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Corpus Amylaceum (Polyglucosan Body) in the Peripheral Olfactory System

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The peripheral part of the olfactory system (bulb and tract) was investigated for the occurrence of corpus amylaceum (CA) (polyglucosan body) in 296 (281 pathological and 15 control) autopsied human brains. No significant differences were found respect to the incidence between the various age groups above 40 or between the different disease groups and the controls. The predominance of CA in the olfactory tract and its loose correlation with the over 40 age group at this localization could be, the result of various factors, including the extremely rich astrocytic and capillary network in the intermediate zone, as well as the proximity of the olfactory tract to the external environment, which may result in the protective role of CA. The role of stress was proved by the HSP-60 positivity of CA. (Pathology Oncology Research Vol 4, No 3, 212–216, 1998)

Key words: corpus amylaceum, olfactory tract

Introduction

The olfactory tract (OT) is one of the predilection sites for corpus amylaceum (CA). Previous studies have revealed that the quantity of CA at certain sites is related to aging, the volume of the cerebrospinal fluid, recurrent disturbances of various barriers and different diseases of the spinal cord (amyotrophic lateral sclerosis, multiple sclerosis, etc).[8,10,12,14] Certain conditions, such as diabetes mellitus, favour the development of CA.[13] Correlation has been observed between age and the incidence of CA at almost all predilection sites in a study of 1409 cases, but it was not found to be strong for the olfactory system (OS).[10] Since this finding was somewhat surprising, different parts of the OS were studied in search of an explanation.

The olfactory bulb (OB) and OT are a special appendage of the brain (the smelling organ). The olfactory nerve comes from the olfactory epithelium of the nasal mucosa involving the smelling receptors. Hundreds of axons wrapped into single Schwann cells run through the holes of the cribriform plate of the cranium. The first synapses in the OB form a rather complex neuronal network (small and large mitral cells, tufted cells and granule cells), the functional significance of which is not understood. The axons of the neurons constituting the OT are arranged into medial and lateral striae, with no connection to the trigonal area. The anterior olfactory nucleus is always found at a distance of 1.0-1.5 cm caudally from the OB and may present itself as a retrobulbar nucleus.[2] A few small groups of scattered neurons are sometimes detected near the anterior olfactory nucleus. The striae advance to different parts of the limbic system via interneurons forming rich connections. In humans, olfaction plays an important role in the secondary and even tertiary motivations, meaning a psychological link between the olfactory and limbic functions. For this reason, pathological examination of the peripheral part of the OS may yield important morphological and physiological information facilitating a better understanding of the aging process, the role and signíficance of stress proteins (HSP-60) and other processes.[5]

It has already been found that – *a*. CA is a special element of the central nervous system, mainly in humans;

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***Table 1.* Occurrence of CA in the OT in various diseases**

	Grade					
Diagnosis	*0*	*1*	*2*	*3*	*4*	*Total*
Infarct	–	2	8	31	25	66
Vascular dementia	–	–	–	3	27	30
Haemorrhage	–	–	8	9	13	30
Aneurysma rupt. + SAH	–	2	5	7	12	26
Parkinson disease	–	–	–	3	3	
Multiple sclerosis	–	–	–	–	2	2
Amyotrophic lat. sclerosis	–	–	–	1	1	2
Alzheimer disease	–	–	1	2	5	8
Senile dementia Alzh. t.	–	–	4	2	16	26
Trauma ac.	–	3	2	13	13	31
Cerebral tumor	1		6	7	9	23
Inflammatory disease		–	–	4	2	6
Chronic alcoholism	–	–	2	4	6	12
Others	6	–	1	3	6	16
Total						281

ordinarily occurring in relation to aging, but with certain chronic vascular, degenerative and demyelinating disease processes promoting its development. A simple method for semiquantitative determination of CA is available.[10] *b.* CA is most frequently formed in the cytoplasm of the astrocytes and also in processes, e.g. around vessels, subpially,

***Table 2.* Sex and age distributions of the pathological cases and controls studied**

	Pathological cases						
Sex / age	*>39*	*40–49*	*50–59*	*60–69*	*70–79*	*80<*	*Total*
Females	16	15	17	17	30	20	115
Males	9	29	35	43	31	19	166
Total	25	44	52	60	61	39	281
	Controls						
Females	1	–	–	–	–	–	1
Males	–	2	4	4	3	1	14
Total							15

characterized by a similar mechanism as for other inclusion bodies.[1,3,7,11,17] Regarding chemical properties, CA belongs to the polyglucosan-glycan (mucopolysaccharid) complexes containing 4% protein.[15,18,20] PAS staining is the best, but silver impregnation has also proved to be useful in certain cases in revealing CA.[4] There is a search regarding stress proteins, such as ubiquitin and many types of HSPs.[4,16]

Materials and Methods

296 autopsied human brains (281 pathological and 15 control cases) were used to obtain different parts of the peripheral OS. Both the OB and OT were removed with or without the trigonal area in 30% of the cases, whereas only one or the other was removed in the remaining 70%. The brains were randomly selected from routine brain autopsy material involving different diagnoses and death causes *(Table 1)*. Age and sex distribution are shown in *Table 2*. The 15 controls had suffered a fatal acute trauma of the brain without any previous central nervous system disease. The OB and OT were carefully separated from the sulcus olfactorius, embedded in paraffin and generally cut in longitudinal planes; however, sometimes various OT segments were examined in several transverse planes. The longitudinal preparation was difficult due to the variable thickness of the individual parts of the OS *(Figure 1)*. Tissue samples of 12–24 μm thickness were stained with haematoxylin + PAS and cresyl violet, as well as with the Woelcke or Spielmeyer myelin methods and were impregnated with silver according to Bielschowsky (neurons and axons), Hortega (microglia) and Gallyas (astrocytes). NSE (neuron specific enolase) was also applied to prove the neuronal character of the cells in the OT. The GFAP (glial fibrillary acidic protein) revealed large numbers of astrocytes, but was negative for CA.[12] No differences were found between the various age ranges concerning the structure and staining properties of the CA. Only the size of the CA displayed some systematic variation, a smaller CA being characteristic under the age of 40. As CA showed a positive reaction with mouse anti-HSP-60 monoclonal antibody, this was also used in certain cases. The HSP-60 positivity displayed similar intensity as in the case of PAS staining.[17]

CA was evaluated semiquantitatively on different segments of the OS.[12] *(Figure 1)*. Samples containing large quantities of CA did not allow for exact counting, impossible and the samples were classified as grade 4.

Results

In the OS, CA showed a distribution similar to the other predilection sites in the various age groups. Grade 4 occurred practically with the same frequency in all of the above 40 age groups *(Figs 2 and 3)*. A relatively low number of CAs appeared over the age of 70 *(Figure 4. a and b)*.

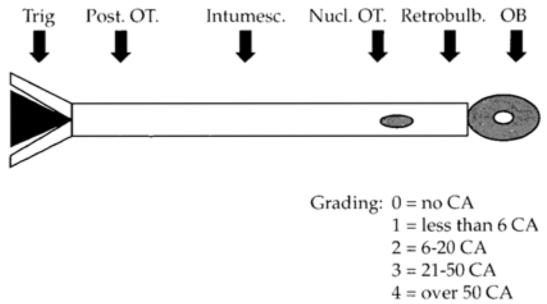

Figure 1. *Different parts of the peripheral OS examined for CA in longitudinal or transversal sections, and the grading system.*

The distribution of CA in the different parts of the OS and in the various segments of the OT showed considerable variation. Grades in the OB were very similar to those in other cortical areas of the brain, with the exception of the pyramidal cell layer of the Ammon's horn and piriform cortex. There were differences between the individual segments of the OT as concerns the anterior, medial and lateral levels. However, this seemed to be uniform in all cases, i.e. the sites of predilection for CA were constant.

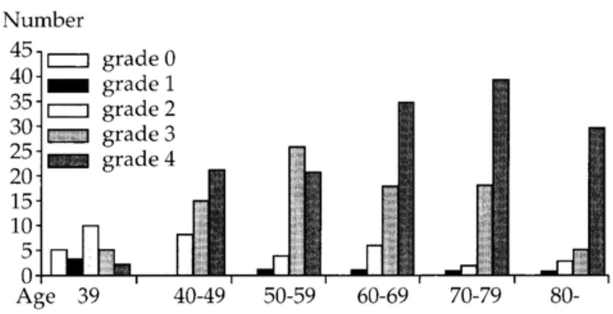

Figure 2. *Age-related distribution of the different CA grades.*

These sites were localized in the following parts of the OT: 1.0-1.5 cm caudally to the OB, a small intumescence in the middle of the OT, and the thinnest segments before the trigonal area. The arrangement of CA in the transverse sections exhibited some variation. A majority of the bodies occurred mainly in the central (intermediate) part of the OT and in the subpial zone. Similar zonal distribution was only observed above 70 years of age. Subpial localization was rather rare in younger age groups.

The intermediate zone between the two striae proved to be a special predilection site for the development of CA; this zone was characterized by a richness in capillaries and the perivascular arrangement of CA. It displayed less ordered myelin sheaths and fibrous astrocytes. When a large amount of CA was found in this zone, the myelinated fibres were decreased in number and the remaining ones were degenerated, with granular and swollen appearance. The trigonal part had the same distribution as was found in the OB, i.e. CA could hardly be seen between the neurons, in the glomeruli or in the cortical areas. The axons and glia impregnated by silver demonstrated a rather complex structure of fibres. A transverse arrangement of axons appeared besides the longitudinal pattern; which could possibbly provide further anatomical proof of the interaxonal and retrograde feed-back systems in the OT.

Occasionally the anterior olfactory nucleus contained the same amount of CA as in the OB. The bulbar part sometimes extended to a certain distance into the OT (0.5 cm), but never with a direct connection with this nucleus or with other smaller groups of neurons in the OT.

A search for senile plaques and neurofibrillary tangles was accomplished in a few Alzheimer disease cases or very elderly persons. These were found in the OB as well as the nucleus of the OT, in the same proportion as in the entorhinal areas and in the Ammon's horn.

The relatively low number of patients in the different disease groups involved in this study *(Table 1)* did not permit the correlatation of the occurrence of CA with definite lesions of the central nervous system. However, the predominance of vascular diseases in the material may be a further indication of an enhanced tendency for the development of CA in these diseases, as suggested earlier.[10]

One outstanding phenomenon was the positive immunostaining of CA for HSP-60, which exhibited the same constancy and intensity as for PAS staining. This must be taken into consideration when explaining the frequent occurrence of CA in the OS *(Figure 5.a and b).*

Discussion

The more frequent occurrence of CA in the peripheral OS even at younger age as compared with other predilection sites may be explained by a number of factors. The OT is characterized by an extremely rich astrocytic fibre

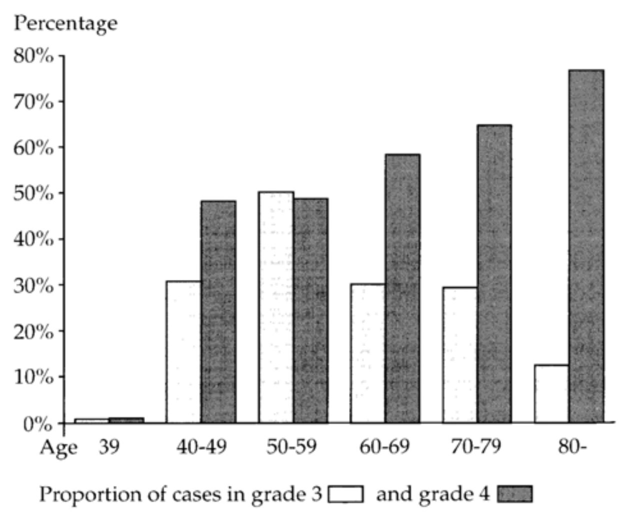

Figure 3. *Percentage of CA – Grade 3 and 4 – in various age groups.*

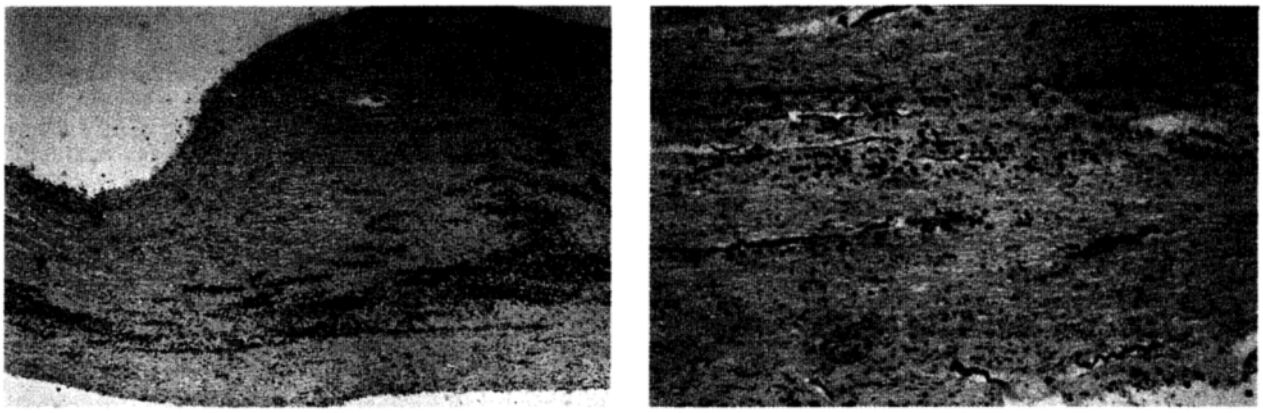

Figure 4. a. Grade 4 CA in a 44-year-old woman (cause of death: subarachnoideal haemorrhage caused by a ruptured aneurysma). b. Grade 2 CA in a 72-year-old woman (cause of death: vascular encephalopathy. PAS+haematoxylin

network and a degenerative process can therefore induce more and more CA in the cytoplasm of these cells. The intermediate zone in which the highest amountig CA occured supports the development because it contains many capillaries enveloped by a thick ring of astrocytic feet. This is similar in the cortical, subpial surfaces, but here the glial layer greatly predominates over the number of capillaries. The glial feet at both predilection sites probably comprise the main morphological component of the blood-brain or brain-cerebrospinal fluid barrier. If certain processes disturb the barrier function, the perivascular glia will form CA. The development of CA may be degenerative in nature, but may also play a protective role in this region. The peripheral part of the OS is exposed to many harmful environmental influences. The distance between the olfactory mucosa and the OB is only a few millimetres, much less than the other cranial nerves have from the peripheral to the central part. Another difference is the virtually direct contact with air in the uppermost part of the nasal cavity; with separatating by only the mucosa. Previous observations has clearly demonstrated the entry of viruses and bacteria or inhaled materials: As the OB and OT are protruding parts of the brain, it seems very probable for the structure of the barrier to differ from those regions where the endothelial system plays an outstanding role.

The variation in frequency of CA at the different levels of the OT may be explained by the variety in the capillarization of the different .parts of the tract. The low number or almost complete lack of CA in the OB may be due to the same barrier structure as that in the cerebral cortex, where CA occurs at a similar rate. The incidence of CA is age-related in the well known predilection sites, however this was not observed in the OT in either our control or pathological samples.

Aging and various conditions associated with recurring disturbances of the barrier functions in the perivascular, subependymal and subpial regions promote the formation of CA. Other factors, such as the greater volume of the cerebrospinal fluid, the products of degenerated parenchymal elements or the insufficiency due to the damage of fibrous astrocytes may also play role in the

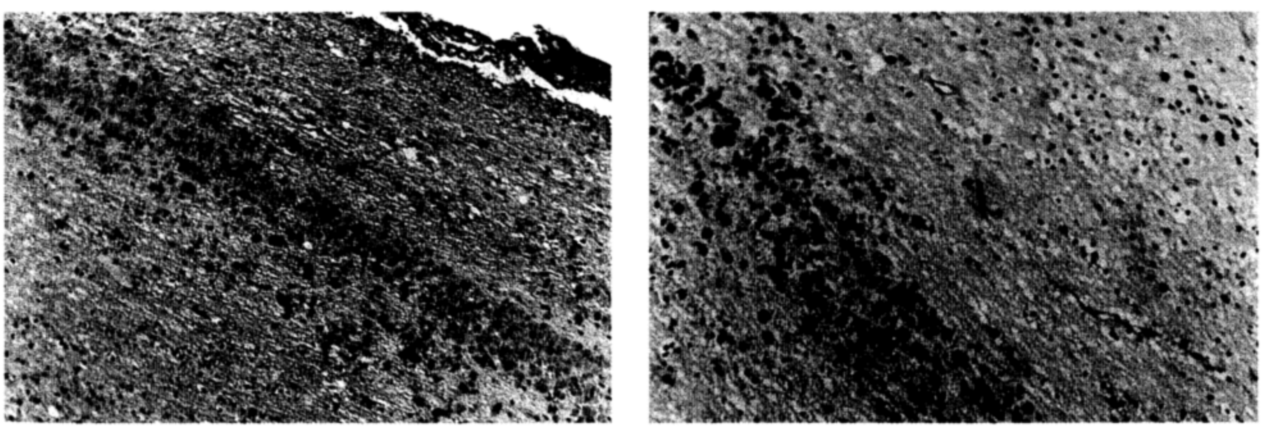

Figure 5. a. HSP-60 positivity of CA in the OT. b. Strong PAS-reaction in the same region.

development of CA. Further mechanisms may be presumed in connection with the retrograde and anterograde axonal flows in the OT.

All these factors could also exert a protective role against harmful effects gaining ready access to the central nervous system. The considerable quantity of perivascular CA observed after various noxae may be interpreted as a morphological sign of barrier disturbances; however, an "impenetrable" layer is formed later, providing protection against further harmful agents and mechanisms. This functional role of CA has been supported by Shingrao et al,[19] suggesting that CA can take part in the recognition of these immunogenic proteins by lymphocytes and microglia. The OT has an extremely rich astrocytic fibre network participating in the formation of CA as a first line of defence against harmful influences. This may explain why the age-related incidence of CA over the age of 40 does not exhibit the almost linear correlation in the OT that it does in other predilection sites. The amount of CA in the OT may also depend on the quantitative and qualitative aspects of different noxae reaching the central nervous system via the peripheral OS, and especially via the OT.

Despite being primarily polyglucosan in nature, CA showed a strong HSP-60 positivity. The factors stimulating the synthesis of stress proteins show wide variation: environmental agents (heat, heavy metals, amino acid analogues, drugs, smoking etc.), diseases (viral and other infections, fever, ischaemia, processes producing free radicals, tumours, etc.) and certain biological functions of normal cells (mitosis, growing factors and aging). The induction of CA-like inclusions by cysteamine in rats may indicate the role of harmful agents in promoting the formation of stress proteins and the development of CA.[6]

As there are ubiquitin immunoreactive structures in the normal central nervous system, positive immunostaining may similarly occur with various HSP (27, 47, 60, 70, 90 and 110) antigens. However, the present study suggests that the large quantity of HSP-60 immunoreactive CA in the OT and in other pathologically affected sites of the central nervous system may be a non-specific phenomenon. The biological role, if there is any, of the high amount of anti-HSP-60 in the CA requires further study.

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