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39 Inositol Phospholipid Kinases in Alzheimer's Disease

J. BOTHMER, J. JOLLES, M. MARKERINK AND R. RAVID

INTRODUCTION

There are an increasing number of papers which suggest that membrane phospholipids might be involved in the pathogenesis of Alzheimer's disease (AD). Various papers stress the possible relevance of phosphatidylcholine (1). In addition, an increased degradation of phosphatidylcholine but also phosphatidylethanolamine in AD brain was found (2). Pettegrew & Klunk (3) suggested on the basis of ^{31}P NMR studies that the earliest molecular/metabolic changes in AD result in elevated levels of phosphomonoesters in the neocortex and allocortex, followed by a cortical and subcortical elevation of phosphodiesters later in the course of the disease, which reflects cellular degeneration and death.

Changes in other phospholipids, notably phosphatidylinositol (PI) and its breakdown product myo-inositol have also been found in AD and in normal ageing (4,5). Such findings may have functional significance because the interconversion of PI into phosphatidylinositol phosphate (PIP) and phosphatidylinositol bisphosphate (PIP_2), and the breakdown of the latter substance into diacylglycerol and inositolphosphate, are key processes in neuronal function (6). These findings are of special relevance because of our recent demonstration that AD brain is different from controls in that PI kinase activity but not PIP kinase activity is decreased in four neocortical locations in demented patients (7).

The present study was undertaken to investigate whether inositol phospholipid phosphorylating activity is different in temporal cortex from patients with AD as compared with brains from non-demented control subjects and to determine the possible relevance of the factor 'age'. In addition, kinase activities were measured in blood platelets obtained from AD patients and controls in order to assess peripheral differences between these groups. Enzyme activities were measured rather than phospholipid contents, in order to circumvent the problem that the levels of PIP and PIP_2—and to a lesser extent PI—are dependent upon the post-mortem interval (8).

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MATERIALS AND METHODS

Subjects

For study 1 and 2, brain samples from patients with AD and non-demented controls were obtained from the Netherlands Brain Bank in Amsterdam. In the first study, five patients with AD were taken (3M, 2F; mean age 65.2 years) and five control subjects (3M, 2F; mean age 69 years). For the second study, an age range of 18 AD patients was studied (7M, 11F; mean age 75.0 years; range 55–88 years). All AD patients were characterized by an onset of 75 years or below. The duration of illness did not differ between relatively young (under 80) and very old AD subjects (above 80). The post-mortem interval ranged between 4 and 6 hours; there were no differences between AD patients and controls. The patients with AD were clinically diagnosed as 'probable Alzheimer's disease' (9,10) and this was verified by post-mortem neuropathological examination. Control subjects had no history of dementia or any other neurological or psychiatric disorder.

With respect to experiments with blood platelets (study 3), five patients with AD were studied (3M, 2F; mean age 69.2 years) and five normal elderly controls (3M, 2F; mean age 70.6 years). The patients and controls were individually matched for age and sex. The mean age at onset of first symptoms in the AD patient group was 63.6 years with a maximum of 72 years. Prior to entry in this study, all subjects underwent thorough medical, neurological and psychiatric examinations. All patients met ADRDA-NINCDS criteria for 'probable Alzheimer's disease' (9). Control subjects were healthy and had no history of any neurological or psychiatric disorder.

Preparation of crude enzyme fractions

As to brain dissection, the leptomeninges were removed. Samples were taken from the medial temporal cortex (right hemisphere) measuring approximately 1 cm^3, and stored at −80°C until use. Pieces of approximately 0.5 g were excised from these tissue samples and thawed in a water bath at 0°C (20 min). The tissue was homogenized in medium consisting of 0.32 M sucrose, 1 mM EGTA, 50 mM Tris-HCl (pH 7.4) and centrifuged for 60 min at 100 000*g* (for details see Ref. 7). The resulting membrane-free supernatant was used as the enzyme fraction. With respect to blood platelets, preparation and homogenization were according to Bothmer et al. (11). Briefly, crude cytosolic and salt-solubilized protein fractions were prepared by the addition of an equal volume of ice-cold Tris-buffer (20 mM, 1 mM EGTA, 1 mM DTT, pH 7.4) containing 2 M NaCl to the homogenate. After thorough mixing, the sample was allowed to stand for 1 h at 4°C, and was then spun down at 15 000*g* for 15 min at 4°C. The resulting supernatant was dialysed (1:100) on microdialysis filters against Tris-buffer for 2 h at 4°C.

PI kinase and PIP kinase assay

Inositol phospholipid kinase activity was measured in supernatant fractions of 15 or 30 µl (10 and 20 µg of protein, respectively) in the PI kinase and PIP kinase assay respectively. They were preincubated for 2 min. Lipid precursors (20 µM PI or 20 µM PIP) were solubilized in 0.1% Triton X-100, 50 mM Tris-HCl and 1 mM EGTA (pH 7.4) and added to the incubation mixture 15 s before the phosphorylation reaction was started by addition of ATP. The reaction lasted 1 min. Incubations were performed under the following conditions: 7.5 µM ATP, 2–3 µCi [gamma-^{32}P] ATP (approximately 3000 Ci/mmol), 50 mM Tris-HCl pH 7.4, 10 mM $MgCl_2$, 1 mM EGTA and 0.02% Triton X-100. The reaction was terminated and the extraction and further analysis of the ^{32}P, incorporated into PIP and PIP_2, were performed as described elsewhere (7,12).

RESULTS AND DISCUSSION

In study 1, PI and PIP kinase activities were measured in the temporal cortex of AD patients and age-matched controls (Figure 1). The AD group was characterized by a reduction in [^{32}P]PIP formation by 50% (MANOVA $P < 0.01$), which is consistent with findings in other neocortical regions (7). In contrast, no alterations were found in PIP kinase activity (Figure 1).

In the second study, an age-gradient for AD patients showed no change in PI kinase activity (Figure 2). In contrast, an age-related decrease in PI kinase activity was found in the control group (13). Remarkably, old AD patients (age above 80 years) appear to be characterized by higher PI kinase activities

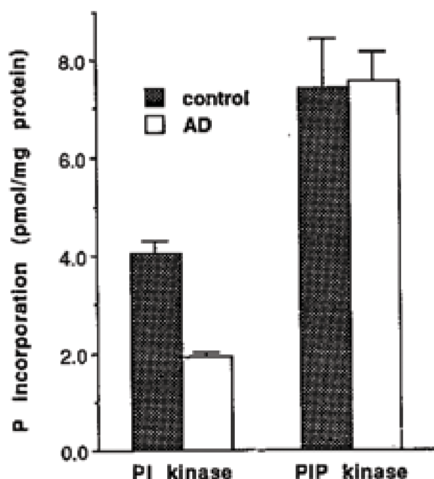

Figure 1. PI kinase and PIP kinase activities in crude cytosolic fractions from temporal cortex of AD brains and age-matched controls, expressed as picomoles of P_i incorporated

compared to young AD patients. This finding is discussed more fully elsewhere (13) in a study where the possible influence of the factor 'onset' was investigated more deeply. In that study, very old AD patients with onset above 75 and age above 80 years had increased PI kinase activity compared with AD patients with earlier onset. Measurements of ^{32}P incorporation into PIP_2 did not show any difference when onset after 75 was compared to earlier onset AD (13). These results are taken to indicate that there is heterogeneity in AD and that relatively young AD patients with early onset are characterized by a pathogenesis different to old AD patients with (very) late onset.

Studies of the biochemical mechanisms underlying the reduced incorporation of ^{32}P into PIP have been performed with early-onset AD patients. It appeared, first, that no inhibiting or stimulating compounds are involved in the mechanism underlying the lower PI kinase activity in AD brains because chelation of ions did not increase PI kinase activity and combination of AD and control cytosolic fractions did not result in stimulation or inhibition of PI kinase activity (14). Secondly, questions as to the type of PI kinase involved are also relevant in view of the fact that different types of PI kinases are known which differ from each other in the phosphorylation position of the inositol ring, in size, in some kinetic properties and also in the physiological functions in the cell (15). The PI kinase that is specifically affected in AD is probably the PI 3-kinase, or type 1 PI kinase, because of (a) the cytosolic localization; (b) the inhibition by the non-ionic detergent Triton X-100; and (c) the insensitivity to adenosine inhibition (14); these are characteristic features

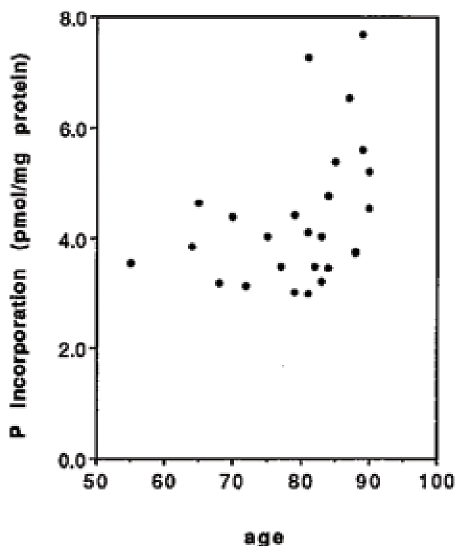

Figure 2. Effect of age on PI kinase activity in cytosolic fractions from temporal cortex samples of AD patients. Data were treated using regression analysis

of type 1 PI kinase. Thirdly, there is growing evidence that AD may be a systemic disease and not just a disease with central changes. For this reason, PI kinase activity was measured in the present study in blood platelets from AD patients and age-matched controls. Platelets were chosen as the peripheral marker because disease-specific abnormalities in the brain, such as those occurring in diseases like Parkinson's disease, Huntington's disease, and depression can be reflected in platelets as has been shown previously (16). Furthermore, platelets were chosen in this study because they contain a complete phosphoinositide metabolizing system with both PI 4-kinase and PI 3-kinase (17). However, despite these indications, the present study revealed no differences in PI kinase activity in platelets from AD patients as compared to controls (Table 1). PI kinase activity was measured in both platelet homogenate and platelet cytosolic/salt-solubilized fractions because of the cytosolic location of the type 1 PI kinase which seems to be specifically affected in AD.

CONCLUSIONS

The change in PI kinase activity measured in this study is physiologically of potential relevance because of the fact that neocortical tissue can be expected to contain cell types which do not degenerate in AD. Thus, if the reduced PI kinase activity is confined to a particular cell type, the effect may be still greater. Furthermore, inositol phospholipids play a key role in impulse initiation and propagation, and thus with intrinsic neuronal and brain functions (see Ref. 18 for review). A large decrease in PIP formation can therefore be expected to have a widespread influence on membrane function, because the pathway which leads to PIP_2 formation is blocked. In addition, the results found with an age gradient of AD patients suggest that PI kinase activity can divide AD patients into subgroups characterized by the age at onset of the disease. Interestingly, there is increasing evidence that early- and late-onset AD differ in neurobiological parameters (19). Even age at death appears to be a relevant variable in the differentiation of AD patients in view of the findings reported by Rossor et al. (20): these authors found clearcut differences in neurotransmitter systems in AD patients younger than 79 years versus AD

Table 1. PI kinase activity (pmol/min mg protein) in homogenates and cytosolic/salt-solubilized protein fractions of blood platelets from patients with probable Alzheimer's disease (AD) and age-matched controls. Values shown are means ± SEM

Group	*n*	Homogenate	Cytosol
Controls	5	8.43 ± 0.81	12.91 ± 0.95
AD	5	9.72 ± 1.36	12.51 ± 0.81

patients dying at older ages. The present findings corroborate these observations for another enzyme system. In addition to age at death, also age at onset of first symptoms appeared to be relevant (13).

With respect to which type of PI kinase is involved, if type 1 PI kinase (PI 3-kinase) is specifically affected in AD, then protein tyrosine kinase activity could be involved. Several receptors that induce cytoskeletal rearrangements in the cell after stimulation, contain intrinsic protein tyrosine kinase activity (15). Activation of these receptors results in stimulation of protein tyrosine activity resulting in the coupling of PI 3-kinase followed by activation of PI 3-kinase activity and cytoskeletal rearrangements. This possible involvement of PI 3-kinase and protein tyrosine kinase in the regulation of cytoskeletal rearrangements in addition to the decline of PI kinase activity and tyrosine kinase activity (21) in AD patients suggest that these enzyme activities may be involved in the cellular pathology of AD. This is because neurofibrillary tangles, which are a prominent feature of AD, are composed predominantly of wrongly metabolized cytoskeletal components.

The present data, and the data presented in our previous publications provide new avenues for investigating the biochemical mechanisms underlying the well-known cellular pathology of AD but also indicate that more attention should be given to the factors of age and onset in future research on Alzheimer's disease.

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