^{31}P NMR of Phospholipid Metabolites in Postmortem Schizophrenic Brain

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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 NMR spectroscopy has been used to measure the metabolic precursors of PL metabolism, namely the phosphomonoesters (PMEs) phosphoethanolamine (pe) and phosphocholine (pc), as well as PL degradation products, the phosphodiesters (PDEs) glycerophosphoethanolamine (gpe) and glycerophosphocholine (gpc) (2). Most studies report reduced PMEs and elevated PDEs in first-episode schizophrenia (2). Reduced PMEs are also seen for chronic, medicated schizophrenia, but the results for PDEs are mixed. Frontal and temporal cortex are primarily the affected regions.

Typically only the broad PME and PDE bands, or partly resolved individual resonances, all of which may include other components, are measured *in vivo* in the ^{31}P spectrum. This renders even relative quantitation difficult. Here we determine absolute concentrations of these individual PL metabolites with good precision in aqueous extracts of tissue from three regions of postmortem brain for schizophrenics, controls, and psychiatric controls using high-resolution ^{31}P NMR spectroscopy.

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), 20 controls (age 71±8), and 7 psychiatric controls (age 63±12) (4 bipolar disorder, 3 mixed other). The groups were matched for age, race, and postmortem interval (PMI), except the PMI of the psychiatric control group (14.6 hours) was not matched to that of either the schizophrenic (4.1 hours) or control (3.9 hours) groups. Controls had no history of neurologic or psychiatric disorders. Samples were extracted using a modification of the Bligh-Dyer method that has been shown to be superior to perchloric-acid extraction of water-soluble metabolites (3,4). Phosphorus-31 NMR was performed at 121.6 MHz (GE GN300) or 161.9 MHz (Varian Mercury 400) using methods similar to those described previously (5). Resonance areas of the four PL metabolites were quantified using the program NUTS (Acorn NMR, Palo Alto, CA) relative to a known amount of added 3',5'-cyclic AMP. Statistical analyses (t-test for independent samples) were performed using Statistica (Statsoft, Tulsa, OK).

Results: The ^{31}P NMR spectrum of the extracts yielded totally resolved resonances for the PL metabolites given above, as well as resonances from other metabolites such as 5'-AMP and α-glycerophosphate that are not considered here. The Table gives the results for the primary comparison, that between schizophrenics and controls, for the PL metabolites. Also given are the results for the pertinent metabolite ratios pe/gpe and pc/gpc, summed phosphomonoesters (pe + pc = "PME"), and summed phosphodiesters (gpe + gpc = "'PDE"). These sums approximate the corresponding quantities measured in the *in vivo* ^{31}P NMR spectra of brain. There was no statistically significant ($p<0.05$) difference by t-test for schizophrenics vs. controls for any metabolite measure in any of the three brain regions studied. Trends ($0.05<p<0.10$) were noted for increased gpc in schizophrenia in all three regions. Trends were also noted for increased temporal and occipital "PDE" and decreased occipital pc/gpc, all of which probably arose from the gpc component in these measures.

Some differences were noted for the schizophrenic and control groups vs. the psychiatric control group (data not shown). However, these are considered less significant because of the smaller size of the psychiatric control group (N=7), and the fact that the PMIs were not matched. However, we previously determined that PL (and presumably PL metabolite) concentrations do not vary with PMI for up to 18 hours (5). For the comparison of controls and psychiatric controls, statistically significant differences were seen for frontal pe/gpe and temporal pc/gpc, with trends for frontal and occipital pc, and occipital "PME". For the comparison of schizophrenics and psychiatric controls, a statistically significant difference was seen for occipital pc/gpc, with trends for frontal pc, and temporal pe and gpc.

Discussion: Our results for postmortem brain do not support the consensus finding *in vivo* that PMEs decrease in schizophrenia, even for chronically medicated subjects. Nor do they strongly support a significant increase in PDEs in schizophrenia, although a trend was observed for higher gpc in all three brain regions studied. The lack of agreement may be due to the fact that here we are clearly measuring only the compounds of interest, while *in vivo* there are additional contributions to the broad, partially resolved PME and PDE bands. In particular, Potwarka et al. (6) found that a broad resonance due to partially detected PLs contributes significantly to the PDE band, and that it is responsible for the increased PDE in schizophrenia. Alternatively, the trends seen here for increased gpc in schizophrenia may arise from the neurotoxic effects of long-term medication with typical, first-generation antipsychotics (7). This is consistent with the fact that similar changes in gpc are seen for all three regions, including occipital cortex, which is generally considered to be minimally affected in schizophrenia. Future studies will explore differences in the PLs themselves, and in the relation between PL and metabolite concentrations in postmortem schizophrenic brain.

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Table. Mean tissue concentrations (μmol/g) ± standard deviations for phospholipid precursors and degradation products

Brain Region→	Frontal		Temporal		Occipital	
Metabolite ↓	Control	Schizophrenia	Control	Schizophrenia	Control	Schizophrenia
pe	0.97 ± 0.18	0.98 ± 0.24	1.22 ± 0.24	1.20 ± 0.17	0.72 ± 0.11	0.72 ± 0.14
pc	0.30 ± 0.06	0.31 ± 0.05	0.38 ± 0.08	0.39 ± 0.10	0.32 ± 0.08	0.33 ± 0.08
gpe	0.71 ± 0.17	0.78 ± 0.28	0.76 ± 0.17	0.84 ± 0.22	0.61 ± 0.18	0.72 ± 0.25
gpc	*0.67 ± 0.16*	*0.90 ± 0.51**	*0.95 ± 0.31*	*1.23 ± 0.61**	*0.53 ± 0.19*	*0.76 ± 0.52**
pe/gpe	1.42 ± 0.28	1.35 ± 0.54	1.66 ± 0.40	1.55± 0.57	1.30 ± 0.51	1.10 ± 0.40
pc/gpc	0.47 ± 0.14	0.46 ± 0.28	0.44 ± 0.17	0.42± 0.30	*0.69 ± 0.27*	*0.54 ± 0.23**
"PME"	1.27 ± 0.20	1.29 ± 0.24	1.59 ± 0.26	1.59 ± 0.20	1.05 ± 0.13	1.05 ± 0.19
"PDE"	1.38 ± 0.32	1.68 ± 0.77	*1.71 ± 0.46*	*2.07 ± 0.81**	*1.14 ± 0.36*	*1.48 ± 0.76**

*Different from controls with $0.05<p<0.10$.