Developmental regulation of genes mediating murine brain glucose uptake

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Developmental regulation of genes mediating murine brain glucose uptake. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R892–R900, 1999.—We examined the molecular mechanisms that mediate the developmental increase in murine whole brain 2-deoxyglucose uptake. Northern and Western blot analyses revealed an age-dependent increase in brain GLUT-1 (endothelial cell and glial) and GLUT-3 (neuronal) membrane-spanning facilitative glucose transporter mRNA and protein concentrations. Nuclear run-on experiments revealed that these developmental changes in GLUT-1 and -3 were regulated posttranscriptionally. In contrast, the mRNA and protein levels of the mitochondrially bound glucose phosphorylating hexokinase I enzyme were unaltered. However, hexokinase I enzyme activity increased in an age-dependent manner suggestive of a posttranslational modification that is necessary for enzymatic activation. Together, the postnatal increase in GLUT-1 and -3 concentrations and hexokinase I enzymatic activity led to a parallel increase in murine brain 2-deoxyglucose uptake. Whereas the molecular mechanisms regulating the increase in the three different gene products may vary, the age-dependent increase of all three constituents appears essential for meeting the increasing demand of the maturing brain to fuel the processes of cellular growth, differentiation, and neurotransmission.

GLUCOSE, an essential substrate for brain oxidative metabolism, is transported across the blood-brain barrier from the circulation into the brain interstitium (10, 20). Interstitial glucose is then transported into neurons and glial cells via a process of facilitated diffusion (23). Facilitative glucose transport is mediated by isoforms of a family of membrane-spanning glycoproteins called facilitative glucose transporters (3, 13). Whereas other facilitative glucose transporter isoforms (GLUT-2, -4, -5, and -7) and sodium-glucose cotransporter isoform-1 are expressed and limited to specific cell types within certain regions of the brain (4, 28), GLUT-1 and -3 are the two facilitative glucose transporter isoforms that are widely expressed by the mammalian brain (11, 29, 30, 37). GLUT-1 [Michaelis constant (Km) 1–5 mM] is expressed in the blood-brain barrier, forming microvessels, choroid plexus, ependymal cells, and glial cells (11, 16, 37), and GLUT-3 (Km 1.8 mM) is primarily a neuronal glucose transporter isofrom (3, 29, 30). Once the substrate is transported into the intracellular compartment, glucose is phosphorylated into glucose 6-phosphate by a rate-limiting reaction catalyzed by the mitochondrial hexokinase I enzyme (34). This reaction forms the initial step in the cascade of glycolysis, a necessary process for ATP production (34). Both transport and phosphorylation constitute brain glucose uptake (19). The normal process of mammalian brain development from the late-gestation fetus to the adult demonstrates a gradual increase in brain glucose uptake to meet the increasing metabolic demand of the brain of the postsuckling mammal (18, 22, 43). The two processes that contribute to the developmental increase in brain glucose uptake have not been entirely elucidated in the context of each other. Most studies to date have examined the transporter proteins alone, and these studies have been undertaken in the rat, rabbit, and human (11, 16, 29, 31, 37, 47). However, the mouse is the species of choice for investigations involving genetic manipulation. Before undertaking investigations of this nature, it is essential to determine if a similar developmental increase in murine brain glucose uptake exists and then to characterize the mechanisms responsible for this change. In the present study, we demonstrate the molecular mechanisms behind a postnatal increase in the entire murine brain glucose uptake.

MATERIALS AND METHODS

Animals. BALB-C mice were purchased from Jackson Laboratories and housed in individual cages under 12:12-h light-dark cycles with free access to water and standard chow. The care and use of animals followed the guidelines established by the National Institutes of Health and were approved by the Magee-Womens Research Institutional Committee for Animal Care and Use.

Sample collection. Male and female mice were mated, and day 0 of gestation was confirmed by the presence of a vaginal plug. The pregnancy was dated, and pooled 18-day gestation fetal brain tissue per litter was obtained after maternal hysterotomy under intraperitoneal pentobarbital sodium anesthesia. Postnatal animals at 1, 7, 14, 21, 35, and 60 days received intraperitoneal pentobarbital sodium, and the brain tissue was obtained for various analyses and stored as described under the different assays.

Nuclear run-on experiments: Isolation of nuclei. Brain nuclei from 1, 21, and 60-day ages were isolated by differential centrifugation using a sucrose gradient. Brain tissue was minced in 1 ml of ice-cold PBS, resuspended in a total volume of 10 ml of PBS, and centrifuged at 500 g for 3 min. The pellet was resuspended in 8 ml of ice-cold homogenization buffer [10 mM Tris pH 7.5, 10 mM NaCl, 3 mM MgCl2, 0.5 mM dithiothreitol (DTT), and 100 mM sucrose] and disrupted
with five strokes of pestle A then five strokes with pestle B of a Dounce homogenizer. Nuclei were pelleted by centrifugation at 700 g for 6 min at 0°C. The nuclear pellet was resuspended in 10 ml of ice-cold sucrose buffer (10 mM Tris pH 7.5, 10 mM NaCl, 3 mM MgCl₂, 0.5 mM DTT, and 170 mM sucrose) and loaded onto a low-sucrose cushion (330 mM sucrose, 5 mM MgCl₂, 10 mM Tris pH 8.0, and 0.5 mM DTT) and centrifuged at 700 g for 10 min at 0°C. The resultant pellet was then resuspended in 2 ml of a lysis buffer (10 mM Tris pH 7.5, 10 mM NaCl, 3 mM MgCl₂, 0.5% Igapel CA630) and loaded onto a high-sucrose cushion (2 M sucrose, 10 mM Tris pH 8.0, 5 mM magnesium acetate, and 0.1 mM EDTA) and centrifuged at 30,000 g for 30 min at 0°C. The final nuclear pellet was resuspended in 200 µl of a storage buffer (50 mM Tris pH 8.3, 5 mM MgCl₂, 0.1 mM EDTA, and 40% glycerol) and divided into 100-µl aliquots between 1.5 and 5 × 10⁶ nuclei and stored at −70°C.

In vitro transcription reaction. The nuclei were thawed on ice, and 90 µl of a transcription buffer (50 mM Tris pH 7.8, 5 mM MgCl₂, 150 mM KCl, 1 mM MnCl₂, 750 µM rATP, 250 µM rCTP and rGTP, 2.5 mM EDTA, and 10% glycerol) with 10 µl of 40 mCi/ml (specific activity = 6,000 Ci/mmole) [32P]UTP (New England Nuclear, Boston, MA) were added. This mixture was incubated at 30°C for 30 min. The transcription reaction was terminated by adding 100 U of DNase I and further incubated for 5 min at 30°C. The proteins were removed by treating the reaction mixture with 100 µg/ml proteinase K in 250 mM NaCl, 10 mM Tris pH 7.4, 25 mM EDTA, and 1% SDS for 30 min at 37°C. Total RNA was isolated as previously described (8), and the nascent transcripts were precipitated in one volume of isopropanol with 100 µg of Escherichia coli tRNA serving as a carrier. Precipitates were centrifuged for 15 min at 4°C at 12,000 rpm, washed with 70% ethanol, and suspended in 50 µl of RNAase buffer (20 mM Tris pH 7.5, 5 mM MgCl₂, and 1 mM CaCl₂) containing 100 U of DNase I and incubated at 37°C. An equal volume of proteinase K buffer was then added and incubated at 37°C for 30 min. Total RNA was extracted (8) and suspended in 30 µl of the TES buffer (20 mM TES pH 7.4, 10 mM EDTA, and 0.2% SDS) to which another 30 µl of TES with 0.45 M NaCl was added. One microfilter of this resuspended RNA was precipitated with tricarbonylacid in the presence of 1 µg of E. coli tRNA, and the incorporated radioactivity was assessed in a scintillation counter.

Slot blot analysis. Two micrograms of 1,432-bp (188–1,620 bp) mouse GLUT-1 (12), 1,841-bp (80–1,561 bp) mouse GLUT-3 (30), 1,800-bp mouse β-actin (44), and 475-bp (58–533 bp) rat leptin (42) (negative control) cDNAs were applied to Gene screen nylon membranes (Dupont-NEN) using a slot blot manifold and cross-linked with an ultraviolet Stratalinker 2400 (Stratagene, La Jolla, CA). These nylon filters were subjected to prehybridization for 1 h at 60°C in 800 µl of a hybridization buffer containing 20 mM TES pH 7.4, 10 mM EDTA, 0.2% SDS, 225 mM NaCl, and 5× Denhardt’s reagent (0.1% polyvinylpyrrolidone, 0.1% BSA, 0.1% Ficoll). We added 3 × 10⁶ cpm of 32P-labeled nascent transcripts obtained from the brain nuclei at different ages (1, 21, and 60 days) to this prehybridization solution, and hybridization continued at the same temperature for 36 h. The filters were then washed one time in 2× saline sodium citrate (SSC; 300 mM NaCl and 30 mM sodium citrate, pH 7.0) and 0.1% SDS for 5 min at 60°C and two times in 2× SSC and 0.1% SDS for 15 min at 60°C, then treated with 8 ml of RNAase A solution (10 mM Tris pH 7.5, 0.3 M NaCl, 2 mM EDTA, and 50 µg/ml RNAase A) for 30 min at 37°C. Finally the filters were washed once with 2× SSC and 0.1% SDS for 10 min at 60°C. The radioactivity in the filters was initially quantitated by a BioRad GS-525 Molecular Phosphorimager System (Hercules, CA), and subsequently the filters were exposed to Kodak BioMax MS film for 72 h with intensifying screens. Transcription/elongation rates for a gene were expressed as nascent radioactive mRNA synthesized by 1 × 1⁶ isolated nuclei that hybridized to a given cDNA.

Northern blot analysis. Poly (A⁺) enriched RNA was extracted from murine brains that had been quick frozen in liquid nitrogen and stored at −70°C by the Mini Ribo sep ultra mRNA extraction kits (Collaborative Research, Bedford, MA). RNA was quantitated spectrophotometrically, and the purity of the samples was assessed as a ratio of 1.8–2.0 at 260/280 nm wavelength. Five micrograms of poly (A⁺) enriched RNA were loaded on 1.2% agarose-2.2 M formaldehyde slab gels and run at 22 V for 16 h. The gels were stained with ethidium bromide and visualized under ultraviolet light to confirm the absence of severe degradation in the RNA samples. The RNA from the gels was transferred to Nytran membranes (Micron Separations, Westboro, MA) and covalently bound by an ultraviolet light in a Stratalinker at 1,200 × 10⁶ mJ for 45 s (Stratalinker 1800; Stratagene, La Jolla, CA). The filters were prehybridized for 2 h at 42°C in 50% formamide, 0.1% polyvinylpyrrolidone, 0.1% BSA, 0.1% Ficoll, 75 mM sodium citrate, 50 mM sodium monobasic phosphate, 1.25 mM EDTA, 0.2% SDS, and 200 µg denatured salmon sperm DNA. The filters were subjected to hybridization with 1 × 1⁶ counts·min⁻¹·ml⁻¹ of 32P-labeled randomly primed 1.5-kb mouse GLUT-1 cDNA (12), 1.6-kb mouse GLUT-3 cDNA (30), or 3.3-kb rat hexokinase I cDNA fragments (40) for 16 h. The filters were washed four times at room temperature in 2 × SSC (1 × SSC = 0.15 M sodium chloride and 0.015 M sodium citrate, pH 7.0) and 0.1% SDS for 5 min each, then an additional two washes were performed in 0.1 × SSC and 0.1% SDS for 15 min at 50°C. The filters were subsequently exposed to X-ray film using screen intensifiers at −70°C for differing lengths of time until optimal resolution was achieved. The same filters were stripped and reprobed under the same conditions with a 32P-labeled randomly primed 1.8-kb rat 18S ribosomal RNA cDNA (7) to correct for the interlane loading variability. The GLUT-1, GLUT-3, and hexokinase I mRNA levels were quantitated by densitometry, the values were first calculated as a ratio to the 18S rRNA optical density, and then they were expressed as a percent of the value at the 35-day developmental age to overcome interblot variability. Initially GLUT-1 and GLUT-3 were assessed at ages between 18 days gestation and 60 days old. On the basis of these results, key stages (1, 14, and 35 days) alone were examined for hexokinase I mRNA levels.

Western blot analysis. One hundred micrograms of brain homogenates were solubilized in Laemmli’s buffer. Protein content was determined by Bradford’s dye-binding assay (39). The samples were then separated by 10% discontinuous SDS-PAGE. The proteins were transferred to nitrocellulose filters by electroblothing, and the filters were subjected to Western blot analysis as described previously (39). The primary antibodies used were affinity purified rabbit anti-rat GLUT-1 (2 µg/ml) and anti-mouse GLUT-3 COOH-terminal peptides (16 amino acids; 1.6 µg/ml) that were produced, purified, and characterized by Rajakumar et al. (36). A 1.25 dilution of the mouse-anti-rat hexokinase I antibody (Chemicon International, Temecula, CA) was used to detect the hexokinase I antigen. The filters were subjected to autoradiography and an optimal exposure time determined within the range of linearity. The autoradiograms were subsequently subjected to densitometry. GLUT-1, GLUT-3, and hexokinase I protein levels were expressed as a ratio to the optical density.
of an internal control protein and presented as a percent of the mean of values obtained at the 35-day developmental age to provide consistency for comparison of the glucose transporter and hexokinase I results. All ages between the 18-day gestation and 60 days were examined for GLUT-1 and GLUT-3 protein levels. On the basis of these initial results, only key stages (1, 14, and 35 days) were examined for hexokinase I protein concentrations.

Hexokinase enzyme activity. Brain tissues from the 1, 14, and 35-day-old mice were homogenized in triethanolamine buffer, pH 7.6. An aliquot (50 µl) of homogenate was assayed for hexokinase I enzyme activity as previously described (5). Hexokinase I enzyme activity was determined by the following indirect method. d-Glucose was converted to glucose 6-phosphate (G-6-P)

\[
glucose + ATP \rightarrow \text{hexokinase} \rightarrow G-6-P + ADP
\]

The generated glucose 6-phosphate was further converted to 6-phospho-D-gluconate (6-P-gluconate) in the presence of glucose-6-phosphate dehydrogenase (G-6-PDH)

\[
G-6-P + NADP^+ \rightarrow 6-P-gluconate + NADPH + H^+
\]

The conversion of NADP to NADPH was then determined spectrophotometrically by measuring the increase in absorbance at 340 nm. Hexokinase I enzyme activity was calculated as described previously (5) and expressed in units per milligrams brain protein per minute.

Glucose uptake. Cerebral glucose uptake (CGU), which includes glucose transport and phosphorylation, was measured using a modified Sokoloff's technique using the 2-deoxy-

\[ ^{3}H \]glucose as the tracer (43, 46). The study was performed at 1, 14, and 35 days. Animals received an intraperitoneal injection of 1 µCi/g body wt of the tracer, and, at 90 min postinjection, brains were quickly removed and immediately placed in liquid nitrogen to stop intermediary metabolism. The brains were weighed and powdered under liquid nitrogen. Perchloric acid brain extracts were neutralized with 2 M KHC\textsubscript{2}O\textsubscript{4} to a pH of 6.8. KClO\textsubscript{4} was then removed by centrifugation at 5,000 g. From the final supernatant, 10 µl was used to measure radioactivity by a scintillation spectrophotometer, whereas 20 µl was used to determine glucose concentration by the glucose oxidase method (39).Simultaneously, blood was also collected on ice and centrifuged at 3,000 g for 5 min to separate plasma from red blood cells, and glucose concentrations were measured as described above, allowing derivation of the specific activity of glucose. CGU was calculated as per a modification (46) of the original Sokoloff's report (43) taking into account the specific activity of glucose in circulation and brain tissue at the 90-min time point. CGU was expressed in micromoles per 100 grams per minute and calculated as follows

\[
\text{cerebral [}^{3}H\text{]deoxyglucose (disintegrations min}^{-1}\text{g}^{-1}\text{)} \times \frac{100}{\text{plasma concentration of [}^{3}H\text{]deoxyglucose (disintegrations min}^{-1}\text{ml}^{-1}\text{)} / \text{plasma glucose concentration (}\mu\text{mol} / \text{ml})}
\]

Data analysis. All data are shown as means ± SE. One-way ANOVA was used to determine the presence of differences, and intergroup differences were validated by the Newman-Keuls test.

### RESULTS

Table 1 provides the mouse body and brain weights at the different ages examined. As expected, an age-dependent increase is noted. An ~45-kDa GLUT-1 protein band and its corresponding ~2.8-kb mRNA band were noted in brain homogenates from the different developmental ages (Fig. 1A). Quantitation of brain GLUT-1 protein and mRNA was observed in Fig. 1B. GLUT-1 mRNA levels paralleled those of the correspond-

### Table 1. Animal demographics

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Values are means ± SE; all n = 11. F, fetal; D, day; P, postnatal.

### References

- Sokoloff's report (43)
- 2-deoxy-

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### Fig. 1. GLUT-1 analyses. A: representative Western (top) and Northern (middle and bottom) blots demonstrating ~45-kDa GLUT-1 protein (top), ~2.8-kb GLUT-1 mRNA (middle), and 1.8-kb 18S ribosomal mRNA (bottom) bands in brain parenchymal homogenates obtained from 18-day (d) fetal (f) and 1, 7, 14, 21, 35, and 60-day-old postnatal mice. B: densitometric quantitation assessing brain GLUT-1 mRNA and protein levels at 18-day fetal (f) (n = 5 and 6 for mRNA and protein, respectively) and 1 (n = 5 and 6), 7, 14, 21, 35, and 60-day-old postnatal mice. GLUT-1 mRNA and protein levels at 18-day fetal (f) (n = 5 and 6) and 1, 7, 14, 21, 35, and 60-day-old postnatal mice are shown. *P < 0.05 compared with corresponding 35-day-old mouse value.
ing protein. A gradual increase in GLUT-1 mRNA and protein concentrations of 75% by 14 days and a 150% increase by 35–60 days is noted. Figure 2A demonstrates the 50-kDa GLUT-3 protein and 4.1-kb GLUT-3 mRNA bands. Quantitation of GLUT-3 mRNA and protein levels revealed a parallel threefold increase between 1 and 14 days and a two- to fourfold increase by 35–60 days (Fig. 2B). Nuclear run-on experiments to determine the molecular level at which these developmental changes occur in brain GLUT-1 and GLUT-3 concentrations revealed no change in GLUT-1 and GLUT-3 transcription/elongation rates among the three ages examined (Fig. 3, A and B).

Hexokinase I enzyme protein (100 kDa) and mRNA (4.3 kb) levels were not significantly different at the three ages examined, namely 1, 14, and 35 days, except for a trend toward higher mRNA levels at 1 day (Fig. 4, A and B). In contrast, hexokinase I enzyme activity increased threefold between 1 and 14 days and fivefold between the 1- and 35-day murine brain (Fig. 5A). Paralleling this developmental increase in brain hexokinase I activity, a progressive increase in 2-deoxyglucose uptake was observed between 1, 14, and 35 days (Fig. 5B).

**DISCUSSION**

The developmental increase in murine brain glucose uptake was similar to that reported previously in the rat and human brain (9, 18, 43, 46). This increase was paralleled by an increase in murine brain GLUT-1 and GLUT-3 levels, suggesting that the mechanism of transport is responsible for the progressive increase in global brain glucose uptake. Previous investigations in the rat have demonstrated two differentially glycosylated species of GLUT-1 in brain (11, 47): the 55-kDa form expressed in the blood-brain barrier forming endothelial cells and the 45-kDa form present mainly in brain parenchymal homogenates (11, 47). Immunolocalization in the rat and rabbit brain has unequivocally shown GLUT-1 only in glial cellular components of the parenchyma, the choroid plexus, and the ependymal lining of the ventricles (11, 16, 37). Our previous
studies in the mouse confirmed the presence of GLUT-1 in the endothelial cells of the brain microvessels in this species as well (38). However, in contrast to previous observations in rat (11, 47), our present studies using murine brain homogenates do not demonstrate two distinctly separate or a widely spread GLUT-1 protein band(s); instead a single compact ~45-kDa band was noted. This suggests that the extent of glycosylation of the murine brain endothelial GLUT-1 protein is no different from that of the glial cell GLUT-1, making them indistinguishable by SDS-PAGE. Thus total estimation in the murine brain homogenate reflects changes in both the endothelial and parenchymal glial cell glucose transporter proteins.

In our present study, both GLUT-1 mRNA and protein increased significantly at 21 days of life, remaining steady thereafter until 60 days. This developmental increase in brain GLUT-1 levels coincides with the time of weaning, suggestive of the high-fat milk intake causing a suppression in brain GLUT-1 levels. On weaning and intake of a relatively higher carbohydrate-containing diet that is characteristic of the standard laboratory chow, an increase in brain GLUT-1 levels was noted. Alternatively, GLUT-1, a glucose-regulated protein (48), is altered by available circulating glucose concentrations (11). During the immediate postnatal period, glucose availability as a substrate to the brain may be overshadowed by the abundance of ketones that are used by the rodent/murine brain (24). Furthermore, GLUT-1 is expressed abundantly in all proliferating cells. During the postsuckling phase of development, the mass of microvasculature is greater than that present in the fetus, immediate postnatal stages, and the suckling phase of development (2, 11). Furthermore, glial proliferation with myelination occurs around the 21-day postnatal age (11, 53). Thus this developmental increase in GLUT-1 may merely parallel the expanding microvascular and glial cell mass, thereby meeting the increasing metabolic demands of these cell types. In addition to fueling the energy needs of cells that express GLUT-1, this facilitative glucose transporter

Fig. 4. Hexokinase (Hk) I analyses. A: representative Western (top) and Northern (middle and bottom) blots demonstrating ~100-kDa hexokinase I protein (top), ~4.3-kb hexokinase I mRNA (middle), and 1.8-kb 18S ribosomal mRNA (bottom) bands in brain homogenates obtained from 1-, 14-, and 35-day-old mice are depicted. B: densitometric quantitation of hexokinase I mRNA and protein levels in brain homogenates obtained from 1 (n = 6 for both mRNA and protein), 14 (n = 6), and 35 (n = 6)-day-old mice is shown.

Fig. 5. Hexokinase enzyme activity and 2-deoxyglucose uptake studies. A: brain hexokinase enzyme activity (U·mg⁻¹·min⁻¹) is shown in brain extracts obtained from 1 (n = 6), 14 (n = 6), and 35 (n = 6)-day-old mice. *P < 0.05 compared with 35-day-old mouse value. B: brain 2-deoxyglucose uptake (µmol·100 g⁻¹·min⁻¹) is shown in whole brains at 1 (n = 6), 14 (n = 6), and 35 days (n = 6). *P < 0.05 compared with 35-day-old mouse value.
isoform acts at both the luminal and abluminal surfaces of the endothelial cells lining the microvasculature, thereby mediating the transfer of glucose from the circulation into the brain interstitium (17). The interstitial availability of glucose helps meet the metabolic demands of neuronal cells. Because the total number of neurons do not change postnatally, the process of synaptogenesis that occurs at 10–14 days postnatally (6) is dependent on the constant availability of glucose.

Closer examination of the mechanism by which GLUT-1 levels increase in brain revealed involvement of posttranscriptional processes, perhaps related to conferring increased mRNA stability with age. Although the limitation of determining the transcriptional rate in nuclei obtained from the entire brain is that there may be a developmentally differing contribution from the various cell types in the brain, this may not be a major problem in the case of GLUT-1. This is because the number of contaminating neurons that do not express GLUT-1 is not different at the various developmental stages examined (6, 25). Our present findings are congruent with previous investigations that have demonstrated the presence of particular consensus sequences necessary for mRNA stability in the 3'-untranslated region of the GLUT-1 gene (15, 45).

GLUT-3, on the other hand, is expressed mainly by mammalian neuronal cells (29, 30). Unlike GLUT-1, GLUT-3 mRNA and protein levels increase dramatically at day 14, while the mice are still consuming a high-fat milk diet. A similar increase has previously been observed in the rat, rabbit, and human brain as well (29, 37, 47). This developmental stage coincides with the rapid increase in synaptogenesis (1) and the acquisition of bioelectrical activity by these specialized cells (14). Previous investigations have demonstrated the presence of GLUT-3 on plasma membranes of cultured rat cerebellar granule (neuronal) cells and processes (26). However, in the human brain, GLUT-3 was particularly noted in cerebral cortical neuronal processes within brain sections (29). Our preliminary investigations in mouse brain sections confirm this subcellular localization pattern, revealing GLUT-3 immunostaining mainly in axonal processes (17a). Thus the process of cell migration with elongating axons and synaptogenesis along with neuronal depolarization may set the stage for an increased substrate demand, thereby leading to an increased demand for GLUT-3 (14, 27). Brain interstitial glucose concentrations are considerably lower than those found in circulation (35, 41); thus the facultative glucose transporter isoform expressed by neuronal cells must possess a low $K_m$ to be able to function in this low range of glucose concentrations. In addition, unlike astrocytes, neuronal cells are incapable of glycogen storage and depend on glycolysis to supply ATP to fuel the cellular metabolic needs. Therefore, GLUT-3, by virtue of its having the lowest $K_m$ for 2-deoxyglucose among all facilitative glucose transporter isoforms, is the ideal neuronal glucose transporter candidate (3).

Similar to GLUT-1, the murine brain GLUT-3 protein band was a compact 50-kDa band, indicating that it is uniformly glycosylated. The glycosylation site has been shown to reside in the NH$_2$-terminal region of the peptide (30, 31). The developmental increase in the GLUT-3 protein is paralleled by a similar increase in the corresponding mRNA levels, which in turn is associated with no increase in the transcription/elongation rate of GLUT-3. Whereas we have previously demonstrated transcriptional regulation of murine brain GLUT-3 gene by Sp1/Sp3 (36), the major regulatory mechanism responsible for the developmental increase in brain GLUT-3 expression appears to be posttranscriptional. Thus it appears that neuronal GLUT-3 levels increase due to enhanced synthesis of this peptide secondary to mRNA stability or increased translational rates. This posttranscriptional increase in GLUT-3 parallels the previously described rapid increase in synapses (1) and may correlate with the functional acquisition of cognition and specialized motor coordination.

Whereas the glucose transporter proteins regulate cellular glucose entry at the plasma membrane, the mitochondrially bound hexokinase I enzyme converts glucose to glucose 6-phosphate in preparation for glycolysis (34, 51). In the adult rat brain, under certain conditions of hyperglycemia and in cultured neurons, glucose phosphorylation was determined to be the rate-limiting step rather than glucose transport (19, 49). Furthermore, hyperglycemia was associated with an increase in fetal murine brain hexokinase I enzyme activity with no major changes in glucose transporter protein levels (38). Hexokinase I enzyme is noted in most brain parenchymal cells, which include the ependymal cells, the choroid plexus, the neurons, and glial cells. A large portion of the enzyme is detected in the nucleus (33, 50), where the NH$_2$-terminal portion of the peptide is tethered to the outer mitochondrial membrane (52). The enzyme is inhibited by glucose 6-phosphate; however, the low levels of brain interstitial glucose concentrations ensure against a build-up of neuronal glucose 6-phosphate to a level that would inhibit hexokinase I activity, thereby adversely affecting glycolysis (35, 41, 51). This mitochondrial enzyme demonstrates a slight decline in mRNA levels between the 1- and 14-day ages in the mouse, remaining unchanged at 35 days. This developmental decline in hexokinase I mRNA levels failed to translate into changes in the protein concentrations, which remained unaffected during the process of development. Although there was no change in protein concentrations, enzyme activity demonstrated a developmental increase. This dichotomy between the enzyme concentrations and activity suggests alterations in posttranslational modifications of the enzyme necessary for enhancing intrinsic functional activity. Previous studies have demonstrated the need for posttranslational modification to activate the enzyme in other cellular systems (32) and in murine brain under hyperglycemic conditions (38). A similar process may be necessary for the developmental increase in the brain hexokinase I enzyme activity.
Despite the differing mechanisms by which developmental changes in the genes mediating murine brain glucose uptake are regulated, the glucose transporter levels and hexokinase I enzyme activity parallel the age-dependent changes in brain glucose uptake. Brain glucose uptake measured by the 2-deoxyglucose analog assesses transport and phosphorylation of glucose (19). Brain glucose uptake measurements in the present study were modified from the classical Sokoloff method described in the rat (43). These modifications included not assessing brain 2-deoxy-[3H]glucose separate from 2-deoxy-[3H]glucose-6-phosphate and not deriving a “lumped constant” (21, 43, 46). The reason for these modifications is the fact that despite the use of ion exchange columns to separate the 2-deoxyglucose from the 2-deoxyglucose-6-phosphate (21), there is a potential for error based on the inherent variability in recovery rates. Whereas the 3-O-methylglucose analog assesses glucose transport alone and not phosphorylation, the efflux of this analog leads to assessment of kinetics during equilibrium, which does not accurately reflect the maximum uptake or K_m of brain glucose transport (19, 21, 43). Because our goal was merely to assess total brain glucose uptake, which includes transport into the interstitium (across blood-brain barrier) and parenchymal cells mediated by the glucose transporters, and intracellular phosphorylation mediated by hexokinase I, we elected to measure total brain 2-deoxyglucose uptake.

Other studies previously have demonstrated that brain glucose uptake is dependent on the ratio of the distribution volumes of 2-deoxyglucose and native glucose, the Michaelis-Menten constants and maximal velocities of brain hexokinase for 2-deoxyglucose and glucose, and the relative activity of brain glucose 6-phosphatase that leads to dephosphorylation of 2-deoxyglucose-6-phosphate and glucose 6-phosphate, the latter being negligible (21, 43, 46). These variables define the lumped constant, which has been assessed and noted to remain unchanged in physiologically normal steady-state conditions (21) in the newborn and adult rat brains (21, 43, 46). However, whereas no similar measurements exist in the mouse (41), the technical difficulty of gaining continuous vascular (systemic arterial and cerebral venous) access, let alone accurately making these assessments in the adult and postnatal mouse, led us to use intraperitoneal access with 90 min to achieve steady state and precluded the use of the lumped constant in our calculations. This omission can overestimate the absolute CGU (21). To overcome this potential, we included the ratio of brain 2-deoxyglucose to glucose concentrations along with the specific activity of plasma glucose in our calculations (21, 43, 46). Thus, with the stated modifications, murine brain glucose uptake increased with advancing postnatal age. Parenchymal GLUT-1 (endothelial cells and glia) and GLUT-3 (neurons) proteins, which mediate transport, and hexokinase I enzyme activity, which mediates phosphorylation, collectively regulated the developmental increase in brain glucose uptake.

In summary, three critical proteins necessary for mediating brain glucose uptake are increased developmentally to meet the increasing metabolic demands and energy requirements of the postnatal/poststuckling maturing murine brain. Transmembrane transport into endothelial cells, glial cells, and neurons, along with intracellular glucose phosphorylation, dictate the elevation in brain glucose uptake by the adult murine brain as opposed to the neonatal brain. Whereas hexokinase I enzyme is regulated at a posttranslational level, GLUT-1, the endothelial cell and glial cell transporter, and GLUT-3, the neuronal glucose transporter, are posttranscriptionally regulated through development. Although cis elements regulating GLUT-1 mRNA stability have been described (15, 45), the cis elements and cytoplasmic RNA-binding factors necessary for exerting GLUT-3 translational control and/or providing GLUT-3 mRNA stability remain unknown and need to be characterized.

Perspectives

We have demonstrated the developmental regulation of glucose transporters (GLUT-1 and GLUT-3) and the hexokinase I enzyme in murine brain. The posttranscriptional increase in glucose transporter expression and the posttranslational increase in hexokinase I enzyme activity lead to the developmental increase in brain glucose uptake necessary for meeting the increasing cellular demands. Any aberration in this developmental process can interfere with the substrate delivery necessary to meet the energy requirements of cellular growth, differentiation, myelination, and neurotransmission. Future investigations should focus on conditions that interfere with this developmental process to determine the effect on acquisition of neurologic function. Furthermore, the present studies in wild-type mice set the stage for future gene manipulation experiments in which the effect of overexpression or absence of one or more of these three proteins on brain cellular development and function will provide the mechanistic basis for neural dysregulation.

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