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Phospholipid-Metabolizing Enzymes in Alzheimer's Disease: Increased Lysophospholipid Acyltransferase Activity and Decreased Phospholipase A_2 Activity

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Abstract: Damage to brain membrane phospholipids may play an important role in the pathogenesis of Alzheimer's disease (AD); however, the critical metabolic processes responsible for the generation and repair of membrane phospholipids affected by the disease are unknown. We measured the activity of key phospholipid catabolic and anabolic enzymes in morphologically affected and spared areas of autopsied brain of patients with AD and in matched control subjects. The activity of the major catabolic enzyme phospholipase A_2 (PLA_2), measured in both the presence and absence of Ca^{2+}, was significantly decreased (−35 to −53%) in parietal and temporal cortices of patients with AD. In contrast, the activities of lysophospholipid acyltransferase, which recycles lysophospholipids into intact phospholipids, and glycerophosphocholine phosphodiesterase, which returns phospholipid catabolites to be used in phospholipid resynthesis, were increased by ~50–70% in the same brain areas. Brain activities of enzymes involved in de novo phospholipid synthesis (ethanolamine kinase, choline kinase, choline phosphotransferase, phosphoethanolamine cytidylyltransferase, and phosphocholine cytidylyltransferase) were either normal or only slightly altered. The activities of PLA_2 and acyltransferase were normal in the degenerating cerebellum of patients with spinocerebellar atrophy type 1, whereas the activity of glycerophosphocholine phosphodiesterase was reduced, suggesting that the alterations in AD brain were not nonspecific consequences of neurodegeneration. Our data suggest that compensatory phospholipid metabolic changes are present in AD brain that reduce the rate of phospholipid loss via both decreased catabolism (PLA_2) and increased phospholipid resynthesis (acyltransferase and glycerophosphocholine phosphodiesterase). **Key Words:** Phospholipase A_2—Lysophospholipid acyltransferase—Glycerophosphocholine—Phosphodiesterase—Choline kinase—Phosphocholine cytidylyltransferase—Choline phosphotransferase—Alzheimer's disease.
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Several lines of evidence suggest that phospholipid metabolism is abnormal in the brain of patients with Alzheimer's disease (AD). Thus, in vitro ^{31}P magnetic resonance spectroscopy and brain biochemical studies have revealed decreased levels of one or more classes of brain phospholipids in cerebral cortical areas of postmortem AD brain (Ellison et al., 1987; Perry et al., 1987; Stokes and Hawthorne, 1987; Brooksbank and Martinez, 1989; Nitsch et al., 1992; Kienzl et al., 1993; Ginsberg et al., 1995; Wells et al., 1995), with concomitant increases in the abundance of glycerophosphodiester phospholipid breakdown products, such as glycerophosphocholine and glycerophosphoethanolamine (Pettegrew et al., 1988; Blusztajn et al., 1990; Nitsch et al., 1992; Smith et al., 1993; Klunk et al., 1996). Altered phospholipid synthesis in AD is suggested by increased levels in vivo and in vitro of phosphomonoester intermediates of de novo phospholipid synthesis in the cerebral cortex of both early- and late-stage AD patients (Pettegrew et al., 1988, 1995; Smith et al., 1993; Cuenod et al., 1995; Klunk et al., 1996). Moreover, preliminary data suggest a beneficial therapeutic effect of CDPcholine in the disorder (Cacabelos et al., 1996), a compound that, in addition to promoting acetylcholine synthesis, also facilitates the formation of neural membrane phospholipids (López-Coviella et al., 1995). These findings may be related to the experimental observations that in AD the β-amyloid protein may induce damage to the cell membrane via activation of membrane phospholipases (Kanfer et al., 1996; Lehtonen et al., 1996).

In principle, altered activities of the enzymes that make up the phospholipid degradative and synthetic pathway may be responsible for abnormal levels of

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Abbreviations used: AD, Alzheimer's disease; GPCPD, glycerophosphocholine phosphodiesterase; PCCT, phosphocholine cytidylyltransferase; PECT, phosphoethanolamine cytidylyltransferase; PLA_2, phospholipase A_2; SCA-1, spinocerebellar ataxia type 1.

phospholipids and their metabolites in AD brain. Membrane phospholipid breakdown can be initiated by phospholipase A_2 [PLA_2; a family of enzymes including calcium-stimulated and -independent enzymes (Mayer and Marshall, 1993), with both forms being found in brain (Rordorf et al., 1991; Hirashima et al., 1992)], which catalyzes the removal of fatty acid residues esterified at the *sn*-2 position of the phospholipid molecule to form toxic lysophospholipids and free fatty acids. Lysophospholipids formed in this reaction are metabolized primarily by either lysophospholipase, which removes the remaining fatty acid residue forming a glycerophosphodiester such as glycerophosphocholine, or lysophospholipid acyltransferase (acyltransferase), which esterifies fatty acids to the lysophospholipid reforming an intact phospholipid. Glycerophosphodiesters are further broken down by diesterases, such as glycerophosphocholine phosphodiesterase (GPCPD), to form, in human brain (Ross et al., 1995*b*), phosphomonoesters such as phosphocholine. De novo synthesis of the two quantitatively major phospholipid classes, phosphatidylethanolamine and phosphatidylcholine, begins with ethanolamine or choline phosphorylation by their respective kinases, followed by reaction of the phosphobases with CTP to form CDPethanolamine or CDPcholine, as catalyzed by phosphoethanolamine cytidylyltransferase (PECT) or phosphocholine cytidylyltransferase (PCCT), respectively. Ethanolamine or choline phosphotransferases (phosphotransferase) then esterify the CDPbases to 1,2-diacyl- or 1-alkyl-2-acyl-glycerol to form phosphatidylethanolamine or phosphatidylcholine, respectively.

Despite the potential clinical and mechanistic importance of phospholipid metabolism in AD, to date there has been no examination of phospholipid synthetic capability, either de novo or via acyltransferase, in AD brain. Furthermore, studies of phospholipid catabolism have been fragmentary in terms of the enzymes and brain regions examined and have yielded largely contradictory results. Thus, activities of PLA_2 and phospholipase D are reported to be either decreased or normal in AD cerebral cortex (Kanfer et al., 1986, 1993; Gattaz et al., 1996), with GPCPD activity both increased (Kanfer et al., 1993) and unaltered (Nitsch et al., 1992). These discrepancies might have arisen from a failure to characterize adequately the human brain enzymes, leading to the use of suboptimal or inappropriate assay conditions.

In the present study, we used the knowledge gained from our recent studies of enzyme characteristics of key phospholipid-metabolizing enzymes in normal human brain (Ross and Kish, 1994; Ross et al., 1995*a,b*, 1997) to examine, in a comprehensive manner, the components of this pathway in AD. Our simultaneous investigation of the 10 key enzymes of phospholipid metabolism in degenerating and morphologically spared brain regions of patients with AD suggests an important involvement of membrane phospholipid metabolism in the disease.

MATERIALS AND METHODS

Patients

Brain tissue [parietal (Brodmann area 7b), temporal (Brodmann area 21), and occipital (Brodmann area 17) cortices, hippocampus (Ammon's horn), and cerebellar cortex] was obtained at autopsy from 10 end-stage clinically demented and histopathologically confirmed patients (five men and five women) with AD and 10 neurologically and histopathologically normal control subjects (five men and five women). The mean ± SEM ages of each group (75 ± 3 years for AD patients and 73 ± 2 years for controls), interval between death and autopsy (10 ± 2 h for AD patients and 10 ± 1 h for controls), and, as a measure of premortem anoxia, tissue pH, measured as described (Mastrogiacomo et al. (1993), in the cerebral cortex (6.3 ± 0.1 for AD patients and 6.2 ± 0.1 for controls) were not significantly different ($p > 0.5$ by two-tailed Student's t test). At autopsy one half brain was fixed in formalin for detailed neuropathological examination as previously described (Bergeron and Pollanen, 1989). The criteria for the diagnosis of AD included the presence of both neuritic plaques and neurofibrillary tangles in hippocampus and neocortex in the absence of other degenerative processes. In the AD group the extent of the neuropathological changes was severe (n = 8) or moderate to severe (n = 2). The cause of death varied among subjects [myocardial infarction (n = 4), pneumonia (n = 4), peritonitis (n = 1), and renal failure (n = 1) in AD cases; myocardial infarction (n = 5), pneumonia (n = 2), artheroembolism (n = 1), pulmonary artherosclerosis (n = 1), and pulmonary embolism (n = 1)].

Cerebellar cortex was also obtained from nine patients, previously described in detail (Robitaille et al., 1995), with genetically confirmed (Orr et al., 1993) spinocerebellar ataxia type 1 (SCA-1) who exhibited atrophy of the cerebellar cortex with moderate to severe neuronal loss and gliosis, and from 11 controls matched with respect to age (37 ± 3 years for SCA-1 patients and 34 ± 1 years for controls), postmortem interval (12 ± 2 h for SCA-1 patients and 11 ± 2 h for controls), and cerebral cortical pH (6.2 ± 0.1 for SCA-1 subjects and 6.3 ± 0.1 for controls).

Chemicals

All radiochemicals were obtained from Amersham (Oakville, Ontario, Canada), New England Nuclear (Toronto, Ontario, Canada), or American Radiolabelled Chemicals (St. Louis, MO, U.S.A.). Other chemicals were purchased from Sigma Chemical Co. (Toronto). TLC plates were Whatman silica gel type LK5D (Chromatographic Specialties; Brockville, Ontario, U.S.A.).

Biochemical analyses

Tissue to be used in enzyme assays was first disrupted by probe sonication in 5 volumes of 50 m*M* HEPES, 1 m*M* EDTA, and 1 m*M* EGTA as previously described (Ross and Kish, 1994). Choline phosphotransferase activity was assayed as described (Cornell, 1992) with some modifications to optimize assay conditions for the human brain enzyme, which had not been characterized previously. Choline phosphotransferase was routinely assayed by incubating 200 μg of homogenate protein at 37°C for 45 min along with 10

TABLE 1. *Summary of assay characteristics*

Enzyme	Substrate and ionic requirements	Product	pH	Protein (μg)	Time (min)
PLA_2	50 μM 1-palmitoyl,2-[^{14}C]arachidonylPE,	Fatty acid	8.5	20	45
Calcium-stimulated	1 mM calcium chloride				
Calcium-independent	50 μM 1-palmitoyl,2-[^{14}C]arachidonylPE	Fatty acid	7.0	20	45
Lysophospholipase	50 μM 1-[^{14}C]palmitoyl lysoPC	Fatty acid	8.0	2	45
Acyltransferase	10 mM 1-palmitoyllysoPC,	PC	6.0	20	5
	100 μM [1-^{14}C]arachidonylPC				
GPCPD	0.5 mM glycerol-3-phospho-[*methyl*-^{3}H]choline,	Phosphocholine	10.5	200	45
	5 mM zinc acetate				
Choline kinase	2 mM [^{14}C]choline, 10 mM ATP	Phosphocholine	8.0	200	45
Ethanolamine kinase	0.5 mM [^{14}C]ethanolamine, 10 mM ATP	Phosphoethanolamine	8.0	100	45
PCCT	2 mM [^{14}C]phosphocholine, 10 mM ATP	CDPcholine	7.5	200	60
PECT	0.5 mM [^{3}H]phosphoethanolamine, 10 mM ATP	CDPethanolamine	7.5	200	60

PC, phosphatidylcholine; PE, phosphatidylethanolamine.

mM 1,2-dioleoyl-*sn*-glycerol, 100 μM cytidine diphospho-[*methyl*-^{14}C]choline (15 Ci/mol), 10 mM magnesium chloride, and 100 mM HEPES (pH 7.5) in a final volume of 50 μl. The reaction was terminated by addition of 500 μl of chloroform/methanol (1:2 vol/vol) followed by sequential addition of 300 μl of chloroform and 500 μl of water. The tubes were vortex-mixed for 1 min and centrifuged, and the aqueous phase was removed. The lower phase was then washed twice with 1 ml of chloroform/methanol/water (3:48:47 by volume), and radioactivity in the lower phase was quantified by liquid scintillation counting. In preliminary experiments the lower phase was dried by vacuum centrifugation, resuspended in a small volume of chloroform/methanol (1:2 vol/vol), and analyzed by TLC on silica gel developed in either chloroform/methanol/water (65:35:4 by volume) or chloroform/methanol/acetic acid/water (75:45:8:2 by volume) (Ross and Kish, 1994). Using either solvent, the radioactive product migrated as a single spot, as assessed by autoradiography, comigrating with that of an authentic phosphatidylcholine standard. Using homogenate preparations of autopsied human temporal cortex, choline phosphotransferase activity was found to possess a pH optimum of 7.5, falling to ~50% of activity recorded using optimal pH at pH 6.5 and pH 8.5 (data not shown). CDPcholine and 1,2-dioleoyl-*sn*-glycerol possessed K_m values of 17 ± 3 μM (mean ± SEM; n = 3) and 1.8 ± 0.2 mM, respectively, for choline phosphotransferase (data not shown). Under the V_{max} conditions routinely used, the rate of phosphatidylcholine formation was linear with respect to time for at least 1 h and with respect to protein up to at least 300 μg of homogenate protein.

Assays for PLA_2, lysophospholipase, acyltransferase, GPCPD, choline kinase, ethanolamine kinase, PCCT, and PECT were carried out as described previously (Ross and Kish, 1994; Ross et al., 1995*a,b*, 1996, 1997). The assay conditions, altered slightly for use in tissue homogenates rather than subcellular fractions, are summarized in Table 1.

RESULTS

All enzyme activities were assayed in homogenate preparations of parietal, temporal, and occipital cortices, hippocampus, and cerebellum of 10 patients with AD and 10 controls matched with respect to age, postmortem interval, pH, and sex.

PLA_2

As compared with the controls, activity of PLA_2, assayed in the presence of Ca^{2+} at pH 8.5, was significantly decreased in AD patients by 41% in the parietal cortex ($p < 0.01$), 38% in the temporal cortex ($p < 0.01$), and 18% in the hippocampus ($p < 0.05$) but was normal in the cerebellum ($p > 0.7$). Calcium-stimulated PLA_2 activity was also reduced by 26% in the occipital cortex, but the reduction was not statistically significant ($p = 0.058$; Fig. 1). Similar decreases in AD brain were observed in PLA_2 activity assayed in the absence of Ca^{2+} at pH 7.0, with activity being reduced by 35% in parietal ($p < 0.01$) and 44% in temporal ($p < 0.05$) cortices, whereas decreases of 22% in the hippocampus ($p = 0.057$) and 17% in the occipital cortex ($p = 0.092$) approached statistical significance.

Kinetic analysis was performed using preparations of parietal cortex from three AD patients possessing low PLA_2 activity and in three randomly chosen controls. The affinity of phosphatidylethanolamine for PLA_2 did not differ significantly ($p > 0.6$ by Student's *t* test) between AD patients and controls (K_m = 8.2 ± 2.1 μM in AD patients vs. 10 ± 3.1 μM in controls when assayed at pH 8.5 in the presence of 1 mM calcium chloride and 16 ± 2.8 μM in AD patients vs. 14 ± 4.6 μM in controls when assayed at pH 7.0 in the presence of 5 mM EDTA), although, as expected, V_{max} was reduced under both assay conditions (V_{max} = 0.32 ± 0.15 nmol/h/mg of protein in AD patients vs. 1.1 ± 0.41 nmol/h/mg of protein in controls when assayed at pH 8.5 in the presence of 1 mM calcium chloride and 0.11 ± 0.07 nmol/h/mg of protein in AD patients vs. 0.33 ± 0.16 nmol/h/mg of protein in controls when assayed at pH 7.0 in the presence of 5 mM EDTA).

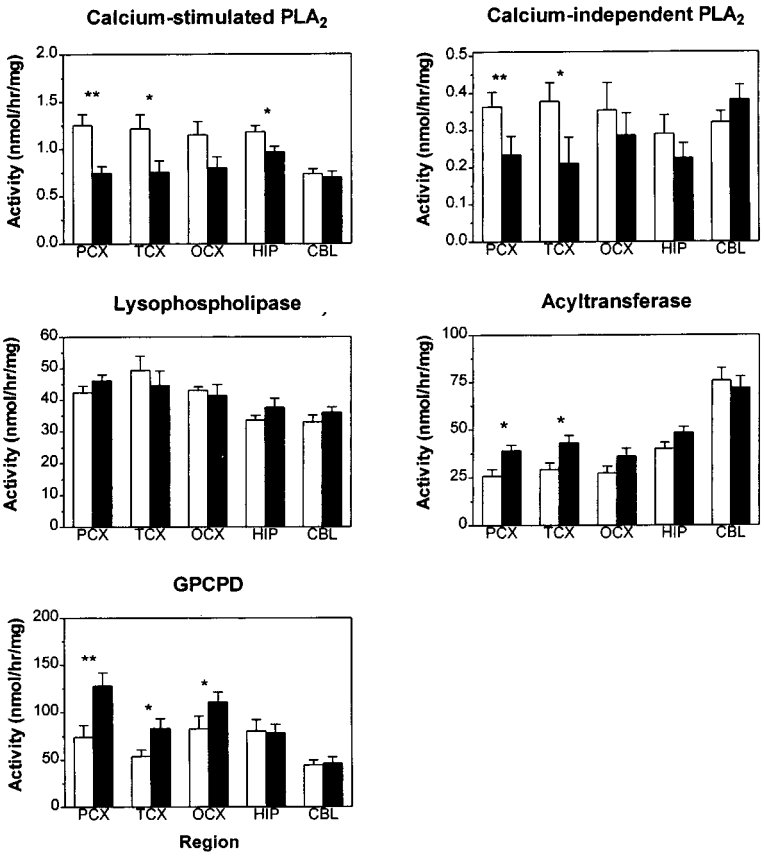

FIG. 1. Activity of enzymes of the phospholipid catabolic pathway along with acyltransferase in patients with AD (■; n = 10) and matched controls (□; n = 10). Data are mean ± SEM (bars) activity values. PCX, parietal cortex; TCX, temporal cortex; OCX, occipital cortex; HIP, hippocampus; CBL, cerebellar cortex. The activity in AD patients was compared with that in controls using a two-tailed Student's *t* test: $^{*}p < 0.05$, $^{**}p < 0.01$.

Acyltransferase, lysophospholipase, and glycerophosphodiesterase

Acyltransferase activity was increased by 48–51% in the parietal ($p < 0.01$) and temporal ($p < 0.05$) cortices of AD patients, with increases approaching statistical significance of 25% in the occipital cortex ($p = 0.112$) and 22% in the hippocampus ($p = 0.059$), but was normal in the cerebellum (Fig. 1). The activity of GPCPD was increased by 73% in parietal ($p < 0.01$), 56% in temporal ($p < 0.05$), and 35% in occipital ($p < 0.05$) cortices of AD patients but was normal in the cerebellum and hippocampus (Fig. 1). In contrast, lysophospholipase activity did not differ significantly between AD and control patients in any brain region (Fig. 1).

De novo pathway

PCCT activity was increased by ~20% in the temporal cortex ($p < 0.05$) and by 18% in the parietal cortex ($p < 0.05$) of AD patients but was normal in the other areas (Fig. 2). Ethanolamine kinase, choline kinase, PECT, and phosphotransferase activities did not differ significantly between AD and control patients in any of the brain regions examined (Fig. 2).

To determine the specificity of the changes observed in AD brain, we used the cerebellar cortex of patients with SCA-1, a severe neurodegenerative disease of the cerebellum and lower brainstem. In contrast to affected regions of AD brain, PLA_2 and acyltransferase activities were normal, and GPCPD activity was reduced, in the cerebellar cortex of the SCA-1 patients (Table 2).

Enzyme activities were not significantly correlated with postmortem interval, age, or tissue pH in any brain region with the exception of lysophospholipase activity, which was significantly correlated with time between death and autopsy in the occipital cortex of control subjects used as a comparison group for the AD patients (Pearson correlation coefficient = 0.82; $p < 0.01$). Lysophospholipase activity was not correlated with this variable in any other brain region of control, AD, or SCA-1 patients.

DISCUSSION

We have analyzed, for the first time in a comprehensive manner, the activities of all the major enzymes of phospholipid metabolism in brain of patients with AD. We found that calcium-stimulated and -independent activities of the major phospholipid-catabolizing enzyme, PLA_2, were decreased, whereas activities of two enzymes involved in the recycling of phospholipid catabolites to reform intact phospholipids, namely, acyltransferase and GPCPD, were elevated relative to age,

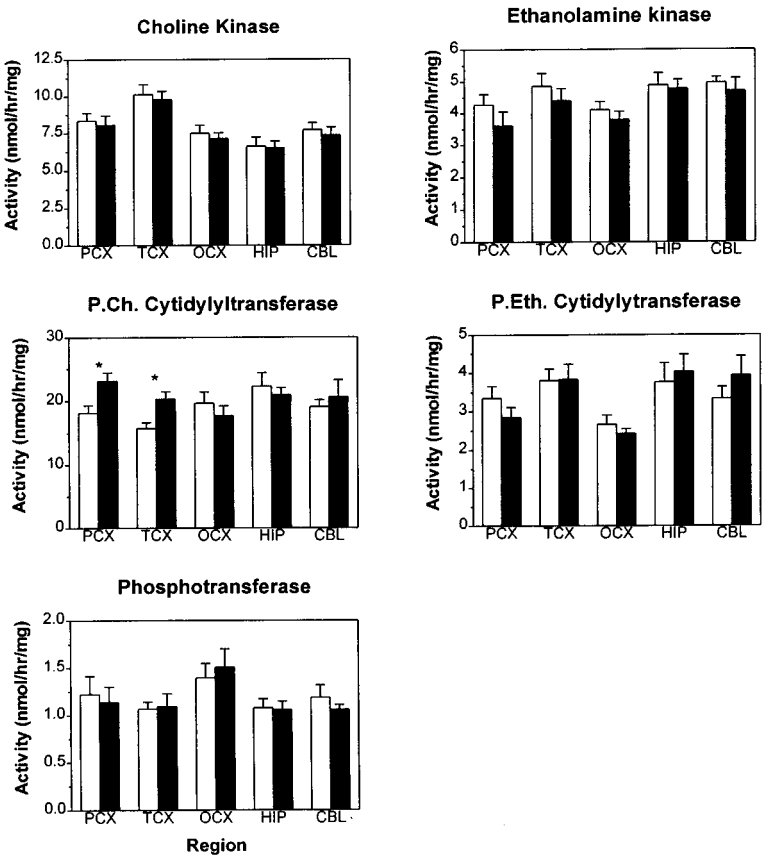

FIG. 2. Activity of enzymes of the de novo phospholipid synthetic pathway in patients with AD (■; n = 10) and matched controls (□; n = 10). Data are mean ± SEM (bars) activity values. PCX, parietal cortex; TCX, temporal cortex; OCX, occipital cortex; HIP, hippocampus; CBL, cerebellar cortex; P.Ch., phosphocholine; P.Eth., phosphoethanolamine. The activity in AD patients was compared with that in controls using a two-tailed Student's *t* test: $^*p < 0.05$.

postmortem interval, agonal status, and sex-matched controls. These changes were in contrast to normal PLA_2 and acyltransferase activities and reduced GPCPD activity in the cerebellum of patients with SCA-1. Because degenerating areas of the brain of SCA-1 patients are characterized, like brain in AD, by marked neuronal loss and gliosis (Robitaille et al., 1995), it appears unlikely that the alterations in phospholipid-metabolizing enzymes in AD occur purely as a nonspecific consequence of the neurodegenerative process. In addition, our observations that the activity of each enzyme in biopsy samples of human brain is not significantly different from that in ~10-h postmortem autopsy samples (Ross and Kish, 1994; Ross et al., 1995*a,b,* 1997), as well as our finding that the activities of the enzymes examined are not correlated with either an index of agonal status (brain pH) or postmortem interval, make it unlikely that pre- and/or postmortem factors have had a significant influence on enzyme activity.

Catabolic enzymes: decreased PLA_2 and increased GPCPD activity may represent compensatory changes

The activity of PLA_2 was reduced in cerebral cortex and hippocampus of AD patients, whereas lysophospholipase activity was normal. The reduction in cerebral cortical PLA_2 activity in AD is in agreement with the observation of Gattaz et al. (1996), who reported a similar magnitude of reduction in calcium-stimulated PLA_2 activity in AD parietal and frontal cortices of patients with AD. However, our results differ from those of Kanfer et al. (1993) who reported that, in AD, PLA_2 activity, assayed in the absence of Ca^{2+}, was normal in the temporal cortex. The different assay conditions used in the present study (phosphatidylethanolamine substrate at pH 7.0) and Kanfer et al. (1993) (phosphatidylcholine substrate at pH 8.0) to measure calcium-independent PLA_2 activity may have resulted in the detection of different enzyme subtypes and suggest that not all forms of PLA_2 are similarly affected in AD. In our investigation, PLA_2 changes were limited to degenerating areas of AD brain (frontal and parietal cortices and hippocampus). This provides no support for the notion that reduced PLA_2 activity is a systemic deficiency, as was suggested by the observation of decreased platelet PLA_2 activity in AD (Gattaz et al., 1996).

Previous studies of AD have indicated a small to moderate loss of membrane phospholipids in affected brain regions (Ellison et al., 1987; Perry et al., 1987; Stokes and Hawthorne, 1987; Brooksbank and Martinez, 1989; Nitsch et al., 1992; Kienzl et al., 1993;

TABLE 2. *Activities of PLA_2, acyltransferase, and GPCPD in cerebellar cortex of patients with SCA-1 and in healthy controls*

Enzyme	Controls (n = 11)	SCA-1 (n = 9)
PLA_2		
Calcium-independent	0.34 ± 0.02	0.31 ± 0.03
Calcium-stimulated	0.55 ± 0.04	0.60 ± 0.07
Acyltransferase	62 ± 7	72 ± 9
GPCPD	82 ± 9	50 ± 7[a]

Data are mean ± SEM activities, in nmol/h/mg of protein.
[a] $p < 0.05$ by two-tailed Student's t test.

Ginsberg et al., 1995; Wells et al., 1995), accompanied by marked increases in levels of glycerophosphodiester phospholipid catabolites (Pettegrew et al., 1988; Blusztajn et al., 1990; Nitsch et al., 1992; Smith et al., 1993; Klunk et al., 1996). These observations are consistent with an accelerated rate of membrane breakdown in the disease, which might take place as a consequence of phospholipase(s) activation, occurring in response to, for example, activation of excitatory amino acid receptors and/or elevated intracellular Ca^{2+} levels (reviewed by Farooqui et al., 1995). Indeed, stimulation of PLA_2 is associated with increased levels of glycerophosphocholine in both Madin–Darby canine kidney cells and H-*ras* transformed cells (Teegarden et al., 1990; Kwon et al., 1995). Furthermore, because PLA_2 more rapidly hydrolyzes oxidized as compared with undamaged phospholipid molecules (Baba et al., 1993; Van den Berg et al., 1993), the postulated increase in free radical-mediated oxidative damage in AD (reviewed by Olanow, 1993) may also result in increased phospholipid breakdown. Most recently, in vitro studies have suggested that β-amyloid protein may directly activate PLA_2 (Kanfer et al., 1996; Lehtonen et al., 1996) and that PLA_2-derived second messengers may modify the processing of the amyloid precursor protein (Emmerling et al., 1996). With these findings in mind, our observation that the in vitro activity of PLA_2 is *decreased* in degenerating areas of AD brain can be viewed as a compensatory change to slow the rate of phospholipid breakdown. It is possible that increased levels of phospholipid-derived intracellular messengers lead to an altered transcription of PLA_2-encoding genes and/or posttranslational modification of the enzyme leading to reduced activity, although it is presently unclear whether protein levels of PLA_2 are reduced or that the specific activity of the enzyme is lower in AD brain. We do not rule out the possibility that low PLA_2 activity in AD brain might be due to increased levels of an endogenous PLA_2 inhibitor. Our finding of increased GPCPD activity in multiple brain regions confirms and extends the findings of Kanfer et al. (1993), who observed increased activity of this enzyme in the temporal cortex of AD patients, but differs from those of Nitsch et al. (1992), who, in a preliminary study of five cases, reported normal GPCPD activity in parietal, frontal, and primary auditory cortices in AD. Increased GPCPD activity in AD may represent a compensatory mechanism to recycle above-normal levels of glycerophosphodiesters (see Nitsch et al., 1992) into the de novo synthesis pathway, thereby preventing an excessive increase in the abundance of these osmotically active compounds.

Enzymes involved in phospholipid synthesis: increased acyltransferase activity may result in more rapid recycling of lysophospholipids

In contrast to the catabolic pathway, activities of those enzymes involved in de novo phospholipid synthesis were either normal or showed only modest alterations in AD. Such observations are consistent with the notion that decreased PLA_2 activity occurs in response to increased phospholipid breakdown occurring in vivo because, if the overall rate of phospholipid catabolism were actually reduced as a primary event, one might have expected the activity of the synthetic enzymes to be similarly decreased. Indeed, we actually detected a small increase in the activity of PCCT, but not PECT, in frontal and parietal cortices of AD patients. Potentially, reduced levels of the methyl donor *S*-adenosylmethionine in AD (Morrison et al., 1996) may diminish the rate of phosphatidylcholine formation via methylation of phosphatidylethanolamine, resulting in activation of de novo phosphatidylcholine synthesis through PCCT.

Investigations using in vivo and in vitro ^{31}P magnetic resonance spectroscopy have indicated that levels of brain phosphomonoesters, specifically phosphocholine, are increased in AD (Pettegrew et al., 1988, 1995; Smith et al., 1993; Cuenod et al., 1995; Klunk et al., 1996). However, our finding of increased PCCT activity might have been expected to result in *reduced* levels of phosphocholine. Thus, it is unlikely that the elevated phosphomonoester levels in AD derive from changes in the activity of the synthetic enzymes. More likely, as suggested by Kanfer et al. (1993), an elevated rate of conversion of glycerophosphocholine into phosphocholine in AD brain, due to increased GPCPD activity, may be responsible.

Although de novo synthetic capability appears largely normal in AD, activity of acyltransferase, a synthetic enzyme that recycles lysophospholipids into intact phospholipids, was markedly increased. Increased acyltransferase activity in AD, in a similar manner to that of reduced PLA_2 activity, may represent a compensatory measure that serves to reduce the rate of phospholipid loss. Thus, elevated acyltransferase activity could result in a greater proportion of lysophospholipid molecules being reacylated to form intact phospholipids, rather than being further degraded by lysophospholipase. Both in vivo and in vitro animal models of lipid peroxidation have indicated that free radical damage leads to an *inhibition* of brain and reti-

nal acyltransferase activity (Anfuso et al., 1994; Alberghina et al., 1995). However, our observation of increased acyltransferase activity in AD indicates that any brain oxidative stress in this disease was not sufficient to inhibit enzyme activity irreversibly.

In summary, we have obtained evidence of altered phospholipid metabolism in AD and suggest that this may constitute an attempt by the brain to reduce membrane loss. Furthermore, these data support the hypothesis that membrane dysfunction might play an important role in the pathogenesis of AD.

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