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Schizophrenia Research 117 (2010) 83–91

Contents lists available at ScienceDirect

Schizophrenia Research

journal homepage: www.elsevier.com/locate/schres

GABA concentration in schizophrenia patients and the effects of antipsychotic medication: A proton magnetic resonance spectroscopy study

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ARTICLE INFO

Article history:
Received 17 November 2008
Received in revised form 18 November 2009
Accepted 21 November 2009
Available online 22 December 2009

Keywords:
Schizophrenia
GABA
Magnetic resonance spectroscopy (MRS)
Atypical antipsychotics
Typical antipsychotics

ABSTRACT

Gamma-amino butyric acid (GABA) is thought to play a role in the pathophysiology of schizophrenia. High magnetic field proton magnetic resonance spectroscopy (^{1}H-MRS) provides a reliable measurement of GABA in specific regions of the brain. This study measured GABA concentration in the anterior cingulate cortex (ACC) and in the left basal ganglia (ltBG) in 38 patients with chronic schizophrenia and 29 healthy control subjects.
There was no significant difference in GABA concentration between the schizophrenia patients and the healthy controls in either the ACC (1.36 ± 0.45 mmol/l in schizophrenia patients and 1.52 ± 0.54 mmol/l in control subjects) or the ltBG (1.13 ± 0.26 mmol/l in schizophrenia patients and 1.18 ± 0.20 mmol/l in control subjects). Among the right handed schizophrenia patients, the GABA concentration in the ltBG was significantly higher in patients taking typical antipsychotics (1.25 ± 0.24 mmol/l) than in those taking atypical antipsychotics (1.03 ± 0.24 mmol/l, $p = 0.026$). In the ACC, the GABA concentration was negatively correlated with the dose of the antipsychotics ($rs = -0.347$, $p = 0.035$). In the ltBG, the GABA concentration was positively correlated with the dose of the anticholinergics ($rs = 0.403$, $p = 0.015$).
To the best of our knowledge, this is the first study to have directly measured GABA concentrations in schizophrenia patients using ^{1}H-MRS. Our results suggest that there are no differences in GABA concentrations in the ACC or the ltBG of schizophrenia patients compared to healthy controls. Antipsychotic medication may cause changes in GABA concentration, and atypical and typical antipsychotics may have differing effects. It is possible that medication effects conceal inherent differences in GABA concentrations between schizophrenia patients and healthy controls.

1. Introduction

1.1. The Gamma-amino butyric acid (GABA) system in schizophrenia

Gamma-amino butyric acid (GABA) is thought to play a role in the pathophysiology of schizophrenia (Guidotti et al., 2005; Wassef et al., 2003).

1.1.1. Postmortem studies

Postmortem studies of $GABA_A$ receptors in chronic schizophrenia have reported inconsistent findings. Some

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doi:10.1016/j.schres.2009.11.011

case–control studies have reported increased $GABA_A$ receptor binding in the cingulate cortex (Hanada et al., 1987; Benes et al., 1992), whereas others have found it to be decreased (Squires et al., 1993) or unchanged (Pandey et al., 1997). The $GABA_A$ receptor is composed of various subunits. Ishikawa et al. (2004) found a higher density of alpha 1 and beta 2/3 subunits in the prefrontal cortex (PFC) of schizophrenia patients compared to control subjects.

The 65 and 67 kDa isoforms of glutamic acid decarboxylase (GAD) are key enzymes in GABA synthesis, and a number of studies have investigated their significance in schizophrenia. Bird et al. (1977) found that GAD levels were decreased in the nucleus accumbuns, amygdala, hippocampus, and putamen of schizophrenia patients. Benes et al. (2000) observed no change in GAD density in the anterior cingulate cortex (ACC) of schizophrenia patients. Woo et al. (2004) found a decrease in the number of GAD67 mRNA-containing neurons in the ACC of schizophrenia patients compared to control subjects. Dracheva et al. (2004) reported an increased expression of GAD65 and GAD67 mRNA in the dorsolateral PFC and in the occipital cortex of schizophrenia patients compared to control subjects.

1.1.2. In vivo neuroimaging studies

Neuroimaging studies using radio active ligands have also reported inconsistent findings. Some studies using single photon emission computed tomography (SPECT) have failed to find any evidence of $GABA_A$ receptor binding abnormalities in the brains of schizophrenia patients compared to healthy controls (Busatto et al., 1997; Verhoeff et al., 1999; Abi-Dargham et al., 1999). One study, however, found a significant correlation between task performance and $GABA_A$/benzodiazepine receptor binding in the frontal and occipital cortices of schizophrenia patients (Ball et al., 1998). Using positron emission tomography (PET), Asai et al. (2008) reported no differences in [11C] Rol5-4513 binding (which represents the density of the alpha 5 subunit of the $GABA_A$/benzodiazepine receptor) in the PFC and the hippocampus of schizophrenia patients compared to control subjects; among the schizophrenia patients, the degree of binding was found to be negatively correlated with negative symptom scores.

1.1.3. The effects of antipsychotic medication on the GABA system in the basal ganglia and cingulate cortex

Gunne et al. (1984) reported an inhibition of GAD activity in monkeys following treatment with antipsychotics. Studies in rats have reported that treatment with typical antipsychotic drugs such as haloperidol (Jolkkonen et al., 1994; Delfs et al., 1995a,b; Laprade and Soghomonian, 1995; Sakai et al., 2001), fluphenazine (Chen and Weiss, 1993; Johnson et al., 1994), and sulpiride (Laprade and Soghomonian, 1995) increased the expression of GAD67 and GAD67 mRNA in the basal ganglia, whereas atypical antipsychotic drugs such as clozapine (Delfs et al., 1995a) and olanzapine (Sakai et al., 2001) did not. These changes may be reflected in the dyskinetic and antipsychotic actions of typical antipsychotics (Delfs et al., 1995b; Sakai et al., 2001). Zink et al. (2004) reported that both haloperidol and clozapine increased [^{3}H]-muscimol binding to $GABA_A$ receptors in the ACC, whereas increased $GABA_A$ receptor binding in the basal ganglia was only induced by haloperidol. Although the underlying mechanism is unclear, these results suggest that antipsychotics may affect the GABA system, and that typical and atypical antipsychotics may have differing effects.

1.2. The role of the ACC and the basal ganglia in schizophrenia

Several changes in the ACC of schizophrenia patients have been reported: (1) alterations in GAD levels (Woo et al., 2004), (2) morphological change (Baiano et al., 2007; Fujiwara et al., 2007; Zetzsche et al., 2007), and (3) activation deficits during cognitive tasks (Liddle et al., 2006; Yücel et al., 2007; Brüne et al., 2008; Koch et al., 2008). Menzies et al. (2007) found that GABA-modulating drugs affected working memory performance and induced activation changes in the ACC of schizophrenia patients. The basal ganglia contain the striatum, the globus pallidus, and other structures. The striatum is thought to receive GABAergic interneurons from other regions of the brain, in particular the globus pallidus and the cerebral cortex (Bolam et al., 2000). The PFC is thought to be involved in the pathophysiology of schizophrenia on three levels: morphologically (Meda et al., 2008), functionally (Lee et al., 2006), and histologically (Woo et al., 2008). The PFC tonically inhibits striatal dopamine projections, and it is thought that this is mediated by GABA interneurons (Carlsson, 2001; Akil et al., 2003; Perlman et al., 2004). The globus pallidus is also thought to be involved in the pathophysiology of schizophrenia (Galeno et al., 2004; Spinks et al., 2005). An increase in $GABA_A$ receptor binding in the basal ganglia following the administration of antipsychotics has been reported (Zink et al., 2004).

1.3. Magnetic resonance spectroscopy

Proton magnetic resonance spectroscopy (^{1}H-MRS) provides an in vivo measurement of brain metabolites such as myo-inositol, N-acetylaspartate, choline-containing compounds, Glx (glutamate plus glutamine), creatine, and phosphocreatine in the human brain. The recent introduction of high magnetic field MRS has enabled the reliable measurement of GABA in specific brain regions. Reduced concentrations of GABA in depressed patients (Hasler et al., 2007) and unchanged concentrations of GABA in panic disorder patients (Hasler et al., 2008) have been reported in areas of the frontal lobe. To the best of our knowledge, no previous ^{1}H-MRS study has examined GABA concentrations in schizophrenia patients. In the present study, ^{1}H-MRS was used to compare GABA concentrations in medicated chronic schizophrenia patients with those of healthy controls using a high magnetic field device. The regions of interest (ROIs) were located in the ACC and in the left basal ganglia (ltBG); the ltBG contain the striatum, globus pallidus, and other structures. The effects of typical and atypical antipsychotic medication on GABA concentrations in the basal ganglia and cingulate cortex were also examined.

2. Method

2.1. Subjects

Thirty-eight patients with chronic schizophrenia and twenty-nine healthy control subjects participated in this

study. All study participants gave written informed consent in accordance with the guidelines of the ethics committee of the University of Tokushima. All diagnoses were assigned according to DSM-IV TR criteria. The schizophrenia patients were classified into the following two groups in order to compare the effects of the type of antipsychotic regime: (1) the TYP group, who were taking typical antipsychotics with or without concomitant atypical antipsychotics, and (2) the ATY group, who were taking atypical antipsychotics without concomitant typical antipsychotics. The patients were also classified according to schizophrenia subtype: paranoid schizophrenia ($n=36$), undifferentiated schizophrenia ($n=1$), and disorganized schizophrenia ($n=1$). Epidemiological data, including age, duration of illness, handedness, Positive and Negative Syndrome Scale scores (PANSS, Kay et al., 1987), and dose of antipsychotics at the time of the scan, are shown in Table 1. The healthy control subjects had no history of any Axis I psychiatric disorder according to DSM-IV TR criteria. None of the patients or healthy control subjects had a serious medical illness, history of head injury, or history of drug or alcohol abuse prior to the scan. All of the schizophrenia patients and healthy control subjects were Japanese and came from the same region, and their native language was Japanese. Handedness was assessed using the Edinburgh Handedness Inventory (Oldfield, 1971). With the exception of two schizophrenia patients, all study participants were right handed.

2.2. The ^{1}H-MRI/MRS procedures

^{1}H-MRS was performed using MEGA-PRESS according to previously reported methods (Mescher et al., 1998). A 3 T clinical magnetic resonance imaging (MRI) scanner was used (Sigma 3T Excite, GE, Milwaukee, WI, USA). Gradients were employed surrounding the frequency selective pulses at 1.9 ppm to diphase transverse magnetization. Water suppression involved three conventional CHESS pulses after manual optimization. The sequence parameters were as follows: (1) TR = 1500 ms, (2) TE = 68 ms, (3) size of ROI = $3.0\times3.0\times3.0$ cm^3 (27 ml), (4) summation = 256 signals for each spectrum, and (5) total acquisition time = 13 min. Alternate measurements with and without frequency selective pulses were taken; J evolution for GABA was refocused during odd-numbered acquisitions but not during even-numbered acquisitions. The difference in the acquired spectra provided an edited spectrum of GABA. The in vitro data for GABA were acquired from MEGA-PRESS, and were used as a basis for the Linear combination model (LC Model) (Provencher, 1993). The quantification of signals from the different MEGA-PRESS spectra was performed with the LC Model. An axial cut approximately 1 cm above the upper end of the body of the lateral ventricles was chosen as a reference slice of the ROI in the ACC. The center of the interhemispheric fissure was 3 cm in front of the central fissure and 2 cm above the corpus callosum (Fig. 1a). An axial cut approximately 1 cm above the genu of the corpus callosum, which provided a continuous view of the anterior and posterior horns of the lateral ventricles, was chosen as a reference slice of the ROI in the ltBG. The ROI of the ltBG was located between the sylvian fissure and the lateral ventricles in order to incorporate the lenticular nucleus (Fig. 1b). The representative ^{1}H-MRS spectra from the MEGA-PRESS sequence are shown in Fig. 2.

T1-weighted three dimensional images were acquired using the following parameters: (1) TE = 4.2 ms, (2) TR = 10 ms, (3) slice thickness = 0.8 mm, (4) matrix 512×512, (5) FOV = 24×24 cm, and (6) Flip angle = 15°. The brain images were thus composed of voxels that were $0.47\text{mm}\times0.47\text{mm}\times0.8\text{mm}$ in size. On the basis of the histogram of voxel intensity, each voxel in the 3D-SPGR brain images was classified as gray matter (GM), white matter (WM), or cerebrospinal fluid (CSF) using the "ImageJ Ver. 1.38" software package (http://rsb.info.nih.gov/ij). The voxels that were considered GM, WM, and CSF in each ROI were counted using the "3D-Slicer Ver.2.6" software package (http://www.slicer.org) in order to obtain the ratio of these tissues. Metabolite concentrations in the ROI were corrected for CSF by dividing the percentage of brain tissues in each ROI, under the assumption that the metabolite concentrations in CSF were equal to zero (Bustillo et al., 2001).

The criteria for selecting the reliable metabolite concentrations were based on the %SD of the fit for each metabolite, reflecting the Cramer–Rao lower bounds (CRLB) for LC Model analysis. The data included in the present study showed %SD of less than 20%. In our previous study, the intraclass correlation of two measurements in the same study participant was greater than 0.7, indicating acceptable reliability as a clinical instrument.

Table 1
Epidemiologic data.

		Schizophrenia	Controls	p
Age	(all)	34.0 ± 10.0	34.0 ± 10.2	n.s.[a]
(mean ± S.D. years old)	(rt handed)	34.7 ± 10.0	34.0 ± 10.2	n.s.[a]
Number (male/female)	(all)	38 (20/18)	29 (17/12)	n.s.[b]
	(rt handed)	36(19/17)	29 (17/12)	n.s.[b]
Duration of illness (years)		11.1 ± 9.4		
PANSS total score (mean ± S.D.)		54.0 ± 14.4		
PANSS positive score (mean ± S.D.)		13.2 ± 6.0		
PANSS negative score (mean ± S.D.)		15.1 ± 5.3		
PANSS general score (mean ± S.D.)		26.7 ± 6.9		
Dose of antipsychotics[c] (mean ± S.D.)		423.7 ± 362.3		

Abbreviation: rt handed, right handed; PANSS, Positive and Negative Syndrome Scale; n.s., not significant.
[a] Two sample Student's *t*-test.
[b] χ square test.
[c] Chlorpromazine equivalent.

2.3. Statistical procedures

Statistical tests were performed using the "SPSS Version 11.5" software package (SPSS Japan Inc., Tokyo, Japan). The absolute GABA levels, as measured with ^{1}H-MRS, were analyzed.

For both ROIs, two sample *t*-tests were used to compare the mean values of the GABA concentrations in schizophrenia patients and healthy control subjects. The GABA concentration of the TYP group was compared to that of the ATY group using analysis of covariance (ANCOVA), with covariance for age.

For both ROIs, the correlation between GABA concentration and each of the clinical measures (e.g., age, dose of benzodiazepine, dose of antipsychotics, dose of anticholinergics, and the positive, negative, and general PANSS scores) was

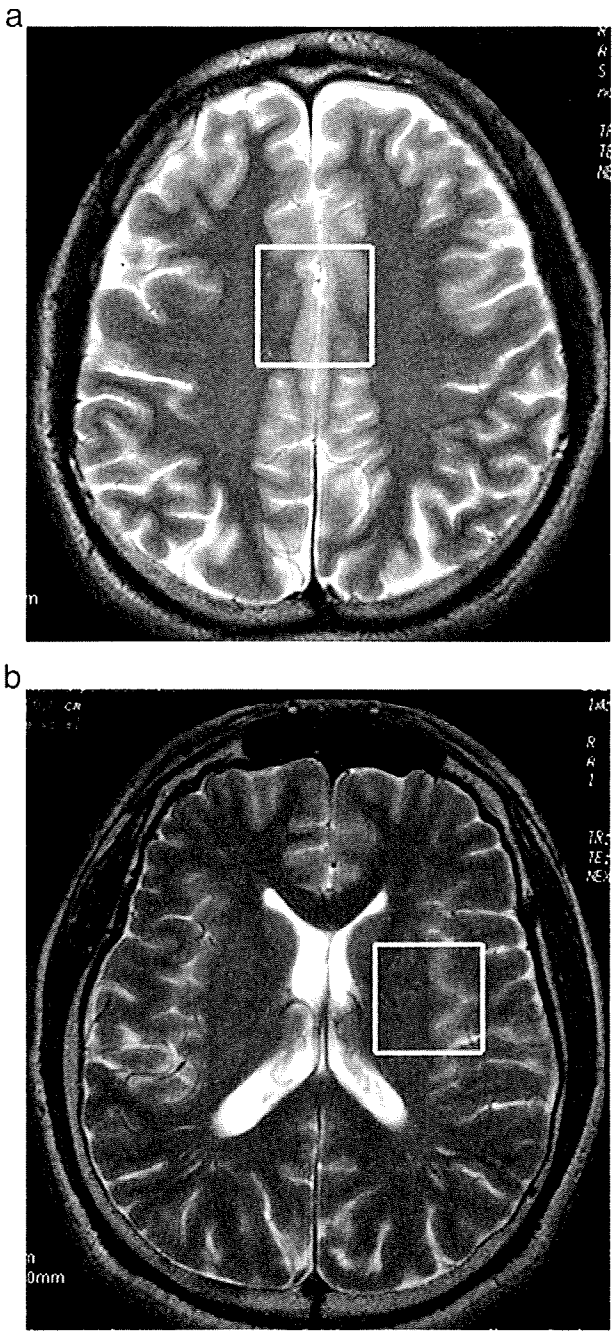

Fig. 1. The region of interest (ROI) positions in the anterior cingulate cortex (ACC) (a) and left basal ganglia (ltBG) (b) for spectroscopic measurement by MEGA-PRESS sequence. The white box represents the location of the ROI (3 cm × 3 cm × 3 cm) that was used in the MEGA-PRESS sequence in the horizontal image.

evaluated using Spearman's rank correlation test. Since handedness could have influenced the results, the analysis in the ltBG was only performed for right handed patients i.e. the two left handed schizophrenia patients were excluded.

Statistical significance was set at $p<0.05$.

Since the partial volume effects of the GM and the WM may influence the GABA concentration in ^{1}H-MRS (Choi et al., 2006), a two sample t-test was used to make the following comparisons of the GM:WM ratio for each ROI: (1) schizophrenia patients and healthy control subjects, and (2) male study participants and female study participants.

3. Results

There were no significant differences between the schizophrenia patients and the healthy control subjects in age range or male:female ratio. No significant difference in GABA concentration was observed between schizophrenia patients and healthy control subjects in either the ACC (1.36 ± 0.45 mmol/l in schizophrenia patients and 1.52 ± 0.54 mmol/l in healthy control subjects, $p=0.18$) or the ltBG (1.13 ± 0.26 mmol/l in schizophrenia patients and 1.18 ± 0.20 mmol/l in healthy control subjects, $p=0.36$).

For the schizophrenia patients, the clinical data of the TYP group and the ATY group are shown in Table 2. The TYP group consisted of 16 patients and the ATY group consisted of 22 patients. For each patient, the name and dose of the antipsychotic medication prescribed at the time of the scan are provided in Tables 3a and b. Eight of the TYP group and 8 of the ATY group were prescribed benzodiazepines, and 13 of the TYP group and 9 of the ATY group were prescribed anticholinergic medication (Tables 3a and b). Three patients were prescribed concomitant mood stabilizers, and a further three patients were prescribed antidepressants (Tables 3a and b). There were no significant differences between the TYP group and the ATY group for the following factors: (1) PANSS positive, negative, and general scores, (2) dose of benzodiazepine (diazepam equivalent dose), and (3) dose of antipsychotics (chlorpromazine equivalent dose). However, age and duration of illness were significantly higher in the TYP group than the ATY group. The TYP group were prescribed anticholinergic medication significantly more often than the ATY group ($X^2=6.18$, $p=0.013$). The mean dose of anticholinergic medication (biperiden equivalent dose) did not differ between the TYP group and the ATY group.

ANCOVA was performed using the type of antipsychotic (i.e. TYP versus ATY) and the use of anticholinergic medication (i.e. patients with versus patients without anticholinergic medication) as two independent factors and with age as a covariate. In the ltBG, ANCOVA revealed a significant effect for the type of antipsychotic ($F=5.48$, $p=0.026$) but not for the use of anticholinergic medication; no interaction between the type of antipsychotic and the use of anticholinergic medication was observed. In the ACC, no significant effect was observed for the type of antipsychotic or the use of anticholinergic medication, and no interaction between these two factors was observed. In the ltBG, the mean ± SD of the GABA concentrations for the TYP group and the ATY group were 1.25 ± 0.24 mmol/l and 1.03 ± 0.24 mmol/l, respectively. In the ACC, the mean ± SD of the GABA concentrations for the TYP group and the ATY group were 1.40 ± 0.48 mmol/l and 1.27 ± 0.51 mmol/l, respectively.

A significant negative correlation was found between the dose of the antipsychotics and the GABA concentration in the ACC ($rs=-0.387$, $p=0.016$) but not in the ltBG. A significant positive correlation was found between the dose of the anticholinergic medication and the GABA concentration in the ltBG ($rs=0.399$, $p=0.016$) but not in the ACC. There was no correlation between the remaining clinical measures (i.e.

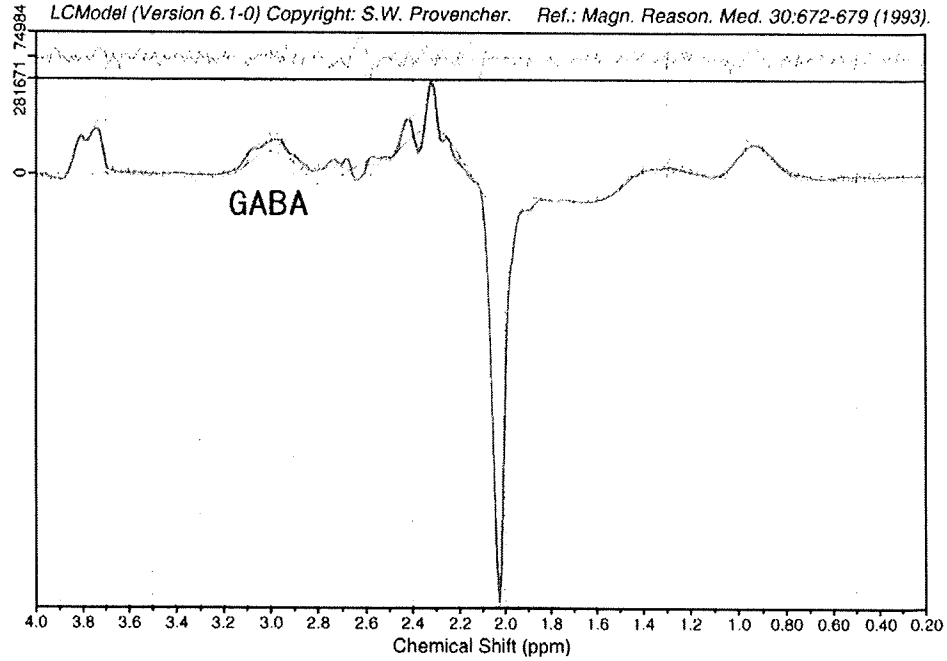

Fig. 2. Representative ^{1}H-MRS spectra from a study participant before the MEGA-PRESS sequence. The peaks that represent GABA are shown.

age, PANSS scores, and dose of benzodiazepine) and the GABA concentrations in the ACC and the ltBG.

The GM:WM ratio did not differ significantly between the schizophrenia patients and the healthy control subjects, or between the TYP group and the ATY group in either of the two ROIs (Table 4).

4. Discussion

4.1. Main findings

This study contributes four main findings. Firstly, no difference in GABA concentration was observed between the schizophrenia patients and the healthy control subjects. Secondly, among the schizophrenia patients, the GABA concentration in the ltBG was significantly higher in the TYP group than in the ATY group. Thirdly, there was a significant negative correlation between the GABA concentration in the ACC and the dose of antipsychotics. Finally, there was a positive correlation between the GABA concentration in the ltBG and the dose of anticholinergic medication.

Table 2
The clinical indices of the schizophrenic patients and types of medication.

		TYP	ATY	*p*
Number	(all)	16	22	
	(rt handed)	15	21	
Age	(all)	38.4 ± 10.7	30.8 ± 8.3	.025*
	(rt handed)	38.7 ± 11.1	31.6 ± 8.2	.046*
Duration of illness	(all)	16.2 ± 11.0	7.1 ± 6.2	.005*
	(rt handed)	16.2 ± 11.0	7.2 ± 6.5	.010*
PANSS positive		12.4 ± 2.6	13.7 ± 7.5	n.s.
PANSS negative		15.0 ± 4.0	15.2 ± 6.1	n.s.
PANSS general		27.0 ± 6.3	27.0 ± 7.3	n.s.
Dose of antipsychotics		435.9 ± 432.9	414.8 ± 311.7	n.s.
Dose of benzodiazepine		6.6 ± 8.3	3.3 ± 4.7	n.s.

Abbreviation: TYP, patients taking typical antipsychotics with or without concomitant atypical antipsychotics; ATY, patients taking atypical antipsychotics without concomitant typical antipsychotics; rt handed, right handed; PANSS, Positive and negative syndrome scale.
* Significantly different in two sample *t*-test ($p<0.05$).

4.2. No difference in the GABA concentration between schizophrenia patients and healthy control subjects

Analysis of GABA receptor binding sites and measurement of GAD have frequently been used to investigate the GABA

Table 3a
Typical antipsychotic group.

Age (years)	Sex	Antipsychotic drugs	BZD	Anticholin
40	F	HPD 0.75 mg	0	0
30	F	HPD 3 mg	0	3
50	F	HPD 1.5 mg	0	3
36	M	HPD 3 mg	0	3
26	F	HPD 2.25 mg	10	2
48	F	HPD 4 mg	10	2
54	M	HPD 2.25 mg	0	6
46	F	SPD 150 mg	10	0
24	F	OLZ 10 mg, HPD 4.5 mg	30	4
39	M	OLZ 30 mg, LP 100 mg	10	3
21	F	PER 16 mg, BPD 1.5 mg	0	2
33	M	QTP 400 mg, RIS 6 mg, LP 150mg	10	4
24	M	RIS 2 mg, LP 25 mg	0	1
36	M	RIS 3 mg, CP 100 mg, LP 100 mg	15	1
52	M	RIS 6 mg, BPD 6 mg	0	3
46	M	RIS 9 mg, LP 50 mg, ZTP 75 mg	10	0

Abbreviations: BZD, benzodiazepine dose expressed as diazepam equivalent dose; Anticholin, anticholinergic drugs expressed as biperiden equivalent dose; HPD, haloperidol; SPD, sulpiride; LP, levomepromadine; BPD, bromperidol; CP, chlorpromadine; ZTP, zotepin.

Table 3b
Atypical antipsychotic group.

Age (years)	Sex	Antipsychotic drugs	BZD	Anticholin
16	F	APZ 6 mg	0	0
25	M	APZ 12 mg	0	0
37	M	APZ 12 mg	10	1
30	F	APZ 6 mg, OLZ 2.5 mg	0	0
21	F	APZ 6 mg, RIS 1 mg	10	1
27	M	OLZ 5 mg	10	0
25	M	OLZ 5 mg	0	0
35	M	OLZ 15 mg	0	0
40	M	OLZ 20 mg	5	0
20	F	OLZ 20 mg	10	0
21	M	OLZ 25 mg	6.7	1
38	F	OLZ 25 mg	0	0
27	M	PER 2 mg	0	0
28	F	PER 16 mg	8.33	0
38	F	PER 20 mg	0	2
28	M	QTP 750 mg	0	2
25	F	RIS 2 mg	0	2
37	F	RIS 2 mg	0	0
36	M	RIS 2 mg	0	0
48	M	RIS 5 mg	0	1
25	F	RIS 8 mg	0	4
41	M	RIS 8mg	13	4

Abbreviations: BZD, benzodiazepine dose expressed as diazepam equivalent dose; Anticholin, anticholinergic drugs expressed as biperiden equivalent dose; HPD, haloperidol; SPD, sulpiride; LP, levomepromadine; BPD: bromperidol; CP, chlorpromadine; ZTP, zotepin.

system of the brain. Findings from postmortem studies of GABAA receptor binding have been inconsistent, and have included reports of an increase (Hanada et al., 1987; Benes et al., 1992), a decrease (Squires et al., 1993), and no change (Pandey et al., 1997). Studies that have used SPECT (Busatto et al., 1997; Verhoeff et al., 1999; Abi-Dargham et al., 1999) or PET (Asai et al., 2008) to measure GABA receptor binding have reported that it is unchanged. GAD is the key enzyme in GABA synthesis. Postmortem studies of schizophrenia patients have reported that GAD levels are decreased in the nucleus accumbuns, amygdala, hippocampus, and putamen (Bird et al., 1977), and unchanged in the ACC (Benes et al., 2000). It has been shown that GAD65 and GAD67 mRNA are increased in the dorsolateral PFC and in the occipital cortex of schizophrenia patients (Dracheva et al., 2004). A reduction in GAD67 mRNA-containing neurons in the ACC has been reported in schizophrenia patients (Woo et al., 2004).

Table 4
The ratio of gray matter and white matter in each ROI.

		Schizophrenia		Control	p
		TYP	ATY		
GM in ACC	(all)	0.38 ± 0.08		0.37 ± 0.05	n.s.
	(all)	0.39 ± 0.08	0.37 ± 0.07		n.s.
WM in ACC	(all)	0.22 ± 0.07		0.22 ± 0.07	n.s.
	(all)	0.22 ± 0.07	0.21 ± 0.08		n.s.
GM in ltBG	(rt handed)	0.34 ± 0.11		0.35 ± 0.10	n.s.
	(rt handed)	0.37 ± 0.13	0.32 ± 0.08		n.s.
WM in ltBG	(rt handed)	0.62 ± 0.12		0.63 ± 0.11	n.s.
	(rt handed)	0.59 ± 0.13	0.65 ± 0.10		n.s.

Abbreviations: GM, gray matter; WM, white matter; rt handed, right handed; ACC, anterior cingulate cortex; ltBG, left basal ganglia; TYP, the patients taking typical antipsychotics with or without concomitant atypical antipsychotics; ATY, the patients taking atypical antipsychotics without concomitant typical antipsychotics; n.s., not significantly different in two sample *t*-test.

Our study has demonstrated that the in vivo GABA concentrations of the ACC and the ltBG did not differ significantly between medicated chronic schizophrenia patients and healthy control subjects.

4.3. Higher ltBG GABA concentration in the TYP group than the ATY group

Animal studies have reported that typical antipsychotics increased the expression of GAD67 and GAD67 mRNA in the basal ganglia (Chen and Weiss, 1993; Johnson et al., 1994; Jolkkonen et al., 1994; Delfs et al., 1995a,b; Laprade and Soghomonian, 1995; Sakai et al., 2001), but that administration of atypical antipsychotic drugs did not (Delfs et al., 1995a; Sakai et al., 2001). Our observation of significantly higher GABA concentrations in the TYP group compared to the ATY group is therefore compatible with the findings of these animal studies. This difference in GABA concentration may be reflected in the dyskinetic effects of typical antipsychotics (Delfs et al., 1995b; Sakai et al., 2001), although the severity of dyskinesia did not differ significantly between the two groups of patients in the present study (data not shown). The true extent of the influence of typical and atypical antipsychotics on GABA concentration in the present patient sample remains unknown since we did not include unmedicated patients. Unmedicated schizophrenia patients might be expected to have lower GABA concentrations than patients treated with typical antipsychotics, but further studies would be necessary to test this hypothesis.

4.4. Relationship between GABA concentration and the dose of antipsychotics in the ACC, and the dose of anticholinergics in the ltBG

Results from animal studies have suggested that antipsychotic medication may influence the GABA system in the cingulate cortex (Sharp et al., 1994, 2001; Vincent et al., 1994; Squires and Saederup, 2000). Zink et al. (2004) reported that both clozapine and haloperidol increased $GABA_A$ receptor binding in the ACC, while only haloperidol increased $GABA_A$ receptor binding in the basal ganglia. These animal studies indicated that both atypical and typical antipsychotics have an effect on the GABA system in the cingulate cortex.

Previous MRS studies have examined the effect of antipsychotic treatment on the combined signals of glutamate, glutamine and GABA (Glx). Although one study reported a decrease in Glx levels following antipsychotic treatment (Choe et al., 1996), another reported no significant difference in the PFC following treatment with risperidone (Szulc et al., 2005). A further study reported no change in the cingulate cortex following a switch from typical antipsychotics to olanzapine (Goff et al., 2002). It is difficult to estimate the exact effect of antipsychotics on GABA concentrations from these studies. In a previous study, we found no significant correlation between glutamate or glutamine concentrations and the dose of antipsychotics (Tayoshi et al., 2009).

The present study found a significant negative correlation between the GABA concentration in the ACC and the dose of antipsychotics i.e. a higher dose of antipsychotics was associated with a lower concentration of GABA in the ACC.

All antipsychotic medications, including both typical and atypical antipsychotics, may decrease the GABA concentration in the ACC. Although no difference was found in the GABA concentration in the ACC of medicated patients compared to healthy control subjects, it would be reasonable to hypothesize that unmedicated patients might show a higher GABA concentration in the ACC. Studies of unmedicated patients would provide valuable insights into this issue.

The significant positive correlation found between the dose of anticholinergic medication and the GABA concentration in the ltBG may be of clinical significance. The striatum receives dense cholinergic innervations from local interneurons, and the main synaptic targets of these cholinergic interneurons are GABAergic projection neurons (Graybiel, 1990; Izzo and Bolam, 1988). Muscarinic receptors on the GABA neurons are known to reduce GABA-mediated synaptic potentials and GABA release (Calabresi et al., 2000). It has been proposed that blockade of muscarinic stimulation by anticholinergic medication may increase the GABA concentration in the basal ganglia of schizophrenia patients.

4.5. Limitations

The present study has certain limitations. Firstly, the volume of the ROIs was set relatively high in order to measure the GABA concentrations. The volume was 27 ml, and the ROIs might therefore have contained heterogeneous tissues. Different results might have been obtained if the GABA concentration had been measured with smaller ROIs containing more homogenous tissues. Secondly, schizophrenia is thought to be a heterogeneous disorder, and the inherent heterogeneity of the schizophrenia patients might have affected the results, although the fact that most of the schizophrenia patients in our study were diagnosed as paranoid type may have minimized this effect. However, had the schizophrenia patients been divided into subgroups on the basis of other factors (e.g., genetic factors), significant differences in the GABA concentration between some of the schizophrenia subgroups and healthy control subjects might have been revealed. Thirdly, since antipsychotic medication may influence GABA concentrations, measurements in patients not taking antipsychotic medication would have provided valuable insights. Finally, although around half of the participants in the present study were female, we did not control for the effect of the menstrual cycle, a factor which may have an effect on GABA concentrations (Epperson et al., 2005).

5. Conclusion

Using ^{1}H-MRS, we have been the first to measure GABA concentrations in schizophrenia patients, but have found no differences in either the ACC or the ltBG between patients and healthy control subjects. Among the schizophrenia patients, the GABA concentration in the ltBG was higher in those taking typical antipsychotics than in those taking atypical antipsychotics. The GABA concentration in the ACC was found to be negatively correlated with the dose of the antipsychotics. Although the underlying mechanism is unclear, our results suggest that antipsychotic medication may induce changes in GABA concentration, and that these changes are dependent upon the type of medication. Antipsychotic medication may conceal inherent differences in GABA concentrations between schizophrenia patients and healthy controls.

Role of funding source

The funding sources had no involvement in the study design; collection, analysis and interpretation of the data; or writing of the report and the decision to submit it for publication.

Contributors

Author Tayoshi designed the study, wrote the protocol, managed the literature search, and undertook the statistical analysis. Author Nakataki recruited study participants and undertook additional statistical analysis. Authors Sumitani, Taniguchi, Shibuya-Tayoshi, Numata, and Iga recruited study participants. Author Harada operated the ^{1}H-MRS. Author Ueno also managed the literature search and recruited study participants. Author Ohmori managed the progress of the entire study.

Conflict of interest

The authors have no conflict of interest to declare.

Acknowledgement

This work was supported in part by a Grant-in-Aid for Scientific Research from the Japanese Ministry of Education, Culture, Sports, Science, and Technology (No. 19790819 and No. 21591520).

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Schizophrenia Research 108 (2009) 69–77

Contents lists available at ScienceDirect

Schizophrenia Research

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Metabolite changes and gender differences in schizophrenia using 3-Tesla proton magnetic resonance spectroscopy (^{1}H-MRS)

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ARTICLE INFO

Article history:
Received 12 July 2008
Revised 10 November 2008
Accepted 10 November 2008
Available online 20 December 2008

Keywords:
Magnetic resonance spectroscopy (MRS)
Glutamate (Glu)
Myo-inositol (mI)
Anterior cingulate cortex (ACC)
Left basal ganglia (ltBG)
Gender difference
Benzodiazepines

ABSTRACT

A change in the glutamatergic system is thought to play an important role in the pathophysiology of schizophrenia. The aim of this study was to investigate the changes in metabolites, including glutamate (Glu), in the anterior cingulate cortex (ACC) and the left basal ganglia (ltBG) of patients with chronic schizophrenia using proton magnetic resonance spectroscopy (^{1}H-MRS). In addition, since gender differences in this illness were known, we examined the effects of gender on these metabolites.

The ^{1}H-MRS was performed on the ACC and ltBG of 30 patients with schizophrenia and 25 healthy individuals who acted as the control group. The levels of Glu, glutamine (Gln), creatine plus phosphocreatine (Cre), myo-inositol (mI), N-acetylaspartate (NAA), and choline-containing compounds (Cho) were measured.

Two-way analysis of variance revealed that the illness significantly affected the levels of Glu and mI in the ACC; both metabolites were lower in the patients with schizophrenia as compared to the control subjects. The results also revealed that gender significantly affected the level of Gln in the ACC and the levels of Cre and NAA in the ltBG; the level of Gln in the ACC were higher in male subjects versus female subjects, whereas Cre and NAA levels in the ltBG were lower in male subjects as compared to female subjects.

These results confirmed a change in the glutamatergic system and suggested an involvement of mI in the pathophysiology of schizophrenia.

1. Introduction

Pathological changes in the brain may be an underlying cause of schizophrenia. Proton magnetic resonance spectroscopy (^{1}H-MRS) is a promising method that may be used to investigate such changes to clarify the pathophysiology of this illness.

Glutamate (Glu) is thought to play an important role in the pathophysiology of schizophrenia (Lang et al., 2007). According to the glutamatergic hypothesis of schizophrenia, the glutamatergic system becomes hyperactive in the acute stage and causes neuroinflammation and apoptosis of neurons through excitotoxity. This hypothesis suggests that the Glu concentration may become higher in the acute stage and lower in the chronic stage. The use of a low magnetic field device for evaluation makes separating the signal of Glu and glutamine (Gln) difficult; thus, the combined signals of these compounds (Glx, or the combiniation of Gln and Glu) or the ratio of Glx/Cre (i.e., ceratine plus phosphocreatine) were often reported. The

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doi:10.1016/j.schres.2008.11.014

Table 1a
Epidemiology of schizophrenic subject and healthy controls

	Control		Schizophrenia		p
	Male	Female	Male	Female	
Number	25		30		
	13	12	14	16	n.s.
Age	34.9±10.7		33.8±9.5		n.s.
	36.8±12.1	32.8±9.1	34.9±9.2	33.1±10.1	n.s.

Ages are shown as mean±S.D. (range).
Abbreviation: n.s., no significant difference.

increase (Choe et al., 1994; Chang et al., 2007), lack of change (Block et al., 2000; Yamasue et al., 2003; Ohrmann et al., 2007; Wood et al., 2007) and decrease (Choe et al., 1996) of the level of Glx or Glx/Cre ratios have been reported. Previous studies with high-magnetic magnetic resonance spectroscopy (MRS) reported that in first-episode schizophrenia patients, Glu concentration significantly increased in the left anterior cingulate cortex (ACC) (Theberge et al., 2002), whereas in medicated, chronic schizophrenia patients, Glu significantly decreased in the left ACC (Theberge et al., 2003).

Since changes in the ACC and basal ganglia were reported in patients with schizophrenia (Molina et al., 2003; Siever and Davis, 2004; Baiano et al., 2007; Harrison et al., 2007; Meda et al., 2008), we measured the levels of Gln, Glu, Cre, myo-inositol (mI), N-acetylaspartate (NAA), and choline-containing compounds (Cho) in the ACC and the left basal ganglia (ltBG) of patients with chronic schizophrenia. Additionally, we paid special attention to the gender differences in patients with schizophrenia. Seeman (1997) reviewed the influence of gender differences in the pathology of schizophrenia during onset, severity, effects of drugs, and typical course of the illness. These differences may be caused by biological factors (e.g., the hormones estrogen and progesterone), but this has not been fully confirmed. Although a few previous MRS studies reported differences between males and females in terms of the manifestation of schizophrenia (e.g., Buckley et al., 1994), very few follow-up studies were attempted. In most MRS studies, the percentage of female subjects was small, thereby making a consideration of the gender differences as related to the pathology of schizophrenia difficult. In this study, we ensured that about half of the participants were female subjects, and we reexamined this issue.

Table 1b
Epidemiology of schizophrenic subject

	Male		Female	p
Age at onset		23.3±7.3		
	24.2±9.0		22.8±5.5	n.s.
Duration of illness (years)		10.2±8.2		
	10.3±8.7		10.0±7,9	n.s.
Duration of therapy (years)		8.6±7.3		
	8.7±8.4		8.5±6.5	n.s.
PANSS Positive		12.5±3.5		
	13.1±3.9		119±3.1	n.s.
PANSS Negative		15.0±5.4		
	16.0±6.4		14.1±4.1	n.s.
PANSS General		27.7±7.2		
	28.1±8.4		26.4±6.1	n.s.
Dose of antpychotics (mg)		383.3±341.0		
	548.0±398.1		239.1±199.6	.011*
Other medications (number)				
Benzodiazepine	6		5	
Antidepressant	1		2	

Age at onset, duration of illness, duration of therapy, PANSS and dose of antipsychotics are shown as mean ± S.D.(range).
Dose of antipsychotics is shown as chloropromazine equivalent.
*Significant in two group t-test ($p<.05$).
Abbreviation: PANSS, Positive and Negative Syndrome Scale; n.s., no significant difference.

2. Materials and methods

2.1. Subjects

Thirty patients with chronic schizophrenia and twenty-five healthy control subjects participated in this study after

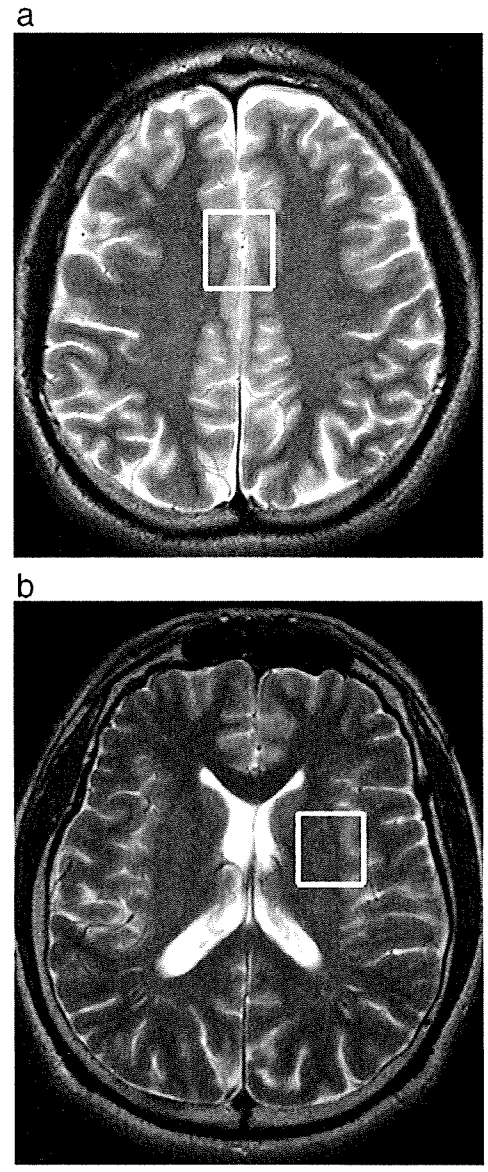
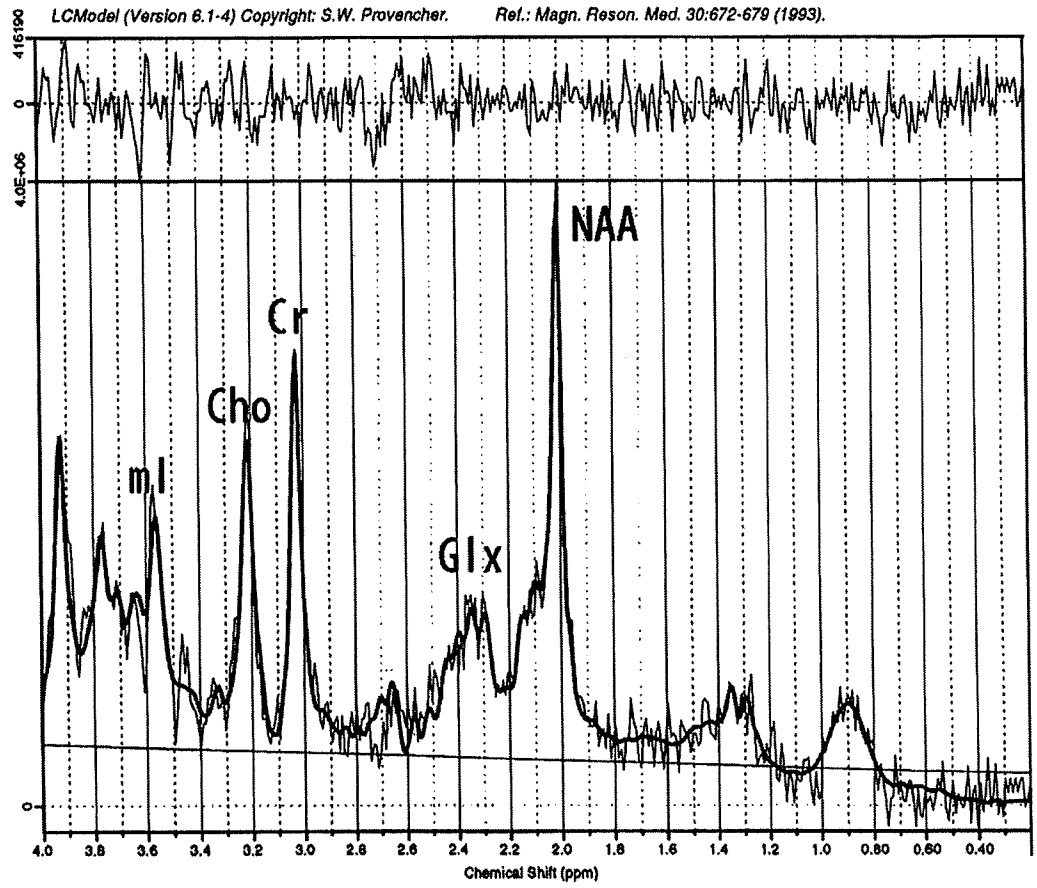
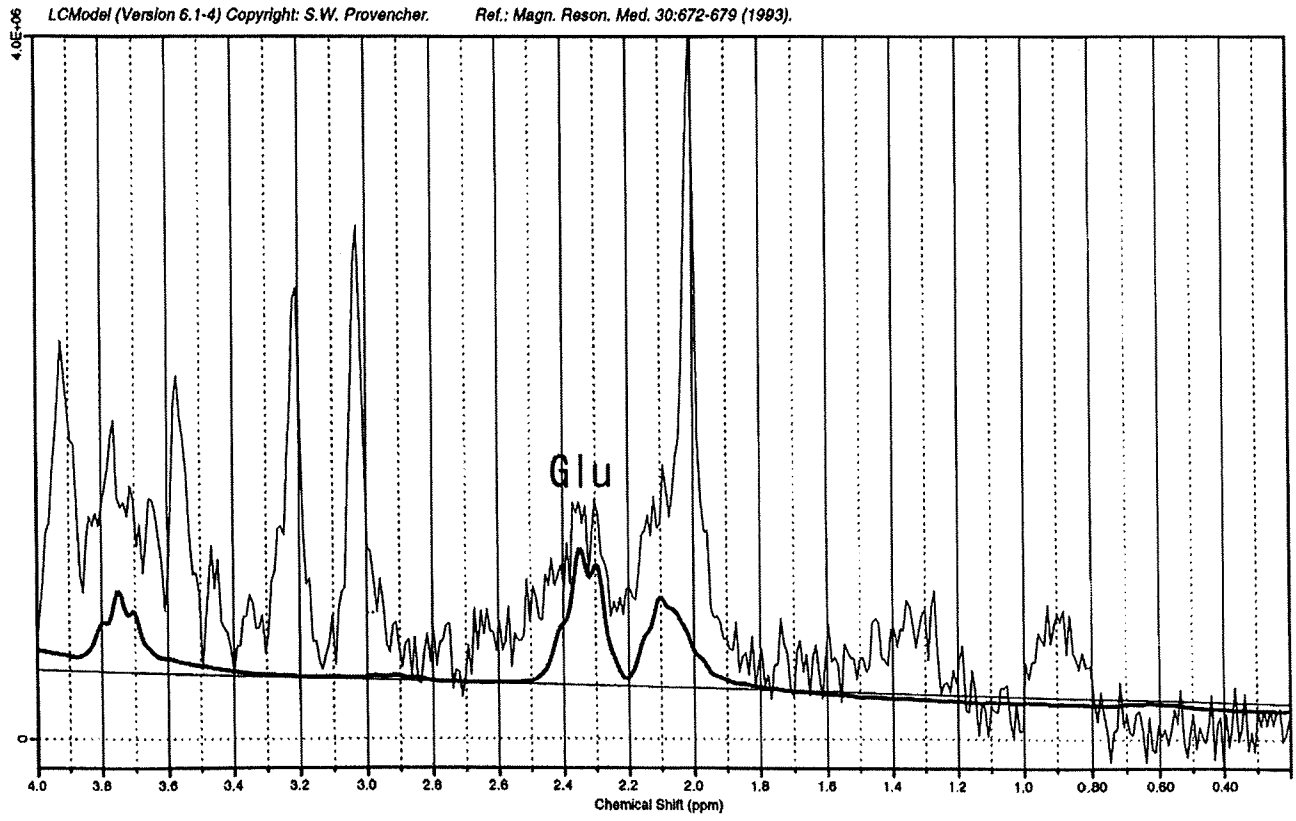


Fig. 1. Region of interest (ROI) positions for spectroscopic measurement by STEAM sequence in the anterior cingulate cortex (ACC) (a) and the left basal ganglia (ltBG) (b). The white box represents the location of the ROI that was used in the STEAM sequence in the horizontal image.

a


LCModel (Version 6.1-4) Copyright: S.W. Provencher. Ref.: Magn. Reson. Med. 30:672-679 (1993).
NAA
Cr
Cho
mI
Glx
Chemical Shift (ppm)

b


LCModel (Version 6.1-4) Copyright: S.W. Provencher. Ref.: Magn. Reson. Med. 30:672-679 (1993).
Glu
Chemical Shift (ppm)

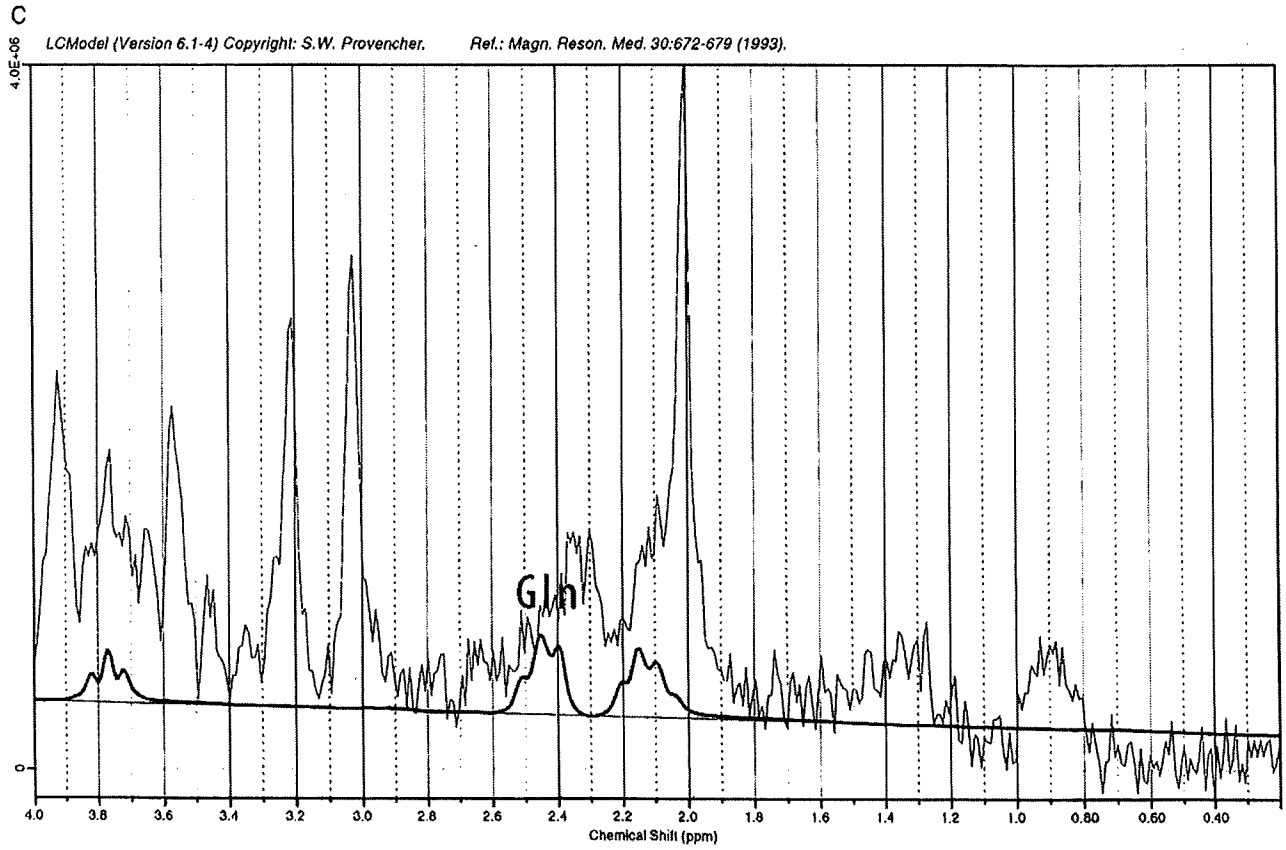


Fig. 2. Representative ^{1}H-MRS spectra of the anterior cingulate cortex (ACC) obtained from a subject in STEAM sequence. (a) shows the peaks that represent each compound. (b) and (c) show the separate peaks for Glu and Gln.

providing written informed consent in accordance with the guidelines of the ethical committee at Tokushima University. The epidemiological data from the subjects are shown in Tables 1a and 1b. All patients were assessed with the DSM-IV TR (American Psychiatric Association, 2000). All schizophrenic patients were outpatients or inpatients of the Tokushima University Hospital. Twenty-eight of the patients (thirteen males and fifteen females) were classified as having paranoid schizophrenia, one female patient was classified as having undifferentiated schizophrenia, and one male patient was classified as having disorganized schizophrenia. All patients were assessed using the Positive and Negative Syndrome Scale (PANSS, Kay et al., 1987). Eleven patients (five male and six female) received benzodiazepines (the 12.0±6.3 mg diazepam equivalent) and three patients (one male and two female) received paroxetine. All healthy volunteers were recruited from the same region and had no history of an Axis I psychiatric illness as determined by the DSM-IV TR. Both the schizophrenic patients and the healthy volunteers were Japanese and spoke Japanese as their mother tongue. None of the patients or healthy volunteers had a serious medical illness, or history of head injury or drug or alcohol abuse before the scan. All subjects were right-handed.

2.2. ^{1}H-MRS procedure

Employing a 3 Tesla clinical magnetic resonance imaging (MRI) instrument (Signa 3T Excite, GE Healthcare, Milwaukee, WI, USA), ^{1}H-MRS was performed using the STEAM sequence with water suppression by CHESS pulses (TE=18 ms, TR=5000 ms, acquisition=64 times) to minimize longitudinal and transverse relaxation efforts. Neurochemical metabolites that can be identified in short-echo ^{1}H-MRS include Cre, Gln, Glu, ml, NAA, and Cho. The area under the peak for each magnetic resonance is proportional to the concentration of that particular compound. Metabolite levels were estimated using linear combination model software (Provencher, 1993). Our basis-set was constructed from original in vitro data for each metabolite. On the basis of previous reports of functional anomalies, the region of interest (ROI) for ^{1}H-MRS was set for the ACC and the ltBG (the ROI size=1.7 cm×1.7 cm×1.5 cm) using three oriented images. For a reference slice of the ACC, an axial cut approximately one cm above the upper end of the body of the lateral ventricle was chosen. The center of the ROI was centered on the frontal interhemispheric fissure, 3 cm in front of the central fissure and 2 cm above the corpus callosum. A reference slice of the ltBG was placed between the Sylvian fissure and the lateral ventricles to encompass the lenticular nucleus (Fig. 1a and b). Representative ^{1}H-MRS spectra of the ACC obtained from a subject with the peaks that represent each compound are shown in Fig. 2a, b, and c.

T1-weighted images (3D-SPGR) were acquired using the following parameters: TE=4.2 ms, TR=10 ms, slice thickness=0.8 mm, matrix 512×512, FOV=24×24 cm, and Flip angle=15°. Thus, the brain images were composed of voxels (voxel size=0.47 mm×0.47 mm×0.8 mm). Based on

Table 2a
Effect of illness in ACC

		Control (mmol)	Schizophrenia (mmol)		p
Cre		10.5±3.1	8.9±3.0	F=3.82,df=1	n.s.
	Male	11.1±3.0	8.1±3.2	t=2.48	.021
	Female	9.8±3.1	9.6.9±2.8	t=0.22	n.s.
Gln		5.6±2.3	4.9±1.8	F=1.38,df=1	n.s.
	Male	6.6±2.3	5.1±2.0	t=1.74	n.s.
	Female	4.5±1.7	4.7±1.6	t=−0.3.6	n.s.
Glu		11.5.±3.6	9.8±2.7	F=4.07,df=1	.049*
	Male	12.3±3.6	9.8±2.8	t=2.43	.022
	Female	10.7.±3.5	10.3±2.5	t=−0.03	n.s.
mI		8.2±2.3	6.8±2.2	F=5.71,df=1	.021*
	Male	8.6±2.2	6.3±2.2	t=2.61	.015
	Female	7.8±2.5	7.1±2.2	t=0.79	n.s.
NAA		11.7±3.3	100±3.3	F=3.82, df=1	n.s.
	Male	12.3±3.3	9.4±3.4	t=2.23	.035
	Female	11.1±3.4	10.5±3.1	t=0.487	n.s.
Cho		3.1±1.0	2.7±1.0	F=2.27,df=1	n.s.
	Male	3.4±0.9	2.5±1.1	t=2.36	0.27
	Female	2.8±0.9	2.8±1.0	t=−0.25	n.s.

The concentration of metabolite are shown as mean±S.D.
Abbreviations: Cre, creatine+phosphocreatine; Gln, glutamine; Glu, glutamate; mI, myo-inistol; NAA, N-acetylaspartate; Cho, choline containing compounds; n.s., no significant difference.
*Significant main effect of illness in two way ANOVA (p<.05).
†Significant in two sample t-test (p<.05).

Table 2b
Effect of illness in two way ANOVA and two sample t-test between schizrophrenic subject and healthy controls in each gender group in ltBG

		Control (mmol)	Schizophrenia (mmol)		p
Cre		5.4±0.6	5.4±0.6	F=0.33, df=1	n.s.
	Male	5.2±0.5	5.2.±0.6	t=0.03	n.s.
	Female	5.7.±0.6	5.5.±0.6	t=0.76	n.s.
Gln		2.7±0.6	2.7±0.7	F=0.01, df=1	n.s.
	Male	2.6.±0.7	2.9.±0.7	t=−1.20	n.s.
	Female	2.7±0.5	2.4.±0.7	t=1.23	n.s.
Glu		4.8.±0.8	4.8±0.8	F=0.13, df=1	n.s.
	Male	4.7±1.0	5.0±0.8	t=−0.88	n.s.
	Female	4.9.±0.4	4.7±0.7	t=−0.72	n.s.
mI		3.3±0.4	4.7±0.7	F=0.13, df=1	n.s.
	Male	3.0±0.4	3.1±0.5	t=−0.62	n.s.
	Female	3.5±0.3	3.0±0.9	t=1.99	n.s.
NAA		5.8±0.9	5.8±0.6	F=0.08, df=1	n.s.
	Male	5.3.±0.9	5.8.±0.6	t=−0.98	n.s.
	Female	6.3±0.4	5.9±0.6	t=2.06	n.s.
Cho		1.5±0.1	1.5±0.2	F=0.00, df=1	n.s.
	Male	1.5±0.1	1.5±0.2	t=−0.58	n.s.
	Female	1.5±0.2	1.5.±0.2	t=−0.51	n.s.

Abbreviation: n.s., no significant difference.
*Significant main effect of illness in two way ANOVA (p<.05). †Significant in two sample t-test (p<.05).

the histogram of voxel intensity, each voxel in the 3D-SPGR brain images was classified into gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF) using the ImageJ Ver.1.38 software package (http://rsb.info.nih.gov/ij). The voxels that were regarded as GM, WM and CSF in each ROI were counted using the 3D-Slicer Ver.2.6 software package (http://www.slicer.org) to obtain the desired ratio of these tissues. Metabolite concentrations were corrected for CSF in the ROI by dividing by the percentage of brain tissue in each ROI, assuming that metabolite concentrations in the CSF were equal to zero (Bustillo et al., 2001).

2.3. Statistical procedures

Statistical tests were performed using the SPSS version 11.5J software package (SPSS Japan Inc., Tokyo, Japan). Absolute metabolite levels measured with ^{1}H-MRS were analyzed. In ROIs for both the ACC and the ltBG, the mixed models approach for repeated two-way analysis of variance (ANOVA), including group (i.e., schizophrenia patients versus healthy volunteers) and gender (i.e., male versus female), was used to reveal the effects of these factors on each metabolite.

Since the partial volume effects of the GM and the WM may influence the metabolite levels in ^{1}H-MRS (Hetherrington et al., 1994; Kim et al., 2005; Pan et al., 2006), the ratios of the GM and the WM for each ROI were compared for either the schizophrenic subjects and the healthy controls or the male subjects and the female subjects using two sample t-tests.

Among the schizophrenic patients, correlations between clinical indices and each metabolite level were evaluated using Spearman's rank correlation test in both ROIs. In the eleven patients who received benzodiazepine, the correlations between the benzodiazepine dose and the metabolite levels were evaluated in both ROIs.

3. Results

Among the metabolites, ANOVA revealed that the illness significantly affected the Glu concentration (F=4.07, df=1, p=.049) and the mI concentration (F=5.70, df=1, p=.021) in the ACC; both metabolites were lower in schizophrenia patients than in control subjects (Tables 2a and 2b). Gender was shown to significantly affect the Gln concentration in the ACC (F=5.88, df=1, p=.019) and the concentrations of Cre

Table 2c
Effect of gender in ACC

		Control (mmol)	Schizophrenia (mmol)		p
Cre		9.5±3.4	9.7±2.9	F=0.03, df=1	n.s.
	Ctrl	11.1±3.0	9.8.±3.1	t=1.00	n.s.
	Sc	8.1.±3.2	9.6±2.8	t=−1.33	n.s.
Gln		5.8±2.2	4.6±1.6	F=5.88, df=1	.019*
	Ctrl	6.6.±2.3	4.5.±1.7	t=2.62	.016†
	Sc	5.1±2.0	4.7.±1.6	t=0.62	n.s.
Glu		10.7.±3.5	10.5±3.0	F=0.09, df=1	n.s.
	Ctrl	12.3±3.6	10.7±3.5	t=1.13	n.s.
	Sc	9.2.±2.9	10.3±2.5	t=−1.09	n.s.
mI		7.4±2.4	7.4±2.3	F=0.00, df=1	n.s.
	Ctrl	8.6±2.2	7.8±2.5	t=0.82	n.s.
	Sc	6.4±2.2	7.1±2.2	t=−0.92	n.s.
NAA		10.8±3.6	10.8±3.2	F=0.00, df=1	n.s.
	Ctrl	12.3.±3.3	11.1.±3.4	t=0.89	n.s.
	Sc	9.4±3.4	10.5±3.1	t=−0.91	n.s.
Cho		2.9±1.1	2.8±0.9	F=0.25, df=1	n.s.
	Ctrl	3.4±0.9	2.8±0.9	t=1.66	n.s.
	Sc	2.5±1.0	2.8.±1.0	t=−0.97	n.s.

Abbreviation: Ctrl, Healthy Controls; Sc, Schizophrenic subjects; n.s., no significant difference.
*Significant main effect of gender in two way ANOVA (p<.05). †Significant in two sample t-test (p<.05).

Table 2d
Effect of gender in ltBG

		Male (mmol/l)	Female (mmol/l)		p
Cre		5.2±0.6	5.5±0.6	F=4.58, df=1	.037*
	Ctrl	5.2±0.5	5.7±0.6	t=−1.89	n.s.
	Sc	5.2±0.6	5.5±0.6	t=−1.16	n.s.
Gln		2.8±0.7	2.5±0.6	F=1.75, df=1	n.s.
	Ctrl	2.6±0.7	2.7±0.5	t=−0.29	n.s.
	Sc	3.0±0.7	2.4±0.7	t=2.17	.039†
Glu		4.8±0.9	4.8±0.6	F=0.00, df=1	n.s.
	Ctrl	4.7±1.0	4.9±0.4	t=−0.77	n.s.
	Sc	5.0±0.8	4.7±0.7	t=0.81	n.s.
mI		3.1±0.4	3.2±0.7	F=1.10, df=1	n.s.
	Ctrl	3.0±0.4	3.5±0.3	t=−3.01	.007†
	Sc	3.1±0.5	3.0±0.9	t=0.48	n.s.
NAA		5.5±0.7	6.1±0.5	F=12.69, df=1	.001*
	Ctrl	5.3±0.9	6.3±0.4	t=−3.42	.003†
	Sc	5.7±0.6	6.0±0.6	t=−1.36	n.s.
Cho		1.5±0.2	1.5±0.2	F=0.02, df=1	n.s.
	Ctrl	1.5±0.1	1.5±0.2	t=−0.71	n.s.
	Sc	1.5±0.2	1.5±0.2	t=0.42	n.s.

Abbreviation: n.s., no significant difference.
*Significant main effect of gender in two way ANOVA (p<.05). †Significant in two sample t-test (p<.05).

(F=4.58, df=1, p=.037) and NAA (F=12.7, df=1, p=.001) in the ltBG. Gln levels in the ACC were significantly higher in male subjects as compared to female subjects (Table 2c and 2d). Among male subjects, Cre, Glu, mI, NAA, and Cho were significantly lower in the schizophrenic subjects than in the control subjects (Table 2a). Among the control subjects, Gln levels were significantly higher in the ACC of the male subjects as compared to the female subjects (Table 2c), and mI and NAA levels were significantly lower in the ltBG of the male subjects as compared to the female subjects (Table 2d). There was no significant illness (i.e., schizophrenic patients vs. control subjects)×gender (i.e., male subjects vs. female subjects) interaction in any metabolite level in either ROI (Table 3). The ratio of GM and WM for each ROI did not significantly differ either between the schizophrenic patients and the healthy controls or between the male subjects and the female subjects (Table 4).

Among the schizophrenic patients, the clinical indices and PANSS positive, negative, general, and total scores did not significantly correlate with the level of any specific metabolite. in addition, treatment with antipsychotics did not correlate with the level of any of the metabolites. The result

Table 3
The F values for illness × gender interaction in each metabolite level in both ROI's

	ACC		ltBG	
	F value (illness illness×gender)	p	F value (illness illness×gender)	p
Cre	2.71	n.s.	0.29	n.s.
Gln	2.61	n.s.	3.00	n.s.
Glu	2.54	n.s.	1.23	n.s.
mI	1.52	n.s.	3.29	n.s.
NAA	1.63	n.s.	3.73	n.s.
Cho	3.44	n.s.	0.57	n.s.

Abbreviations: ACC, anterior cingulate cortex; ltBG, left basal ganglia; n.s., no significant difference.

Table 4
The ratio of GM, WM in the ROIs of ACC and the ltBG

	Control	Schizophrenia	p
GM ratio in the ACC	0.36±0.11	0.42±0.13	n.s.
WM ratio in the ACC	0.17±0.09	0.16±0.09	n.s.
GM ratio in the ltBG	0.49±0.16	0.48±0.17	n.s.
WM ratio in the ltBG	0.49±0.18	0.50±0.18	n.s.
	Male	Female	p
GM ratio in the ACC	0.41±0.14	0.38±0.11	n.s.
WM ratio in the ACC	0.14±0.09	0.18±0.09	n.s.
GM ratio in the ltBG	0.52±0.17	0.45±0.16	n.s.
WM ratio in the ltBG	0.45±0.18	0.53±0.17	n.s.

Abbreviations: GM, gray matter; WM, white matter; n.s., no significant difference.

was similar when analyzed for each gender group. Treatment with benzodiazepine correlated with mI levels (r=0.66, p=.027) in the ACC and Cre (r=0.73, p=0.11), Gln (r=.73, p=.011) and Glu (r=.66, p=.029) levels in the ltBG.

4. Discussion

4.1. Glutamate change

In the ACC, Glu levels were significantly decreased in schizophrenic patients. Because of the difficulty in isolating the Glu signal from Gln and other signals using a low magnetic field MR device, most previous MRS studies reported the combined signals of these compounds (Glx: Glu plus Gln). In previous studies that used a high-magnetic MR device, Glu levels in the left ACC were significantly increased in first-episode schizophrenia patients (Theberge et al., 2002), whereas these levels were significantly decreased in medicated patients with chronic schizophrenia (Theberge et al., 2003). Wood et al. (2007) reported that Glx levels in the ACC did not change in relation to schizophrenia. Zavitsanou et al. (2002) reported that ionotropic glutamate receptors were observed in increased levels in the postmortem ACC of patients with chronic schizophrenia. This result suggests a postsynaptic compensation for the impaired glutamatergic neurotransmission. Oni-Orisan et al. (2008) reported that in the postmortem ACC of chronic schizophrenia patients, transcription of the vesicular glutamate transporter (VGLUT) – which is known to package Glu into vesicles in the presynaptic terminal for subsequent release into the synaptic cleft – increased, and the expression of VGLUT protein decreased. This study suggested that the Glu release in the ACC decreased in chronic schizophrenic subjects. The result of our study is compatible with this postmortem study. Since Glu concentration showed no significant correlation with age, duration of illness, duration of therapy, or dosage of antipsychotics, these factors are not the likely cause for the reduction of Glu.

4.2. Myo-inositol change

The mI concentration was significantly lower in schizophrenic patients as compared to the healthy subjects. Previous ^{1}H-MRS studies with schizophrenic patients did not show consistent results regarding mI concentration. Block

et al. (2000), a study of the left frontal lobe using 1.5-Tesla imaging; Delamillieure et al. (2000), a study of the bilateral thalamus using 1.5-Tesla imaging; and Theberge et al. (2003), a study of the left ACC and the left thalamus using 4.0-Tesla imaging, reported no significant change in mI levels. Bluml et al. (1999) reported that the mI level did not change in the parietal cortex in either drug-naïve patients or medicated patients, while Szulc et al. (2005) reported that the mI concentration in the thalamus of drug-naïve patients increased after they received risperidone. Since researchers have suggested that antipsychotics may be effective via a dampening action on an overactive phosphatidylinositol second messenger system, where mI plays an important role (Kim et al., 2005), the influence of medication might contribute to the inconsistency among these studies. In a post-mortem study, Shimon et al. (1998) reported that the mI concentration decreased in chronic schizophrenia patients in the frontal and occipital cortex. The result of our study is consistent with this postmortem study.

4.3. N-acetylaspartate stability

Several studies using low magnetic field devices reported a change in NAA levels in chronic schizophrenia patients (Deicken et al., 2000; Auer et al., 2001; Ende et al., 2000), while studies using high magnetic MR devices did not have results consistent with these findings. Theberge et al. (2003) reported that NAA levels in chronic schizophrenia patients did not significantly differ in the left ACC and the left thalamus, whereas Chang et al. (2007) reported a significant NAA decrease in the bilateral, frontal, and temporal white matter of elderly schizophrenia patients. In our study, the NAA level was not significantly decreased in schizophrenic subjects. However, a trend of decrease ($F=3.82$, $p=.056$) was observed, and if we had assembled a larger sample, we may have observed a statistically significant decrease.

4.4. Gender differences

Gender differences in the clinical features of schizophrenia are widely known (Seeman, 1997), but the biological differences have not been fully confirmed. Although MRS is a useful method to investigate these gender differences in schizophrenia, only a few MRS studies have referred to these issues. Buckley et al. (1994) reported that male schizophrenic patients showed significant decreases in NAA levels and increases in Cho levels in the frontal cortex as compared to both male controls and female patients, but these differences were concealed within overall patient-control comparisons. In recent ^{1}H-MRS studies that used high magnetic MR devices on chronic schizophrenia patients, the ratios of male subjects to female subjects were quite large, but gender differences were not examined (Theberge et al., 2003; Tang et al., 2007; Matsuzawa et al., 2008). In our study, ANOVA revealed that gender significantly affected the Gln level in the ACC and the Cre and NAA levels in the ltBG. In male subjects, the levels of Cre, Glu, mI, and NAA in the ACC were significantly decreased in the ACC of schizophrenic patients as compared to the control subjects, and no compound level significantly differed in the ltBG of schizophrenic patients versus control subjects. However, in female subjects, no compound level significantly differed in the ACC, while the NAA concentration significantly decreased in the ltBG of schizophrenic patients as compared to the control subjects (Tables 2a and 2b). Part of these gender differences might be caused by the menstrual cycle (Rasgon et al., 2001; Batra et al., 2008). Since the morphological changes in schizophrenic patients are more prominent in male subjects (Waddington, 1993; Moreno et al., 2005) and the gender difference in the morphology might be attributable to a greater vulnerability among males to neurodevelopmental forms of schizophrenia (Waddington, 1993), the more prominent metabolite changes in male subjects may relate to these morphological findings. The gender differences in our study suggest that the male/female ratio may influence the results of MRS studies and that previous findings in studies with predominantly male subjects may not be generalized to female patients.

4.5. PANSS scores and metabolite level

Within the patient groups, the PANSS positive, negative, general, and total scores did not significantly correlate with the levels of any of the metabolites in either ROI. Some previous studies reported significant correlations between the NAA concentration and the PANSS negative scores (Sigmundsson et al., 2003; Tanaka et al., 2006), but Wood et al. (2007) reported that a PANSS negative syndrome did not correlate with the NAA level in the ACC. No previous study reported a correlation between the NAA level in the ltBG and the PANSS scores. The functional difference in the region where the ROI was placed may cause the inconsistent result.

4.6. Effects of benzodiazepines

The dose of benzodiazepine positively correlated with the mI level in the ACC and with Cre, Gln and Glu levels in the ltBG. A few MRS studies regarding acute benzodiazepine administration have been conducted. After acute benzodiazepine administration, Brambilla et al. (2002) found no significant change in the levels of Cre, Glx, mI, NAA, and Cho, whereas Goddard et al. (2004) found significant GABA reduction and speculated that benzodiazepines may have an inhibitory effect on glutamic acid decarboxylase (GAD), which is involved in the synthesis of γ-aminobutyric acid (GABA) from Gln and Glu. In preclinical observations, Izzo et al. (2001) reported that a withdrawal from chronic diazepam administration is associated with a marked increase in cortical GAD_{65} mRNA expression, indicating that benzodiazepine exposure may tend to suppress GAD gene expression. Raol et al. (2005) reported that long-term treatment with benzodiazepine suppresses the level of mRNA expression of both GAD_{65} and GAD_{67}. These results suggest that benzodiazepine administration may produce an increase in Gln and Glu levels and a decrease in GABA levels. The positive correlation of benzodiazepine dosage with Gln and Glu concentrations may be partly accounted for by these mechanisms. However, no MRS study has reported on chronic benzodiazepine use to our knowledge. Additionally, the mechanisms for the change in Cre and mI levels by chronic benzodiazepine use are not clear. Since the dosage of benzodiazepine did not significantly correlate with the PANSS scores, which did not significantly correlate with the level of any compound, this result may not

simply be caused by the severity of the illness. Although the mechanisms are unknown, our result suggests that long-term benzodiazepine use may increase ml levels in the ACC and Cre, Glu and Gln levels in the ltBG.. In addition, benzodiazepines are often used in various psychiatric illnesses for different purposes. Future MRS studies should consider that long-term benzodiazepine use may become a confounding factor in the interpretation of results.

4.7. Limitations

Several limitations can be identified in our study. Some confounding factors are present and might influence the results. In MRS studies, the results might be influenced by the region or the size of the ROI, which is not necessarily composed of uniform tissue. The participants include different clinical types of schizophrenic patients. The therapies with which they were treated were not equal.

5. Conclusion

Using a high-magnetic field MR device, our study revealed significant decreases in the levels of Glu and ml in the ACC of schizophrenic patients. It also demonstrated the existence of gender differences in some brain metabolites and dose-dependent benzodiazepine effects on the levels of certain metabolites.

Role of the funding source

This work was supported in part by a Grant-in-Aid for Scientific Research from the Japanese Ministry of Education, Culture, Sports, Science, and Technology (No.19790819) and a Grants-in-Aid for Scientific Research from the 21st Century COE Program, Human Nutritional Science on Stress Control Tokushima, Japan. The ministry had no further role in the study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Contributors

The author Tayoshi designed the study, wrote the protocol, managed the literature search, and undertook the statistical analysis. Authors Sumitani, Taniguchi, Shibuya-Tayoshi, Numata, Iga, and Nakataki recruited the subjects. Author Harada operated the MRS. Author Ueno also managed the literature searches and recruited subjects. Author Ohmori managed the progress of the entire study.

Conflict of interest

All authors declare that they have no conflicts of interest.

Acknowledgement

This work was supported in part by a Grant-in-Aid for Scientific Research from the Japanese Ministry of Education, Culture, Sports, Science, and Technology (No.19790819) and a Grants-in-Aid for Scientific Research from the 21st Century COE Program, Human Nutritional Science on Stress Control Tokushima, Japan.

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Neuromol Med
DOI 10.1007/s12017-009-8106-x

ORIGINAL PAPER

Association Study Between the Pericentrin (*PCNT*) Gene and Schizophrenia

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Received: 30 July 2009 / Accepted: 6 November 2009

Abstract Disrupted-in-schizophrenia 1 (*DISC1*), a known genetic risk factor for schizophrenia (SZ) and major depressive disorder (MDD), interacts with several proteins and some of them are reported to be genetically associated with SZ. Pericentrin (*PCNT*) also interacts with *DISC1* and recently single-nucleotide polymorphisms (SNPs) within the *PCNT* gene have been found to show significant associations with SZ and MDD. In this study, case-controlled association analysis was performed to determine if the *PCNT* gene is implicated in SZ. Nine SNPs were analyzed in 1,477 individuals (726 patients with SZ and 751 healthy controls). No significant difference was observed between the controls and the patients in allelic frequencies or genotypic distributions of eight SNPs. Although allelic distribution of rs11702684 was different between the two groups ($P = 0.042$), the difference did not reach statistical significance after permutation correction for multiple comparisons. In the haplotypic analysis, we could not find any significant association in our subjects, either. This gene may not play a major role independently in the etiology of SZ in the Japanese population.

Keywords Schizophrenia · PCNT · Kendrin · Case–control association study · DISC1

Electronic supplementary material The online version of this article (doi:10.1007/s12017-009-8106-x) contains supplementary material, which is available to authorized users.

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Introduction

Schizophrenia (SZ) is a complex psychiatric disorder that afflicts approximately 1% of the population throughout the world and has high heritability (Craddock et al. 2005). The pericentrin gene (the official symbol; *PCNT* and also called kendrin) is located at 21q22.3, which is one of chromosomal lesions prevalent in SZ by cytogenetic analysis (Demirhan and Tastemir 2003). *PCNT* is a coiled-coil protein localized specifically to the centrosome throughout the cell cycle (Flory et al. 2000) and an integral component of the pericentriolar material (Li et al. 2001). This protein provides sites for microtubule nucleation in the centrosome by anchoring gamma-tubulin complex (Takahashi et al. 2002), then it plays an important role in microtubule organization, spindle organization, and chromosome segregation (Doxsey et al. 1994; Purohit et al. 1999). Disrupted-in-schizophrenia 1

Published online: 24 November 2009

Humana Press