Magnetic Resonance in Medicine 61:28–34 (2009)

Phospholipid Composition of Postmortem Schizophrenic Brain by ^{31}P NMR Spectroscopy

John M. Pearce,[1,2] Richard A. Komoroski,[1,2,3,*] and Robert E. Mrak[3]

Cell membrane abnormalities due to changes in phospholipid (PL) composition and metabolism have been implicated in schizophrenia pathogenesis. That work has generally assessed membrane phospholipids from nonneural tissues such as erythrocytes and platelets. High-resolution ^{31}P NMR spectroscopy was used to characterize PLs of gray matter in postmortem brain for 20 schizophrenics, 20 controls, and 7 patients with other mental illnesses (psychiatric controls). Tissues from frontal, temporal, and occipital cortices were extracted with hexane–isopropanol, and ^{31}P NMR spectra were obtained in an organic–solvent system to resolve the major PL classes (based on headgroups) and subclasses (based on linkage at the *sn* − 1 position). Surprisingly, repeated-measures multivariate analysis of variance revealed no overall differences among the groups. There were no significant differences ($P < .05$) among the three groups for any individual PL subclass, including lysophospholipids. The sum of all phosphatidylethanolamine headgroups was significantly lower for schizophrenics than for controls or psychiatric controls in the frontal cortex. The present results are minimally correlated with previous results for aqueous PL metabolites on these same samples. The metabolite changes measured by in vivo ^{31}P MRS in schizophrenia do not appear to reflect PL concentration changes. The present results offer very little support for the phospholipid hypothesis of schizophrenia. Magn Reson Med 61:28–34, 2009. © 2008 Wiley-Liss, Inc.

Key words: schizophrenia; phosphatidylcholine; phosphatidylethanolamine; lysophospholipids

Cell membrane abnormalities associated with changes in phospholipid (PL) composition and metabolism have been implicated in schizophrenia pathogenesis (1–3). Studies of erythrocytes and platelets from schizophrenic patients have suggested that alterations in membrane PL composition may play a role in the pathophysiology of schizophrenia (4,5). The results of these previous findings have been mixed, but they have generally suggested altered concentrations of phosphatidylserine (PS), phosphatidylcholine (PC), phosphatidylethanolamine (PE), and/or phosphatidylinositol (PI) in schizophrenics compared to controls (5–8). Although erythrocytes and platelets are convenient models for some aspects of neurometabolism, changes measured in peripheral tissue may not reflect changes in neuronal membranes, and may be influenced more readily by extraneous factors. For example, Hibbeln et al. (9) found that smoking status and dietary intake predicted erythrocyte membrane fatty-acid composition for schizophrenic patients. Moreover, membrane PL composition varies greatly among different body tissues, consistent with widely different functions. Thus, it is essential to measure PL composition and metabolism in the brain itself.

In vivo ^{31}P MRS can be used to measure compounds involved in PL metabolism, primarily low molecular weight, phosphomonoester (PME) precursors and phosphodiester (PDE) degradation products (10). Although the detailed findings of various studies are not totally consistent, in general, in vivo ^{31}P MRS studies do suggest alterations of PL metabolism in schizophrenia (11). Because PLs themselves in intact cell membranes are solid-like and have reduced molecular mobility, their MR signals are very broad and typically not visible in vivo.

An alternative to in vivo MRS is to perform high-resolution NMR spectroscopy on extracts of samples from postmortem brain. Tissue PLs can be characterized using ^{31}P NMR in vitro after organic solvent extraction and suspension in a special reagent to prevent aggregation and yield narrow resonances (12,13). Although high-performance liquid chromatography (HPLC) is often used for PL analysis, it is labor intensive, and can require substantial sample modifications and multiple analyses. High-resolution ^{31}P NMR has the advantages of detection and quantitation of numerous PLs in a single assay, the detection of unsuspected and unidentified PLs, and its nondestructive nature. Because the number of P-atoms per molecule is known for generic PLs, molar concentrations can be determined for PLs of unknown side-chain composition. Although there are obvious disadvantages to analysis of postmortem samples (14), unlike in vivo, precise quantitation of well-resolved resonances of individual PLs or their metabolites can be performed.

In an earlier, preliminary study on nine subjects (15), we used ^{31}P NMR of tissue extracts from the frontal cortex of the postmortem brain to detect PL differences between schizophrenics and controls. Here we used high-resolution ^{31}P NMR spectroscopy in an organic–solvent system

[1]Department of Radiology, University of Arkansas for Medical Sciences, Little Rock, Arkansas, USA.

[2]Department of Psychiatry and Center for Imaging Research, University of Cincinnati, Cincinnati, Ohio, USA.

[3]Department of Pathology, University of Arkansas for Medical Sciences, Little Rock, Arkansas, USA.

Grant sponsor: NIH; Grant number: MH064553 (to R.A.K.).

Current address for Robert E. Mrak: Department of Pathology, University of Toledo Health Science Campus, 3000 Arlington Avenue, Toledo, OH 43614-2598, USA.

*Correspondence to: Richard A. Komoroski, Department of Psychiatry and Center for Imaging Research, University of Cincinnati College of Medicine, 231 Albert Sabin Way, P.O. Box 670583, Cincinnati, Ohio 45267-0583, USA. E-mail: richard.komoroski@uc.edu

Received November 30, 2007; revised June 24, 2008; accepted August 19, 2008.

DOI 10.1002/mrm.21820
Published online in Wiley InterScience (www.interscience.wiley.com).

to characterize PLs by class (headgroup) and subclass (linkage at the *sn* − 1 position) in gray matter of frontal, temporal, and occipital cortices of postmortem brain for 20 schizophrenics, 20 controls, and 7 patients with other mental illnesses (psychiatric controls). We chose gray matter because schizophrenia is considered to be largely a gray-matter disease, although white-matter abnormalities have been detected (16).

In another previous study (17) we measured the concentrations of the metabolic precursors of PL metabolism, the PMEs phosphoethanolamine (pe) and phosphocholine (pc), as well as the PDE degradation products glycerophosphoethanolamine (gpe) and glycerophosphocholine (gpc) in the same samples that are the subject of the present study. Increased gpc was found in all three brain regions for schizophrenics relative to controls. Here we also examine possible correlations between the present PL results and those previous results on aqueous PL metabolites in the same samples (17).

MATERIALS AND METHODS

The protocol was approved by the Human Research Advisory Committee of the University of Arkansas for Medical Sciences. Autopsies were obtained with the informed consent of next of kin. Written informed consent was obtained from each subject assessed premortem. Diagnoses were made postmortem using the Diagnostic Evaluation after Death (DEAD) protocol (18). DEAD uses information from multiple sources, including a structured chart review and interviews with kin. The diagnosis of schizophrenia in this instrument relies on the criteria of Feighner et al. (19). Information obtained from family members and treating physicians was incorporated when available. For the majority of cases the medical record was the major or sole source of information. Diagnoses were made independently by two research-trained, board-certified psychiatrists. A previous study (20) confirmed that schizophrenia diagnoses made with DEAD in a large group of elderly subjects agreed with those made according to *DSM-III-R* (21). Many patients also had been administered the Brief Psychiatric Rating Scale (BPRS), the Abnormal Involuntary Movement Scale, and/or the Neurobehavioral Cognitive Status Examination.

The patient groups consisted of 20 schizophrenics (S) (age 71 ± 12 years, 15 males, 5 females), 20 controls (C) (age 71 ± 8 years, 20 males, 0 females), and 7 psychiatric controls (PC) (age 63 ± 12 years, 6 males, 1 females) (four bipolar disorder; one each posttraumatic stress disorder, personality disorder, substance abuse). The groups were matched for age, race, and postmortem interval (PMI), except the PMI of the psychiatric control group (14.6 ± 10.0 h) was not matched to that of either the schizophrenic (4.1 ± 1.7 h) or control (3.9 ± 0.6 h) groups. The 11 schizophrenics whose medication status was known definitively had all been on first-generation antipsychotics. Four of five psychiatric controls whose medication status was known had been on first-generation antipsychotics, and one on second-generation antipsychotics. Controls had no history of neurologic or psychiatric disorders.

All brains were examined by a neuropathologist (R.E.M.) for pathological changes that would preclude inclusion in this study. At the time of gross autopsy, the brainstem and the left cerebellar and cerebral hemispheres were processed for special studies (see below). The right cerebrum and cerebellum were fixed for 10–14 days in 20% phosphate-buffered formalin, and then sectioned for gross examination and extensively sampled for paraffin embedding and histological examination. Sections were stained with hematoxylin-and-eosin stain, and with the Sevier-Munger modification of Bielschowski's stain for evaluation of senile changes (neuritic plaques and neurofibrillary tangles). Subjects with neuropathological changes, including Alzheimer's disease as based on Consortium to Establish a Registry for Alzheimer's Disease (CERAD) criteria (22), were excluded.

Tissues used in this study were obtained fresh at autopsy. These were dissected fresh, frozen in liquid nitrogen, and stored at −85°C until used. Samples were taken from the left hemisphere in frontal, temporal, and occipital cortices. Frontal and temporal cortices were chosen because they have been strongly implicated in the pathophysiology of schizophrenia. The occipital cortex was chosen as a control region, because it has not been strongly implicated in schizophrenia. All samples were taken by a single individual with extensive experience in brain anatomy to ensure that identical regions were sampled for all brains.

For the frontal cortex, samples were taken from Brodmann area 10. The section was approximately anterior to a vertical cut 2-cm posterior to the apex of the frontal pole and superior to the orbitofrontal gyrus. For the temporal cortex, samples were taken from the central region of left superior temporal gyrus in Brodmann area 22. For the occipital cortex, samples were taken from Brodmann area 18, superior to primary visual cortex. As much white matter as possible was removed before the solvent extraction procedure. It is impossible to measure white matter contamination exactly, but for the cortical regions studied here, we estimated it to be on the order of 5–15%, consistent with a previous study (23).

Based on a visual inspection of the partially thawed surface of each frozen sample, 0.2–0.5 g of primarily gray matter was isolated and deposited into a centrifuge tube on ice. Cold hexane–isopropanol (3:2) was then added at a volume ratio of 18:1, based on an assumed tissue density of 1.0 g/mL. The mixture was homogenized in the tube by manual grinding with a teflon rod for 5 min, followed by sonication in a water-cooled (~15°C) cup-horn flow cell assembly (Fisher Model 50 Sonic Dismembrator) for 5 min. Homogenates were then centrifuged at 2500 × *g* for 10 min at 8°C. The supernatants were then removed, subdivided into two separate glass vials, and the headspaces filled with nitrogen. The samples were stored at −85°C. One of each set of extract subsamples was used for immediate (within a few days) ^{31}P NMR analysis (see below), and the other was reserved for possible future analyses, with 0.1% butylated hydroxytoluene added to retard oxidation.

Samples were prepared for analysis in the $CDCl_3$–CH_3OH–H_2O (10:4:2) solvent system. After removing hexane–isopropanol under a stream of nitrogen and weighing the dried extract, 0.94 mL of $CDCl_3$:CH_3OH (10:4) was added. The mixture was vortexed briefly, then 134 μL of deionized H_2O containing 0.2 M Cs–EDTA at pH 6.0 was added. The total volume was determined so as to obtain a

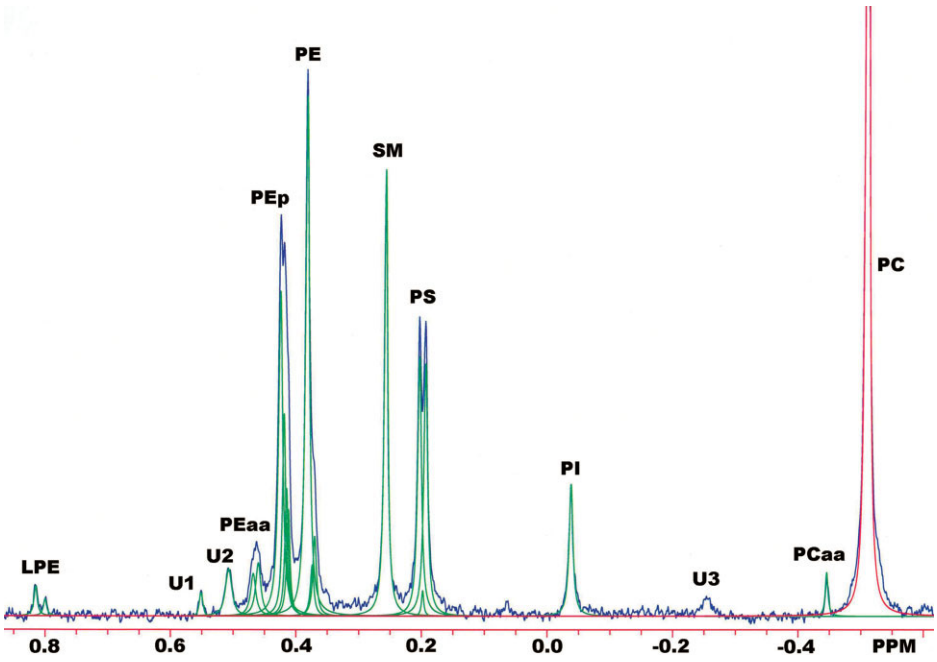

FIG. 1. A 161.9-MHz ^{31}P NMR spectrum of a hexane–isopropanol extract of the occipital cortex from a schizophrenic patient. The PC peak is off-scale. Chemical shifts are referenced to 85% H_3PO_4 at 0.00 ppm by setting the chemical shift of PC to −0.51 ppm (see text). U1–U3 are unidentified PLs. LPC usually occurred at the same chemical shift as PI under the conditions used here. Also shown are the individual lines used to fit the various PL class and subclass resonances. The numbers of lines used were: one each for U1, U2, SM, LPC, PI, PC_{aa}, PC; two each for LPE, PE_{aa}; three for PS; four each for PE_p, PE. U3, which appeared in only a few spectra, was not fit.

minimum 5-cm height (700 μL) of organic phase in the NMR tube and to exclude the aqueous phase from detection and facilitate shimming. The mixture was vortexed briefly again and the two phases were allowed to settle for at least 15 min before transferring them, lower phase first, to a 5-mm NMR tube, which was allowed to settle for at least an hour and subsequently equilibrated to a temperature of 25.0°C in the NMR probe.

Phosphorus-31 NMR was performed at 161.9 MHz on a Varian Mercury 400 spectrometer using methods similar to those described previously (24). Spectra were acquired with WALTZ-16 ^{1}H decoupling (no nuclear Overhauser enhancement) with minimum postacquisition delay. Typical conditions were: tip angle 84°; rf-pulse width, 5.6 μs; TR, 4.1 s; spectral width, 1 kHz in 8K points; typical number of transients, 496.

The resonances were quantified relative to the summed areas of all PL resonances using the program NUTS 2D (Acorn NMR, Palo Alto, CA). The FIDs were zero-filled once, multiplied by an exponential function (0.2 Hz line narrowing for resolution enhancement), Fourier transformed, and the resulting spectra phased and fit with Lorentzian-Gaussian lineshapes using a Simplex optimization algorithm. Because the resonances from PL classes often display complex shapes or even splitting due to the presence of molecular species with slightly different chemical shifts (24,25), each resonance band was fit as the sum of one to four individual lines (Fig. 1). However, even though these individual lines represent potentially identifiable molecular species of the various PL classes (24,25), we report only the relative concentrations of PL classes without regard to molecular species. Chemical shifts were referenced to 85% H_3PO_4 at 0.00 ppm by setting the native PC peak to −0.51 ppm (25,26). Intensity corrections for partial saturation were not required because this is a comparison of relative resonance intensities between subject groups, and absolute concentrations were not determined. The ^{31}P spin-lattice relaxation times of PLs in this solvent system generally ranged from 1.2 to 2 s. Thus, the TR and tip angle used here provide good overall signal-to-noise ratio (SNR). As long as the SNR is good, the measured resonance intensities will be usable for relative quantitation (27). Absolute quantitation is usually not performed in this two-phase system due to variable partitioning effects for PI, PS, lyso-PLs and many quantification reference compounds (24). In our laboratory absolute quantitation is usually performed on PLs dispersed at low concentration in a single phase of aqueous detergent, although that system does not resolve PL subclasses as well as the present one (24). The aqueous-detergent analysis could not be performed here because, unlike the current analysis at higher PL concentration, it requires a ^{31}P NMR probe for 10-mm sample tubes, which is currently not available to us.

Statistical analyses (*t*-tests for independent samples, Pearson and Spearman correlations, repeated-measures multivariate analysis of variance [MANOVA], and post hoc tests [Bonferroni and Scheffe]) were performed using Statistica (Statsoft, Tulsa, OK). The statistical significance of univariate results was confirmed by post hoc testing.

RESULTS

Phospholipid Classes and Subclasses

Figure 1 shows the 161.9-MHz ^{31}P NMR spectrum of a hexane-isopropanol extract of the occipital cortex from a schizophrenic patient. The identities of the resonances of the PL classes and subclasses were confirmed by spiking an extract sample with standard compounds (24). Resonances were seen for lysophosphatidylethanolamine (LPE), alkyl, acyl-phosphatidylethanolamine (PE_{aa}), phosphatidylethanolamine plasmalogen (PE_p), PE, sphingomyelin (SM), PS, PI, lysophosphatidylcholine (LPC), alkyl, acyl-phosphatidylcholine (PC_{aa}), and PC. LPC usually oc-

Table 1
Phospholipid Concentrations (± standard deviations)[a] in Three Regions of Postmortem Brain for Schizophrenics, Controls, and Psychiatric Controls

Brain region →	Frontal			Temporal			Occipital		
Metabolite ↓	Control	Schizophrenia	Psychiatric control	Control	Schizophrenia	Psychiatric control	Control	Schizophrenia	Psychiatric control
LPE	0.036 ± 0.11	0.077 ± 0.16	0.16 ± 0.30	0.036 ± 0.11	0.056 ± 0.14	0.094 ± 0.17	0.67 ± 0.77	0.65 ± 0.63	0.62 ± 0.43
PE_{aa}	2.78 ± 0.74	2.52 ± 0.67	3.72 ± 1.37	2.27 ± 0.51	2.34 ± 0.62	2.53 ± 0.97	3.41 ± 2.16	2.91 ± 1.11	2.42 ± 1.04
PE_p	19.30 ± 1.60	18.76 ± 1.82	18.17 ± 3.47	19.41 ± 1.68	19.00 ± 1.71	18.94 ± 1.48	18.67 ± 3.15	18.65 ± 1.95	18.18 ± 1.87
PE	16.22 ± 1.52	16.38 ± 1.40	16.91 ± 2.50	16.11 ± 1.69	16.44 ± 1.40	17.13 ± 1.41	15.71 ± 1.94	15.90 ± 1.63	16.75 ± 1.70
total PE	38.30 ± 0.79	37.67 ± 1.12[b,c]	38.80 ± 0.53	37.80 ± 1.07	37.78 ± 0.76[d]	38.60 ± 0.80	37.78 ± 1.19	37.47 ± 0.93	37.35 ± 0.75
SM	9.27 ± 0.79	9.51 ± 0.67	9.24 ± 0.97	9.27 ± 0.74	9.60 ± 0.54	9.31 ± 1.00	8.79 ± 0.99	9.29 ± 0.74[e]	9.10 ± 0.59
PS	13.70 ± 0.90	13.67 ± 0.84	13.17 ± 1.30	13.37 ± 0.98	13.30 ± 0.76	13.04 ± 0.79	13.01 ± 1.22	12.70 ± 0.96	12.74 ± 0.84
PI	3.35 ± 0.34	3.31 ± 0.37	3.26 ± 0.23	3.24 ± 0.34	3.22 ± 0.37	3.31 ± 0.19	3.36 ± 0.38	3.48 ± 0.48	3.46 ± 0.37
PC_{aa}	0.59 ± 0.16	0.58 ± 0.15	0.51 ± 0.11	0.57 ± 0.15	0.55 ± 0.16	0.55 ± 0.07	0.74 ± 0.19	0.73 ± 0.17	0.66 ± 0.25
PC	33.13 ± 1.64	33.51 ± 2.00	32.96 ± 1.99	33.99 ± 2.22	33.75 ± 1.59	33.75 ± 1.20	33.36 ± 2.09	33.54 ± 1.39	33.86 ± 1.02
total PC	33.72 ± 1.57	34.08 ± 1.93	33.47 ± 1.98	34.56 ± 2.17	34.31 ± 1.48	34.30 ± 1.13	34.10 ± 1.95	34.26 ± 1.30	34.51 ± 0.85
SM/total PE	0.242 ± 0.021	0.253 ± 0.018[e]	0.238 ± 0.026	0.245 ± 0.018	0.254 ± 0.014[e]	0.242 ± 0.030	0.233 ± 0.027	0.248 ± 0.021[e]	0.244 ± 0.020

[a]As percent of total PL measured in the ^{31}P NMR spectrum.
[b]Significantly different from control at $P = 0.047$.
[c]Significantly different from psychiatric control at $P = 0.017$.
[d]Significantly different from psychiatric control at $P = 0.022$.
[e]Different from control at $0.05 < P < 0.10$.

curred at the same or similar chemical shift as PI under the conditions used here. Because the amount of LPC is very small when unambiguously resolved from PI, which was seldom under our circumstances, it was not quantified. Unidentified resonances U1–U3 were also observed. The resonance U2 probably arises from cardiolipin (24). The resonance U3, which appeared in only a few spectra, was not fit in the quantitation. Also shown are the individual lines used to fit the various PL-class and -subclass resonances. Table 1 gives the average, relative PL concentrations for the subject groups in the three brain regions as the percent of total PL measured in the ^{31}P NMR spectrum.

The results for the individual PLs for the combined schizophrenic and control groups did not correlate with PMI, age, sex, or race, except for temporal LPE, which correlated with sex ($r = 0.39$, $P = 0.012$), and occipital PC, which correlated with age ($r = 0.34$, $P = 0.030$). Metabolite concentration did not correlate with PMI in the unmatched PC group alone. When considering all three subject groups (S, C, PC), repeated-measures MANOVA did not show a significant effect of subject group, but did show a highly significant effect of brain region ($P < 10^{-5}$, $F = 8.4$). Univariate tests for individual PLs showed no statistically significant group effect after post hoc correction.

When considering only schizophrenics and controls, repeated-measures MANOVA showed a trend ($P = 0.092$, $F = 1.9$) for the effect of subject group, and a highly significant effect of brain region ($P < 10^{-5}$, $F = 10.9$). Univariate tests showed no statistically significant difference between schizophrenics and controls for any *individual* PL in any of the three brain regions studied. However, schizophrenics showed a trend toward higher occipital SM than controls ($P = 0.081$) that survived post hoc testing.

The resonances from PL classes display complex shapes or splitting due to the presence of molecular species (differing alkyl side chains at the *sn* − 1 and/or *sn* − 2 positions) with slightly different chemical shifts (24,25). Here, each resonance band was fit as the sum of one to four individual lines (Fig. 1). These individual lines represent potentially identifiable molecular species or unresolved clusters of species. As seen before for PS (24), molecular species are well resolved under the present solution conditions. Assignments for these resolved PS species have been suggested previously (24), but are not of particular interest here. Because these individual resonances are generally not resolved (except for PS and partly for PE_p), we do not report these individual molecular species results. However, statistical comparisons by *t*-test for these component resonances revealed no differences between schizophrenics and controls.

To compare with previous results in peripheral tissue, we summed PE and PC subclasses to yield total PE and total PC measures (Table 1), respectively, which we subjected to MANOVA analysis. Total PE was significantly lower for schizophrenics than for either controls ($P = 0.047$) or psychiatric controls ($P = 0.017$) in the frontal cortex, and lower than for psychiatric controls ($P = 0.022$) in the temporal cortex.

Because the ratio SM/PE in erythrocytes was found to be sensitive to psychopathology (5,7), we include the ratio SM/total PE in our analysis (Table 1). This ratio trended ($0.05 < P < 0.10$) higher for schizophrenics in all three brain regions.

In the previous study (17) of aqueous PL metabolites in these samples, a males-only comparison yielded a significant result different from that of the total analysis. Hence, a comparison of the schizophrenic and control groups that included only males (15 S, 20 C) was made. There were no statistically significant differences at $P < 0.05$ for any PL. Total frontal PE headgroups trended lower, and temporal SM higher, in male schizophrenics relative to controls.

Correlation of PL and PL–Metabolite Concentrations

We also performed Pearson correlations for selected PLs from the present work with related PL metabolites from a previous study (17) of these same samples in each of the three brain regions for the schizophrenic group. Specifically, correlations were examined for ethanolamine-based PLs (LPE, PE_{aa}, PE_p, PE, and total PE [all PE headgroups]) with pe

and gpe. A similar analysis was performed for choline-based PLs with pc and gpc. Total occipital PE correlated with gpe ($r = 0.645$, $P = 0.002$) and total occipital PC inversely correlated with gpc ($r = -0.638$, $P = 0.002$).

DISCUSSION

Phospholipid Classes and Subclasses

Studies on erythrocytes and platelets suggest that alterations in membrane PL composition may play a role in the pathophysiology of schizophrenia (4,5). Early findings have been summarized (5,6). These findings were mixed, but generally suggested decreases in PC and PE, and increases in PS and SM in schizophrenics relative to controls. Decreases in PC, PE, and PS were seen for skin fibroblasts of schizophrenics (28). Ponizovsky et al. (7) found increased SM, decreased PE, and a correlation of the ratio of these PLs with symptomatology, in erythrocytes from schizophrenic patients. Perhaps the most consistent findings are reduced PE and increased SM in peripheral measures of PL concentrations (5,29). Results are also mixed for PI in peripheral tissues (30–32).

Yao et al. (33) found significantly lower concentrations of PC and PE from the caudate in schizophrenia. Trends were also noted for a reduction in total PL content, and for increases in PI and SM. Schmitt et al. (34) saw reduced PC and SM, increased PS, and unchanged PE, PI, and LPC in left thalamus postmortem for schizophrenia.

Because of its central role in PL metabolism, considerable attention has been directed to the enzyme phospholipase A_2 (PLA_2). Gattaz and coworkers (8,35,36) found increased PLA_2 activity and the expected product, LPC, in serum or platelets from drug-naive schizophrenics.

In a preliminary ^{31}P NMR study (15) we found increased PI, total PL content, and PC molecular species with one saturated and one unsaturated acyl chain in the frontal cortex for schizophrenics relative to controls. No differences were seen for other PLs, including LPC and LPE. Unlike the present study, that preliminary study was performed in a single-phase, aqueous-detergent solution, which permitted absolute quantitation (see above).

Surprisingly, repeated-measures MANOVA revealed no overall differences among the groups. However, differences found in previous studies were never particularly large or encompassing. Taken as a whole, they were quite mixed. Here we detect few differences among the PL class and subclass compositions of schizophrenics, psychiatric controls, and controls. When considering only schizophrenics and controls, repeated-measures MANOVA showed a trend for the effect of subject group on PL composition. Schizophrenics showed reduced frontal total PE relative to both normal and psychiatric controls, and a trend toward higher occipital SM than controls. Reduced frontal total PE and increased SM in schizophrenics are consistent with a number of previous studies in peripheral tissue (5), and the observations in caudate by Yao et al. (33). In erythrocytes, the derivative ratio SM/PE was found to be sensitive to the severity of psychopathology as measured by BPRS scores (5,7). Here, the ratio SM/total PE trended higher for schizophrenics relative to controls for all three brain regions.

As in our preliminary study (15), we observe low levels of LPC and LPE, which do not differ between schizophrenics and controls. Primarily because of their low levels, LPC and LPE are difficult to measure by ^{31}P NMR. They can also be generated by hydrolysis during sample workup. Although absolute quantitation of lysophospholipids is problematic in the solvent system used here, the concentrations appear to be substantially lower as a fraction of total PC than those reported by Pangerl et al. (8) for platelets. Our observations again provide no evidence for increased PLA_2 activity or LPC in schizophrenic brain.

Although primarily directed at schizophrenia, the phospholipid hypothesis has been studied and invoked in the context of all psychiatric illnesses (2). Thus, a psychiatric control group was included in this study to confirm that any differences seen between the schizophrenic and control groups were specific to schizophrenia. Because of its smaller size and mixed diagnosis, the psychiatric control group is less useful than the normal control group for comparison to the schizophrenic group. However, our results for the psychiatric control group are consistent with the primary results comparing schizophrenics to a normal control group. Interestingly, the major difference we see between schizophrenics and normal controls, that for total PE in the frontal cortex, is also seen between schizophrenics and psychiatric controls. This suggests that this reduction in total PE may be specific to schizophrenia.

Both frontal and temporal cortices are implicated in schizophrenia (37). The occipital cortex was chosen as a control region because it has not been strongly implicated in schizophrenia. However, the phospholipid hypothesis of schizophrenia postulates global changes in PL composition and metabolism (1,2), which are also suggested by changes in peripheral PL measures in schizophrenia (4–6). Thus, our observation of trends toward higher SM and SM/total PE in the occipital cortex is not inconsistent with the phospholipid hypothesis.

It is reasonable to expect PL metabolism and concentrations to be altered by antipsychotic medications (38). Acute neurotoxic effects of antipsychotic medications have been observed (38). Our schizophrenic subjects were old and had been on (primarily first-generation) antipsychotic medications for many years. In functional neuroimaging measures in schizophrenics, changes with age, and presumably with antipsychotic treatment, have been characterized as somewhat similar to the effects of normal aging (37). Although anatomic changes are often seen after years of both disease burden and antipsychotic treatment, the effects on membrane PL composition have not been well studied. Over 6 months of treatment in schizophrenics, Schmitt et al. (39) saw fluctuating, but statistically significant, changes in platelet PC, PE, and LPC relative to the neuroleptic-free baseline. No changes were observed in brain PL fatty-acid composition in rats treated with either a typical or atypical antipsychotic medication (40). Very recently the effects of atypical antipsychotics on lipid metabolism were studied using metabolomic mapping (41). Atypical antipsychotics raised plasma PE levels that had been suppressed in schizophrenic patients (41).

Unfortunately, here we can shed no light on the effects of antipsychotic medication. The 11 schizophrenics whose medication status was known definitively had all been on

a variety of first-generation antipsychotics. However, the other nine schizophrenics were likely on a variety of first-generation antipsychotics also.

It is not unreasonable to expect external factors such as diet or smoking to affect PL composition, although less so for brain than for peripheral measures. Most schizophrenics are heavy smokers, which may influence brain PL composition. Hibbeln et al. (9) found that smoking status and dietary intake predicted erythrocyte membrane fatty-acid composition for schizophrenic patients. Unfortunately, we do not know either the smoking or dietary status of our subjects, whether schizophrenics or controls.

Although the schizophrenic and control groups are matched for PMI, it is still desirable to minimize PMI. The average PMIs for our schizophrenic and control groups were about 4 h, which is relatively short for a study of human postmortem tissue. We previously determined that PL concentrations do not vary with PMI for up to 18 h (24). The evidence suggests that any PL-concentration changes with PMI will be nonexistent or minor for the schizophrenic and control groups. For our psychiatric control group the average PMI was greater, but even so we found no changes in PL concentrations with PMI. Metabolite concentration did not correlate with PMI in the unmatched PC group.

Because schizophrenia is generally considered to be a gray-matter disease, we decided to study pure gray-matter samples. White matter contamination should not significantly affect the present results. It is known that the PL headgroup and fatty-acid compositions of gray and white matter in human brain are different (42). A 15% contamination of white in gray matter would, relative to a 0% contaminated sample, result in about a 5% error at most in relative PL composition. Because samples from different groups were treated identically, the contamination level would be similar for the same anatomic regions across groups. For example, if there were a 15% contamination in a temporal-cortex sample from a control, a similar contamination is likely in a temporal-cortex sample from a schizophrenic. There is no reason to expect a systematic difference in the white matter contribution between schizophrenics and controls. Ventricular enlargement and cortical atrophy have been studied extensively in schizophrenia (37), but are not always present. Because there is always some small level of white-matter contamination for both controls and schizophrenics, systematic differences, if present, would be small.

We are sensitive to the limitations surrounding the use of postmortem tissue. Lewis (14) describes a number of criteria characteristic of good studies employing postmortem tissue in psychiatric research. Our present study largely fulfills Lewis' criteria. Our samples are well characterized according to clinical diagnosis, neuropathology, postmortem factors, and toxicology. We have well-characterized specimens pertinent to the disease, and that group is well matched to a control group. To the extent possible, relevant factors such as PMI have been matched and optimized. Postmortem studies often have perimortem confounds that cannot be eliminated. Comparison of matched groups compensates for this to some extent, as we have done here. Antemortem and some demographic factors are difficult to characterize, but should be relatively uniform.

Unfortunately, particularly for schizophrenia, patients are very likely to be elderly, institutionalized, and have a long history of treatment with antipsychotic medication. That is the case with our study. It is extremely difficult to obtain sufficient numbers of brains of younger, drug-naive, or unmedicated psychiatric patients. In most in vivo studies, the ages of chronic schizophrenia patients are typically under 50, whereas in our study the average age is about 71.

Because we did not see major changes in any individual PL subclass, it is appropriate to ask whether the ^{31}P NMR method is sufficiently sensitive for relative quantitation. For the major resonances the SNR is relatively high. Quantitative NMR is a longstanding and well-understood technique that is suitable for this type and level of quantitation (27). It also remains possible that there are differences in molecular-species distributions between schizophrenics and controls, and that such differences may be detectable using an alternative ^{31}P NMR analysis method with these same samples (15,25). Other techniques may very well be applicable to this analysis. In particular, matrix-assisted laser desorption and ionization time-of-flight (MALDI-TOF) mass spectrometry seems promising (43). ^{31}P NMR at higher magnetic field should also be valuable for better resolution and detection of minor PLs, PL subclasses, and molecular species.

Correlation of PL and PL–Metabolite Concentrations

The results of the present study do not appear to coincide with those of our previous study (17) of aqueous PL metabolites. Here we observe differences in total frontal PE and occipital SM in schizophrenia, whereas before we observed increased gpc in all three brain regions. Consistent with the implications of the membrane hypothesis of schizophrenia, we had predicted that PL concentrations would correlate with concentrations of related PL metabolites in schizophrenia in affected brain regions. For example, it might be expected that an increase in gpc would accompany a decrease in PC.

We performed Pearson correlations for selected PLs from the present work with related PL metabolites from the previous study for each of the three brain regions for the schizophrenic group. We did find the expected inverse correlation of gpc and PC in the occipital cortex, but not in either the frontal or temporal cortex, the regions that are primarily implicated in schizophrenia. The only other correlation found was the positive one between total occipital PE and gpe, which does not fit our prediction. The metabolite changes measured by in vivo ^{31}P MRS in schizophrenia do not appear to reflect PL concentration changes, at least directly.

CONCLUSIONS

Surprisingly, repeated-measures MANOVA revealed no overall differences in PL composition among schizophrenics, normal controls, and psychiatric controls. Except for total PE in the frontal cortex, there were no statistically significant differences between schizophrenics and controls for any of the major individual PL classes or subclasses. In addition, there was minimal correlation of the present results for the PLs themselves with results for the

relevant PL metabolites obtained for these same samples. The inverse correlation of gpc and PC was seen in the occipital cortex, but not in either the frontal or temporal cortex. The metabolite changes measured by in vivo ^{31}P MRS in schizophrenia do not appear to reflect PL concentration changes. The present results offer very little support for the phospholipid hypothesis of schizophrenia.

ACKNOWLEDGMENT

We thank Sue Woodward for obtaining the frozen brain samples.

REFERENCES

1. Horrobin DF, Glen AIM, Vaddadi K. The membrane hypothesis of schizophrenia. Schizophr Res 1994;13:195–207.
2. Peet M, Glen I, Horrobin DF, editors. Phospholipid spectrum disorder in psychiatry. Carnforth: Marius Press; 1999. 339 p.
3. Fenton WS, Hibbeln J, Knable M. Essential fatty acids, lipid membrane abnormalities, and the diagnosis and treatment of schizophrenia. Biol Psychiatry 2000;47:8–21.
4. Mahadik SP, Evans DR. Is schizophrenia a metabolic brain disorder? Membrane phospholipid dysregulation and its therapeutic implications. Psychiatr Clin North Am 2003;26:85–102.
5. Ponizovsky AM, Barshtein G, Bergelson LD. Biochemical alterations of erythrocytes as an indicator of mental disorders: an overview. Harv Rev Psychiatry 2003;11:317–332.
6. Rípová D, Strunecká A, Nemcová V, Farská I. Phospholipids and calcium alterations in platelets of schizophrenic patients. Physiol Res 1997;46:59–68.
7. Ponizovsky AM, Modai I, Nechamkin Y, Barshtein G, Ritsner MS, Yedgar S, Lecht S, Bergelson LD. Phospholipid patterns of erythrocytes in schizophrenia: relationships to symptomatology. Schizophr Res 2001;52:121–126.
8. Pangerl AM, Steudle A, Jaroni HW, Rüfer R, Gattaz WF. Increased platelet membrane lysophosphatidylcholine in schizophrenia. Biol Psychiatry 1991;30:837–840.
9. Hibbeln JR, Makino KK, Martin CE, Dickerson F, Boronow J, Fenton WS. Smoking, gender, and dietary influences on erythrocyte essential fatty acid composition among patients with schizophrenia or schizoaffective disorder. Biol Psychiatry 2003;53:431–441.
10. Reddy R, Keshavan MS. Phosphorus magnetic resonance spectroscopy: its utility in examining the membrane hypothesis of schizophrenia. Prostagland Leukotri Essen Fatty Acids 2003;69:401–405.
11. Williamson PC, Drost DJ. ^{31}P magnetic resonance spectroscopy in the assessment of brain phospholipid metabolism in schizophrenia. In: Peet M, Glen I, Horrobin DF, editors. Phospholipid spectrum disorder in psychiatry. Carnforth: Marius Press; 1999. p 45–55.
12. London E, Feigenson GW. Phosphorus NMR analysis of phospholipids in detergents. J Lipid Res 1979;20:408–412.
13. Glonek T. ^{31}P NMR in the analysis of extracted tissue phospholipids. In: Quin LD, Verkade JG, editors. Phosphorus-31 NMR spectral properties in compound characterization and structural analysis. New York: VCH; 1994. p 283–294.
14. Lewis DA. The human brain revisited: opportunities and challenges in postmortem studies of psychiatric disorders. Neuropsychopharmacology 2002;26:143–154.
15. Komoroski RA, Pearce JM, Griffin STW, Mrak RE, Omori M, Karson CN. Phospholipid abnormalities in postmortem schizophrenic brains detected by ^{31}P nuclear magnetic resonance spectroscopy: a preliminary study. Psychiatry Res Neuroimaging 2001;106:171–180.
16. Lim KO, Adalsteinsson E, Spielman D, Sullivan EV, Rosenbloom MJ, Pfefferbaum A. Proton magnetic resonance spectroscopic imaging of cortical gray and white matter in schizophrenia. Arch Gen Psychiatry 1998;55:346–352.
17. Komoroski RA, Pearce JM, Mrak RE. ^{31}P NMR spectroscopy of phospholipid metabolites in postmortem schizophrenic brain. Magn Reson Med 2008;59:469–474.
18. Salzman, S, Endicott J, Clayton P, Winokur G, editors. Diagnostic Evaluation After Death (DEAD). Rockville, MD: NIMH Neurosciences Research Branch; 1983.
19. Feighner JP, Robins E, Guze SB, Woodruff RA, Winokur G, Munoz R. Diagnotic criteria for use in psychiatric research. Arch Gen Psychiatry 1972;26:57–63.
20. Karson CN, Casanova MF, Kleinman JE, Griffin WST. Choline acetyltransferase in schizophrenia. Am J Psychiatry 1993;150:455–459.
21. Diagnostic and Statistical Manual of Mental Disorders, 3rd rev. ed. Washington, DC: American Psychiatric Press; 1987.
22. Mirra SS, Heyman A, McKeel D, and participating CERAD neuropathologists. The consortium to establish a registry for Alzheimer's disease (CERAD): Part II. Standardization of the neuropatholgic assessment of Alzheimer's disease. Neurology 1991;41:479–486.
23. Omori M, Pearce J, Komoroski RA, Griffin ST, Mrak RE, Husain MM, Karson CN. In vitro ^{1}H-magnetic resonance spectroscopy of postmortem brains with schizophrenia. Biol Psychiatry 1997;42:359–366.
24. Pearce JM, Komoroski RA. Analysis of phospholipid molecular species in brain by ^{31}P NMR spectroscopy. Magn Reson Med 2000;44:215–223.
25. Pearce JM, Komoroski RA. Resolution of phospholipid molecular species by ^{31}P NMR. Magn Reson Med 1993;29:724–731.
26. Edzes HT. Determination of the resonance frequency ratio of the ^{31}P and ^{1}H NMR resonance shift standards 85% orthophosphoric acid and tetramethylsilane. Magn Reson Chem 1992;30:850–854.
27. Malz F, Jancke H. Validation of quantitative NMR. J Pharmaceut Biomed Anal 2005;38:813–823.
28. Mahadik SP, Mukherjee S, Correnti EE, Kelkar HS, Wakade CG, Costa RM, Scheffer R. Plasma membrane phospholipid and cholesterol distribution of skin fibroblasts from drug-naive patients at the onset of psychosis. Schizophrenia Res 1994;13:239–247.
29. Ryazantseva NV, Novitskii VV, Kublinskaya MM. Changes in the lipid phase of erythrocyte membranes in patients with paranoid schizophrenia. Bull Exp Biol Med 2002;1:84–86.
30. Keshavan MS, Mallinger A, Panchalingam KS, Pettegrew JW. Erythrocyte membrane phospholipid alterations in schizophrenia. Biol Psychiatry 1992;31:62A–63A.
31. Demish L, Heinz K, Gerbaldo H, Kirsten R. Increased concentrations of phosphatidylinositol (PI) and decreased esterification of arachidonic acid into phospholipids in platelets from patients with schizoaffective disorders or atypic phasic psychoses. Prostaglan Leukotri Essen Fatty Acids 1992;46:47–52.
32. Yao JK, Yasaei P, van Kammen DP. Increased turnover of platelet phosphatidylinositol in schizophrenia. Prostaglan Leukotri Essen Fatty Acids 1992;46:39–46.
33. Yao JK, Leonard S, Reddy RD. Membrane phospholipid abnormalities in postmortem brains from schizophrenic patients. Schizophrenia Res 2000;42:7–17.
34. Schmitt A, Wilczek K, Blennow K, Maras A, Jatzko A, Petroianu G, Braus DF, Gattaz WF. Altered thalamic membrane phospholipids in schizophrenia: a postmortem study. Biol Psychiatry 2004;56:41–45.
35. Gattaz WF, Schmitt A, Maras A. Increased platelet phospholipase A_2 activity in schizophrenia. Schizophr Res 1995;16:1–6.
36. Tavares H, Yacubian J, Talib LL, Barbosa NR, Gattaz WF. Increased phospholipase A2 activity in schizophrenia with absent resonse to niacin. Schizophr Res 2003;61:1–6.
37. Berman KF, Weinberger DR. Neuroimaging studies of schizophrenia. In: Charney DS, Nestler EJ, Bunney BS, editors. Neurobiology of mental illness. Oxford: Oxford University Press; 1999. p 246–257.
38. Dean CE. Antipsychotic-associate neuronal changes in the brain: toxic, therapeutic, or irrelevant to the long-term outcome of schizophrenia? Prog Neuro-Psychopharmacol Biol Psychiatry 2006;30:174–189.
39. Schmitt A, Maras A, Petroianu G, Braus DF, Scheuer L, Gattaz WF. Effects of antipsychotic treatment on membrane phospholipid metabolism in schizophrenia. J Neural Transm 2001;108:1081–1091.
40. Levant B, Crane JF, Carlson SE. Sub-chronic antipsychotic drug treatment does not alter brain phospholipid fatty acid composition in rats. Prog Neuro-Psychopharmacol Biol Psychiatry 2006;30:728–732.
41. Kaddurah-Daouk R, McEvoy J, Baillie RA, Lee D, Yao JK, Doraiswamy PM, Krishnan KRR. Metabolomic mapping of atypical antipsychotic effects in schizophrenia. Mol Psychiatry 2007;12:934–945.
42. O'Brien JS, Sampson EL. Lipid composition of the normal human brain: gray matter, white matter, and myelin. J Lipid Res 1965;6:537–551.
43. Schiller J, Süß R, Fuchs B, Müller M, Zschörnig O, Arnold K. Recent applications of MALDI-TOF mass spectrometry and ^{31}P NMR spectroscopy in phospholipid research. Future Lipidology 2006;1:115–125.