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SPECIAL ISSUE

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Decreased phospholipase A_2 activity in the brain and in platelets of patients with Alzheimer's disease

Abstract Phospholipase A_2 (PLA_2) is a key enzyme in the metabolism of membrane phospholipids. PLA_2 influences the processing and secretion of the amyloid precursor protein, which give rise to the β-amyloid peptide, the major component of the amyloid plaque in Alzheimer's disease (AD). We investigated the PLA_2 activity in two samples: in post-mortem brains from 23 patients with AD and 20 non-demented elderly controls, and platelets from 16 patients with a diagnosis of probable AD, 13 healthy controls and 14 elderly patients with a major depression. In AD brains PLA_2 activity was significantly decreased in the parietal, and to a lesser degree in the frontal, cortex. Lower PLA_2 activity correlated significantly with an earlier onset of the disease, an earlier age at death and higher counts of neurofibrillary tangles and senile plaques. In platelets PLA_2 activity was also significantly reduced in the AD group as compared with healthy and depressed controls. The reduction of the enzyme activity in platelets correlated with an early disease onset and with the severity of cognitive impairment, indicating a relationship between abnormally low PLA_2 activitiy and a more severe form of the illness. The present results provide new evidence for a disordered phospholipid metabolism in AD brains and suggest that reduced PLA_2 activity may contribute to the production of amyloidogenic peptides in the disease. Further studies are needed to examine whether PLA_2 activity in platelets may be useful as a peripheral marker for a subgroup of patients with AD.

Key words Alzheimer's disease · phospholipase A_2 · Brain phospholipids · Platelets · Amyloid precursor protein · β-amyloid

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Introduction

Alterations in the processing of the amyloid precursor protein (APP) may give rise to the β-amyloid peptide (βA), the major component of the amyloid plaque in Alzheimer's Disease (AD). Phospholipase A_2 (PLA_2) is a key enzyme in the metabolism of membrane phospholipids, cleaving the *sn*-2-fatty acid ester to produce lysophospholipids and free fatty acids. Emmerling et al. (1993) reported that the inibition of PLA_2 decreased the carbachol-stimulated secretion of APP from cells transfected with the human m1-muscarinic receptor, whereas the activation of PLA_2 increased APP secretion. Experimental evidence suggests that increased APP secretion by cells reduces the production of amyloidogenic peptides (Caporaso et al. 1992).

In light of this evidence, we investigated the activity of PLA_2 in brain tissue and in platelets from patients with AD compared with non-demented controls. Patients with AD showed a significant reduction in enzyme activity in both tissues. The data presented here on brain tissue have been reported previously (Gattaz et al. 1995 a).

Methods

Brain samples

Autopsied brain samples of parietal and frontal cortex (Brodman areas 7 and 32, respectively) were obtained from the Medical Research Council Alzheimer's Disease Brain Bank, Institute of Psychiatry, London. Samples from 23 cases of neuropathologically confirmed AD (7 males and 16 females; mean age ± SD 81.0 ± 7.5 years) and 20-non demented controls (10 males and 10 females; mean age ± SD 75.6 ± 9.8 years) were investigated. The degree of dementia was assessed within 12 months before death in all but 2 AD patients with the Mini-Mental State Examination (MMSE; Folstein et al. 1975). The causes of death were similar in patients with AD and controls and were usually due to terminal circulatory or respiratory failure. In patients with AD the means ± SD for the age at disease onset was 72.9 ± 6.9 years, the duration of illness 9.5 ± 5.8 years and the MMSE score 3.1 ± 4.9.

Histological counts were obtained from 16 patients with AD. Histological and counting methods for senile plaques (SPs) and

Table 1 Demographic and neurochemical data in Alzheimer's disease patients and non-demented controls (means ± SD). PLA_2 activity is given in pmol/mg per 45 min arachidonic acid

	Alzheimer (n = 23)	Controls (n = 20)
Age	81.0 ± 7.5*	75.6 ± 9.8
Gender	7 M; 16 F	10 M; 10 F
Post-mortem time (h)	30.7 ± 14.5	38.1 ± 11.1
PLA_2 parietal	27.4 ± 20.2**	43.4 ± 23.8
PLA_2 frontal	26.9 ± 16.4*	37.9 ± 19.8

*$P < 0.10$
**$P < 0.005$ (compared with controls)

Table 2 Age, gender and PLA_2 activity (in pmol/mg protein/min arachidonic acid) in patients with AD, healthy and psychiatric controls (means ± SD)

	Alzheimer (n = 16)	Healthy controls (n = 13)	Major depression (n = 14)
Age	70.2 ± 11.3	62.6 ± 9.7	62.3 ± 12.2
Gender	4 M; 8 F	9 M; 4 F	6 M; 8 F
PLA_2 activity	14.3 ± 4.6*	19.4 ± 6.4	21.3 ± 6.0

*$P < 0.03$ compared with healthy controls and $P < 0.002$ compared with depressed controls

neurofibrillary tangles (NFTs) have been described elsewhere (Förstl et al. 1992). The mean counts/mm^2 ± SD (range) were: SPs parietal 5.0 ± 6.3 (0–22), SPs frontal 4.1 ± 7.2 (0–27), NFTs parietal 4.7 ± 4.6 (0.4–18) and NFTs frontal 5.9 ± 7.6 (0.1–24).

The demographic and biochemical data and the post-mortem interval are given in Table 1. There was a trend for the AD group to have a higher age at death than the control group ($P < 0.10$). The difference in post-mortem interval, with shorter mean time from death to storage in the AD group, did not reach statistical significance ($P = 0.15$).

Platelets

We determined the platelet PLA_2 activity in 16 patients with a "probable" AD (NINCDS-ADRDA criteria) as compared with 13 healthy controls and to 14 psychiatric patients with a major depression (Table 2). There were no significant differences between the three groups regarding age and gender distribution. In patients with AD the cognitive performance was assessed with the CAMCOG and the MMSE.

The methods for tissue preparation and determination of PLA_2 activity have been described in detail elsewhere (Gattaz et al. 1995 a and b for brain and platelets; respectively).

Data were analysed by non-parametric tests (Mann-Whitney, Wilcoxon and Spearman correlation coefficients = r_s).

Results

Brain tissue

The AD group showed significantly lower parietal PLA_2 activity (p-PLA_2) than controls ($P < 0.005$). Frontal PLA_2 activity (f-PLA_2) was also reduced in the AD group, but the difference failed to reach statistial significance ($P = 0.051$; Table 1). There was a significant correlation between p-PLA_2 and f-PLA_2 in patients with AD ($r_2 = 0.55$, $P < 0.005$), but not in controls ($r_s = 0.28$, n.s.).

In the AD group both p-PLA_2 and f-PLA_2 correlated positively with age at death ($r_s = 0.37$, $P < 0.05$ and $r_s = 0.25$, n.s.). In controls the correlations with age were negative, but non-significant. Because patients with AD were slightly older than controls ($P < 0.10$). PLA_2 values were corrected for age by the regression coefficient in patients and controls for further comparisons and partial correlations. Age correction increased the significance of the difference for p-PLA_2 ($P < 0.001$) and the decrement of f-PLA_2 in the AD group became significant ($P < 0.05$).

Both p-PLA_2 and f-PLA_2 correlated positively with age of onset ($r_s = 0.60$, $P < 0.005$ and $r_s = 0.58$, $P < 0.005$, respectively). F-PLA_2 correlated negatively with counts of NFTs ($r_s = -0.56$, $n = 16$, $P < 0.01$) and SPs ($r_s = -0.47$, $n = 16$, $P < 0.05$). No significant correlations were found between p-PLA_2 and histological counts.

No correlations were found between both p-PLA_2 and f-PLA_2 and Mini-Mental State scores, duration of illness and post-mortem interval. No significant differences were found between p-PLA_2 and f-PLA_2 within the AD group or within the control group (Table 1). Within both groups there were also no differences in the enzyme activity between males and females.

Platelets

Platelet PLA_2 activity was significantly reduced in patients with AD as compared with healthy controls ($P < 0.03$) and to patients with major depression ($P < 0.002$; Table 2). The reduction in enzyme activity correlated with an early onset of illness ($r_s = 0.43$, $P < 0.10$) and with the cognitive impairment in the CAMCOG ($r_s = 0.55$, $P < 0.05$). The AD patients with an MMSE score lower than 10 (median) showed significantly lower PLA_2 activity (11.8 ± 3.1) than patients with an MMSE score higher than 10 (16.2 ± 4.6, $P < 0.05$).

Discussion

Reduced PLA_2 activity in the brain suggests a decreased breakdown of membrane phospholipids by PLA_2 in patients with AD. The degree of the PLA_2 reduction was related to the severity of the pathological process, because in our AD sample the low enzyme activity was correlated with an earlier onset of the disease, an earlier age at death and higher NFTs and SPs numbers.

The hypothesis of a decreased breakdown of membrane phospholipids in AD is supported by the results of an in vivo ^{31}P NMR spectroscopy investigation of the phosphomonoesters and phosphodiesters in patients with AD (Brown et al. 1989). Phosphomonoesters are the precursors and phosphodiesters are the breakdown products of membrane phospholipds in the brain. Patients with AD showed increased phosphomonoesters and phosphomonoesters/phosphodiesters ratios in the temporoparietal region. Reduced PLA_2 activity, as demonstrated in our study, may contribute to this increment of phosphomonoesters in AD.

In the brain docosahexanoate (22:6, n-3) is one of the principal free fatty acids released by the deacylation of membrane phospholipids through PLA_2 (Bazan et al. 1986). Skinner et al. (1989) found a marked reduction in the proportion of docosahexanoate in the parietal cortex (but not in the frontal cortex) from patients with AD, which is compatible with reduced PLA_2 as observed in the present study. Changes in the activity of other enzymes related to the membrane phospholipid turnover have already been described in AD brains, such as increased lipases and lysophospholipase activity (Farooqui et al. 1990) and decreased phospholipase D activity (Kanfer et al. 1986). The present finding of reduced PLA_2 activity provides new evidence for a disordered membrane phospholipid metabolism in AD.

A characteristic neuropathological finding in AD is the deposition of fibrillar aggregates of the β-amyloid protein, a 39–43 amino acid peptide produced by the cleavage of a large, membrane-bound amyloid precursor protein (APP; Dyrks et al. 1988). Emmerling et al. (1993) reported that the inhibition of PLA_2 decreased the carbachol-stimulated secretion of APP from cells transfected with the human m1-muscarinic receptor, whereas the activation of PLA_2 increased APP secretion. Experimental evidence suggests that increased APP secretion by cells reduces the production of amyloidogenic peptides (Coporaso et al. 1992). Conversely, it is conceivable that the reduction of APP secretion, as caused by decreased PLA_2 activity, may represent one possible route for amyloidogenesis in AD. This assumption is supported by the correlation between the reduction of PLA_2 activity and the counts of senile plaques.

Because PLA_2 activity is under genetic control (Sharp et al. 1991), it is not unlikely that the enzyme activity in the brain is related to the activity in blood cells. To test this assumption we investigated the enzyme activiy in platelet membranes from patients with AD compared with healthy and psychiatric controls. Platelets are interesting peripheral models in AD research because they contain and secrete APP (Smith et al. 1990). Our finding of reduced PLA_2 activity in platelets is consistent with the results in brain tissue. In both studies decreased PLA_2 activity was related to a more severe form of AD (early onset, more severe cognitive impairment and higher number of plaques and tangles). Moreover, reduced platelet PLA_2 activity was specific for AD as compared with age-matched psychiatric controls. Further studies should clarify the potential importance of PLA_2 activity in platelets as a peripheral marker for a subgroup of patients with AD.

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