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^{31}P magnetic resonance spectroscopy of the medial temporal lobe of schizophrenic patients with neuroleptic-resistant marked positive symptoms

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Abstract ^{31}P magnetic resonance spectroscopy was performed in 16 medicated schizophrenic patients with neuroleptic-resistant marked positive symptoms and in 16 healthy volunteers matched for age and sex in order to determine what changes in phosphorus metabolites are detected in such patients as compared to the controls. The schizophrenic patients showed an increased level of phosphodiesters in the bilateral medial temporal lobes. They also showed a decrease in the level of β-ATP in the left medial temporal lobe. These findings suggest that schizophrenic patients with prominent positive symptoms refractory to neuroleptics may have a disturbance of bilateral membrane phospholipid and left-sided high-energy phosphate metabolism in the medial temporal lobe.

Key words Phosphorus magnetic resonance spectroscopy · Schizophrenia · Medial temporal lobe Prominent positive symptoms

Introduction

The involvement of the temporal lobe in the pathophysiology of schizophrenia has been suggested by neurophysiological studies, using electroencephalography (Flor-Henry 1976; Morihisa et al. 1983), event related potential (McCarley et al. 1989; Shagass and Roemer 1991), positron emission tomography (DeLisi et al. 1989; Liddle et al. 1992), single photon emission computed tomography (Paulman et al. 1990; Kawasaki et al. 1992) and magnetic resonance imaging (Suddath et al. 1989; Barta et al. 1990). There is particular interest in the medial temporal lobe and superior temporal gyrus (Barta et al. 1990; Kawasaki et al. 1992; Shenton et al. 1992; Weinberger et al 1992; Bogerts et al. 1993; Fukuzako et al.).

Advances in magnetic resonance spectroscopy (MRS) now permit the noninvasive study of brain metabolism in vivo. The phosphorus spectrum offers information on the brains's metabolism of phospholipid and of high-energy phosphate (Bottomley 1989; Lock et al. 1990; Keshavan et al. 1991). Using in vivo ^{31}P-MRS, three groups of investigators found metabolic abnormalities in the temporal lobe (Calabrese et al. 1992; Fujimoto et al. 1992) or slight elevation of pH in the left temporoparietal region (O'Callaghan et al. 1991) in schizophrenia.

Schizophrenia is characterized by the heterogeneity of its clinical symptoms, response to treatment, and clinical course, as well as other features. Neurophysiological differences have been demonstrated between schizophrenic subtypes (Kishimoto et al. 1987; Paulman et al. 1990; Buchanan et al. 1993). An association between an increase in cerebral blood flow in the medial temporal lobe and distortion of reality such as auditory hallucinations and delusions was pointed out by Liddle et al. (1992). We selected a relatively homogeneous group of patients with neuroleptic-resistant marked positive symptoms. We hypothesized that metabolic abnormalities of their medial temporal region might be detected by ^{31}P-MRS.

Subjects and Methods

Subjects

The psychiatrists in charge of the patients selected 34 patients from 486 inpatients in the psychiatric wards in the Fujimoto Hospital and the Kagoshima University Hospital. The patients selected were those who (1) met the DSM-III-R criteria for schizophrenia (American Psychiatric Association 1987); (2) had no history of neurologic disorder, metabolic disorders (e.x. diabetes, hyperthyroidism), drug abuse or alcoholism; (3) had no history of electroconvulsive therapy or leukotomy; (4) had not been relieved from positive symptoms such as auditory hallucinations and delusions for more than two years, despite neroleptic treatment. Two psychiatrists (K.T. and T. Fukuzako) evaluated the 34 patients on the Scale for the Assessment of Negative Symptoms (SANS) (An-

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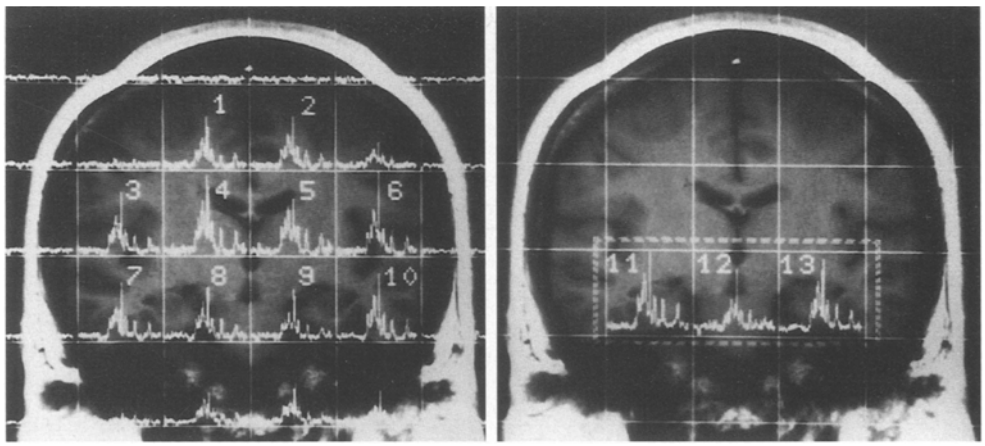

Fig. 1 Voxel placement for obtaining phosphorus spectra from 10 voxels *(left)*. Voxel placement for obtaining phosphorus spectra from medial temporal region *(right)*. Spectra were produced by re-calculating the acquired signals

dreasen 1984a) and the Scale for the Assessment of Positive Symptoms (SAPS) (Andreasen 1984b). The score employed for each item was the mean of the two raters. In this study, "a patient with neuroleptic-resistant marked positive symptoms" was defined as a patient who had a total score of over 20 on SAPS and 4 of 5 on auditory hallucinations or delusions. Twenty-one patients fulfilled this definition. Five of the patients did not give written informed consent to participate in the study or did not keep resting during the MRS examination. As a result, 16 patients (eight men and eight women) aged 39.6 ± 8.1 years were selected for this study.

Average scores were 44.1 ± 23.0 (range 13–89) on SANS and 45.6 ± 13.3 (26–71) on SAPS. Such symptoms had prevented them from leaving hospital and/or of gaining employment for more than 5 years. All patients were given neuroleptics (863.8 mg/day, chlorpromazine equivalents) (Davis 1976) and anticholinergic agents (5.7 mg/day, benztropine equivalents) (Klett and Caffey 1972) at the time of the MRS examination. Thirteen had participated in MRI and/or MRS examination before this study. Seven were subjected to repeated MRS because of movement artifacts. The control group consisted of 16 healthy subjects matched for age and sex who were members of our hospital staff and their relatives. Their mean age was 40.1 ± 7.4. None had a history of psychiatric or neurological illness or of alcohol or drug abuse. All subjects were right-handed according to the Edingburgh inventory (Oldfield 1971); a laterality score of greater than 80 was designated as right-handed (Schachter et al. 1987).

MR spectroscopy

Investigations were conducted on a MR system (Siemens-Asahi Meditec, Erlangen, Germany) with a magnetic field strength of 2.0 tesla. A circular polarizing (quadrature detection) head coil with a diameter of 29 cm was used. The head coil was tuned to 84.5 MHz for proton imaging and 34.2 MHz for in vivo multivoxel ^{31}P-MRS (three-dimensional chemical shift imaging; 3DCSI).

T1-weighted spin-echo images with a repetition time (TR) of 500 msec and an echo time (TE) of 15 msec were acquired, and unlocalized shimming was performed over the whole head using proton observation. Voxel placement is shown in Fig. 1. The field of view was 24 cm used in combination with an 8 × 8 data matrix and sections 4 cm thick. The volume of each voxel was 36 ml (3 × 3 × 4 cm^3). On the midsagittal slice, the placement of voxels was determined as the posterior line of volume of interest (VOI) passing through the superior and inferior colliculus. On the coronal plane, the placement of the voxels was determined as the midline of voxels passing through the interhemispheric fissure and third ventricle. A total of 10 voxels showing typical spectral patterns were obtained. Local shimming of the magnetic field was carried out for all VOIs by optimizing the proton signal from water. Usually a half-height water signal line width of 0.1 ppm was achieved. The spectra from 3 voxels (voxel 11, 12, 13) were produced by recalculating the signals already obtained. On ^{31}P-MRS, the TR was

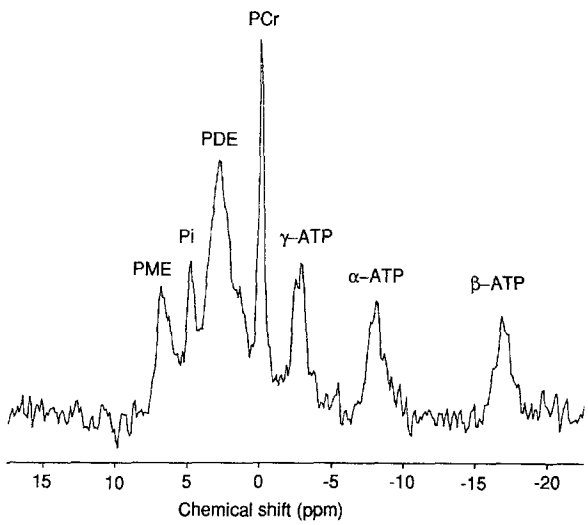

Fig. 2 ^{31}P NMR spectrum of the left medial temporal region (voxel 13) in a patient with schizophrenia

2 sec, the number of sample points 1024, and acquisition delay 1.72 msec. Twelve measurements were obtained for each spectrum. Starting with unlocalized shimming the entire procedure took 45–60 minutes. Data were processed with exponential multiplication (16 Hz) and Fourier transformed. Phase correction was then applied.

Spectral peaks were obtained for phosphomonoesters (PME), inorganic orthophosphate (Pi), phosphodiesters (PDE), phosphocreatine (PCr) and the γ, α and β phosphates of 5′-adenosine triphosphate (ATP) (Fig. 2). The spectra were quantified by peak area measurements. Peak areas were automatically calculated by means of a Lorentzian curve fitting procedure after baseline correction, using a computer program (Miyazaki 1992). For each spectrum the integrated areas of the PME, Pi, PDE, PCr and γ-, α- and β-ATP were measured and the mole percentages were calculated. The β-ATP peak was adopted as an ATP reference, in that γ- and α-ATP contain other phosphate metabolites such as adenosine diphosphate and dinucleotide phosphate.

In addition to the voxel 11 and 13, we tested possible changes of metabolites in the voxel 1 and 2 that contained frontal and parietal lobes.

Statistics

The Wilks-Shapiro test was used to test the normality of distribution of data, and the normality was not denied ($P < 0.05$, positively

Table 1 Mole percentage of phosphorus metabolites

		Patients ($N = 16$)	Controls ($N = 16$)	MANOVA		
				Source	F-value	P-value
PME	Left	10.5 ± 1.8	9.5 ± 2.0	Diagnosis	< 0.01	0.97
	Right	10.0 ± 1.8	11.0 ± 1.8	Side	1.21	0.28
				Diagnosis by side	5.37	0.02
Pi	Left	4.6 ± 1.3	5.2 ± 1.4	Diagnosis	3.88	0.06
	Right	4.6 ± 1.6	5.5 ± 1.4	Side	0.15	0.70
				Diagnosis by side	0.22	0.64
PDE	Left	42.9 ± 2.6	38.1 ± 2.8	Diagnosis	35.3	< 0.01
	Right	40.9 ± 2.8	37.5 ± 2.8	Side	3.65	0.06
				Diagnosis by side	0.96	0.33
PCr	Left	10.8 ± 1.3	11.2 ± 1.8	Diagnosis	2.29	0.14
	Right	10.4 ± 1.2	11.1 ± 1.2	Side	0.49	0.49
				Diagnosis by side	0.11	0.75
β-ATP	Left	8.7 ± 1.9	11.3 ± 2.4	Diagnosis	7.60	< 0.01
	Right	10.0 ± 1.9	10.3 ± 1.9	Side	0.10	0.75
				Diagnosis by side	5.17	0.03

Values are represented as mean ± SD

skewed). Multivariate analysis of variance (MANOVA) with a between-subject factor of diagnosis (schizophrenics vs controls) and a within-subject factor of side (left vs right) was applied to mole percentages of the five metabolites. Differences between groups were tested by the two-tailed t-test. Analyses yielding a P value < 0.05 were considered statistically significant. Pearson's product moment (r) was used to determine the relationship between mole percentage and neuroleptic dose. Kendall's rank correlation coefficient (τ) was used to test the relationship between mole percentage and anticholinergic dose.

Results

Table 1 presents the mole percentages of phosphorus metabolites in the two groups and the results of MANOVA. Significant effects of diagnosis were seen for PDE and β-ATP. A significant bilateral increase in the % PDE was present in the schizophrenic group (left, $t = 4.94$, $P < 0.0001$; right, $t = 3.48$, $P < 0.005$). A significant diagnosis by side interaction emerged for β-ATP. Subsequent analysis revealed that schizophrenic patients showed a % β-ATP reduction in the left temporal lobe $t = 3.33$, $P < 0.005$). A significant diagnosis by side interaction was also observed for PME, with higher values in schizophrenics in the left side and in controls in the right side. However, these differences in the % PME did not reach statistical significance by t-test (left., $t = 1.63$, $P = 0.11$; right, $t = 1.64$, $P = 0.11$). There were no significant correlations between the % PDE and the daily dose of neuroleptics (left, $r = 0.02$, $P = 0.94$; right, $r = 0.09$, $P = 0.73$) and the daily dose of anticholinergic agents (left, $\tau = 0.01$, $P = 0.94$; right, $\tau = 0.19$, $P = 0.41$). No significant correlations were seen between the % β-ATP and the daily dose of neuroleptics (left, $r = 0.35$, $P = 0.18$; right, $r = 0.08$, $P = 0.76$) and the daily dose of anticholinergic agents (left, $\tau = 0.23$, $P = 0.33$; right, $\tau = -0.30$, $P = 0.20$). There were no significant differences in mole percentages of the five metabolites in the voxel 1 and 2 between schizophrenic and control groups (PME, left 9.5 ± 2.9 cf. 10.6 ± 2.3, right 9.5 ± 2.5 cf. 9.9 ± 1.7; Pi, left 6.1 ± 1.3 cf. 6.8 ± 1.7, right 6.5 ± 1.9 cf. 6.5 ± 2.7; PDE, left 38.9 ± 3.4 cf. 39.0 ± 1.9, right 40.6 ± 3.7 cf. 39.5 ± 3.4; PCr, left 9.9 ± 1.5 cf. 10.1 ± 1.4, right 9.6 ± 1.6 cf. 10.2 ± 1.4; β-ATP, left 10.6 ± 2.1 cf. 9.9 ± 2.1, right 10.5 ± 1.8 cf. 9.9 ± 2.3; $0.01 < t < 1.41$, $0.17 < P < 0.99$).

Discussion

We observed an increase in PDE in the bilateral medial temporal lobes of schizophrenic patients as compared with normal controls. Calabrese et al. (1992) found a trend towards an increased PDE in the bilateral temporal lobes of schizophrenic patients relative to conrols. Fujimoto et al. (1992) found a higher level of PDE in the left temporal lobe of male patients with chronic schizophrenia than in normal controls, results that are consistent with our findings.

The effects of neuroleptics and anticholinergic agents must be considered when discussing the significance of the increase in PDE level. There was no significant correlation between the level of PDE and the daily dose of neuroleptics or anticholinergics. Nevertheless, we need to be cautious about interpreting our results, in that 4 weeks of neuroleptic administation multiplied the increased level of PDE in the dorsal prefrontal cortex (Pettegrew et al. 1989). Further study is needed to confirm whether the PDE level is increased in the temporal lobe of drug-naive schizophrenics. Such an investigation is currently underway in our laboratory.

PDE resonance in in vitro experiments is composed mainly of glycerol 3-phosphoethanolamine and glycerol 3-phosphocholine. These two metabolites appear exclu-

sively in the catabolic pathways of phospholipids (Pettegrew et al. 1987). However, PDE resonance obtained by in vivo MRS was considered to have different components from in vitro experiments. The resonance in vivo is reported to come largely from uncoupled membrane phospholipids and in part from breakdown products (Murphy et al. 1989; Lock et al. 1990). PDE is more concentrated in the white than the gray matter (Kilby et al. 1990). Therefore, the increase in PDE seems to reflect a decreased ratio of gray-to-white matter volume in the VOI, changes in mobility of membrane phospholipids, and/or an increase in catabolic activity of bilayer phospholipids. The increased catabolic activity of the membrane phospholipids might be due to the accelerated activity of phospholipase A2 (Gattaz et al. 1990). Volumetric measurements of gray and white matter in the VOIs are required to determine how the results are affected by structural changes in the VOIs.

The level of β-ATP was reduced in the left medial temporal lobe of the patients studied. Fujimoto et al. (1992) demonstrated a decrease of ATP in the left temporal lobe, similar to our findings. A trend towards a side by group interaction of β-ATP was reported, with higher values on the left than on the right in medicated schizophrenics (Calabrese et al. 1992). The discrepancy between their results and ours may be due to differences in characteristics of patients, location of VOI and MRS procedures. In the Calabrese study, subjects were not matched for age and sex. In addition, we selected patients with markedly positive symptoms. They used image-selected in vivo spectroscopy with 87 ml VOI which contained other structures besides temporal lobes. We used 3DCSI as a method of measurement with 36 ml VOI. The VOI was mainly locazied in the medial temporal lobe but did not eliminate brain structures other than temporal lobe, because we did not obtain a phosphorus spectrum of acceptable signal-to-noise ratio when the VOI became smaller (unpublished data).

Based on the theoretical metabolic pathways in the mammalian cell (Keshavan et al. 1991) any definite interpretation of the reduction in β-ATP does not seem to be warranted, because no significant changes were detected in the level of Pi or PCr. We can only suggest that there may be some abnormality of energy metabolism in the left medial temporal lobe and not on the right side, as measured by MRS. Kawasaki et al. (1992) found an increase in cerebral blood flow of the left hippocampal region of schizophrenics. Liddle et al. (1992) showed an association between increased regional cerebral blood flow in the left medial temporal lobe and hallucinations and delusions. DeLisi et al. (1998) demonstrated a positive correlation between hallucinations and increased glucose use in the left temporal lobe. Predominantly, a left-sided functional disturbance in the temporal lobe of these studies is consistent with the results of our study.

Relationships between clinical symptoms and abnormalities in PDE or ATP were not tested because the distribution of characteristics of the patients in this study was skewed due to our selection criteria described above. Studies employing many patients with various characteristics are needed for determining such relationships. In conclusion, our results suggest that schizophrenic patients who have prominent positive symptoms refractory to neuroleptic treatment may have a disturbance of bilateral membrane phospholipid and left-sided high-energy phosphate metabolism in the medial temporal lobe.

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