Vol. 126, No. 1, 1985 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 16, 1985 Pages 289-294

NEUROPEPTIDE Y-LIKE IMMUNOREACTIVITY IN NEURITIC PLAQUES OF ALZHEIMER'S DISEASE

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Received November 12, 1984

SUMMARY: Alzheimer's disease, a form of senile dementia, is characterized by the presence of neuritic plaques and neurofibrillary tangles throughout the cortex and hippocampus. This study demonstrates the presence of neuropeptide Y-like immunoreactivity within 10-20% of neuritic plaques. Neuropeptide Y is a 36 amino acid peptide which is distributed unevenly throughout the brain and has an interneuronal location.

Senile dementia of Alzheimer type (SDAT) is characterized and diagnosed by the presence of neuritic plaques and neurofibrillary tangles throughout the cortex and hippocampus. The mechanism of formation of either neuritic plaques or neurofibrillary tangles is unknown, although the severity of the disease has been correlated with the number of both of these pathological manifestations (1). At the light microscopic level neuritic plaques appear as spherical structures consisting of a central amyloid core surrounded by argentophilic processes. Golgi studies have shown that the neuritic elements within plaques represent abnormally distended neuronal varicosities which may represent sprouting within the plaque or damaged terminals (2). Neurochemical studies of Alzheimer brain have demonstrated a decreased content of choline acetyltransferase (ChAT) in post-mortem cerebral cortex (3-5). The ChAT deficit is thought to represent cholinergic cell loss within the nucleus basalis of Meynert which projects to the cortex. Besides a cholinergic deficit the noradrenalin content of SDAT cortex is

0006-291X/85 $1.50

also reduced (6,7). This loss of noradrenaline is due to a degeneration of noradrenergic cell bodies in the locus coeruleus. A decreased content of the neuropeptide somatostatin (SRIF) has also been reported in SDAT (8,9). The SRIF containing cells represent intrinsic cortical neurones. Some of these SRIF containing cells of the human cerebral cortex have recently been shown to contain neuropeptide Y (NPY)-like immunoreactivity (10).

NPY is a 36 amino acid peptide recently isolated and sequenced from porcine brain (11) and human phaeochromocytoma (12). The distribution of NPY in human brain by radioimmunoassay and immunohistochemistry revealed a high cortical content and an interneuronal localization (13). This study also showed what appeared to represent NPY-LI within a neuritic plaque. Since previous studies have shown AChE staining within neuritic plaques the present study was designed to determine if neuritic plaques characteristic of SDAT contain NPY-LI.

MATERIALS AND METHODS: Brains from patients dying with SDAT and control brains were immersion fixed in 4% formaldehyde in 100 mM phosphate buffer pH 7.4. Prior to sectioning, blocks of cortex and hippocampus were transferred to 30% sucrose in phosphate buffer and left for at least 12 hours to ensure sucrose impregnation. Sections were cut on a freezing microtome at 35 µM thickness, collected in phosphate buffer and processed to visualize NPY-LI using the peroxidase-antiperoxidase technique of Sternberger (14). NPY antiserum (code 011) was used at a final dilution of 1:500. Absorption control experiments were also undertaken. NPY antiserum was incubated with synthetic NPY (2 nmoles/ml) for 60 minutes prior to incubation of the antiserum with tissue sections. After immunostaining the sections were dried onto gelatinized slides and dipped into a 1% solution of thioflavine S for 10 minutes. The sections were then differentiated in 80% ethanol and coverslipped with phosphate buffered saline containing glycerol. Thioflavine S stains amyloid and can be visualized by fluorescent microscopy. The sections wre examined using a Leitz microscope equipped with both fluorescent and bright field optics.

RESULTS AND DISCUSSION: Neuropeptide Y staining in both the cortex and hippocampus revealed a variety of cell types and fibres as previously described (13). In the SDAT brains

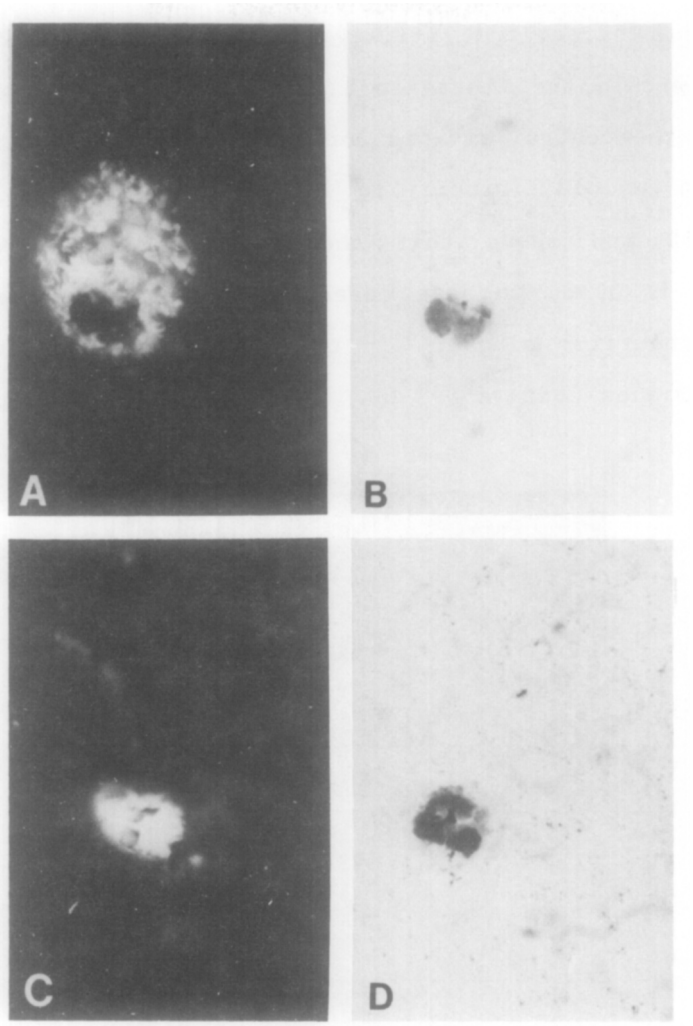

Fig. 1. Fluorescent neuritic plaques (A,C) from hippocampus of SDAT brains stained with thioflavine S. The same sections under bright field optics (B,D) show dense NPY-LI demonstrated by the peroxidase-antiperoxidase technique. Scale 10 mm = 98 µm.

thioflavine S fluorescently labelled neuritic plaques and tangles. Two types of neuritic plaque can be distinguished. The classical plaque (Fig. 1A) is composed of a central core of tightly packed amyloid fibres that is surrounded by a margin of more loosely arranged amyloid fibres that are intermingled with dystrophic neurites. The "burned out" plaque (Fig. 1C) consists solely of tightly packed amyloid. Fig. 1B shows the same field of view as Fig. 1A, under bright field optics. The dark staining

represents neuropeptide Y-like immunoreactivity and is co-localized within the fluorescent neuritic plaque. The staining is outside the central amyloid core and occupies a portion of the surrounding amyloid fibres. Fig. 1D shows the same field of view as Fig. 1C and shows that the neuropeptide Y-like immuno-reactivity is also, in some cases, seen in the central amyloid core of a neuritic plaque. In the hippocampus about 10-20% of neuritic plaques contain NPY-LI. Fig. 2A shows a low power view

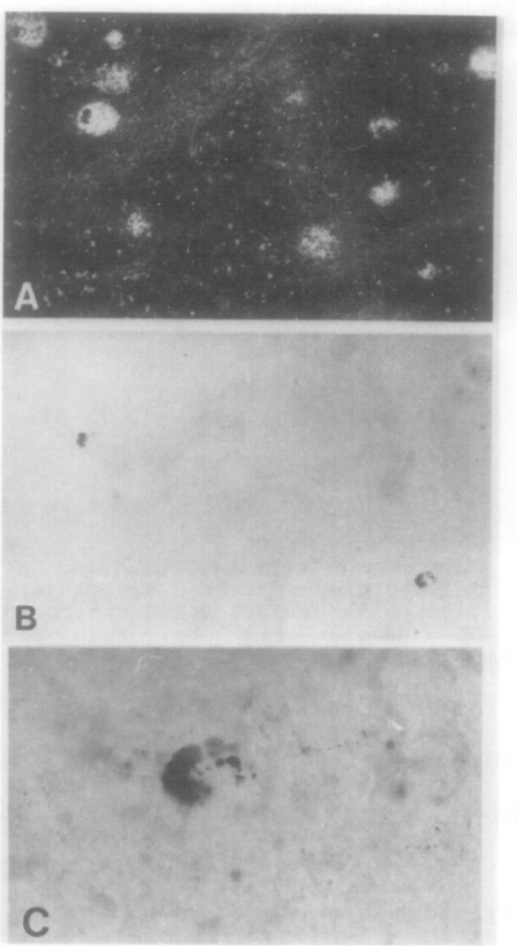

Fig. 2. Lower power view of section through hippocampus of SDAT brain stained with thioflavine S showing numerous neuritic plaques. B: Same section as A under bright field optics demonstrting NPY-LI in two of the neuritic plaques. C: High power view of NPY-LI of bottom right hand section of B. Scale A,B 10 mm = 98 µm; C 10 mm = 24 µm.

of hippocampus stained with thioflavine S. The same section is shown in Fig. 2B under bright field optics. Two of the plaques shown in Fig. 2B can be shown to contain NPY-LI. The immunoreactivity in the bottom right hand corner of Fig. 2B is shown enlarged in Fig. 2C. Note the bulbous varicosities entering the denser immunoreactive material in the plaque.

This type of NPY-LI was not seen in any of the cerebral cortex or hippocampal material taken from control brains. In addition preabsorption of NPY antisera with synthetic NPY abolished all staining. The presence of NPY-LI within neuritic plaques may represent degeneration of any passing fibres suggesting that any fibre within the vicinity of a plaque may be involved in the pathology. This may not, however, always be the case since other studies have shown apparently normal neuronal processes passing through neuritic plaques. Another possible explanation would be that processes of peptide containing neurones may degenerate first causing the neuritic plaques to form around them. In support of this explanation it is known that amyloid is formed from peptides in endocrine polypeptide tumours (15). Whether or not the same is true of neuritic plaque amyloid remains to be determined.

ACKNOWLEDGMENTS

We would like to thank Dr G.P. Reynolds and Dr C.Q. Mountjoy for providing post-mortem brain. D.D. acknowledges support from the Wellcome Foundation and the Hereditary Disease Foundation.

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