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Increased Expression of c-Jun Transcription Factor in Cerebellar Vermis of Patients with Schizophrenia

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In the cerebellar vermis of schizophrenic patients, our previous studies have revealed alterations in the mitogen-activated protein (MAP) kinase signaling cascade and downstream transcription factors within the c-fos promoter. Since the proteins of the Fos and Jun families of immediate-early genes dimerize to form activating protein (AP)-1, the present study was conducted to examine the expression of Jun transcription factors in schizophrenic and control subjects. Using Western blot analysis, we determined the protein levels of c-Jun, Jun B, and Jun D as well as the levels of c-jun mRNA by relative RT-PCR in post-mortem samples from cerebellar vermis. The expression of c-Jun protein and c-jun mRNA was significantly increased in the cerebellar vermis of patients with schizophrenia, whereas no significant differences were found in the expression of Jun B or Jun D proteins. Studies in rats indicated that the abnormal expression of c-Jun transcription factor observed in schizophrenic patients was not related to post-mortem intervals or chronic treatment with antipsychotic medications. This study provides new insights into cerebellar abnormalities of schizophrenia at the level of expression of c-Jun that target key genes associated with the MAP kinase cascade.
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INTRODUCTION

Dysfunction of glutamatergic *N*-methyl-D-aspartate (NMDA) receptor-mediated neurotransmission, defect in the regulator of G-protein signaling 4 (RGS4) expression, and neurotrophic abnormality have been implicated in schizophrenia (Olney and Farber, 1995; Coyle, 1996; Mirnics *et al*, 2001; Takahashi *et al*, 2000). In neurons, extracellular stimuli, such as NMDA receptors, nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and NT-4 can trigger activation of the mitogen-activated protein (MAP) kinase signal transduction cascades, and these signals are then relayed to the nucleus to activate transcription factors and gene expression (Fiore *et al*, 1993; Kurino *et al*, 1995; Xia *et al*, 1996; Vanhoutte *et al*, 1999; Boulton *et al*, 1991; Bonni *et al*, 1999; Kaplan and Miller, 2000; Cavanaugh *et al*, 2001). Three classes of MAP kinases, referred to as extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK), and p38, have been identified in mammals. One of the most widely studied nuclear targets of the MAP kinase pathways is the activator protein (AP)-1 transcription factor (Whitmarsh and Davis, 1996). AP-1 is a homodimer and/or heterodimeric complex composed of members of the Jun (c-Jun, Jun B, and Jun D) and Fos (c-Fos, Fos B, Fra-1, and Fra-2) families. Dimerization can also occur with other transcription factor families, such as CREB and ATF (Herdegen and Leah, 1998).

Accumulating evidence suggests that transcriptional regulation of genes plays a key role in the molecular mechanisms of critical cellular events, including neuronal plasticity, synaptic transmission, and morphological differentiation. Recent studies have also indicated that immediate-early genes jun and fos, which are widely expressed in the central nervous system, may play an important role in long-term potentiation, learning, and memory (Dragunow, 1996). Despite the increasing number of studies linking the immediate-early genes to important brain functions, their role in various neuropsychiatric disorders including schizophrenia is still poorly understood.

Previously, we reported elevated expression of several intermediates of the ERK pathway in post-mortem brain tissue from individuals with schizophrenia. We found increased protein levels of MAP kinase ERK2 in cerebellar vermis, but not in mesopontine tegmentum or frontal pole (Brodmann area 10) (Kyosseva *et al*, 1999). Furthermore, we demonstrated that the levels of several transcription factors within the promoter region of c-fos gene, that is, Elk-1,

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cyclic adenosine monophosphate (cAMP) response element binding protein (CREB), and activating transcription factor-2 (ATF-2) were elevated in the cerebellar vermis in schizophrenic patients as well (Kyosseva *et al*, 2000). In this study, we have determined whether members of the Jun family of transcription factors are altered in the brain of patients with schizophrenia. The studies were conducted to measure the expression of c-Jun, Jun B, and Jun D proteins by Western blot in cerebellar vermis of schizophrenic and control subjects, as well as the expression of c-jun mRNA by relative reverse transcription-polymerase chain reaction (RT-PCR). Our focus was the cerebellar vermis, since post-mortem and neuroimaging studies have described abnormalities in cerebellum in schizophrenia. Thus, patients with schizophrenia were shown to have a smaller anterior vermis (Weinberger *et al*, 1980) and smaller Purkinje cell size (Tran *et al*, 1998). Magnetic resonance imaging (MRI) studies have also described a significant reduction in the volume of the vermis as well (Nopoulos *et al*, 1999, 2001; Ichimiya *et al*, 2001). Several recent post-mortem studies have reported increased expression of nitric oxide synthase (Karson *et al*, 1996; Bernstein *et al*, 2001), decreased expression of synaptic proteins synaptophysin, complexin II (Eastwood *et al*, 2001b), and SNAP-25 (Mukaetova-Ladinska *et al*, 2001), as well as abnormalities in the expression of serotonin 5-HT_{2A} receptors in the cerebellum in schizophrenia (Eastwood *et al*, 2001a). In addition, new evidence suggests that the cerebellum may play a role in cognition, behavior, and psychiatric illness (Allen *et al*, 1997; Rapoport *et al*, 2000).

MATERIALS AND METHODS

Subjects

Post-mortem brain tissue was obtained after consent for autopsy from the Central Veterans Healthcare System (Little Rock, AR) and the University of Arkansas for Medical Sciences (UAMS). All the procedures were approved by the Human Research Advisory Committee of UAMS.

Psychiatric diagnosis was made post mortem by using the Diagnostic Evaluation After Death (Salzman *et al*, 1983). This is a structured chart review for which the main source of information is the medical record. In addition, information obtained from family members and treating psychiatrists was incorporated when available. Diagnostic Evaluation After Death was used whether the patient was being evaluated pre- or post mortem. Many of the elderly psychiatric patients were interviewed pre mortem, and diagnoses were established independently by two trained psychiatrists according to DSM-III-R criteria. Clinical and pathological data for schizophrenic and control subjects are summarized in Table 1. Post-mortem brain tissue was obtained from nine patients with schizophrenia (eight men and one woman) and 12 control subjects (10 men and two women). Most patients with schizophrenia were receiving antipsychotic medications at the time of death. Two patients with schizophrenia had histories of alcohol abuse. Medical records from control subjects were reviewed to determine whether they had an active psychiatric disorder at the time of their death and/or earlier in their lives. Two control subjects had histories that were consistent with alcohol dependence, which ceased 8–9 years prior to death. A third subject had an uncertain history of alcoholism that may have been ongoing at the time of his death; two control subjects were prescribed very low doses of amitriptyline (50 mg at bedtime) and mesoridazine, respectively, for sleep or pain control. In both the groups (schizophrenic and control), the cause of death was generally cancer, cardiac failure, or a terminal respiratory condition.

Neuropathology

All brain specimens were subjected to standard gross and microscopic neuropathological examination by a board-certified neuropathologist. In addition to the routine hematoxylin and eosin staining, selected sections were stained with the Sevier–Munger modification of Bielschowski's stain to evaluate neuritic plaques and neurofibrillary tangles. All cases used in this study were free of significant neuropathological change, including Alzheimer's disease as determined by using CERAD criteria (Mirra *et al*, 1991). Mild senile changes (normal for age) were presented in six subjects (two schizophrenic and four controls), and one schizophrenic showed a small infarction in the occipital lobe (Table 1). After collection during autopsy, the brain tissue was dissected and stored at -80°C. Cerebellar vermis was taken from the anterior vermis just of midline.

Effect of Post-Mortem Interval (PMI)

Adult male Sprague–Dawley rats (approximately 250 g) were purchased from Harlan Sprague–Dawley, Inc. (Indianapolis, IN) and were allowed to acclimate to their new environment for several days before the experiments. Animal use procedures were in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of UAMS. The rats were euthanized by carbon dioxide, and then decapitated either immediately or after 3, 6, 9, 12, and 24 h. All the killed rats were kept 1 h at room temperature and then transferred to a refrigerator maintained at a temperature of 4–8°C, in which they remained until decapitation and dissection. This procedure is analogous to our post-mortem human procedures, in which all bodies are transferred to the morgue within 1.5 h of death and kept under refrigeration until autopsied. The rat brains were rapidly removed and cerebellar vermis was dissected, and stored at -80°C until use.

Effect of Haloperidol and Risperidone

Sprague–Dawley rats (approximately 250 g) were given subcutaneous injections of haloperidol in concentrations of 0.15 or 1.5 mg/kg, rispredone in concentrations of 0.05 or 0.5 mg/kg, or saline daily for 21 days. High doses for both drugs were representative of human treatments for severe psychosis. The rats were killed by decapitation 24 h after the last injection. The brains were rapidly removed and cerebellar vermis was dissected, and stored at -80°C until use.

Table 1 Clinical and pathological data for schizophrenic and control subjects

Age (years)/sex/ time ill (years)	Medication at death (mg/day)	PMI (h)	Weight (g)	Neuropathology[a]	Cause of death
Schizophrenic patients					
82/M/37	Trifluoperazine (10) Trihexyphenidyl (4)	9.0	1250	Normal	Myocardial infarction
80/M/51	Haloperidol (20)	9.0	—	Mild senile change[b]	Pneumonia
72/M/48	Mesoridazine (25)	3.0	—	Mild senile change[b]	Renal failure
68/M/32	Carbamazepine (200) Haloperidol (40)	12.5	—	Normal	Cardiac arrest
65/M/32	Haloperidol (15)	16.0	1275	Normal	Pneumonia
58/M/34	Fluphenazine (20) Haloperidol (11)	4.0	1240	Single small acute infarction in occipital lobe	Respiratory failure
52/M/20	Loxapine (40)	7.0	1520	Normal	Myocardial infarction
47/M/18	Haloperidol (20)	3.0	1380	Normal	Chronic obstructive pulmonary disease
42/F/21	Clozapine (450)	5.5	1190	Normal	Pulmonary thrombotic embolism
Normal controls					
79/M	None	13.0	1270	No tissue available	Cancer
76/M	None	5.0	1470	Normal	Cancer
76/M	None	3.0	1480	Normal	Cardiac arrest
73/M	None	4.5	1180	Mild senile change[b]	Pneumonia
72/M	Mesoridazine	5.0	1180	Mild senile change[b]	Cancer
68/M	Amitriptyline (50)	4.0	1220	Mild senile change[b]	Pulmonary thrombotic embolism
68/M	None	5.0	1415	Normal	Sepsis
66/M	None	3.5	1360	Normal	Cancer
66/M	None	4.0	1430	Normal	Respiratory failure
58/M	None	4.5	1150	Mild senile change[b]	Heart failure
57/F	Unknown	14.0	1100	Normal	Cancer
49/F	None	7.0	1275	Normal	Pulmonary thrombotic embolism

PMI indicates post-mortem interval; M, male; F, female.
[a]Determined using Consortium to Establish a Registry for Alzheimer's Disease (CERAD) criteria.
[b]Diagnosis not definitive for Alzheimer's Disease by CERAD criteria.

Isolation of Nuclear Fractions and Western Blot Analysis

Nuclear fractions from human and rat tissue were isolated as previously described (Kyosseva *et al*, 2000). Nuclear extracts (25 μg protein) were separated by 10% sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis. After electrophoresis, the proteins were electrophoretically transferred to nitrocellulose membranes in a buffer containing 25 mM Tris-HCl, pH 7.4, 190 mM glycine, and 20% methanol for 2 h. The nonspecific binding of proteins was performed by blocking the membranes with 5% nonfat dry milk in Tris-buffered saline/0.1% Tween-20 (TBST), pH 7.5 for 1 h. The blots were incubated with a primary c-Jun polyclonal antibody corresponding to residues 55–67 of human c-Jun from Cell Signaling Technology (Beverly, MA), diluted 1 : 1000 in TBST containing 5% bovine serum albumin overnight at 4°C or polyclonal Jun B (N-17), and Jun D (329) antibodies from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA) diluted 1 : 1000 in TBST containing 5% milk. After several washings with TBST, the blots were incubated for 1 h at room temperature with horseradish peroxidase-conjugated anti-rabbit IgG, diluted 1 : 2000 in TBST containing 5% milk. After further washings, the immunoblots were detected using the enhanced chemiluminescence (ECL) detection system provided with the antibody kit. The blots were stripped of detection antibodies by washing the membranes several times with water and then incubated in a buffer containing 100 mM 2-mercaptoethanol, 2% SDS, 62.5 mM Tris-HCl, pH 6.7 at 55°C for 30 min. After several washes in TBST, the blots were reprobed with a polyclonal actin (I-19) antibody from Santa Cruz Biotechnology, Inc. The autoradiograms were scanned with a Computerized Laser Densitometer model 300A (Molecular Dynamics, Sunnyvale, CA). Densities of the immunoreactive bands were analyzed with Image Quant software (Molecular Dynamics). The optical density of each band is expressed in arbitrary densitometric units. All the experiments were performed at least three times with similar results.

Relative Reverse Transcription Polymerase Chain Reaction

Total RNA was isolated from 0.1 g samples of frozen (−80°C) human brain tissue taken from the cerebellar vermis using RNease Mini Kit (Qiangen, Valencia, CA). RNA concentrations were calculated from the optical density at 260 nm, and the purity was determined by A_{260}/A_{280}. The integrity of the isolated RNA was evaluated by gel electrophoresis on 1.25% precast agarose gel (Sigma) in MOPS buffer stained with ethidium bromide. Total RNA (300 ng) from each sample was reverse transcribed using 'Ready-to-Go You-Prime-First Strand' Beads (Amersham Pharmacia Biotech, Piscataway, NJ) and oligo $d(T)_{15}$ (Promega, Madison, WI). A volume of 2 μl of each RT products were PCR amplified in a final volume of 20 μl using Taq PCR master mix (Qiagen, Valencia, CA) and

0.5 μl of each c-jun gene-specific primers (final concentration 1 μM). The levels of 28S rRNA served as an endogenous control. The sequences of c-jun and 28S rRNA primers were as follows: c-jun 5′-primer: bases 2514–2540; c-jun 3′ primer: bases 2675–2651; expected PCR fragment: 162 bp; 28S rRNA 5′-primer: bases 4535–4564; 28S rRNA 3′-primer: bases 4667–4638, expected PCR fragment: 133 bp (Puntschart *et al*, 1998).

The reactions were run on a Gene Amp PCR System 2400. After initial denaturing at 94°C for 2 min, the subsequent cycles for c-jun were as follows: denaturing for 10 s at 94°C; annealing for 90 s at 37°C (five cycles), at 45°C (five cycles), and at 65°C (23 cycles); extension for 30 s at 72°C; and final extension for 5 min at 72°C. In all 16 cycles of PCR, each consisting of denaturing at 94°C for 10 s, annealing at 60°C for 90 s, and extension at 72°C for 10 s were performed for 28S rRNA. Total PCR products were electrophoresed on 1.25% agarose gel in TBE and stained with ethiduim bromide. The area and density of PCR product bands were measured using Scion Image Program for IBM (Scion Corporation) and the resulting measures were expressed as arbitrary densitometry units and were statistically analyzed.

Statistical Analysis

Results are presented as mean ± standard deviation (SD) values. For human post-mortem studies, data from experimental groups were compared using analysis of variances ANOVA *post hoc* test. Correlations were carried out using the Spearman rank correlation. For experiments examining the effects of antipsychotic medications in rats, data were analyzed using a Friedman two-way analysis of variance test and the results are expressed as χ^2 statistic. The statistical analysis was conducted using software StatView for Windows, version 4.5 (Abacus Concepts, Inc., Berkley, CA). *p*-Values less than 0.05 were considered to be statistically significant.

RESULTS

Effect of Age, PMI, and Duration of Illness on C-Jun Expression

Demographic data for schizophrenic and control subjects are shown in Table 1. Schizophrenic patients did not significantly differ from controls in age (mean ± SD, 63 ± 14 *vs* 67 ± 9 years; $p = 0.39$), PMI (mean ± SD, 7.2 ± 4.7 *vs* 6.7 ± 4.1 h; $p = 0.77$), or brain weight (mean ± SD, 1309.2 ± 120.9 *vs* 1294 ± 132 g; $p = 0.81$). There was no significant correlation between c-Jun protein levels and age ($r = -0.25$, $p = 0.27$) or PMI ($r = -0.08$, $p = 0.74$). Similarly, there was no significant correlation between c-jun mRNA and age ($r = 0.16$, $p = 0.51$) or PMI ($r = -0.19$, $p = 0.45$). We also did not observe a correlation between duration of illness and c-Jun protein ($r = 0.24$, $p = 0.43$) or mRNA expression ($r = -0.22$, $p = 0.53$).

c-Jun, Jun B, and Jun D Levels in Post-Mortem Cerebelar Vermis

Nuclear fractions of cerebellar vermis from patients with schizophrenia and age-matched control subjects were electrophoresed on SDS polyacrylamide gel electrophoresis and blots were probed with antibodies to c-Jun, Jun B, and Jun D. The c-Jun antibody recognized a protein of approximately 46 kDa, whereas for Jun B and Jun D, proteins of about 39 kDa were detected on the autoradiograms. Representative immunoblots showing data for c-Jun, Jun B, and Jun D levels from one schizophrenic and one control subject are presented in Figure 1a. The immunoblots were reprobed with the housekeeping protein actin to verify equal loading and to help control for nonspecific differences. Differences in the levels of Jun proteins were quantified by densitometry of immunoblots from nine patients with schizophrenia and 12 control subjects. As seen in Figure 1b, the schizophrenics had c-Jun levels significantly higher than those of control subjects (mean ± SD, 1044 ± 469 *vs* 350 ± 346 arbitrary densitometric units, $p = 0.0009$, respectively). Four patients with schizophrenia showed relatively low expression of c-Jun protein, but there was no direct association with any demographic characteristics or antipsychotic treatment (eg one patient was on haloperidol, second received haloperidol in combination with carbamazepine, the third was on loxapine, and the fourth received trifluoperazine and trihexylphenidyl). In contrast to the elevated c-Jun levels, the protein expression of the other two members of Jun family, Jun B (mean ± SD, 724 ± 124 *vs* 660 ± 116 arbitrary densitometric units, $p = 0.24$), and Jun D (mean ± SD, 2410 ± 334 *vs* 2389 ± 254 arbitrary densitometric units, $p = 0.87$)

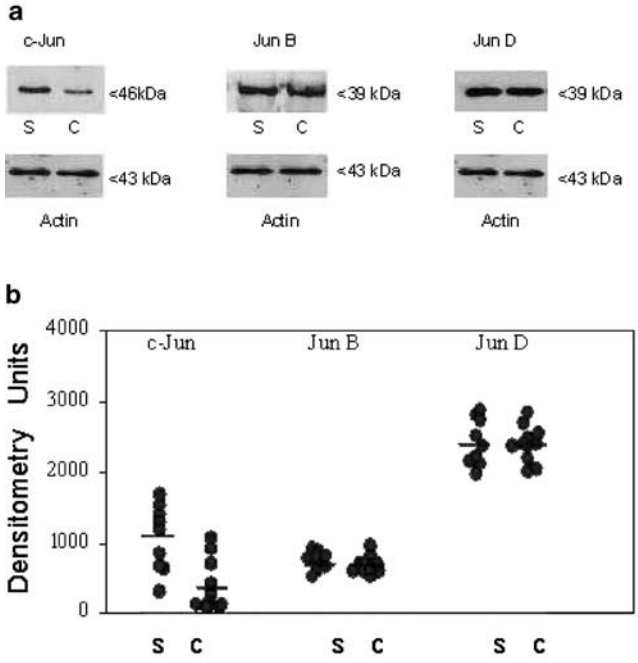

Figure 1 Representative immunoblots of c-Jun, Jun B, and Jun D (a) in cerebellar vermis from one patient with schizophrenia (s) and one control subject (c). Samples (25 μg protein) from nuclear fractions were subjected to SDS polyacrylamide gel electrophoresis, and the resulting gels were processed for Western blot analysis as described in 'Materials and methods' section. The blots were stripped and reprobed with actin antibody. Actin was used as a loading control. Autoradiograms from nine schizophrenic and 12 control subjects were scanned by densitometer and data are expressed in densitometry units (b). Values are means ± SD.

appeared to be unaltered in schizophrenic patients as compared to the control subjects.

c-jun mRNA Expression in Post-Mortem Cerebelar Vermis

To further determine whether the increased protein levels of c-Jun were due to increased gene expression, we examined the mRNA levels of c-jun in nine patients with schizophrenia and nine control subjects by relative RT-PCR. Representative gel electrophoresis of PCR products from one schizophrenic and one control subject using gene-specific c-jun (162 bp) and 28S rRNA (133 bp) primers is presented in Figure 2a. The levels of 28S rRNA served as an internal standard to ensure equal loading of RNA. The results showed statistically significant elevation of c-jun mRNA in the vermis of schizophrenic brains (2853 ± 646 *vs* 1573 ± 488, $p < 0.0002$, Figure 2b) and thus confirmed the established elevation of c-Jun protein levels.

Effect of PMI and Antipsychotic Medications on c-Jun Expression

We have previously shown that the protein levels of transcription factors Elk-1, CREB and ATF-2 did not change significantly in rat brain during storage of the tissue for 24 h at 4°C (Kyosseva *et al*, 2000). In this study, we also determined the post-mortem stability of c-Jun protein and mRNA levels at time intervals of 0, 3, 6, 9, 12, and 24 h. As shown in Figure 3, we did not detect significant changes of c-jun expression after the rat brains were left for 24 h at 4°C.

Next, we determined whether the increased protein levels of transcription factor c-Jun could result from treatment with antipsychotic drugs, since most of the patients included in this study had an extensive lifetime exposure to medications. The typical antipsychotic haloperidol (0.15 and 1.5 mg/kg) or the atypical antipsychotic risperidone (0.05 and 0.5 mg/kg) was administered to rats (six per experimental group) by injection for 21 days. Densitometry of the immunoblots presented in Figure 4 shows that chronic treatment with two different doses of either haloperidol or risperidone does not have any effect on the protein levels of c-Jun ($\chi^2_4 = 2.67$, $p = 0.61$).

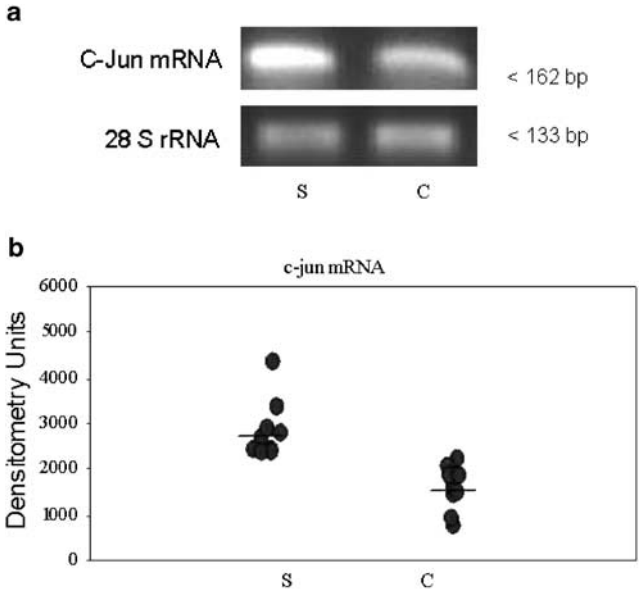

Figure 2 Relative RT-PCR of c-jun mRNA in cerebellar vermis from patients with schizophrenia and control subjects. Total RNA (300 ng) were reverse transcribed and amplified with polymerase chain reaction using gene-specific c-jun (162 bp) and 28S rRNA (133 bp) primers. 28S rRNA served as an internal standard. The upper panel (a) shows representative electrophoresis on a 1.25% agarose gel stained with ethidium bromide of PCR products from 1 schizophrenic (s) and one control (c) subject. Data for c-jun mRNA from nine schizophrenic and nine control subjects were analyzed by measuring the area and density of electrophoretic bands, and are expressed in densitometry units (b). Values are means ± SD.

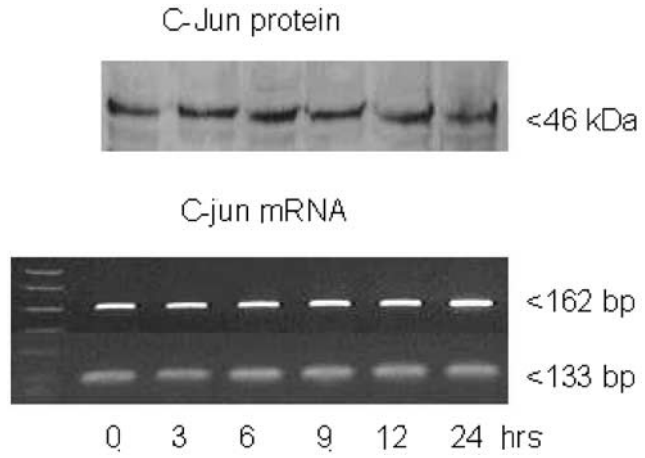

Figure 3 Effect of PMI on c-Jun protein and mRNA levels in rat cerebellum. Rats (three per experimental group) were killed and their brains were removed for dissection at 0 time, 3, 6, 9, 12, 24 h. Nuclear fractions (25 μg protein) were subjected to SDS polyacrylamide gel electrophoresis and Western blot analysis. The c-jun mRNA levels were determined by relative RT-PCR using gene-specific primers for c-jun (162 bp) and 28 S rRNA (133 bp).

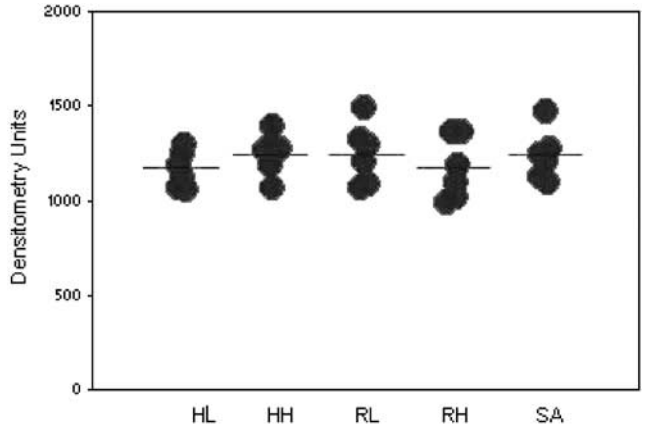

Figure 4 Effect of haloperidol and risperidone on c-Jun protein levels in rat cerebellum. Rats (six per treatment group) received haloperidol by injections at low (HL, 0.15 mg/kg/day) or high (HH, 1.5 mg/kg/day), risperidone at low (RL, 0.05 mg/kg/day) or high (RH, 0.5 mg/kg/day) doses, or saline for 21 days. Nuclear fractions were isolated and subjected to SDS polyacrylamide gel electrophoresis and Western blot analysis. The resulting autoradiograms were scanned by densitometer and data are expressed as densitometry units. Values are means ± SD.

DISCUSSION

We have recently provided evidence that schizophrenia is associated with alterations of the ERK signal transduction pathway in the cerebellar vermis in both patients and a rodent phencyclidine (PCP) model of schizophrenia (Kyosseva *et al*, 1999, 2001). We also showed that downstream transcription factor targets within the promoter of c-fos were upregulated in post-mortem cerebellar vermis (Kyosseva *et al*, 2000). These earlier findings prompted us to elucidate the possible involvement of Jun transcription factors in the cerebellar abnormalities in schizophrenia, since proteins of jun and fos leucine zipper families dimerize to form AP-1 transcription factor that has been shown to be involved in CNS disorders and development (Pennypacker, 1995). In this study, we examined by Western blot analysis the protein levels of c-Jun, Jun B, and Jun D in cerebellar vermis of patients with schizophrenia and control subjects. We found significant elevation in the expression of c-Jun protein in schizophrenic patients. We also showed, by relative RT-PCR, that the expression of c-jun mRNA was significantly increased in the vermis of schizophrenic subjects compared with control subjects. Neither Jun B nor Jun D protein was increased in the cerebellar vermis from patients with schizophrenia. Such selective alterations of Jun family of transcription factors in post-mortem brain of schizophrenics may be of major interest, since the transcriptional regulation of genes plays an important role in the disease process.

Our findings of increased protein levels of c-Jun in patients with schizophrenia could suggest increased phosphorylation of this transcription factor. However, using commercially available antibodies, we were unable to detect the phosphorylated active form of c-Jun. The phosphorylation of proteins cannot be reliably determined in post-mortem tissue due to rapid dephosphorylation, which can affect the expression of phophorylated active forms. At this point, however, we cannot comment if the observed overexpression of c-Jun protein levels is caused by increased phosphorylation.

Treatment with the typical antipsychotic drug haloperidol is shown to increase the expression of the immediate-early genes in the striatum and nucleus accumbens and the therapeutic affect of this agent is modulated by NMDA-dopamine D2 receptor interactions (Leveque *et al*, 2000; Hussain *et al*, 2001). The atypical antipsychotic drugs including risperidone have different affinities for several neurotransmitter receptors, such as dopamine, serotonin, muscarinic, and adrenergic receptors (Lieberman *et al*, 1998; Meltzer, 1999; Svensson, 2000). Therefore, in the present study, we tested whether the induced c-Jun expression in the cerebellar vermis could be increased by effects of antipsychotic treatment, because most of the schizophrenic patients included in our study (Table 1) had received antipsychotic medications. However, in contrast to the observations in humans, there were no significant differences in the levels of c-Jun protein in haloperidol- or risperidone-treated rats and controls. A very recent study has shown that chronic 17-day treatment with haloperidol and clozapine increased the expression levels of jun and fos family immediate-early genes in the rat prefrontal cortex using *in situ* hybridization (Kontkanen *et al*, 2002). Taken together, these results indicate a differential and brain region-specific regulation of jun and fos genes by antipsychotic drug treatment. Although further work is required to determine the precise mechanisms by which different antipsychotics exert their effects on gene expression, the results of elevated c-jun expression in cerebellar vermis in schizophrenic patients do not seem to be directly related to the chronic antipsychotic drug treatment and thus may represent a neuropathological feature of the disease itself.

The finding of abnormal protein and gene expression of c-Jun in cerebellar vermis of patients with schizophrenia may be relevant to the notion that the cerebellum may be an important neroanatomic region in the pathophysiology in psychiatric disorders, including schizophrenia (Katsetos *et al*, 1997). Until recently, little attention has been paid to the role of the cerebellum, presumably due to its primary role in motor control (Martin and Albers, 1995). However, increasing numbers of studies have found evidence that links the cerebellum with nonmotor functions (Middleton and Strick, 1994, 1998; Paradiso *et al*, 1997; Schmahmann, 1998). These findings support a role for the cerebellum in diverse cognitive functions, including attention, working memory, verbal learning, and memory. Recent evidence derived from anatomical, physiological, and functional neuroimaging studies has suggested cognitive function impairments in schizophrenic patients (Jacobsen *et al*, 1997; Wassink *et al*, 1999). In addition, several PET studies have found abnormalities in cerebellar blood flow in a variety of cognitive tasks in patients with schizophrenia (Andreasen *et al*, 1996, 1997). Based on the above new evidence, it has been hypothesized that in schizophrenia, there is a disturbance in the cortical–cerebellar–thalamic–cortical circuit, producing a fundamental cognitive deficit in the disease (Wassink *et al*, 1999). In addition, cerebellar abnormalities, particularly a decrease in tissue volume, abnormalities in cerebellar blood volume and expression of tyrosine receptor kinase B, reelin, and glutamic acid decarboxylase (GAD) 67 mRNA levels have been found in patients with mood disorder (Soares and Mann, 1997; Loeber *et al*, 2002; Bayer *et al*, 2000; Guidotti *et al*, 2001).

It is increasingly recognized that transcription factors play a critical role in the development and functioning of the normal nervous system, as well as adaptation of the nervous system to various stimuli such as injury, growth factors, and drug treatment, as well as physiological and pathological events (Morgan and Curran, 1991; Herdegen and Leah, 1998; Duman *et al*, 1999). Transcription factors are nuclear proteins recognizing specific DNA sequences in gene promoters that modulate transcriptional activity in response to extracellular stimuli. The MAP kinase pathways provide a common route by which signals from different receptors, including heterotrimeric G-protein coupled receptors, converge at major regulatory elements of promoters of the immediate-early genes including jun and fos. Several studies have shown changes in G-protein levels in post-mortem brain in schizophrenics, although the results are somewhat contradictory (Okada *et al*, 1990, 1991, 1994; Nishino *et al*, 1993; Yang *et al*, 1998). A very recent study provides evidence of elevated dopamine receptor-coupled G proteins measured in mononuclear leukocytes of patients with schizophrenia (Avissar *et al*,

2001). Moreover, abnormalities were found in the expression of the regulator of RGS4 in schizophrenia using cDNA microarrays (Mirnics *et al*, 2001).

Despite a growing body of data demonstrating that immediate-early genes play an important physiological role in CNS, there is no evidence to our knowledge about the role of Jun family of transcription factors in psychiatric disorders. Whether the observed c-jun alterations are specific to schizophrenia or also can be found in major psychosis is still unclear. We are currently designing experiments to evaluate the expression of immediate-early genes jun and fos in patients with mood disorders, since schizophrenia and bipolar disorder are similar in several epidemiological aspects, such as age of onset, lifetime risk, course of illness, and genetic susceptibility (Berrettini, 2000). It has been shown that Jun proteins are differentially expressed in human neurological diseases. For example, several studies reported increased expression of c-Jun protein in Alzheimer's disease (Anderson *et al*, 1994, 1996; MacGibbon *et al*, 1997; Marcus *et al*, 1998) and multiple sclerosis (Martin *et al*, 1996), while decreased Jun D protein was found in brains of patients with Down's syndrome (Labudova *et al*, 1998). The lack of c-Jun elevation in the cerebellum of Alzheimer's and multiple sclerosis subjects, in contrast to that observed in patients with schizophrenia, suggests a regional and disease-specific alteration in c-Jun expression.

In conclusion, we found evidence of specific overexpression of c-Jun protein and mRNA levels in cerebellar vermis in schizophrenia. These results show that c-Jun may be involved in the neuropathological changes in the brains of patients with schizophrenia because the immediate-early genes encode transcription factors that serve to regulate the expression of other so-called late respond genes and therefore can trigger a cascade of events that may lead to functional and morphological alterations of neurons (Morgan and Curran, 1991). The above data, as well as data from our previous studies suggest the involvement of novel target genes and signal transduction pathways in cerebellar abnormalities in schizophrenia. In an attempt to elucidate the molecular mechanisms underlying these alterations in the brains of schizophrenic patients, future studies will concentrate on determining the mRNA expression and DNA binding activity of the corresponding transcription factors forming the AP-1 complex in both patients and PCP model of schizophrenia.

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