Alpha Synuclein Protein Levels are Increased in Serum from Recently Abstinent Cocaine Abusers

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Abstract

Alpha synuclein is increased in dopamine neurons of cocaine abusers and in rats whose alcohol preference is inbred. Recent studies have shown increased alpha-synuclein protein expression in serum of alcoholic patients that is linked with severity of alcohol craving. The aim of this study was to analyze the serum levels of alpha synuclein in healthy subjects and in recently abstinent cocaine abusers. Alpha synuclein protein expression was measured by enzyme-linked immunosorbent assay in serum specimens obtained from 38 recently abstinent cocaine dependent patients and 14 control subjects. The presence of cocaine dependence disorder was based on the Structured Clinical Interview (DSM-IV). Drug severity was assessed by the Addiction Severity Index ratings and composite measures. Scores of the intensity and frequency of cocaine craving episodes were obtained from the Minnesota Cocaine Craving Questionnaire. The serum concentrations of alpha synuclein in cocaine dependent patients were significantly higher as compared with age-matched drug-free controls (p < 0.001). Alpha synuclein levels in blood were significantly correlated with the intensity (r = 0.60, p < 0.001) and frequency (r = 0.64, p < 0.001) of cocaine craving and with thirty days of cocaine use in the prior month before entry to treatment (r = 0.56, p < 0.005). However, there was no correlation between the serum protein levels of alpha synuclein and age in either group. This report is the first demonstration of altered alpha synuclein levels in peripheral blood from cocaine abusers. These data agree with previous reports in postmortem brain of cocaine abusers and provide support for an association between alpha synuclein and cocaine dependence.

Keywords

Cocaine; serum; dopamine; craving; synuclein

1. Introduction

Alpha synuclein is abundantly expressed in various brain regions and is particularly enriched in presynaptic terminals (Lavedan, 1998). Intracellular aggregates of the protein have been associated with synucleinopathies of Alzheimer's and Parkinson's disease (Eriksen et al., 2003; Sidhu et al., 2004). Animal models developed in mice and Drosophila have shown that...
pathological inclusions of alpha synuclein resulted in degeneration of dopaminergic neurons and motor deficits (Masliah et al 2000; Feany and Bender, 2000). In its normal context, alpha synuclein participates in diverse synaptic functions, including neuronal development, differentiation and neuroplasticity (Jenco et al., 1998; Maroteaux and Scheller, 1991; Papachroni et al., 2005). Alpha synuclein is expressed during development and maturation of dopaminergic neurons, and the protein regulates dopamine homeostasis in the adult brain by affecting neurotransmitter biosynthesis, the activity of the dopamine transporter, and presynaptic recruitment of dopamine for vesicular storage and release (Lee et al., 2001; Perez et al., 2002; Yavich et al., 2004).

Alpha synuclein is elevated in rats whose alcohol preference is inbred (Liang et al., 2003) and high levels are associated with an increase in alcohol craving in patients during withdrawal (Bonsch et al., 2005a; Bonsch et al., 2004). Peripheral blood alpha synuclein mRNA levels are elevated in cynomolgus monkeys that chronically self-administer ethanol (Walker and Grant, 2006). Alpha synuclein protein and mRNA is increased in midbrain dopaminergic neurons of cocaine abusers (Mash et al., 2003). Although the involvement of dopamine in reinforcement to drugs and alcohol is well established (Volkow et al., 1999), these findings link dysregulated alpha synuclein in addictions to cocaine and alcohol. The aim of this study was to determine whether the levels of alpha synuclein in blood were altered in recently abstinent cocaine addicts compared to non-drug using controls. We further correlated the serum levels of alpha synuclein with days of cocaine use in the prior month and with measures of the intensity and frequency of cocaine craving.

2. Methods and Materials

2.1 Subjects

Residual serum specimens were obtained from thirty-eight recently abstinent patients seeking treatment for cocaine dependence (34 male; 4 female). Each subject underwent a physical examination, blood tests and full psychiatric evaluation at treatment admission. Detailed demographic information was obtained and patients completed the Addiction Severity Index (McLellan et al., 1992). The average age was 36.8 ± 9.86 and the average duration of use of cocaine was 12.7 ± 7.1 (Table 1). All patients were diagnosed as cocaine dependent according to the DSM-IV criteria using a Structured Clinical Interview for DSM-IV diagnoses (SCID-IV; (Spitzer and First, 2005). The clinical interviews were carried out by a psychiatrist trained for application of the SCID-IV (F.R.E.). All of the subjects stopped cocaine and alcohol at program admission. Urinalysis, which tested for the presence of cocaine, opiates, amphetamine, and benzodiazepines was conducted at program intake. Urine specimens tested positive for cocaine in 90% of the patients. There was no other current substance dependence, but six subjects met DSM-IV criteria for alcohol dependence. None of the subjects had a history of a major medical illness or current use of prescription drugs. The intensity and frequency of cocaine craving was assessed at program entry using the Minnesota Cocaine Craving Scale (Halikas et al., 1991). The years of cocaine use and number of days of cocaine use in the last month were obtained by retrospective chart review. The retrospective chart review of patient clinical records was approved by the Institutional Review Board of the University of Miami Miller School of Medicine. Fourteen age-related control subjects (10 male; 4 female) were recruited from the general population. Control subjects were free from psychiatric or physical disturbances. The age range for the control subjects was 35.6 ± 5.2 years (male/female, 10/4).

2.2 Immunoassay of alpha synuclein in serum

Fasting blood was obtained at 8:00 AM from patients 24 to 72 hours after the last use of cocaine (28.8 ± 16.7 hours, mean ± S.D.). The blood samples were centrifuged after collection and residual serum specimens were stored at −80°C. Alpha synuclein was measured in serum by
an enzyme-linked immunosorbent assay performed according to the directions of the supplier (Biosource, Immunoassay Kit #KHB0061, Camarillo, CA 93012). The commercial kit uses a monoclonal antibody raised against full length human alpha synuclein to detect and quantify the level of total protein monomers. Purified recombinant protein standards of alpha synuclein were assayed in parallel with human serum. Standard curves with human recombinant alpha synuclein in control serum ($r^2 = 0.96$) were compared to the standard diluent buffer ($r^2 = 0.97$). The spiked sera from pooled controls gave results that were linear across the concentration range. Serum samples from patients and control subjects were analyzed together in batch runs. For some subjects, multiple assays were done on diluted serum from a single subject to verify that low or high concentrations were in the linear range of the assay. All measurements were repeated twice and the average value was determined.

2.3 Data analysis
Statistical calculations were carried out using SPSS Statistical Software Package (SPSS for Windows - version 14, Chicago, IL). Differences between cocaine dependent patients and control subjects were calculated using ANOVA and the Student's $t$ test. Correlation studies were obtained with the Pearson correlation coefficient. Values are expressed as mean ± standard deviation and a significance level of $p < 0.05$ was used.

3. Results
A total of 38 cocaine dependent subjects with long-standing histories of cocaine use and 14 control subjects were evaluated. Table 1 shows the demographic and clinical data of the cocaine dependent group. All of the subjects met DMS-IV criteria for cocaine dependence. The groups of patients and control subjects were similar in age with male predominance. Psychiatric comorbidity was 21.6% and none of the participants met DMS-IV criteria for dependence to cannabis, opiates, sedatives or other drugs. By self-report, 84% of the cocaine addicts were drug users who smoked crack cocaine. None of the cocaine dependent subjects in this sample were injectors. The number of days of cocaine use in the 30 days prior to treatment admission was 9.0 (S.D. 7.9) days.

As shown in Figure 1A, alpha synuclein protein expression was significantly elevated in recently abstinent cocaine dependent patients (35.7 ng/ml; SD, 26.6 ng/ml) as compared to age-matched control subjects (4.1 ng/ml; SD, 2.5 ng/ml). These values agree with previous reported serum levels by Bonsch and coworkers (2005a) for alpha synuclein in healthy controls (N = 50; 5.92 ng/ml; SD, 9.72 ng/ml). Alpha synuclein concentrations in human plasma samples in 44 normal donors gave median of 5.6 ng/ml (Fjorback et al,. 2007). These observations demonstrate a narrow concentration range for alpha synuclein in control subjects using a quantitative ELISA. In recently abstinent cocaine dependent patients, the serum levels ranged from 3.3 to 82.2 ng/ml with mean values elevated approximately 8-fold as compared to age-matched and drug-free controls (Fig. 1A). Patients who met criteria for cocaine and alcohol dependence had serum levels of alpha synuclein that were in the same range (N = 6; 32.4 ng/ml, SD 26.5). Age was not significantly correlated with the level of alpha synuclein in either controls ($r = 0.30$, $p = 0.31$) or abstinent cocaine dependent patients ($r = 0.16$, $p = 0.34$). The levels of alpha synuclein did not differ between male and female subjects in both the control and cocaine groups (data not shown; $p > 0.05$) in agreement with a recent study (Fjorback et al., 2007).

Independent variables at program admission were selected for determining their possible influences on craving measures (alpha synuclein protein, age, years of cocaine use, and days of use in the prior month). We observed a positive correlation between alpha synuclein protein levels and the intensity and frequency of cocaine craving. Individual patient scores on the reported intensity of craving were significantly associated with serum alpha synuclein levels.
The individual values for alpha synuclein are shown as a function of the intensity of cocaine craving (Fig. 1B) and the days of use in the prior month (Fig. 1C). The mean intensity rating for craving at program admission was 45.8 (SD 31.2). The frequency of craving episodes was correlated with individual levels of alpha synuclein ($r = 0.64$, $p < 0.001$). Not surprisingly, a positive correlation was observed between serum levels of alpha synuclein and the number of days of cocaine use in the prior month ($r = 0.56$, $p < 0.005$).

4. Discussion

We have measured alpha synuclein in serum from recently abstinent cocaine dependent patients using the ELISA method. The expression of alpha synuclein protein concentrations was significantly elevated when compared with age-matched control subjects. Furthermore, there was a significant association between alpha synuclein and cocaine craving scores. The serum levels of alpha synuclein were correlated with days of cocaine use in the prior month, providing additional evidence for increased levels of alpha synuclein with cocaine exposure.

Bonsch et al (Bonsch et al., 2004) reported the first study evaluating the expression of alpha synuclein in blood in patients with alcoholism. Later work demonstrated a correlation between the levels of alpha synuclein in serum with the intensity of alcohol craving in addicted patients during withdrawal (Bonsch et al., 2005a; Bonsch et al., 2004). Monkeys that chronically self-administered ethanol for 18 months had 3-fold higher peripheral blood levels of alpha synuclein mRNA, suggesting that peripheral alpha synuclein upregulates with chronic exposure to alcohol (Walker and Grant, 2006). We observed an elevated expression of alpha synuclein in recently abstinent cocaine dependent patients that is linked to measures of craving and days of use in the past month. Cocaine and alcohol are frequently used together (Hearn et al., 1991) and this combination is associated with enhanced CNS toxicity and dependence severity (Bolla et al., 2000). These results suggest that alpha synuclein may be a biomarker of the intensity of withdrawal-induced cravings in both cocaine abusers and alcoholics.

The gene for alpha synuclein maps to chromosome position 4q21.3–22 (Chen et al., 1995; Shibasaki et al., 1995; Spillantini et al., 1995). This region has been associated with drug abuse through a genome-wide SNP genome scan (Uhl et al., 2001). Genomic variation in SNCA has been associated with susceptibility to alcohol (Bonsch et al., 2005b), methamphetamine psychosis/dependence (Kobayashi et al., 2004) and alcohol craving (Bonsch et al., 2005a; Bonsch et al., 2005b; Foroud et al., 2000; Foroud et al., 2007). Using a family based test of association in multiplex alcoholic pedigrees, analyses in the 3′ end of SNCA identified a haplotype overestimated in European-American individuals who crave alcohol and a different haplotype, which was preferentially transmitted to noncravers (Foroud et al 2007). These observations are consistent with a previous report in alcohol preferring P rats, which proposed that SNPs in the 3′UTR of *Snca* contribute to alcohol preference (Liang et al., 2003). However, a more exhaustive analysis in two American Indian populations failed to find an association of alpha synuclein with alcohol and drug dependence (Clarimon et al., 2007). Although several SNPs initially returned significant p values, none of the results remained significant after correction for multiple testing, suggesting that SNCA was not a factor for addiction vulnerability in these populations. Altered DNA methylation patterns in the alpha synuclein promoter have been associated with alcoholism, suggesting that epigenetic factors may contribute to differential expression of the protein in certain vulnerable populations (Bonsch et al., 2005c). Whether addicted individuals with high alpha synuclein concentrations are at increased risk for craving and relapse due to promoter polymorphisms requires further study.

Peripheral blood alpha synuclein is reactive with epitope specific antibodies and is indistinguishable from the human brain protein (Li et al., 2007). How alpha synuclein reaches serum from the brain in unknown, but blood levels of alpha synuclein may reflect the gene expression in the brain.
dosage and/or altered protein expression, since triplication of the alpha synuclein gene causes increased brain expression and a doubling of blood alpha synuclein (Miller et al., 2004). Cocaine abuse leads to state dependent increases in the levels of alpha synuclein in postmortem brain (Mash et al., 2003; Qin et al., 2005) and peripheral blood as shown in this report. Dysfunction of central dopaminergic neurotransmission has been proposed as a mechanism for withdrawal severity and craving in drug and alcohol dependence (Self and Nestler, 1995) and changes in dopamine are associated with the level of craving reported by cocaine abusers with the largest effects seen in the most severely dependent (Volkow et al., 1999). Because alpha synuclein is involved in the modulation of dopaminergic activity, these preliminary results support a role for alpha synuclein gene expression in the intensity of craving for drugs or alcohol. The relationship between alpha synuclein and the risk for withdrawal-induced craving requires study in a large cohort. Further study is needed to determine if repeated measures of alpha synuclein in serum correlate with changes in cravings and rates of relapse in abstinent cocaine abusers.

References

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Walker SJ, Grant KA. Peripheral blood alpha-synuclein mRNA levels are elevated in cynomolgus monkeys that chronically self-administer ethanol. Alcohol (Fayetteville, NY 2006;38:1–4.
Figure 1.
Measures of alpha synuclein in serum samples from recently abstinent cocaine abusers. A. Median and quartiles (box plots) as well as 10th and 90th percentiles (whiskers) of the of the concentration of alpha synuclein in cocaine dependent (N = 38) and aged-matched drug-free control subjects (N = 14). Alpha synuclein serum concentrations were significantly increased in the cocaine abusers compared with those of the aged-matched drug-free control group (t-test, p < 0.0001) Thick lines represent the mean value. Correlation between alpha synuclein serum protein concentrations and self-reported ratings for craving intensity and the pattern of use in cocaine dependent patients (N = 38). A significant positive correlation was demonstrated between alpha synuclein serum concentrations and B) intensity of desire to use (Pearson $r =$
0.60, \( p<0.0001 \), and C) days of use in month prior to treatment (Pearson \( r = 0.56, p<0.0005 \)). Similar significant results were obtained for the frequency rating of cocaine craving as described in the Results section.
### Table 1

Demographic Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years); mean (S.D.)</td>
<td>36.8 (9.86)</td>
</tr>
<tr>
<td>Male (%)</td>
<td>86.5</td>
</tr>
<tr>
<td>Race/ethnic group (%)</td>
<td></td>
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<tr>
<td>White</td>
<td>83.8</td>
</tr>
<tr>
<td>Hispanic</td>
<td>16.2</td>
</tr>
<tr>
<td>Years of Education; mean (S.D.)</td>
<td>14.1 (1.96)</td>
</tr>
<tr>
<td>Years of Cocaine Use; mean (S.D.)</td>
<td>12.7 (7.1)</td>
</tr>
<tr>
<td>Days of Use in Last Month; mean (S.D.)</td>
<td>9.0 (9.9)</td>
</tr>
<tr>
<td>ASI Composite Scores; mean (S.D.)</td>
<td></td>
</tr>
<tr>
<td>Medical</td>
<td>0.17 (0.31)</td>
</tr>
<tr>
<td>Educational</td>
<td>0.32 (0.29)</td>
</tr>
<tr>
<td>Alcohol</td>
<td>0.20 (0.25)</td>
</tr>
<tr>
<td>Legal</td>
<td>0.23 (0.09)</td>
</tr>
<tr>
<td>Family</td>
<td>0.41 (0.27)</td>
</tr>
<tr>
<td>Psychiatric</td>
<td>0.36 (0.23)</td>
</tr>
<tr>
<td>Current Alcohol Dependence %</td>
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<tr>
<td>Current Co-morbid Axis I Disorder %</td>
<td>21.6</td>
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<tr>
<td>Current Axis II Disorder %</td>
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