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The decreased level of casein kinase 2 in brain cortex of schizophrenic and Alzheimer's disease patients

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The content of casein kinase 2 is considerably decreased in ribosome-free extracts of the frontal cortex of schizophrenic and Alzheimer's disease patients in comparison to normal brains as has been demonstrated by means of immunoblotting. The activity of casein kinase 2 towards endogenous substrates and casein is also diminished in the cases of mental pathologies examined. This phenomenon may explain the well-known aberrations in the phosphorylation of structural proteins of human brain which are intrinsic for the mental diseases.

Casein kinase 2; Brain; Schizophrenia; Alzheimer's disease

1. INTRODUCTION

The presence of paired helical filaments in Alzheimer's disease (AD) brains indicates the existence of marked neuronal cytoskeletal pathology, which is accompanied by the severe disturbances in the phosphorylation of tubulin, τ-protein and microtubular-associated proteins (MAPs) [1–5]. The role of casein kinase 2 in the phosphorylation of these proteins is well known [6–8].

Casein kinases 2 are found in all eukaryotic cells studied. All these enzymes (except for casein kinase 2 from yeast and myxomycetes) share a common polypeptide structure and consist of four subunits ($\alpha_2\beta_2$ or $\alpha\alpha'\beta_2$) with α/α' having a molecular mass of 35–44 kDa and β of 24–29 kDa [9,10]. Activity of casein kinase 2 in vitro is not regulated by the known second messengers or covalent modifications. However, administration of different hormones or growth factors to competent cells changes activity and amount of casein kinase 2 via yet unknown mechanisms [10]. These events may lead to variations in the phosphorylation status and activity of numerous protein substrates of casein kinase 2. So the disorders in the activity or expression of this enzyme may cause different cellular aberrations.

In this article we demonstrate that the Alzheimer's disease and schizophrenia are accompanied by the decreased level of soluble casein kinase 2 and alterations in the phosphorylation pattern of some of its endogenous substrates. A part of our results confirm the data of Iimoto et al. [11] who have demonstrated aberrant casein kinase 2 in Alzheimer's disease a few months before.

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2. MATERIALS AND METHODS

Research was carried out on postmortem materials: samples of frontal brain cortex (area 10 by Brodman) were taken from patients aged 50–70 years, who died from sudden heart failure. The postmortem delay was less than 6 h in each case. All material was divided into 3 groups: mentally normal controls (3 cases), schizophrenic patients (5 cases) and Alzheimer's disease patients (3 cases). The value of average age and average postmortem delay were similar for all groups.

After isolation the tissue samples were placed into plastic cryotubes and frozen in liquid nitrogen. Ribosome-free extracts were prepared in standard buffer which contained 10 mM triethanolamine, 10 mM KCl, 0.1 M NaCl (unless otherwise indicated), 5 mM $MgCl_2$, 1 mM EDTA, 6 mM 2-mercaptoethanol, 0.2 mM phenylmethylsulfonyl fluoride, 10% glycerol, pH 7.8.

SDS-electrophoresis was performed according to the method of Laemmli [12], immunoblotting – according to the method of Towbin [13]. The antiserum to casein kinase 2 from calf thymus was kindly provided by Prof. Dahmus (Department of Biochemistry and Biophysics, University of California, Davis, CA).

For isolation of heparin-binding proteins ribosome-free extract of human brain was applied to a heparin-Sepharose column (3–5 ml of the extract to 1 ml of heparin-Sepharose), washed thoroughly with the standard buffer and eluted with 0.6 M NaCl in the standard buffer.

Protein kinase assay was carried out as described earlier [14]. Protein content was measured by the method of Schaffner and Weismann [15].

3. RESULTS AND DISCUSSION

Fig. 1 demonstrates the results of immunoblotting of ribosome-free human brain extracts stained with an-

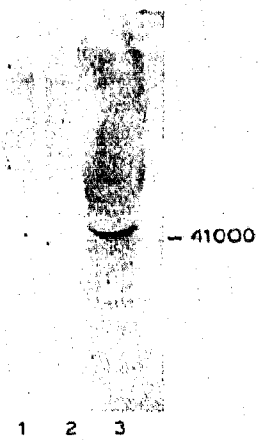

Fig. 1. Immunoblot of human frontal cortex extracts stained with antiserum to casein kinase 2. (1) Alzheimer's disease; (2) schizophrenia; (3) normal brain.

tiserum to casein kinase 2. The single line of 41 kDa (which corresponds to the α-subunit of casein kinase 2) has been detected in all studied extracts of normal cortex. We failed to register β-subunit of the enzyme perhaps due to its low immunogenicity or individual structural peculiarities between species [16]. The line of 41 kDa was absent in all cases examined of schizophrenic and AD brains. It means that schizophrenia and AD are connected by the decreased level of soluble casein kinase 2. Our next step was to examine whether the activity of casein kinase 2 correlates with its low content in mental brains.

It has been shown earlier that casein kinase 2 and some of its endogenous substrates possess polyanion-binding activity and can be isolated on the columns with heparin or poly(U) coupled to Sepharose [14,17]. This fraction of polyanion binding proteins is suitable for measurement of casein kinase 2 activity since it contains no other protein kinases nor phosphatases and proteases ([18,19] and unpublished data). We have isolated heparin binding proteins from normal human brain and from brains of patients with schizophrenia and AD and demonstrated that the polypeptide composition of these protein fractions from normal and schizophrenic brains is almost similar (except for the p150 which is absent in the mental brain) while the heparin binding proteins from AD brain lack a few more polypeptide chains (p105, p98, p36, p35 and p32) (Fig. 2A).

The activity of casein kinase 2 towards endogenous substrates and casein in mental brains is decreased in comparison to the normal brain (Table I) and in AD it is even 1.5 less than in schizophrenia. This result is confirmed by the autoradiography which demonstrates the endogenous substrates of casein kinase 2. As is shown on Fig. 2B, heparin binding proteins from mental brains contain less substrates for the endogenous casein

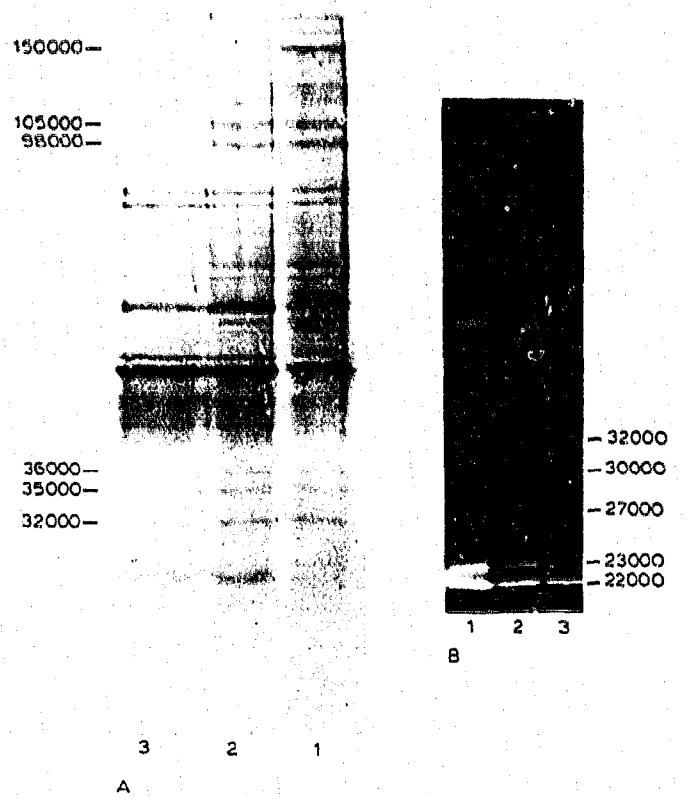

Fig. 2. Electrophoresis of in vitro phosphorylated heparin binding proteins isolated from normal (1), schizophrenic (2) and Alzheimer's disease (3) brains. 20 μg of heparin binding proteins were incubated with 0.1 mM [γ-^{32}P]ATP (250 pm/pm) for 1 h at 22°C in a total volume of 100 μl. (A) The gel was stained with Coomassie blue R-250. (B) Autoradiograph of the dried gel. Exposure time was 14 days.

kinase 2 than the analogous protein fraction from normal brain. However, there are 5 polypeptide chains which are permanently phosphorylated in all cases (p32, p30, p27, p23 and p22).

Thus we have demonstrated the decrease of casein kinase 2 content and activity in the frontal cortex of mental disease patients. Such phenomena may be intrinsic for the aberrations in the phosphorylation of

Table I

Casein kinase 2 activity in normal and mental brains

Brain	Specific phosphorylation of heparin binding proteins (pmol ^{32}P/μg protein)[a]	Specific phosphorylation of casein (pmol ^{32}P/μg casein)[a]
Normal	1.00 ± 0.18	2.83 ± 0.13
Schizophrenic	0.44 ± 0.14	2.09 ± 0.20
Alzheimer's disease	0.32 ± 0.10	1.49 ± 0.18

[a] The mean value was calculated according to Student's criteria with $P < 0.05$

tubulin, MAPs, τ, heparin binding and other proteins and may lead to severe disturbances in brain functioning.

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