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Glucose transporter 5 (GLUT5)-like immunoreactivity is localized in subsets of neurons and glia in the rat brain

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Abbreviations: 3, principal oculomotor nucleus; 3V, third ventricle; 4n, trochlear nerve; 4V, fourth ventricle; 7, facial nucleus; 10, dorsal motor nucleus of the vagus; 12, hypoglossal nucleus; 12n, root of the hypoglossal nerve; AA, anterior amygdaloid area; ACg, anterior cingulate cortex; ACo, anterior cortical amygdaloid nucleus; AD, anterodorsal thalamic nucleus; AF, amygdaloid fissure; AHi, amygdalohippocampal area; AHy, anterior hypothalamic area; alv, alveus hippocampus; AM, anteromedial thalamic nucleus; Amb, ambiguus nucleus; APTD, dorsal anterior pretectal area; APTV, ventral anterior pretectal area; Aq, cerebral aqueduct; Arc, arcuate hypothalamic nucleus; asc7, ascending fibers of the facial nerve; AV, anteroventral thalamic nucleus; B, cells of basal nucleus of Meynert; bas, basilar artery; bic, brachium of the inferior colliculus; BL, basolateral amygdaloid nucleus; BLV, ventral basolateral amygdaloid nucleus; BM, basomedial amygdaloid nucleus; bp, brachium pontis; bsc, brachium superior colliculus; BST, bed nucleus of the stria terminalis; BSTPO, preoptical bed nuleus of the stria terminalis; CA1, field CA1 of Ammon's horn; CA2, field CA2 of Ammon's horn; CA3, field CA3 of Ammon's horn; CA4, field CA4 of Ammon's horn; Cb, cerebellum; cc, corpus callosum; Ce, central amygdaloid nucleus; CeL, lateral central amygdaloid nucleus; CeM, medial central amygdaloid nucleus; CG, central grey; cg, cingulum; CGD, dorsal central grey; CGM, medial central grey; CICDM, dorsomedial central nucleus of the inferior colliculus; CICVL, ventrolateral central nucleus of the inferior colliculus; CL, centrolateral thalamic nucleus; CLi, caudal linear nucleus of the raphe; Cl, claustrum; CM, central medial thalamic nucleus; Cnf, cuneiform nucleus; Cop, copula of the pyramis; cp, cerebral peduncle; CPu, caudate putamen; CxA, cortex-amygdala transition zone; d, dorsal nucleus of the inferior olive; DA, dorsal hypothalamic area; DCo, dorsal cochlear nucleus; df, dorsal fornix; DG, dentate gyrus; dhc, dorsal hippocampal commissure; Dk, nucleus of Darkschewitsch; DLG, dorsolateral genuculate nucleus; DLL, dorsal nucleus of the lateral lemniscus; DM, dorsomedial hypothalamic nucleus; DPB, dorsal parabrachial nucleus; DpG, deep grey layer of the superior colliculus; DpMe, deep mesencephalic nucleus; DPO, dorsal periolivary region; DR, dorsal raphe nucleus; dsc, dorsal spinocerebellar tract; DTg, dorsal tegmental nucleus; dtgx, dorsal tegmental decussation; ec, external capsule; ECu, external cuneate nucleus; EIC, external nucleus of the inferior colliculus; eml, External medullary lamina; En, endopiriform nucleus; Ent, entorhinal cortex; EP, entopeduncular nucleus; f, fornix; FC, fasciola cinereum; fi, fimbria hippocampus; fmj, forceps major corpus callosum; fr, fasciculus retroflexus; FrPaM, motor area of the frontoparietal cortex; FrPaSS, somatosensory area of the frontoparietal cortex; FStr, fundus striati; G, gelatinosus nucleus of the thalamus; Gem, gemini nuclei; Gi, gigantocellular reticular nuclues; GP, globus pallidus; GrCo, granule cell layer of the cochlear nucleus; GrDG, granular layer of the dentate gyrus; hbc, habenular commissure; HiF, hippocampal fissure; I, intercalated nuclei of the amygdala; ic, internal capsule; icp, inferior cerebellar peduncle; ICPC, intracommissural nucleus of the posterior commissure; IF, interfascicular nucleus; IG, indusium griseum; IMCPC, interstitial magnocellular nucleus of the posterior commissure; IMD, intermediodorsal thalamic nucleus; In, intercalated nucleus; InC, interstitial nucleus of Cajal; Inf, infracerebellar nucleus; InG, intermediate grey layer of the superior colliculus; Int, interpositus cerebellar nuclues; IntG, intermediate geniculate nucleus; InWh, intermediate white layer of the superior colliculus; IO, inferior olive; IPA, interpeduncular nucleus; IPIP, interpeduncular nucleus; IPF, interpeduncular fossa; IPOP, interpeduncular nucleus; IPP, interpeduncular nucleus; K, nucleus of K; KF, Kölliker-Fuse nucleus; La, lateral amgdaloid nucleus; lab, longitudinal association bundle; LatC, lateral cerebellar nucleus; LC, locus coeruleus; LD, laterodorsal thalamic nucleus; LDTg, laterodorsal tegmental nucleus; LH, lateral hypothalamic area; LHb, lateral habenular nucleus; ll, lateral lemniscus; LM, lateral mammillary nucleus; lo, lateral olfactory tract; LOT, nucleus of the lateral olfactory tract; LOTD, dorsal nucleus of the lateral olfactory tract; LP, lateral post thalamic nucleus; LRt, lateral reticular nucleus; LSO, lateral superior olive; LTz, lateral nuclues of the trapezoid body; LV, Lateral ventricle; LVe, Lateral venstibular nucleus; m, medial nucleus of the inferior olive; m5, motor root of the trigeminal nerve; mcp, middle cerebellar peduncle; MD, mediodorsal thalamic nucleus; MDL, lateral mediodorsal thelamic nucleus; ME, median eminence; Me, medial amygdaloid nucleus; Me5, nucleus of the mesencephalic tract trigeminal nerve; MG, medial geniculate nucleus; MHb, medial habenular nucleus; ML, lateral part of the medial mammilay nucleus; ml, medial lemniscus; mlf, medial longitudinal fasciculus; MM, medial part of the medial mammillary nucleus; Mo5, motor trigeminal nucleus; MP, posterior part of the medial mammillary nucleus; mp, mammilary peduncle; MPO, medial preoptic area; MSO, medial superior olive; MT, medial terminal nucleus of accessory optic tract; MTz, medial nucleus of the trapezoid body; mt, mammillothalamic tract; mtg, mammillotegmental tract; MVe, medial vestibular nucleus; oc, olivocerebellar tract; Op, optic nerve layer of the superior colliculus; OPT, olivary pretectal nucleus; opt, optic tract; OT, nucleus of the optic tract; ox, optic chiasma; Pa, paraventricular hypothalamic nucleus; PaS, parasubiculum; PBP, parabrachial pigmented nucleus; PC, paracentral thalamic nucleus; pc, posterior commissure; PCg, posterior cingulate cortex; PCRt, parvocellular reticular nucleus; Pe, periventricular hypothalamic nucleus; PFl, paraflocculus; PGi, paragigantocellular reticular nucleus; PLCo, posterolateral cortical amygdaloid nucleus; PMCo, poseromedial cortical amygdaloid nucleus; PN, paranigral nucleus; Pn, pontine nuclei; PnO, pontine reticular nucleus; PO, primary olfactory cortex; Po, posterior thalamic nuclear group; PP, peripeduncular nucleus; PPT, posterior pretectal nucleus; PR, prerubral field; pr, principal nucleus of the inferior olive; Pr5, principal sensory trigeminal nucleus; PrF, primary fissure; PrH, prepositus hypoglossal nucleus; PrS, presubiculum; PT, paratenial thalamic nucleus; PV, paraventricular thalamic nucleus; PVA, anterior paraventricular thalamic nucleus; py, pyramidal tract; Re, reuniens thalamic nucleus; ReIC, recess of the inferior colliculus; RF, rhinal fissure; Rh, rhomboid thalamic nuclues; RLi, rostral linear nucleus of the raphe; RMg, raphe magnus nucleus; ROb, raphe obscurus nucleus; RPa, raphe pallidus nucleus; RPC, parvocellular part of the red nucleus; RPn, raphe pontis nucleus; RRF, retrorubral field; rs, rubrospinal tract; RSpl, retrosplenial cortex; Rt, reticular thalamic nucleus; S, subiculum; s5, sensory root of the trigeminal nerve; Sag, sagulum nucleus; SC, superior colliculus; scc, splenium corpus callosum; SCh, suprachiasmatic nucleus; SCO, subcommissural organ; scp, superior cerebrellar peduncle; SFO, subfornical organ; SG, suprageniculate thalamic nucleus; SI, substantia innominata; sm, stria medullaris thalamus; SNC, compacta of the substantia nigra; SNL, lateralis of the substantia nigra; SNR, reticularis of the substantia nigra; SO, supraoptic hypothalamic nucleus; SOR, retrochiasmatic area of the supraoptic hypothalamic nucleus; Sol, nucleus of the solitary tract; SolL, lateralis of the nucleus of the solitary tract; SolM, medialis of the nucleus of the solitary tract; sox, supraoptic decussation; sp5, spinal tract of the trigeminal nerve; Sp5I, interpolar of the nucleus of spinal tract of the trigeminal nerve; Sp5O, oralis of the nucleus of spinal tract of the trigeminal nerve; SpVe, spinal vestibular nucleus; st, stria terminalis; str, superior thalamic radiation; Str17, area 17 of the striate cortex; Str18, area 18 of the striate cortex; Str18a, area 18a of the striate cortex; Su7, suprafacial nucleus; SuG, superficial grey layer of the superior colliculus; SuM, supramammillary nucleus; sumx, supramammillary decussation; SuVe, superior vestibular nucleus; Te, temporal cortex; TeAud, auditory area of the temporal cortex; tfp, transverse fibers pons; TS, triangular septal nucleus; tz, trapezoid body; VCoA, anterior ventral cochlear nucleus; VCoP, posterior ventral cochlear nucleus; vhc, ventral hippocampal commissure; VL, ventrolateral thalamic nucleus; VLGMC, magnocellular part of the ventrolateral geniculate nucleus; VLGPC, parvocellular part of the ventrolateral geniculate nucleus; VLL, ventral nucleus of the lateral lemniscus; VM, ventromedial thalamic nucleus; VMH, ventromeidla hypothalamic nucleus; VTA, ventral tegmental area; VP, ventral pallidum; VPL, ventral posterolateral thalamic nucleus; VPB, ventral parabrachial nucleus; VPM, medialis of the ventroposterior thalamic nucleus; vsc, ventral spinocerebellar tract; xscp, decussation of the superior cerebellar peduncle; ZI, zona incerta; Zo, zonal layer of the superior colliculus.

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ABSTRACT

This study aimed at examining the distribution of glucose transporter 5 (GLUT5), which preferentially transports fructose, in the rat brain by immunohistochemistry and Western blotting. Small immunoreactive puncta (less than 0.7 μm) were sparsely distributed all over the brain, some of which appeared to be associated with microglial processes detected by an anti-ionized calcium-binding adapter molecule 1 (Iba-1) monoclonal antibody. In addition, some of these immunoreactive puncta seemed to be associated with tanycyte processes that were labeled with anti-glial fibrillary acidic protein (GFAP) monoclonal antibody. Ependymal cells were also found to be immunopositive for GLUT5. Furthermore, several noticeable GLUT5 immunoreactive profiles were observed. GLUT5 immunoreactive neurons, confirmed by double staining with neuronal nuclei (NeuN), were seen in the entopeduncular nucleus and lateral hypothalamus. Cerebellar Purkinje cells were immunopositve for GLUT5. Dense accumulation of immunoreactive puncta, some of which were neuronal elements (confirmed by immunoelectron microscopy), were observed in the optic tract and their terminal fields, namely, superior colliculus, pretectum, nucleus of the optic tract, and medial terminal nucleus of the optic tract. In addition to the associated areas of the visual system, the vestibular and cochlear nuclei also contained dense GLUT5 immunoreactive puncta. Western blot analysis of the cerebellum indicated that the antibody used recognized the 33.5 and 37.0 kDa bands that were also contained in jejunum and kidney extracts. Thus, these results suggest that GLUT5 may transport fructose in subsets of the glia and neurons for an energy source of these cells.

1. Introduction

Glucose is the principal source of energy for the mammalian brain; a continuous supply of this substrate is essential for maintenance of normal brain functions (Maher et al., 1994). Glucose transporter proteins (GLUTs) are highly homologous integral membrane proteins with 12 membrane-spanning domains and a single glycosylation site, and are responsible for the facilitative uptake of glucose and other monosaccharaides into mammalian cells (Bell et al., 1993; Gould and Holman, 1993). To date, 14 GLUT family members have been identified (Mueckler and Thorens, 2013). Among them, GLUT5 (molecular weight, 50–60 kDa in rats; Maher et al., 1994) is highly expressed in the small intestine, kidney, testis and brain (Bell et al., 1993; Gould and Holman, 1993; Cui et al., 2003; Horikoshi et al., 2003). Although GLUT5 is classified as a glucose transporter, it is known to function as a high-affinity fructose transporter with a poor glucose transporting ability (Kayano et al., 1990; Burant et al., 1992; Funari et al., 2007). Most tissues expressing GLUT5 have been found to be rich in fructose, with the exception of the brain (Maher et al., 1994).

Ambient fructose levels are low in the brain (Maher et al., 1994), suggesting the minor contribution of GLUT5 for neuro-energetics. However, a number of studies have shown that GLUT5 is expressed in the Purkinje cells of the cerebellum (Funari et al., 2005), microglia (Payne et al., 1997; Horikoshi et al., 2003) and epithelial cells of choroid plexus, and the ependymal cells in the brain (Ueno et al., 2014). Despite the considerable interest in understanding fructose metabolism in the brain owing to increased consumption of dietary fructose, the overall distribution of GLUT5 in the brain has not been reported so far. In the present study, we investigated the distribution of GLUT5 in the rat brain, and found the more extensive distribution of GLUT5 in subsets of neurons and glia of the rat brain than one might expect.

2. Materials and methods

This study was carried out with the permission from the Ethics Committee of Kanagawa Dental University, and in accordance with the guidelines established by the committee. Male Wistar rats (n = 7) were deeply anesthetized with pentobarbital sodium (Wako Pure Chemical Industries, Ltd., Osaka, Japan), and perfused with 0.9% NaCl and, subsequently, with 4% formaldehyde and 0.2% picric acid in 0.1 M sodium phosphate buffer (PB, pH 6.9). The brain was rapidly dissected and fixed, in a same fixative, for one or two days at 4 °C. After washing in PB and immersing in 20% sucrose, the frozen samples were cut into 20-μm-thick transverse sections using a sliding microtome equipped with a freezing stage and immunostained using the free-floating method.

Immunohistochemistry was performed according to the method routinely used in our laboratory (Yamamoto et al., 2011). Briefly, the sections were washed overnight in 0.1 M PB (pH 7.4) containing 0.9% saline (PBS), and incubated with rabbit anti-GLUT5 antibody (Immuno-Biological Laboratories Co., Takasaki, Japan) diluted in 1.5 μg/ml in PBS containing 1% bovine serum albumin (BSA) and 0.3% Triton X-100 (PBS-BSAT) for 2 days at 4 °C. This antibody was raised against a part of the C-terminal region (SEVYPEKEELKELPPVTSEQ) of human GLUT5 and affinity purified (company's description). After washing in PBS, the sections were incubated with a secondary antibody (biotinylated goat anti-rabbit IgG, Vector Laboratories, Burlingame, CA, USA) diluted in PBS-BSAT (1:200) for 1 h at room temperature. The sections were then washed again in PBS and incubated with avidin-biotin-horseradish peroxidase complex (ABC; Vector Laboratories) diluted in PBS-BSAT (1:200) for 30 min at room temperature. After a final wash in PBS, the sections were reacted with 3, 3′-diaminobenzidine tetrahydrochloride (DAB; 0.02%) and hydrogen peroxide (0.005%) in 0.05 M Tris–HCl buffer solution (pH 7.4). Thereafter, the sections were counterstained with thionin and coverslipped with Malinol (Muto Pure Chemicals, Tokyo, Japan). Some sections were coverslipped without counterstaining. Negative controls were prepared by omitting the antibody in the first incubation or by using antibody pre-absorbed with a GLUT5 antigen peptide (20 μg/ml; Immuno-Biological Laboratories Co.). Cytoarchitecture and line drawing of the rat brain were referred to a rat brain atlas (Paxinos and Watson, 2007).

To identify the types of immunoreactive cells, double immunofluorescence staining was performed. Neurons, astrocytes and microglia were labeled using mouse, anti-neuronal nuclei (NeuN) monoclonal antibody (1:500; Chemicon International, Temecula, CA, USA; Yamamoto et al., 2011), mouse, anti-glial fibrillary acidic protein (GFAP) monoclonal antibody (1:10; American Research Products, Inc., Belmont, MA, USA; Aoki et al., 2010), and mouse, anti-ionized calcium-binding adapter molecule 1 (Iba-1)