Maastricht University

Phosphatidylinositol kinase is reduced in Alzheimer's disease

Document status and date:
Published: 01/01/1992

DOI:
10.1111/j.1471-4159.1992.tb10981.x

Document Version:
Publisher's PDF, also known as Version of record

Please check the document version of this publication:

• A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
• The final author version and the galley proof are versions of the publication after peer review.
• The final published version features the final layout of the paper including the volume, issue and page numbers.

Link to publication

Rapid Communication

Phosphatidylinositol Kinase Is Reduced in Alzheimer's Disease

J. Jolles, J. Bothmer, M. Markerink, and *R. Ravid

*Department of Neuropsychology and Psychobiology, University of Limburg, Maastricht; and
The Netherlands Institute for Brain Research, Amsterdam, The Netherlands

Abstract: Phosphatidylinositol (PI) kinase and PI phosphate (PIP) kinase activities were measured in postmortem samples of brain tissue from patients with Alzheimer's disease and nondemented control subjects. A membrane-free cytosolic fraction from four neocortical locations, with exogenous inositol lipids as the substrate, was used. Tissue from patients with Alzheimer's disease was characterized by reduced PIP formation; the reduction was 50% in prefrontal cortex, temporal cortex, and parietal cortex and 40% in precentral gyrus. In contrast, no alterations were found in PI bisphosphate formation in these four neocortical locations. The specific changes in PI kinase but not PIP kinase activity suggest that the findings may have functional relevance to the involvement of brain membrane processes in Alzheimer's disease. **Key Words:** Phosphatidylinositol kinase—Phosphatidylinositol phosphate kinase—Brain cortex—Alzheimer's disease. **Jolles J. et al.** Phosphatidylinositol kinase is reduced in Alzheimer's disease. *J. Neurochem.* **58,** 2326–2329 (1992).

Alzheimer's disease (AD) is a neurodegenerative disease and the most common cause of adult-onset dementia. The etiology and pathogenesis are presently not known. Interest in brain membrane phospholipids was aroused several years ago in relation to the hypothesis of a cholinergic dysfunction in AD (see, e.g., Bartus et al., 1982) and the notion that these neurons utilize choline for the formation of the membrane phospholipid phosphatidylcholine (Wurtman et al., 1990). Alterations in phosphatidylcholine and its metabolites and in other phospholipids in AD brains have now been described (see, e.g., Miatto et al., 1986; Pettegrew et al., 1989; Blusztajn et al., 1990). Changes in other phospholipids, notably phosphatidylinositol (PI) and its breakdown product, *myo*-inositol, have also been investigated in AD and in normal aging (Stokes et al., 1983; Stokes and Hawthorne, 1987). Such findings may have functional significance because the interconversion of PI into PI phosphate (PIP) and PI bisphosphate (PIP_2) and the breakdown of the latter substance into diacylglycerol and inositol trisphosphate are key processes in neuronal function (for review, see Abdel-Latif, 1986; Berridge, 1987; Downes and MacPhee, 1990).

The present study was undertaken to investigate whether inositol phospholipid phosphorylating activity is different in brains from patients with AD as compared with brains from nondemented control subjects. Considering the rapid breakdown of inositol phospholipids after death (Lin et al., 1990) and after low-energy periods in vitro (Jolles et al., 1981b), we chose to measure enzyme activity rather than analyze phospholipid contents. Brain samples were obtained by rapid autopsy (within 4–6 h after death). AD and control subjects were matched for age to reduce possibly confounding factors such as duration of disease and interaction between age and disease. Four neocortical locations were compared to detect possible regional variations like those observed in old animals (Bothmer et al., 1990a). PI kinase and PIP kinase were studied in a membrane-free supernatant of AD and control brain, with exogenous PI and PIP as lipid substrates.

MATERIALS AND METHODS

Subjects

Brain samples from five patients with AD (three males and two females; mean age, 65.2 years) and five control subjects (three males and two females; mean age, 69 years) were used in this study (Table 1). Patients and controls were individually matched for age and postmortem interval. Brain tissue was obtained from The Netherlands Brain Bank (The Netherlands Institute for Brain Research). The mean postmortem interval was 5 h for AD subjects and 5 h 15 min for the controls. The patients with AD were clinically diagnosed as "probable Alzheimer's disease" (McKhann et al., 1984; American Psychiatric Association, 1987), and this was verified by postmortem neuropathological examination. Control subjects had no history of dementia or any other neurological or psychiatric disorder.

Brain dissection

Brain specimens for analysis of inositol phospholipid kinase activity were obtained from four neocortical locations

Abbreviations used: AD, Alzheimer's disease; PI, phosphatidylinositol; PIP, phosphatidylinositol phosphate; PIP_2, phosphatidylinositol bisphosphate.

TABLE 1. *Autopsy data of subjects used in the study*

Subject, experimental no.	Patient no.	Age (years)	Sex	Brain weight (g)	Postmortem delay (h min)	pH	Cause of death
Controls							
C1	88–29.4	60	M	1,350	6 00	6.45	Septic shock following aorta valve inplantation
C2	88–194.3	65	M	1,310	5 15	6.18	Heart failure
C3	88–328.1	66	F	1,100	6 15	6.24	Metastases of breast cancer
C4	88–366.0	71	F	1,240	5 05	6.28	Sepsis and cardiogenic shock
C5	88–255.2	73	M	1,340	4 15	6.89	Cerebral infarct without evident cause
C6	88–231.2	73	M	1,410	5 20	6.93	Postoperative heart failure
AD							
A1	89–154	59	F	780	5 05	6.59	Cardiac arrest
A2	89–112	64	M	1,210	5 30	6.21	Collum fracture; died after operation
A3	89–10	68	F	895	5 45	6.52	Bronchopneumonia
A4	88–209	70	M	1,075	4 30	6.38	Cachexia
A5	86–272	65	M	1,360	4 00	—	Cachexia/lung emboli/dehydration
A6	88–216	72	M	1,040	4 45	6.89	Pneumonia and cachexia

Crude enzyme fractions from the frontal superior gyrus and the parietal lobe were prepared from subjects C1–C5 and A1–A5, crude enzyme fractions from the precentral gyrus were prepared from subjects C1–C4 and C6 and A1–A5, and crude enzyme fractions from the medial temporal gyrus were prepared from patients C1–C5 and A2–A6.

in the right hemisphere: (a) the frontal superior gyrus, (b) the precentral gyrus, (c) the medial temporal gyrus, and (d) the parietal lobe. The leptomeninges were removed, and samples were excised. These samples were sealed in plastic, rapidly frozen by immersion in liquid nitrogen, and stored at $-80°C$ until use.

Preparation of crude enzyme fraction

Pieces of ∼0.5 g were excised from the tissue samples and thawed in a water bath at $0°C$ (20 min). The tissue was homogenized in a medium consisting of 0.32 M sucrose, 1 mM EGTA, and 50 mM Tris-HCl (pH 7.4) in a total volume 10 times the brain tissue volume, by 12 up-and-down strokes of a Potter–Elvehjem Teflon–glass homogenizer (radial clearance, 0.125 mm; 700 rpm), followed by homogenization by hand in a glass–glass homogenizer with 3 up-and-down strokes. The homogenate was centrifuged for 60 min at 100,000 g, and the resulting membrane-free supernatant was used as the crude enzyme fraction. This fraction was stored at $-80°C$. There was no decline in enzyme activity after 1 month of storage.

PI kinase and PIP kinase assay

Inositol phospholipid kinase activity was measured as described before (van Dongen et al., 1986; Moritz et al., 1990), with some modifications. The incubation volume (normally 25 μl) was doubled for the PIP kinase assay to reduce interassay variability. Supernatant fractions of 15 or 30 μl (10 and 20 μg of protein, respectively) were preincubated for 2 min. Lipid precursors [20 μM PI or 20 μM PIP (Sigma), solubilized in 0.1% Triton X-100, 50 mM Tris-HCl, and 1 mM EGTA, pH 7.4] were added 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 of [γ-^{32}P]ATP (∼3,000 Ci/mmol; Amersham, U.K.), 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 (Jolles et al., 1981a; Bothmer et al., 1990b). Protein content was determined according to the method of Lowry et al. (1951).

RESULTS

The results of the PI kinase assay in brain samples from patients with AD and controls are shown in Fig. 1A. A significant difference between AD and control patients was found in the four neocortical locations tested [$p < 0.01$ by multivariate analysis of variance (MANOVA)]. The AD group was characterized by a reduction in [^{32}P]PIP formation by
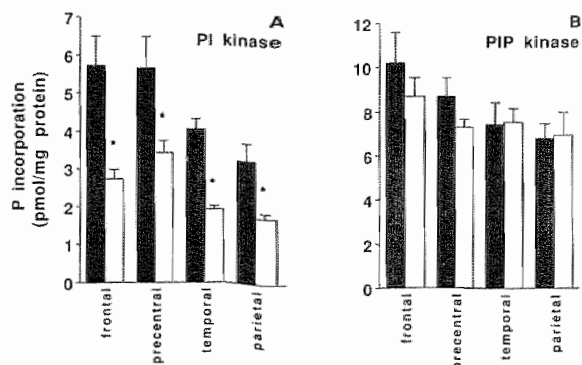

FIG. 1. PI kinase (**A**) and PIP kinase (**B**) activities in crude cytoplasmic fractions from four neocortical regions of AD brains and age-matched controls, expressed as picomoles of P_i incorporated into PI monophosphate per milligram of protein. Data were analyzed by one-way analysis of variance: *$p < 0.01$.

50 (prefrontal cortex, temporal cortex, and parietal cortex) or 40% (precentral cortex). In the control samples, there was a regional variation in PIP formation: [^{32}P]PIP formation was significantly lower in the temporal and parietal cortex than in the prefrontal and precentral cortex [$p < 0.01$ by the procedure of Siegel and Catellan (1988)]. There were no such differences in the AD samples.

The results of the PIP kinase assay with tissue from patients with AD and controls are shown in Fig. 1B. There were no differences between AD and control subjects in any of the four structures; the decreased [^{32}P]PIP$_2$ formation in the four structures did not reach statistical significance.

DISCUSSION

The present study shows that a crude cytosolic PI kinase fraction obtained from neocortical structures taken from brains of patients with AD has ~50% less activity than similar fractions obtained from control brains. This effect appears to be quite specific as there was no decrease in PIP kinase activity. The difference between the two patient groups cannot be ascribed to factors such as age and postmortem delay because the groups had been matched for these factors. The postmortem interval (4–6 h) was very short, which is an advantage considering the rapid breakdown of the various compounds after death. The large SD values in the studies of Stokes and Hawthorne (1987), in which they measured the absolute concentrations of free *myo*-inositol and inositol phospholipid-bound inositol in brains from patients with AD and control subjects, are probably caused by the postmortem interval (7–83 h), as they suggested. In this study we measured enzyme activities and thus circumvented the problem that the levels of PIP and PIP$_2$—and to a lesser extent PI—are dependent on the postmortem interval. A rapid postmortem breakdown of the polyphosphoinositides in rat brain has been described in previous studies (Dawson and Eichberg, 1965; Jolles et al., 1981b); Jolles et al. (1981b) showed that 50% of the labile form of PIP and PIP$_2$ was no longer detectable within 2 min. Likewise, Lin et al. (1990) provided evidence for a very rapid hydrolysis of rat brain PIP$_2$ within minutes after decapitation.

Both the present results and the findings of Stokes and Hawthorne (1987) suggest that the inositol phospholipid system may be involved in the pathogenesis of AD. It is of importance in this respect that other investigators have also found evidence for an involvement of membrane phospholipids and their metabolites in AD. Several research groups, using ^{31}P NMR spectroscopy, have found elevations in levels of phosphomonoesters, e.g., phosphocholine, early in the course of the disease, followed by an elevation in levels of phosphodiesters, e.g., glycerol-3-phosphorylcholine (Barany et al., 1985; Miatto et al., 1986; Brown et al., 1989; Blusztajn et al., 1990). In addition, AD brain appears to be characterized by a decrease in levels of phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and cholesterol (Pettegrew et al., 1989). Apart from phospholipids, phosphorylated proteins are implicated in AD as well. For example, the phosphorylation of protein tyrosine (Shapiro et al., 1991) and of microtubule-associated proteins (Hanger et al., 1991) has received much attention over the last years. It remains to be established whether there is any relation between these changes and the findings reported in the present article.

The change in PI kinase activity is of potential relevance because of the large effect found: A reduction of 50% in enzyme activity is substantial in view of the fact that neocortical tissue can be expected to contain cell types that do not degenerate in AD. Thus, if the reduced PI kinase activity is confined to a particular cell type or types, 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 (for reviews, see Abdel-Latif, 1986; Berridge, 1987). A large decrease in PIP formation can therefore be expected to have a widespread influence on membrane function because the pathway that leads to PIP$_2$ formation is blocked. This may result in a blockade of PIP$_2$ hydrolysis and thus inhibit the formation of the second messengers inositol trisphosphate and diacylglycerol. It is relevant in this respect that reduced numbers of inositol trisphosphate binding sites have been found in the parietal cortex and hippocampus of patients with AD (Young et al., 1988).

With respect to the biochemical mechanisms underlying the reduced incorporation of ^{32}P into PIP, it is possible that the AD brain samples are characterized by a reduced quantity of PI kinase, by changes in enzyme kinetics, or by the presence or absence of cofactors. Questions as to the type of PI kinase involved are also relevant in view of the fact that two different types of PI kinase are known, namely, the kinase that phosphorylates PI at the 4 position of the inositol ring (the so-called "PI 4-kinase") and the one that phosphorylates the 3 position ("PI 3-kinase"). This difference is of importance because the two kinases are implicated in different functions in the cell (Downes and MacPhee, 1990). Involvement of the PI 3-kinase would be of great relevance because this kinase is suggested to be involved in cell growth and in the maintenance of the cytoskeleton (Carpenter and Cantley, 1990). The fact that the present findings were obtained with a membrane-free supernatant suggests that the kinase responsible for these findings might be the PI 3-kinase. Research is in progress to investigate whether the present findings are due to changes in the activity of PI 3-kinase or PI 4-kinase.

The finding that the differences between AD brains and control brains were quite similar for the four structures tested is indicative of a global degeneration of the neocortex. Indeed, the patients were in the terminal stage of the disease, i.e., stage 7 on the Global Deterioration Scale (Reisberg et al., 1983) when most neocortical tissue is known to be involved. Research is planned to compare the tertiary association zones investigated in the present study with the primary sensory cortex and limbic zones and with the cerebellum. A comparison with the latter structure is judged to be especially relevant because this region is not pathologically affected in AD. In addition, other neurological disease groups (Parkinson's disease and multi-infarct dementia) are investigated in order to ascertain the specificity of the observed changes in PI kinase activity. In conclusion, specific alterations in inositol phospholipid phosphorylation have been found in the present study. The findings may be important because of the key role played by inositol phospholipids in neuronal functioning.

REFERENCES

Abdel-Latif A. A. (1986) Calcium-mobilizing receptors, polyphosphoinositides, and the generation of second messengers. *Pharmacol. Rev.* **38**, 227–272.

American Psychiatric Association (1987) *Diagnostic and Statistical Manual of Mental Disorders,* 3rd ed., revised. American Psychiatric Association, Washington, DC.

Barany M., Chang Y., Arus A., Rustan T., and Frey W. H. (1985) Increased glycerol-3 phosphorylcholine in post mortem Alzheimer brain. *Lancet* **1,** 517.

Bartus R. T., Dean R. L. III, Beer B., and Lippa A. S. (1982) The cholinergic hypothesis of geriatric memory dysfunction. *Science* **217,** 408–417.

Berridge M. J. (1987) Inositol triphosphate and diacylglycerol: two interacting second messengers. *Annu. Rev. Biochem.* **56,** 159–193.

Blusztajn J. K., Lopez-Gonzales-Coviella L., Logue M., Growdon J. H., and Wurtman R. J. (1990) Levels of phospholipid catabolic intermediates, glycerolphosphocholine and glycerolphosphoethanolamine, are elevated in brains of Alzheimer's disease but not of Down's syndrome patients. *Brain Res.* **536,** 240–244.

Bothmer J., Markerink M., and Jolles J. (1990*a*) Age related changes in the interconversion of inositol phospholipids in the rat brain cortex, in *From Gene to Man* (Van Bezooijen C. F. A., Ravid R., and Verhofstad A. A. J., eds), pp. 186–189. J. H. Pasmans Publishers, The Hague, The Netherlands.

Bothmer J., Markerink M., and Jolles J. (1990*b*) Phosphatidic acid and polyphosphoinositide formation in a broken cell preparation from rat brain: effects of different incubation conditions. *Neurochem. Int.* **17,** 27–33.

Brown G. G., Levine S. R., Gorell J. M., Pettegrew J. W., Gdowski B. J., Bueri J. A., Helpern J. A., and Welch K. M. A. (1989) In vivo ^{31}PNMR profiles of Alzheimer's disease and multiple subcortical infarct dementia. *J. Neurol.* **39,** 1423–1427.

Carpenter C. L. and Cantley L. C. (1990) Phosphoinositide kinases. *Biochemistry* **29,** 11147–11156.

Dawson R. M. C. and Eichberg J. (1965) Diphosphoinositide and triphosphoinositide in animal tissue: extraction, estimation and changes post-mortem. *Biochem. J.* **96,** 634–643.

Downes C. P. and MacPhee C. H. (1990) *myo*-Inositol metabolites as cellular signals. *Eur. J. Biochem.* **193,** 1–18.

Hanger D. P., Brion J.-P., Gallo J.-M., Cairns N. J., Luthert P. J., and Anderton B. H. (1991) Tau in Alzheimer's disease and Down's syndrome is insoluble and abnormally phosphorylated. *Biochem. J.* **275,** 99–104.

Jolles J., Zwiers H., Dekker A., Wirtz K. W. A., and Gispen W. H. (1981*a*) ACTH1–24 affects protein phosphorylation and polyphosphoinositide metabolism in the rat brain. *Biochem. J.* **194,** 283–291.

Jolles J., Schrama L. H., and Gispen W. H. (1981*b*) Calcium-dependent turnover of brain polyphosphoinositides in vitro after prelabelling in vivo. *Biochim. Biophys. Acta* **666,** 90–98.

Lin T. N., Sun G. Y., Premkumar N., MacQuarry R. A., and Carter S. R. (1990) Decapitation-induced changes in inositol phosphates in rat brain. *Biochem. Biophys. Res. Commun.* **167,** 1294–1301.

Lowry O. H., Rosebrough N. J., Farr A. L., and Randall R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193,** 265–275.

McKhann G., Drachman D., Folstein M., Katzman R., Price D., and Stadlan E. M. (1984) Clinical diagnosis of Alzheimer's disease: report of the NINCDS–ADRDA Work Group under auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* **34,** 939–945.

Miatto O., Gonzalez R. G., Bounanno F., and Growden J. H. (1986) In vitro ^{31}PNMR spectroscopy detects altered phospholipid metabolism in Alzheimer's disease. *Can. J. Neurosci.* **13,** 535–539.

Moritz A., De Graan P. N. E., Ekhart P. F., Gispen W. H., and Wirtz K. W. A. (1990) Purification of a phosphatidylinositol 4-phosphate kinase from bovine brain membranes. *J. Neurochem.* **54,** 351–354.

Pettegrew J. W., Moossy J., Panchalingham K., Martinez J., Strychor S., McKeag G., Brantoover G., and Boller F. (1989) Correlation of phospholipids and senile plaques in Alzheimer's disease. (Abstr) *Neurology* **39** (Suppl 1), 396.

Reisberg B., Ferris S. H., DeLeon M. J., and Crook T. (1983) Clinical presentation, diagnosis and symptomatology of age-associated cognitive decline and Alzheimer's disease, in *Alzheimer's Disease* (Reisberg B., ed), pp. 173–183. The Free Press, New York.

Shapiro I. P., Masliah E., and Saitoh T. (1991) Altered protein tyrosine phosphorylation in Alzheimer's disease. *J. Neurochem.* **56,** 1154–1162.

Siegel S. and Castellan N. J. (1988) *Nonparametric Statistics for the Behavioral Sciences.* McGraw-Hill, New York.

Stokes C. E. and Hawthorne J. N. (1987) Reduced phosphoinositide concentrations in anterior temporal cortex of Alzheimerdiseased brains. *J. Neurochem.* **48,** 1018–1021.

Stokes C. E., Gillon K. R. W., and Hawthorne J. N. (1983) Free and total lipid *myo*-inositol concentrations decrease with age in human brain. *Biochim. Biophys. Acta* **753,** 136–138.

van Dongen C. J., Kok J. W., Schrama L. H., Oestreicher A. B., and Gispen W. H. (1986) Immunochemical characterization of phosphatidylinositol 4-phosphate kinase from brain. *Biochem. J.* **233,** 859–864.

Wurtman R. J., Blusztajn J. K., Ulus I. H., Lopez Gonzalez-Conviella I., Buyukuysal L. R., Growdon J. H., and Slack B. E. (1990) Choline metabolism in cholinergic neurons: implications for the pathogenesis of neurodegenerative diseases, in *Advances in Neurology, Vol. 51: Alzheimer's Disease* (Wurtman R. J., Corkin S. H., Growdon J. H., and Ritter-Walker E., eds), pp. 117–125. Raven Press, New York.

Young L. T., Kish S. H., Li P. P., and Warsh J. J. (1988) Decreased brain [^{3}H]inositol 1,4,5-trisphosphate binding in Alzheimer's disease. *Neurosci. Lett.* **94,** 198–202.