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An antiserum to the C-terminus of the Alzheimer amyloid precursor recognizes a soluble 70 kDa protein

James Simpson, Christine M. Bladon, Celia M. Yates and Anthony J. Harmar

MRC Brain Metabolism Unit, Department of Pharmacology, University of Edinburgh, 1 George Square, Edinburgh EH8 9JZ, Scotland

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A rabbit antiserum to the C-terminus of the putative brain amyloid precursor was used to probe Western blots of tissue proteins separated by SDS-PAGE. The antiserum specifically labelled a protein of approx. 70 kDa in the Tris buffer-soluble fraction of brain samples from rat, Alzheimer subjects, cases of young and old Down's syndrome, and age-matched controls. The 70 kDa protein was present in low concentrations in human liver and kidney, and was undetectable in human skeletal muscle. The 70 kDa protein may be a metabolite of the amyloid precursor.

Amyloid; Antiserum; Western blotting; Alzheimer-type dementia; Down's syndrome

1. INTRODUCTION

In ATD, and in Down's syndrome cases aged >40 years, abnormal protein deposits (amyloid) are frequently present in brain plaques and blood vessels [1]. The amino acid sequence of the amyloid peptide (A4) has been determined, and oligonucleotide probes used to derive the structures of possible precursors (see [2]). The processing of amyloid precursor proteins (proA4), and the factors influencing the liberation of A4, are largely unknown. We have, therefore, searched for ProA4-related proteins in human and rat brain and in peripheral tissues using an antiserum to the C-terminus of proA4.

2. MATERIALS AND METHODS

Polyacrylamide gradient gels (PAA 4/30) and low molecular mass protein standards were obtained from Pharmacia (Milton Keynes, England).

Correspondence address: J. Simpson, MRC Brain Metabolism Unit, Department of Pharmacology, University of Edinburgh, 1 George Square, Edinburgh EH8 9JZ, Scotland

Abbreviations: A4, amyloid polypeptide; ATD, Alzheimer-type dementia; ProA4, amyloid precursor protein; SDS-PAGE, SDS-polyacrylamide gel electrophoresis

2.1. *Production of antiserum to the C-terminal pentadecapeptide of proA4*

The proA4 peptide H_2N-GYENPTYKFFEQMQN-CO_2H [3] was synthesised by the solid-phase method using Fmoc-*t*-butyl-polyamide chemistry [4]. The peptide was coupled to succinylated thyroglobulin and an antiserum raised in rabbits [5].

2.2. *Preparation of tissue samples*

Human autopsy tissues from neuropathologically confirmed cases of ATD, Down's syndrome and age-matched controls were stored at −70°C; rat brain samples were used immediately after dissection. Homogenates in Tris buffer were separated by centrifugation into soluble and particulate fractions and boiled in SDS and 2-mercaptoethanol [6] in preparation for SDS-PAGE. Rat cerebral grey matter was rapidly homogenised in the presence of SDS and mercaptoethanol, boiled for 5 min, and the supernatant separated by centrifugation for 15 min at 14 000 × *g*. Extracts of human skeletal muscle were prepared as described by Zimmermann et al. [7].

2.3. *SDS-PAGE and Western blotting*

Tissue samples containing 30 μg protein [8] or 3 μl protein standards were separated in Tris/glycine/SDS buffer [6] by SDS-PAGE [9] and electrophoretically transferred to nitrocellulose [6].

2.4. *Identification of proA4-related proteins*

Electroblotted nitrocellulose was incubated overnight at 4°C with the proA4 antiserum (diluted 1:1000), and reactivity to protein bands visualised with biotinylated anti-rabbit Ig (1:100) and streptavidin-biotinylated peroxidase (1:300) with diaminobenzidine as substrate [10]. The specificity of antibody binding to separated proteins was examined using antiserum to which proA4 peptide (300 μg/ml antiserum) had been added, and by

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use of an antiserum raised to a peptide (somatostatin-14) unrelated to proA4 [5]. Protein standards were stained with amido black.

3. RESULTS

When homogenates of human temporal cortex were separated by SDS-PAGE and transferred to nitrocellulose, the antiserum to proA4 stained several protein bands including a strongly stained band at approx. 70 kDa. The 70 kDa protein was not stained by a non-relevant antiserum (section 2.4). When the proA4 antiserum was blocked with the proA4 C-terminal pentadecapeptide, labelling of the 70 kDa protein was abolished. This 70 kDa protein was present in the Tris buffer-soluble fraction of brain homogenates, but very little was detected in the particulate fraction (fig.1).

No marked differences in the staining intensity of the 70 kDa protein in soluble extracts of temporal cortex were observed, either between groups or within groups, of (a) six ATD subjects aged 65–89 years and six age-matched controls, and (b) Down's syndrome subjects aged 1.5, 27 and 63 years and controls aged 1.2 and 65 years. The 70 kDa protein was also present in the soluble fraction of white matter (corpus callosum) and cerebellum from post-mortem human brain, and in the hypothalamus and cerebral grey matter of rat brain (fig.1). The staining intensity of the 70 kDa protein was much lower in the soluble fraction of human liver, and even lower in kidney, compared with brain. No specific staining was observed in the soluble fraction of human skeletal muscle, the particulate fractions of human liver, kidney and muscle, or in plasma from two patients with ATD.

4. DISCUSSION

We have demonstrated that an antiserum to the C-terminal pentadecapeptide of the putative

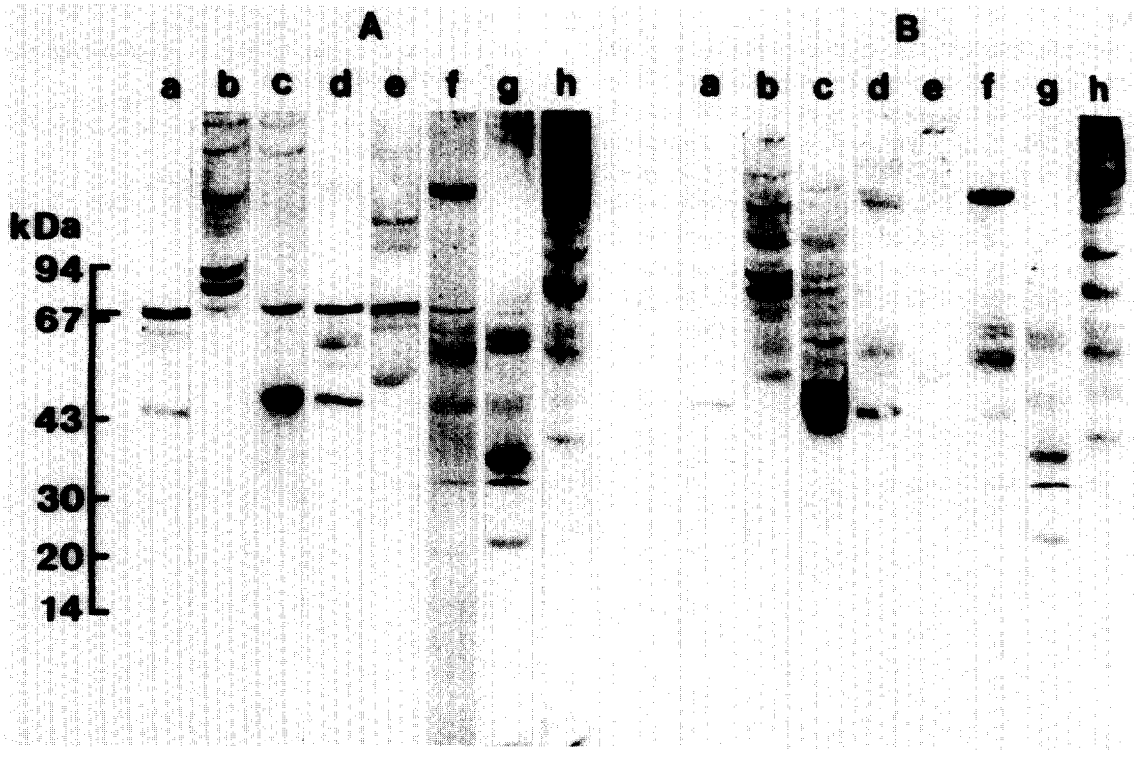

Fig.1. Labelling of brain proteins on electroblotted nitrocellulose with (A) an antiserum to the proA4 C-terminus, and (B) antiserum blocked with proA4 C-terminal pentadecapeptide. (a) Temporal cortex, soluble fraction, (b) temporal cortex, particulate fraction, (c) corpus callosum, soluble fraction, (d) cerebellum, soluble fraction, (e) rat hypothalamus, homogenate, (f) liver, soluble fraction, (g) muscle, soluble fraction, (h) muscle, particulate fraction. 30 μg protein/sample (18.5 μg for e). Molecular mass calibration from experiment a.

amyloid precursor [3] recognizes a soluble protein of approx. 70 kDa in post-mortem brain from cases of ATD, Down's syndrome and control subjects. This protein was probably not a consequence of autolysis, because a 70 kDa immunoreactive protein was observed in fresh rat brain which was rapidly prepared for SDS-PAGE.

The 70 kDa protein may relate to amyloid in one of three ways: (i) Kang et al. [3] isolated a cDNA clone encoding a 78.6 kDa protein which contained the A4 sequence, and which had characteristics of a glycosylated cell-surface receptor. The 70 kDa protein may be a product of post-translational processing of the 78.6 kDa protein. (ii) In situ chromosome hybridisation studies using A4 cDNA as probe [11] have indicated that there are several chromosomes containing A4-related sequences. Our 70 kDa protein may be encoded by a gene carrying the message for a similar, but not identical, protein to proA4. (iii) The 70 kDa protein may contain an epitope present in proA4, but the 70 kDa protein may be otherwise unrelated to proA4.

Little information is available concerning brain proteins immunoreactive to proA4 antisera. Using antisera to different peptide sequences of proA4, Shivers et al. [12] identified a 92 kDa protein associated with the plasma membrane of neurons in rat brain. In order to investigate the relationship of our 70 kDa protein to proA4, we propose to peptide-map the 70 kDa protein and compare its sequence with the published sequences of proA4.

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