ORIGINAL ARTICLE

Increased Phospholipid Breakdown in Schizophrenia

Evidence for the Involvement of a Calcium-Independent Phospholipase A_2

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Background: Magnetic resonance spectroscopy studies have suggested above-normal turnover of membrane phospholipids in brains of patients with schizophrenia. One possible explanation for these findings is increased activity of the phospholipid-catabolizing enzyme phospholipase A_2 (PLA_2). However, attempts to demonstrate higher PLA_2 activity in the serum of subjects with schizophrenia have led to conflicting results. We hypothesized that this was due to serum PLA_2 activity consisting of a family of different enzymes, with each group of investigators measuring activity of different PLA_2 forms.

Design: Activity of PLA_2 in serum samples obtained from 24 individuals with schizophrenia was compared with serum obtained from 33 age- and sex-matched control subjects, using both fluorometric and radiometric assays with different substrates. Each method had previously yielded conflicting results concerning the status of the enzyme in schizophrenia.

Results: With the fluorometric assay, serum PLA_2 activity in individuals with schizophrenia was markedly increased by 49% compared with control subjects ($P<.001$). In contrast, radiometric assay of the same serum samples resulted in PLA_2 activity not significantly different between patients and control subjects. Further investigations demonstrated that, whereas the radiometric assay measured activity of a calcium-dependent enzyme, the fluorometric assay detected a calcium-insensitive enzyme possessing an acid-neutral pH optimum.

Conclusions: Increased calcium-independent PLA_2 activity was seen in the serum of patients with schizophrenia. This change, if present also in the brain, may well explain the increased levels of phosphodiesters observed using magnetic resonance spectroscopy and therefore may contribute to the pathophysiological features of the disorder.

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A SUBSTANTIAL body of data suggests that membrane phospholipid metabolism is altered in patients with schizophrenia.[1,2] For example, most investigators,[3-9] although not all,[10,11] observed decreased levels of 1 or both major membrane phospholipids, phosphatidylcholine and phosphatidylethanolamine (PE), in serum and fibroblasts of patients with schizophrenia. In addition, altered levels of specific fatty acids have been observed in erythrocytes of patients with schizophrenia,[12] whereas the turnover of platelet phosphatidylinositol[13] and arachidonic acid[14] is increased. Taken together, these findings suggest elevated phospholipid breakdown in schizophrenia.

Recently, direct evidence for perturbed phospholipid metabolism in the brain of subjects with schizophrenia was obtained using phosphorus 31 magnetic resonance spectroscopy, which allows the abundance of phosphorous-containing compounds to be measured in vivo. Several independent investigators have reported increased levels of membrane breakdown products (phosphodiesters) in the frontal and temporal cortices of drug-naive[15-17] and drug-treated[18,19] subjects with schizophrenia. Furthermore, the abundance of metabolites used in membrane synthesis (phosphomonoesters) is decreased consistently in the frontal cortex of drug-treated and drug-naive patients,[16,17,20-23] implying more rapid use of these compounds for membrane biosynthesis. Taken together, these data suggest that membrane turnover is accelerated in schizophrenia. The consequences of such a perturbation are unclear, but may include membrane breakdown and altered interneuronal communication, in particular the production of phospholipid-derived second messengers (eg, prostaglandins, leukotrienes, and inositol phosphates),[24-26] ul-

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SUBJECTS AND METHODS

SUBJECTS

Subjects with schizophrenia were recruited by advertisement from the patient population of the Clarke Institute of Psychiatry, Toronto, Ontario. All received diagnoses according to *DSM-III-R* criteria[34] using the Structured Clinical Interview for *DSM-III-R* (SCID), and their psychopathological states were assessed using the Positive and Negative Syndrome Scale (PANSS). Control subjects had no history of mental illness as assessed during the SCID interview and were recruited by advertisement from the greater Toronto area. The sample consisted of 24 hospitalized individuals suffering from paranoid schizophrenia (15 male and 9 female patients; mean±SEM age, 40±2 years) as described in **Table 1** and 33 healthy control subjects (20 male and 13 female subjects; mean±SEM age, 38±2 years), all of whom had given written informed consent. There were no significant differences between the mean ages (P=.26, Student t test) or relative numbers of male and female subjects (P=.64, Pearson χ^2 goodness of fit) in each group. The subjects with schizophrenia were all receiving neuroleptic therapy at the time of blood withdrawal (397±75 chlorpromazine equivalents). Both control subjects and subjects with schizophrenia showed no signs of physical illness and had no recent history of alcohol or other drug abuse based on information collected during the SCID interview. In addition, subjects had not taken any anti-inflammatory medication for at least 2 weeks before blood withdrawal. Venous blood (10 mL) was withdrawn into Vacutainers without anticoagulant and allowed to clot for approximately 30 minutes at room temperature. Blood was centrifuged at $1500g_{average}$ for 20 minutes at room temperature, and the resulting serum samples were stored at −80°C until assay.

MATERIALS

The 1-octasanyl-2-(pyren-1-yl)-hexanoyl-*sn*-phosphatidylmethanol (C28-O-PHPM) was purchased from Oy Academ Ab, Helsinki, Finland. The 1-alk-1′-enyl-2-[^{14}]C-arachidonyl phosphatidylcholine (choline plasmalogen) was a gift from Ernest Do, PhD, New England Nuclear, Boston, Mass. All other radiochemicals were purchased from New England Nuclear, Toronto, Ontario. Porcine pancreatic PLA_2 ($pPLA_2$) was purchased from Sigma Chemical Company, St Louis, Mo, whereas secretory ($sPLA_2$) and cytosolic PLA_2 ($cPLA_2$) were a gift from Ruth Kramer, PhD, Lilly Research Laboratories, Indianapolis, Ind.

DETERMINATION OF PLA_2 ACTIVITY

Serum PLA_2 activity was assayed fluorometrically,[34] based on the procedure used by Gattaz et al[28,29] in their studies of serum and plasma PLA_2 in schizophrenia, and radiometrically, based on the procedure used by Albers et al[32] in their study of PLA_2 in the disorder. Assays were performed in duplicate, with the investigator unaware of the diagnosis.

The fluorometric assay measures the rate of hydrolysis of the pyrene-labeled phospholipid analogue, C28-O-PHPM. The *sn*-1 ether bond of this compound makes it resistant to hydrolysis by serum lipases.[35] Briefly, 40 μL serum was incubated in the presence of 30 μmol/L C28-0-PHPM and 14 mmol/L Tris(hydroxymethyl) aminomethane (Tris)/hydrogen chloride (pH, 7.4) (final assay volume, 200 μL) for 90 minutes at 37°C, and the released pyrenyl-hexanoic acid was extracted and quantified as described.[35] "Blank" reactions (to measure nonenzymatic hydrolysis) contained serum but were stopped at zero time. No difference in blank values was observed between control subjects and subjects with schizophrenia. Hydrolysis of the *sn*-1 ester bond by incubation in the presence of 1 mol/L hydrogen chloride did not lead to increased release of fluorescent product (data not shown). The assay was linear with respect to time for at least 2 hours, and with respect to volume of serum up to 70 μL. We were concerned that the low ionic strength of Tris in the assay buffer would be

timately leading to the behavioral abnormalities observed in the disorder.

It is therefore of great interest to discover the mechanisms that underlie the observed membrane abnormalities. We and others[22] suggest that a likely mechanism, which offers a simple explanation for the schizophrenia-associated increase in phosphodiester levels, is an increase in the activity of phospholipase A_2 (PLA_2), the rate-limiting enzyme of phospholipid breakdown.[27] However, whereas 2 groups of investigators have found that PLA_2 activity is increased in serum of subjects with schizophrenia,[28-31] another group did not.[32] These conflicting results may relate to the fact that PLA_2 is not a single protein species, but a family of different enzymes.[33] We hypothesized that different investigators, using various assay procedures, have detected different PLA_2 subtypes, and that a change in the activity of the enzyme is confined to a specific isoform. In an attempt to resolve this controversy, PLA_2 activity was assayed in the serum of control subjects and patients with schizophrenia using 2 different methods characterized with respect to the distinct PLA_2 isoforms that each detects.

RESULTS

Phospholipase A_2 activity, measured using the fluorometric assay (**Figure 1**, left), was significantly (P<.001) and markedly increased by 49% in the subjects with schizophrenia (41.1±2.2 pmol fatty acid per minute per milliliter of serum) compared with control subjects (27.6±1.5 pmol fatty acid per minute per milliliter of serum). To confirm the reproducibility of the assay, a subset of subjects (n=21) underwent reassay, with similar results (data not shown; r_s=0.94). Kinetic analysis (**Figure 2**) in 3 subjects with schizophrenia possessing high serum PLA_2 levels and in 3 randomly selected control subjects revealed that elevated activity was due to significantly increased maximal velocity (69.6±1.6 vs 32.6±3.6 pmol fatty acid per minute per milliliter of serum in subjects with schizophrenia and control sub-

insufficient to adequately buffer the incubation mix. However, measurement of the assay buffer pH following the addition of serum did not reveal any significant fluctuation from 7.4 (3 control subjects and 3 patients with schizophrenia).

The radiometric method used autoclaved *Escherichia coli* bacteria labeled with hydrogen 3–labeled arachidonic acid at the *sn*-2 position as substrate,[32] modified to make use of commercially available reagents. Specifically, 30 μL serum was incubated in the presence of approximately 60 000 disintegrations per minute of ^{3}H-arachidonyl–labeled *E coli* (specific activity, 407 MBq/mmol phosphate), 1 mmol/L calcium chloride, and 100 mmol/L N-[2-hydroxyethyl]piperazine-*N*′-2-ethanesulfonic acid/sodium hydroxide (pH, 7.4) (final assay volume, 75 μL) for 30 minutes at 37°C. The reaction was terminated by the addition of 75 μL ethanol containing 1% acetic acid and 100 μg/mL arachidonic acid, and the release of radiolabeled free fatty acid was quantitated using thin-layer chromatography and liquid scintillation spectroscopy.[36] Blank reactions contained serum but were stopped at zero time. The reaction was linear with respect to time up to 40 minutes and with respect to serum up to 60 μL. Since specific radioactivity of the *E coli* was given in terms of moles of phosphate, activities are described in terms of picomole (pmol) of phosphate equivalents of fatty acid. However, since almost all the radioactivity is associated with the fatty acid fraction, the specific radioactivity per mole of phosphate is very similar to that per mole of fatty acid. In experiments using synthetic radiolabeled phospholipids, substrates were dried by vacuum centrifugation and resuspended in ethanol. One hundred microliters serum was incubated with 10 mmol/L ethylenediaminetetraacetic acid (EDTA), 100 mmol Tris/hydrogen chloride (pH, 7.4), and 6.5 μmol/L phospholipid substrate (specific activity, approximately 1850 MBq/mmol) in 5 μL ethanol (final assay volume, 400 μL) at 37°C for 60 minutes. The reaction was terminated by the addition of 400 μL butanol, followed by vortexing and centrifugation at 13 000$g_{average}$ for 5 minutes.[37] An aliquot of the butanol phase was dried by vacuum centrifugation, the residue was resuspended in ethanol, and radiolabeled free fatty acid was quantitated as described above.

PURIFIED PLA_2 SUBTYPES

To determine the PLA_2 subtypes detected by each assay, purified enzymes were used. Each enzyme was diluted in 20 mmol/L Tris/hydrogen chloride (pH, 7.4) containing 1 mg/mL bovine serum albumin (essentially fatty acid free) immediately before use. In preliminary experiments, the linear range of fatty acid release with respect to protein concentration was determined, and concentrations were chosen that gave similar activities to those obtained using human serum. The protein concentrations used for the fluorometric assay were 130, 40, and 1000 ng protein per tube of $pPLA_2$, $sPLA_2$, and $cPLA_2$, respectively, and for the radiometric assay were 0.65, 200, and 1000 ng protein per tube of $pPLA_2$, $sPLA_2$, and $cPLA_2$, respectively.

STATISTICAL ANALYSIS

Data from the control subjects and subjects with schizophrenia were compared using a 2-tailed, unpaired, Student *t* test, after first confirming normality of the data using a Liliefors test. Interaction between subject group and assay type was investigated by 2-way analysis of variance (ANOVA). Spearman correlation coefficients were used throughout (r_s), as was an α level of .05.

DETERMINATION OF pH OPTIMUM

The buffers used at each pH were MES (2-[*N*-Morpholino]ethanesulfonic acid]), 5.0 to 6.5; HEPES (*N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid]), 7.0 to 8.0; and borate, 8.5 to 10.0.

Data are given as mean±SEM.

jects, respectively; $P<.001$), whereas the substrate Michaelis constant was not significantly altered (6.9±2.0 μmol/L in subjects with schizophrenia vs 3.9±0.1 μmol/L in control subjects; $P=.21$). Fluorometrically assayed PLA_2 activity was significantly correlated with overall severity ($r_s=0.44$; $P=.03$) and positive symptom scores ($r_s=0.42$; $P=.04$) of the PANSS scale, but not with the negative symptom ($r_s=0.25$) or general psychopathology score ($r_s=0.33$) (**Figure 3**).

In contrast, no significant difference ($P>.99$) was apparent between control subjects and subjects with schizophrenia when PLA_2 activity was assayed radiometrically (Figure 1, right), using the same serum samples as for the fluorometric assay (mean activities, 91.4±7.4 vs 91.3±4.2 pmol phosphate equivalents of fatty acid per minute per milliliter of serum in subjects with schizophrenia and control subjects, respectively). The discordance between both methods was further revealed by the lack of correlation between the activity measured in each subject by the different assays ($r_s=0.091$; **Figure 4**), and the statistically significant ($P=.007$) interaction between assay type and subject group as assessed by 2-way ANOVA. In addition, no significant correlation was observed between radiometrically assayed PLA_2 activity and any PANSS scores ($-0.15<r_s<0.22$).

Measured PLA_2 activity was not significantly different in male and female subjects and was not correlated with age, treatment duration, or chlorpromazine equivalents of neuroleptic medication ($P>.25$ in all cases). Furthermore, the addition of 20 nmol/L haloperidol (a concentration typical of that found in serum of patients receiving neuroleptic therapy[38]) to each assay had no significant effect on activity (n=5 control subjects; data not shown).

The dissimilarity between the results of both PLA_2 assays was further investigated by an examination of the calcium ion requirements of the activity revealed by each method. As expected,[32,39] activity measured by the radiometric assay was almost completely abolished by the addition of the calcium ion chelator EDTA to the assay

Table 1. Clinical Details of Subjects With Schizophrenia

Subject No./ Sex/Age, y*	Duration of Treatment, y†	PANSS Scale Scores‡			Medication at Time of Blood Withdrawal
		Positive	Negative	General Psychopathology	
1/M/42	22	22	22	30	Fluphenazine hydrochloride; 50 mg/2 wk
2/M/42	21	20	11	27	Perphenazine; 80 mg/d
3/F/41	15	7	11	16	Flupenthixol; 60 mg/4 wk
4/M/38	20	7	7	17	Flupenthixol; 20 mg/3 wk
5/M/28	3	13	20	25	Fluphenazine; 5 mg/d
6/M/46	20	12	12	23	Trifluoperazine hydrochloride; 20 mg/d
7/M/36	14	15	12	25	Haloperidol; 100 mg/2 wk
8/M/42	22	10	14	27	Perphenazine; 12 mg/d
9/M/33	15	28	10	27	Fluspirilene; 4 mg/wk
10/M/33	15	35	24	50	Clozapine; 250 mg twice daily
11/F/25	6	15	16	29	Risperidone; 6 mg/d
12/F/65	41	7	14	22	Thioridazine hydrochloride; 100 mg twice daily
13/F/43	24	8	13	21	Fluphenazine; 13 mg/3 wk
14/F/40	23	16	11	ND	Pipotiazine palmitate; 100 mg/2 wk
15/M/43	25	16	10	ND	Flupenthixol; 50 mg/2 wk
16/F/46	28	18	18	31	Flupenthixol; 2 mg/2 wk
17/F/48	30	17	25	20	Pimozide; 6 mg/d
18/M/32	13	16	13	25	Pimozide; 4 mg/d
19/F/43	21	7	7	16	Haloperidol; 0.5 mg/d
20/F/43	25	8	8	24	Fluphenazine; 100 mg/4 wk
21/M/34	16	ND	ND	ND	Fluphenazine; 24 mg/d
22/M/30	6	8	11	21	Risperidone; 4 mg/d
23/M/31	15	19	8	28	Flupenthixol; 3 mg twice daily
24/M/49	30	29	29	42	Clozapine; 300 mg twice daily

*Mean±SEM age was 40±2 years.
†Mean±SEM duration of treatment was 18±2 years.
‡PANSS indicates Positive and Negative Syndrome Scale; ND not determined. For positive symptoms, mean±SEM score was 15±2; for negative symptoms, 14±1; and for general psychopathology, 26±2.

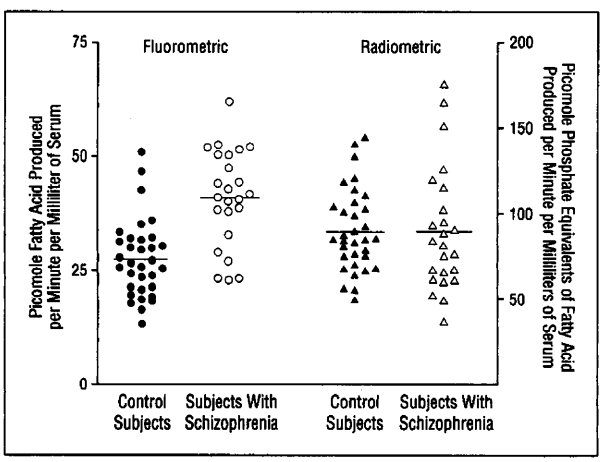

Figure 1. *Phospholipase A_2 (PLA_2) activity in the serum of subjects with schizophrenia (n=24) compared with that in control subjects (n=33). The PLA_2 activity was significantly (P<.001) increased in subjects with schizophrenia compared with control subjects when assayed fluorometrically (left) but unchanged (P>.99) when assayed radiometrically (right). Bars indicate the mean activity in each group.*

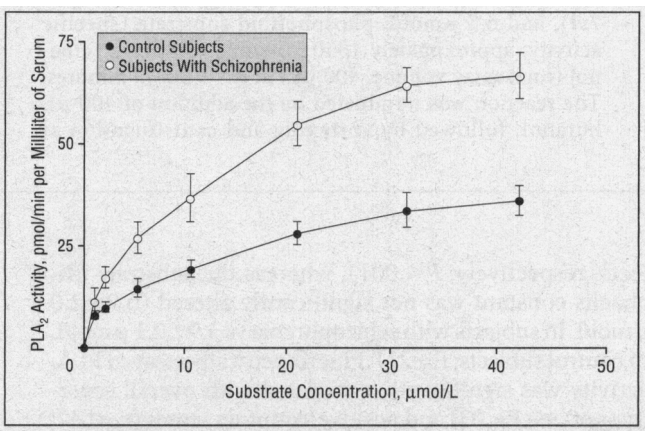

Figure 2. *Effect of substrate concentration on serum phospholipase A_2 (PLA_2) activity in control subjects and subjects with schizophrenia. The PLA_2 activity was measured fluorometrically under varying substrate concentrations in control subjects or subjects with schizophrenia. Each point represents the mean values of 3 subjects analyzed in separate experiments. Bars indicate the SEM.*

buffer, although activity in the presence of 1 mmol/L calcium chloride was not significantly different from that without added calcium, indicating that PLA_2 is fully activated by endogenous calcium ions (**Table 2**). In contrast, the inclusion of EDTA in the fluorometric assay did not significantly alter the rate of hydrolysis (Table 2). The EDTA-insensitive activity was investigated further and found to possess a slightly acidic pH optimum of 6.0 to 7.0 (**Figure 5**) and was moderately inhibited (by 29.1%±5.5% in 5 control subjects) by 5 mmol/L dithiothreitol.

We next compared the characteristics of 3 purified PLA_2s in each assay, ie, porcine $pPLA_2$, human low-molecular-weight $sPLA_2$, and human high-molecular-

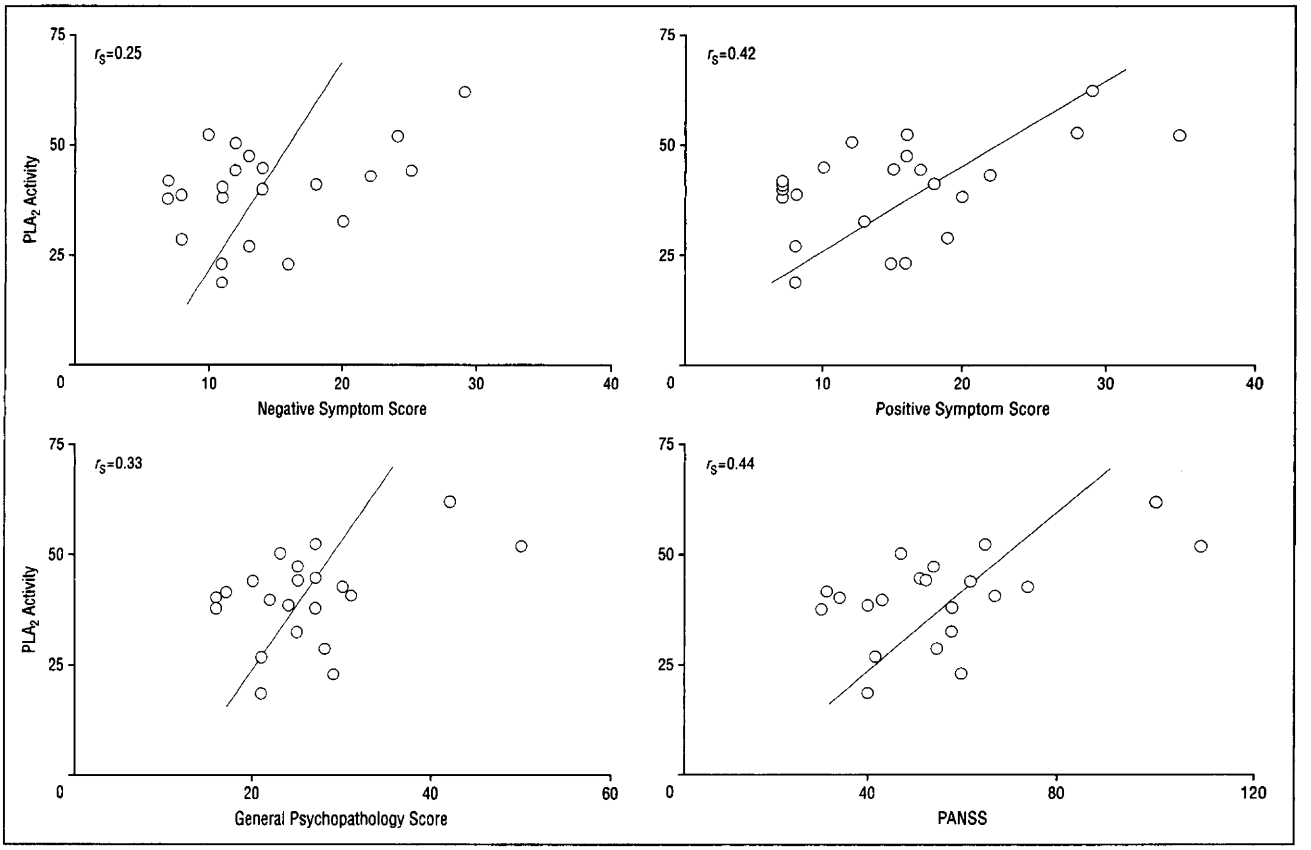

Figure 3. *Correlation between phospholipase A_2 (PLA_2) activity and Positive and Negative Syndrome Scale (PANSS) scores. Serum PLA_2 activity in subjects with schizophrenia measured fluorometrically was significantly correlated ($P<.05$) with positive symptom and overall PANSS scores but not with negative symptom or general psychopathology score. Each panel also shows the best-fit line and the Spearman regression coefficient (r_s).*

weight $cPLA_2$. In the radiometric assay, $pPLA_2$ was much more active than $sPLA_2$ or $cPLA_2$, while in the fluorometric assay $cPLA_2$ was completely inactive, the highest rate of hydrolysis being catalyzed by $sPLA_2$ followed by $pPLA_2$ (Table 2). Furthermore, irrespective of assay type, no hydrolysis was detected in the presence of EDTA. It is therefore unlikely that the enzymatic activity in human serum that catalyses the hydrolysis of C28-O-PHPM in the fluorometric assay derives from $cPLA_2$, $sPLA_2$, or $pPLA_2$.

Finally, we investigated whether human serum contained a calcium-insensitive PLA_2 able to hydrolyze phospholipid species normally found in the body, as opposed to the atypical phospholipid analogue C28-O-PHPM. No evidence was found for such an enzyme active against phosphatidylinositol or diacylphosphatidylcholine. However, PE was hydrolyzed in the presence of 10 mmol/L EDTA, with low rates of hydrolysis of 1-alk-1′-enyl-2-acyl phosphatidylcholine (choline plasmalogen) also being observed (**Table 3**). Furthermore, we also detected the presence of a lysophospholipase activity (the enzyme that hydrolyzes the lysophospholipid product of PLA_2 catalysis) in serum (Table 3).

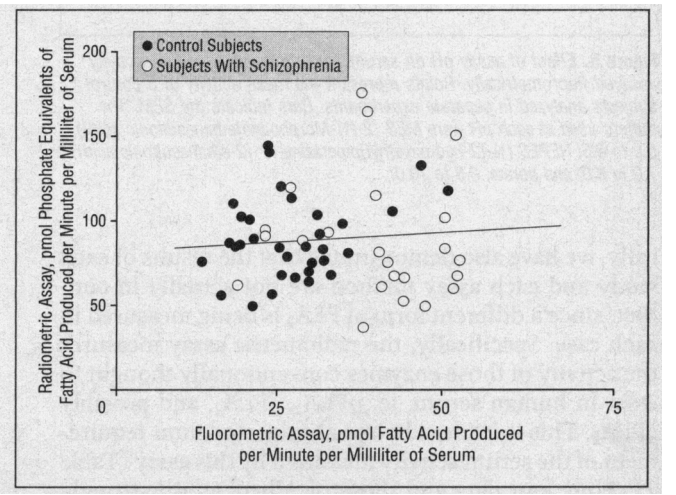

Figure 4. *Lack of correlation between phospholipase A_2 activity measured fluorometrically and radiometrically in control subjects and subjects with schizophrenia. The best-fit line through all points is shown.*

COMMENT

To our knowledge, this is the first investigation in which multiple forms of PLA_2 have been simultaneously assayed in the serum of patients with schizophrenia. Our major finding was that calcium-independent PLA_2 ($iPLA_2$) activity was increased in schizophrenia, whereas calcium-dependent activity was unaltered.

In agreement with Gattaz et al,[29] if serum PLA_2 is assayed by the fluorometric procedure, we found activity to be markedly elevated in patients with schizophrenia, whereas if the enzyme is assayed radiometrically we found, like Albers et al,[32] the activity to be unaltered. Criti-

Table 2. Calcium Ion Requirements of Purified and Human Serum Phospholipase A_2*

Source	Radiometric Assay			Fluorometric Assay		
	No Exogenous Calcium Chloride	1 mmol/L of Calcium Chloride	1 mmol/L Calcium Chloride Plus 5 mmol/L EDTA	No Exogenous Calcium Chloride	1 mmol/L of Calcium Chloride	1 mmol/L Calcium Chloride Plus 5 mmol/L EDTA
Control serum (n=5)†	103.2±7.1	104.2±13.9	8.8±4.0	26.2±4.1	27.3±2.5	30.0±3.4
Schizophrenic serum (n=5)†	96.8±7.4	108.3±11.2	8.8±2.2	40.3±3.7	42.2±5.0	38.9±3.0
Pancreatic PLA_2 (n=3)‡	Not determined	4225±126	Not detectable	Not determined	16.2±2.1	Not detectable
Secretory PLA_2 (n=3)‡	Not determined	18.2±2.6	Not detectable	Not determined	55.5±4.2	Not detectable
Cytosolic PLA_2 (n=3)‡	Not determined	4.1±0.8	Not detectable	Not determined	Not detectable	Not detectable

*EDTA indicates ethylenediaminetetraacetic acid.

†Data are given as phospholipase A_2 (PLA_2) activities of human serum in mean picomoles of fatty acid per minute per milliliter of serum (SEM) of 5 randomly selected subjects. This experiment was repeated in a second set of 5 control subjects and 5 subjects with schizophrenia, with qualitatively similar results (data not shown).

‡Data are given as mean picomoles of fatty acid per minute per microgram of protein (±SEM) in 3 independent experiments. Not detectable describes activities below the detection limits of the assay, ie, 0.2 picomole of fatty acid per minute per microgram of protein for the radiometric assay and 1.5 picomoles of fatty acid per minute per microgram of protein for the fluorometric assay.

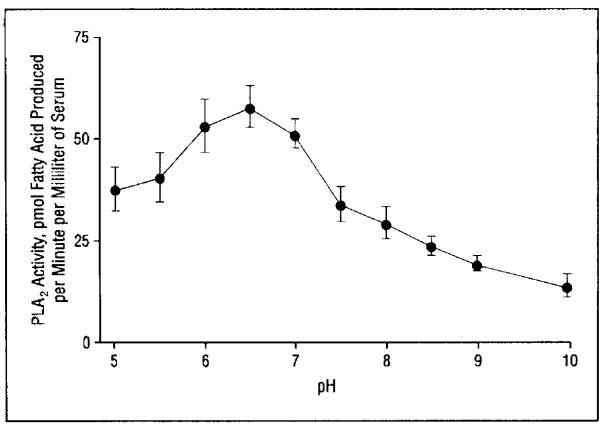

Figure 5. *Effect of assay pH on serum phospholipase A_2 (PLA_2) activity assayed fluorometrically. Points represent the mean activity of 3 control subjects analyzed in separate experiments. Bars indicate the SEM. The buffers used at each pH were MES (2-[N-Morpholino]ethanesulfonic acid]), 5.0 to 6.5; HEPES (N-[2-hydroxyethyl]piperazine-N' -[2-ethanesulfonic acid]), 7.0 to 8.0; and borate, 8.5 to 10.0.*

cally, we have also demonstrated that the results of each study and each assay method are not actually in conflict, since a different form of PLA_2 is being measured in each case. Specifically, the radiometric assay measures the activity of those enzymes conventionally thought to exist in human serum, ie, $pPLA_2$, $sPLA_2$, and possibly $cPLA_2$. This is evident by the absolute calcium requirement of the serum activity measured by this assay (Table 2). Thus, our data and those of Albers et al[32] strongly suggest that the activity of "classical" calcium-sensitive serum PLA_2 is unchanged in serum of subjects with schizophrenia. This finding firmly differentiates schizophrenia-associated alterations in serum PLA_2 activity from those observed in, for example, acute pancreatitis, rheumatoid arthritis, and multiple injuries, which are due to increased serum levels of $sPLA_2$ occurring as part of the acute-phase response in these diseases.[40] We suggest that enhanced PLA_2 activity in schizophrenia, assayed using the substrate C28-O-PHPM, may involve an $iPLA_2$ activity. Moreover, since no correlation was apparent between the calcium-dependent and calcium-independent activities of human serum (Figure 4), it appears that whatever the cause of increased serum $iPLA_2$ activity, the calcium-dependent enzymes are unaffected. This conclusion is at odds with that of Noponen et al,[31] who demonstrated increased calcium-dependent[39] PLA_2 activity in schizophrenia and other psychiatric disorders. Again, it is possible that a different activity is detected by each assay. Unfortunately, we were unable to study the characteristics of the enzyme assayed by Noponen et al[31] since, despite using identical assay conditions to those described in their report, no significant hydrolysis of the phospholipid substrate could be detected in 8 of 10 serum samples assayed.

Although purified $sPLA_2$ and $pPLA_2$ are capable of hydrolyzing C28-O-PHPM in a calcium-dependent manner, the lack of inhibition of serum activity by calcium chelators (EDTA) suggests that they do not contribute significantly to serum PLA_2 activity as measured fluorometrically (Table 2). This can be explained by reference to the levels of these enzymes present in normal human serum, approximately 4 ng/mL for $pPLA_2$ and 3 ng/mL for $sPLA_2$.[40] Based on the specific activities of the purified enzymes (Table 2), the enzyme levels in normal serum are below the detection limit of the assay. Admittedly, the activity of our $pPLA_2$ preparation was lower than that used in the original characterization of the fluorometric assay.[35] However, even using this higher specific activity (2 pmol of fatty acid per minute per nanogram of protein), the rate of fatty acid release would be only 8 pmol of fatty acid per minute per milliliter of serum (given that serum contains 4 ng/mL $pPLA_2$), which in our hands would be only slightly above blank values. Thus, the fluorometric assay is simply not sensitive enough to detect the low levels of $pPLA_2$ and $sPLA_2$ normally present in human serum.

The identity of the $iPLA_2$ we detected in serum is unclear. Blood is known to contain at least 4 calcium-independent lipases, ie, hepatic endothelial lipase,[41] lipoprotein lipase,[42] platelet activating factor acetylhydrolase,[43] and lecithin-cholesterol acyltransferase.[44] However, the characteristics of these enzymes are dissimilar to the $iPLA_2$ activity we have identified, suggesting that the en-

Table 3. Calcium-Independent Phospholipid Hydrolyzing Activities in Human Serum

Substrate	Activity*
1-Palmitoyl, 2-[^{14}C]-arachidonyl, phosphatidylethanolamine	1.21±0.21
1-Stearoyl, 2-[^{3}H]-arachidonyl, phosphatidylcholine	Not detectable
1-Alk-1′-enyl, 2-[^{14}C]-arachidonyl, phosphatidylcholine	0.20±0.02
1-Stearoyl, 2-[^{14}C]-arachidonyl, phosphatidylinositol	Not detectable
1-[^{14}C]-Palmitoyl, lysophosphatidylcholine	1.57±0.90

**Activity was measured at a pH of 7.5 in the presence of 10 mmol/L of ethylenediaminetetraacetic acid. Values are given as mean picomoles of fatty acid released per minute per milliliter of serum (±SEM) of 6 control subjects analyzed in 3 separate experiments. Not detectable describes activities below 0.2 picomole of fatty acid produced per minute per milliliter of serum, the detection limits of the assay.*

zyme described herein may be of a novel form. Interestingly, a phosphatidylmethanol hydrolyzing iPLA$_2$ activity has recently been detected in platelets.[45] A platelet source for serum iPLA$_2$ is also supported by the observation that platelet PLA$_2$ activity is increased, albeit marginally, in schizophrenia.[30]

Importantly, we have also demonstrated that the natural phospholipids PE and choline plasmalogen can be hydrolyzed in a calcium-independent manner by an enzyme present in human serum, suggesting that the actions of serum iPLA$_2$ are not limited to the hydrolysis of atypical phospholipids such as C28-O-PHPM (Table 3). This is of particular interest given that many,[3-6] although not all,[7,9,10] investigators have reported reduced platelet or erythrocyte PE levels in schizophrenia.

Our findings differ from those of Gattaz et al[28] with respect to the effect of neuroleptic medication on serum PLA$_2$ in schizophrenia, in that all of our patients were treated with neuroleptics and those observed by Gattaz et al with increased activity were not. Indeed, they found that PLA$_2$ activity was reduced after the commencement of neuroleptic therapy to the point where there was no longer a difference between the activities of patients with schizophrenia and control subjects.[28] However, whereas the duration of drug treatment of our subjects always exceeded 1 year, and in most cases was considerably longer, Gattaz et al[28] only monitored PLA$_2$ activity for 3 weeks after the commencement of therapy. Thus, the neuroleptic-associated reduction in serum PLA$_2$ activity described by Gattaz et al[28] may be a temporary effect reversed after more extended treatment. It is worth noting that addition of haloperidol to the assay at a concentration typical of that found in the serum of neuroleptic-treated patients with schizophrenia (20 nmol/L[38]) did not affect PLA$_2$ activity. Clearly, longer-term studies of the effect of neuroleptic treatment will be required to resolve this issue.

It is presently unclear the degree to which perturbed phospholipid metabolism is specific to schizophrenia. Gattaz et al[28-30] have consistently reported unaltered PLA$_2$ activity in subjects suffering from psychiatric disorders (primarily major depression) other than schizophrenia. However, recent reports have indicated that phospholipid metabolites in the brains of patients suffering from bipolar disorder are changed in a similar manner to those in schizophrenia.[46-48] To address this issue, we are currently investigating serum iPLA$_2$ activity in bipolar disorder, as well as other well-defined psychiatric and neurological conditions.

Interestingly, the increase in serum PLA$_2$ activity in schizophrenia was positively, although weakly, correlated with overall psychopathological severity, a finding due mainly to a correlation between the positive symptom component of the PANSS scale and PLA$_2$ activity (Figure 3). In contrast, negative symptom scores correlated poorly with PLA$_2$ activity. Thus, if increased PLA$_2$ activity is the basis for the elevated levels of brain phosphodiester observed during brain imaging, one would expect to find a correlation between phosphodiester levels and positive, but not negative, symptoms. Indeed, although increased brain phosphodiester levels are present in groups of patients possessing marked positive symptoms,[18] phosphodiester levels are unchanged in subjects with mainly negative symptoms.[22,23] Differences in degree of positive symptoms also may explain why some researchers report increased phosphodiester levels in individuals with schizophrenia receiving long-term neuroleptic treatment,[18,19] whereas others only detect a change in neuroleptic-naive patients,[16] although other interstudy variables, such as differing anatomical regions or stages of illness, may also account for the contrasting findings of individual studies. A causative role of iPLA$_2$ in increasing levels of brain phosphodiesters and in the pathophysiological features of the disorder is dependent on a serumlike iPLA$_2$ being present in the brain. To this end, our experiments have indicated that human brain contains iPLA$_2$ activities capable of hydrolyzing PE[49] and C28-O-PHPM (B.M.R., unpublished data, July 1995).

In summary, we have shown that the failure by some groups to detect increased PLA$_2$ activity in the serum of individuals with schizophrenia is due to methodological differences and that the affected activity is attributable, at least in part, to a calcium-independent enzyme. If a similar increase occurs in the brains of subjects with schizophrenia, this may explain why brain phosphodiester levels are increased, especially in those with pronounced positive symptoms. Currently, it is unknown whether increased PLA$_2$ activity represents a primary detrimental phenomena, eg, increased membrane breakdown and production of eicosanoids, or is a beneficial compensatory change in response to other biochemical abnormalities such as decreased prostaglandin synthesis.[2] Future experiments using PLA$_2$ inhibitors to reduce the rate of membrane turnover in patients with schizophrenia will likely resolve this issue.

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