Functional Alterations in Alzheimer's Disease: Decreased Glucose Transporter 3 Immunoreactivity in the Perforant Pathway Terminal Zone

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Abstract. Positron emission tomography (PET) studies measuring glucose utilization have demonstrated cerebral hypometabolism in Alzheimer's disease (AD). The anatomic and biochemical basis for this observation remains unknown. We have examined the distribution in the hippocampal formation of the neuron-specific glucose transporter 3 (Glut3) protein. Using quantitative immunohistochemistry, we find a large reduction (49.5%) in Glut3 immunoreactivity in the outer portion of the molecular layer of the dentate gyrus in AD brains. This region corresponds to the terminal zone of the perforant pathway, whose cells of origin in layer II of the entorhinal cortex are selectively destroyed in AD. Because glucose uptake reflects metabolic demand, these results suggest a decrement of functional activity in the deafferented dentate gyrus granule cells. Generalizing from this observation, decreased glucose uptake seen on PET studies may reflect, in part, decreased glucose transport and utilization in functionally deafferented cortical fields.

Key Words: Alzheimer's disease; Cerebral metabolism; Glucose transport; Hippocampal formation; Immunohistochemistry.

INTRODUCTION

Studies of glucose transport and utilization show that glucose influx varies in different cerebral areas and that glucose supplies in various brain regions change in accord with their metabolic needs (1–3). As a polar molecule, glucose requires a carrier system to cross the lipid bi-layer of cellular membranes. Five different glycoprotein glucose transporters (Glut1–5) have been identified in mammalian species, each specific to different tissues and cell types within the body (for review see 4). Glut1 and Glut3 are the only two glucose transporters present in the central nervous system (5–7). Glut1 transports glucose across the endothelial cells of the blood–brain barrier and is also the glucose transporter for glial cells (8–10), whereas Glut3 is present in neurons (6, 7, 11–14). The amount of glucose, and possibly insulin, in the brain regulates Glut1 (15–18). Mechanisms of Glut3 regulation are unknown.

Glucose transport to neurons is therefore dependent on two glucose transporters. Under normal conditions Glut1 transport is not rate-limiting, with only one-half of the glucose transported across the blood–brain barrier metabolized by the brain (19). Glut3 has a higher affinity for hexoses than Glut1 (20), thus ensuring efficient glucose uptake by neurons, even in an environment of low extracellular glucose concentrations. glucose phosphorylation, and not glucose transport, is believed to limit glucose utilization within the individual cell (21, 22).

In patients with Alzheimer's disease (AD), positron emission tomography (PET) scans show decreased glucose uptake in association cortices (23–25). A primary dysfunction in glucose transport, the loss of neurons and synapses and/or decreased functional activity in the remaining neural systems could be the cause of the loss of regional glucose uptake. Kalaria and Harik (26) have reported a decrease in glucose transporter activity at the blood–brain barrier in AD. Recently, Simpson et al (27) used Western blot analysis to demonstrate a decrease in both Glut1 and Glut3 protein levels in the parietal, occipital, and temporal cortices, the caudate nucleus, and hippocampus in AD. The reduction in Glut3 was larger and highly significant. The reduction in Glut3 was larger than the reduction in synaptic protein (as measured by SP14 content).

We now have studied the pattern of Glut3 immunostaining in the AD hippocampal formation to test the hypothesis that expression of Glut3, and hence neuronal glucose transport, is diminished in deafferented areas of the AD brain.

MATERIALS AND METHODS

Sixteen individuals were studied. Eight had a clinical and neuropathological diagnosis of AD (age = 74.1 ± 9.4, mean ± SD). Eight were controls who died of non-neurological conditions and did not meet Khachaturian criteria for AD by neuropathological evaluation (age = 80.7 ± 13.8). The Alzheimer Disease Brain Bank independently performed all neuropathological diagnostic evaluations on paraffin-embedded sections using the Bielschowsky silver stain to visualize senile plaques and neurofibrillary tangles.

The hippocampal formation was fixed in paraformaldehyde-lysine metapetidate at 4°C for 24 to 48 hours, and then cryoprotected in a solution of 15% glycerol in phosphate buffered saline (PBS) (pH = 7.4) for 24 additional hours. Fifty micron thick sections were cut using a freezing sledge microtome and stored in a 15% glycerol in PBS solution at −80°C until use.

Sections were processed for immunohistochemistry using a free-floating procedure. Glut3 immunoreactivity was identified using a polyclonal antibody from East Acres Laboratory (Southbridge, MA) raised against a 12 amino acid peptide cor-
Fig. 1. Glut3 immunoreactivity in the dentate gyrus of (A) control and (B) AD patients (bar = 100 μm). (dg = dentate gyrus, hf = hippocampal fissure)

responding to the carboxy terminus of the human Glut3 sequence. Immunoreactivity was detected using a goat anti-rabbit secondary antibody (Jackson Immunoresearch, West Grove, PA) and visualized with 3,3'-diaminobenzidine as the chromagen. Preabsorption of the polyclonal antibody with 500-fold excess antigen (East Acres) abolished staining.

The optical density of Glut3 immunohistochemical product in subfields of the hippocampal formation was measured with the Bioquant Image Analysis System. Measurements were made of the inner and outer portions of the molecular layer of the dentate gyrus, CA4, CA3, CA1, and the subiculum under identical optical conditions. The observer was unaware of the diagnostic category. Optical density measurements were standardized using a translucent strip of increasing grades of grey density (Kodak, Rochester, NY) as an external standard. The darkness of the immunohistochemical product is directly proportional to optical density, which is converted to diffuse density using a negative log function.

Control and AD hippocampal subfields were compared using a mixed between-within subject ANOVA (SAS program, Statistical Analytical Systems, Inc., Cary, NJ).

RESULTS

Pattern of Glut3 Immunoreactivity in the Human Hippocampal Formation

Glut3 immunostaining is diffuse throughout the neuropil, but it is not homogenous in all brain regions. In both control and AD brains, the mossy fiber zone of area CA3 stains more intensely than other hippocampal subfields. This corresponds well to the high metabolic activity in the mossy fiber zone also reflected by cytochrome oxidase histochemistry (28).

There are two distinct bands of staining in the dentate gyrus in control brains (Fig. 1A). The granule cell layer (stratum granulosum) is nearly unstained, whereas the molecular layer contains a homogenous band of Glut3 immunoreactivity. In contrast, in all cases of AD the dentate gyrus shows three distinct bands. The granule cells remain nearly unstained, but the molecular layer contains an inner band with preserved staining and an outer band of diminished immunostaining (Fig. 1B). Other areas of the hippocampal formation appear unchanged in AD.

Analysis of the diffuse density of Glut3 immunoreactivity in the hippocampal subfields revealed a significant reduction in the density of immunoreactivity (49.5%) in the outer portion of the molecular layer of the dentate gyrus in AD versus control subjects, \( F(1,14) = 15.34, p < 0.005 \) (Fig. 2). No significant differences in Glut3 immunoreactivity were found in the inner molecular layer, CA4, CA3, CA1, or the subiculum. Neither postmortem interval nor age correlate with Glut3 immunoreactivity.

DISCUSSION

We report a marked decrease in Glut3 immunoreactivity in the outer portion of the molecular layer in AD,
while activity in the inner portion remains intact. The inner and outer portions of the dentate gyrus molecular layer receive afferent input from different sources. The inner portion receives afferents primarily from CA4 and the basal forebrain area. About 85% of the synaptic terminals in the outer portion of the molecular layer are derived from the perforant pathway, whose cells of origin are in layer II of the entorhinal cortex (29). In AD, these entorhinal neurons are selectively vulnerable to neurofibrillary tangles and to neuronal death and are destroyed early in the course of the disease (30–32).

It is well established in experimental systems that diminished afferent activity is associated with alterations in several metabolic and biochemical activities (33). For example, cytochrome oxidase activity (34) and glucose utilization (35) are reduced in the visual cortex after monocular visual deprivation. Consistent with destruction of the perforant pathway in AD, we have previously shown several structural and biochemical changes in the perforant pathway terminal zone. Levels of glutamate, the perforant pathway's neurotransmitter, are decreased in the molecular layer of the dentate gyrus (36), there is a remodeling of remaining afferents to the deafferented terminal zone (37–39), there is a loss of synaptic markers in the terminal zone (29, 40, 41), and there is a decrease in cytochrome oxidase activity in the terminal zone (28). The loss of cytochrome oxidase activity also occurs in downstream targets of dentate gyrus projections (28) and appears to be associated with downregulation of mitochondrial subunits of the cytochrome oxidase complex (42).

The disruption of the perforant pathway in AD destroys cortical-hippocampal feedforward projections, thereby interrupting a crucial component of a memory-related neural system (43). This is perhaps the clearest example of the more general phenomenon of selective destruction of cortico-cortical projection neurons leading to the loss of feedforward and feedback connections throughout memory-related neural systems (44).

It is important to note that the dentate gyrus granule cells are not affected by neurofibrillary tangles or other neuropathological changes of AD (43, 44). These results emphasize that neurons that remain structurally intact in AD may nonetheless undergo substantial changes in chemical and metabolic parameters as neural systems fail. By contrast, and somewhat surprisingly, we did not see a change in Glut3 density in cytoarchitectonic fields such as CA1/subiculum that contain substantial numbers of neurofibrillary tangles and senile plaques. This is in accord with the observation that topographic maps of neurofibrillary tangles and senile plaques (45) do not match closely to the regional distribution of diminished glucose utilization seen on PET (23–25).

We propose that the decrease in Glut3 immunoreactivity in the perforant pathway terminal zone reflects a loss of metabolic activity, and hence glucose utilization, in the dentate gyrus granule cells secondary to deafferentation. Together with the observation from Western blots that Glu1 and Glut3 are downregulated in multiple brain regions in AD (27), our anatomical data may provide insight at the neural cell and system level to the decreased glucose transport observed at the regional level by PET scans in AD (23, 25).

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