The Effect of Treatment with Antipsychotic Drugs on Brain N-Acetylaspartate Measures in Patients with Schizophrenia

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**Background:** The specific intracellular effects of antipsychotic drugs are largely unknown. Studies in animals have suggested that antipsychotics modify the expression of various intraneuronal proteins, but no analogous in vivo data in humans are available. The objective of the present study was to assess whether antipsychotics modify N-acetylaspartate (an intraneuronal marker of neuronal functional integrity) measures in brains of patients with schizophrenia.

**Methods:** We used proton magnetic resonance spectroscopic imaging to study 23 patients with schizophrenia (DSM-IV diagnosis) using a within-subject design. Patients were studied twice: once while on a stable regimen of antipsychotic drug treatment (for at least 4 weeks) and once while off medication for at least 2 weeks. Several cortical and subcortical regions were assessed, including the dorsolateral prefrontal cortex and the hippocampal area.

**Results:** Analysis of variance showed that, while on antipsychotics, patients had significantly higher N-acetylaspartate measures in the dorsolateral prefrontal cortex ($p = .002$). No other region showed any significant effect of treatment.

**Conclusions:** These results indicate that antipsychotic drugs increase N-acetylaspartate measures selectively in the dorsolateral prefrontal cortices of patients with schizophrenia, suggesting that these drugs modify in a regionally specific manner the function of a population of cortical neurons. N-Acetylaspartate measures may provide a useful tool to further investigate the effects of antipsychotics at the intracellular level. Biol Psychiatry 2001;49:39–46 © 2001 Society of Biological Psychiatry

**Key Words:** Schizophrenia, dorsolateral prefrontal cortex, N-acetylaspartate, typical antipsychotics, atypical antipsychotics

**Introduction**

Considerable evidence has accumulated over several decades that the efficacy of antipsychotic drugs in patients with schizophrenia involves antagonistic effects at dopamine D$_2$ receptors (Seeman and Lee 1975). However, it is also clear that not all therapeutic actions are explained by D$_2$ receptor blockade. For example, clozapine is an effective antipsychotic and may have superior efficacy in treatment-refractory patients with schizophrenia, but it exerts relatively little D$_2$ antagonism at clinical doses (Meltzer 1991). It has been proposed that D$_2$ blockade is only part of a cascade of events that eventually lead to more fundamental intraneuronal changes (Weinberger and Lipska 1995). In this regard, experiments in animals have indicated that antipsychotics modify immediate early gene expression in a regionally specific fashion (Deutch et al 1992; Dragunow et al 1990; Fink-Jensen and Kristensen 1994; Robertson et al 1994) and, though not binding to glutamate receptors, change the expression of glutamate receptor genes (Eastwood et al 1995; Fitzgerald et al 1995; Lidsky et al 1997; Ulas et al 1993). Therefore, it is conceivable that the antipsychotic properties of these drugs ultimately lie in their ability to modify the activity of specific populations of nondopaminergic neurons (Weinberger and Lipska 1995).

Evidence from animal studies of intraneuronal effects are not easily extended to in vivo human studies. Available human studies that have explored the brain effects of antipsychotics have involved electroencephalography or neuroimaging techniques investigating brain glucose metabolism or cerebral blood flow. These latter studies have generally shown cortical reductions and subcortical increases in brain metabolism or blood flow consequent to
treatment with antipsychotics (Buchsbaum and Hazlett 1998; Holcomb et al 1996; Miller et al 1997). However, these techniques have not been able to investigate neuronal-specific events, and the findings are difficult to interpret at the cellular level.

Proton magnetic resonance spectroscopy (1H-MRS) is an in vivo assay technique that provides information about some aspects of brain biochemistry and neuronal function. It identifies N-acetyl–containing compounds (mostly N-acetylaspartate [NAA]), choline-containing compounds (CHO), and creatine+phosphocreatine (CRE). Whereas CHO and CRE are present in neurons and in glial cells, NAA is found almost exclusively in neurons and their processes (Urenjak et al 1993) and in higher concentrations in rat pyramidal glutamatergic neurons (Moffett and Namboodiri 1995). The precise function of NAA is still unclear. Although until relatively recently NAA was referred to as a marker of neuronal structural integrity, a number of more recent studies have demonstrated that NAA reductions are reversible, suggesting that NAA is sensitive to processes affecting the functioning of neurons (Cendes et al 1997; De Stefano et al 1995; Vion-Dury et al 1995).

Several studies have been performed with 1H-MRS and 1H-MRS imaging (1H-MRSI) in schizophrenia to assess putative neuronal pathology. The majority of these studies have investigated the hippocampal area and the dorsolateral prefrontal cortex (DLPFC), two anatomic regions long believed to play an important role in the pathophysiology of schizophrenia (Goldman-Rakic 1994; Lewis et al 1999; Weinberger 1999). With some exceptions (Bartha et al 1997; Fukuzako et al 1995; Stanley et al 1996), the large majority of the experiments have reported NAA reductions in the hippocampal area and in the DLPFC of patients with schizophrenia relative to normal control subjects (Bertonino et al 1996, 1998a, 1998b; Brooks et al 1998; Buckley et al 1994; Callicott et al 1998; Deicken et al 1997, 1998; Maier et al 1995; Nasrallah et al 1994; Thomas et al 1998; Yurgelun-Todd et al 1996). Most of these studies were confounded by the fact that patients were treated with antipsychotics. However, a few studies in first-break or in drug-free patients have also found NAA reductions in the hippocampal area and in the DLPFC (Bertolino et al 1998a; Cecil et al 1998; Renshaw et al 1995), strongly suggesting that the findings are not an epiphenomenon of pharmacologic treatment.

As NAA measures can be affected by various medical interventions (Cendes et al 1997; De Stefano et al 1995; Vion-Dury et al 1995), it is conceivable that NAA levels in the brain may be affected by treatment with antipsychotic drugs. Only three prior studies have attempted to answer this question. Okumura et al (1959) reported that 30–60 days of intraperitoneal administration of chlorpromazine in rats did not alter NAA levels as measured with chromatographic analysis of whole brain extracts. Moreover, Lindquist et al (2000) have shown that, whereas 7 days of treatment with haloperidol or clozapine does not cause any change in subcortical NAA levels in vivo in rats, the same treatment regimen with olanzapine causes small reductions of NAA in the same brain region. However, neither of these studies addressed the possibility that antipsychotic drugs could affect specific populations of neurons and, thus, regionally specific NAA levels. Recently, Heimberg et al (1998) reported higher NAA levels in the frontal lobe of patients treated with atypical antipsychotics versus patients on typical antipsychotics. However, these results depended upon a between-subjects study design and, therefore, may not reflect drug-specific effects.

The purpose of this study was to assess whether treatment with antipsychotic medications affects NAA levels in patients with schizophrenia. In particular, we hypothesized that antipsychotics might change NAA levels in the hippocampal area and DLPFC, regions in which previous studies have reported NAA reductions in schizophrenia. We used a within-subject design that is well suited to address this question.

Methods and Materials

Subjects

Twenty-three patients (18 male and three female; age [mean ± SD] = 36.9 ± 8.1 years) who met DSM-IV criteria for schizophrenia and had been chronically ill (illness duration = 10.7 ± 5.9 years) gave informed consent after the procedure had been fully explained and volunteered to participate in this study. Diagnoses based on DSM-IV criteria were determined by a diagnostic conference utilizing data from a structured diagnostic interview, clinical interview by a research psychiatrist, past psychiatric and medical records, and informant interviews. Patients are extensively evaluated for their capacity to provide informed consent. This process includes an interview by an ethics specialist who is not part of the Clinical Brain Disorders Branch and is not involved in the research. A structured interview aimed at determining the capacity to understand the research and its risks, and which has been reviewed by the National Institutes of Health ethics department, is completed. Patients’ capacity to provide informed consent is reviewed on multiple occasions during their stay in the research program. Exclusion criteria included history of significant drug abuse (no active drug use in the past 5 years), head trauma with loss of consciousness, and any significant medical condition. All patients underwent at least two scans, one while drug free and the other while on drugs. Two of the patients were drug naive; the others were studied after they had been drug free for at least 2 weeks (mean ± SD = 24.21 ± 9.12, range = 14–60 days, excluding the two drug-naive patients) and restudied after they had been on stable doses of neuroleptics for at least 4 weeks.
(mean ± SD = 511.2 ± 930.2 days). Table 1 provides further clinical details about the patients studied, including the antipsychotics with which they were treated. To avoid order effects, seven of the patients received the first scan while off drugs and the remaining while on drugs. Four patients who had received the on-drugs scan first received a third scan (again on drugs for at least 2 weeks [mean 48 ± 36 days] after they had been drug free). Therefore, these four patients received three scans in the following order: on, off, on. The drug-free data of six patients have been reported in a previous article (Bertolino et al 1998a).

Psychopathology of each subject was also assessed at the two time points by means of the Scale for the Assessment of Negative Symptoms (Andreasen 1989) and the Positive Symptoms Assessment Scale (Bigelow and Berthot 1989).

### 1H-MRSI Procedure

The same 1H-MRSI procedure was used for all the scans. Proton magnetic resonance spectroscopy imaging was performed on a GE-SIGNA 1.5-T MR scanner (GE Medical Systems, Milwaukee) as in earlier reports (Bertolino et al 1996; Duyn et al 1993). The 1H-MRSI slices were positioned parallel to the sylvian fissure and encompassing the hippocampal formation as visualized on T1-weighted MRI scans (Spin Echo, repetition time [TR] = 500 msec, echo time [TE] = 12 msec). Briefly, the 1H-MRSI sequence involves a spin echo slice selection with TR of 2200 msec and TE of 272 msec and includes suppression of water and most of the signal arising from lipids in the skull marrow and in surface tissues (outer volume saturation; Duyn et al 1993). Phase encoding procedures were used to obtain a 32 × 32 array of spectra from volume elements in each selected slice. Each volume element (“voxel”) had nominal dimensions of 7.5 mm × 7.5 mm × 15 mm (0.84 mL). Actual volume, based on full width at half maximum after filtering of k-space, was 1.4 mL (Duyn et al 1993). The filter was a radial cosine filter starting at half-maximum radius. At the end of each 1H-MRSI scan a second T1-weighted MRI scan was acquired at the same level as the first. This further scan was used for visually checking for gross motion that may have intervened during the 1H-MRSI scan.

To produce metabolite maps, location and integration of the signal strength (range 5.0 ppm and 0.1 ppm on each side of the center of the peak) of NAA, CHO, and CRE peaks in all brain voxels were automatically computed. Metabolite signals are reported as ratios of the area under the peaks NAA/CRE, NAA/CHO, and CHO/CRE. Regions of interest (ROIs) were drawn blindly with reference to standard anatomic atlases by two raters on coplanar structural MRI scans (intraclass correlation coefficient for the two raters = .9). The method for drawing the ROIs is described in detail elsewhere (Bertolino et al 1996). These ROIs were then transferred by computer on the 1H-MRSI maps and the average NAA, CHO, and CRE values were computed. Regions of interest were identified in the DLPFC, hippocampal area, superior temporal gyrus, inferior frontal gyrus, occipital cortex, anterior and posterior cingulate gyrus, thalamus, putamen, prefrontal white matter, and centrum semiovale (CSO).

### Morphometry

To exclude the possibility that 1H-MRSI findings were due to morphometric changes, we measured the volume of the prefrontal lobe. The T1-weighted axial MRI scans used to localize the MRSI slices were resliced coronally using Medex software (version 3.21, Sensor Systems, Stirling, VA). The same software package was then used to manually outline prefrontal lobe areas.
on the coronal images. Measurements of frontal lobe were done by outlining the area of each slice rostral to the genu of the corpus callosum.

**Statistical Analysis**

Statistical analysis to assess the effects of drugs on \(^1\)H-MRSI and morphometric measures was performed with repeated-measures analysis of variance (ANOVA) for each ROI with treatment and hemisphere (left and right) as within-subject factors. Correlations between NAA measures and clinical variables were performed with Spearman analysis.

**Results**

Figure 1 shows the on–off comparison for all ROIs. By repeated-measures ANOVA, the only significant effect of treatment for NAA/CRE was in the DLPFC \([F(1,22) = 11.5, p = .002]\). All other \(p\) values were >.2. Post hoc analysis with Tukey honest significant difference showed that patients off neuroleptics had lower NAA/CRE in the DLPFC (mean on = 2.78, mean off = 2.54, \(p = .002\)). Figure 2 provides each individual’s NAA/CRE data in the DLPFC while on and off antipsychotics. Nineteen of 23 patients have lower NAA/CRE while off antipsychotics (Fisher exact test, \(p = .05\)). No effect of side or interaction of side by treatment was found in the DLPFC. No other significant main effect of treatment, of side, or of their interaction was found in any of the other ROIs for any of the metabolite ratios.

To evaluate whether the ratio changes (NAA/CRE) arise because of changes in the numerator or denominator terms, the mean integrated areas of NAA and CRE resonances were normalized to the corresponding mean integrated areas in the CSO (i.e., NAA DLPFC/NAA CSO, CRE DLPFC/CRE CSO). We used the CSO as a reference because in a previous study (Bertolino et al 1996) its metabolite ratios showed a low coefficient of variation among several other ROIs and because it is probably not involved in the primary pathophysiology of schizophrenia. This further analysis in the DLPFC showed a trend of NAA to be lower in patients while they are drug free \([\text{ANOVA, } F(1,22) = 3.4, p < .07]\), whereas no difference was found for normalized CRE \([\text{ANOVA, } F(1,22) = .14, p > .7]\), indicating that the ratio differences previously seen arise due to differences in NAA.

To evaluate whether the effect seen on NAA/CRE in the DLPFC could have been influenced by order effects, we performed a further ANOVA with order as an additional within-subject factor. No main effect of order was found.
The effect of drug was found on the volume of the prefrontal cortex. Illness duration, drug treatment duration, or duration of withdrawal from antipsychotics were not significant main effects. These data further suggest that the neuroleptic effect is reversible.

There were no significant correlations between NAA/CRE changes in the DLPFC and in symptomatology, illness duration, drug treatment duration, or duration of withdrawal from antipsychotics. No significant main effect of drug was found on the volume of the prefrontal lobe ($F = 1.8, p > .1$).

**Discussion**

The results of this study show that NAA measures in the DLPFC are selectively increased by treatment with antipsychotic drugs in patients with schizophrenia. This effect seems to be produced by both typical and atypical antipsychotics, though the effect may be slightly greater with the atypicals. In addition, antipsychotic treatment did not affect NAA measures in any of the other regions surveyed, including the hippocampal area, the other region where previous studies have consistently shown NAA reductions in schizophrenia.

This report is the first in vivo evidence that treatment with antipsychotics in patients with schizophrenia selectively affects a population of neurons in the DLPFC. Although the origins of these NAA changes in the DLPFC are not clear, it is tempting to implicate glutamatergic neurons. N-Acetylaspartate concentration in the rat brain is highest in pyramidal, glutamatergic neurons (Moffett and Namboodiri 1995), and NAA synthesis in the mitochondria is dependent on glutamate and pyruvate or 3-hydroxybutyrate (Clark 1998). Therefore, it is logical to expect that changes in glutamate may in turn affect NAA synthesis and concentration, if only as a surrogate measure of neuronal metabolic activation. Reductions in NAA concentration are seen in association with various pathologic conditions involving glutamatergic neurons (Cendes et al 1997; Hugg et al 1996; Tedeschi et al 1996). Other data also suggest that NAA concentration varies with the structural and functional state of glutamatergic cells (Cendes et al 1997; Hugg et al 1996; Najim et al 1998). Several studies have now shown that NAA levels are reduced in patients with schizophrenia in the hippocampal area and the DLPFC, two areas in which glutamatergic pathology has been implicated (Akbarian et al 1996; Eastwood et al 1995; Weinberger 1999). N-Acetylaspartate reductions in the DLPFC predict several neurobiological phenomena associated with schizophrenia, such as dopamine regulation in striatum and working memory–related cortical activation, which depend on corticofugal and intracortical glutamate projections, respectively (Bertolino et al 1999a, 2000a, 2000b; Callicott et al, in press). Consistent with the data in schizophrenia, neonatal lesions of the hippocampal area in rodents and primates produce developmental impairment of the DLPFC that predicts dysregulation of the dopaminergic system (Bertolino et al 1997, 1999a, 1999b). Taken together, these various data suggest that NAA concentrations, at least in the context of neuronal pathology, may vary as a correlate of the activity of glutamate neurons.

These speculations are consistent with an emerging basic science literature suggesting that both typical and atypical antipsychotics affect glutamatergic metabolism in a regionally specific fashion. In this regard several basic science studies have shown that both typical and atypical antipsychotics increase the expression of glutamate receptor subunits (GluR2) messenger RNA (Eastwood et al 1996), GluR1 protein levels (Fitzgerald et al 1995), and N-methyl-D-aspartate (NMDA) activity in the rat prefrontal cortex (Lidsky et al 1997), as well as stimulate glutamate release in the striatum (Bardgett et al 1993; See and Lynch 1995, 1996; Yamamoto and Cooperman 1994), a region that receives glutamatergic inputs from the prefrontal cortex. Therefore, both typical and atypical antipsychotics seem to affect glutamatergic activity, even though they do not “bind” to NMDA receptors, per se. It is yet to be determined whether the NAA changes in the prefrontal cortex reflect any of these effects.

There is another possible explanation for the NAA increases in the DLPFC. N-Acetylaspartate is expressed in all neuronal processes, including synapses. Even though the literature is quite controversial on the subject (Glantz and Lewis 2000), chronic treatment with antipsychotics seems to affect synaptic spine density (Kelley et al 1997; Kerns et al 1992; Rodriguez and Pickel 1999). Therefore, it is theoretically possible that NAA changes reflect these structural changes. However, there is other evidence that makes this seem unlikely. Synaptic density is affected by chronic treatment with antipsychotics primarily in the striatum, where we do not find any difference in our study. Moreover, structural synaptic changes are accompanied by increased membrane turnover that in 1H-MRSI should be reflected in CHO changes. Notwithstanding the limitations associated with interpreting a negative result (especially in a relatively small sample of patients) and with vagaries in the interpretation of the CHO signal, lack of increased...
CHO may suggest that there is no significant membrane turnover.

There are several limitations of this study. The change in NAA measures in the DLPFC associated with antipsychotic treatment is about 9%. In a previous study in which we assessed the reproducibility of NAA measures in 10 patients with schizophrenia on stable treatment with antipsychotics, we reported a coefficient of variation of about 9% in the DLPFC (Bertolino et al. 1998b). Thus, the magnitude of our findings here does not exceed the degree of expected variability. However, as 19 of the 23 (p < .05, Fisher exact text) patients studied in this experiment showed NAA changes in the same direction (higher NAA on antipsychotics), and as all four of the patients studied on three occasions showed consistent effects, the results appear to be determined by the effect of drugs. It is also important to remember that, whereas other anatomic regions have shown similar or even higher coefficients of variation, the DLPFC was the only region with a significant change. It also should be noted that, as both the treatment and the off-medication phases of this study were relatively brief, it is conceivable that a longer period would have resulted in greater changes in NAA measures.

Another limitation of this study is the rather gross volumetric measurement of the prefrontal lobe. We performed this measurement to exclude any major volume change in the prefrontal lobe that could have confounded the NAA measurements. However, the images we had acquired did not allow us to measure more specifically the volume of the DLPFC, which is where we found the NAA changes. Therefore, we cannot definitively exclude volumetric DLPFC changes. Nonetheless, the use of metabolite ratios (which should be less sensitive to volume changes) along with these data does not support any gross volumetric change of the prefrontal lobe.

Another possible confound of our data is motion. Indeed, patients off antipsychotics are not sedated and are therefore more anxious about any procedure. We cannot exclude that any subtle motion may have affected the data. However, as noted above, we screened for motion that may have affected the spectra. Moreover, it is unlikely that the results would have been so regionally specific if they were a result of motion artifacts. It is also worth underscoring that antipsychotic treatment may have contributed to some of the inconsistencies in the \textsuperscript{1}H-MRS literature. Even though the majority of studies have shown NAA reductions in the frontal cortex of patients with schizophrenia, some studies have failed to do so. It is possible that the latter studies have not found differences in NAA measures in the frontal cortex because they studied patients under treatment with antipsychotics.

In conclusion, treatment with antipsychotics, both typical and atypical, increases NAA measures selectively in the DLPFC of patients with schizophrenia. Even though the neurobiological mechanism responsible for these changes is unclear, \textsuperscript{1}H-MRSI may be an ideal tool to further investigate in vivo changes induced by antipsychotics at the neuronal level.

References


Bertolino A, Roffman J, Lipska BK, van Gelderen P, Olson P,
Weinberger DR (1999b, October): Postpubertal emergence of prefrontal neuronal deficits and altered dopaminergic behaviors in rats with neonatal hippocampal lesions. Abstract presented at the annual meeting of the Society for Neuroscience, Miami.


Glantz LA, Lewis DA (2000): Decreased dendritic spine density on prefrontal cortical pyramidal neurons in schizophrenia. *Arch Gen Psychiatry* 1:65–73.


