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Increased PLA_2 activity in the hippocampus of patients with temporal lobe epilepsy and psychosis

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ABSTRACT

Objective: The aim of this work was to investigate whether increased activity of the enzyme phospholipase A_2 (PLA_2) in the brain, as frequently reported in schizophrenia, is also related to psychosis in epilepsy. Our working hypothesis was based on the increased prevalence of schizophrenia-like psychosis in patients with temporal lobe epilepsy (TLE) secondary to mesial temporal sclerosis (MTS), as compared to patients with other forms of epilepsy.
Methods: We determined PLA_2 activity in hippocampal tissue from 7 patients with TLE-MTS and psychosis, as compared to 9 TLE-MTS patients without psychosis. Hippocampal tissue was obtained from patients who underwent an anterior temporal lobectomy due to therapy-resistant epilepsy.
Results: We found that patients with TLE-MTS and psychosis had a significantly increased calcium-independent PLA_2 activity as compared to patients without psychosis ($p = 0.016$).
Conclusion: Our finding suggest that an increment in brain PLA_2 activity is not specific for schizophrenia, but rather may be associated to the manifestation of schizophrenia-like psychotic symptoms in general.

1. Introduction

Temporal lobe epilepsy (TLE) secondary to mesial temporal sclerosis (MTS) is the most frequent cause of focal refractory epilepsy in adults (Qin et al., 2005). The prevalence of schizophrenia-like psychotic symptoms in patients with TLE ranges from 7% to 11% and is thus higher than the expected 0.5%–1% in the general population (Elliott et al., 2009). Some clinical variables, such as duration of epilepsy and presence of refractory epilepsy with frequent seizures, may be implicated in the occurrence of psychosis, although this issue remains controversial (Elliott et al., 2009; Nobuo et al., 2004). Psychosis in TLE patients is mostly characterized by Schneiderian first rank symptoms (thought insertion, thought broadcast), paranoid delusions, and hallucinations (visual and auditory) (Marsh and Rao, 2002).

Given the clinical similarities, it is conceivable that psychosis in epilepsy may share some common biological substrate with schizophrenia. One consistent biochemical finding in schizophrenia is an increased activity of the enzymes phospholipases A_2 (PLA_2). This family of enzymes is responsible for the metabolism of membrane phospholipids and is composed by three main groups: calcium-dependent cytosolic PLA_2 ($cPLA_2$), calcium-dependent secretory PLA_2 ($sPLA_2$) and calcium-independent intra-cellular PLA_2 ($iPLA_2$) (Kudo and Murakami, 2002). PLA_2 hydrolyzes the fatty-acid at the sn-2 position of phospholipids, releasing free fatty-acids and lysophospholipids. These are substrates to the synthesis of prostaglandins and arachidonic acid, which are important mediators of neuronal transmission and signaling processes. Moreover, PLA_2 activity controls the physicochemical properties of neuronal membranes, affecting for instance receptor function and the release and reuptake of neurotransmitters (Piomelli, 1993; Bazan et al., 1993).

We first reported increased PLA_2 activity in serum, plasma and in platelets of drug-free schizophrenic patients (Gattaz et al., 1987, 1990, 1995; Tavares et al., 2003), and this finding was replicated by other laboratories in blood and in *postmortem* brain tissue (Ross et al., 1997, 1999; Noponen et al., 1993; Smesny et al., 2005). Moreover, treatment with anti-psychotic drugs was found to reduce significantly PLA_2 activity, restoring the enzyme activity in schizophrenic patients to levels similar as those observed in control subjects (Gattaz et al., 1987; Tavares et al., 2003; Schmitt et al., 2001).

The role of phospholipases has also been investigated in experimental models of epilepsy. Whereas seizures in rats induced an increase in $sPLA_2$ activity (Yegin et al., 2002), intracerebral

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injections of $sPLA_2$ induced seizures in rodents (Dorandeu et al., 1998). There are so far no investigations of PLA_2 activity in patients with TLE, although an increment of the enzyme activity in this disease has been theoretically hypothesized (Simonato, 1993). It is possible that increased PLA_2 activity is not specific for schizophrenia, but may rather reduce the threshold for the onset of psychotic symptoms in general. In this case, one would expect in TLE that the enzyme activity would be increased only in those patients with psychotic symptoms. To test this hypothesis we studied the activity of PLA_2 subgroups in the hippocampal tissue of patients with refractory TLE with MTS who underwent a lobectomy due to therapy-resistant epilepsy.

2. Methods

2.1. Subjects

The sample comprised 16 patients with TLE-MTS who underwent an anterior temporal lobectomy at the Functional Neurosurgery Center, Institute of Psychiatry, University of São Paulo. The indication of surgery was the refractory epilepsy. Diagnosis of TLE was done according to ILAE criteria (Commission on Classification and Terminology of the International League Against Epilepsy, 1989) based on clinical anamnesis, EEG and video-EEG exams. These neurophysiological exams were carried on at the Laboratory of Clinical Neurophysiology of the Institute of Psychiatry. Diagnosis of MTS was confirmed by 1.5 T MRI. Patients were submitted to a psychiatric evaluation and psychiatric diagnosis was established according to DSM-IV TR criteria (American Psychiatric Association, 2000).

Exclusion criteria were comorbidity with mood disorders, alcohol and drug abuse, and with other organic or metabolic disease.

Seven patients presented TLE-MTS and interictal chronic psychosis and nine patients presented TLE-MTS without psychotic symptoms during their lifetime. At the time of epilepsy surgery, patients with psychosis were under remission and thus, no psychopathological ratings were assessed. Both groups were comparable regarding demographic variables. All patients were on anti-epileptic drug (AED) treatment, and all but one patient with psychosis received treatment with anti-psychotic drugs (APDs)(Table 1).

All 16 patients included in this study underwent a standard surgical procedure with an anterior temporal lobectomy performed with the same protocol and by the same neurosurgeon (Wen et al., 2006, 1999).

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 hippocampus samples were collected during the surgery and immediately frozen at −80 °C. Tissue was defrost to 0 °C, homogenized in 20 vol. 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 (Lowry et al. 1951).

2.2.2. Radioenzymatic assay

Aliquots of hippocampus homogenates were used to determine the activity of PLA_2 subtypes ($cPLA_2$, $sPLA_2$ and $iPLA_2$) by radioenzymatic assays as described elsewhere (Schaeffer and Gattaz, 2005). Briefly, the substrate used was L-α-1-palmitoyl-2-arachidonyl-phosphatidyl-choline labeled with [l-^{14}C] in the arachidonyl tail at position sn-2 (^{14}C-PC) (48 mCi/mmol specific activity, PerkinElmer, MA). Prior to the enzymatic reaction, a mixture of ^{14}C-PC and toluol–ethanol–butylhydroxytoluol antioxidants (1:10, v/v) was evaporated under a nitrogen stream (0.075 μCi/sample), resuspended in 0.3 mg/mL BSA in ultra pure water and homogenized by sonication. 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 (BEL) (Biomol, USA), 300 μg of protein from diluted homogenates, and 0.075 μCi of ^{14}C-PC. After 15 min incubation at 37 °C, the reactions were interrupted by adding a mixture of HCl–isopropanol (1:12, v/v). The released [l-^{14}C] arachidonyl was extracted and 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

Table 1
Clinical and demographic data of patients with Temporal Lobe Epilepsy with Mesial Temporal Sclerosis with and without psychosis.

	Patient	Age (yrs)	Lateralization	Age of onset yrs	Duration yrs	Seizure type	Frequency of seizures	Current AEDs	Antipsychotic Drugs
With Psychosis	F	49	Left	1	44	SPS/CPS/GTC	3/month	PHT/LMT/CLZ	Risperidone 4 mg/d
	M	50	Right	21	22	SPS/CPS/GTC	12/month	CBZ/CLB	Risperidone 6 mg/d
	M	40	Right	16	16	SPS/CPS/GTC	2/month	CBZ	Risperidone 1 mg/d
	F	48	Left	13	31	SPS/CPS	4/month	CBZ/VPA	Trifluoperazine 15 mg/d
	M	19	Left	4	13	SPS/CPS	1/month	CBZ/TPM/CLB	Risperidone 4 mg/d
	F	48	Left	7	38	SPS/CPS/GTC	3/month	CBZ	Risperidone 4 mg/d
	M	55	Right	16	34	SPS/CPS	3/month	CBZ/LTG	
Means	4M/3F	44.14 ± 1.94	4L/3R	11.1 ± 7.3	28.3 ± 11.6		4.0/month		
Without Psychosis	M	35	left	5	24	SPS/CPS	2/month	CBZ/VPA/CLB	
	F	62	right	31	27	CPS/GTC	2/month	OXC/CLB	
	M	22	right	10	9	SPS/CPS		CBZ/PB	
	F	30	left/right	15	12	SPS/CPS/GTC	12/month	CBZ/CLB	
	F	44	right	25	15	SPS/CPS/GTC	12/month	CBZ	
	F	55	right	8	40	SPS/CPS/GTC	8/month	PHT/TPM/PB/CLB	
	F	36	right	21	13	SPS/CPS/GTC	1/month	CBZ/CLB	
	M	26	left	4	19	SPS/CPS	30/month	GBP/CBZ/VPA/CLB	
	M	30	right	3	24	CPS/GTC	1/month	CBZ/PB	
Means	4M/5F	37.79 ± 13.4	3L/6R/1LR	13.6 ± 10.1	20.3 ± 9.6		8.5/month		

SPS, simple partial seizures; CPS, complex partial seizures; GTC, generalized tonic clonic seizures; AED, anti-epileptic drugs; PHT, phenytoin; PB, Phenobarbital; CBZ, carbamazepine; CLZ, clonazepam; CLB, clobazam; TPM, topiramate; OXC, oxcarbazepine; VPA, valproate; yrs, years; LTG, lamotrigine; GBP, gabapentin; M, male; F, female; R, right; L, left.