

# Phospholipids and Calcium Alterations in Platelets of Schizophrenic Patients

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## Summary

Alterations in phospholipid metabolism in blood elements have been proposed as the possible biochemical marker of schizophrenia. In the present study, we investigated the composition and membrane distribution of phospholipids in platelets of drug-free schizophrenic patients and controls. We have demonstrated that platelets of drug-free schizophrenics have significantly higher cytosolic  $Ca^{2+}$  levels in comparison with healthy controls. Platelets of drug-free schizophrenic patients have a lower content of phosphatidylinositol (PI). After thrombin activation, PI is the target of phospholipase C instead of phosphatidylinositol 4,5-bisphosphate ( $PIP_2$ ), which is hydrolyzed in platelets of controls. Alterations in the distribution of phospholipids were found in the plasma membrane of platelets of schizophrenic patients. We suggest that alterations in phospholipid metabolism might be evoked by a disturbance of calcium homeostasis in schizophrenic patients.

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## Key words

Schizophrenia – Platelets – Phospholipids – Calcium – Phosphoinositide

## Introduction

Phospholipids and their metabolites have attracted much interest in studies of cellular signalling systems in the last decade. Although phospholipids are the major component of all cell membranes, they have not been in the mainstream of schizophrenia research. Phospholipids are no longer viewed as structural matrix for proteins, but rather as dynamic components of cell membranes. Metabolism and distribution of phospholipids in cell membranes are the result of very complex and subtle regulatory mechanisms.

Phospholipid composition and metabolism in the erythrocytes and platelets of schizophrenic patients were studied intensively in the search for biological and biochemical markers of schizophrenia (Rotrosen and Wolkin 1987). It is suggested that the alterations in phospholipid metabolism detected in platelets of schizophrenics may also take place in the brain and thus influence the processes of neurotransmission (Rana and Hokin 1990, Kaiya 1992). It can be hypothesized that a systemic metabolic disorder, common for blood elements and neurones, may take place in the etiology of schizophrenia.

The first report about the abnormalities in the phospholipid composition of red blood cells demonstrated increased content of phosphatidylserine (PS) and a smaller decrease in phosphatidylcholine (PC) and phosphatidylethanolamine (PE) in schizophrenics as compared to healthy controls (Stevens 1972). Changes in the phospholipid composition of red blood cells and platelets studied by many authors are summarized in Table 1. The heterogeneity of results reflects not only the well-known heterogeneity of the clinical manifestation of schizophrenia, but also of investigated patient groups, and probably the effect of different therapies. However, these data show that significant alterations in phospholipid composition of blood elements can be detected in schizophrenic patients. The major phospholipids analysed in these studies play an important role in the activity of many membrane enzymes, for which they form a membrane lipid microenvironment. It has been demonstrated that plasma membrane of eukaryotic cells exhibits an asymmetric distribution of phospholipids across the lipid bilayers (Gordesky and Marinetti 1973).

Table 1  
Phospholipids in schizophrenia

Diagnostic categories	Diagnostic criteria	Tissue	Method	PC	PE	PS	PI	SPM	LPC	Reference
SCHIZOPHRENICS (untreated and phenothiazine-medicated)	n.r.	RBCs	TLC	↓	↓	↑	n.r.	-	n.r.	Stevens (1972)
SCHIZOPHRENICS	Feighner criteria	RBCs	TLC	↓	↓	↑	n.r.	-	n.r.	Henn (1980)
SCHIZOPHRENICS (2 week washout p.)	Feighner criteria	RBCs	TLC	↑	↓	↑	-	-	n.r.	Sengupta et al (1981)
platelets		↑		↓	↑	-	-	n.r.		
after 2 month - 1 year thioridazine and/or CPZ treatment		RBCs	TLC	-	-	-	-	-	n.r.	
platelets	-	-		-	-	-	n.r.			
SCHIZOPHRENICS	n.r.	RBCs	TLC	-	-	-	n.r.	-	n.r.	Cordasco et al (1982)
SCHIZOPHRENICS (2 week washout p.)	DSM-III	RBCs	TLC	↓	-	-	-	↑	n.r.	Hitzemann, Garver (1982)
SCHIZOPHRENIFORM (2 week washout p.), 4 from 9 patients drug-naive				↓	-	-	-	↑	n.r.	
SCHIZOPHRENICS (neuroleptic medication, duration of illness 6 months - 32 years)	DSM-III and Research diagnostic criteria for schizophrenia	RBCs	TLC	-	-	-	n.r.	-	n.r.	Lautin et al (1982)
			HPLC	-	-	-	n.r.	-	n.r.	
SCHIZOPHRENICS (psychotropic medication of various types)	DSM-III	RBCs	TLC	↓	™	↑	n.r.	-	n.r.	Tolbert et al (1983)
SCHIZOPHRENICS (2 week washout p.)	DSM-III and Research diagnostic criteria (RDC) and SADS	RBCs	TLC	↓	-	-	-	-	n.r.	Hitzemann et al (1984)
SCHIZOPHRENIFORM disorder (2 week washout p.)				↓	-	-	-	-	n.r.	
SCHIZOPHRENICS (2 week washout p.)	DSM-III and Research diagnostic criteria (RDC)	RBCs	TLC	↓	n.r.	n.r.	n.r.	n.r.	n.r.	Hitzemann et al (1985)
SCHIZOPHRENIFORM disorder (2 week washout p.)				↓	n.r.	n.r.	n.r.	n.r.	n.r.	
CHRONIC PARANOID SCHIZOPHRENICS (antipsychotic drugs, at least 3-year diagnosis of the disorder)	DSM-III and Schedule for affective disorders and schizophrenia (SADS)	RBCs	HPLC	↓	-	↑	-	n.r.	n.r.	Orologas et al (1986)
		Platelets		↓	-	↑	-	n.r.	n.r.	
PARANOID SCHIZOPHRENIC (mean duration of illness 26 ± 11 months, 5 neuroleptic-naive patients, 4 patients at least 2 weeks drug free)	DSM-III-R, Brief psychiatric rating scale (total score 57,9 ± 4,0) and negative symptom rating scale (score 26,5 ± 4,1)	Platelets	HPTLC	-	-	-	-	-	↑	Pangerl et al (1991)
SCHIZOPHRENIA and SCHIZOARECTIVE DISORDERS (drug free)	n.r.	RBCs	HPTLC	-	↓	n.r.	™	↑	-	Keshavan et al (1993)

Notes: RBCs = red blood cells, TLC = thin-layer chromatography, HPLC = high-performance liquid chromatography, HPTLC = high-performance thin-layer chromatography, ↑ = Schiz > control, ↓ = schiz < control, - = schiz = control, n.r. = not reported, PC = phosphatidylcholine, PE = phosphatidylethanolamine, PS = phosphatidylserine, PI = phosphatidylinositol, SPM = sphingomyelin, LPC = lysophosphatidylcholine.

The aminophospholipids are vastly confined to the inner leaflet of red blood cells and the platelet plasma membrane. Most of sphingomyelin and 45 % of PC is located in the outer leaflet (Bever *et al.* 1983, Zachowski 1993). The asymmetric distribution of phospholipids in the plasma membrane of non-activated cells is relatively stable. In contrast, there are important changes in the transbilayer movement of phospholipids in activated platelets. The substantial amount of negatively charged PS becomes exposed at the outer surface of the membranes. The activity of phospholipid translocase is influenced by cytosolic  $\text{Ca}^{2+}$  concentration (Bever *et al.* 1983, Schroit and Zwaal 1991).

Phospholipids and their metabolites have evoked interest in the studies of cellular signalling. Phosphatidylinositol 4,5-bisphosphate ( $\text{PIP}_2$ ) is the target of phospholipase C and the source of second messenger molecules – inositol 1,4,5-trisphosphate ( $\text{IP}_3$ ) and diacylglycerol (DAG) in the phosphoinositide signalling system.  $\text{IP}_3$  mobilizes calcium from intracellular stores and elevates the cytosolic  $\text{Ca}^{2+}$  level. DAG is the activator of protein kinase C, but also serves as the precursor for *de novo* phospholipid biosynthesis (Berridge and Irvine 1984, Berridge 1993). Newly produced phosphatidic acid (PA) can either be reincorporated into phospholipids, or attacked by phospholipase  $\text{A}_2$  and yield lysophosphatidic acid, or serve as a potential messenger alone (Eichholtz *et al.* 1993).

In platelets of schizophrenics, the accumulation of DAG was observed (Kaiya *et al.* 1989). Platelets have been used as a model for the study of altered phosphoinositide metabolism in the research of schizophrenia. The initial observation of Kayia *et al.* (1989) shifted attention to the study of abnormal phosphoinositide turnover in schizophrenia (Tandon and Greden 1991, Kayia 1992). Changes in inositol phosphates formation and in the metabolism of PA as well as the alterations in prostaglandins synthesis were found in platelets of patients treated with neuroleptics (Rotrosen and Wolkin 1987, Essali *et al.* 1990, Yao *et al.* 1992). Kayia *et al.* (1989) suggested that the abnormal metabolism of PI is a state-dependent biological marker for the subgroup of acute schizophrenic patients. Later, Kayia (1992) postulated that the imbalance of second messenger systems may be related to positive schizophrenic symptoms in some patients with a relatively good prognosis.

The cytosolic  $\text{Ca}^{2+}$  level affects many pathways of phospholipid metabolism, and knowledge about the role of phospholipids and  $\text{Ca}^{2+}$  in signal transduction processes has expanded enormously during the last decade (Rana and Hokin 1990). It is evident that many pathophysiological changes are closely connected to disturbances of calcium homeostatic mechanisms. In resting platelets, there are

no experimental data available about the changes in the cytosolic  $\text{Ca}^{2+}$  level in schizophrenia. Das *et al.* (1992) reported decreased intraplatelet  $\text{Ca}^{2+}$  mobilization after thrombin stimulation in schizophrenic patients treated with neuroleptics in comparison to healthy controls.

Our paper brings evidence about the increased  $\text{Ca}^{2+}$  level in platelets of drug-free schizophrenics. It describes alterations in the composition, distribution and turnover of phospholipids as the consequence of  $\text{Ca}^{2+}$  homeostasis imbalance.

## Methods

### Subjects

The investigated groups for phospholipid content and asymmetry estimation consisted of 14 drug-free schizophrenic patients (7 males, 7 females; mean age  $27.0 \pm 2.6$  years; duration of the disease  $1.7 \pm 0.43$  years) and of 13 control healthy volunteers (7 males, 6 females; mean age  $32.0 \pm 3.8$  years).

The groups for the  $^{32}\text{P}$  incorporation study consisted of 10 drug-free schizophrenics (5 males, 5 females; mean age  $26.6 \pm 1.3$  years; duration of the disease  $2.4 \pm 0.85$  years) and of 24 control healthy volunteers (8 males, 16 females; mean age  $31.9 \pm 2.0$  years).

The groups for the intracellular calcium concentration estimation consisted of 5 drug-free schizophrenic patients (2 males, 3 females; mean age  $25.2 \pm 1.8$  years; duration of the disease  $0.63 \pm 0.23$  years), of 19 control healthy volunteers in the case of unstimulated platelets (6 males, 13 females; mean age  $29.4 \pm 2.1$  years) and of 10 control healthy volunteers in the case of thrombin-stimulated platelets (2 males, 8 females; mean age  $31.9 \pm 2.5$  years).

All control subjects were mentally healthy, without any family history of mental or somatic illness (e.g. hypertension or diabetes).

### Diagnostics

Schizophrenic patients were hospitalized, diagnosed and treated in the Prague Psychiatric Centre (Third Medical Faculty) and in the Department of Psychiatry of the First Medical Faculty of Charles University. The DSM-III-R diagnoses (American Psychiatric Association 1987) were made independently by two trained psychiatrists. The DSM-III-R diagnosis of schizophrenia was confirmed after a six months follow-up period.

Inclusion criteria: age 18–60 years, no concurrent somatic illness (e.g. hypertension or diabetes), no evidence of organic brain damage, no substance abuse in the history, written informed consent. The schizophrenic patients were drug-free. Most of them were drug-naïve patients who had never been exposed to neuroleptic medication, the remaining

patients were drug-free for at least 6 months before the examination. Control subjects and patients were not on any special diet.

#### Preparation of samples

Citrated blood samples were always collected at 07:30 h before breakfast and immediately centrifuged twice at 130 x g for 15 min. Platelet-rich plasma was combined and centrifuged at 500 x g for 15 min.

For the  $^{32}\text{P}$  incorporation study, platelets were resuspended in a incubation medium containing (in mmol/l): NaCl 113.0, KCl 5.6,  $\text{Na}_2\text{HPO}_4$  0.2,  $\text{MgCl}_2$  1.0, Hepes 40.0, glucose 8.0, EDTA 1.0, pH 7.2. The mixture was incubated at 37 °C for 90 min. Human plasma thrombin (Calbiochem, U.S.A.) was added 90 s before the end of the incubation period (2 U/ml).

For the phospholipid asymmetry estimation, the isolated platelets were resuspended in a medium containing (in mmol/l): Hepes 40.0, glucose 9.0,  $\text{Na}_2\text{HPO}_4$  0.2, KCl 5.6, NaCl 112.0,  $\text{Mg}^{2+}$  0.15,  $\text{Ca}^{2+}$  2.0, pH 7.4. and incubated with Merocyanine 540 0.5  $\mu\text{g}/\text{ml}$  (MC 540 Sigma) for 15 min at room temperature.

After 5-min incubation of platelet suspension with ionophore A 23187 (Eli Lilly), to a final concentration 3.8  $\mu\text{mol}$ , the same incubation with Merocyanine 540 followed as described above.

#### Phospholipid asymmetry measurements

The fluorescence of thrombocyte samples was measured using flow cytometry by FACS can (Becton Dickinson). In our experiments, fluorescence in the 585 nm area and comparison with the control was expressed by a statistical value  $D/s(n)$ , according to Kolmogorov-Smirnov. The Kolmogorov-Smirnov two-sample test calculates the probability that two histograms are different. The calculation is based on computing the summation of the curves and finding the greatest difference between the summation curves.  $D/s(n)$  is a value which is indicative of the similarity of the curves compared. The closer  $D/s(n)$  is to zero, the more alike the two curves are. (Terstappen and Thompson 1991).

#### Phospholipid content estimations

Platelets were extracted twice with chloroform: methanol (2:1, v/v). The pooled extract was vortexed with 0.9 % KCl (20 % of extract). The lower phase was evaporated under a stream of nitrogen to dryness. High performance liquid chromatography for the phospholipid separation and estimation was performed on silica columns with UV detection by absorbance at 203 nm using isocratic elution. The solvent mixture was acetonitrile: 85 % phosphoric acid (38:2, v/v) at a flow rate of 1.5 ml/min.

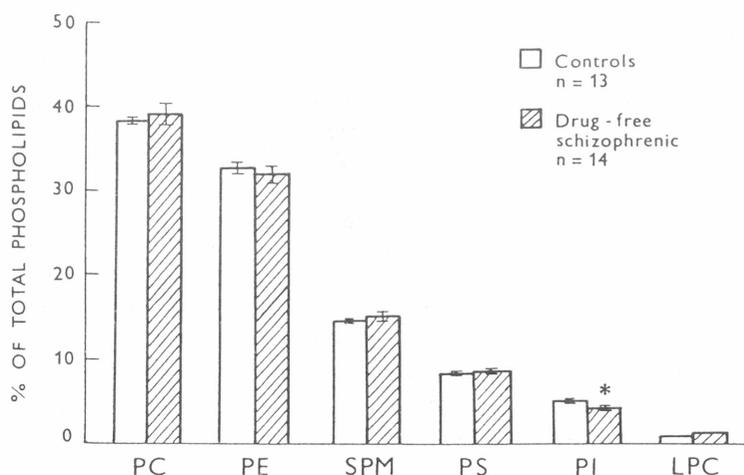
#### Phosphoinositide and phosphatidic acid estimations

$^{32}\text{P}$  (carrier-free, Polatom, Poland) was added at the beginning of the period studied (3.7 MBq/ml). The incubation was stopped by adding two volumes of the cold incubation medium, platelets were centrifuged at 900 x g, and washed again. Phosphoinositides and PA were extracted using acidified chloroform: methanol as described earlier (Struneck et al. 1987), separated (Jolles et al. 1979) on TLC plates with silica gel H (Merck, Germany) and detected autoradiographically.

Radioactivity was assayed in the liquid scintillation counter LS-1801 (Beckman, U.S.A.). Protein determinations (Lowry et al. 1951) and phospholipid estimations (Struneck et al. 1987) were done in triplicate.

#### Cytosolic calcium estimations

$[\text{Ca}^{2+}]_i$  was determined using long wavelength indicator Fluo-3AM (excitation 490 nm; emission 530 nm) according to a modified method (Molecular Probes, Report No. MP 1240, 1992);  $1-3 \times 10^8$  platelets/ml were loaded by incubation with 2.0  $\mu\text{M}$  Fluo-3AM (Molecular Probes, U.S.A.) for 30 min at 37 °C. Samples were then centrifuged, washed and resuspended in 2.5 ml of buffer containing (in mM): NaCl 113.0, KCl 5.6,  $\text{Na}_2\text{HPO}_4$  0.2,  $\text{MgCl}_2$  1.0, Hepes 40.0, glucose 8.0, pH 7.2. Thrombin (2U/ml) was added and changes in  $[\text{Ca}^{2+}]_i$  were immediately recorded. A spectrofluorophotometer RF-5000 (Shimadzu, Japan) was used for the fluorescence measurement.



**Fig. 1.** Phospholipid composition of platelets from control subjects and drug-free schizophrenic patients. PC - phosphatidylcholine, PE - phosphatidylethanolamine, SPM - sphingomyelin, PS - phosphatidylserine, PI - phosphatidylinositol, LPC - lysophosphatidylcholine.

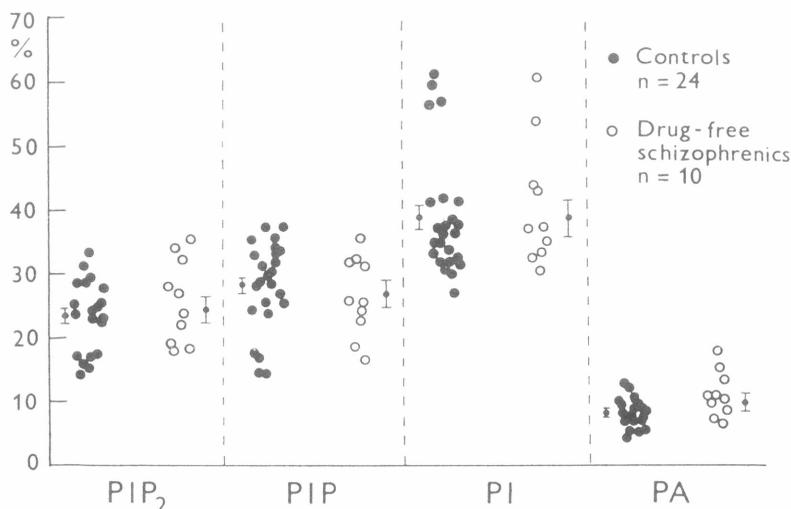
### Statistical analysis

The results are expressed as mean values  $\pm$  S.E.M. for a given number of independent experiments. Pairwise group comparisons were performed using the separate variance version of the t-test. The BMDP software package (Dixon *et al.* 1992) was used for the analyses.

## Results

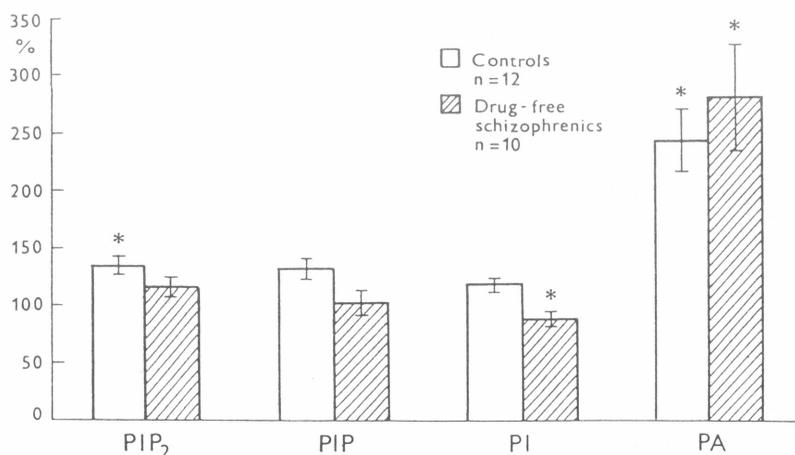
### Phospholipid content in platelets of drug-free schizophrenic patients

No differences in the content of PC, PE, PS, SPM and lysophosphatidylcholine (LPC) were found in platelets of drug-free schizophrenic patients and healthy control subjects. However, there was a significant decrease in the PI content ( $p < 0.01$ , Fig. 1). The content of PI represents  $5.02 \pm 0.23\%$  of the total phospholipid content in platelets of the controls and  $4.04 \pm 0.27\%$  in drug-free patients. According to our estimation, controls platelets contained  $896.4 \pm 40.9$  nmol of PI/100 mg protein ( $n = 13$ ), while platelets of drug-free schizophrenic patients only  $720.7 \pm 47.3$  nmol of PI/100 mg of protein ( $n = 14$ ).



**Fig. 2.** Radioactivity (cpm/mg prot.) detected in phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>), phosphatidylinositol 4-phosphate (PIP), phosphatidylinositol (PI) and phosphatidic acid (PA) in platelets of controls and drug-free schizophrenic patients. 100 % is the sum of radioactivity detected in PIP<sub>2</sub>, PIP, PI and PA; means  $\pm$  S.E.M.

**Fig. 3.** Effect of thrombin on <sup>32</sup>P incorporation into phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>), phosphatidylinositol 4-phosphate (PIP), phosphatidylinositol (PI) and phosphatidic acid in platelets of controls and drug-free schizophrenic patients. Data are expressed in % of unstimulated control values, mean  $\pm$  S.E.M.; \* $P < 0.01$ .



### <sup>32</sup>P incorporation into phosphoinositides and phosphatidic acid

The time course of <sup>32</sup>P incorporation into PA and PIP<sub>2</sub> shows that these phospholipids reach isotope steady-state after 90 min incubation in resting platelets and their labelling does not change for the next 30 min. This also makes it possible to use the

incorporation changes observed as an indicator of mass changes of PIP<sub>2</sub> and PA. The incorporation of <sup>32</sup>P into PI and PIP increased steadily throughout the whole period investigated (data not shown). In order to compare individual measurements, we expressed the total radioactivity (in cpm/mg of protein) detected in phosphoinositides and PA as 100 %. No differences in

the incorporation of  $^{32}\text{P}$  into phosphoinositides and PA were found in the platelets of drug-free schizophrenics in comparison with the controls (Fig. 2).

Stimulation of platelets with thrombin significantly increased  $^{32}\text{P}$  incorporation into PA in the controls ( $p < 0.01$ ) as well as in drug-free schizophrenic patients ( $p < 0.01$ ). While in the platelets of controls

thrombin significantly increased ( $p < 0.01$ ) the  $^{32}\text{P}$  incorporation into  $\text{PIP}_2$  without affecting the  $^{32}\text{P}$  incorporation into PI, the response of platelets of schizophrenic patients was different. After thrombin stimulation, we observed a significant decrease of  $^{32}\text{P}$  incorporation into PI and no change in the  $^{32}\text{P}$  incorporation into  $\text{PIP}_2$  (Fig. 3).

Table 2

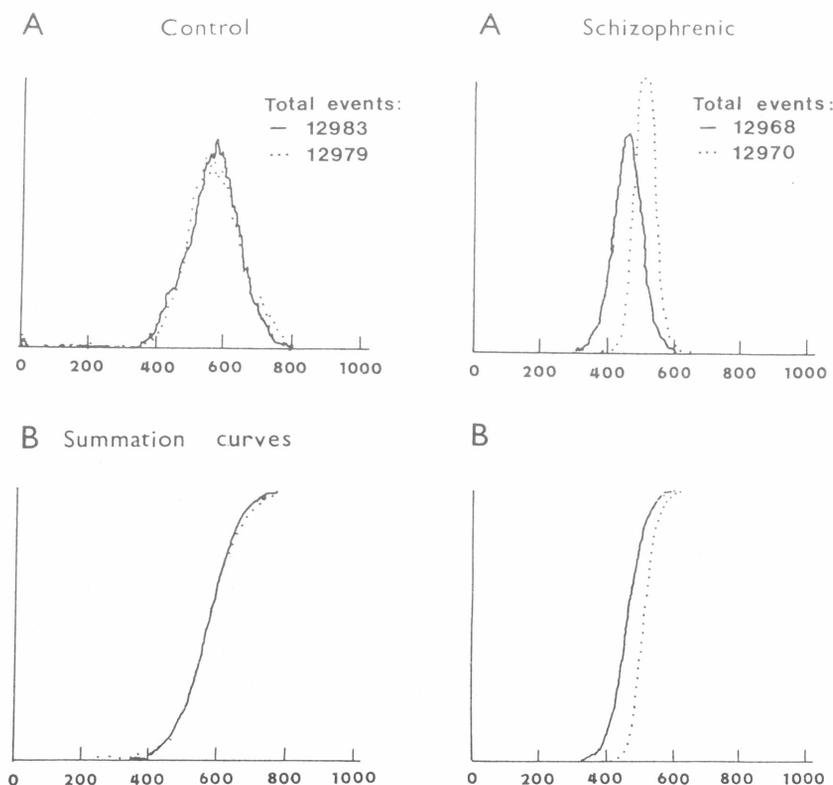
Intracellular calcium level in resting and thrombin stimulated platelets of drug-free schizophrenics and controls (mean  $\pm$  S.E.M.)

	RESTING	THROMBIN STIMULATED	
	[nM]	[nM]	% OF UNSTIMULATED CONTROL VALUE
SCHIZOPHRENICS (n = 5)	188.4 $\pm$ 12.5	267.4 $\pm$ 25.8 P < 0.03	143.6 $\pm$ 14.0
CONTROLS (n = 10)	86.8 $\pm$ 7.9	132.0 $\pm$ 10.5 P < 0.01	154.7 $\pm$ 7.9

#### Cytosolic calcium levels

Platelets of drug-free patients had a significantly higher ( $p < 0.001$ ) cytosolic calcium level as compared to the controls. In resting platelets of controls there was  $86.8 \pm 7.9$  nM of  $[\text{Ca}^{2+}]_i$ , while this value increased to  $188.4 \pm 12.5$  nM in platelets of drug-

free schizophrenic patients. The cytosolic calcium level increased 90 s after platelet stimulation with thrombin in both investigated groups, but there were no differences in calcium mobilization between the controls and schizophrenic patients (Table 2).



**Fig. 4.** Histogram of fluorescence in 585 nm area in non-activated (solid curve) and  $\text{Ca}^{2+}$ -activated (dotted curve) platelets (A) and summation curves expressed by statistical value  $D/s(n)$  according to Kolmogorov-Smirnov (B) in controls and drug-free schizophrenics.

### Flow-cytometric analysis of merocyanine 540 binding

A different merocyanine 540 binding was found in the group of schizophrenic patients ( $D/s(n) = 20.2 \pm 4.8$ ;  $p < 0.05$ ) as compared to the controls ( $D/s(n) = 9.1 \pm 1.9$ ). Figure 4 shows the comparison of two representative examples of flow-cytometric analysis in platelets of a control subject and drug-free schizophrenic patient.

## Discussion

In the past, platelets and red blood cells have been used in the investigation of phospholipid alterations in schizophrenia. The multiple biochemical and pharmacological similarities with neurons have been well documented in platelets (Siess 1991). The data summarized in Table 1 demonstrate that alterations in phospholipid composition were found in blood elements of schizophrenic patients. The hypothesis that the phospholipid content determination can be a biochemical marker for schizophrenia was not always confirmed due to the heterogeneity of the results. However, changes in the phospholipid content as reported by numerous authors suggest that a systemic metabolic disorder takes place in cells of schizophrenics. We propose that the systemic metabolic disorder could be associated with disturbances in calcium homeostasis.

Changes in cytosolic  $Ca^{2+}$  levels have been widely demonstrated in connection with many cellular events and pathological situations, including mental diseases. To date, these changes have not been reported in the platelets of schizophrenics. Our data demonstrate a significantly higher cytosolic  $Ca^{2+}$  level in platelets of drug-free schizophrenic patients in comparison with the controls. The increased cytosolic  $Ca^{2+}$  concentration could represent the common denominator of all alterations of phospholipid metabolism and could evoke diverse biochemical and clinical manifestations of schizophrenia, although the elevation of calcium cytosolic concentration is not specific for schizophrenia (Dubovsky *et al.* 1991, Le Qang Sang *et al.* 1993).

The phospholipid membrane composition is the result of many metabolic pathways and phospholipid interconversions. Changes in the cytosolic  $Ca^{2+}$  concentration markedly affects the turnover of plasma membrane phospholipids. Strunecká and Folk (1990) observed that increased  $Ca^{2+}$  concentration evoked the increased incorporation of U- $^{14}C$ -serine into PS in human red blood cells. The increased content of PS or changes in its redistribution in the plasma membrane can have serious consequences (Devaux *et al.* 1987, Strunecká *et al.* 1990). In our experiments we have observed that platelets of drug-free schizophrenics have enhanced merocyanine 540 binding as compared with the controls. A fluorescent

lipophilic probe, merocyanine 540, binds preferentially to the loosely packed and disorganized phospholipid bilayer. It was suggested that this probe shows an enhanced fluorescence in the presence of anionic phospholipids (Williamson and Schlegel 1984). The parallel between binding of merocyanine 540 and the redistribution of PS into the outer leaflet of the plasma membrane confirmed with a 2,4,6-trinitrobenzenesulfonic acid probe and after phospholipase A<sub>2</sub> degradation has been demonstrated in red blood cells (Strunecká *et al.* 1990). Despite the fact that merocyanine 540 binding is not specific, there is a difference in phospholipid distribution between platelets of drug-free schizophrenics and platelets of the controls. The increase of cytosolic  $Ca^{2+}$  induces the transfer of aminophospholipids from the inner to the outer leaflet; it also inhibits the activity of translocase in human erythrocytes and platelets (Bitbol *et al.* 1987, Devaux *et al.* 1988, Schroit and Zwaal 1991). Changes in the lipid microenvironment can affect the activity of many enzymes and their association with membranes.

Because some reports indicated alterations of phosphoinositide metabolism in platelets of schizophrenics, we investigated  $^{32}P$  incorporation into inositol lipids in platelets of drug-free schizophrenics. No difference between the total amount of  $^{32}P$  incorporated into PIP<sub>2</sub> in platelets of schizophrenics and controls was found in our study. On the other hand, the conclusion can be made that PI has a higher turnover in platelets of schizophrenic patients.

The reactivity of the phosphoinositide signalling system can be investigated using stimulation of platelets with thrombin (Rink and Hallam 1984). Phosphodiesterase cleavage of phosphatidylinositol 4,5-bisphosphate is the earliest biochemical event following this stimulation. The hydrolysis of all three inositol lipids due to phospholipase C has been observed in many tissues (Rittenhouse 1982). A long time ago it has been recognized that calcium is an important regulator of phospholipase C activity (Lapetina 1983). The hydrolysis of PIP<sub>2</sub> preceding that of PI and calcium mobilization has been observed in platelets (Rana and Hokin 1990, Cocroft and Thomas 1992). However, the  $Ca^{2+}$  ionophore is capable to stimulate PI breakdown in platelets. Addition of  $Ca^{2+}$  chelators inhibits platelet function and PI hydrolysis. Thus, the hydrolysis of PIP<sub>2</sub> is  $Ca^{2+}$ -independent, whereas hydrolysis of PI is  $Ca^{2+}$ -dependent in platelets.  $Ca^{2+}$  is a probable trigger of PI breakdown. Early studies on phospholipase C had clearly established the existence of a multiple isoform of this enzyme in various tissues (Cocroft and Thomas 1992). The increased cytosolic  $Ca^{2+}$  can cause a shift of phospholipase C activity or activate various phospholipase C isoforms.

In platelets of control subjects, we observed an increase of  $^{32}P$  incorporation into PIP<sub>2</sub> after

thrombin stimulation to 134 % of control unstimulated values reflecting an increased PIP<sub>2</sub> breakdown and resynthesis. These data are in agreement with the data reported by other authors (Rittenhouse 1982, Lapetina 1983). The pattern observed in thrombin-stimulated platelets of drug-free schizophrenics is different. There is no significant change of <sup>32</sup>P incorporation into PIP<sub>2</sub> and PIP. <sup>32</sup>P incorporation into PI decreases to 87 % of unstimulated values. These findings indicate that PI is the target of phospholipase C in platelets of drug-free schizophrenics.

There are no theories about the physiological role of IP<sub>1</sub>. Sherman *et al.* (1981) found good correlation between seizure duration and the IP<sub>1</sub> level in the cerebral cortex and in hippocampus. Hokin (1970) observed a loss of PI *in vitro* upon brain stimulation and inhibition of <sup>32</sup>P incorporation into PI caused by dopamine. It cannot be excluded that the generation of IP<sub>1</sub> can induce pathophysiological changes in the brain and affect neurotransmission processes.

The investigations of phospholipid metabolism alterations in the blood elements of

schizophrenic patients broaden our knowledge about the dynamics of phospholipid metabolism. Despite the detailed knowledge about biochemical pathways, the conceptual framework for a full understanding of their role in the cell metabolism is not known at this point. Disturbances in calcium homeostasis may be the common denominator of the observed biochemical heterogeneity. In accordance with the theories about the etiology of schizophrenia, the disturbance in calcium homeostasis may be genetically linked or induced by an infection. Haemolytic viruses or bacterial toxins induce changes in the permeability of plasma membranes to cations, namely Ca<sup>2+</sup> (Pasternak 1987). Diverse pathological stimuli may evoke an increase of cytosolic Ca<sup>2+</sup> levels. This alteration may then evoke the diverse biochemical and clinical manifestations of schizophrenia.

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