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Short communication

Correlation between platelet and brain PLA_2 activity

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ABSTRACT

The phospholipase A_2 (PLA_2) enzymes have been implicated in several neuropsychiatry disorders and activity alterations have been described in brain and platelet. Since brain tissue is not readily available for the measurement of PLA_2 activity, it would be of interest to test directly whether PLA_2 activities in both tissues are correlated. We performed this task assessing PLA_2 activity in platelets and hippocampus collected simultaneously from 19 patients undergoing temporal lobectomy for treatment of refractory epilepsy. Our findings suggest that *total* PLA_2 activity in platelets may reflect the *total* activity of the enzyme in the brain (r_s=0.59, p=0.008). However in our sample no correlations were found between the subgroups of the enzyme in brain and in platelets. This lack of correlations may be due to different effects of drug treatment on the PLA_2 subtypes. In face of the difficulty to obtain brain tissues from living patients, further studies with larger drug-free samples are warranted to clarify whether the use of platelets is a reliable strategy to reflect the subtypes of PLA_2 activity in the brain.

1. Introduction

The phospholipase A_2 (PLA_2) enzymes catalyze the hydrolysis of membrane phospholipids at *sn*-2 position, releasing fatty acids and lysophospholipids, which mediate the transmission and processing of neuronal signals [1,2]. Increased PLA_2 activity has been described in platelets and in brain of schizophrenic and psychotic patients [3–10], whereas reduced enzyme activity was found in platelets and in post mortem brain of patients with Alzheimer's disease [11–13].

Platelets share many similar biologic properties to neurons and have been widely used in the search for biomarkers related to neuropsychiatric disorders [14–16]. There is indirect evidence suggesting that abnormal PLA_2 activity in platelets may reflect the enzyme activity in the brain. Since brain tissue is not readily available for the measurement of PLA_2 activity, it would be of interest to test directly whether PLA_2 activities in both tissues are correlated.

We performed this task in the present study, by assessing PLA_2 activity in platelets and hippocampus collected simultaneously from patients undergoing temporal lobectomy for treatment of refractory epilepsy.

2. Methods

2.1. Subjects

The sample comprised 19 patients (11 male/eight female, mean age 39.9 ± 14.2 years) with Temporal lobe epilepsy–mesial temporal sclerosis (TLE–MTS) who underwent an anterior temporal lobectomy at the Functional Neurosurgery Center, Institute of Psychiatry, University of São Paulo, for treatment of refractory epilepsy. Diagnosis of TLE was done according to ILAE criteria (Commission on Classification and Terminology of the International League Against Epilepsy 1989) [17] based on clinical anamnesis, EEG and video-EEG exams. Diagnosis of MTS was confirmed by 1.5 T MRI. Nine subjects of the study presented psychiatry comorbidity (five with psychoses and four with major depression). Exclusion criteria were comorbidity with alcohol and drug abuse, and with other organic or metabolic disease. Part of this sample (psychotic patients and individuals without psychiatric comorbidity) was described elsewhere [18]. All patients were on antiepileptic drug (AED) treatment.

All 19 patients included in this study underwent a standard surgical procedure with an anterior temporal lobectomy with hippocampus removal performed with the same protocol and by the same neurosurgeon [19,20].

This study was approved by the local ethics committee of the University of São Paulo.

2.2. Determination of PLA_2 activity

2.2.1. Tissue preparations

The hippocampi and blood samples were collected at the same time during the surgery. The hippocampi were immediately frozen

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at −80 °C. Tissue was defrost to 0 °C, homogenized in 20 volume of 5 mM Tris–HCl buffer (pH 7.4) and again stored at −80 °C until analysis. Prior to PLA_2 activity determination, total protein levels were determined in the homogenates by the Bio-Rad DC Protein Assay (Bio-Rad, Hercules, CA) modified from the Lowry assay [21].

2.2.2. Platelet preparation

Samples of fresh blood were obtained through the puncture of a peripheral vein in the forearm. Platelets were isolated by centrifugation at 515*g* for 15 min at 20 °C with acid citrate dextrose solution (ACD-NIH-formel-A solution: glucose 123.8 mM, tri-sodium citrate 83.9 mM, citric acid 41.3 mM) (1:10v:v). Platelet-rich plasma (PRP) was collected and pH was adjusted to 6.5 by titration with ACD-NIH-formel-A. PRP was centrifuged at 1159*g* for 10 min at 20 °C and the supernatant was carefully removed. A washing solution (30 mM sodium citrate pH 6.5; 5 mM potassium chloride; 2 mM calcium chloride; 1 mM magnesium chloride; 5 mM glucose; 500 μg/mL albumin; 50 μg/mL apyrase) was added to the supernatant and incubated for 10 min. The solution was centrifuged at 1159*g* for additional 8 min at 20 °C and the pellet was re-suspended with tris-sucrose solution. Platelets aliquots were frozen immediately at −70 °C until experimentation. Under these conditions, there was no evidence of platelet activation, (data not shown).

2.2.3. Radioactive substrates preparation

The radioactive substrates L-α-1 palmitoyl-2-arachidonyl-phosphatidylcholine (PC-AA-[1^{14}C])—(Perkin Elmer Life Science, USA) and L-3 phosphatidylcholine, 1-palmitoyl-2-palmitoyl—PC-PA-[1^{14}C]—(Amersham Biosciences, UK) both labeled in position 2 with [1^{14}C], were processed similarly, i.e., diluted 1:10 in a 1:2 toluene–ethanol solution containing 140 mg/mL of antioxidant buthylatedhydroxytoluene (BHT). The solution was then evaporated under stream of nitrogen (12 μl or 0.06 μCi per sample), re-suspended with 150 μL of ice-cold 0.3 mg/mL BSA solution and homogenized using a sonicator to form liposomes. The count of total radioactivity was standardized for both substrates (120,000 CPM). All assays were conducted with a blank sample. The assays were considered viable when the blank sample values were below 300 CPM.

2.2.4. Radioenzymatic assays for brain tissue

Aliquots of hippocampus homogenates were used to determine the activity of PLA_2 subtypes (cytosolic $cPLA_2$, secretory $sPLA_2$ and calcium-independent $iPLA_2$) by radioenzymatic assays as described elsewhere (Schaeffer and Gattaz, 2005). Briefly, the substrate used was PC-AA-[1^{14}C]. Brain tissue homogenates were diluted to a final protein concentration of 1.5 mg/mL with 50 mM Tris–HCl (pH 8.5 for $sPLA_2$ and $cPLA_2$ or pH 7.5 for $iPLA_2$). The assays contained 100 mM Tris–HCl buffer (pH 8.5 or pH 7.5), 1 μM (for $cPLA_2$ and $iPLA_2$) or 2 mM $CaCl_2$ (for $sPLA_2$), 100 μM bromoenol lactone (Biomol), (USA), 300 μg of protein from diluted homogenates, and 150 μL of PC-AA-[1^{14}C]. After 15 min incubation at 37 °C, the reactions were interrupted by the addition of 700 μl isopropanol–hydrochloric acid.

2.2.5. Radioenzymatic assays for platelets

This assay has been previously standardized by our group [22]. Briefly, the assay mixture contained 50 μl of 1.0 M Tris–HCl buffer pH 7.5, 200 μl of platelet (0.2 mg of homogenate protein), 150 μl of PC-AA-[1^{14}C]. Calcium concentrations of 5 Mm and 100 μM were used to measure $sPLA_2$ and $cPLA_2$ activity respectively. To measure $iPLA_2$ activity were used 5 Mm EDTA and 150 μl of PC-PA-[1^{14}C] (0.06 μCi). The solution was incubated for 30 min at 37 °C and the reaction was stopped by the addition of 700 μl isopropanol–hydrochloric acid.

For both procedures i.e. hippocampus and platelets samples the ^{14}C-labeled fatty acid released by PLA_2 was extracted with n-heptane, followed by adsorption of the unbroken phospholipids and the lysophospholipids on 60 mg of silica. The radioactivity was measured in a liquid scintillation counter (Tri-Carb 2100 TR; Packard, USA) for calculating PLA_2 activities (pmol/mg protein/min−1). All PLA_2 activities were performed in triplicate. Inter-and intra-assay variation coefficients were below 5%. totalPLA_2 activities were calculated by the sum of the three group activity.

2.3. 3-Statistical analysis

Correlations between enzyme activities were calculated through Spearman Coefficients. All statistical analyses were done with the software Statistical Package for Social Science (SPSS, Chicago, USA), version 14.0 and significance level was set at $p < 0.05$.

3. Results

The correlations were tested with all samples and only in patients without psychiatric comorbidity. Table 1.

Total PLA_2 ($tPLA_2$) in the brain was significantly correlated with $tPLA_2$ in platelets (r_s=0.59, p=0.008). Moreover, $tPLA_2$ in the brain was also significantly correlated with $sPLA_2$ and $cPLA_2$ in platelets (r_s=0.60, p=0.007 and r_s=0.57, p=0.01 respectively)(Fig. 1). No correlations were found among the subtypes $iPLA_2$, $sPLA_2$ and $cPLA_2$ in the brain and in platelets. Table 2.

In the ten subjects without psychiatric comorbidity, the correlation remained significant among $tPLA_2$ in brain and $tPLA_2$, $sPLA_2$ and $cPLA_2$ in platelets (r_s=0.82, p=0.004; r_s=0.87, p=0.001 and r_s=0.84, p=0.002 respectively). Moreover, $cPLA_2$ in brain showed a correlation with $tPLA_2$ in platelets (r_s=0.65, p=0.043). No correlations were found among the subtypes $iPLA_2$, $sPLA_2$ and $cPLA_2$ in the brain and in platelets. No effects of age and sex were found on the correlations.

Table 3 describes the demographic data and PLA_2 group's activities of patients with and without psychiatric comorbidity.

4. Discussion

In this study we tested the correlations between PLA_2 activity in the hippocampus and in platelets of TLE–MTS patients. This is the first investigation in human subjects to correlate PLA_2 activity in platelets and in the brain in vivo, with both collected simultaneously during neurosurgical procedure for therapy-resistant TLE–

Table 1
Correlations between PLA_2 activity in the brain and in platelets in all patients (n=19).

		Platelets			
		$iPLA_2$	$sPLA_2$	$cPLA_2$	$tPLA_2$
Brain	$iPLA_2$	r_s=−0.14 p=0.285	r_s=0.38 p=0.112	r_s=0.35 p=0.068	r_s=0.39 p=0.098
	$sPLA_2$	r_s=−0.21 p=0.191	r_s=0.34 p=0.154	r_s=0.31 p=0.205	r_s=0.30 p=0.22
	$cPLA_2$	r_s=−0.14 p=0.571	r_s=0.45 p=0.05	r_s=0.42 p=0.072	r_s=0.46 p=0.046
	$tPLA_2$	r_s=−0.27 p=0.290	**r_s=0.60** **p=0.007**	**r_s=0.57** **p=0.011**	**r_s=0.59** **p=0.008**

r_s=Spearman's rho correlation coefficient.