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Strong immunoreactivity of β-amyloid precursor protein, including the β-amyloid protein sequence, at human neuromuscular junctions

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At the postsynaptic domain of the human neuromuscular junction (NMJ), we have demonstrated strong concentrations of the N-terminus 45–62, C-terminus 676–695 and β-amyloid protein sequences of β-amyloid precursor protein (βAPP). We used well-characterized monoclonal and polyclonal antibodies for co-localization with three other postsynaptic proteins, applying double and triple fluorescence labeling. Strong immunoreactivity of all three βAPP sequences was found at all NMJs identified by bound α-bungarotoxin (αBT), where they co-localized with αBT and with immunoreactive desmin and dystrophin, which are postsynaptic proteins of human NMJs. This appears to be the first demonstration of βAPP sequences concentrated postsynaptically at human NMJs. βAPP may have a role in normal junctional biology and possibly in some diseases affecting NMJs.

β-Amyloid precursor protein (βAPP), a product of a chromosome-21 gene [28], exists in virtually all tissues [6, 8, 9, 26, 32, 34]. βAPP is a glycoprotein cell surface component containing a large extracellular N-terminus domain, a transmembrane domain and a short cytoplasmic carboxyl-terminus domain [9, 15, 21]. The βAPP gene produces at least three alternatively spliced transcripts encoding βAPP containing 695, 751 or 770 amino acids; last two contain in their extracellular region an insert of a Kunitz-type protease inhibitor [9, 15, 19, 21, 31]. The biological functions of βAPP are unknown, but it may play a role in mediating: cell-to-cell and cell-to-matrix interaction [7, 16, 22, 28], neurite growth [7], and maintenance of cell integrity and shape [20]. Its presynaptic localization at central and peripheral synapses has been reported [23].

β-Amyloid protein (βAP) is a 39–42 residue polypeptide that is a part of βAPP [9, 15, 21]. βAP is the major component of amyloid fibrils in blood vessels and senile plaques in the brain of patients with Alzheimer's Disease, Down syndrome, Dutch hereditary cerebrovascular amyloidosis, and very advanced age [13, 18, 25] [reviewed in ref 24]. We have recently demonstrated pathologic accumulation of βAP [3, 4] and two other βAPP sequences [5], along with ubiquitin [2], in vacuolated muscle fibers of inclusion body myositis. We now demonstrate that the N-terminus 45-62, C-terminus 676–695, and βAP sequences of βAPP are highly concentrated at the postsynaptic domain of normal human neuromuscular junctions (NMJs).

Immunolocalization was performed on 10 μm transverse sections of fresh-frozen diagnostic human muscle biopsies. The biopsies were screened with our 18 routine histochemical reactions [10]. Fifteen that showed no abnormality and contained pan-esterase-positive NMJs were selected for study. More than 200 NMJs were analyzed. Four antibodies against βAPP sequences were used: (a) rabbit polyclonal antiserum C8, directed against a synthetic peptide of C-terminal amino-acids 676–695 (C-βAPP) [26], diluted 1:1000; (b) rabbit polyclonal antiserum, anti-SP18, against the N-terminal sequence 45–62 (N-βAPP) [29], diluted 1:500; (c) rabbit polyclonal antiserum R1280, against sequence 1–40 of synthetic βAP [14], diluted 1:1000; and (d) mouse monoclonal antibody G-OP-1, against sequence 8–17 of synthetic βAP, diluted 1:200 [33]. In all experiments, NMJs were identified by the binding of FITC- or rhodamine-labeled α-bungarotoxin (αBT) (Molecular Probes) to the nicotinic acetylcholine receptors (AChRs).

To co-localize βAPP with other postsynaptic proteins situated at human NMJs [1, 11, 27], monoclonal antibodies against desmin (Chemicon), ubiquitin (Chemicon), and the C-terminus portion of dystrophin (Novocastra), were used in double or triple fluorescence-labeling utilizing FITC, Texas red, Rhodamine, and AMCA

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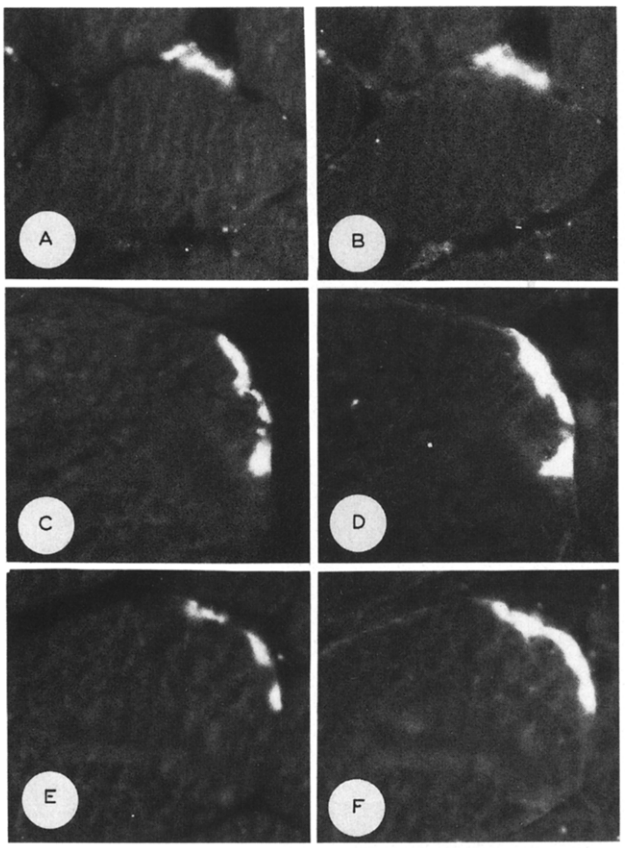

Fig. 1. Double labeling of the human NMJs demonstrates close co-localization of αBT bound to nicotinic acetylcholine receptors with the immunolocalization of 3 sequences of βAPP, all × 1375. A,C,E: αBT; B: N-βAPP; D: βAP; F: C-βAPP. The βAP and C-βAPP immunoreactivities occupy a slightly larger area than those of αBT and N-βAPP. Lipofuscin granules are autofluorescent in A,B.

[1, 27]. Specificity of the βAPP immunoreactivites were determined by: (a) omitting the primary antibody, (b) replacing the primary antibody with non-immune serum, and (c) absorbing the anti-βAP primary monoclonal antibody with synthetic βAP sequence 8–17.

In all biopsies, all the NMJs identified by αBT binding had very strong immunoreactivity of all the βAPP sequences. The muscle non-junctional sarcolemma was very faintly immunoreactive with all antibodies. The immunoreactive topographic pattern of the N-terminus sequence compared exactly to the localization of bound αBT (Fig. 1). The βAP and C-βAPP sequences typically extended slightly deeper into the muscle fiber than the αBT location (Figs. 1 and 2). The C-βAPP and βAP immunoreactivities closely co-localized with the postsynaptic cytoplasmic desmin immunoreactivity of the same triple-labeled NMJs (Fig. 3A,B,C and G,H,I). C-βAPP and βAP also co-localized with the concentration of dystrophin immunoreactivity at triple-labeled NMJs (Fig. 3D,E,F and J,K,L), as did N-βAPP (not illustrated). For all these immunolocalizations, when the primary antibody was omitted, absorbed, or replaced by a non-immune serum, the immunoreaction did not take place.

The co-localization of βAP and C-βAPP with αBT, desmin and dystrophin suggests that they are located at the postsynaptic membrane folds and the subsynaptic domain inside the muscle fiber. The close co-localization of N-βAPP with bound αBT and dystrophin suggests that it may be mainly confined to the postsynaptic folds. Precise localization of the βAPP sequences awaits ultrastructural immunocytochemistry (in progress).

At the NMJ, the molecular composition of the muscle fiber's extracellular matrix, plasmalemma, and subsynaptic cytoplasmic domain are different from those of its non-synaptic region [reviewed in ref. 12]. By demonstrating that sequences of βAPP are strongly concentrated at the NMJs, our study raises the question of its normal function there*. For example, βAPP may be involved in: (a) maintaining stability and configuration of the postsynaptic membrane by associating with postsynaptic cytoskeletal components of the muscle fiber, e.g. dystrophin and desmin; (b) maintaining adhesion between the postsynaptic and presynaptic components; (c) the interaction between the postsynaptic plasmalemma and basal lamina (and perhaps junctional acetylcholinesterase); (d) inhibiting excessive or unwanted serine proteases that may exist at the NMJ; or (e) binding and internalization of synaptic substances (analogous to the demonstrated internalization of βAPP/βAP into lysosomes [17]. The strong concentration of βAPP at human NMJs also raises the possibility that it could have a pathogenic role in some diseases affecting those junctions.

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*In this discussion we are assuming that the immunolocalized βAB, C-βAPP, and N-βAPP are residing within intact βAPP, but conceivably a portion of them could also be separate from that parent molecule.

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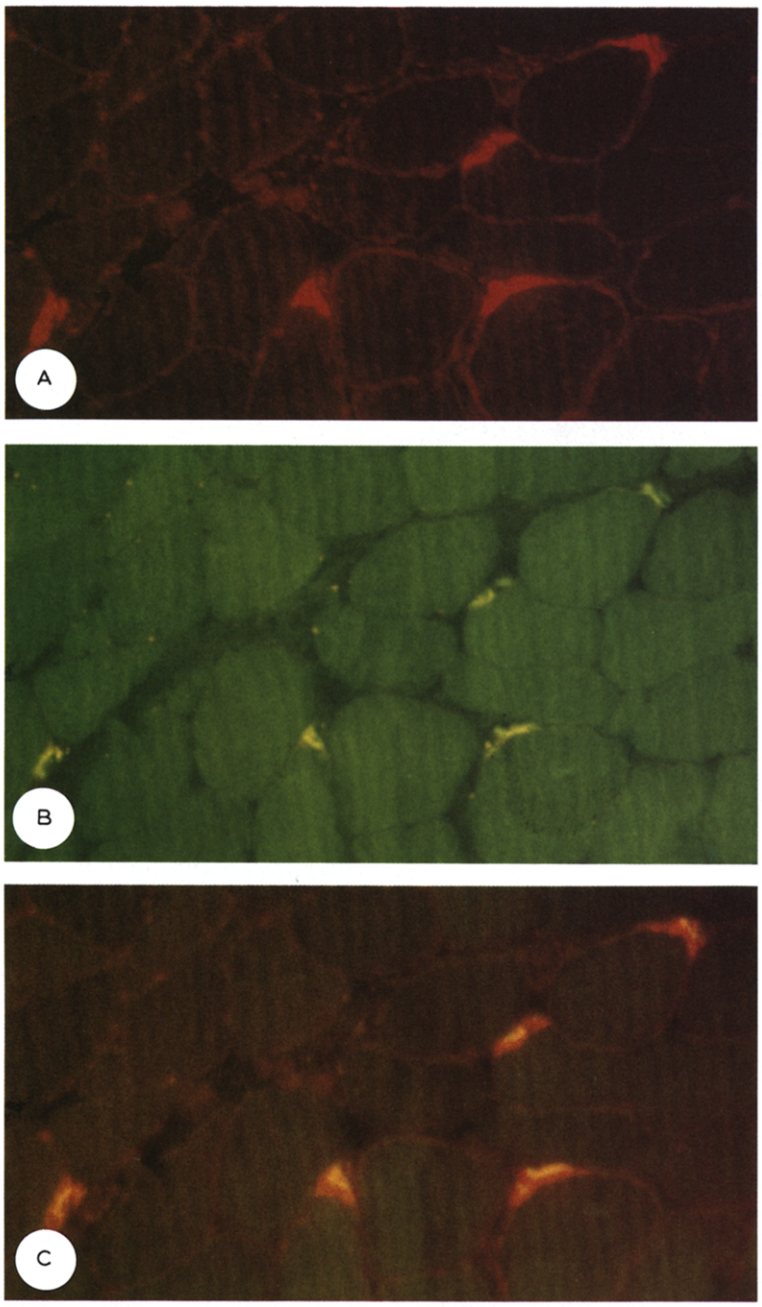

Fig. 2. Double labeling of the NMJs on the same section demonstrates close co-localization of βAP (Texas red) (A) and FITC-labeled αBT (B). Double photographic exposure (C) demonstrates that βAP immunoreactivity occupies a slightly larger area than bound αBT, extending slightly deeper into the muscle fiber. × 570.

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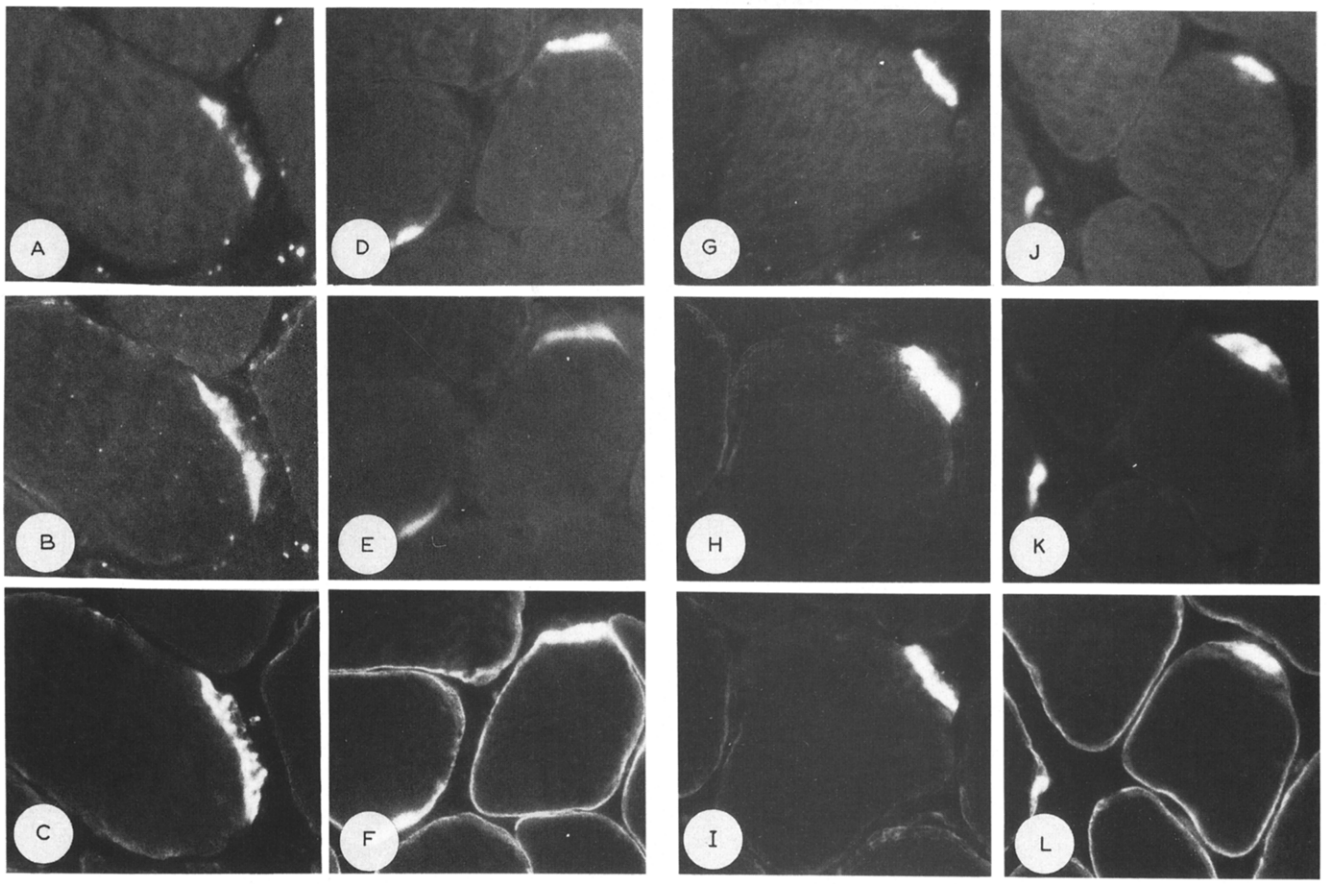

Fig. 3. Triple labeling of the human NMJs with αBT, C-βAPP antibody, βAP antibody, and antibodies against desmin and dystrophin, all × 1000. A,B,C: αBT, C-βAPP and desmin. D,E,F: αBT, C-βAPP and dystrophin. G,H,I: αBT, βAP and desmin. J,K,L: αBT, βAP and dystrophin. There is a close co-localization of C-βAPP and βAP with the 3 postsynaptic membrane components αBT, desmin and dystrophin. In addition, dystrophin shows strong localization around the entire perimeter of the muscle fibers. Lipofuscin granules are autofluorescent in A,B.

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