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Effect of the TaqIA polymorphism on ethanol response in the brain

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ABSTRACT

Acute ethanol administration increases striatal dopamine release and decreases cerebral glucose metabolism. The A1 allele of the ANKK1 TaqIa polymorphism is associated with lower dopaminergic tone and greater risk for alcoholism, but the mechanisms are unclear. We hypothesized that ethanol would be more reinforcing in men with the A1 allele (A1+) than in men without it (A1−), as indicated by decreased anxiety and fatigue and altered activity in associated brain regions. In a pilot study, A1+ and A1− men (6/group) drank ethanol (0.75 ml/kg) or placebo beverages on each of 2 days. Positron emission tomography with [F-18] fluorodeoxyglucose (FDG) was used to assess regional cerebral glucose metabolism as a measure of relative brain activity while participants performed a vigilance task. Significant findings were as follows: Ethanol decreased anxiety and fatigue in A1+ men but increased them in A1– men. Ethanol increased activity in the striatum and insula of A1+ men, but reduced activity in the anterior cingulate of A1– men. Reduced anxiety and fatigue in A1+ men were significantly associated with greater activity within a right orbitofrontal region previously implicated in cognitive control, and less activity in structures associated with anxiety (amygdala), fatigue (thalamus), and craving/reinforcement (striatum). In contrast, anxiety and fatigue changes were unrelated to brain activity in A1− men. Although these results require replication in a larger sample, alcohol-induced negative reinforcement may explain the greater risk for alcoholism associated with the A1 allele.

1. Introduction

A growing literature points to an important role of genotype in vulnerability to substance abuse, with much of this work centering on the role of central dopamine systems in motivation and reinforcement (Volkow et al., 2007). Reduced density of striatal D2 dopamine receptors has been associated with reinforcer-induced impulsivity in rats (Dalley et al., 2007), and with presence of the TaqIA A1 allele of the *ANKK1* gene (previously reported as located in the D2 dopamine receptor gene) (Neville et al., 2004) in humans, both through *in vitro* (Noble et al., 1991; Thompson et al., 1997) and *in vivo* (Jonsson et al., 1999; Pohjalainen et al., 1998) studies. Although A1 prevalence differs between ethnic groups (Barr and Kidd, 1993), raising the possibility of stratification bias, studies both across and within diverse ethnic groups have reported robust associations of the polymorphism first with misuse of alcohol (Blum et al., 1990) and subsequently with other forms of substance abuse (Li et al., 2004a; Noble 2003; Young et al., 2004). In a meta-analysis of 3329 Caucasian adults, the prevalence of the Al allele (AlAl and A1A2 genotypes) was significantly higher ($P = 1.54 \times 10^{-8}$) in alcoholics (38.9%) than nonalcoholics (29.4%) (Noble, 2003). Effect sizes are often small, however (Berggren et al., 2006), requiring large samples or meta-analyses to show associations in the absence of more strongly linked endophenotypes (White et al., 2008).

These associations have led to the hypothesis that the lower dopaminergic tone of the mesocorticolimbic reinforcement circuits in individuals with the A1 allele produces reinforcement deficiencies or anhedonia (Blum et al., 2000; Noble, 2000). Low D2 function is thought to 1) increase the reward value of direct D2 agonists, including all drugs of abuse, 2) decrease the reward value of less potent natural reinforcers, and 3) decrease the capacity for frontocortical inhibition, all of which contribute to compulsive drug taking (Volkow et al., 2004).

A report consistent with this view indicated that individuals with the A1 allele are characterized by less activation of the mesocorticolimbic reinforcement system than those without the allele (Cohen et al., 2005). In contrast, treatment with the D2 agonist bromocriptine enhanced

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activation of the reward system in individuals with, but not without, the A1 allele (Kirsch et al., 2006).

We have previously shown that presence of the A1 allele in adolescents is associated with abnormal levels of, or abnormal relationships between, constructs related to hedonic tone, including family stress (Berman and Noble, 1997), the personality characteristics of novelty seeking and harm avoidance (Berman et al., 2002), and negative affect (Berman et al., 2003). In PTSD-afflicted veterans, we found greater anxiety/insomnia, social dysfunction and depression in those with than without the A1 allele (Lawford et al., 2006). In combination with a gene coding for reduced dopamine transporter density, presence of the A1 allele has recently been associated both with higher levels of anxiety (Kulikova et al., 2008b) and mental fatigue (Kulikova et al., 2008a), as compared with individuals who did not combine these genotypes. In sum, the studies to date suggest that individuals with the A1 allele may be more susceptible to stress-induced anxiety and other negative affective states, and that these individuals are more likely to self-medicate negative affective states with dopamine-releasing drugs, such as alcohol.

The role of the A1 allele in mediating acute effects of alcohol is unknown. Alcohol consumption increases arousal and reduces anxiety in some individuals, while others primarily report increased fatigue. It increases striatal dopamine release, which has been associated with drug-mediated reward (Koob and Le Moal, 2008; Yoder et al., 2009), and decreases cerebral glucose metabolism (de Wit et al., 1990; Volkow et al., 2006b). To test the hypothesis that alcohol is more reinforcing in men with the A1 allele, we assessed self-reported anxiety and fatigue and cerebral metabolic responses to acute ethanol administration. We hypothesized that in men with the A1 allele ethanol would show reduced anxiety, fatigue, and activity in brain structures associated with these unpleasant states compared with men without it.

2. Methods

2.1. Study design

Effects of ethanol were compared between social drinkers with no copies of the A1 allele (A1− group) and those with one or two copies (A1+ group). Cerebral glucose metabolism was assessed using positron emission tomography (PET) while participants were engaged in an auditory continuous performance test (CPT) in two sessions, counterbalanced for order, 1–21 days apart (mean ± S.D. = 10.4 ± 7.8 days). In one session, participants drank a 250-ml mixture of caffeine-free diet soda plus a moderate dose of ethanol (0.75 g/kg body weight) equivalent to 1.4 ml of ethanol/ml of body water (Savoie et al., 1988). In the other session, a non-pharmacologically relevant amount of ethanol was floated on top of the soda to simulate the taste and smell of a mixed beverage. In each session, anxiety and fatigue were self-reported. [F-18]Fluorodeoxyglucose (FDG) was administered to assess cerebral glucose metabolism, a measure of regional brain function (Phelps et al., 1979; Reivich et al., 1979), and raw counts from FDG, scaled to the global mean, were used as a surrogate index of regional cerebral glucose metabolism (rCMRglc). Relative activity, as used in this report, refers to this measure. Blood-alcohol content was assessed via a breathalyzer at least once per hour until recording a reading of 0.02% or lower. If all other study procedures were completed, the subject was then released.

2.2. Research participants

Participants were recruited through advertisements in local newspapers. Volunteers who met initial criteria in a telephone screening came to UCLA. After giving written informed consent, as approved by the UCLA Office for Protection of Research Subjects, they underwent a comprehensive evaluation, including medical history, physical examination, psychiatric interview (Structured Clinical Inventory for DSM-IV (SCID-I) (First et al., 1996), and completion of the Beck Depression Inventory (Beck et al., 1961), the Wender Utah Rating Scale for attention-deficit hyperactivity disorder (ADHD) (Ward et al., 1993), and a drug use survey.

Symptoms of depression (scores > 18 on the Beck Depression Inventory) or ADHD (scores ≥ 46 on the Wender Utah Rating Scale) were exclusionary, as were history of neurological disease, head trauma with loss of consciousness > 5 min, claustrophobia, systemic disease, HIV-seropositive status, lifetime history of any DSM-IV Axis I or II psychiatric diagnosis, and history of dependence on alcohol or an illicit drug of abuse. Habitual use of caffeine or nicotine and light use of marijuana (≤ 1 cigarette per week) or alcohol (< 90 g absolute alcohol per week) were allowed. Twelve right-handed (score > 20 on a modified version of the Edinburgh Handedness Test [Oldfield, 1971]) Caucasian men aged 21–39 (mean [S.D.] = 29.0 [5.2]) completed the study and were paid for their participation.

2.3. Genotype analysis

A 10-ml blood sample was drawn from each subject. Genomic DNA was extracted and used as a template for determination of TaqI A alleles by the polymerase chain reaction (Grandy et al., 1993). The amplification of DNA was carried out using a Perkin-Elmer GeneAmp 9600 Thermocycler. Approximately 500 ng of amplified DNA was digested with 5 U of TaqI restriction enzyme (New England Biolabs) at 65 °C overnight. The resulting products were separated by electrophoresis in a 2.5% agarose gel containing ethidium bromide and visualized under ultraviolet light. Three genotypes are revealed: A1A2 (three fragments: 310, 180 and 130 bp), A2A2 (two fragments: 180 and 130 bp), and A1A1 (one uncleaved 310 bp fragment). A1+ allele subjects had either the A1A1 or A1A2 genotype; A1− allele subjects had the A2A2 genotype only.

2.4. Experimental sessions

Participants abstained from alcohol and marijuana for 48 h before each test session but used coffee and/or tobacco as usual. At each session, recent drug and alcohol use were evaluated by a urine drug screen and breathalyzer test. All participants received a standard non-ketogenic breakfast (egg, juice, toast) upon arriving at UCLA. After breakfast, a venous catheter was inserted for infusing FDG. PET images were acquired with a Siemens ECAT EXACT HR+ tomograph (CTI, Knoxville, TN) in 3D mode. A plastic facemask (Scrypton Systems, Annapolis, MD) was fitted to each subject to minimize head motion. A 3-min ^{68}Ge transmission scan verified the position of the brain, and a 20-min ^{68}Ge transmission scan was performed for attenuation correction.

Participants were removed from the gantry, and trained on the CPT, which requires a button press prompted by a tone of designated pitch within a sequence of nontarget tones (inter-stimulus interval = 2 s). This task was used to standardize the cognitive set during assessment of rCMRglc. After training, participants drank the experimental beverage over 5 min and then provided self-ratings of the tension–anxiety and fatigue-inertia subscales from the Profile of Mood States (McNair et al., 1971). The 30-min CPT was then initiated. Five minutes later, FDG (≤ 5 mCi, ≤ 185 MBq) was injected. When the CPT ended, the subject was repositioned in the scanner gantry, and brain images were acquired for 30 min. Since the FDG was injected within the first half hour after alcohol consumption, and peak alcohol concentration is attained in 40–50 min (Sammi et al., 2000), uptake of the radiotracer presumably occurred during the ascending limb of the blood-alcohol curve.

We reconstructed 128 × 128 pixel images using a Hann filter (cut-off frequency = 0.5 cycles/pixel). The average transverse resolutions at 1 and 10 cm from the center of the field-of-view (FOV), measured in