BRAIN LEVELS OF CYTOPLASMIC CASEIN KINASE 2 AND ITS SUBSTRATE PROTEINS IN ALZHEIMER'S DISEASE

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Considerable disturbances in the protein phosphorylation system have now been discovered in Alzheimer's disease. For instance, increased phosphorylation of protein P61, abnormal phosphorylation of tau-protein [4, 6], and disturbances of phosphorylation of tubulin and MAP proteins [7, 12] have been demonstrated. Concentrations of two protein kinases, whose role in the phosphorylation of these proteins is sufficiently well known, namely protein kinase C [5] and casein kinase 2 [8], in the cytosol of the nerve cells also are lowered. We also have demonstrated a decrease in the activity and concentration of casein kinase 2 in the frontal cortex of patients with schizophrenia and Alzheimer's disease [3]. Since maximal changes were found in Alzheimer's disease, in the investigation described below our attention was focused on the temporal cortex in Alzheimer's disease, in which the most characteristic disturbances of this disease are located (discovery of senile plaques and neurofibrillary tangles, disappearance of neurons, etc.).

EXPERIMENTAL METHOD

Experiments were carried out on postmortem material: samples of the temporal cortex (area 21) from patients aged 50-70 years, dying from acute heart failure, were taken not later than 8 h after death. The control group consisted of five persons with no mental pathology (average age 64.0 ± 1.8 years, time after death 5.8 ± 0.5 h), and the group of patients with Alzheimer's disease consisted of seven persons (average age 62.8 ± 1.1 years, time after death 6.5 ± 0.8 h). Histological analysis of the cerebral cortex of the patients with Alzheimer's disease, aimed at detecting neurofibrillary tangles, was undertaken by I. G. Makarenko, on the staff of the Neuromorphology Laboratory.

Tissue samples were placed in cryomicroscopy tubes and frozen in liquid nitrogen. Ribosome-free extracts were prepared in standard buffer containing 10 mM triethylamine, 10 mM KCl, 0.1 M NaCl, 5 mM $MgCl_2$, 1 mM EDTA, 6 mM 2-mercaptoethanol, 0.2 mM phenylmethylsulfonyl fluoride, and 10% glycerol, pH 7.8.

SDS-electrophoresis was carried out by Laemmli's method [11] and immunoblotting by Towbin's method [14]. Antiserum to casein kinase from calf thymus was generously provided by Professor Dahmus (University of California, USA).

To isolate heparin-binding proteins the ribosome-free extract of human brain proteins was applied to a column with heparin-sepharose (50 mg extract to 5 ml sepharose), which was washed with standard buffer, after which the bound proteins were eluted with 0.6 M NaCl in the same buffer.

Protein kinase activity was measured by the method described in [10]. The protein concentration was measured by the method of Schaeffner and Weismann [13].

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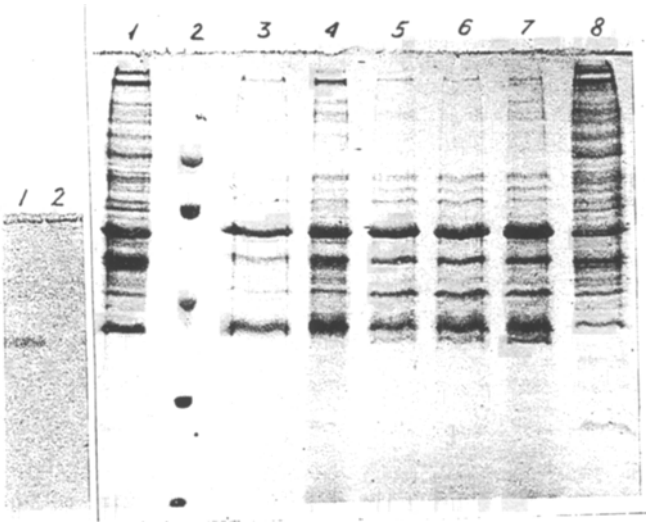

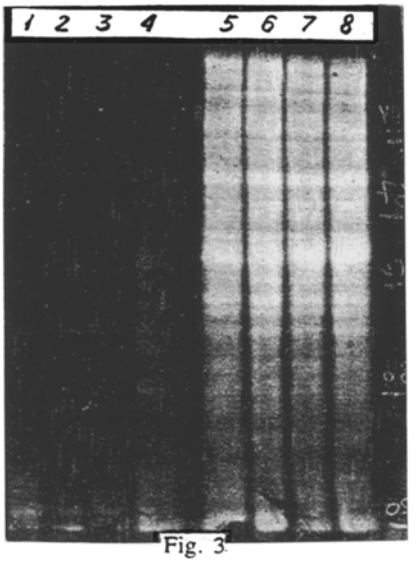

Fig. 3

Fig. 1. Immunoblotting of extracts of human temporal cortex, developed with antiserum to casein kinase 2: 1) brain of mentally healthy persons; 2) brain of patient with Alzheimer's disease.

Fig. 2. Electrophoresis of fractions of heparin-bound proteins: 1, 8) brain of mentally healthy persons, 3-7) brain of patients with Alzheimer's disease, 2) markers of molecular weight (from top to bottom: 20, 30, 43, 67, and 94 kD).

Fig. 3. Autoradiograph of gel after electrophoresis of fractions of heparin-bound proteins: 1-4) brain from patients with Alzheimer's disease, 5-8) brain of mentally healthy persons.

EXPERIMENTAL RESULTS

Like the results obtained on the frontal cortex of psychiatric patients, a considerable decrease in casein kinase 2 concentration was found in the temporal cortex in Alzheimer's disease.

Figure 1 shows the results of immunoblotting of a ribosome-free protein extract from the human temporal cortex, developed with antiserum to casein kinase 2. A line corresponding to 41 kD (the α-subunit of casein kinase 2) was discovered in all extracts from the control human brain. The β-subunit of the enzyme could not be found,

possibly because of its low immunogenicity or individual structural differences between species. In all cases of Alzheimer's disease studied the 41 kD line was absent from the blots, evidence of a marked decrease in the soluble casein kinase content in the brain of these patients. It is to be expected that as a result of this decrease, phosphorylation of the substrate proteins for casein kinase 2 will be disturbed in the patients' brain.

It was shown previously that casein kinase 2 and some of its endogenous substrates possess polyanion-binding activity and can be isolated on a column with heparin or poly(U), attached to sepharose [1, 2]. The fraction of polyanion-bound proteins contained neither other protein kinases nor phosphates and proteases [9, 10]. Electrophoresis of heparin-bound proteins from normal brain and from the brain of patients with Alzheimer's disease is illustrated by the gels in Fig. 2. They show that in Alzheimer's disease a whole series of protein zones is absent from the fraction of isolated heparin-bound proteins, especially in the high-molecular-weight region above 60 kD. The differences we found, incidentally, cannot have been due to the different ages of the subjects whose tissue was investigated post mortem, nor to the time elapsing after death. Besides the difference in the protein spectrum of the fractions, it was also found that the total quantity of heparin-bound proteins isolated from the brain in Alzheimer's disease was reduced by almost half of the normal level: under normal conditions 2.34 ± 0.19 mg of heparin-bound proteins was isolated from 50 mg of the ribosome-free extract, compared with only 1.44 ± 0.11 mg in the case of Alzheimer's disease. We cannot yet explain this decrease, but it may be the result of diminished expression of these proteins in the patients' brain, the result of their redistribution among the cytosol and membrane fractions, and also the result of a change in the properties of the heparin-bound proteins, which may cause a change also in their binding with heparin-sepharose. It must be pointed out that in one patient (Fig. 2, lane 4) the spectrum of heparin-bound proteins closely resembled the normal spectrum. Histological analysis in this case revealed only single neurofibrillary tangles in the cortex of this patient, unlike in the other patients studied.

Casein kinase 2 activity relative to endogenous substrates and to casein in the patients' brain was 2-5 times lower than in the normal brain. It is interesting to note that in patient A2 the level of specific phosphorylation of casein did not differ from normal, but phosphorylation of heparin-bound proteins was significantly depressed. These results are confirmed by those of autoradiography, which demonstrate endogenous substrates of casein kinase 2. As Fig. 3 shows, heparin-bound proteins from the patients' brain contained significantly less of the substrates for endogenous casein kinase 2 than the corresponding fraction from the control brain. Only the four main polypeptide chains with mol. wt. of 75, 50, 37, and 20 kD were phosphorylated in the patients' brain in all cases, but less intensively than in the normal brain.

Thus the level of soluble casein kinase 2 was significantly depressed in the temporal cortex of patients with Alzheimer's disease and the composition of the fraction of heparin-bound proteins was modified and phosphorylation of the proteins contained in this fraction in vitro was reduced. This phenomenon may have a very important effect on normal phosphorylation of the microtubular proteins, namely MAP, tau-protein, and heparin-bound and other proteins, and in turn this may lead to serious disturbances of nerve tissue metabolism in Alzheimer's disease.

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