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

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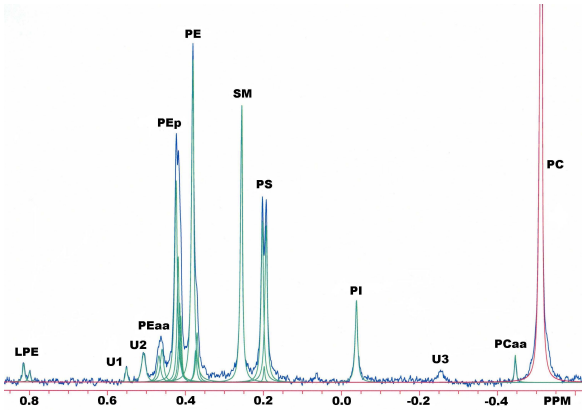
Introduction: Evidence is accumulating that schizophrenia involves abnormalities in the composition and metabolism of cell membrane phospholipids (PLs) (1). *In vivo* ^{31}P MRS can be used to measure compounds involved in PL metabolism, primarily the precursors phosphocholine (pc) and phosphoethanolamine (pe), and the degradation products glycerophosphocholine (gpc), and glycerophosphoethanolamine (gpe) (2). *In vivo* ^{31}P MRS studies suggest alterations of PL metabolism in schizophrenia (2). 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 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 yield narrow resonances (3). Here we used high resolution ^{31}P NMR spectroscopy in an organic-solvent system 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. We also examine possible correlations between the present PL results and previous results on aqueous PL metabolites in these same samples (4).

Methods: Gray matter samples were taken from frontal [Brodmann area (BA) 10], temporal (BA 22), and occipital (BA 18, a control region) cortex of frozen, postmortem, left hemispheres of 20 DSM-III-R schizophrenics (age 71±12) and 20 controls (age 71±8). The groups were matched for age, race, and postmortem interval (PMI) (schizophrenics, 4.1 hours; controls, 3.9 hours). Controls had no history of neurologic or psychiatric disorders. Samples were extracted with hexane-isopropanol, prepared for analysis in the $CDCl_3$-CH_3OH-H_2O (10:4:2) solvent system, and ^{31}P NMR performed at 161.9 MHz on a Varian Mercury 400 spectrometer using methods similar to those described previously (5). Spectra were acquired with inverse-gated, WALTZ-16 ^{1}H decoupling (no nuclear Overhauser enhancement) with minimum post-acquisition delay. Typical conditions were: tip angle 84º; rf-pulse width, 5.6 µs; TR, 4.1 s; spectral width, 1 kHz in 8K points; typical number of transients, 496. Resonance areas of the PL peaks were quantified using the program NUTS (Acorn NMR, Palo Alto, CA). 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 (3,5), each resonance band was fit as the sum of 1 to 4 individual lines (Figure).

Results: The Figure shows the 161.9-MHz ^{31}P NMR spectrum of a hexane-isopropanol extract of occipital cortex from a schizophrenic patient. Chemical shifts were referenced to 85% H_3PO_4 at 0.00 ppm by setting the native PC peak to –0.51 ppm (3,5). The identities of the resonances of the PL classes and subclasses were confirmed by spiking an extract sample with standard compounds. Resonances were seen for lysophosphatidylethanolamine (LPE), alkyl, acyl-phosphatidylethanolamine (PE_{aa}), phosphatidylethanolamine plasmalogen (PE_p), phosphatidylethanolamine (PE), sphingomyelin (SM), phosphatidylserine (PS), phosphatidylinositol (PI), alkyl, acyl-phosphatidylcholine (PC_{aa}), and phosphatidylcholine (PC). Three resonances were unidentified. The Table gives the average PL concentrations for the subject groups in the three brain regions as the percent of total PL measured in the ^{31}P NMR spectrum. 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 (after post-hoc testing) 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. Total PE ($PE+PE_p+PE_{aa}+LPE$) in frontal cortex was significantly lower for schizophrenics than for controls ($p=0.047$). We performed Pearson correlations for selected PLs with related PL metabolites from a previous study (4) of these same samples for the schizophrenic group. Correlations were examined for ethanolamine-based PLs relative to pe and gpe, and 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: Except for total PE in frontal cortex, there were no statistically significant differences between schizophrenics and controls for any of the major PL classes or subclasses in frontal or temporal cortex, regions strongly implicated in the neuropathology of schizophrenia. With that exception, our results do not strongly support the notion that schizophrenia arises from the altered composition of PLs in brain. However, it 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 (3,5). There was minimal correlation of the present PL results with results for the relevant aqueous PL metabolites for these same samples. We did find the expected inverse correlation of gpc and PC in occipital cortex, but not in either frontal or temporal cortex, the regions that are primarily implicated in schizophrenia. The metabolite changes measured by *in vivo* ^{31}P MRS in schizophrenia do not appear to reflect underlying PL concentration changes.

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Table. Phospholipid Concentrations (± standard deviations) in Three Regions of Postmortem Brain

Brain Region→	Frontal		Temporal		Occipital	
Metabolite ↓	Control	Schizophrenia	Control	Schizophrenia	Control	Schizophrenia
LPE	0.036±0.11	0.077±0.16	0.036±0.11	0.056±0.14	0.67±0.77	0.65±0.63
PE_{aa}	2.78±0.74	2.52±0.67	2.27±0.51	2.34±0.62	3.41±2.16	2.91±1.11
PE_p	19.30±1.60	18.76±1.82	19.41±1.68	19.00±1.71	18.67±3.15	18.65±1.95
PE	16.22±1.52	16.38±1.40	16.11±1.69	16.44±1.40	15.71±1.94	15.90±1.63
total PE	38.30± 0.79	37.67± 1.12[a]	37.80± 1.07	37.78± 0.76	37.78± 1.19	37.47± 0.93
SM	9.27±0.79	9.51±0.67	9.27±0.74	9.60±0.54	8.79±0.99	9.29±0.74[b]
PS	13.70±0.90	13.67±0.84	13.37±0.98	13.30±0.76	13.01±1.22	12.70±0.96
PI	3.35±0.34	3.31±0.37	3.24±0.34	3.22±0.37	3.36±0.38	3.48±0.48
PC_{aa}	0.59±0.16	0.58±0.15	0.57±0.15	0.55±0.16	0.74±0.19	0.73±0.17
PC	33.13±1.64	33.51±2.00	33.99±2.22	33.75±1.59	33.36±2.09	33.54±1.39
total PC	33.72± 1.57	34.08± 1.93	34.56± 2.17	34.31± 1.48	34.10± 1.95	34.26± 1.30

[a]Significantly different from Control at $p=0.047$. [b]Different from Control at $p=0.081$.

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