PC12 Cells
Release Stimulatory Factors After Transfection with β/A4-C-Terminal DNA of the Alzheimer Amyloid Precursor Protein

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Received March 12, 1992; Accepted May 6, 1992

ABSTRACT

The β/A4 region of the amyloid precursor protein (APP) accumulates in brains of victims of Alzheimer disease (AD) where it is a major component of senile plaques. We examined the pathophysiological consequences of overexpression of the β/A4-C-terminal DNA in PC12 cells. Serum-free conditioned media (SFCM) from positive transfectants stimulated control PC12 cells to extend neurites and increase in size. Unlike the factor that affected cell size, neurite lengthening activity was significantly decreased after immunoabsorption with anti-β/A4 monoclonal antibodies (MAb) and changes in pH. The data support the view that among the consequences of β/A4-C-terminal DNA overexpression in PC12 cells is the release of factors that stimulate nontransfected cells to undergo morphological transformations that include differentiation to a neuronal phenotype. It is hypothesized that similar activities that may contribute to the molecular pathophysiology of the disorder may be present in the AD brain.

Index Entries: Amyloid, Alzheimer disease, PC12 cells, neurite extension, transfection.

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INTRODUCTION

β/A4 amyloid is a 4.2 kDa polypeptide that is the major protein component of senile plaques and affected blood vessels of the Alzheimer disease (AD) brain (Glenner and Wong, 1984a,b; Masters et al., 1985). The peptide is derived from one or more amyloid precursor proteins (APP) of 695, 751, or 770 amino acids (Goldgaber et al., 1987; Kang et al., 1987; Robakis et al., 1987; Tanzi et al., 1987; Vitek et al., 1988; Zain et al., 1988) the larger of which contain a protease inhibitor domain (Tanzi et al., 1988; Ponte et al., 1988; Kitaguchi et al., 1988).

The precise role of β/A4 amyloid in AD pathology is incompletely defined and whether it is an active or passive agent during senile plaque formation is unsettled. There are two reported normal proteolytic processing mechanisms for APP. The first cleaves within the β/A4 region and yields a secreted product (Esch et al., 1990; Sisodia et al., 1990). The second utilizes the endosomal/lysosomal pathway and produces C-terminal derivatives among which are at least two fragments, containing the entire β/A4 peptide at or near their amino termini, which may be intermediates during the deposition of amyloid (Estus et al., 1992). Consistent with the view that certain APP domains are functionally active are observations on APP fragments containing portions of the β/A4 peptide which appear to have either trophic or toxic activity under defined primary cell or cultured cell conditions (Majocha et al., 1991; Marotta et al., 1992; Whitson et al., 1989; Yankner et al., 1989, 1990).

Based on these considerations, which indicate the potential importance of the β/A4 region to neurodegeneration in the AD brain, the present studies were undertaken to help clarify the functional consequences of β/A4 over-representation. We report here that incorporation and overexpression of DNA encoding the β/A4-C-terminal region of APP in transfected PC12 cells is associated with secretion of one or more factors that stimulate nontransfected PC12 cells to undergo morphological differentiation.

MATERIALS AND METHODS

Preparation of Transfected PC12 Cells

PC12 cells were permanently transfected with an SV40-based vector containing an Eco RI fragment of APP that contains a 1.1-kb segment extending from a site near the beginning of the β/A4 domain to within the 3' terminal coding region. The complete details were reported earlier (Marotta et al., 1989). The cells were propagated as described by Greene and Tischler (1976) except that medium contained DMEM, 10% calf serum, 5% fetal bovine serum. Transfected cells that were positive for β/A4 DNA (AC126 and AC127) were compared in subsequent studies to control cells.