Brief Report

Increased Expression of Activity-Dependent Genes in Cerebellar Glutamatergic Neurons of Patients With Schizophrenia

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Objective: The purpose of this study was to evaluate the functional state of glutamatergic neurons in the cerebellar cortex of patients with schizophrenia.

Method: The authors measured messenger ribonucleic acid (mRNA) levels of three activity-dependent genes expressed by glutamatergic neurons in the cerebellar cortex (GAP-43, BDNF, and $GABA_A$-δ subunit) in the tissues of 14 patients with schizophrenia and 14 matched nonpsychiatric comparison subjects. Since its level of expression does not change in response to neuronal activity, gamma-aminobutyric $acid_A$-α6 subunit mRNA was used as a control.

Results: The levels of GAP-43 and BDNF mRNAs were significantly elevated in patients with schizophrenia, and a similar finding was observed for $GABA_A$-δ mRNA. In contrast, the levels of the $GABA_A$–α6 subunit mRNA, which is expressed in cerebellar granule cells in an activity-independent manner, did not differ from comparison subjects.

Conclusions: These results suggest that glutamatergic neurons may be hyperactive in the cerebellar cortices of patients with schizophrenia.

(Am J Psychiatry 2006; 163:1829–1831)

Three independent studies have reported that in resting conditions or after mild demanding cognitive challenges, patients with schizophrenia exhibit increased regional cerebral blood flow and glucose uptake in the cerebellum (1–3). To explore the hypothesis that this metabolic hyperactivity involves increased activity of glutamatergic neurons in the lateral hemispheres of the cerebellar cortex, we took advantage of the relative simplicity of cortical circuits in this part of the cerebellum, in which granule cells are by far the most abundant glutamatergic neuron. To evaluate the functional state of these neurons, the levels of growth-associated protein-43 (GAP-43), brain-derived neurotrophic factor (BDNF), and gamma-aminobutyric acid $(GABA)_A$-δ receptor subunit messenger ribonucleic acid (mRNA) were measured in cerebellar tissue obtained from patients with schizophrenia and matched nonpsychiatric comparison subjects. These genes were chosen because in the cerebellar cortex they are selectively expressed by granule cells (4–6) and their expression is regulated by the level of neuronal activity (6, 7). To assess whether changes in the expression of granule cell-specific genes were restricted to activity-dependent genes, the levels of the $GABA_A$-α6 subunit mRNA were also measured. This mRNA is expressed by granule cells but in an activity-independent manner (7).

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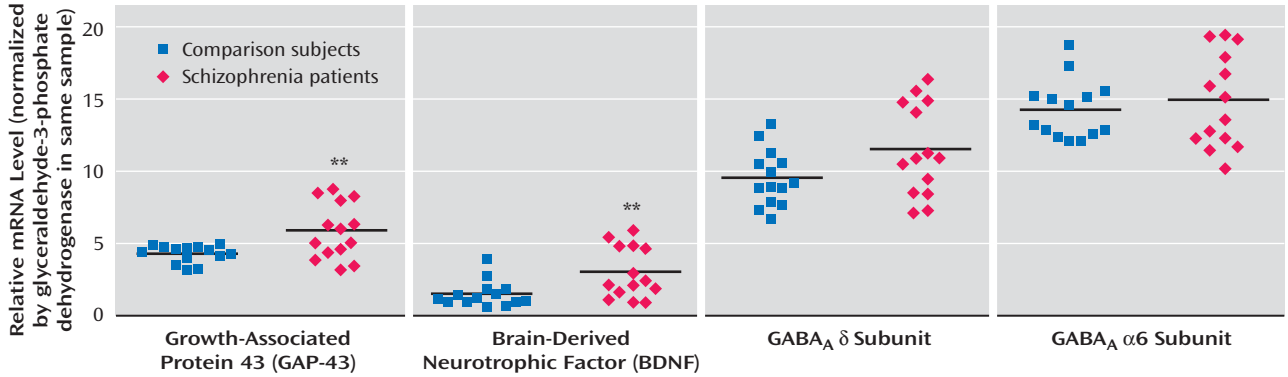
FIGURE 1. Increased Expression of Activity-Dependent Genes in Cerebellar Cortices of Schizophrenia Patients



[a] Graphs show the relative levels of each mRNA normalized by glyceraldehyde-3-phosphate dehydrogenase in the same sample. Values represent the average of three independent determinations. Data were analyzed by analysis of covariance.
** p<0.01 (N=14 in each group).

Method

Cerebellar tissues from patients with schizophrenia and comparison subjects without psychiatric history were obtained from the Maryland Brain Collection. Tissues from 14 patients with a diagnosis of schizophrenia according to DSM–IV criteria and 14 sex-, age- and postmortem interval-matched comparison subjects were included in this study. All the specimens were from male subjects between 25 and 65 years of age at the time of death with a postmortem interval lower than 24 hours. None of the subjects in this study had a history of alcohol abuse or dependence. No significant differences were found between patients and comparison subjects in the mean age (45 [SD=12] years versus 43 [SD= 10] years), postmortem interval (12 [SD=5] hours versus 16 [SD=6] hours) or pH (6.54 [SD=0.2] versus 6.49 [SD= 0.1]). The cause of death was suicide in three patients with schizophrenia but none of the comparison subjects. All patients with schizophrenia were receiving antipsychotics at the time of death. No patients were receiving medications for mood disorders. Samples of cortical areas corresponding to the superior portion of the semilunar lobule (crus I) of cerebellar hemispheres were dissected at –20°C and frozen at –80°C. Total RNA was isolated using Tri reagent (Sigma, St. Louis, Mo.). The levels of GAP-43, BDNF, GABA$_A$-δ, and GABA$_A$-α6 subunit mRNAs were measured on an ABI Prism 7000 using TaqMan® Assays-on-Demand probes (Applied Biosystems, Foster City, Calif.) according to the manufacturer's protocols. The levels of all mRNAs were normalized to that of glyceraldehyde-3-phosphate dehydrogenase in the same study group, and the relative levels of the mRNA were calculated as described by Livak and Schmittgen (8). Glyceraldehyde-3-phosphate dehydrogenase was selected for normalization because no significant differences were found in the levels of this mRNA in the cerebellum of patients with schizophrenia and comparison subjects. All experimental procedures and data analyses were performed blind to the diagnosis. Statistical analyses were performed by analysis of covariance using SPSS version 13.0, with diagnosis as the independent factor and brain pH and age as covariates. Pearson's correlation analyses were used to correlate the expression of activity-dependent genes with each other and with the activity-independent GABA$_A$-α6 subunit gene. To evaluate the effect of the medication on gene expression, the levels of the same mRNAs were measured in the cerebellum of adult male rats (N=12) exposed to haloperidol (1 mg/kg/day, i.p.) for 6 months.

Results

Relative to comparison subjects without psychiatric history, cerebellar cortices from patients with schizophrenia contained significantly higher levels of GAP-43 (F=8.817, df=1, 24, p=0.007) and BDNF mRNAs (F=9.408, df=1, 24, p= 0.005) (Figure 1). A similar finding was observed for GABA$_A$-δ, although differences in the levels of this mRNA did not reach significance (F=3.323, df=1, 24, p<0.09). In contrast, the levels of the GABA$_A$-α6 subunit mRNA in patients with schizophrenia were not significantly different from comparison subjects (F=0.013, df=1, 24, p=0.92). The observed differences remained significant when the three patients with schizophrenia who died by suicide were excluded from the analysis. No correlations were found between the levels of any of the mRNAs analyzed and postmortem interval, pH, or age, except for GAP-43, which showed a significant effect of brain pH (F=4.810, df=1, 24, p<0.04). In addition, a positive correlation was found between the levels of expression of the three activity-dependent genes across all samples (GAP-43 versus GABA$_A$-δ subunit: r=0.58, p=0.0001; GAP-43 versus BDNF: r=0.37, p=0.04; and BDNF versus GABA$_A$-δ subunit: r=0.54, p= 0.002). In contrast, there was no correlation between activity-dependent and activity-independent gene expression (GAP-43 versus GABA$_A$-α6: r=0.03, p=0.88; GABA$_A$-δ subunit versus GABA$_A$-α6 subunit: r=0.04, p=0.81; and BDNF versus GABA$_A$-α6 subunit: r=0.17, p=0.40). Finally, we observed no differences in the levels of BDNF, GAP-43, GABA$_A$-δ subunit, or GABA$_A$-α6 subunit mRNAs between rats that were chronically treated with haloperidol and vehicle-treated animals.

Discussion

In this study, we found that the levels of GAP-43 and BDNF, two mRNAs whose expression is dependent on the activity of glutamatergic neurons, are increased in the lateral cerebellar cortices of patients with schizophrenia.

Since in this brain region these genes are uniquely expressed by cerebellar granule cells (4–6), our findings suggest that this particular subtype of glutamatergic neuron may be hyperactive in schizophrenia. Consistent with this interpretation, the levels of GAP-43 and BDNF mRNAs were positively correlated with those of $GABA_A$-δ, another mRNA regulated by neuronal activity. In contrast, the levels of $GABA_A$-α6 subunit mRNA, which is selectively expressed by granule cells but in an activity-independent manner (7), did not differ from comparison subjects. Although it is difficult to draw parallels between molecular and neuroimaging studies, it is of interest to note that our findings of increased activity-dependent gene expression in the cerebellum are consistent with the reported increases in regional cerebral blood flow and glucose uptake in this brain region (1–3). Given the role of cerebellar granule cells in processing sensory information (9), a dysfunction in these cells could explain the abnormalities in sensory-motor integration observed in patients with schizophrenia (10).

Presented at the International Congress on Schizophrenia Research (ICOSR), Savannah, GA, April 2-7, 2005. Received April 18, 2005; revisions received Aug. 24 and Sept. 26, 2005; accepted Oct. 19, 2005. From the Departments of Neurosciences and Psychiatry, University of New Mexico School of Medicine, Albuquerque; Departamento de Psiquiatría y Neurociencias Universidad Diego Portales, Chile; Instituto Psiquiátrico Dr. José Horwitz Barak, Chile; The MIND Institute, UNM-MIND Imaging Center, Albuquerque; Department of Psychiatry, Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City; and the Maryland Psychiatric Research Center, University of Maryland School of Medicine, Baltimore. Address reprint requests and correspondence to Dr. Perrone-Bizzozero, Department of Neurosciences, University of New Mexico School of Medicine, Albuquerque, NM, 87131; nbizzozero@salud.unm.edu (e-mail).

The authors report no competing interests.

Supported by the Mental Illness and Neuroscience Discovery (MIND) Institute.

The authors thank Brian Reinert and Melissa Merhege for technical assistance, Dr. Andrea Allan for help with statistical analyses, and Drs. Fernando Valenzuela, Federico Bolognani, and Daniel Tanner for their insightful comments.

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