

## Heat Shock Protein 60 in Corpora Amylacea

István GÁTI,<sup>1</sup> Lóránt LEEL-ÔSSY<sup>2</sup>

<sup>1</sup>Department of Neurology, University Medical School, Pécs, <sup>2</sup>Laboratory of Neuropathology, Department of Pathology, "St. Borbála" County University Hospital, Tatabánya, Hungary

Heat shock protein 60 representation in the corpora amylacea of the brain was investigated in five different neurological diseases. In the cases with cerebral infarct, amyotrophic lateral sclerosis, multiple sclerosis, acute disseminated encephalomyelitis and primary tumors of the nervous system the corpora amylacea showed similar appearance with strong HSP-60 positivity in all investigated disorders at the predilection sites. In the inflammatory diseases, besides corpora amylacea, several cellular elements exhibited HSP-60 immunostaining too. In these cases, the widespread HSP-60 immunoreactivity associated with relative moderate corpora amylacea production as compared to other diseases. From this contradiction we concluded the corpora amylacea participate in the cellular stress reaction but stress

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protein synthesis certainly is not the primary event in corpora amylacea formation. In the development of the corpora amylacea the incipient process is most probably degenerative in nature, which later on is accompanied by stress protein synthesis and slow growing of these round structures designated for a protective role in the brain. However, the role of the stress protein synthesis in the corpora amylacea formation and growth was not unequivocally answered in this study. It is necessary to perform further comparative investigations of the stress protein representation and corpora amylacea formation in different diseases which may help in discovering useful pathogenetic data and the biological role of this degenerative structure. (Pathology Oncology Research Vol 7, No 2, 140–144, 2001)

### Introduction

The cells of the nervous system produce peculiar materials with special morphology in the elderly, and especially in some degenerative disorders. These structures are known as Bunina,- Hirano,- Lewy-bodies, Rosenthal fibres, Corpora amylacea (CA), Alzheimer' neurofibrillary tangles, etc. The source and the role of these structures has not yet been quite clearly explained. Since these inclusions or degenerative products usually develop in the ageing brain and in some kinds of chronic degenerative brain diseases it is plausible to assume that repetitive, or chronic local cellular stress reactions might participate in their origin and development.

Heat shock proteins (HSP) are important members of the substances synthesised in the cells under stress conditions.<sup>1</sup>

These proteins are ubiquitous and, they are also present in the cells of the nervous system under normal conditions.

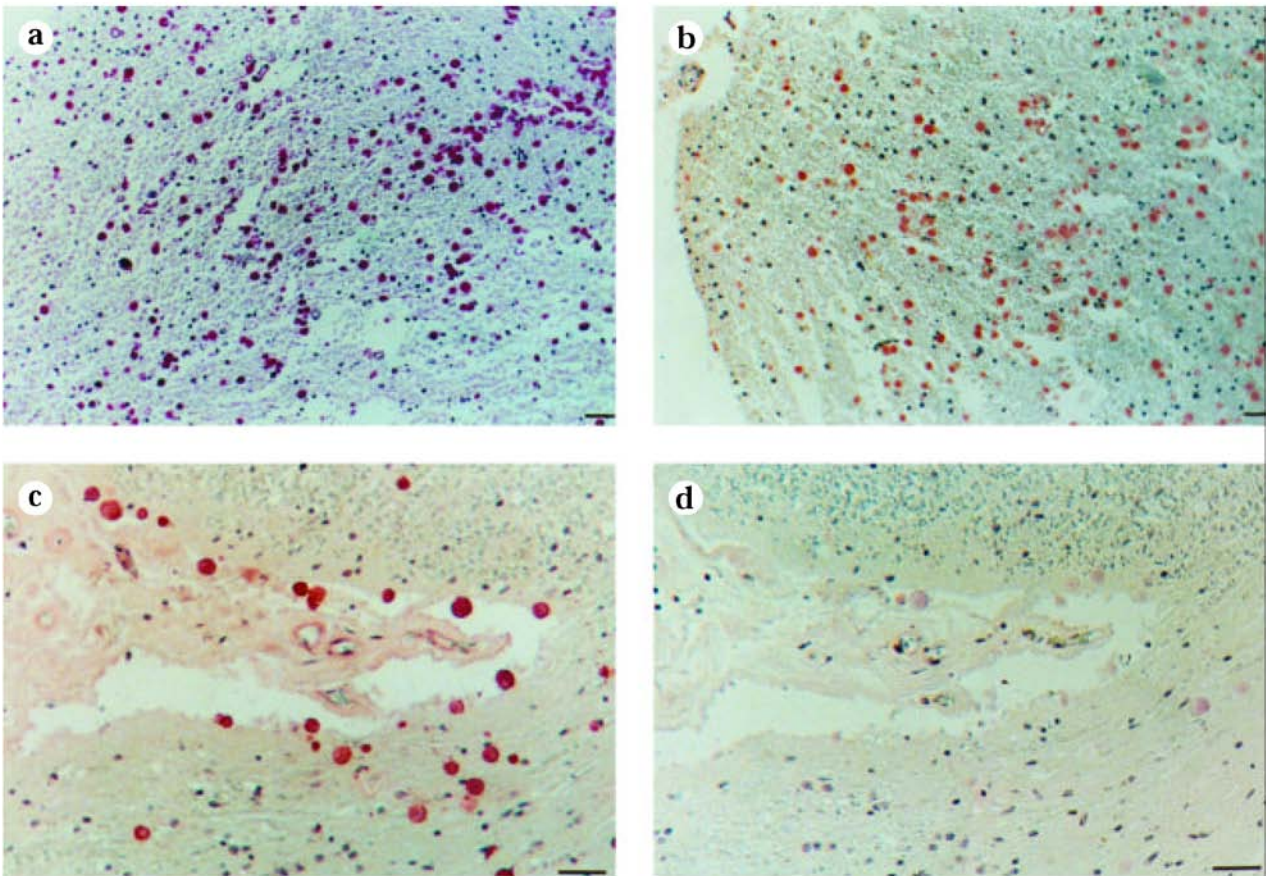
Several reports have accumulated during the last few years about their central nervous system representation and speculation arose about their significance.<sup>5,18,21,22</sup> Numerous publications analysed the relationship between the stress proteins and the degenerative products mentioned above.<sup>6,15</sup> We also investigated the heat shock protein positivity in different neurological diseases and observed that the CA were strongly labeled by HSP-60 antibody. We thought it might be worthy to demonstrate these data and that we may be able to draw some conclusions from the findings.

### Materials and Methods

The cases to be studied were selected from the brain autopsies of the Laboratories of Neuropathology of the Departments of Neurology, University Hospital Pécs, further from the University Hospital of Tatabánya and Miskolc. The brains were removed in 48 hours after death. Formalin fixation and paraffin embedding was established after the macroscopical neuropathological investigation in

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*Correspondence:* István GÁTI, MD, PhD. Laboratory of Neuropathology, Department of Neurology, University Medical School, Rét 2, H-7623 Pécs, Hungary; Tel: (36) 72 314 344



**Figure 1a.** Corpora amylacea in the olfactory tract in a case of cerebral infarct. – **1b.** Corresponding section stained with HSP-60 – **1c.** HSP-60 positive corpora amylacea in the medulla oblongata of an ALS case – **1d.** Corresponding section without use of the primary antibody (negative control). The bars represent 50  $\mu$ m.

every cases. The histological diagnosis was based on routine neuropathological staining and different impregnation methods including haematoxylin-eosin, PAS, Klüver-Barrera, Woelcke stainings and Gallyas-astrocyte, Horte-ga-microglia, Bielschowsky- axon impregnations as well as GFAP immunocytochemistry. The HSP-60 immunohistochemical reactions were performed in cases of the following disorders: 1) Cerebral infarct. 2) Acute disseminated encephalomyelitis. 3) Multiple sclerosis. 4) Glioblastoma multiforme. 5) Amyotrophic lateral sclerosis. Five cases were selected from every group for examination and randomly assorted.

The HSP-60 immunohistochemical reactions were performed by peroxidase method. The 5  $\mu$ m sections were incubated in bovine serum albumin then 3% hydrogen peroxide was applied. HSP-60 monoclonal antibody (Sigma H 4149) was administered as primary antibody in 1:250 dilution for 2 hours. only higher Butter solution was used for the negative control sections. After the primary antibody, biotinylated secondary antibody was added which was followed by the ExtrAvidin-Peroxidase reaction. The reaction was visualised by addition of an AEC

substrate mixture. The monoclonal antibody used in our studies reacts with the 383-447 amino acids of the human HSP-60 but it does not exhibit cross reaction with the stress protein of bacterial origin. The sections from different neurological diseases were investigated for HSP-60 immunoreactivity and the HSP-60 representation was analysed as a possible marker of degeneration and CA formation.

### Results

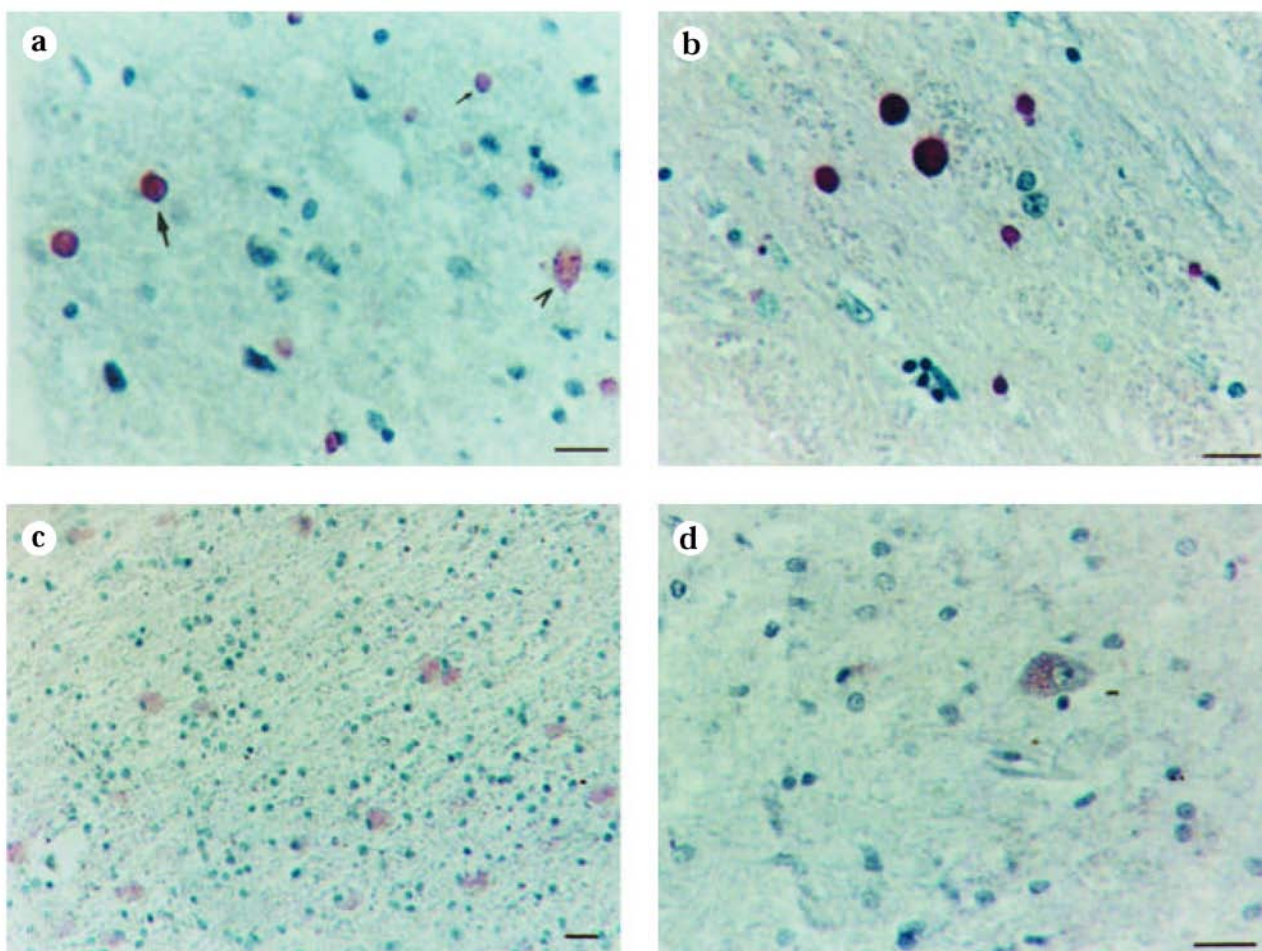
The results demonstrated unequivocal HSP-60 positivity of the CA in all samples (*Figure 1*). There were no significant differences found between the intensity of the reactions. While there was a pronounced HSP-60 positivity in the CA the immunohistochemical reaction was relatively weak in other structures of the brains, except the cases of inflammatory diseases. It was possible to detect punctuate HSP-60 representation in the astrocytes, and cytosolic HSP-60 positivity in the neurons, oligodendrocytes and inflammatory cells (lymphocytes) in the cases of multiple sclerosis and disseminated encephalomyelitis.

*Figure 2* demonstrates the HSP-60 positivity of the glial and inflammatory cells in a multiple sclerosis case (*Figure 2a*) which however correlated with a relative low density of CA in the same sample (*Figure 2b*). The special granular reaction in neurons of a case with acute disseminated encephalomyelitis seemed to correspond to lipofuscin pigment (*Figure 2c*). In the cases of cerebral infarction, besides the CA HSP-50 positivity, we were able to find only some scattered HSP-60 immunoreactive neurons and microglia. The CA showed HSP-60 positivity in the cases of amyotrophic lateral sclerosis and malignant brain tumour while there was no positive reaction noticed in other structures.

### Discussion

During the last few years data have been reported on different members of the heat shock protein families. These proteins are ubiquitous and, they must have important

roles in degeneration, immunological and malignant processes.<sup>7,9,10,11,13,14</sup> The small molecular weight ubiquitin (HSP-8-28 kD) appears during normal ageing of the brain.<sup>16</sup> In pathological conditions, this ubiquitin is accumulated in Lewy bodies, Rosenthal fibers, neurofibrillary tangles, in damaged neurons of amyotrophic lateral sclerosis, furthermore in lysosomes of the neurons in Prion diseases.<sup>6,16</sup> These data still advanced not enough to understand the role of ubiquitin, but it was postulated that this protein must be an important indicator of the proteolytic processes of the cells.<sup>4</sup> Ubiquitin was also found in the CA although another observation demonstrated the lack of CA in cases of amyotrophic lateral sclerosis.<sup>8</sup> Lowe and his coworkers found understandable that – in spite of the low protein content of CA (4%) – stress proteins might be important components of these inclusions which proved to be astrocytic in origin.<sup>3</sup> They referred to the observation of others who demonstrated some homology with ubiquitin of CA protein components by using HPLC method.<sup>4</sup> The



**Figure 2a.** Corpora amylacea in the pons of a multiple sclerosis case (arrow) and weak HSP-60 reaction of the inflammatory cells (small arrow). – **2b.** Several CA in the white matter in multiple sclerosis. – **2c.** HSP-60 immunoreactivity of oligodendrocytes in acute disseminated encephalomyelitis. – **2d.** Granular HSP-60 positivity of the neurons of the cerebellar dentate nucleus in acute disseminated encephalomyelitis. The bars represent 50  $\mu$ m.



other main stress protein family HSP-70, -72 also were represented in the central nervous system.<sup>2,3,22</sup> These stress proteins have also been found in the CA.<sup>15</sup> The findings attributed protective role to this stress protein family for cell survival in different stress conditions. These stress proteins seem primarily to protect against the malignant cell transformation and tumour growth.<sup>14,20</sup>

Our knowledge has also increased on the HSP-60 stress protein family during the last few years. It has been suggested that this stress protein is basically bound to the mitochondrial proteins<sup>17</sup> in stress conditions and they participate mainly in the chronic inflammatory processes.<sup>19</sup> A few data were only found in the literature on the HSP-60 representation in the CA.<sup>10</sup> In our material, we were able to demonstrate strong HSP-60 positivity in CA in all investigated diseases. Besides the strong HSP-60 immunoreactivity of the CA we could also demonstrate positive reactions in the cellular elements of the inflammatory diseases. The HSP-60 positivity of the cellular elements in our cases correspond to the data of the previous publications.<sup>3,19</sup> However the CA formation in cases of the multiple sclerosis and in the acute disseminated encephalomyelitis did not correlate with the obvious HSP-60 immunoreactivity since these inflammatory diseases displayed a relative low CA density as compared to other disease processes. The abundant CA occurrence was not associated with obvious HSP-60 representation in other investigated cases. This finding may indicate that stress protein synthesis is probably not a primary event in the development of the CA. We were able to demonstrate HSP-60 positivity in the astrocytes in cases of multiple sclerosis in contrast to the other diseases, but this phenomenon was not accompanied by enhanced formation of CA.

It has been well documented that corpora amylacea have no pathognostic significance, but that they accumulate in certain conditions and pathological processes.<sup>11,12</sup> CA is especially enhanced in the ageing brains, in the processes, which may cause blood-brain barrier disturbances, especially in chronic vascular diseases, diabetes mellitus. The other specific property of CA that they develop mainly at some predilection sites which are in the proximity of structures possessing barrier function perivascular space, subpial and subependymal localizations. Several publications analysed the structure of the CA. Their rich acid polysaccharide content make them best demonstrable by the PAS stain. As has been mentioned above, CA is thought to be derived from astrocytes. Certainly many different factors contribute to the formation and development of these structures, as the components of the degraded cells, metabolites originated from the cerebrospinal fluid, blood and even from the mesenchyma of pia mater and adventitia of the vessel wall.<sup>10</sup> However, although a few communications demonstrated ubiquitin and HSP-60 positivity of CA, previous publications also emphasised the surprising

lack of their immunoreactivity by using many other different antigens.<sup>11</sup> Our recent observations suggested an unequivocal HSP 60 representation in corpora amylacea. It appeared that the stress protein synthesis probably not a primary event in the CA formation but a secondary process induced by some products of a degenerative process (ageing, amyotrophic lateral sclerosis etc.) or by a recurrent functional disturbances of the barriers. These data definitely indicate that CA participate in the stress reaction. Can we suppose that the astrocytes may synthesize stress proteins during their degeneration induced by some pathological conditions which increase further in the well developed CA?

The stress protein positivity of CA arises several questions regarding the role and significance of these structures which have yet remained unanswered: Why is HSP-60 confined so much to the CA irrespective of the pathological processes? Why was a relative lack of the HSP-60 positivity found in other structures of the central nervous system such as neuron, myelin and axon especially in chronic, non inflammatory diseases where they appeared in large quantities. It seems the stress protein reactions do not explain the origin and source of CA by themselves. Future extended experiments should be done to provide a better understanding of the relationships of the stress protein reactions and the development of the degenerative brain products, which also might help to understand the biological role and significance of the CA. These further investigations may also be helpful in the better understanding of the ageing and the pathogenesis of different neurological diseases.

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