Reduced Phosphodiesters and High-Energy Phosphates in the Frontal Lobe of Schizophrenic Patients: A $^{31}$P Chemical Shift Spectroscopic-Imaging Study

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**Background:** $^{31}$Phosphorous magnetic resonance spectroscopy has been widely used to evaluate schizophrenic patients in comparison to control subjects, because it allows the investigation of both phospholipid and energy metabolism in vivo; however, the results achieved so far are inconsistent. Chemical shift imaging (CSI) has the advantage that instead of only one or a few preselected voxels the tissue of a whole brain slice can be examined. The aim of the present investigation was to determine whether the results of previous studies of our group, showing that phosphodiesters (PDE) are decreased in the frontal lobe of schizophrenic patients as compared to control subjects, might be confirmed in an independent unmedicated patient sample using the CSI technique.

**Methods:** A carefully selected new cohort including 11 neuroleptic-free schizophrenic patients and 11 age- and gender-matched healthy control subjects was recruited. CSI was applied and an innovative analysis method for CSI data based on a general linear model was used.

**Results:** PDE, phosphocreatine, and adenosine triphosphate (ATP) were found to be significantly decreased in the frontal lobe of patients with schizophrenia.

**Conclusions:** Because PDE was decreased in schizophrenic patients, the membrane phospholipid hypothesis of schizophrenia could not be corroborated. Further results indicate decreased ATP production in the frontal lobe of patients with schizophrenia. Biol Psychiatry 2000;47:954-961 © 2000 Society of Biological Psychiatry

**Key Words:** Schizophrenia, $^{31}$phosphorous magnetic resonance spectroscopy, chemical shift imaging, phospholipid membrane hypothesis

**Introduction**

$^{31}$Phosphorous magnetic resonance spectroscopy (MRS) is a noninvasive technique that allows in vivo evaluation of phosphorous molecules in tissues (e.g., in brain tissue). Two major independent groups of molecules containing phosphor can be differentiated by MRS: the phospholipids and the high-energy phosphates. The phospholipids comprise phosphomonoesters (PME), which in turn are mainly composed of phosphocholine (PC), phosphoethanolamine (PE), and L-phosphoserine (PS) on the one hand, and phosphodiesters (PDE) on the other hand. The PDE peak of the MR spectrum is a sum peak of glycerol-3-phosphocholine (GPC), of glycerol-3-phosphoethanolamine (GPE), and of mobile phospholipids present in the cell membrane (Murphy et al 1989). PME are regarded as precursors of membrane phospholipids, whereas PDE are believed to represent products of membrane breakdown.

The high-energy phosphates comprise phosphocreatine (PCr) and nucleoside triphosphates, mainly adenosine triphosphate (ATP). Another metabolite important in energy metabolism that can be detected by MRS is inorganic phosphate (Pi). PCr, ATP, and Pi are regarded as markers of cellular energy metabolism and are believed to stay in an equilibrium. As recently summarized by Magistretti et al (1999), ATP consumption is directly linked to neuronal, probably glutaminergic activity.

Thus, MRS allows the investigation of both cell membrane turnover and cellular energy metabolism in the tissues in vivo. It is therefore not surprising that MRS has become a frequently applied spectroscopy technique in schizophrenia research since the pioneering work of Pettegrew et al in 1991.

The results obtained by MRS, however, are inconsistent, both with respect to the amount of phospholipid compounds and the markers of brain energy metabolism. Pettegrew et al (1991) originally reported increased PDE and decreased PME levels in neuroleptic-naive, first-episode schizophrenic patients in the dorsolateral prefrontal cortex. They suggested that these alterations are com-
Table 1. Demographic Data of 11 Schizophrenic Patients and 11 Control Subjects

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>33.4 ± 9.5</td>
<td>33.7 ± 9.1</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>8/3</td>
<td>8/3</td>
</tr>
<tr>
<td>Duration of illness (years)</td>
<td>7.2 ± 7.6</td>
<td>—</td>
</tr>
<tr>
<td>Range (years)</td>
<td>1–22</td>
<td>—</td>
</tr>
<tr>
<td>Neuroleptic-free period prior to assessment (days)</td>
<td>4.3 ± 0.4</td>
<td>—</td>
</tr>
<tr>
<td>BPRS score</td>
<td>57.4 ± 8.4</td>
<td>—</td>
</tr>
<tr>
<td>CGI score</td>
<td>5.8 ± 0.6</td>
<td>—</td>
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Values are given ± SD. BPRS, Brief Psychiatric Rating Scale; CGI, Clinical Global Impression.

*Seven of the patients were neuroleptic naive; data refer to the four patients medicated before the wash-out period.

Methods and Materials

Subjects

Eleven schizophrenic inpatients and 11 age- and gender-matched control subjects were included in the study. Ten of the patients had a diagnosis of schizophrenia (four paranoid type, three catatonic type, two residual type, one undifferentiated type) and one patient had a diagnosis of schizoaffective disorder according to DSM-IV criteria (American Psychiatric Association 1994). Diagnoses were made independently by the treating psychiatrist and by another experienced psychiatrist. Only if both diagnoses were the same and fulfilled the inclusion criteria was the respective patient included. At the end of the hospitalization period (mean duration: 9 weeks) the diagnoses were verified according to the hospital charts. The main demographic variables together with the individual wash-out and pretreatment data of the four pretreated patients are given in Tables 1 and 2.

Seven of the patients had never been medicated with neuroleptics before, and four had discontinued neuroleptic treatment for a mean of 4.3 ± 0.4 days. Using gas chromatography–mass spectroscopy (detection limit 10 ng/mL), neuroleptics in plasma could not be detected in any patient prior to MRS. Patients were assessed using the Clinical Global Impression (CGI; National Institute of Mental Health 1970) and the Brief Psychiatric Rating Scale (BPRS; Overall and Gorham 1962). Using the Edinburgh
inventory (Oldfield 1971), all schizophrenic patients and 10 of the 11 control subjects were found to be right handed. The clinical interview was performed immediately before the MRS measurement. All control subjects were recruited by newspaper advertisements and were paid for their participation. Control subjects and patients were thoroughly investigated for symptoms of internal and neurological disease; control subjects or patients who had evidence of neurological disorders or who had first-degree relatives with such disorders were excluded from the study, as were subjects who had a history of substance abuse. Control subjects also underwent a psychiatric examination. Those who showed any psychiatric symptoms or had a history of psychiatric symptoms or had first-degree relatives suffering from a major psychiatric disorder were excluded. The study was approved by the ethical review board of the University of Jena. After a detailed description of the study protocol to the subjects, patients and control subjects gave written informed consent.

Method of $^{31}P$-MRS Chemical Shift Imaging

Two-dimensional $^{31}P$-CSI data were acquired with a conventional magnetic resonance imaging (MRI) scanner at 1.5 T (Gyroscan ACSII, Philips, Hamburg, Germany) and a quadrature transmit/receive $^{31}P$ head coil. Prior to the measurement, the magnetic field homogeneity was improved by an automatic shim procedure including a volume indicated by the bold line in Figure 1. A PRESS sequence is used to measure the line broadening of the water signal. After shimming, the half-height line width (full width at half maximum) of the water signal had to be less than 15 Hz. Then, a phase-encoded image selective CSI sequence using free induction decay volume selection was applied. The Philips Gyroscan ACS II used has no option to adjust a preacquisition delay time; however, in the scan record the sample offset time was specified. For the CSI measurements, the offset time was $\Delta t_0 = 8.3 \times 10^{-4}$ sec. An $8 \times 8$ CSI matrix was acquired with a repetition time of 2000 msec, a sampling rate of 2 kHz, and a sampling time of 0.5 msec. Data were averaged over 16 measurements. To position the obliquely located slice (Figure 2) in a reproducible and reliable fashion, $T_1$-weighted scout images were recorded with the body coil of the scanner. On a midsagittal slice, the commissura anterior (CA) and commissura posterior (CP) were identified. The points where the line through CA and CP crosses the posterior cerebral surface and where a perpendicular to the CA–CP line through CA crosses the cerebral surface were identified. A line parallel to the line through these two reference points and crossing CP determined the location of the measured slice. The mean angles between the measured slice and the CA–PC line did not differ between both groups (mean angle in schizophrenic patients: 36.4° ± 2.8°, in control subjects: 37.6° ± 4.2°, $p = .56$, Mann–Whitney U test). The acquired slice had a thickness of 4 cm, with 2 cm being located below and 2 cm above this line (Figure 2). In the transversal orientation, the size of the measured VOI was adjusted to the individual size of the brain. The mean size of the VOI was 18.4 $\times$ 18.4 $\times$ 4 cm$^3$, resulting in $8 \times 8$ voxels of about 19 cm$^3$ each (Figure 1). Because the VOI was adjusted to the individual size of the brain, one might speculate that the mean voxel volumes were different between both groups; however, this was not the case (mean voxel volume in schizophrenic patients: 181 ± 13 cm$^3$, control subjects: 186 ± 8 cm$^3$, $p = .47$, Mann–Whitney U test). The slice covered the frontal, temporal, and occipital lobe, the cingulum, the thalamus, and the cerebellum.

The acquired time domain signals from each voxel were direct current corrected, zero filled to 2,048 data points, and exponentially multiplied (8 Hz). After Fourier transformation, an automatic zero- and first-order phase correction were applied. After this preprocessing, an iterative nonlinear last-square fit in the frequency domain was applied to the spectrum using the Pearch Research (PERCH) software (view online at http://www.uku.fi/ perch). Details of the fitting process and a figure of a typical simulated spectrum have already been published by Riehemann et al (1999b). The received values were converted into greyscale values and stored as CSIs. To minimize global distortions due to altered amplifier adjustments or varying shimming fields in different measurements, the greyscale values of each CSI were normalized for the mean total integrated area of the spectrum (Bottomley 1991; Rudin and Sauter 1992). This CSI preprocessing was based on the software Xunspecl (Philips, Best, Netherlands). Automatic calculation was established using batch processing and corresponding filter programs on a Sun workstation. No user interaction was necessary during the whole procedure of image generation. The resulting CSIs were finally converted into the “Analyze format.”

Statistical analysis of the CSI of the respective metabolites was performed by means of a general linear model (GLM) (Riehemann et al 1999a) implemented in the software package SPM96 (Statistical Parametric Mapping, version 96; Friston et al 1995). A subtractive GLM design allowing the detection of group differences was used. Two linear contrasts of effects in each voxel were used, the first contrast depicting higher values in control subjects compared to schizophrenic patients (or lower values in schizophrenic patients), the second contrast depicting higher values in schizophrenic brains. Each contrast resulted in a map of the $t$ statistic, which was transformed to the unit normal distribution and thresholded at 1.69, corresponding to $p < .05$. The obtained Z(1.69) map was finally overlaid on the corresponding anatomic image.
Results

The numbers of the voxels used in the text refer to those marked on the grid overlaid on the corresponding anatomic slice (see Figure 1). Tentative allocation of anatomic structures underlying each voxel is also given in Figure 1. In the following only the voxel numbers will be referred to.

In the frontal lobe, PDE was found to be decreased in schizophrenic patients compared to control subjects in voxel 13 (Figure 3a). PME was decreased in voxel 28 (Figure 3d). In the cerebellum, both PDE and PME were found to be decreased, but in different voxel locations. In voxel 45, PDE was lower in brains of schizophrenic patients, whereas in voxels 37, 46, and 53 (Figure 3d) PME appeared to be lower in patients with schizophrenia than in control subjects. Significant differences in ATP content (sum of all ATP peaks) between schizophrenic patients and control subjects were observed only in the frontal lobes. ATP was lower in voxels 12, 13, 14, and 22 (Figure 3b). There were no significant differences in PCr levels in any of the voxels except voxel 12, where PCr was decreased. Schizophrenic patients did not show higher values for either PME,
Discussion

Phospholipids in Schizophrenic Brain Tissue

The present study revealed that alterations of phospholipid levels were present throughout the brain in patients with schizophrenia. Significant differences in comparison to control subjects were observed in the frontal lobes, in the region of the basal ganglia and in the cerebellum.

Our finding of decreased PDE in the right frontal lobe is not in accordance with the majority of previous studies reporting either increased or unchanged values in schizophrenic patients (Deicken et al 1994; Fujimoto et al 1992; Pettegrew et al 1991; Potwarka et al 1999; Shiori et al 1994, Stanley et al 1994, 1995; Williamson et al 1991). These discrepancies may partly be due to methodological differences, because increased PDE values in schizophrenia have mainly been reported in those studies where the measured frontal VOIs included mostly grey and not white matter. Because PDE and PME levels are higher in white than in grey matter (Buchli et al 1994), one should rather have expected PDE decreases in schizophrenic patients as a result of studies investigating a relatively high amount of white matter. Just the opposite is the case, however. Thus, the results do not seem to be primarily dependent on the localization methods used.

A given voxel contains an admixture of grey and white matter. Because in schizophrenic patients cortical grey matter seems preferentially to be reduced (e.g., Falkai and Bogerts 1986; Goldstein et al 1999; Suddath et al 1989), the proportion of grey to white matter is in favour of white matter in schizophrenic patients compared to control subjects. As mentioned above, however, the relative concentration of PDE is about 40% higher in white compared to grey matter (Buchli et al 1994). Therefore, the finding of a reduced PDE cannot be explained solely by a reduction of grey matter in schizophrenia.

The region of the basal ganglia has as yet only been examined in two studies by MRS (Deicken et al 1995a; Fujimoto et al 1992). Fujimoto et al (1992) reported reduced PDE and increased PME on the left side in 16 neuroleptic-medicated schizophrenic patients as compared to control subjects and interpreted these results as indicating higher anabolic activity of membrane phospholipids in this region. Deicken et al (1995b), however, found no differences in phospholipids in the basal ganglia when comparing chronically ill, mostly neuroleptic-treated patients with control subjects. In the present study, PME was lower in schizophrenic patients than in control subjects on the left side, indicating decreased membrane anabolic activity in schizophrenic patients, which is in contrast to Fujimoto’s study. Because the patients included in our study did not receive neuroleptics and those reported by Fujimoto et al were long-term treated, the observed differences might be due to an effect of neuroleptic medication; however, previous studies addressing this issue in the frontal lobes indicate that PDE increases rather than decreases due to neuroleptic treatment (Volz et al 1999).

To our knowledge, this is the first study to examine cerebellum by means of MRS in schizophrenic patients compared to control subjects. In recent years, the cerebellum has increasingly been suggested to play an important role in the pathophysiology of schizophrenia (e.g., Katsetos et al 1997). Thus, Andreasen et al (1998) attributed a central role to the cerebellum in their model of “cognitive dysmetria” as the underlying defect in schizophrenia. In the right anterior cerebellar region, PDE was decreased in patients with schizophrenia compared to control subjects; however, in right cerebellar regions, schizophrenic pa-
Patients showed lower PME values than control subjects. These results cannot be explained as yet, but may point to an alteration of phospholipid metabolism in patients with schizophrenia, which is not restricted to the frontal lobe but may include the cerebellum as well.

A reduction in PDE might also be due to an effect of neuroleptic treatment via the inhibition of phospholipase A₂ (PLA₂) activity (Gattaz et al. 1987, 1990). PLA₂ leads to the transformation of PC and PE to lysophosphatidylcholine and -ethanolamine, which are then further degraded to GPC and GPE, both major components of the PDE peak. By inhibiting PLA₂ activity, neuroleptics might lower and thus normalize previously increased PDE levels in medicated schizophrenic patients as suggested by Stanley et al. (1995). Neuroleptics have even been suggested to cause a decrease of PDE in medicated patients compared to control subjects (Volz et al. 1997a, 1998); however, PDE values were also reported to be lower in patients who had not been medicated for 3 days up to 10 years (Volz et al. 1997b). In the present study, 7 out of 11 patients were neuroleptic naive, a fact that speaks against a significant influence of neuroleptic treatment on the measured PDE values in schizophrenic patients.

The results confirm those of our earlier studies obtained with a different measurement technique (ISIS) and a conventional analyzing approach. They suggest that membrane catabolism is decreased rather than increased in the frontal lobes of schizophrenic patients. Therefore, our findings do not confirm the original hypothesis of increased membrane phospholipid metabolism (Pettegrew et al. 1991).

High-Energy Phosphates and Pi in Schizophrenic Brain Tissue

Alterations of ATP and PCr in schizophrenia were only present in the frontal lobes of schizophrenic patients, not in other brain regions investigated. The frontal lobes have frequently been suggested to play a major role in the pathogenesis of schizophrenia (i.e., in the concept of hypofrontality). Previous MRS studies also reported on decreased PCr (Deicken et al. 1994; Fujimoto et al. 1992); however, Williamson et al. (1991) and Kato et al. (1994, 1995) reported on increased PCr values in the frontal lobe of schizophrenic patients. PCr and PCr/ATP were increased in neuroleptic treated patients compared to control subjects, whereas there was no difference between neuroleptic-free schizophrenic patients and control subjects (Volz et al. 1997b, 1998). It was therefore hypothesized that neuroleptics might lead to a decrease of energy...
demanding processes in the frontal lobe. With respect to Pi, the majority of studies did not show significant differences between control subjects and patients with schizophrenia (Kegeles et al 1998), whereas Stanley et al (1995) and Potwarka et al (1999) found decreased values in schizophrenic patients.

The results of the present study confirm those of our earlier study using the ISIS and not the CSI technique. PCr and ATP did not show an increase in schizophrenic patients but even appeared to be decreased. This difference may be due to the higher proportion of drug-naive patients in the present group of schizophrenic patients (64%) than in our earlier sample (20%). According to the present results it might be hypothesized that in neuroleptic-naive patients there is a reduction in PCr and ATP, whereas in patients who have been neuroleptic free for a relatively short time there are no differences compared to control subjects, and in patients treated with neuroleptics over a long time there is an increase in high-energy phosphates in brain tissue.

Various studies including postmortem examinations and functional imaging studies have demonstrated an impairment of energy metabolism in schizophrenia. These changes were interpreted as suggesting reduced demand for metabolic energy in the cells. In the present study, ATP and PCr were lower in schizophrenic frontal lobes than in control subjects, whereas there was no change of Pi concentration. Our results therefore indicate that rather than a reduced demand for energy an abnormality in energy generation may be present in brain tissue of unmedicated schizophrenic patients. Our results are in accordance with positron emission tomography (PET) studies that revealed a decreased metabolism in the frontal cortex of schizophrenic patients, which is generally assumed not to be related to neuroleptic medication (Asarnow et al 1990; Cohen et al 1988). Our findings are also consistent with biochemical evidence: In brain tissue, energy is generated almost exclusively through oxidative phosphorylation by means of the mitochondrial respiratory chain. Cavalier et al (1995) reported a 43% reduction of activity of one enzyme complex of the mitochondrial respiratory chain in patients with schizophrenia, which might lead to an impairment of energy generation. Further supporting evidence comes from studies examining brain metabolism during tasks that normally raise glucose utilisation levels. Neurolept-free schizophrenic patients had only attenuated increase in glucose metabolic rate in an auditory discrimination task as compared to control subjects (Asarnow et al 1990; Cohen et al 1987).

In summary, our results with respect to phospholipids confirm alterations, but do not corroborate the membrane phospholipid hypothesis of schizophrenia. The second major finding of decreased ATP and PCr (and no differ-

ences in Pi) in schizophrenic patients compared to control subjects could be interpreted as a marker of altered oxidative phosphorylation mechanisms in schizophrenia.

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