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Research article

Glucose transporter 8 immunoreactivity in astrocytic and microglial cells in subependymal areas of human brains

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HIGHLIGHTS

- Immunoreactivity for GLUT8 is localized in the cytoplasm of subependymal glial cells in autopsied human brains.
- Immunoreactivity for GLUT8 is mainly localized in subependymal vimentin-positive glial cells.
- Immunoreactivity for GLUT8 is sometimes localized in GFAP-positive astrocytes and CD68- or HLA-DR-positive microglial cells.

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ABSTRACT

Glucose transporter 8 (GLUT8), a glucose/fructose transporter, has been shown to be expressed in neuronal cells in several brain areas. A recent immunohistochemical study has shown the presence of GLUT8 in the cytoplasm of epithelial cells of the choroid plexus and ependymal cells. In this study, localization of GLUT8 in glial cells was investigated using immunohistochemical methods. Immunoreactivity for GLUT8 was observed in cells showing astrocytic or microglial structural features located around the lateral ventricles. Confocal microscopic examination revealed that subependymal GLUT8-positive cells with large amounts of cytoplasm mainly show clear immunoreactivity for vimentin, while they were also colocalized with weak immunoreactivity for glial fibrillary acidic protein (GFAP) within the cytoplasm of some cells. In addition, some GLUT8-positive cells with small amounts of cytoplasm and small nuclei showed CD68 or HLA-DR immunoreactivity, indicating them to be cells of microglia/macrophage lineage. These findings suggest that glucose/fructose is transported into the cytoplasm of vimentin- or GFAP-positive astrocytic and CD68- or HLA-DR-positive microglial cells located around the lateral ventricle.

1. Introduction

Glucose or fructose is transported across the cell membrane by a specific transport system and acts as a fueling source in cells. In the brain, some kinds of glucose transporters are known to be located in neuronal or glial cells. It is thought that GLUT2, GLUT3, GLUT4, GLUT6, and GLUT8 are located in neurons, while GLUT1 and GLUT2 are located in astrocytes [1]. In addition, it is known that GLUT5 is located in microglia [2], while GLUT1 is located in endothelial cells [3]. Accordingly, it is likely that neuronal and glial cells can be supplied energy through these transporters.

Fructose is widely produced in many plants in the form of free monosaccharides or as a part of the disaccharide sucrose [4,5]. A large proportion of the intake of fructose is metabolized in the liver by the fructose-1-phosphate pathway. The enzymes involved in this pathway have also been found in the kidneys and small intestine [6,7]. At present, transport of intravascular fructose into the brain remains to be determined. However, Kusmierz et al. [8] reported that concentrations of glucose and fructose in the CSF were 58.7 ± 7 mg/dl and 2.5 ± 1.1 mg/dl, respectively, while concentrations of glucose and fructose in human plasma were 89.7 ± 21.4 mg/dl and 2.3 ± 2.1 mg/dl, respectively. These findings indicate a relatively high concentration of fructose in the CSF com-

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pared with glucose, suggesting transport of fructose from the blood into the CSF via fructose transporters in the endothelial cells of cerebral vessels and/or epithelial cells of the choroid plexus. The localization of GLUT5 and GLUT2, familiar transporters of fructose [1,9,10], has been examined in detail in the jejunum [11]. GLUT5, which only transports fructose, not glucose, is localized in epithelial cells in the intestine, and is activated in response to increased dietary fructose intake [10,12,13]. GLUT2 is known not only to be a low affinity glucose transporter, but also a lower affinity fructose transporter. Concerning the localization of GLUT5 and GLUT2 in the central nervous system, it is known that GLUT5 is situated in microglia [2], epithelial cells of the choroid plexus, and ependymal cells [14], and that GLUT2 is expressed in ependymal cells as well as in neurons in several areas [15,16]. At present, it is believed that GLUT7, GLUT8, GLUT11, and GLUT12, as well as GLUT5 and GLUT2, likely transport fructose into cells [1,9,10].

GLUT8, a cytoplasmic glucose transporter, has been shown to be expressed in the murine intestine [17–19]. In addition, GLUT8 was recently demonstrated to regulate fructose transport and global mammalian fructose utilization [20]. GLUT8 has also been shown to be expressed in neuronal cells in several brain areas, including the cerebral cortex, hippocampus, amygdala, and hypothalamus [1,21]. In addition, it is known that GLUT8 is located in high-density microsomes mainly near the rough endoplasmic reticulum [22]. A recent study [23] showed that immunoreactivity for GLUT8, but not GLUT7, GLUT11, or GLUT12, is located in epithelial cells of the choroid plexus and in the ependymal cells, suggesting the contribution of GLUT8 to the transport of intravascular fructose into the brain.

It is known that astrocytes are mostly glycolytic, expressing enzymes involved in glycolysis, while neurons are oxidative cells [24]. Astrocyte glycolysis principally forms lactate that is then exported from astrocytes to neurons to form pyruvate. However, it remains to be completely clarified which hexose transporters are located in astrocytes. In this study, it was investigated whether GLUT8, which is localized in ependymal cells, is also localized in other glial cells located in the parenchyma.

2. Materials and methods

This study using human brains was approved by the institutional ethics committee of the Faculty of Medicine, Kagawa University. Human brain samples (n = 6) were obtained at autopsy 1–5 h after death from 3 patients with no neurological abnormalities and from 3 patients with a neurological disease in Kagawa University Hospital, as previously reported [14]. The main diagnosis of each case was established according to the clinical and autopsy findings. The brains were fixed in 10% formalin and processed for immunohistochemical examination. The brain samples were embedded in paraffin and sectioned at a 4 μm thickness. The paraffin sections were treated with 2% bovine serum albumin in PBS and incubated with rabbit anti-GLUT8 antibody (1:100, Bioss, Boston, MA, USA) at 4 °C overnight. Staining was achieved with a Simple Stain kit (Nichirei, Tokyo, Japan) and developed with 3,3'-diaminobenzidine tetrahydrochloride (DAB) and hydrogen peroxide (Nichirei) at room temperature for 5–7 min. The sections were counterstained with hematoxylin.

For triple fluorescent staining, antigen retrieval was performed by heating sections in EDTA buffer (pH 9.0) for 20 min. After blocking with 2% bovine serum albumin in PBS, the paraffin sections were incubated with rabbit anti-GLUT8 antibody (1:100, Bioss) at 4 °C overnight, followed by incubation at RT for 60 min in Alexa Fluor 594-conjugated anti-rabbit IgG (1:200, Molecular Probes, OR). After washing in PBS, the sections were incubated with mouse anti-glial fibrillary acidic protein (GFAP) (1:50, Abcam, Cambridge, UK), anti-vimentin (1:50, DAKO, Glostrup, Denmark), anti-CD68 (clone PG-M1) (1:50, DAKO), or anti-HLA-DR (1:25, DAKO) antibody at 4 °C overnight, followed by incubation at RT for 60 min in Alexa Fluor 488-conjugated anti-mouse IgG (1:200, Molecular Probes, Eugene, OR). Cross reactions were not detected after double staining. In addition, the sections were incubated for 60 min at RT in Monomeric Cyanine Nucleic Acid Stains (TO-PRO-3, Molecular Probes), diluted to 2.5 μM in PBS. Fluorescent signals were viewed under a confocal microscope (Carl Zeiss LSM700, Oberkochen, Germany). As a control experiment, we performed an identical immunohistochemical procedure, but with omission of the primary antibody or using normal serum instead of the primary antibody.

3. Results

Immunohistochemical examination using the antibody for GLUT8 revealed clear immunoreactivity for GLUT8 in the cytoplasm of subependymal glial cells as well as ependymal cells in all human brains examined (Fig. 1A–F). There were some differences in the staining density among them. Dense immunostaining for GLUT8 was shown in some sections (Fig. 1B and F), while relatively light immunostaining was shown in the others (Fig. 1A and E). Clear immunoreactivity for GLUT8 was seen in glial cells with large amounts of cytoplasm and fine processes located near ependymal cells in 6 brains. In addition, an enlarged immunohistochemical image in the subependymal area indicates that clear immunoreactivity for GLUT8 was observed in cells with large cytoplasm and fine processes, while strong immunoreactivity for GLUT8 was sometimes observed in cells with small amounts of cytoplasm and small nuclei (Fig. 1G). An enlarged image in another area situated at a distance from ependymal cells indicates that a few cells with large amounts of cytoplasm and fine processes showed clear immunoreactivity for GLUT8 (Fig. 1H).

Confocal microscopic images in areas around the lateral ventricle indicate that immunoreactivity for GLUT8 was sometimes localized to the cytoplasm of astrocytes showing weak GFAP immunoreactivity (Fig. 2A–D). In astrocytes, immunoreacitivity for GLUT8 was observed in cytoplasm showing weak immunoreacitivity for GFAP, but not in cytoplasm showing clear immunoreactivity for GFAP. Some cells with nucleoli, presumably neurons, showed immunoreactivity for GLUT8 without GFAP immunoreactivity, while other astrocytic cells with GFAP immunoreactivity showed almost no or very weak immunoreactivity for GLUT8 (Fig. 2A–D). In subependymal areas, immunoreactivity for GLUT8 was mainly observed in cells with large amounts of cytoplasm showing clear immunoreactivity for vimentin (Fig. 2E–H). In addition, immunoreactivity for GLUT8 was frequently observed in cells with small amounts of cytoplasm showing immunoreactivity for CD68 (Fig. 2I–L), and also in cells with small amounts of cytoplasm showing immunoreactivity for HLA-DR (Fig. 2M–P).

4. Discussion

In this study, immunoreactivity for GLUT8 was observed in glial cells located around the lateral ventricle (Fig. 1). GLUT8 immunoreactivity was mainly observed in the cytoplasm of subependymal vimentin-positive glial cells, and sometimes in cytoplasm showing weak GFAP immunoreacitivity in some astrocytes (Fig. 2) as well as in neurons with nucleoli and no GFAP immunoreactivity (arrowheads in Fig. 2A–D). The expression of vimentin in glial cells is regarded as an immature feature of astrocytes, and glial cells start to express GFAP with maturation [25–27]. Radial glia-like cells with vimentin-positive radial processes were reported in the subependymal area of adult rats [28]. Some processes of these cells showed co-expression of GFAP and vimentin, suggesting that