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ORIGINAL ARTICLE

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Immunolabelling of hippocampal microvessel glucose transporter protein is reduced in Alzheimer's disease

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Abstract Changes in cerebral microvessel ultrastructure have been reported to occur in Alzheimer's disease (AD). In order to investigate whether these changes are associated with compromised blood-brain transport mechanisms, hippocampal formation sections from AD and age-matched normal brains were immunolabelled with an antibody to the GLUT-1 glucose transporter protein. GLUT-1 immunolabelling of microvessel endothelium was significantly reduced in the AD compared to normal hippocampal formation. Thus, AD is associated with a reduction in cerebral microvessel endothelium glucose transporter content, which may result in decreased glucose availability to the brain.

Key words GLUT-1 Glucose transporter protein Immunohistochemistry · Microvessel endothelium Hippocampus · Alzheimer's disease

Introduction

Alzheimer's disease (AD) is a progressive dementing disorder principally affecting those members of the population over 65 years of age [17]. Changes in cerebral microvessel ultrastructure have been reported to occur in AD, including thickening [11, 15] and layering [15] of the basement membrane, the loss of perivascular glial processes [13] and a diminution of nerve plexi [16]. These ultrastructural changes are likely to compromise the blood-brain barrier functions of cerebral microvessels and may result in the deposition of aluminium [2] and serum proteins [20] in the parenchyma of AD brains.

Cerebral microvessel endothelial cells are known to have important carrier functions which include the active transport of glucose from the blood to the brain, through a barrier which is impenetrable to the passive diffusion of polar, water-soluble substances. The brain requires vast amounts of glucose per day and consequently a hexose transport system exists in its microvessel endothelial cells, to facilitate the entry of this substrate of oxidative metabolism into the brain parenchyma [3]. The glucose transporter system of cerebral microvessel endothelial cells is stereo-specific for D-glucose [1, 10], saturable [10] and non-energy dependent [1]. The GLUT-1 isoform accounts for all glucose transporter binding sites at the blood-brain barrier and is expressed selectively in microvessel endothelium with minimal, if any, expression in neurones or glia [14].

Significant reductions in cerebral oxidative metabolism have been demonstrated in vivo in the brains of clinically diagnosed AD patients, using positron emission tomography (PET) scanning [7]. These reductions are most prominent in those cortical regions that are most affected by AD pathology, namely the temporal and parietal lobes [7]. In addition, dynamic PET scanning has been reported to show a significant decrease in the transport of glucose from blood to brain in temporal and frontal regions of the brain in AD compared to controls [8]. The concentration of cerebral glucose transporter measured by [^{3}H] cytochalasin B binding, has also been reported to be significantly reduced in isolated frontal cortex microvessels and whole samples from frontal, temporal and hippocampal cortex of AD compared to control brains [9]. The present study was designed to investigate further the effect of AD on the brain glucose transporter system, utilising immunohistochemistry. The hippocampal formation was the brain region selected for investigation because it is heavily implicated in the pathology of AD [5] and has been suggested to be the primary site of the disease's pathogenesis [12].

Materials and methods

Brains were obtained at necropsy from nine patients with dementia (age range 66–95 years, mean=82 years) clinically assessed using the criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the AD and Related Disorders Association [18]. The histological diagnosis of AD was subsequently established by the presence of hippocampal and neocor-

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Table 1 Mean intensity scores of GLUT-1 immunolabelling (plus individual section scores) of each hippocampal formation, together with subject details (*M* male, *F* female, *AD* Alzheimer's disease)

Brain		Age (years)	Sex	Post mortem delay (h)	GLUT-1 immunolabelling intensity		Cause of death
H1	AD	66	F	24	3	(3,3)	Bronchopneumonia
H2	AD	83	F	12	3	(3,3)	Bronchopneumonia
H3	AD	84	F	72	1	(1,1)	Bronchopneumonia
H4	AD	95	F	50	1.5	(2,1)	Bronchopneumonia
H5	AD	82	M	–	2.5	(2,3)	Bronchopneumonia
H6	AD	74	M	40	3	(3,3)	Bronchopneumonia
H7	AD	83	F	–	1	(1,1)	Chronic bronchitis
H8	AD	86	F	24	1	(1,1)	Bronchopneumonia
H9	AD	81	F	–	2	(2,2)	Bronchopneumonia
H19	Control	80	M	24	3.5	(3,4)	Coronary atheroma
H20	Control	68	M	24	4	(4,4)	Congestive cardiomyopathy
H21	Control	83	M	48	2	(2,2)	Aortic aneurysm
H22	Control	75	F	24	4	(4,4)	Myocardial infarct
H23	Control	78	F	43	3.5	(3,4)	Myocardial infarct
H24	Control	95	F	24	3	(3,3)	Coronary atheroma
H25	Control	84	M	24	3	(3,3)	Pulmonary embolism
H26	Control	78	M	18	2	(2,2)	Chronic bronchitis

tical senile plaques and neurofibrillary tangles [19]. Brains were also obtained from eight subjects (age range 68–95 years, mean=80 years) with no history of dementia and with no abnormality on histological examination of the brain. Details of the age, sex, cause of death and post-mortem delay for each subject are given in Table 1.

The brains were fixed by immersion in neutral formalin solution and the central part of each left hippocampal formation (dentate gyrus, cornu ammonis and subicular complex) was dissected out. Each tissue block was trimmed to approximately 1 cm^3 and processed into paraffin wax. Serial 10 μm sections were then cut perpendicular to the long axis of the hippocampus. Two sections, 100 μm apart, were taken from each brain and mounted onto glass slides. The sections were dewaxed in xylene, immersed in absolute alcohol for 1 min, 0.75% hydrogen peroxide in absolute methanol for 30 min and two changes of phosphate buffered saline (PBS) at pH 7.2 for 3 min each. They were then incubated in normal swine serum diluted 1:5 in PBS, for 15 min at room temperature. Rabbit anti-human GLUT-1 serum, raised against the carboxy-terminal region of GLUT-1 residues 477–492 [4] diluted 1:500 in PBS, was subsequently applied and the sections incubated overnight at 4° C. The sections were then rinsed in PBS and incubated with biotinylated swine anti-rabbit IgG (Dako, Buckinghamshire, UK) diluted 1:100 in PBS for 30 min at room temperature. They were then rinsed in PBS and incubated in peroxidase conjugated streptavidin (Dako) diluted 1:200 in PBS for 30 min at room temperature. After rinsing in PBS, the sections were incubated in a solution of 0.05% diaminobenzidine tetrahydrochloride and 0.01% hydrogen peroxide in PBS for 8–10 min, rinsed in running tap water and counterstained with haematoxylin. The sections were then dehydrated through a series of graded alcohols, cleared in xylene and mounted in DPX. A negative control section, treated by substituting normal swine serum for the primary antibody, was included in every "run".

The sections were coded so that the investigator was unaware of their previous history and examined in a Nikon Optiphot light microscope using a ×10 objective. The whole cross-section of the hippocampal formation was viewed and the overall intensity of the immunoreaction was scored semi-quantitatively. The intensity of GLUT-1 immunolabelling in each section was assigned one of the following scores: 1) negligeable or very weak labelling, 2) weak labelling, 3) medium labelling and 4) strong labelling. The sections were then decoded and the mean staining intensity score calculated for each hippocampal formation.

Mean GLUT-1 immunolabelling intensity scores of AD and control hippocampi were compared by means of a Mann-Whitney U test. A Spearman rank correlation analysis was subsequently performed on the mean intensity scores to investigate their possible relationship to post mortem delay.

Results

GLUT-1 immunolabelling was observed only in microvessel endothelial cell walls and was never observed in sections incubated with normal swine serum instead of primary antibody. In sections from both normal and AD hippocampi, immunolabelled microvessel density appeared greatest in the molecular layers of both the cornu ammonis and dentate gyrus. The stratum pyramidale and stratum oriens of the cornu ammonis also exhibited a marked immunolabelled microvessel density. The stratum radiatum and stratum lacunosum of the cornu ammonis and the stratum granulosum of the dentate gyrus exhibited a typically low density of immunolabelled microvessels. In general, immunolabelled microvessel density appeared greater in CA2 and CA3 than in the other fields of the hippocampal formation. Although the density of GLUT-1 immunolabelled microvessels varied with the layers and regions of the hippocampal formation, the intensity of microvessel endothelial cell immunolabelling was uniform throughout an individual section and identical or very similar between sections from the same brain. The mean and individual GLUT-1 immunolabelling intensity scores for each hippocampal formation are given in Table 1. Overall, GLUT-1 microvessel endothelium immunolabelling intensity was significantly lower ($U_{9,8}$=12 P=0.02) in the hippocampal formation of AD compared to normal age matched brains (Fig. 1). There was no significant correlation between mean immunolabelling intensity score and post mortem delay (r=–0.32,

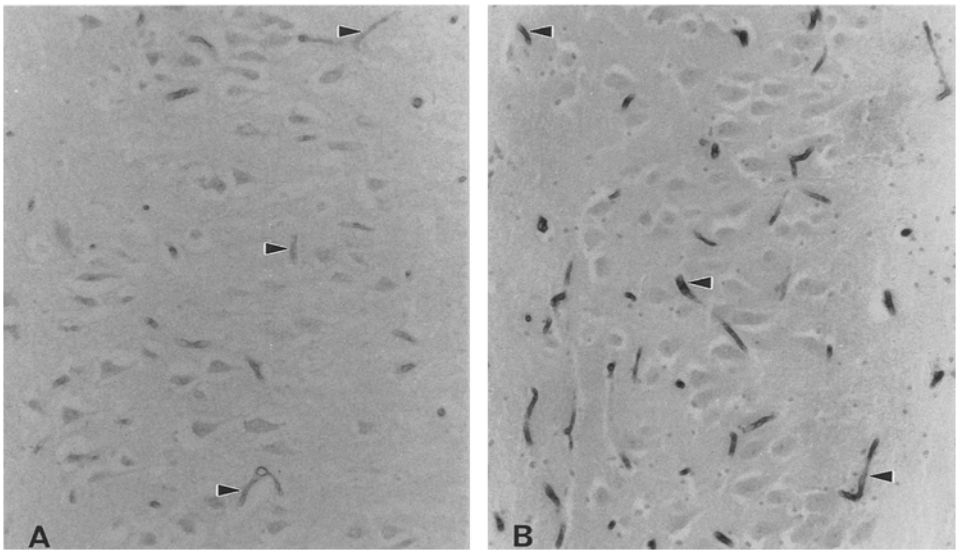

Fig. 1 Photomicrographs illustrating GLUT-1 immunolabelling of CA1 hippocampal microvessels from (**A**) Alzheimer's disease (subject H8) and (**B**) elderly normal (subject H19) brain. *Arrowheads* indicate microvessels. Magnification ×600

P=0.25). Although it is not strictly appropriate to average semi-quantitative data, we consider that the use of the non-parametric statistical analysis based on 'ranking' presented above is justified because: 1) in 13 out of 17 cases the two sections received the same score, 2) in those cases where two identical scores were not assigned, they were only one category apart and it is reasonable to assume that the 'true' score lay between the two individual scores.

Discussion

The reduction of GLUT-1 immunolabelling in the hippocampal formation of AD brains observed in the present study, is likely to reflect a disease related decrease in the amount of glucose transporter protein present in the microvessel endothelium of AD compared to age-matched normal brains, since the intensity of GLUT-1 immunolabelling was not related to post mortem delay. This finding is in agreement with the previously reported reduction of hexose transporter protein, identified by reversible and covalent binding of [^{3}H] cytochalasin B, in isolated AD microvessels and brain tissue [9]. Decreased glucose metabolism has also been reported to occur in the temporal cortex of clinically diagnosed AD patients in vivo [7]. However, the current study is to our knowledge the first to use immunohistochemistry to label GLUT-1 in cerebral microvessel endothelial cells.

Although glucose transport across the blood-brain barrier is not thought to be rate-limiting for metabolism under normal conditions [1], it may be in some disease states and thus, a decrease in microvessel glucose transporter protein would limit the entry of glucose into the brain for oxidative metabolism. Therefore, the reduction in glucose transporter content of AD cerebral microvessel endothelium demonstrated in the present study, may underlie the reduction in glucose transport across the blood-brain barrier reported to occur in the disease in vivo [8].

The immunocytochemistry and light microscopy employed in the present study, did not allow differentiation of the "sub-populations" of GLUT-1 transporter proteins that have been shown to be differentially distributed across the cerebral microvessel endothelial cell [6]. An estimated 40% of the total endothelial cell GLUT-1 protein has been shown to lie within the cytoplasmic space [6] and may represent a reserve pool of transporter protein. Therefore, it is important to determine whether the deficit observed in the present investigation affects the cytoplasmic or plasma membrane compartments, or both.

It is not clear whether the reduction in AD microvessel endothelial cell GLUT-1 demonstrated in the present study, is primary or secondary to the disease process. However, the effect is likely to be deleterious in either case. If a reduction in microvessel endothelial cell GLUT-1 is a primary event in the pathogenesis of AD, reduced glucose availability to the brain may initiate some of the neuronal destruction that occurs. If it is a secondary event, it may serve to exacerbate neurodegeneration.

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