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Drastic reduction of the zinc- and magnesium-stimulated protein tyrosine kinase activities in Alzheimer's disease hippocampus

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Tyrosine phosphorylation of proteins from postmortem hippocampi of five Alzheimer's disease and five control cases have been compared It was found that addition of Zn^{2+} or Mg^{2+} to membrane fractions of control hippocampi caused the phosphorylation of 32-, 40-, 55-, 60-, 80- and 100-kDa proteins or 43-, 55-, 60- and 90-KdA proteins, respectively. The phosphorylation of all these proteins is shown to be drastically reduced in Alzheimer's disease hippocampi Vanadate, an inhibitor of protein tyrosine phosphatases, had no influence on the level of protein phosphorylation. Western blot analysis did not reveal any differences in the anti-phosphotyrosine immunoreactive membrane proteins from Alzheimer's disease and control hippocampi Tyrosine kinase activity of immunoprecipitated $p60^{c\text{-}src}$ from Alzheimer's disease and control hippocampi were the same. In conclusion, the Zn^{2+}- and Mg^{2+}-stimulated tyrosine kinase activities, distinct from activity of $p60^{c\text{-}src}$, are decreased in Alzheimer's disease hippocampus.

Protein phosphorylation, Phosphotyrosine; Zinc, Magnesium, Human hippocampus, Alzheimer's disease

1. INTRODUCTION

Hippocampus is a primary brain region for investigating the synaptic basis of learning and memory in vertebrates [1]. It is an essential component of the memory system damaged in amnesia [2] and one of the brain areas most severely affected by lesion in senile dementia due to Alzheimer's disease [3–5]. Hippocampus accumulates an abundance of zinc [6]. Zinc is actively taken up and released from hippocampal mossy fiber terminals during stimulation of nerve fiber tracts [7–9], but the functional significance of these events is still not clear. It was observed that zinc cations induce tyrosine phosphorylation of $p60^{c\text{-}src}$ and 49-kDa protein in rat hippocampal membranes, therefore, participation of zinc-induced protein tyrosine phosphorylation in hippocampal neurotransmission was proposed [10,11]. In the present work, we have studied tyrosine phosphorylation of proteins from human postmortem hippocampi, comparing non-demented and Alzheimer's disease cases.

2. MATERIALS AND METHODS

2.1. *Materials*

[γ-^{32}P]ATP (3,000 Ci/mmol) was purchased from Claster (Obninsk, Russian Federation). The anti-$p60^{src}$ monoclonal antibody, LAO, was obtained from NCI Repository Microbiological Associates (Bethesda, USA). The phosphotyrosine-specific monoclonal antibody was kindly provided by Dr A I. Kharitonenkov.

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2.2. *Preparation of samples for analysis*

The experiments were carried out on postmortem material. Samples of hippocampus were obtained from male and female patients. Mentally normal controls (5 cases aged 48–82 years, average age 62 years, average postmortem delay 5 4 ± 0 5 h) died of sudden heart failure; patients with Alzheimer's disease (5 cases aged 60–78 years, average age 67 years, average postmortem delay 6 3 ± 0 8 h) died of sudden heat failure and cachexia. The diagnoses of Alzheimer's disease cases were made on the basis of clinical findings and histopathological data Severe clinical symptology in the patients examined in this study and significant brain atrophy were accompanied by an intense formation of tangles and senile plaques, as well as by neuronal loss, especially in the hippocampus. Diffuse plaques and neurofibrillary tangles were detected using Congo red staining.

Pieces of material (approx. 0 5 g) from the central part of hippocampi after coronal section were used for homogenization. Membrane and cytosolic hippocampal fractions were prepared as described before [10], but washing of the membranes with EDTA was omitted.

2.3. *Protein tyrosine phosphorylation assay*

The standard reaction mixture (20 μl) contained 50 mM HEPES-NaOH, pH 6.9, 1 mg/ml of proteins from hippocampal homogenate, membrane or cytosolic fraction, 0.1 mM [γ-^{32}P]ATP (1 Ci/mmol), 0 5 mM $ZnCl_2$ or 10 mM $MgCl_2$ and (or not) 0.1 mM Na_3VO_4. After incubation for 10 min at 30°C the reaction was terminated by adding electrophoresis sample buffer and boiling for 5 min. The samples were subjected to SDS-PAGE on 10% gels followed by alkali treatment of the gels [12], staining, drying and exposure for autoradiography.

Immunoprecipitation and assay of $p60^{c\text{-}src}$ tyrosine kinase activity was as described before [11]

2.4. *Western blot detection of phosphotyrosine-containing proteins*

Samples containing 25 μg of hippocampal membrane proteins were subjected to SDS-PAGE on 10% gels and transferred to nitrocellulose membranes using a Hoefer semi-dry transfer unit TE 70 for 4 h at 120

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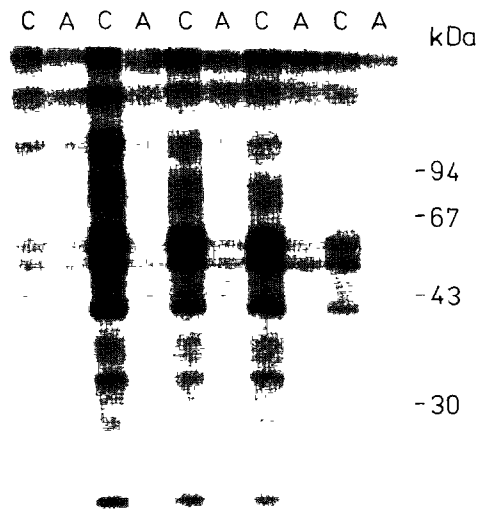

Fig. 1. Zn^{2+}-stimulated protein tyrosine phosphorylation in membrane fractions derived from five control (C) and five Alzheimer's disease (A) hippocampi.

mA. The blots were blocked with Tween-20 and BSA, incubated with phosphotyrosine-specific mouse monoclonal antibody for 2 h at 25°C, followed by anti-mouse IgG peroxidase conjugate (Sigma) for 1 h at 25°C. Detection was made with the use of diaminobenzidine.

3. RESULTS

To compare tyrosine phosphorylation of proteins from Alzheimer's disease and control hippocampi, the samples were incubated with [γ-^{32}P]ATP and $ZnCl_2$ followed by electrophoresis and treatment of the gels with hot alkali. A number of proteins from the control hippocampal membrane fractions were phosphorylated (Fig. 1, lanes C). The phosphorylation of all these proteins in Alzheimer's disease hippocampal membrane fractions was strongly reduced (Fig. 1, lanes A). Protein tyrosine phosphorylation in complete hippocampal homogenates was similar to that in membrane fractions, while phosphorylation of only the 43-kDa protein was observed in cytosolic fractions of both control and Alzheimer's disease cases (data not shown). Mg^{2+}-induced tyrosine phosphorylation of Alzheimer's disease membrane proteins decreased (Fig. 2), similar to that induced by Zn^{2+}. Vanadate, an inhibitor of protein tyrosine phosphatases, had no stimulatory effect on phosphorylation induced by both cations, giving data similar to that illustrated in Figs. 1 and 2. These results indicate a reduction in protein tyrosine kinase activity in Alzheimer's disease hippocampus rather than an increase in protein tyrosine phosphatase activity.

Western blot detection of endogenous phosphotyrosine-containing membrane proteins did not reveal any differences between control and Alzheimer's disease cases (data not shown). Principle phosphotyrosine-containing proteins were in the region of 55–60 kDa. A similar result was obtained earlier in an analysis of antiphosphotyrosine immunoreactivity of particulate fraction proteins from frontal cortex of control and Alzheimer's disease cases [13].

It is known that hippocampus contains a high level of the protein tyrosine kinase, $p60^{c\text{-}src}$ [14], and as such was a reason to suggest that activity of this tyrosine kinase decreases in Alzheimer's disease. We immunoprecipitated $p60^{c\text{-}src}$ from control and Alzheimer's disease hippocampal membranes and measured its activity using enolase as its conventional exogenous substrate (Fig. 3). Excision of the phosphorylated enolase from the gel and counting of its radioactivity revealed no statistically significant differences in the activity between control and Alzheimer's disease hippocampal $p60^{c\text{-}src}$.

4. DISCUSSION

Recently, reduction of the activity responsible for Mg^{2+}/Mn^{2+}-induced tyrosine phosphorylation of the synthetic peptide substrate was demonstrated in Alzheimer's disease frontal cortex [13]. The present work has revealed strong reduction of Zn^{2+}-stimulated and Mg^{2+}-stimulated protein tyrosine kinase activities in postmortem Alzheimer's disease hippocampus. Both cations induce tyrosine phosphorylation of 55- and 60-kDa proteins in control unaffected hippocampus (Figs. 1 and 2, odd lanes). In contrast to the phosphorylation of 32-, 40-, 80- and 100-kDa proteins in the presence of Zn^{2+} (Fig. 1) it is the 43- and 90-kDa proteins that are phosphorylated in the presence of Mg^{2+} (Fig. 2). Furthermore, in the presence of Zn^{2+} there was lower tyrosine phosphorylation in the first control and higher tyrosine phosphorylation in the fifth control (Fig. 1) compared to phosphorylation in the presence of Mg^{2+} (Fig. 2). These data suggest that Zn^{2+} and Mg^{2+} activate different protein tyrosine kinases in human hippocampus,

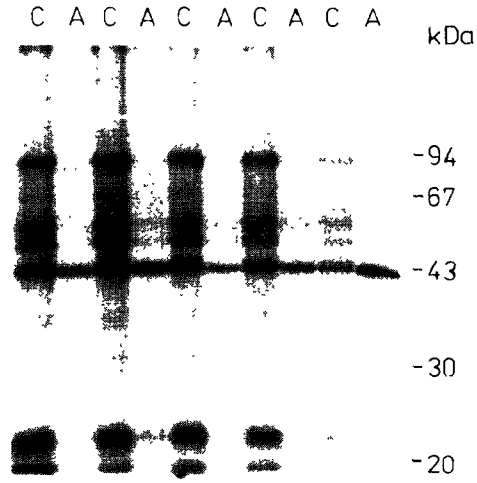

Fig. 2 Mg^{2+}-stimulated protein tyrosine phosphorylation in membrane fractions derived from five control (C) and five Alzheimer's disease (A) hippocampi.

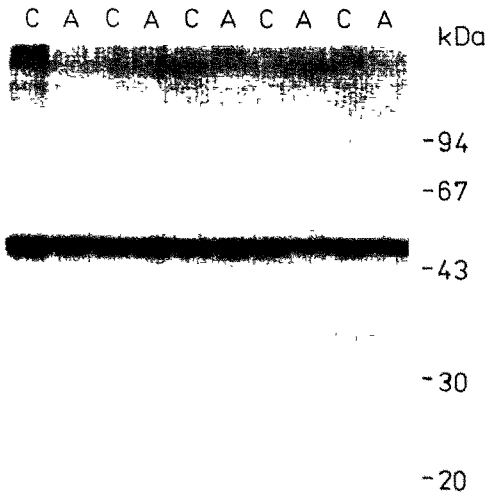

Fig. 3. Tyrosine phosphorylation of enolase by $p60^{c\text{-}src}$ immunoprecipitated from the samples of five control (C) and five Alzheimer's disease (A) hippocampi.

and the activities of both drop in Alzheimer's disease. No change in hippocampal $p60^{c\text{-}src}$ tyrosine kinase activity due to Alzheimer's disease was found (Fig. 3).

The primary experimental model for investigating memory formation is long-term potentiation [1]. Some time ago it was found that long-term potentiation in the hippocampus was blocked by tyrosine kinase inhibitors [15]. Zinc was proposed to participate in hippocampal neurotransmission via protein tyrosine phosphorylation [10]. In the present study we have determined that Zn^{2+} induces tyrosine phosphorylation of at least six proteins from human hippocampus (Fig. 1). Earlier it was observed that zinc cations activate protein tyrosine kinase distinct from $p60^{c\text{-}src}$ which phosphorylates only two rat hippocampal proteins [10,11]. We can speculate that this difference could be a result of a higher declarative memory system organization in human. Reduction of Zn^{2+}- or Mg^{2+}-stimulated protein tyrosine kinase activities in Alzheimer's disease hippocampus indicates a possible connection of neuronal protein tyrosine kinase activity loss to severe memory and intellectual impairment characteristic of Alzheimer's disease. We propose that Zn^{2+}- and Mg^{2+}-activated protein tyrosine kinases distinct from $p60^{c\text{-}src}$ may participate in cellular mechanisms of memory origination in mammalian hippocampus.

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REFERENCES

[1] Bliss, T.V.P. and Collingridge, G.L. (1993) Nature 361, 31–39
[2] Squire, L.R. and Zola-Morgan, S. (1988) Trends Neurosci. 11, 170–175.
[3] Wilcock, G.K and Esiri, M.M. (1982) J. Neurol. Sci 56, 343–356.
[4] Price, J.L., Davis, P.B, Morris, J C and White, D.L (1991) Neurobiol. Aging 12, 295–312.
[5] Armstrong, R.A. (1991) Neurosci Res. Commun 9, 91–97
[6] Frederickson, C.J., Klitenick, M.A., Manton, W.I. and Kirkpatrick, J B (1983) Brain Res 273, 335–339.
[7] Assaf, S.Y. and Chung, S -H. (1984) Nature 308, 734–736.
[8] Howell, G A., Welch, M.G. and Frederickson, C J. (1984) Nature 308, 736–738.
[9] Aniksztejn, L., Charton, G. and Ben-Ari, Y. (1987) Brain Res. 404, 58–64.
[10] Vener, A.V. and Loeb, J. (1992) FEBS Lett 303, 261–264.
[11] Vener, A.V. and Loeb, J. (1992) FEBS Lett 308, 91–93.
[12] Bourassa, C., Chapdelaine, A., Roberts, K D. and Chevalier, S. (1988) Anal. Biochem. 169, 356–362
[13] Shapiro, I.P, Mashiah, E. and Saitoh, T (1991) J Neurochem. 56, 1154–1162
[14] Ross, C.A., Wright, G.E , Resh, M.D. Pearson, C.A and Snyder, S.H. (1988) Proc Natl. Acad. Sci. USA 85, 9831–9835
[15] O'Dell, T.J., Kandel, E R. and Grant, S.G.N. (1991) Nature 353, 558–560.