ANTIBODIES TO CHOLINERGIC CELL BODIES IN ALZHEIMER'S DISEASE

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ABSTRACT

In the present investigation we examined the possibility that patients with senile dementia of the Alzheimer type (SDAT) produce antibodies which interact with cholinergic neurons. The anticholinergic antibody contents of sera obtained from 10 SDAT patients and 5 elderly controls were assayed by means of a solid phase immunoassay (ELISA). Using purely cholinergic cell bodies isolated from the electric lobe of Torpedo brain as antigen, binding of SDAT sera was 156±32% of controls. Statistical analysis of the results by means of Student's t-test and Wilcoxon two-sample test found them to be significantly different (p<0.01). By contrast binding of SDAT sera to purely cholinergic nerve terminals isolated from the Torpedo electric organ did not differ statistically from those of controls. The significance of the SDAT antibodies directed against these antigens in the pathogenesis of SDAT and their potential use in diagnostic procedures is discussed.

INTRODUCTION

Senile dementia of the Alzheimer type (SDAT) is the major dementing disorder of old age. Despite the immense number of clinical cases the etiology and pathogenesis of the disease are still obscure. Furthermore, the definite diagnosis of the disease can only be made based on histopathological evidence obtained at autopsy or by biopsy (McKhann et al. 1984).

Recent studies indicate a decreased cholinergic activity in the CNS in SDAT (Rossor 1982). This is manifested both by a decrease in presynaptic cholinergic parameters (e.g. choline uptake (Rylett et al. 1983) and acetylcholine synthesis and release (Sims et al. 1983)) and by selective degeneration of certain cholinergic neurons (Coyle et al. 1983). The mechanisms underlying these changes are not known.
Several lines of evidence suggest that immunological mechanisms may be involved in the pathogenesis of SDAT. These include the presence of amyloid fibrils in the senile plaques (Eikelenboom & Stam 1982) and elevated serum immunoglobulin and increased blood-cerebrospinal fluid barrier permeability (Alafuzoff et al. 1983). Significantly higher binding of antineuronal antibodies in senile dementia have been described (Nandy 1978). Whether immunoglobulin abnormalities associated with SDAT play a part in inducing the cholinergic dysfunction is not known.

In the present communication we examined the possibility that antibodies in SDAT sera bind specifically to cholinergic neurons. This was performed by means of an enzyme linked immunoadsorbant assay (ELISA) utilizing as antigens the purely cholinergic nerve terminals and cell bodies isolated respectively from the Torpedo electric organ and electric lobe. Our findings suggest that sera of SDAT patients contain antibodies directed specifically against the cholinergic perikarya.

MATERIALS AND METHODS

1. Purification of cholinergic cell bodies and nerve terminals.
   Nerve terminals (synaptosomes) were purified from the homogenates of fresh Torpedo electric organ by differential and density gradient centrifugation as previously described (Michaelson & Sokolovsky 1978). The cell bodies of the cholinergic neurons which innervate the electric organ were isolated from freshly excised Torpedo electric lobes as described by Dowdall et al. (1978). Both preparations (~2 mg protein/ml) were kept at -70°C until used.

2. Preparation of antisera. Antisera to Torpedo synaptosomes and cholinergic perikarya (PK) were prepared by immunizing rabbits with 0.2 mg and 0.5 mg protein respectively twice at a 14-day interval as in Walker et al. (1982). Human sera were obtained from ten patients who fulfilled the criteria of SDAT (mean age 76±8) and from five elderly controls without signs of dementia or immunological disease (mean age 74±5).

3. Detection of anticholinergic antibodies: Plastic ELISA wells (NUNC Immunoplates Type II) were coated for 24 h at 4°C with 200 μl of synaptosomes (15 μg/ml) or cholinergic cell bodies (20 μg/ml) diluted in 50 mM sodium bicarbonate pH 9.6, after which they were washed (x 3) with phosphate buffered saline pH 7.5 (PBS) which contained Brij (0.1%). (This procedure resulted in the absorbance of about 5-10% acetylcholinesterase activity (Ellman et al. 1961) associated with the antigen, which is an estimate of the amount of protein bound to the plate). Plates were then incubated for 60 min at room temperature with PBS + 1% BSA after which they were washed (x 3) with PBS + Brij. Sera to be tested were diluted in PBS containing