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Histochemical Analysis of Senile Plaque Amyloid and Amyloid Angiopathy

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Summary. Histochemical methods were used to obtain information on the chemical constituents of brain amyloid in senile dementia of the Alzheimer type. The staining properties of brain amyloid (senile plaque and amyloid angiopathy) were compared with those of extraneural amyloidosis and endocrine amyloid. We found no histochemical differences between amyloid in senile plaques and in amyloid angiopathy. The content of aromatic amino acids was higher in amyloid of plaques and in amyloid angiopathy than in endocrine amyloid. Furthermore, we found persistent birefringence and affinity of brain amyloid for Congo red after exposure to potassium permanganate, suggesting that AA amyloid is not a major constituent of cerebral amyloid.

Key words: Amyloid – Endocrine amyloid – Senile plaques – Amyloid angiopathy – Alzheimer's disease.

Introduction

Amyloid deposits are commonly found in the core of senile (neuritic) plaques in the brain of patients suffering from senile dementia as well as in old non-demented subjects. Moreover, amyloid is often present in the wall of parenchymatous and leptomeningeal vessels, a condition known as amyloid angiopathy (Mandybur 1975) which is frequently, though not invariably associated with senile dementia (Torack 1975). The origin of amyloid and in particular, its role in the pathogenesis of senile plaques is at present unknown. Amyloid of senile plaques could derive from a circulating precursor e.g. an immunoglobulin or a serum amyloid A protein (SAA protein, Glenner 1978). An alternate possibility is a local origin of the amyloid from constituents of the neuropile

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Table 1

Case	Age (yrs)	Sex	Type	Organ	Tryptophan (10 µm)		Tyrosine (10 µm)		Alcian blue (10 µm)	
					SP	AA	SP	AA	SP	AA
1	72	F		Brain	++	++/+++	++	++	++/+++	++/+++
2	77	M		Brain	++	++	++	++	++/+++	++/+++
3	79	M		Brain	++	++/+++	+/++	+/++	++	++
4	72	F		Brain	++	++	+/++	++	++	++/+++
5	70	M		Brain	+/++	++/+++	+/++	++	++	+++
6	80	F		Brain	+/++	++	+	++	++	++
7	85	M		Brain	o/+	o/+	+/++	++	++/+++	++
8	91	M		Brain	o	o	o/+	o/+	++/+++	+++
9	75	F	Medullary carcinoma	Thyroid	o/+		o		++	
10	41	M	Medullary carcinoma	Thyroid	o/+		o		++/+++	
11	62	M	Secondary	Rectum	++		+/++		+	
12	68	F	Secondary	Rectum	++/+++		−		−	
13	56	M	Secondary	Kidney	++		−		−	
14	62	F	Secondary	Kidney	++/+++		++		+/++	
15	64	M	Myeloma	Rectum	++		++		++	
16	47	M	Primary	Kidney	+++		++		++	

− = not determined; SP = senile plaque; AA = amyloid angiopathy

Case 7 and 8 are control cases, which were fixed in formaldehyde for 14 days. Case 1–6: each value represents an average of at least 10 observations. AA encompasses the two forms of angiopathy, i.e. "plaque-like" and "congophilic angiopathy"

of the cerebral cortex such as the proteinaceous debris of degenerated neurites commonly found at the plaque periphery (Terry and Wisniewski 1970). In a recent histochemical study, Powers and Spicer (1977) found similarities between amyloid of senile plaques and amyloid of endocrine origin (APUD- amyloid, Pearse et al. 1072). These authors stressed the importance of local factors in the genesis of senile plaque amyloid and put forward the hypothesis that the major fibrillar protein of amyloid is derived from some local neural source. Differences of histochemical reactions between amyloid in vessels and that in senile plaques (Powers and Spicer 1977) and the localisation of amyloid deposits in amyloid angiopathy suggest that the latter derives from a circulating amyloidogenic protein (Torack 1975; Glenner 1978). It is therefore conceivable that the heterogeneity of senile cerebral amyloid is due to different origin of the proteins. The purpose of this study was to obtain information of the chemical constituents of brain amyloid by comparison of staining properties of amyloid deposits found in senile plaques and in amyloid angiopathy with those of extraneural amyloidosis and APUD- amyloid.

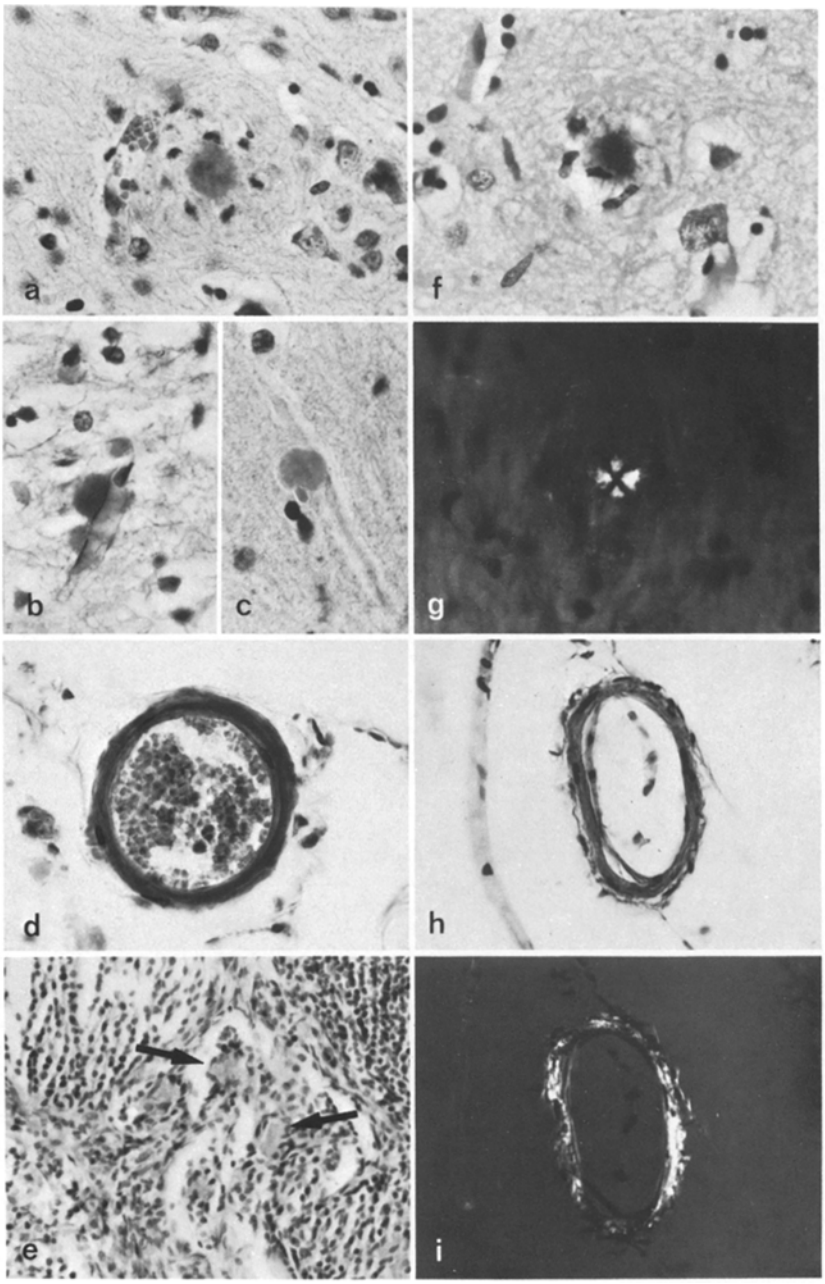

Fig. 1. **a** Case 3. Senile plaque with central amyloid. Congo red (×320). **b** Case 4. "Plaque like angiopathy" of a cortical microvessel. Congo red (×400). **c** Case 3. Drop-like amyloid deposit in the wall of a capillary in the molecular layer of the cerebellum. Congo red (×500). **d** Case 4. Congophilic angiopathy of a leptomeningeal vessel. Congo red (×320). **e** Case 9. Medullary carcinoma of the thyroid. Small amyloid deposits (*arrows*) in the center of some cell clusters. Congo red (×500). **f, g** Case 2. Senile plaque. Persistent Congo red affinity and birefringence after exposure to potassium permanganate (×320). **h i** Case 4. Congophilic angiopathy of a small leptomeningeal vessel. Persistent Congo red affinity and birefringence after exposure to potassium permanganate (×200)

Materials and Methods

Thirty brains from patients suffering from *senile dementia* were fixed in phosphate buffered formaldehyde for 6 to 12 h, from 5 to 22 h after death (Table 1). Two further brains were fixed in unbuffered formaldehyde for 14 days. Tissue blocks from the first temporal gyrus, hippocampus, area 17 and cerebellar cortex were embedded in paraffin. Sections (5 μm) were deparaffinized and stained with HE, PAS and alcaline Congo-red and viewed in polarised light for localisation of amyloid. The blocks from six brains with a high number of amyloid containing plaques and congophilic vessels were selected for this study (Table 1).

Surgical specimens of two *medullary thyroid carcinomas* containing amyloid, biopsies of the kidney of 3 patients suffering from *primary or secondary amyloidosis*, and biopsy samples of the rectum of two patients with *secondary amyloidosis* or *myeloma* were fixed in buffered formaldehyde for approx. 12 h (Table 1).

Series of ten consecutive sections from all blocks were cut at 5 and 10 μm on an optical Leitz microtome. All sections were cut by the same technician. The first section of each series was stained with Congo – red. In the second and third sections the presence of tryptophan and tyrosine was checked, using the dimethylaminobenzaldehyde (DMAB) reaction (Adams 1957) and the diazotation coupling method (Glenner and Lillie 1959). The fourth section was stained with alcian blue at pH 2.5 in the presence of 0.2 M magnesium chloride. The additional 5 μm sections were stained with Congo-red with or without previous exposure to potassium permanganate and dilute sulfuric acid according to Wright et al. (1977). The potassium permanganate technique was also applied to senile plaques in two cases of Down's disease and on Kuru-like plaques in one case of Creutzfeld-Jacob disease (Hayek and Ulrich 1975).

Tryptophan and tyrosine content of all amyloid was estimated semi-quantitatively by one of us, using a 0–3+scale, 12 to 15 h after completion of the staining reaction. Sections of the submandibular glands of male mice were used as positive controls for tryptophan and tyrosine reactions. In the brain, the following amyloid positive structures were evaluated: the central core of senile plaques, the „plaque-like angiopathy" (Scholz 1938) (i.e., the amyloid angiopathy of intrinsic cortical vessels), the „congophilic angiopathy" (Pantelakis 1954) (i.e., amyloid angiopathy of leptomeningeal vessels) (Fig. 1a–d).

The presence of an autofluorescence at 405 nm was examined in deparaffinized sections (5 μm) of formaldehyde fixed material from two brains with senile dementia and from one medullary thyroid carcinoma. A Zeiss UV microscope fitted with a mercury lamp and incident light equipment was used (Filters: G 405, FT 460, LP 475).

Results

Senile plaques and amyloid angiopathy occured often, but not invariably, in the same brain area. Nonetheless amyloid-rich senile plaques were usually seen in areas presenting severe amyloid angiopathy. Distinction between amyloid-rich senile plaques and „plaque-like angiopathy" was often difficult because of blurring of the central capillary by amyloid deposits. In the molecular layer of the cerebellar cortex only small drop like amyloid deposits along capillaries were found (Fig. 1c).

The results of semiquantitative estimation of the histochemical reactions are given in Table 1. The intensity of staining varied somewhat in different areas of the same brain (in the range of 1–2+) but the average intensity of staining showed little variation from one brain to another.

We found no differences in reactivity for tryptophan, tyrosine and alcian blue between the amyloid core of senile plaques and the amyloid deposits of plaque-like angiopathy. In some areas, tryptophan staining of amyloid contain-

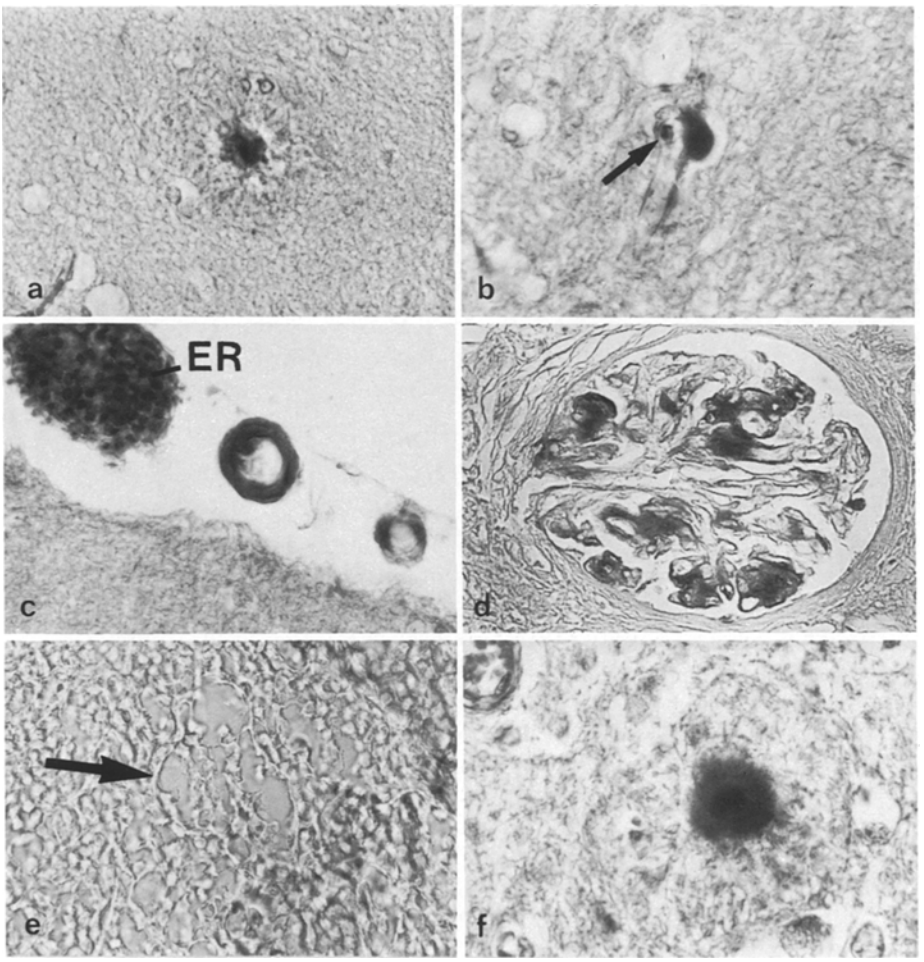

Fig. 2. a Case 3. Senile plaque core with presence of tryptophan. Section 10 µm DMAB-nitrite method (× 320). **b** Case 4. "Plaque-like angiopathy" of a cortical microvessel. The amyloid deposits stain as intensely as an erythrocyte (*arrow*). Section 10 µm DMAB-Nitrite method (× 500). **c** Case 6. "Congophilic angiopathy" of a smal leptomeningeal vessel. The vessel wall stains as intensely for tryptophan as erythrocytes (ER). Section 10 µm DMAB-Nitrite method (× 400). **d** Case 13. Secondary amyloidosis. Strongly positive reaction for tryptophan in amyloid deposits in a glomerulum. Section 10 µm DMAB-Nitrite method (× 200) **e** Case 9. Absence of tryptophan reaction in stromal amyloid (*arrow*) of medullary thyroid carcinoma. Section 10 µm DMAB-Nitrite method (× 400). **f** Case 3. Senile plaque core showing high tyrosine reactivity in it's center. Section 10 µm Diazotation coupling (× 500)

ing leptomeningeal (Fig. 2c) and perforating vessels was slightly more intense than in amyloid of senile plaques and plaque-like angiopathy (Fig. 2a, b).

Amyloid core of senile plaques showed a target-like appearance in the tyrosine stain due to a high tyrosine reactivity in their center (Fig. 2f). Tryptophan and tyrosine reactions were entirely negative in brains fixed in formaldehyde for 14 days. This can be explained by the deleterious effect of prolonged fixation

on these reactions (Powers and Spicer 1977). Tryptophan staining of the amyloid of medullary thyroid carcinoma was entirely negative (Fig. 2e), while tryptophan containing amyloid deposits of secondary amyloidosis in the kidney (Fig. 2d) and large bowel were stained more intensely than that present in senile plaques and plaque-like angiopathy.

The alcian blue reaction displayed a considerable variability in all types of amyloid deposits. There were no appreciable differences in alcianophilia between cerebral amyloid, endocrine and other forms of amyloid deposits. Following $KMnO_4^-$ treatment, only the secondary amyloid lost its Congo red affinity and its green birefringence in polarized light. Senile plaque amyloid, kuru-like plaques and congophilic angiopathy were $KMnO_4^-$ resistant (Fig. 1f–1).

Amyloid in senile plaques and in the wall of cerebral vessels gave a moderately strong yellow autofluorescence while amyloid of medullary carcinomas of the thyroid was negative.

Discussion

At present the chemistry of amyloid occurring in the brain is not known. The approach to this problem has been essentially histochemical, but the results of studies have often been contradictory. Katenkamp and Stiller (1973), using polarized light microscopy after diverse histochemical treatments, found variable behaviour of amyloid deposits of senile plaques, some resembling primary amyloid and others secondary amyloid. Nikaido et al. (1971) found the amino acid composition of amyloid of an enriched senile plaque fraction to be different from that of a control secondary amyloid of the spleen. Powers and Spicer (1977), using carefully controlled histochemical methods, concentrated on specific reactions for aromatic amino acids and demonstrated a paucity of tryptophan and tyrosine in senile plaque amyloid. They found an almost total lack of tryptophan reactivity in their reference cases of endocrine amyloid, thereby confirming the results obtained by Pearse et al. (1972). By contrast, AA and AL amyloid gave a strong reaction for tryptophan and tyrosine. The authors concluded that senile plaque amyloid is histochemically similar to APUD amyloid. However, using similarly controlled histochemical methods we found that the amyloid present in senile plaques and in angiopathy differs from endocrine amyloid because of its consistently more intense tryptophan and tyrosine reaction. Furthermore, senile brain amyloid gave an autofluorescence, whereas amyloid of endocrine origin was entirely negative. The interpretation of Powers and Spicer (1977) is compatible with a derivation of senile plaque amyloid from tryptophan-poor brain constitutents, such as normal or degenerated neurotubules (Davison and Huneeus 1970) but it can hardly explain the origin of the associated amyloid deposits of the brain vessels, which are most likely of serum origin.

We found a persistent affinity of senile brain amyloid for congo-red dye after exposure to permanganate and dilute sulfuric acid. According to Wright et al. (1977) and Van Piiswijk and van Heusden (1979) this affinity is lost only in the presence of AA amyloid (that is, in secondary amyloidosis and in familial mediterranean fever). We, therefore, assume that AA amyloid is not a major constituent of cerebral amyloid.

Recently, immunoglobulin G was localised to the central core and amyloid angiopathy by fluorescent antibody and immunoperoxidase techniques (Ishii and Haga 1975, 1976). This suggests that amyloid fibrils in senile plaque and amyloid angiopathy are of immunoglobulin origin. However, Katenkamp et al. (1970), using fluorescence-immune histochemical method, found globulins in a small percentage of senile plaques only. The positive reaction was thought to be due to an exudation of serum in plaques intimately associated with blood vessels. It is therefore possible that immunoglobulins are a facultative constituent of cerebral amyloid and not the major component of its protein fibril.

Another possibility is the presence of a fibrillar protein of yet unknown chemical composition as a major constituent of brain amyloid. This hypothesis is sustained by the finding of a unique fibrillar protein, not immunologically cross- reacting with other known amyloid proteins in senile cardiac amyloid (Westermark et al. 1977, Cornwell et al. 1978). The chemical composition of amyloid associated with aging could therefore partially depend on its localisation.

More precise information on the chemistry of senile amyloid in the brain will be available with the development of more sensitive immunological and biochemical methods.

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