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IDENTIFICATION OF AMYLOID PRECURSOR PROTEIN IN SYNAPTIC PLASMA MEMBRANE

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Summary : Although the etiology of Alzheimer's disease has not been elucidated yet, dysfunction and loss of synapses are believed to cause dementia. Recent studies suggest that the primary cause of the disease is closely related to the aberrant processing of the amyloid precursor protein (APP). To investigate the localization of APP at synaptic sites, we obtained synaptic plasma membrane and synaptic vesicles from rat brain. Enhanced chemiluminescence (ECL) Western blot analysis using two specific polyclonal antibodies against APP revealed strong APP immunoreactivity in the synaptic plasama membrane, but not in the synaptic vesicle fraction. These data indicate that APP is localized at the synaptic plasma membrane and may play a role in physiological synaptic activity. Alternative localization or aberrant processing of APP at the synaptic site may cause impairment of synaptic function in Alzheimer's disease.

Alzheimer's disease is a progressive neurodegenerative disorder affecting aged people with pathological features such as extracellular senile plaques, intraneuronal neurofibrillary tangles and the loss of neurons and synapses (1). The major component of senile plaque is amyloid β protein (β /A4), which is a 39- to 42-residue, proteolytic peptide of a larger membrane-associated glycoprotein, termed amyloid precursor protein (APP) (2, 3, 4). Although β/A4 accumulates only in aged mammalian brain, expression of APP is ubiquitously observed in many organs and in cultured cells from various origins (5) . APP is a well-preserved protein among various species; however, little is known of its physiological functions. Although previous reports using immunoelectron microscopy demonstrated that APP immunoreactivity is associated with the Golgi apparatus, endoplasmic reticulum, presynaptic vesicular-like structures or postsynaptic membranes in rat cortical neurons (6,7), intracellular localization of APP has not been determined.

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Here we show the localization of full-length APP in rat synaptic plasma membrane by immunoblot analysis using two specific polyclonal antibodies against APP.

MATERIALS AND METHODS

Antibodies The polyclonal antibodies against a synthetic peptide corresponding to the carboxyl-terminal 30 residues (666-695) of APP (8) or the amino-terminal 18 residues (45-62) of APP were gifts from Dr. Yasuo Ihara, the University of Tokyo. We named them CAPP and NAPP, respectively. As a marker for synaptic plasma membrane or synaptic vesicles, we used a commercially available monoclonal antibody (Dakopatts, Denmark) which recognizes synaptophysin, a 38 kDa integral protein of synaptic vesicles (9). We call it anti-SP. In enhanced chemiluminescence (ECL) western blotting, the dilutions of CAPP, NAPP and anti-SP were 1 : 2000, 1 : 2000 and 1 : 5000, respectively.

Purification of synaptic plasma membrane Synaptic plasma membrane fraction was prepared from Sprague-Dawley rat forebrains according to the method of Jones and Matus (10). Briefly, a crude mitochondrial fraction containing mitochondria, synaptic plasma membrane and myelin was isolated from rat forebrain homogenate by centrifugation. Then it was hypotonically lysed and suspended in the two-step sucrose density gradient. Under the influence of applied gravitational force, mitochondria, synaptic plasma membrane and myelin were densitometrically separated. The synaptic plasma membrane-enriched fraction was recovered from the interface of the two-step sucrose density gradient centrifugation. Hypotonical lysis was performed in ice and the other procedures were performed at 4℃.

Purification of synaptic vesicles Synaptic vesicles were isolated and purified according to the methods of Nishiye et al. (11). Briefly, a crude mitochondrial fraction was isolated from the homogenate of Sprague-Dawley rat cerebral cortex. Synaptic vesicles were purified from a crude mitochondrial fraction by hypotonic lysis followed by repeated density-equilibrium centrifugations. All procedures were performed at 4℃.

Sample preparation and gel electrophoresis Protein concentration of each fraction was determined by the BCA protein assay kit (Pierce, Rockford, IL). Equal quantities of proteins from synaptic plasma membrane, synaptic vesicles and rat brain homogenates were separated on 9 % SDS-polyacrylamide gel under reducing conditions (12).

Immunoblotting The separated proteins on polyacrylamide gel were electroblotted onto Immobilon PVDF membranes (Millipore, Bedford, MA), essentially according to the method of Towbin et al. (13). After blocking with Tris-buffered saline with Tween (TBST) containing 5 % skim milk, the membranes were incubated with the primary antibodies for one hour at room temperature. Then the membranes were washed in TBST and incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG antibody (Amersham, U.K.). After thorough washing, the immunoreactive proteins were visualized with ECL system according to the manufacturer's directions (Amersham, U.K.). Specificity of immunolabelling was confirmed with the antigen-absorbed NAPP.

RESULTS

Figure 1 shows immunoblots of various subcellular fractions obtained in the preparation of synaptic plasma membrane. Equal amounts of proteins were applied on each lane. In the synaptic plasma membrane fraction, an intense 38 kDa band was observed with anti-SP (Figure 1, A, lane 4). Furthermore, a 105 kDa band was immunolabelled by both CAPP and NAPP in the same fraction (Figure 1, B, C, lane 4) , suggesting the localization of full-length APP. The molecular weight of APP is consistent with that of previous reports (6, 14, 15). The immunoreactivity of APP in synaptic plasma membrane was stronger than that of all other

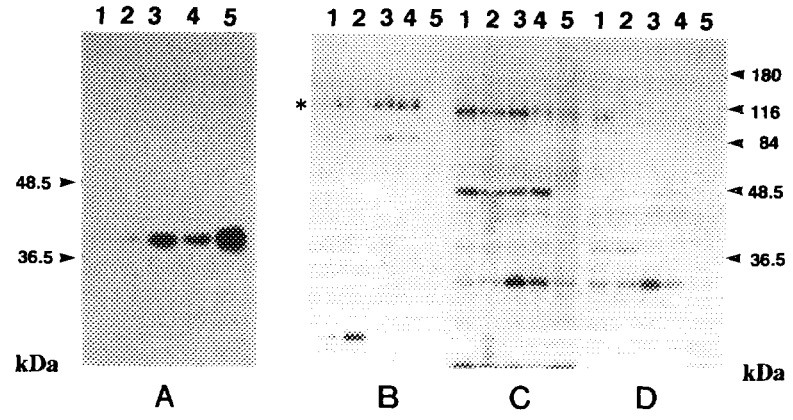

Figure 1 . Immunoblots of subcellular fraction (A : 15 μg per lane, B, C, D : 30 μg per lane) in the preparation of synaptic plasma membrane stained with anti-SP (A), CAPP (B), NAPP (C) or preabsorbed NAPP (D) .
Lanes : 1, rat brain homogenate; 2, crude mitochondrial fraction; 3, myelin fraction; 4, synaptic plasma membrane; 5, mitochondrial fraction.
* : amyloid precursor protein.

fractions. The present result clearly indicates a preferential localization of full-length APP in synaptic plasma membrane.

Figure 2 shows the results obtained in the study of synaptic vesicle fraction. A large quantity of synaptophysin was immunolabelled in synaptic vesicle fraction (Figure 2, A, lane 5). In sharp contrast with the result obtained from the synaptic plasma membrane

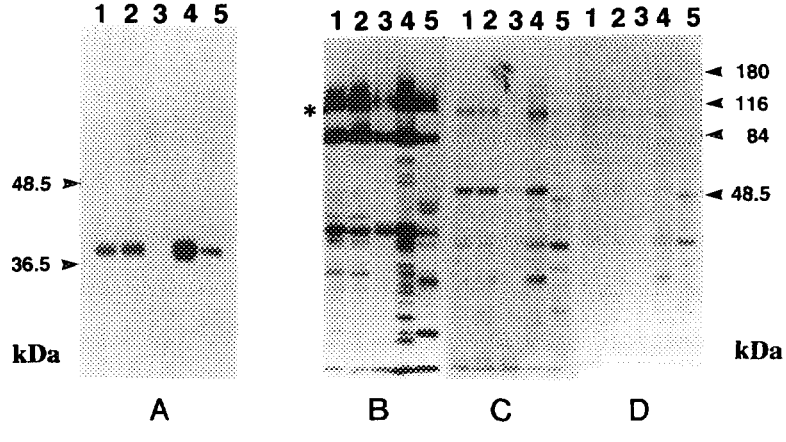

Figure 2 . Immunoblots of subcellular fraction (A : 5 μg per lane, B, C, D : 30 μg per lane) in the preparation of synaptic vesicle stained with anti-SP (A), CAPP (B), NAPP (C) or preabsorbed NAPP (D) .
Lanes : 1, rat cerebral cortex; 2, crude mitochondrial fraction; 3, 4, crude synaptic vesicle fraction; 5, purified synaptic vesicles.
* : amyloid precursor protein.

fraction, the immunoreactivity of full-length APP was drastically decreased in the synaptic vesicle fraction (Figure 2, B, C, lane 5).

When NAPP was preabsorbed with the antigen peptide, the protein bands of 105 kDa were not detected (Figure 1, D, Figure 2, D) .

DISCUSSION

The primary cause of Alzheimer's disease (AD) remains unknown; however, the reports of familial Alzheimer's disease with APP gene mutation (16-20) strongly indicate the involvement of APP in the pathogenesis. In spite of extensive studies of APP, the function and the localization of the protein have remained unclear.

Masliah et al. reported the decrease in the immunoreactivities of synaptophysin in the cortex of the brains with AD, suggesting preferential abnormalities of the synapses preceding neuronal death (21).

The hippocampus, where a marked accumulation of β/A4 is observed in AD, has an extensive capability of synaptic plasticity (22). Based on this, whether or not, APP is localized at synaptic sites and involved in synaptic function should be important issues in the pathogenesis of AD.

Schubert et al. reported a preferential localization of APP at synaptic sites using immunofluorescence, laser confocal microscopy and immunoelectron microscopy (6). They also found negative localization of APP in transmitter-containing, synaptic vesicles. The present study, the first biochemical report of the synaptic localization of APP, provides support to the result of Schubert et al. Our result indicates that APP is localized at the synaptic plasma membrane, and not in the synaptic vesicle, and is involved in surface activation processes at synaptic sites. However, the issue whether the localization of APP is presynaptic or postsynaptic remains to be determined. A recent report by Shigematsu et al. (7) indicating the selective post-synaptic localization of APP, differs from that of Koo et al. who have shown that APP is synthesized in neurons and undergoes axonal transport to the presynaptic terminal (23).

In future studies, the method employed in the present study should enable determination of more detailed localization of APP at synaptic sites using a method which regulates the axonal transport system.

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