Volume 334, number 3, 289–295 FEBS 13259 November 1993

Neuron-specific glucose transporter (NSGT): CNS distribution of GLUT3 rat glucose transporter (RGT3) in rat central neurons

Shinya Nagamatsu[a,*], Hiroki Sawa[b], Kouichi Kamada[b], Yoko Nakamichi[a], Katsuhiko Yoshimoto[a], Takao Hoshino[b]

Departments of [a]Biochemistry and [b]Neurosurgery, Kyorin University School of Medicine, Shinkawa 6-20-2, Mitaka, Tokyo 181, Japan

Received 28 September 1993

The identity of the glucose transporters (GLUT) expressed in neurons in situ has yet to be fully established. In the present study we have isolated the GLUT3 (RGT3) cDNA and produced anti RGT3 polyclonal antibody allowing us to investigate the cellular localization and tissue distributions of RGT3 mRNA and protein in the central nervous system of the rat by the methods of in situ hybridization and immunohistochemistry. Here we demonstrate the direct evidence that RGT3 is present in neurons in adult rat brain. In situ hybridization showed the expression of RGT3 mRNA mostly in the regions of hippocampus, cerebral cortex, striatum, and the granule cell layer of the cerebellum, indicating that RGT3 mRNA is predominantly expressed within neurons. Immunohistochemistry showed that RGT3 protein is widely distributed in the rat brain, and concentrated on the plasma membrane of neurons. Double labeling studies with anti-RGT3, glial fibrillary acidic protein (GFAP), and neuron specific enolase (NSE) antibodies revealed the specific expression of RGT3 protein in neurons. Thus, RGT3 is indicated to be a neuron specific glucose transporter isoform (NSGT), and suggested to play a functionally significant role in rat central neurons.

Glucose transporter; GLUT3; Neuron specific enolase (NSE); Glial fibrillary acidic protein (GFAP)

1. INTRODUCTION

Since the brain cannot store the significant levels of carbohydrate, maintenance of normal cerebral functions is almost exclusively dependent on a sustained supply of glucose from the blood [1,2]. Glucose, being a polar substance, crosses lipid bilayers of cell membranes [3] by a saturable and stereospecific nonenergy dependent process of facilitated diffusion system [4], which consists of a family of closely related membrane associated glycoprotein termed the glucose transporters; GLUT1–GLUT4 [5]; GLUT5 has recently been shown to be a fructose transporter [6].

In the central nervous system, glucose transport is mediated by a facilitative diffusion type transport system [7,8,9], and suggested to be regulated at two levels. First, glucose is transported across the blood brain barrier (anatomically consisting of contiguous brain capillary endothelial cells jointed by zonula occludens type tight junctions [10]) from the circulation into the brain's extracellular space. Once in the extracellular space, glucose is then transported intracellularly into both neuronal and glial cells by a similar carrier-mediated process. GLUT1 is concentrated in cells of blood–brain barrier, which indicates the general role for this transporter isoform is the transport of glucose from the blood into the underlying tissue layers [11,12,13]. On the other hand, the identity of the glucose transporter expressed specifically in neurons has yet to be fully established. Although GLUT1 mRNA and protein can be shown to be present in primary cultures of neonatal neuronal cells [14,15], the expression of GLUT1 in these cells might be due to the cultural conditions. In fact, it has not been possible to show expression of GLUT1 in neuronal cells in situ except the expression in the Purkinje cells of only mouse cerebellum (unpublished results), which indicates that another glucose transporter isoform is responsible for glucose transport in neuronal cells. In humans, GLUT3 mRNA is expressed at highest level in the brain, but, present at variable levels in all tissues and tumors [16,17]. Mantych et al. [18] showed that human GLUT3 protein was present in neuropils and microvascular endothelial cells in human brain, but they could not demonstrate the definite exhibition of GLUT3 protein in neuronal cells. In contrast to the tissue distribution of human GLUT3 mRNA, mouse GLUT3 mRNA, which we have recently cloned from mouse brain [19], was only present at high levels in the brain, suggesting mouse GLUT3 being a neuronal glucose transporter.

In this study we have isolated a cDNA clone from rat brain encoding a rat glucose transporter protein (GLUT3), and produced antibodies using multiple antigen peptide (MAP) of COOH-terminal 17 amino acids of rat GLUT3 (RGT3). Thereby we could have taken advantages of a powerful approach combining in situ

*Corresponding author. Fax: (81) (422) 41-6865.

The nucleotide sequence reported in this paper has been submitted to the GenBank/EMBL Data Bank with accession number D13962.

hybridization and immunohistochemistry to determine the protein localization in expressing neurons. Here, we demonstrate that RGT3 protein is localized on the plasma membrane of rat neurons in situ. Double labeling studies with using neuronal (neuron-specific enolase; NSE) and astrocytic (glial fibrillary acidic protein; GFAP) markers revealed the specific expression of RGT3 in neurons. Thus, we propose rodent GLUT3 as a neuron specific glucose transporter (NSGT).

2. MATERIALS AND METHODS

2.1. *cDNA cloning*

The rat GLUT3 cDNA was isolated from a rat hippocampus λgt10 cDNA library (Clontech) using a portion of the previously described mouse GLUT3 sequence as probe [19]. The probe was a 563 base pair (bp), radiolabeled with [^{32}P]dCTP by nick translation. Hybridization conditions consisted of 50% formamide, 5 × SSC, 1 × Denhardt's solution, 0.1% SDS, salmon sperm at 100 μg/ml, and probe (10^6 cpm/ml) at 42°C. Final wash conditions were 0.1 × SSC, 0.1% SDS at 55°C. Purified pharge clones were subcloned into pGEM4G (Promega, Biotech). DNA sequence analysis was performed by the dideoxynucleotide chain termination procedure [20] using synthetic oligonucleotide primers, and Sequenase 2.0 (US Biochemical) after ligation to M13mp18, or -19.

2.2. *In situ hybridization histochemistry*

Brains from adult male Wistar rats were removed immediately after killing, embedded in OCT compound (Tissue Tek Inc.) in the liquid nitrogen, and stored at −80°C until sectioning. Ten-μm sagittal sections were cut at −20°C and collected throughout the brain. Sections were then air-dried, washed with 0.1 M Phosphate buffered saline (PBS), fixed with 4% paraformaldehyde, and quenched with 2 mg/ml of glycine. Sections were digested with 1 μg/ml of protease K and pre-hybridized with 50% (v/v) deionized formamide/2 × SSC at 42°C for 30 min. Digoxygenin-labeled sense and antisense riboprobes were prepared by transcription of plasmids coding for rat GLUT3 using T7 or SP6 RNA polymerase as described in the manual of RNA labeling kit (Boehringer Mannheim). The cDNA inserts in the plasmid, cloned in pGEM4G (Promega Biotec), encodes amino acids 207–394 of rat GLUT3. Slides were hybridized for a 16 h at 42°C in a 100 μl solution of 20 mM Tris-HCl (pH 7.4), 2.5 mM EDTA, 1 × Denhardt's solution, 300 mM NaCl, 0.5 mg/ml yeast tRNA, 50% formamide and 50–100 μg/ml antisense or sense digoxygenin labeled riboprobes. After washing the slides with 50% formamide and 2 × SSC for 30 min at 45°C and rinsing with 10 mM Tris-HCl (pH 7.4) and 500 mM NaCl, they were treated with 20 μg/ml boiled RNase A solution in the same buffer for 1 h at room temperature. Slides were then washed twice with 50% formamide/2 × SSC for 30 min at room temperature. To detect the signals, slides were washed twice with 50 mM Tris-HCl (pH 8.0), 150 mM NaCl and incubated with 1:500 dilution of anti-digoxygenin Fab fragment conjugated with alkaline phosphatase for 1 h at room temperature. After washing the slides three times with 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, they were rinsed with 100 mM Tris-HCl (pH 9.0), 100 mM NaCl, and 5 mM $MgCl_2$. The color was developed with the incubation of Nitroblue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP) in a dark box. The reaction was stopped with TE buffer (10 mM Tris-HCl (pH 8.0), 1 mM EDTA), and slides were mounted in gelatin/glycine solution, then observed with light microscopy.

2.3. *Antibody production*

Multiple antigen peptide (MAP) containing the 17 amino acid (GVELNSMQPVKETPGNA) carboxy-terminus of the rat GLUT3 protein was synthesized on an Applied Biosystem model 430A peptide synthesizer basically by the method of Tam [21]. Peptides of COOH-terminal amino acids of the human GLUT3 (KDGVMEMNSIEPAKET) or human GLUT1 (SQSDKTPEELFHPL) were also synthesized. MAP of RGT3 was emulsified with complete Freund's adjuvant and injected into rabbits using standard procedures for antiserum production. Rabbits were boosted by a subcutaneous injection of the antigen emulsified in incomplete Freund's adjuvant.

2.4. *Immunoblot analysis*

The whole brain or different organs of the rat were homogenized in 5 ml of a solution of 40 mM Tris-HCl (pH 7.4), 2 mM EDTA, 250 mM Sucrose, 1 mM phenylmethylsulfonyl fluoride (PMSF) and 1% Triton X-100, mixed with Laemmli sampling solution. Westerns were performed essentially as previously described [19]. Approximately 50–100 μg of proteins was subjected to 10% SDS-polyacrylamide gel electrophoresis (PAGE), and electrophoretically transferred to nitrocellulose. The filters were blocked with 5% non-fat milk in 10 mM Tris-buffered saline (TBS), pH 7.4, at 37°C for 1 h followed by incubation for 3 h at room temperature with 1:500 dilutions of the primary antiserum. Filters were washed with TBS containing 0.05% Tween 20. Immunoreactive bands were visualized by alkaline phosphatase-coupled goat anti-rabbit IgG secondary antibody (Dako), using substrates Nitroblue tetrozolium and 5-bromo-4-chloro-3-indolyl-phosphate (Wako Ind. Ltd.). For competition analysis, each peptide of RGT3, human GLUT3, or human GLUT1 was preincubated with the diluted primary antiserum for 30 min prior to incubation with filter.

2.5. *Immunohistochemistry*

Adult rats were sacrificed by ether anesthesia and decapitation, and dissected brains were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer (PB). The paraffin embedded tissues were cut into 5 μm and deparaffinized with xylene and rehydrated with 0.05 M Tris-HCl (pH 7.4) and 0.15 M NaCl in 100% ethanol. They were incubated with 0.3% H_2O_2 before treatment with 10% normal goat serum to reduce a non-specific binding. Tissue sections were reacted with a 1:500 dilution of RGT3 antiserum for 1 h at 37°C in TBS. The immunohistochemical staining was performed according to the avidin-biotin peroxidase (Dako, Denmark) method of Hsu [22]. The peroxidase reaction was done with 3,3′-diaminobenzidine tetrachloride (DAB; Nakarai tesque), which produces the brown color. Certain brain sections were double labeled with RGT3 antiserum (1:500 dilution) tagged with a biotinylated anti-rabbit second antibody (Dako, Denmark) and anti-neuron-specific enolase (NSE) antiserum (1:1000 dilution; a gift from Dr. I. Takeshita, Kyushu University, Japan), a marker for neuronal cells [23–25]; or an anti-glial fibrillary acidic protein antiserum (GFAP; 1:1000 dilution; a gift from Dr. M. Fukui, Kyushu University, Japan), a marker for astrocyte [26,27], tagged with an ALP conjugated anti-rabbit second antibody. In cases where the biotinylated second antibody was employed, after the primary RGT3 antiserum incubation, sections were treated with a biotinylated goat anti-rabbit IgG for 40 min, followed by incubation with the avidin-biotin-peroxidase complex for another 30 min and, finally, with 3′,3′-diaminobenzidine (a brown color-producing reagent). The slides were then incubated with 5% acetic acid for 3 h to remove the excess of RGT3 antiserum, and were incubated with 1:1000 diluted solution of anti-NSE or GFAP antisera for 1 h at RT. After the sections were incubated with anti-rabbit IgG conjugated with alkaline phosphatase (ALP) (1:500 dilution), ALP reaction was done using a Naphtol AS-MX phosphate (Sigma) and a Fast blue BB base (Wako Ind.) (a blue color-producing reagent). Thus, RGT3 was stained brown, NSE and GFAP were stained blue.

3. RESULTS

3.1. *Isolation and sequence of rat GLUT3 cDNA clones*

Initially, three clones were isolated from a rat brain cDNA library by high-stringency hybridization conditions with a 563 base pair (bp) cDNA fragment of mouse GLUT3. The sequences of two clones indicated

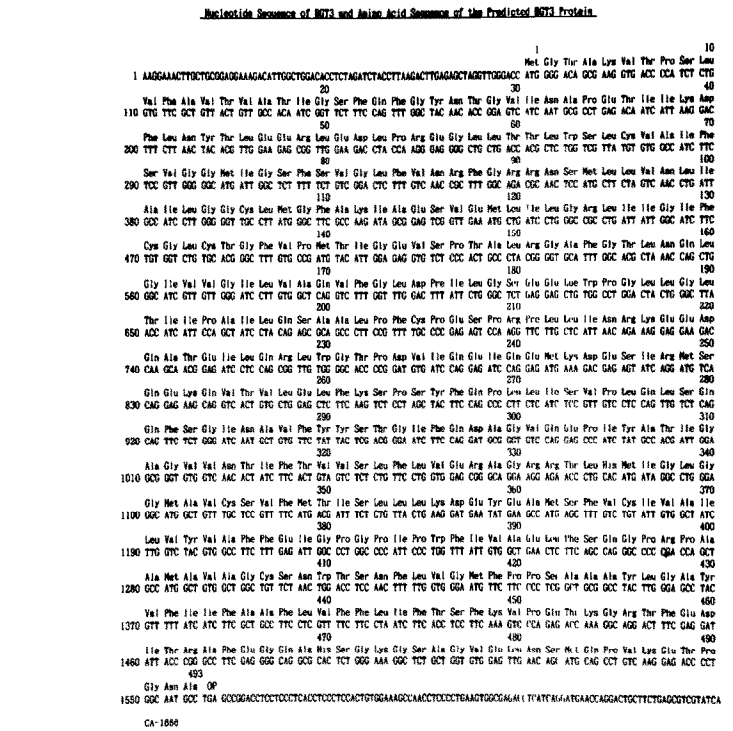


Fig. 1. Nucleotide sequence of RGT3 and amino acid sequence of the predicted RGT3 protein. The number of the nucleotide at the start of each line is indicated. The predicted molecular mass of RGT3 is 53,566 kDa.

that they encoded RGT3, a protein homologous to mouse GLUT3 or human GLUT3. These clones contain the complete protein-coding region, the 5′ and 3′ untranslated region of the mRNA (Fig. 1). The sequences of these clones agreed in overlapping regions, showing them to be derived from the same mRNA.

RGT3 is composed of 493 amino acids and has ~ 94% and ~ 82% identity to the sequences of mouse GLUT3 [19] and human GLUT3 [16], respectively. Like the other facilitated glucose transporters, hydropathic and secondary structure analyses of RGT3 suggest the presence of 12 hydrophobic transmembrane domains with a predominantly α-helical secondary structure, an extracellular loop between M1 and M2 with a potential *N*-glycosylation sites, and a large intracellular hydrophobic loop in the center of the protein. Although data are not shown, RNA blotting studies revealed that the RGT3 cDNA probe hybridized to a single transcript of ~ 4.0 kb in the cerebrum, cerebellum, and brainstem of rat brain, and RGT3 mRNA was only present in the rat brain as well as the results of mouse GLUT3 [19].

3.2. *Cellular localization of rat GLUT3 mRNA in brain*

In situ hybridization (ISH) was used to examine the patterns of expression of RGT3 genes in brain. Hybridization of a digoxigenine-labeled complementary (antisense) RNA probe for RGT3 mRNA to brain sections showed that GLUT3 mRNA was expressed in substantial parts of brain with relatively high levels in the hippocampus, dentate gyrus, cerebral cortex, striatum, and cerebellum (Fig. 2). RGT3 mRNA expression was also observed in the brainstem with low levels. Fig. 2C shows ISH in the cerebellum at higher magnification. Most hybridization signals were observed in the granule cell layer of cerebellum.

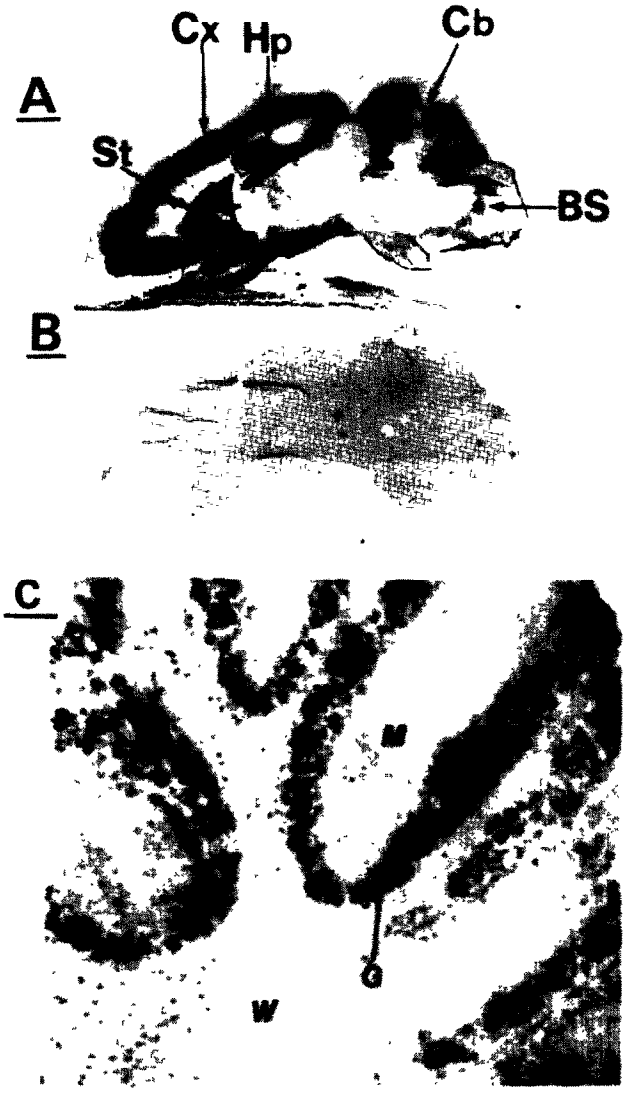


Fig. 2. Distribution of RGT3 mRNA in adult rat brain analyzed by in situ hybridization. Sagittal sections of adult rat brain were hybridized to digoxigenine labeled antisense or sense strand RNA probes for RGT3 mRNA, as shown in A or B, respectively. Note that RGT3 mRNA is present in most parts of the brain with highest levels in the cortex (Cx), hippocampus (Hp), striatum (St), and cerebellum (Cb), but is at low abundance in the brainstem. An adjustant sagittal section hybridized to RGT3 sense probe as a control (B) shows the extent of nonspecific hybridization. C represents a localization of ISH signals of RGT3 mRNA in cerebellum. ISH of the cerebellum cortex, showing dense RGT3 signal diffusely overlying the granule cell layer (G). *Other abbreviations:* M, molecular cell layer; W, white matter of the cerebellum.

3.3. *Characterization of RGT3 specific antibodies*

Specific polyclonal rabbit antibodies were raised to RGT3 protein by immunization with multiple antigen

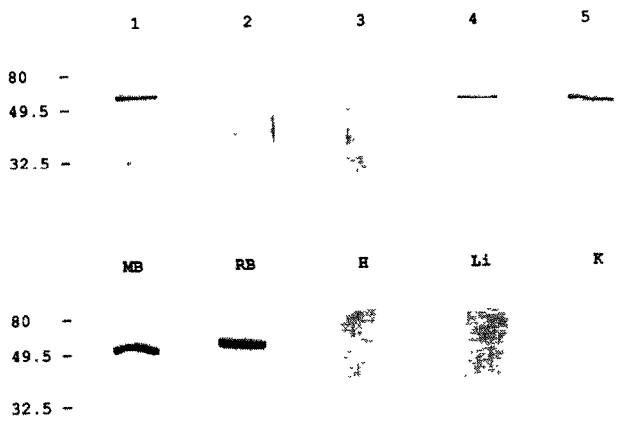


Fig. 3. Characterization of RGT3 protein by immunoblot. (A) Tissue lysates from a whole adult rat brain (50 μg of protein per lane) were separated by SDS 10% polyacrylamide gel electrophoresis and immunoblotted with a rabbit polyclonal antibody to amino acids 477–493 of RGT3 protein, in the absence (lane 1) or presence (lane 2) of excess of the immunizing peptide. An excess of synthetic peptides of C-terminal regions of human GLUT3 or human GLUT1 was also added in lane 4 or lane 5, respectively. Antibody binding was detected using an alkaline phosphatase-conjugated secondary antibody. RGT3 was recognized as a single band of ~ 60 kDa. Lane 3 shows the immunoreactivity of the rat brain proteins with preimmune serum. (B) Tissue lysates from several rat organs and a mouse brain (150 μg of protein per lane) were immunoblotted with RGT3 antiserum. Note that the immunoreactive band from a mouse brain was migrated at ~ 50 kDa, in contrast to a single band of ~ 60 kDa of RGT3. The mobilities of prestained molecular mass markers (size noted in kilodalton) are shown to the left. *Abbreviations:* MB, mouse brain; RB, rat brain; He, heart; Li, liver; K, kidney.

peptides (MAP) deduced from C-terminal coding region generating the specific antiserum. This peptide antiserum specifically recognizes the appropriate rat or mouse GLUT3 proteins in an immunoblot (Fig. 3). The antibody reacted with rat or mouse whole brain lysates of values of ~ 60 and ~ 50 kDa on SDS-polyacrylamide electrophoresis, respectively. Since the protein sequence of C-terminal coding region of RGT3 protein used in the synthetic peptide was exactly same as mouse GLUT3 protein, the antibody reacted with both proteins. Although these size estimates are slightly different from the predicted molecular mass values of 53,488 Da for mouse [19] and 53,566 Da for rat, this may be due to either the mobility on SDS-PAGE or the glycosylation. As well as the result of tissue distribution by RNA blotting study (data not shown), the antibody recognized the protein only in the brain. Preimmune serum did not react with glucose transporter proteins, and absorption of this antiserum with the immunizing peptide completely inhibited its binding. Addition of synthetic peptides of C-terminal coding regions of human GLUT3 protein (KDGVMEMNSIEPAKET) or human GLUT1 protein (SQSDKTPEELFHPL) did not inhibit the binding of this antiserum to RGT3 protein. Taken together, these results suggest that this antiserum specifically recognizes GLUT3 protein in rodent.

3.4. *Immunohistochemical localization of rat GLUT3 in brain*

The distribution of RGT3 protein in the rat brain as revealed by antibody labeling is shown in Fig. 4. The immunoreactivity was present in most parts of the brain. Strong signals were observed in lateral habenular nucleus, amygdaloid nucleus, oculomotor nucleus, and red nucleus (Fig. 4A and B). Labeling of many nucleuses in the brainstem was noted with greater intensities in the raphe pontine nucleus, pontine reticular nuclei V, trigeminal nucleus, lateral superior olive, nucleus of trapezoid body, mesencephalic trigeminal nucleus (Fig. 4C). In the cerebellum, RGT3 immunoreactivities were found in the greatest concentration in the granule cell layer and white matter of the cerebellum, but was absent from molecular cell layer (Fig. 4D). This is in excellent agreement with ISH of cerebellum, which shows that the granule cell layer contains the higher levels of RGT3 mRNA, while the molecular cell layer lacks hybridization signals (see Fig. 2C).

3.5. *RGT3 is neuron specific*

Analyses of 3 μm sections using light microscopy demonstrated that RGT3 immunoreactivity was clearly present on the cell surface membrane of neuronal cell bodies and fine processes (Fig. 5A). Immunoreactive neurons showed strong plasma membrane labeling of neuronal cell bodies and dendrites. Staining was absent from the nuclei and cytoplasm of immunoreactive cells. Fig. 5B shows the neurons in brainstem double-stained for the RGT3 and for the neuron-specific enolase (NSE) antigen. The plasma membrane of neuronal cell body, whose cytoplasmic segment was stained for the NSE antibody, was also stained for the RGT3 antibody. Fig. 5C shows double-stained for the RGT3 and for the glial fibrillary acidic protein (GFAP) antigen. The sequence of the plasma membrane of neuronal cell body not stained for the GFAP antibody were stained the RGT3 antibody.

4. DISCUSSION

In spite of an important role of glucose metabolism in neurons, the presence of glucose transporter proteins in neurons in situ has not yet been shown clearly. Since mouse GLUT3 is predominantly expresses in brain as shown in previous studies [19,28], it was suggested that mouse GLUT3 is a neuronal glucose transporter, however, we and others did not show the direct evidence of the presence of GLUT3 protein in neurons in situ. The present study is the first to demonstrate the distribution of glucose transporter protein (GLUT3) expressed in neurons.

In this study, we isolated the cDNA clone encoding RGT3, which indicates that it is a protein of 493 amino acids and that it has ~ 94% and ~ 82% identity to the sequences of mouse GLUT3 and human GLUT3, re-

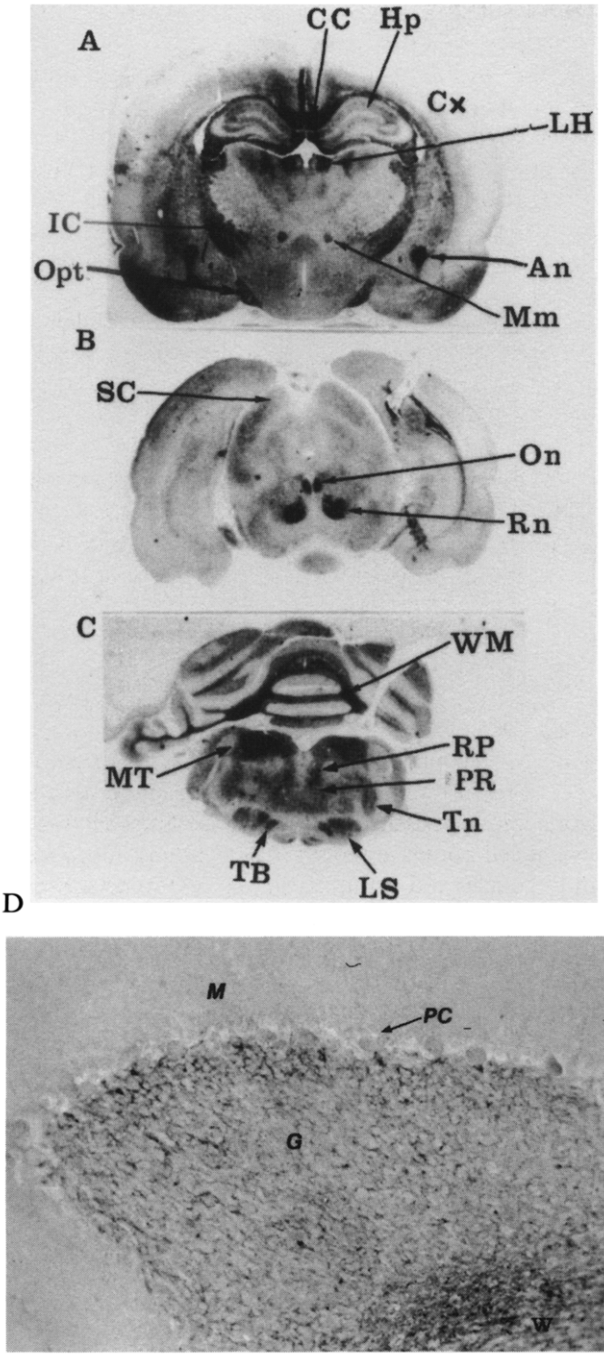


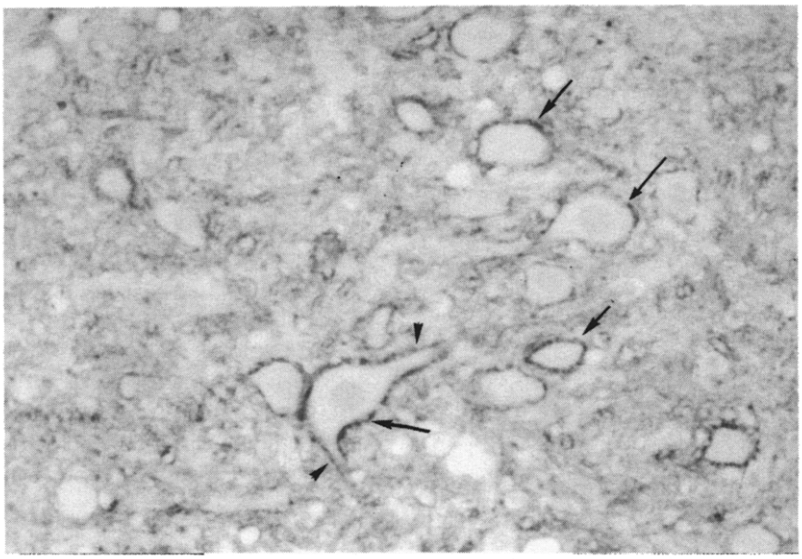
Fig. 4. RGT3 immunoreactivities in coronal sections of rat brain. A series of photomicrographic image shows the overall distribution of RGT3 protein in coronal sections of the rat brain (A,B,C). (D) Immunohistochemistry of cerebellum at higher magnification, showing dense RGT3 staining localized to the granule cell (G) layer, and the white matter (W) of the cerebellar cortex, and to a lesser degree in the Purkinje cells (PC). *Abbreviations:* CC, corpus callosum; Hp, hippocampus; Cx, cortex; LH, lateral habenular nucleus; An, amygdaloid nucleus: Mm, mammillo thalamic tract; IC, internal capsule; Opt, optic tract; On, oculomotor nucleus; SC, superior colliculus; Rn, red nucleus; WM, white matter of cerebellum; RP, raphe pontine nucleus; PR, pontine reticular nuclear V; Tn, trigeminal nucleus; LS, lateral superior olive; TB, trapezoid body; MT, mesencephalic trigeminal nucleus.

spectively. The sequence of GLUT3 is highly conserved between mouse and rat, but is not highly conserved between human and rodent. In contrast to the tissue distribution of human GLUT3 mRNA which is present at variable levels in all human tissue [16,29], expressions of RGT3 mRNA and protein are restricted to the brain as well as to the mouse as previously shown [19,30]. Thus, not only the amino acid sequence but also the tissue distribution pattern are highly conserved between mouse and rat GLUT3, suggesting that rodent GLUT3 is a neuron-specific glucose transporter. ISH revealed that RGT3 mRNA was expressed at relatively high levels in the hippocampus, dentate gyrus, cerebral cortex, striatum, cerebellum, and to a lesser degree in the brainstem, which is almost similar to mouse GLUT3 mRNA expression pattern, as previously shown somewhere [19]. The major difference between mouse and rat was observed in the region of cerebellum, where mouse GLUT3 mRNA was predominantly expressed in the Purkinje cell layer, whereas RGT3 mRNA was observed mostly in the granule cell layer. Physiological meanings of such a species difference are unknown at present.

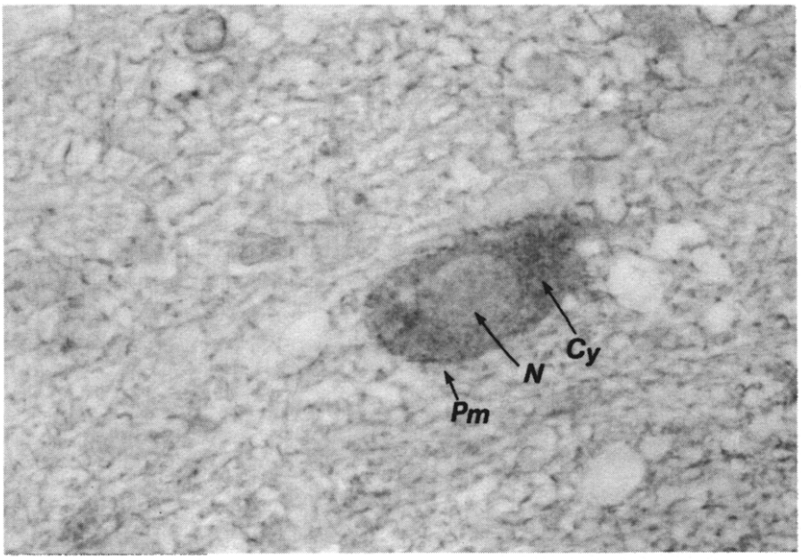
Immunohistochemistry is a powerful tool for the purpose of addressing the question of whether RGT3 is present only in neurons or distributed widely in cells other than neurons (e.g. astrocyte, oligodendrocyte, or endothelial cells of blood brain barrier, etc.), while clarifying the localization of RGT3 protein in the rat brain. In order to produce an anti-RGT3-specific antibody allowing us to do immunohistochemical studies, we synthesized the multiple antigen peptide (MAP) of 17 amino acids (477–493) of RGT3 by the method of Tam [21], and immunized rabbits. RGT3 antiserum produced by MAP was specific for rat GLUT3 proteins by following reasons. (1) Preimmune serum did not react with rat whole brain lysates. (2) ~ 60 kD immunoreactive band was absorbed with the preincubation of 100 μg/ml of immunizing peptide. (3) Synthetic peptides of COOH-terminal regions of human GLUT3 or human GLUT1 did not inhibit the binding of this antibody to RGT3 protein. In fact, in rodent brain the human GLUT3 synthetic peptide antibody do not crossreact with the rodent GLUT3 band [29]. (4) Tissue distribution of RGT3 protein agreed with the result of RNA blotting. Thus, this antibody enabled us to study the distribution of RGT3 proteins in rat central nervous system (CNS).

The RGT3 protein distributions in CNS were very extensive, but not ubiquitous, with detectable expression throughout each major subdivision of the brain. The results of immunohistochemistry in several different parts of the rat brain argue that RGT3 protein is localized on the plasma membrane of neurons. Although the staining densities of RGT3 proteins were not parallel to the levels of RGT3 mRNAs revealed by ISH, the pattern of the distribution correlated roughly with

A



B



C

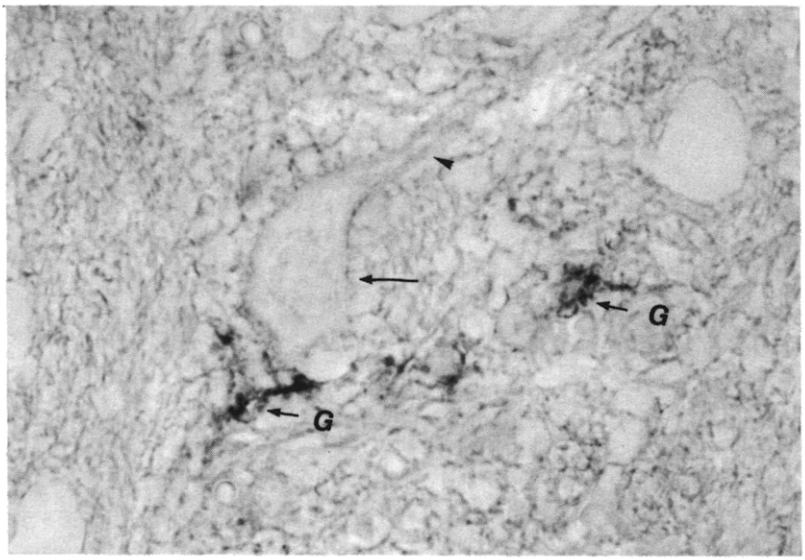


Fig. 5. Immunolocalization of RGT3 in neurons from the rat brain. (A) In 3 μm sections of brainstem, RGT3 immunoreactivity is localized to the plasma membrane (Pm) of neurons (arrows) and to dendrites (arrowheads). (B) Immunodetection of NSE antigen (which labels the cytoplasm (Cy) within neuron) and RGT3 by double-staining technique on the same section as in (A). NSE staining is shown by blue-color, and RGT3 shown by brown. (N = nucleus) (C) Immunodetection of GFAP antigen (which labels the glial cells (G)) and RGT3 on the same section as in (A). GFAP staining is shown by blue-color, and RGT3 shown by brown.

←

each other. In the cerebellum, ISH showed that RGT3 mRNA was heavily concentrated in the granule cell layer of cerebellar cortex, but was not found in the molecular layer and the white matter of cerebellum. On the other hand, RGT3 immunoreactivity was detected in the granule cell layer and the white matter of cerebellum, but not in the molecular layer where the axons (parallel fibers) of the granule cells are packed. Taken together the results of ISH and immunohistochemistry in the cerebellum, it is suggested that RGT3 protein is synthesized in the granule cells, and is concentrated in the neuronal somata.

To directly demonstrate that RGT3 protein is present in neurons, double-stained technique was carried out with the neuron-specific enolase (NSE) and the glial fibrillary acidic protein (GFAP) antibodies, which are the cell markers of neurons [23–25] and glias [26,27], respectively. RGT3 immunoreactivity could be detected only in the neuron in which the cytoplasm was stained with NSE. The γ subunit of enolase (γ-enolase), designated neuron-specific enolase (NSE), is distributed widely in other than neuronal and neuroendocrine cells [31,32]; though, at least in normal brain, NSE is expressed only in neurons. The results thus demonstrate that RGT3 protein is specifically expressed in neurons, but not in glias or blood–brain barriers in brain.

In conclusion, we have cloned rat GLUT3 glucose transporter cDNA and produced specific antibody using multiple antigen peptide (MAP), thereby we have visualized the distribution and localization of RGT3 mRNA and protein by ISH and immunohistochemistry. The examinations of combined ISH and immunohistochemistry identified RGT3 as a neuron-specific glucose transporter. Further investigation of expression or distribution of RGT3 mRNA and protein under different conditions would provide additional information about the significance of the glucose transporter system in physiological and pathological functions in the CNS.

Acknowledgements: This study was supported in part by Grant-in-Aid for Scientific Research (A)02404058, (B)03454353, and (B)04454366 from the Ministry of Education, Science and Culture, Japan and by Grant-in-Aid for the Science Research Promotion Fund, Japan Private School Promotion Foundation.

REFERENCES

[1] Owen, O.E., Morgan, A.P., Kemp, H.G., Sullivan, J.M., Herrera, M.G. and Chahill, G.F. (1967) J. Clin. Invest. 46, 1589–1599.
[2] Lund-Anderson, H. (1979) Physiol. Rev. 59, 305–352.
[3] Wheeler, T.J. and Hinkle, P.C. (1985) Annu. Rev. Physiol. 47, 503–517.
[4] Carruthers, A. (1990) Physiol. Rev. 70, 1135–1176.
[5] Bell, G.I., Kayano, T., Buse, J.B., Burrant, C.F., Takeda, J., Lin, D., Fukumoto, H. and Seino, S. (1990) Diabetes Care 13, 198–208.
[6] Burant, C.F., Takeda, J., BrotLaroche, E., Bell, G.I. and Davidson, N.O. (1992) J. Biol. Chem. 267, 14523–14526.
[7] Crone, C. (1965) J. Physiol. 181, 103–113.
[8] Gjedde, A. (1983) Acta Neurol. Scand. 67, 3–25.
[9] Pardridge, W.M. (1983) Physiol. Rev. 63, 1481–1535.
[10] Reese, T.S. and Karnovsky, M.J. (1967) J. Cell. Biol. 34, 207–217.
[11] Boado, R.J. and Pardridge, W.M. (1990) Biochem. Biophys. Res. Commun. 166, 174–179.
[12] Pardridge, W.M., Boado, R.J. and Farrell, C.R. (1990) J. Biol. Chem. 265, 18305–18040.
[13] Takata, K., Kasahara, T., Kasahara, M., Ezaki, O. and Hirano, H. (1990) Biochem. Biophys. Res. Commun. 173, 67–73.
[14] Walker, P.S., Donovan, J.A., Van Ness, B.G., Fellows, R.E. and Pessin, J.E. (1988) J. Biol. Chem. 263, 15594–15601.
[15] Maher, F., Davies-Hill, T.M., Lysko, P.G., Henneberry, R.C. and Simpson, I.A. (1991) Mol. Cell. Neurosci. 2, 351–360.
[16] Kayano, T., Fukumoto, H., Eddy, R.L., Fan, Y-S., Byers, M.G., Shows, T.B. and Bell, G.I. (1988) J. Biol. Chem. 263, 15245–15248.
[17] Yamamoto, T., Fukumoto, H., Koh, G., Yano, H., Inagaki, N., Yamada, Y., Inoue, K., Manabe, T. and Imura, H. (1990) Biochem. Biophys. Res. Commun. 170, 223–230.
[18] Mantych, G.J., James, D.E., Chung, H.D. and Devaskar, S.V. (1992) Endocrinology 131, 1270–1278.
[19] Nagamatsu, S., Kornhauser, J.M., Burant, C.F., Seino, S., Mayo, K.E. and Bell, G.I. (1992) J. Biol. Chem. 267, 467–472.
[20] Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463–5467.
[21] Tam, J.P. (1988) Proc. Natl. Acad. Sci. USA 85, 5409–5413.
[22] Hsu, S.M., Raine, L. and Fanger, H. (1981) J. Histochem. Cytochem. 29, 577–580.
[23] Rider, C.C. and Taylor, C.B. (1974) Biochim. Biophys. Acta 365, 285–300.
[24] Fletcher, L., Rider, C.C. and Taylor, C.B. (1976) Biochim. Biophys. Acta 452, 245–252.
[25] Schmechel, D.E., Marangos, P.J. and Brightman, M.W. (1978) Nature 276, 834–836.
[26] Bignami, A., Eng, L.F., Dahl, D. and Ueda, C.T. (1972) Brain Res. 43, 429–435.
[27] Mannoji, H., Takeshita, I., Fukui, M., Ohta, M. and Kitamura, K. (1981) Acta Neuropathol. (Berl.) 55, 63–69.
[28] Yano, H., Seino, Y., Inagaki, N., Hinokio, Y., Yamamoto, T., Yasuda, K., Masuda, K., Someya, Y. and Imura, H. (1991) Biochem. Biophys. Res. Commun. 174, 470–477.
[29] Shepherd, P.R., Gould, G.W., Colville, C.A., McCoid, S.C., Gibbs, E.M. and Kahn, B.B. (1992) Biochem. Biophys. Res. Commun. 188, 149–154.
[30] Gould, G.W., Brant, A.M., Kahn, B.B., Shepherd, P.R., McCoid, S.C. and Gibbs, E.M. (1992) Diabetologia 35, 304–309.
[31] Kato, K., Ishiguro, Y., Suzuki, F., Ito, A. and Semba, R. (1982) Brain Res. 237, 441–448.
[32] Haimoto, H., Takahashi, Y., Koshikawa, T., Nagura, H. and Kato, K. (1985) Lab. Invest. 52, 257–263.