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Letters to the Editor

Identification of immunoreactive atrial natriuretic peptide in atrial amyloid

Amyloidosis is a disorder characterised by the deposition of an abnormal proteinaceous material in the extracellular tissues.[1 2] It may occur as a primary disease process or secondary to a variety of conditions characterised by chronic inflammation. It has been described in association with tumours and occurs in heredofamilial forms. It occurs in a localised or systemic distribution.

Amyloid has characteristic staining reactions, but is best defined by its appearance on electron microscopy where fine non-branching fibrils about 10 nm in diameter can be identified. Despite the similarity of appearance the chemical structure of the fibrils varies with site and disease association. In systemic primary and myeloma associated amyloid the fibrils contain protein derived from immunoglobulin light chains. In secondary amyloid the fibrils contain a protein related to the acute phase reactant serum amyloid A. In hereditary or familial amyloidosis and in cerebral amyloid of Alzheimer's disease they contain prealbumin. Amyloid localised to endocrine organs may contain hormone related peptides—for example, immunoreactive calcitonin is found in amyloid associated with medullary carcinoma of the thyroid.[3–5]

Amyloid affects the heart as part of systemic amyloidosis or in a localised form as a manifestation of aging.[6] Two types occur in the aging heart: that affecting both the ventricles and atria and that affecting the atria alone, known as isolated atrial amyloid.[7] Isolated atrial amyloid has been reported in 78% of patients over the age of 80 years: its origin is unknown.

Recent evidence suggests that the heart is an endocrine organ, and peptides arising from the atria have been isolated and characterised. They have potent natriuretic, diuretic, and vasodilating actions and may play an important part in the homeostasis of body fluids.[8 9] In this report we describe the immunohistochemical localisation of human atrial natriuretic peptide to amyloid fibrils in human atrial appendage, indicating that some cardiac amyloid is analogous to that seen in other endocrine organs.

Material and methods

A fresh piece of right atrial appendage removed at coronary bypass surgery was fixed in paraformaldehyde lysine sodium periodate[10] for 24 hours. It was then divided to provide blocks for routine paraffin histology and electron microscopy. Paraffin sections were cut at 4 μm and stained with haematoxylin and eosin, sulphated alcian blue, and alkaline Congo red for amyloid.[11]

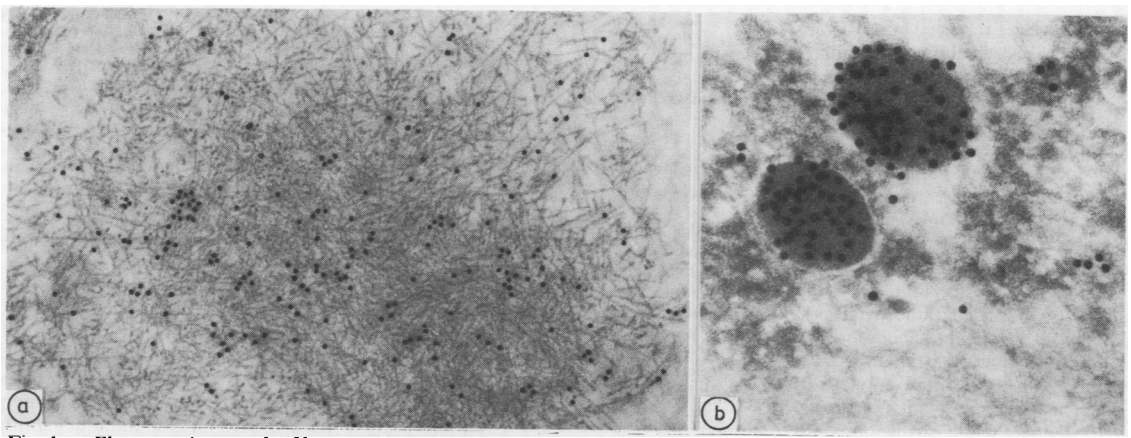

Fig. 1a *Electron micrograph of human atrial appendage showing labelling of amyloid fibrils for human atrial natriuretic peptide. (Immunogold/ uranyl acetate lead citrate, 15 nm gold balls.)* × 56 000.

Fig. 1b *Electron micrograph of human atrial appendage showing labelling of neurosecretary granules for human atrial natriuretic peptide. (Immunogold/uranyl acetate lead citrate, 15 nm gold balls.)* × 112 000.

They were also stained by the indirect immunoperoxidase technique for atrial natriuretic peptide.[12] Tissue for electron microscopy was embedded in LR white resin, and ultrathin sections were stained by indirect immunochemistry for atrial natriuretic peptide, using goat antirabbit immunoglobulin conjugated to 15 nm colloidal gold (Janssen Pharmaceutical) as the second antibody.[5] The antibodies to atrial natriuretic peptide were raised in rabbits using synthetic atrial natriuretic peptide conjugated to bovine thyroglobulin as the immunogen.

Results

Ultrastructural examination of the atrial tissue showed the presence of fine deposits of amyloid fibrils 7–10 nm in diameter around small blood vessels and adjacent to muscle cells. The amyloid fibrils clearly and consistently labelled for immunoreactive atrial natriuretic peptide (Fig. 1a), whereas background labelling of the surrounding connective tissue was negligible. The presence of atrial natriuretic peptide was also shown in neurosecretory granules within atrial muscle cells (Fig. 1b). Immunoperoxidase staining for atrial natriuretic peptide by light microscopy was positive in atrial muscle cells and focally positive around small blood vessels. Reaction with preabsorbed antiatrial natriuretic peptide was consistently negative. The amyloid was not detected by light microscopy, and the Congo red stain was negative.

Discussion

Amyloid has been reported in various endocrine tumours, including medullary carcinomas of the thyroid, carcinoids, phaeochromocytomas, carotid body tumours, insulinomas, and gastrinomas.[13] Endocrine associated amyloid differs from other types in that histochemical staining for tryptophan is negative. Moreover, in some cases the constituent protein of endocrine associated amyloid has been shown to be related to the native hormone product.[3 5]

Studies on the cardiac amyloid associated with aging have subdivided it into two types, which differ both in chemical composition and distribution.[14] Isolated atrial amyloid is similar to endocrine associated amyloid in that it lacks histochemically demonstrable tryptophan, whereas senile cardiac amyloid found in both atria and ventricles contains readily demonstrable tryptophan.

In this report we describe the presence of immunoreactive human atrial natriuretic peptide in atrial amyloid fibrils, which can be seen on electron microscopy, and have also confirmed its presence within neurosecretory granules in atrial cardiocytes.[15] These findings support the view that some atrial amyloid is of an endocrine type and suggest that atrial natriuretic peptide, or an atrial natriuretic related peptide, may be its primary constituent.

The amyloid was only visualised ultrastructurally and was not evident on light microscopy. This phenomenon is not uncommon if deposits are small.[16] In this case it was therefore impossible to confirm the ultrastructural observations at light microscopy level, although there was a little perivascular positivity for atrial natriuretic peptide on indirect immunoperoxidase staining. As deposits of isolated atrial amyloid increase with age and the patient studied was only 64 years old, it may be possible to show amyloid atrial natriuretic peptide on light microscopy more readily in older patients. Further studies are currently being undertaken.

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Manual or radiometric method for detection of bacteraemia

We read with interest the paper by Ganguli *et al*[1] that a manual rather than a radiometric method was more rapid for the detection of bacteraemia. In this laboratory we compared the radiometric technique with the standard method of blood culture examination.[2] No difference between the two techniques was found. The isolation rate was 4% for each method when 5216 blood cultures were examined by the manual