Norepinephrine Transporter Gene Variation Modulates Acute Response to D-Amphetamine

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Background: Individual differences in subjective responses to stimulant drugs such as amphetamine may influence risk of abuse as well as clinical-treatment response to these drugs. Because the effects of amphetamine are mediated in part by the norepinephrine transporter (SLC6A2), we examined interindividual differences in mood response to amphetamine in relation to SLC6A2 gene polymorphisms.

Methods: Ninety-nine healthy volunteers participated in three sessions in which they randomly received either placebo or *D-amphetamine (10 mg or 20 mg) under double-blind conditions. Every subject completed self-report measures on subjective effects (Profile of Mood States). Afterward, all individuals were genotyped for eight SLC6A2 gene polymorphisms. Individual genotypes and haplotypes were investigated.*

Results: The intronic 36001C/C (rs47958) genotype was associated with increases in positive mood and elation after 20 mg of D-amphetamine. Positive mood and elation levels were also found to be associated with the haplotype GCC formed from 28257G/C (rs36017), 28323C/T (rs2270935), and 36001A/C (rs47958). These findings remained significant after adjustment for multiple testing.

Conclusions: Polymorphisms in the SLC6A2 gene were associated with mood responses to *D-amphetamine. If confirmed, this observation may contribute to a better understanding of interindividual variations in the clinical response to amphetamine and in the risk of becoming addicted to amphetamine.*

Key Words: Amphetamine, elation, individual differences, norepinephrine transporter (SLC6A2) gene, polymorphisms, positive mood

Amphetamine is a short-acting psychostimulant drug that increases feelings of positive mood, euphoria, well-being, and alertness (Brauer and de Wit 1996); modulates attention; enhances cognitive performance; and decreases impulsive behavior (de Wit *et al.* 2002). The mood-altering effects appear to make the drug attractive as a drug of abuse, whereas its effects on attention and behavior make it clinically useful for patients with attention-deficit/hyperactivity disorder (ADHD). However, individuals vary in their responses to the drug. One third of ADHD individuals do not respond to amphetamine (Wilens *et al.* 2002), and individuals also appear to differ in their vulnerability to subjective euphorigenic effects of the drug (Brauer and de Wit 1996; de Wit *et al.* 1986; Gabbay 2003; Kuhar *et al.* 2001). Several recent laboratory studies confirm that acute responses to stimulant drugs vary across individuals (Kimberg *et al.* 1997; Mattay *et al.* 2000).

Interindividual differences in response to amphetamine have been associated with several factors, including personality (Hutchison *et al.* 1999), gender (White *et al.* 2002), expectancies (Mitchell *et al.* 1996), and social conditions (de Wit *et al.* 1997). There is growing evidence that genetic factors also contribute to variations in response to stimulants. Twin studies reveal a high concordance in responses to amphetamine in monozygotic twins (Crabbe *et al.* 1983; Nurnberger *et al.* 1982), and several studies have shown that genetic variations in the proteins involved in amphetamine's action may influence responses to the drug (Hohoff *et al.* 2005; Kirley *et al.* 2003; Lott *et al.* 2005; Mattay *et al.* 2003). One protein that has not as yet been investigated is the norepinephrine transporter (SLC6A2), a member of the Na^+/Cl^-–dependent transporter family, which removes norepinephrine from the synaptic cleft. Amphetamine alters norepinephrine transporter function and blocks norepinephrine reuptake from the synaptic cleft into presynaptic noradrenergic neurons (Sulzer *et al.* 2005; Xu *et al.* 2000).

The SLC6A2 gene is mapped to chromosome 16q12.2 (Brüss *et al.* 1993) and is composed of 14 exons that span more than 45 kb (Pörzgen *et al.* 1995; Figure 1). In the central nervous system, the SLC6A2 gene is expressed mainly in the locus ceruleus (Hahn *et al.* 2005) but also in other brain areas, the adrenal medulla and the vas deferens (Brüss *et al.* 1993; Cubells *et al.* 1995; Lorang *et al.* 1993). Polymorphisms in the 5'-flanking region of the SLC6A2 gene influence transcription regulation of the gene (Hahn *et al.* 2005; Kim *et al.* 1999; Meyer *et al.* 1998).

A deficit of norepinephrine in the synaptic cleft has been proposed as a determinant of depressive illness, and several antidepressants are inhibitors of norepinephrine uptake (Lambert *et al.* 2000; Stahl 2000). Mice lacking the SLC6A2 are hyperresponsive to locomotor stimulation by amphetamine, behave like antidepressant-treated wild-type mice (Xu *et al.* 2000), and are protected from generalization of depressive behavior (Haller *et al.* 2002). Genetic or acquired deficits in norepinephrine inactivation cause hyperadrenergic states that can provoke arousal in the individual (Shannon *et al.* 2000).

In the present study, we, therefore, examined interindividual D-amphetamine response in relation to eight SLC6A2 gene polymorphisms. In particular, we focused on the relationship between polymorphisms in the SLC6A2 gene and self-reported levels of arousal and positive mood after amphetamine medication. We hypothesized that variation in the SLC6A2 gene would

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Received March 8, 2006; revised September 11, 2006; accepted September 21, 2006.

0006-3223/07/$32.00
doi:10.1016/j.biopsych.2006.09.031

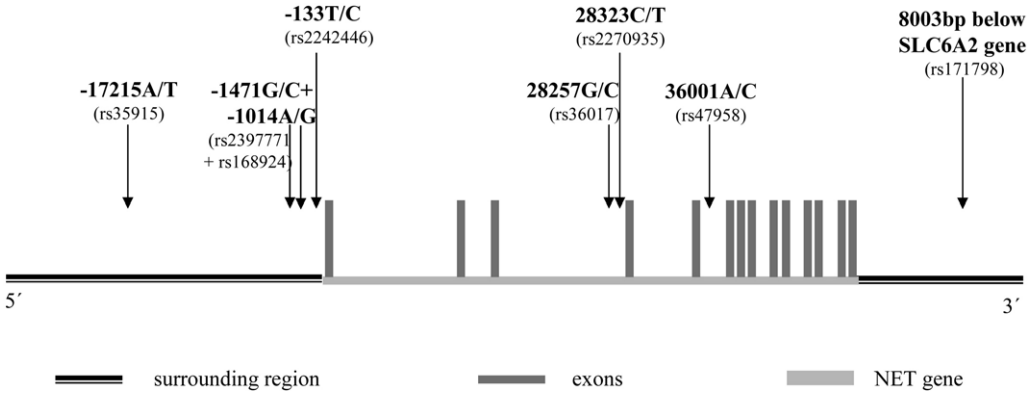

Figure 1. Genomic structure of SLC6A2 gene with 14 exons (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?CMD=search&DB=snp; Pörzgen *et al.* 1995). Meyer et al (1998) described the SLC6A2 gene with 15 exons and the start codon −1,004 bp further upstream than shown.

be associated with differences in subjective reports of positive mood and arousal after D-amphetamine.

Methods and Materials

Subjects

A total of 99 healthy male and female volunteers, 49 women and 50 men, were recruited. To reduce variability caused by withdrawal from caffeine or nicotine, subjects were excluded if they consumed more than three cups of coffee per day or smoked more than 10 cigarettes per week. All subjects participated in a psychiatric screening interview based on DSM-IV criteria (American Psychiatric Association 1994). Individuals were screened for Axis I disorders, but not for personality disorders. They completed several screening questionnaires as well as a psychiatric symptom checklist (SCL-90; Derogatis 1983), the Michigan Alcoholism Screening Test (MAST; Selzer 1971), and a health questionnaire with a detailed section on current and lifetime drug use. Candidates with any Axis I psychiatric disorder, including treatment for a substance-use disorder or a history of legal, personal, or employment problems related to drug use; any current medical condition requiring medication; or any other medical condition considered to be a contraindication for amphetamine (e.g., hypertension, pathological electrocardiogram) were excluded. Volunteers who had less than a high school education, who lacked fluency in English, and who worked a night shift also were excluded, as well as were pregnant or lactating women and women planning to become pregnant during the study.

Design

The study used a three-session, within-subjects design. Subjects participated in three sessions, separated by at least 48 hours, in which they received either placebo or D-amphetamine (10 mg or 20 mg). D-Amphetamine and placebo were administered in randomized order and under double-blind conditions. The study was approved by the University of Chicago's institutional review board and was performed in accordance with the Helsinki Declaration of 1975.

Volunteers first attended an orientation session in which the procedures were scheduled and rules and conditions were explained. Subjects signed the consent form, gave a blood sample for genotyping, and practiced self-report questionnaires and psychomotor tests. Volunteers were instructed to abstain from eating after midnight on the night before the sessions and to abstain from taking any drug, including alcohol, caffeine, and nicotine, for 24 hours before each session. Because of the altered pharmacokinetics of D-amphetamine with a dampened response during the luteal phase of the menstruation cycle (White *et al.* 2002), sessions for women were scheduled for the follicular phase only.

Sessions were conducted from 9:00 AM to 1:00 PM, at least 48 hours apart. At the beginning of each session, subjects provided urine and breath samples to confirm their compliance with drug and alcohol abstinence. They were given a light meal to reduce stomach irritation from amphetamine and completed measures and baseline mood questionnaires of predrug subjective effects. At 9:30 AM, a capsule containing D-amphetamine (10 or 20 mg) or placebo was administered with 100 mL of water. Compared with clinically recommended daily doses of up to 40 mg of amphetamine for school-aged children with ADHD (Greenhill *et al.* 2002; Spencer *et al.* 2006), we used relatively low doses of D-amphetamine to minimize risk to subjects. These doses, however, were sufficient to produce measurable subjective and behavioral effects in the participating healthy volunteers. Subjective, behavioral, and physiologic measures, including heart rate, blood pressure, and temperature, were obtained 30, 60, 90, 150, and 180 min after capsule ingestion. Subjective measurements consisted of ratings of drug effects and mood (see next section). At 1:00 PM, subjects were allowed to leave the laboratory.

Dependent Measures

Subjects completed three standardized questionnaires assessing subjective drug effects: the Drug Effects Questionnaire, the 49-item Addiction Center Inventory (Martin *et al.* 1971) and the Profile of Mood States (POMS; Johanson and Uhlenhuth 1980; McNair *et al.* 1971). For the present study, the POMS was used to examine the association between SLC6A2 gene polymorphisms and amphetamine mood response. The POMS is highly sensitive to the effects of drugs in samples of healthy volunteers and indicates current subjective drug effects. It consists of 72 adjectives that are used to describe momentary mood states on eight primary scales (anger, anxiety, confusion, depression, elation, fatigue, friendliness, and vigor) by using a five-point scale ranging from "not at all" (0) to "extremely" (4). As primary outcome measures, the composite scales for arousal [(anxiety + vigor) − (fatigue + confusion)] and positive mood (elation – Depression) were investigated. Peak change scores were calculated by subtracting the predrug baseline scores from the measured scores after 30, 60, 90, 150, and 180 min after drug ingestion. The value with the greatest distance from baseline was chosen as peak change score. In case of similar positive and negative maximum distances, 0 was used as peak change score.