in named genes between these 2 groups. There were 20 significant Gene Ontology (GO) categories, which included negative regulation of protein kinase activity, anti-apoptosis, and regulation of G-protein coupled receptor signaling. Ingenuity® analysis indicated a network of transcription factors, involving oncogenes (*Fos*, *Jun*, *Junb* had higher expression in the ACB of the CA group), suggesting increased neuronal activity. There were 43 genes located within rat QTLs for alcohol consumption and preference; 4 of these genes (*Tgfa*, *Hspa5*, *Mtus1* and *Creb3l2*) are involved in anti-apoptosis and increased transcription, suggesting that they may be contributing to cellular protection and maintaining high alcohol intakes. Overall, these findings suggest that chronic CA drinking results in genomic changes that can be observed during the early acute phase of ethanol withdrawal. Conversely, chronic MSA drinking, with its associated protracted withdrawal periods, results in genomic changes that may be masked by tight regulation of these genes following repeated experiences of ethanol withdrawal.

1. Introduction

Microarray analysis has emerged as a tool to study the multiple, complex effects of pharmacological treatments on changes in gene expression. Examining innate differences and changes in gene expression in response to ethanol in lines or strains of mice and rats with divergent responses to ethanol has provided important clues toward identifying genes and gene networks involved in vulnerability to high ethanol drinking behavior. Given this, examining changes in gene expression following chronic ethanol drinking will, presumably, provide information to identify genes and gene networks involved in maintaining this behavior, as well as the consequences of chronic ethanol exposure.

Many innate genetic expression differences between high and low ethanol-consuming rodent lines have been identified. For example, Edenberg et al. (2005) examined differences in gene expression in the hippocampus of inbred alcohol-preferring (iP) and inbred alcohol-non-preferring (iNP) rats, and reported differences for genes involved in cell growth and adhesion, cellular stress reduction and anti-oxidation, protein trafficking, cellular signaling pathways, and synaptic function. In a subsequent study, Kimpel et al. (2007) reported on innate differences in gene expression between iP and iNP rats in 5 CNS regions, including the nucleus accumbens (ACB).

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These authors indicated that genes associated with anti-apoptosis, axon guidance, nerve transmission as well as synaptic structure and function displayed expression differences between the rat strains. Worst et al. (2005) reported on the transcriptome analysis of the frontal cortex from ethanol-naïve AA (Alko, alcohol) and ANA (Alko, non-alcohol) rats, with mRNA level differences found that could reflect altered neurotransmitter release. Using a whole brain analysis of inbred long-sleep and inbred short-sleep mice, Xu et al. (2001) detailed expression differences for metabolic-associated genes with higher levels seen in the long-sleep mice. In a comprehensive transcriptome meta-analysis of gene expression differences across a number of different mouse strains, Mulligan et al. (2006) identified several *cis*-regulated candidate genes for an ethanol preference QTL on chromosome 9.

Alterations in gene expression produced by exposure to ethanol have been reported in a few studies. Acute ethanol injections (6 g/kg; i.p.) produced gene expression changes associated with cell signal regulation, gene regulation, and homeostasis/stress responses in the whole brain from C57BL/6 J and DBA/2 J, high- and low-ethanol drinking mice, respectively (Treadwell and Singh, 2004). Kerns et al. (2005) reported that acute i.p. ethanol injections altered the expression of genes involved in glucocorticoid signaling, neurogenesis, myelination, neuropeptide signaling, and retinoic acid signaling in the ACB, prefrontal cortex and VTA of C57BL/6 J and DBA/2 J mice. Differences in the expression levels of genes coding for oxido-reductases and ADP-ribosylation factors have also been found in the dorsal hippocampus of Lewis rats given 12% ethanol or water for 15 months (Saito et al., 2002). In a recent study, Bowers et al. (2006) reported that chronic ethanol consumption, in a liquid diet, altered the expression of over 100 genes in the cerebellum of PKCgamma wild-type and mutant mice. In contrast, Saito et al. (2004) in a previous study found no statistically significant effects of chronic free-choice ethanol drinking on gene expression in the striatum of C57BL/6By mice. The above studies were conducted using ethanol injections or 24-h free- or forced-choice drinking. A recent study from our laboratory (Rodd et al., 2008) reported that operant ethanol self-administration produced approximately 500 significant changes in gene expression in the ACB when measured 24 h after the last 1-h operant session, whereas saccharin self-administration produced less than 60 significant changes, suggesting that chronic ethanol consumption was producing persisting effects on gene expression in the ACB of P rats. However, it is important to determine if the effects of ethanol drinking alone (absence of operant responding) produce similar changes in gene expression in limbic regions that are involved in regulating ethanol drinking.

In an initial study from our laboratory, Bell et al. (2006a) examined protein expression changes in the ACB and amygdala of iP rats given 24-h continuous access (CA) or multiple scheduled access (MSA; four 1-h sessions during the dark cycle) to ethanol for 6 weeks. The results of this study indicated that ethanol drinking conditions differentially changed protein expression in the ACB and amygdala. However, a relatively insensitive 2-dimensional gel electrophoresis procedure was used in this study and only the most abundant proteins found in tissue from the whole ACB or amygdala could be detected. The microarray procedure offers a potentially more sensitive method to measure changes resulting from ethanol drinking under different conditions of availability, which produce different patterns of ethanol intake and associated blood alcohol levels (c.f., Bell et al., 2006a,b). Therefore, the objective of this study was to examine changes in gene expression associated with chronic ethanol drinking under binge-like ethanol drinking conditions. For comparison purposes, the effects of 24-h free-choice drinking on gene expression were also determined. Gene expression changes were determined the next day after the binge-like group's last scheduled access period of the previous day. Ethanol was removed from both groups at the same time to control for the length of ethanol deprivation before brain tissue was harvested. The hypothesis to be tested was that chronic binge-like ethanol drinking would produce significant persisting effects on gene expression in the ACB of P rats that would not be observed with 24-h continuous ethanol access drinking.

2. Method

2.1. Animals and ethanol drinking procedures

Subjects were adult (>90 days old), ethanol-naïve, male P rats from the S52 generation. The rats were single-housed in hanging stainless steel wire-mesh (bottom and front) cages on a reverse 12 h/12 h dark–light-cycle (light offset at 1000 h). Animals had ad libitum access to food. Rats were randomly divided into three groups ($n=9$/group): the 1st group had access to water as their sole fluid, the 2nd group had continuous/daily, concurrent, free-choice access to 15% and 30% (v/v) ethanol and water, and the 3rd group had bout-like, concurrent access to 15% and 30% ethanol, with water available ad libitum. The bout-like group experienced a multiple scheduled access (MSA) protocol, such that they received three 1-h access periods each separated by 2 h starting at the beginning of the dark cycle (i.e., 1000–1100, 1300–1400, and 1600–1700 h). The MSA animals were given ethanol access in 5-day blocks (Monday–Friday), with each block separated by 2 days without ethanol. Measurements of water and ethanol intake, and body weights were taken Monday through Friday at 0900 h; ethanol intakes, for MSA animals, were also taken at the end of each 1-h access period. After the MSA group's 1st day of re-exposure to ethanol access, of the 9th week, both groups of ethanol drinking rats had ethanol removed at 1700 h. To ensure that ethanol blood levels were absent and the deprivation period was equivalent for both groups, all rats were killed the next day (15 h after removing ethanol). Rats were killed by decapitation and their brains processed for microarray analyses, as described below.

2.2. Brain dissections

Rats were killed by decapitation within the same 2-h time frame over 2 days with equal number of animals from each group being killed on each day to minimize differences in time of sacrifice and dissection, and maintain the experimental balance across groups. During the 7th and 8th weeks, the rats in the MSA group dissected on the 2nd day had ethanol access moved to Tuesday through Saturday to preserve the 5-day a week schedule. The head was immediately placed in a cold box maintained at −15 °C, where the brain was rapidly removed and placed on a glass plate for dissection. All equipments used to obtain tissue were treated with RNAse Zap (Ambion, Inc. Austin, TX) to prevent RNA degradation. The ACB was dissected according to the coordinates of Paxinos and Watson (1998). Briefly, the ACB was dissected from a 2-mm section generated by a coronal cut at 2 mm anterior to the optic chiasm (Bregma 1.70 mm) and a coronal cut at the optic chiasm (Bregma −0.26 mm). Dissected tissue was immediately homogenized in Trizol reagent (Invitrogen, Carlsbad, CA) and processed according to the manufacturer's protocol, but with twice the suggested ratio of Trizol to tissue, as discussed previously (Edenberg et al., 2005). Ethanol precipitated RNA was further purified through RNeasy® columns (Qiagen, Valencia, CA), according to the manufacturer's protocol. The yield, concentration and purity of the RNA were determined by running a spectrum from 210 to 350 nm, and analyzing the ratio of large and small ribosomal RNA bands using an Agilent Bioanalyzer. Yields and purity of the RNA were deemed excellent.

2.3. Microarray procedures

Separate preparations of total RNA were made from the ACB of individual animals. Samples were not pooled. Standard Affymetrix protocols (GeneChip® Expression Analysis Technical Manual, Rev. 5 and updates) were used to synthesize biotinylated cRNA, starting with 5 µg total RNA from each region, using the Affymetrix kits for