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Glucose Transporter Expression in Brain: Relationship to Cerebral Glucose Utilization

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Key Words

Glucose transport
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Dehydration

Abstract

Glucose is the principle energy source for mammalian brain. Delivery of glucose from the blood to the brain requires its transport across the endothelial cells of the blood-brain barrier and across the plasma membranes of neurons and glia, which is mediated by the facilitative glucose transporter proteins. The two primary glucose transporter isoforms which function in cerebral glucose metabolism are GLUT1 and GLUT3. GLUT1 is the primary transporter in the blood-brain barrier, choroid plexus, ependyma, and glia; GLUT3 is the neuronal glucose transporter. The levels of expression of both transporters are regulated in concert with metabolic demand and regional rates of cerebral glucose utilization. We present several experimental paradigms in which alterations in energetic demand and/or substrate supply affect glucose transporter expression. These include normal cerebral development in the rat, Alzheimer's disease, neuronal differentiation in vitro, and dehydration in the rat.

Introduction

The uptake of glucose into the mammalian brain is mediated by a family of facilitative glucose transporter proteins (GLUTs) (fig. 1) [for reviews, see Bell et al., 1993; Maher et al., 1994a, b; Vannucci et al., 1997b]. There are two isoforms of GLUT1: the 55-kD isoform mediates transport of glucose across both the luminal and abluminal membranes of the endothelial cells of the blood-brain barrier (BBB), and the less glycosylated 45-kD isoform functions in choroid plexus, ependyma, and also in nonvascular brain, primarily in glia. GLUT3 is the neuronal glucose transporter and GLUT5, a putative fructose transporter, is expressed in microglia, the resident macrophages of brain. GLUT5 is an extremely poor glucose transporter and probably contributes little to cerebral glucose metabolism [Burant et al., 1992].

The brain is heterogeneous with regard to its level of energy demand which is mediated by regional variation in rates of cerebral blood flow and glucose utilization. In the normal adult rat brain, the correspondence between regional cerebral metabolism rate for glucose ($rCMR_{glc}$) and the distribution of both the vascular form of GLUT1 [Rahner-Welsch et al., 1995; Zeller et al., 1997] and GLUT1 mRNA (fig. 2) is fairly good. Most of the mRNA for GLUT1 is localized to cerebral microvessels [Vannucci et al., 1997b]. There is little or no correspondence, however, between $rCMR_{glc}$ and GLUT3 mRNA (fig. 2). This discordance may reflect the difference between the location of mRNA in neuronal cell bodies and the sites of the highest $rCMR_{glc}$ in neuropil. In fact the GLUT3 protein is located predominantly in regions of neuropil [McCall et al., 1994].

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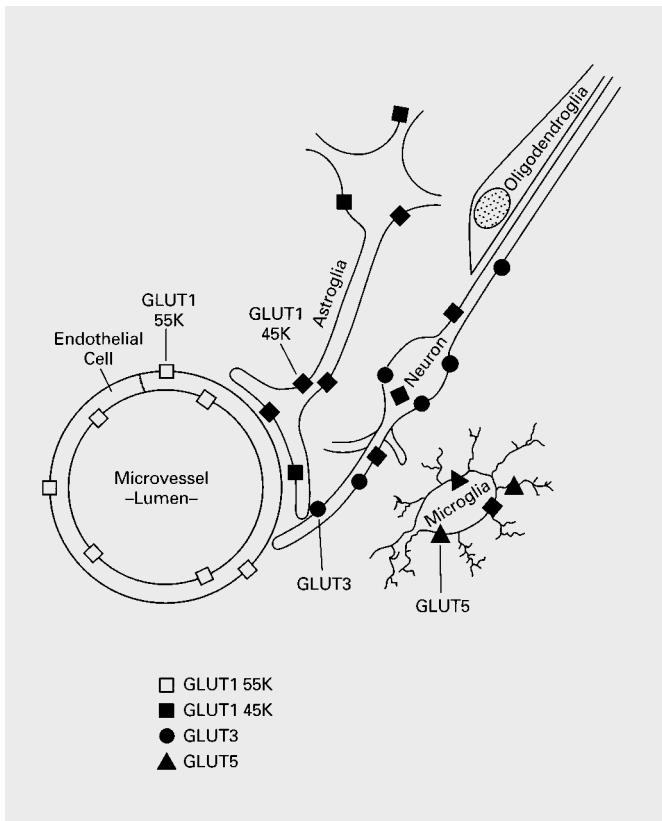


Fig. 1. Schematic representation of cellular distribution of glucose transporters in mammalian brain [Maher et al., 1994a].

In normal adult rat over a broad range of circulating glucose concentrations, the levels of glucose transporter proteins are sufficient to ensure an adequate supply of glucose to meet metabolic demand. The question arises as to whether or not this system for the delivery of substrate to brain is responsive to alterations in levels of either substrate supply or glucose metabolic demand. To address this question, we have investigated several different paradigms in which metabolic demand is altered chronically. What follows represents a compilation of previously published studies [for review, see Vannucci et al., 1997b], as well as recent novel findings.

Methods

All of the following studies employed previously published methods. In the interests of brevity they will not be described in detail but will be referenced here. Cerebral glucose utilization was measured according to the 2-deoxyglucose method of Sokoloff et al. [1977], and in situ hybridization was performed on adjacent sections as de-

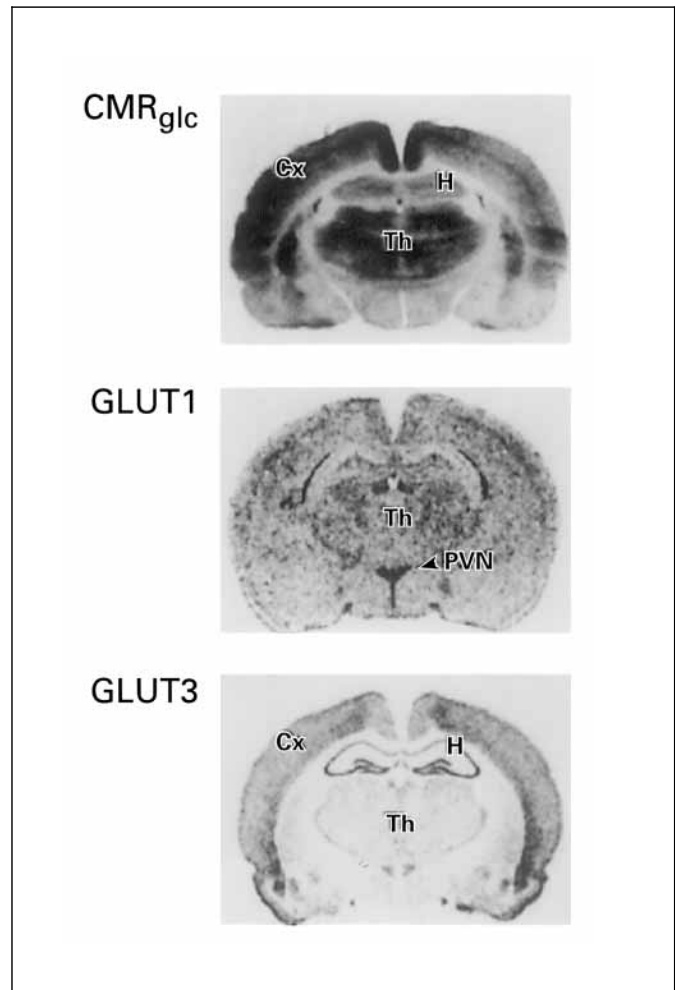


Fig. 2. Cerebral glucose utilization and GLUT1 and GLUT3 mRNA expression. Cerebral glucose utilization was determined in conscious, unrestrained adult male rats according to the 2-deoxyglucose method of Sokoloff et al. [1977]. In situ hybridization for GLUT1 and GLUT3 was performed on adjacent sections using [³⁵S]riboprobe as described by Vannucci et al. [1997a]. According to this methodology, all of the ¹⁴C-compounds are removed from the sections by the prehybridization washes prior to subsequent hybridization with [³⁵S]riboprobe. Hybridization with sense probes routinely yields no positive signal [Vannucci et al., 1997a].

scribed by Vannucci et al. [1997a]. Sample membrane preparation and Western blot analysis were performed using polyclonal antipeptide antibodies: GLUT1–5, generous gifts from Hoffmann-La Roche; Na,K-ATPase, provided by R. Levenson; synaptophysin, Sigma Chemical Co., or monoclonal antibodies, SP-14 for the detection of SNAP-25 [Honer et al., 1993]. Immunodetection and quantitation were performed either with chemiluminescence (DuPont-NEN, Renaissance) and image analysis [Vannucci et al., 1997a] or ¹²⁵I-Protein A and phosphorimage analysis [Simpson et al., 1994].

Paradigms

Developmental Regulation

The close relationship between cerebral glucose transporter expression and utilization is perhaps most clearly seen during normal postnatal development in the rat. Although alternate substrates, such as ketone bodies and lactate, are of quantitative importance as cerebral metabolic fuels during the newborn period, an adequate supply of glucose is essential for normal brain development and function. At birth, rates of metabolic demand and CMR_{glc} in the rat are quite low due to the relative immaturity of the brain. We have previously shown that the levels of both GLUT1 and GLUT3 glucose transporter proteins are also low at birth and increase during postnatal maturation, as do rates of CMR_{glc} [Vannucci, 1994; Vannucci et al., 1994b]. Although at birth, levels of 55 kD BBB GLUT1 are detected in isolated microvessels at concentrations approximately 40% of the adult level, not all capillaries are perfused and thus measured rates of glucose uptake are only 20% that of the adult [Daniel et al., 1978; Fuglsang et al., 1986; Dyve and Gjedde, 1991]. The increase in vascularity between postnatal days 5 and 9 (P5–P9), followed by an increase in 55 kD GLUT1 density in the microvessels, results in full glucose transport capacity by P30. Nonvascular GLUT1 (45 kD) increases gradually and uniformly throughout the first 4 weeks, which is likely reflective of the postnatal maturation of glial elements as well as the overall growth of the brain.

Cortical levels of GLUT3 protein increase sharply between P14 and P21 which coincides with synaptogenesis and neuronal maturation, and most closely matches observed increases in CMR_{glc} . The postnatal expression of both GLUT1 and GLUT3 mRNAs, as determined by in situ hybridization histochemistry, is depicted in figure 3. GLUT1 mRNA expression is initially most prominent in the microvessels, seen as the punctate signals throughout the brain section and in the ventricles. The overall expression of GLUT1 mRNA is relatively low at P1–P10, in keeping with the low levels of protein. GLUT3 mRNA levels also remain relatively low through day 10, but demonstrate a significant increase of 27.5% ($p < 0.001$) at P14. This is a time period of active synaptogenesis as well as the point at which circulating levels of ketone bodies begin to fall and rates of CMR_{glc} rise [Neligi et al., 1988]. The increase in GLUT3 mRNA is however transient and is reduced by 15% at P21 before returning to adult levels of expression by day 30. The regulation of GLUT3 expression in concert with neuronal maturation and func-

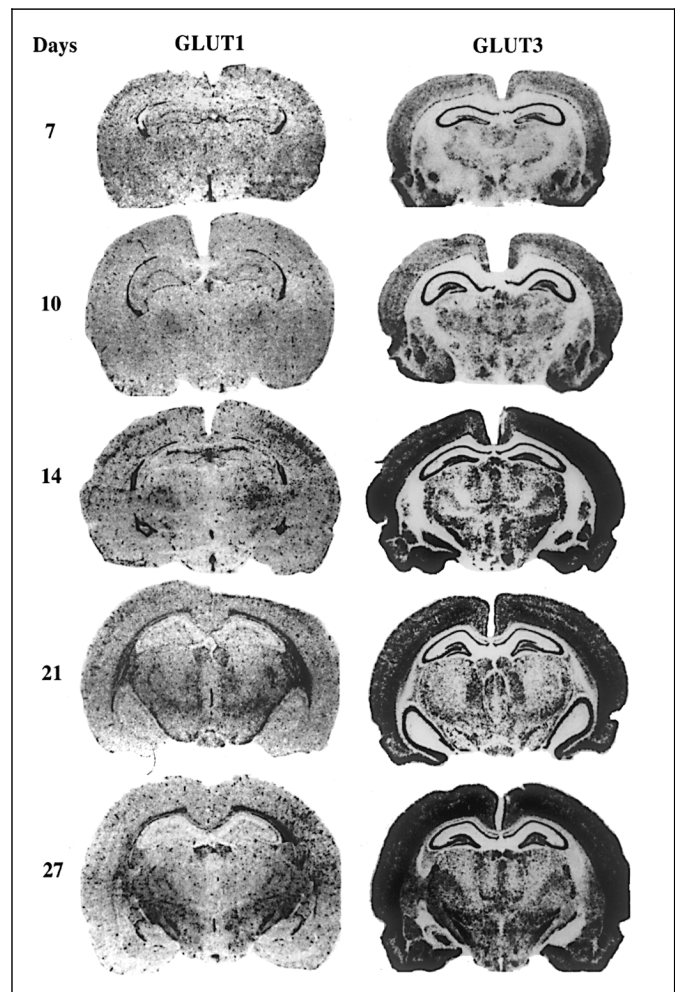
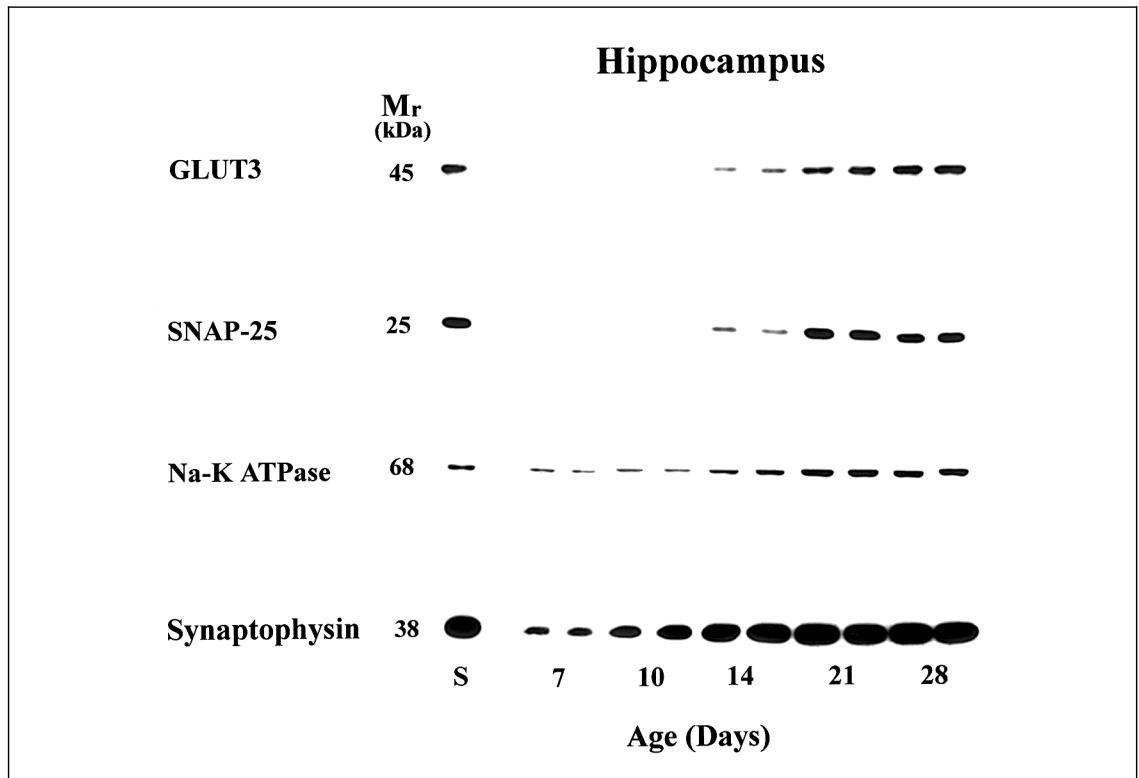


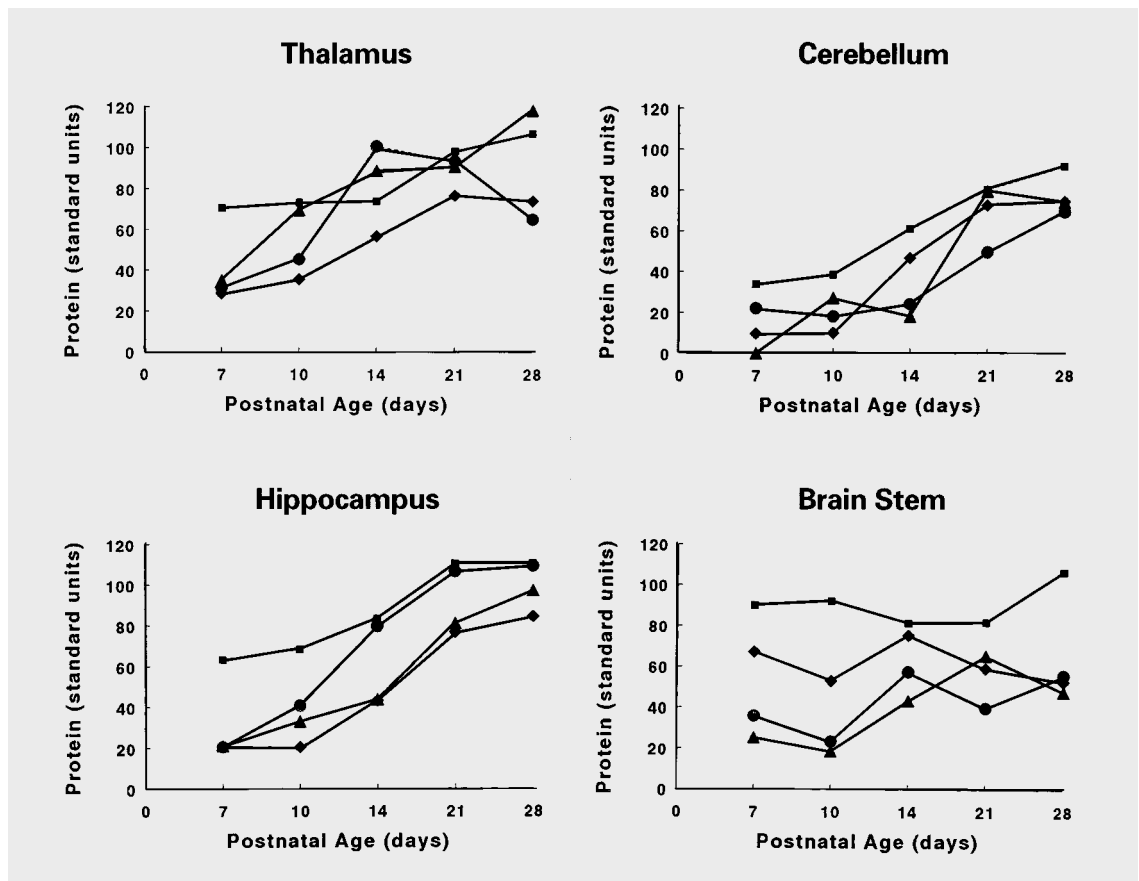
Fig. 3. GLUT1 and GLUT3 mRNA expression during normal postnatal development in rat brain. Autoradiograms of adjacent coronal sections from postnatal rats at 7, 10, 14, 21 and 28 days of age, were analyzed for GLUT1 and GLUT3 mRNA expression by in situ hybridization with [35 S]riboprobes as described by Vannucci et al. [1997a]. Hybridization with sense riboprobes did not produce a positive hybridization signal (data not shown).

tion is further supported by its regional and temporal coexpression with other neuronal proteins.

Representative Western blots of hippocampal membrane samples from postnatal rats at 7, 10, 14, 21 and 28 days of age analyzed for GLUT3, SNAP-25, synaptophysin, and the α_3 subunit of Na,K-ATPase are depicted in figure 4A. Whereas the protein primarily responsible for energy utilization, Na,K-ATPase, is expressed early and reaches near adult levels by P14, the expression of GLUT3 is most closely associated with synaptic proteins, especially SNAP-25. This analysis was repeated with

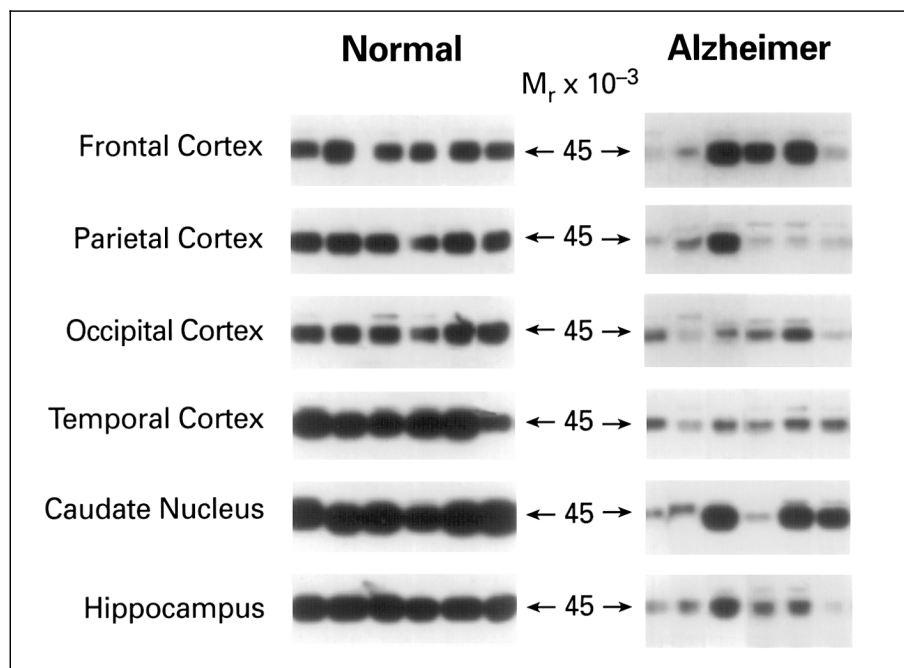


4A



4B

Fig. 5. Regional distribution of GLUT3 in brains of patients with AD. Western blot analysis of brain samples prepared from 6 control patients and 6 patients with AD. Brain samples were prepared from frontal, parietal, occipital, temporal cortex, caudate nucleus, and hippocampus using rabbit polyclonal anti-GLUT3 antibody and ^{125}I -Protein A, visualized with autoradiography [Simpson et al., 1994].



membranes prepared from cortex, thalamus, cerebellum and brainstem of these same animals and quantitated in terms of standard units, as shown in figure 4B. The values for cortex were identical to those observed for hippocampus (data not shown). It is clear from these graphs that GLUT3 expression increases in direct relation to regional neuronal maturation. Thus the brainstem, which is quite mature at birth, does not demonstrate striking increases in GLUT3 or Na,K-ATPase, although levels of SNAP-25 and synaptophysin double between 7 and 28 days. In direct contrast to this is the cerebellum, which is characterized by predominantly postnatal maturation in the rat.

Fig. 4. Developmental expression of neuronal proteins. **A** Western blot analysis of GLUT3, SNAP-25, synaptophysin and α_3 Na,K-ATPase in hippocampal membranes prepared from postnatal rats at 7, 10, 14, 21 and 28 days of age using rabbit polyclonal antibodies to GLUT3 [Maher et al., 1992], synaptophysin (Sigma), and the α_3 subunit of Na,K-ATPase, and a mouse monoclonal antibody to SNAP-25 [Honer et al., 1993], and the Renaissance Chemiluminescence Reagent (DuPont NEN, Boston, Mass., USA) as described in Vannucci et al. [1997a, b]. S, microsomal brain membrane standard, 15 μg , membrane samples, 20 μg . **B** Quantitation of Western blot analysis for hippocampus, thalamus, brainstem and cerebellum prepared from 5 to 6 rats/age and analyzed as described in **A**. Results are expressed relative to the brain standard, S, in arbitrary standard units. ■ = Na,K-ATPase; ● = synaptophysin; ▲ = SNAP-25; ◆ = GLUT3.

In the forebrain, the maturation of thalamus precedes the cortex, which is paralleled by increases in GLUT3 expression [Vannucci, 1994], and is in keeping with higher rates of glucose utilization measured in the neonatal rat and human brain [Chugani and Phelps, 1986; Chugani et al., 1987; Nehlig et al., 1988].

Alzheimer's Disease (AD)

An early and consistent finding in patients with AD is a reduction in rCMR_{glc} in parietal and temporal lobes, as determined by positron emission tomography (PET) [Jagust et al., 1991; Friedland et al., 1989]. The link between reductions in CMR_{glc} and the capacity to transport glucose was first demonstrated by Kalaria and Harik [1989], who reported decreased levels of glucose transporter in cerebral microvessels prepared from Alzheimer patients. These observations have now been extended to include measurements of both 55 and 45 kD GLUT1, and GLUT3 [Simpson et al., 1994]. This study examined 6 forebrain regions from Alzheimer's patients, as compared with patients dying of nonneurologic cause, all obtained from the Brain Bank, Department of Pathology, Albert Einstein College of Medicine. In agreement with the original observations of Kalaria and Harik [1989], a decrease in 55 kD GLUT1 was observed in all regions and was highly significant ($p < 0.01$) in parietal cortex and the caudate nucleus. 45 kD GLUT1 exhibited an overall de-

crease in all cortical structures and in the caudate nucleus. A far more striking finding, however, was the reduction in the levels of the neuronal glucose transporter, GLUT3, in all regions, as illustrated in figure 5. The quantitation of the Western blot analysis is presented in figure 6A. To determine the extent to which these reductions in GLUT3 simply reflect neuronal loss, the samples were also analyzed for the synaptic protein, SNAP-25 (fig. 6B); the ratios of GLUT3/SNAP-25 are expressed in figure 6C. This analysis reveals that, even when the reduction in GLUT3 is corrected for neuronal loss, a significant ($p < 0.05$) decrease in neuronal glucose transport remains in parietal and temporal cortical regions, hippocampus, and caudate nucleus. Thus, the correlation between CMR_{glc} and glucose transporter expression, especially GLUT3, in both parietal and temporal cortex is maintained in this clinical condition. A surprising exception to this was seen in the caudate nucleus, which did not demonstrate a reduced CMR_{glc} , despite sharp reductions in both GLUT1 and GLUT3. This may relate to the observation that this region expresses the highest levels of both transporters, implying a potential redundancy in the normal caudate, not seen in other regions.

Cerebellar Granule Cells

To further investigate the relationship between CMR_{glc} and glucose transporter expression in vitro, we have studied cerebellar granule cells in culture. Cerebellar granule cells are isolated from postnatal day 8 (P8) rat brain and are allowed to differentiate over 8 days in culture (fig. 7). Cytosine arabinoside is routinely added on day 2 to prevent glial proliferation, thus yielding a culture which is >90% neuronal [Maher et al., 1991]. Differentiation is associated with neurite outgrowth, synapse formation, and the expression of excitatory amino acid (NMDA) receptors [Gallo et al., 1986; Novelli and Henneberry, 1987]. The differentiation-dependent increases in functional activity are associated with increased glucose transport activity, as determined by 2-deoxyglucose uptake, increased expression of GLUT1 and GLUT3 mRNAs, and proteins (fig. 7). Peak mRNA expression occurs at day 4, whereas glucose transport activity and protein levels peak at day 6. Subsequent studies in this culture system have demonstrated that quantitatively, GLUT3 is the predominant transporter isoform, being approximately 8 times greater than GLUT1 [Maher and Simpson, 1994]. Levels of metabolic demand in these cells can be manipulated by either altering ambient potassium levels in which the cells are maintained, or by chronic stimulation with the glutamate agonist, NMDA (fig. 8). GLUT3 expression

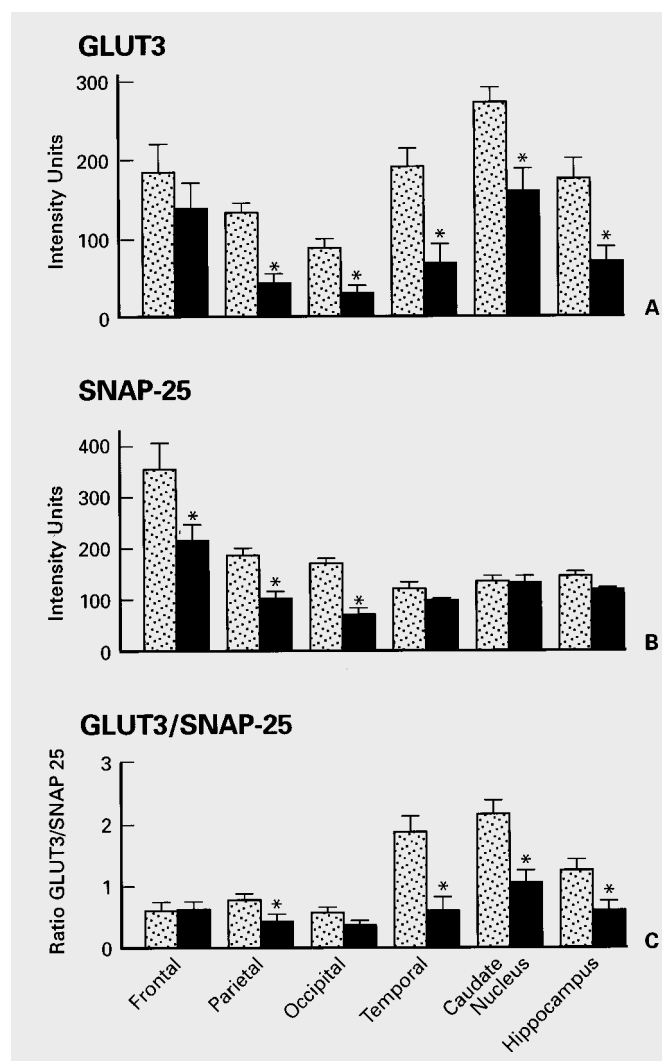
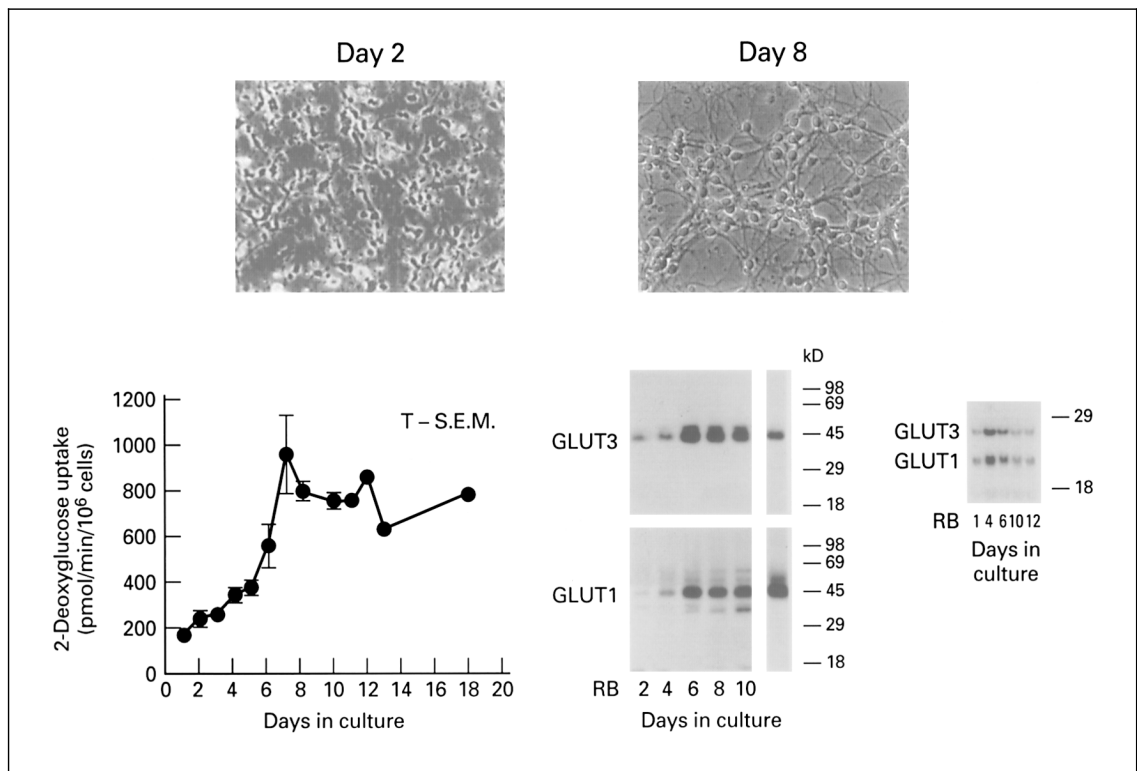
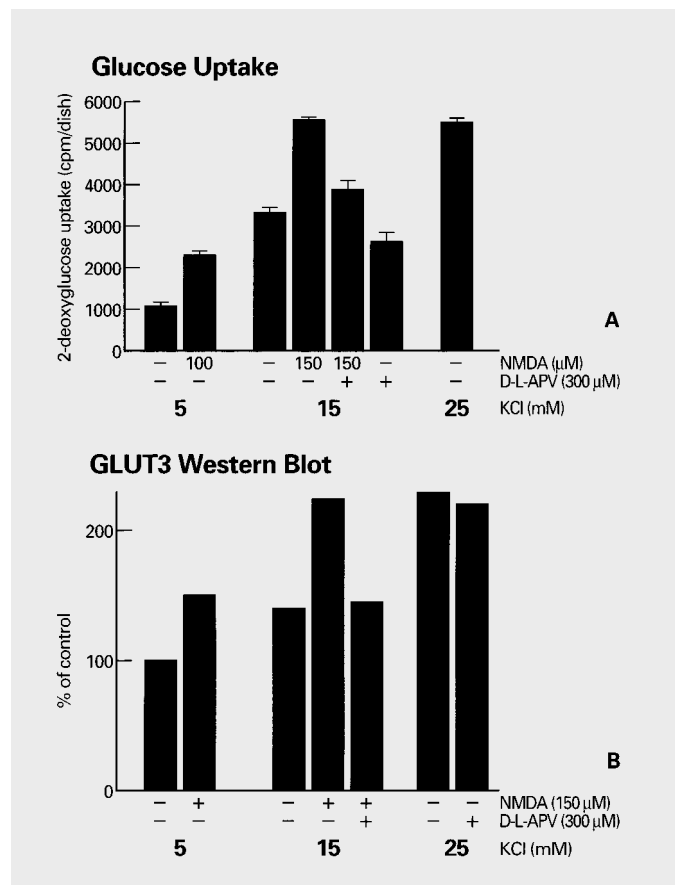


Fig. 6. Regional levels of GLUT3 and SNAP-25 protein in brains of patients with AD. Western blots depicted in figure 5 for GLUT3 (A), and corresponding Western blots for SNAP-25 (B), were quantitated by phosphorimage analysis and mean values are presented, \pm SEM, $n = 12$; (C) the ratio of GLUT3/SNAP-25 was calculated [Simpson et al., 1994]. \square = Control, \blacksquare = Alzheimer patients. * Significant decreases in GLUT3 in samples from Alzheimer's patient compared to controls ($p < 0.05$).

and transport activity have been set to 100% for cultures grown in 5 mM potassium for 6–8 days. Stimulation by NMDA resulted in a 120% increase in transport activity and a 45% increase in GLUT3 expression. Increasing potassium levels to 15 mM resulted in a 33% increase in transport activity and a corresponding 50% increase in GLUT3, which could be augmented by the inclusion of NMDA. The effects of NMDA could be blocked by the addition of the antagonist APV (2-amino-5-phosphono-



7



8

Fig. 7. Differentiation of cerebellar granule cells: GLUT1 and GLUT3 expression and glucose transport activity. Cerebellar granule cells were isolated from day 8 rat pups as described by Maher et al. [1991]. Top panel illustrates phase contrast microscopy of day 2 and day 8 cultures. The alterations in mRNA (Northern blot analysis), protein (Western blot analysis) and glucose transport activity (2-deoxyglucose), over time in culture depicted below [Maher et al., 1991].

Fig. 8. Effects of potassium and NMDA on glucose transport activity and GLUT3 expression in cerebellar granule cells. Cells were maintained in serum-free medium in the presence of varying concentrations of potassium and NMDA as depicted in the figure. **A** illustrates glucose transport activity, measured with 2-deoxyglucose, and **B** GLUT3 levels determined by Western blot analysis [Maher et al., 1994a].

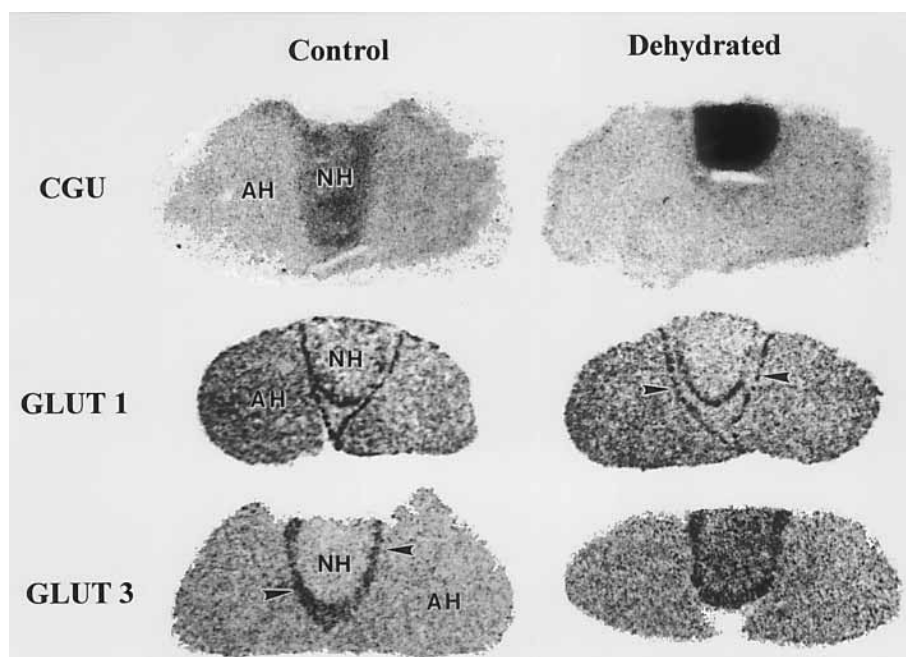


Fig. 9. Cerebral glucose utilization and GLUT1 and GLUT3 mRNA expression in pituitary prepared from control and 3-day dehydrated rats. Adult male rats were dehydrated by 3 days of water deprivation and cerebral glucose utilization was determined with the 2-deoxyglucose autoradiographic method of Sokoloff et al. [1977]. A representative 2-deoxyglucose autoradiogram is shown in the top panels, compared with autoradiograms of pituitary sections analyzed for GLUT1 and GLUT3 mRNA expression by in situ hybridization as described in Vannucci et al. [1997b].

pentanoic acid). Growth in 25 mM potassium elicited maximum (600%) transport activity and 125% increase in GLUT3 expression, which could not be further augmented with NMDA, nor blocked by APV [Maher et al., 1994a, b]. It is important to note, however, that these modulations all required chronic incubations, and attempts to elicit an acute response of either transport activity or GLUT3 levels have been unsuccessful.

Dehydration and the Hypothalamo-Neurohypophysial Axis

The hypothalamo-neurohypophysial axis in the rat is an excellent system in which to study the relationship between neuronal activity (CMR_{glc}) and transporter expression in vivo. The neurohypophysis is an extension of the central nervous system, which contains axon terminals of oxytocin- and vasopressin-secreting hypothalamic neurons and glial-like pituicytes but lacks a BBB [Hatton, 1988]. Early studies by Kadekaro et al. [1992] demonstrated that dehydration, induced by prolonged water deprivation, results in increased vasopressin secretion from the neural lobe of the pituitary and a profound increase in $rCMR_{glc}$ throughout the hypothalamo-neurohypophysial axis in the rat. We have confirmed this increase in CMR_{glc} in the neural lobe and compared it with the measurement of GLUT1 and GLUT3 mRNA levels in adjacent sections (fig. 9). Three days of dehydra-

tion resulted in a 164% increase in CMR_{glc} and a 72% increase in GLUT3 mRNA, but no change or even a small decrease (28%) in GLUT1 mRNA levels. After 48 h of water deprivation, both GLUT1 and GLUT3 protein levels in the neural lobe were significantly increased relative to control. This effect was further enhanced by 3 days of dehydration with a 44% increase in GLUT1 and a 55% increase in GLUT3 (fig. 10) [Vannucci et al., 1994a].

Discussion

The objective of this study was to address the potential relationship between rates of cerebral glucose utilization and the expression of the major glucose transporters in brain, GLUT1 and GLUT3. We have described several diverse paradigms in which we have been able to document just such parallel relationships. During normal post-natal development, increases in $rCMR_{glc}$ correspond to the relative maturity of the brain region, and are mirrored by increases in GLUT3. In contrast, decreases in $rCMR_{glc}$ in brains of patients with AD are reflected in decreases in both GLUT1 (BBB) and GLUT3 proteins. Furthermore, the decrease in GLUT3 is greater than can be accounted for by neuronal loss, suggesting a potential etiological significance in this neurodegenerative disease. Regulation of glucose transport in neurons was further explored in stud-

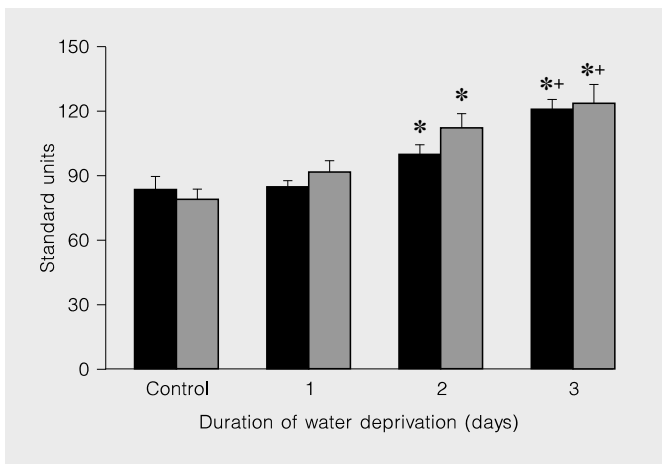


Fig. 10. GLUT1 (■) and GLUT3 (▨) protein levels in the neurohypophyses at 1, 2 and 3 days of water deprivation. Neural lobes were dissected from pituitaries of rats subjected to either 1, 2 or 3 days of water deprivation and control, water-replete, rats and analyzed for GLUT1 and GLUT3 content by Western blot with polyclonal antibodies and ^{125}I -Protein A, and quantitated by phosphor-image analysis and expressed relative to a brain standard in standard units [Vannucci et al., 1994]. GLUT1, GLUT3: Bars represent means \pm SE for groups of 6–10 animals/group. Values were analyzed by one-way analysis of variance (ANOVA) and Tukey test for multiple comparisons. * $p < 0.05$, different from water-sated control; + $p < 0.05$, different from 1-day water-deprived.

ies of cerebellar granule cells which revealed a direct relationship between chronic cell activation, glucose transport activity, and GLUT3 expression. A similar relationship was observed in *in vivo* studies where activation of the hypothalamo-neurohypophysial axis by progressive dehydration resulted in increased GLUT3 mRNA and protein expression in the axon terminals and increased GLUT1 protein in the pituicytes, respectively, that form of the neuronal lobe of the pituitary.

The question now arises as to the precise mechanism(s) of regulation of glucose transporter expression in brain. We have described conditions in which both GLUT1 and GLUT3 levels change in parallel with $r\text{CMR}_{\text{glc}}$, and others in which changes in CMR_{glc} are more closely paralleled by changes in levels of the neuronal glucose transporter, GLUT3. These changes support a coordinate relationship among alterations in levels of substrate supply, metabolic demand, and the capacity for substrate delivery.

Relatively little is known about either the acute or chronic regulation of the levels of expression of these transporters in brain. However, the regulation of GLUT1,

which is ubiquitously expressed in the periphery, has been the subject of several studies [for reviews, see McGowan et al., 1995; Klip et al., 1994]. GLUT1 is uniformly detected in cells in culture and levels of GLUT1 mRNA and protein expression are affected by oncogenic transformation as well as growth factors and activators of protein kinase C [Massa et al., 1996; Merrall et al., 1993; Murakami et al., 1992; Werner et al., 1989]. In addition, GLUT1 mRNA and protein levels respond to ambient glucose concentrations by down-regulation in response to hexose concentrations above the normal range, and are enhanced in response to glucose deprivation [reviewed by Klip et al., 1994]. Such regulation of GLUT1 by glucose places this isoform into the family of glucose-regulated proteins (GRPs), which responds to a variety of cellular stresses [Wertheimer et al., 1991]. The regulation of GLUT1 expression appears to be under both transcriptional and posttranscriptional control [Dwyer et al., 1996; McGowan et al., 1995]. The GLUT1 promoter region contains several sequence elements for transcription factors, including a TATA box, two SpI sites [Dyran and Tijan, 1985], and a region responsive to phorbol esters [Angel et al., 1987]. GLUT1 has recently been shown to be a target protein of the hypoxia-inducible transcription factor (HIF-1), which binds to a HIF-1 binding site in the 5' enhancer site, similar to the regulation of vascular endothelial growth factor (VEGF) by this transcription factor [Levy et al., 1995; Ebert et al., 1995]. An additional posttranscriptional mechanism has been described which involves stabilization of GLUT1 mRNA by the interaction of cytoplasmic proteins with distinct regions within the 3' UTR of the message [Dwyer et al., 1996]. Clearly there is a wide range of options for the regulation of GLUT1 expression. The real key to future studies is the determination of which of these mechanisms are in fact operational in the various experimental paradigms described herein.

Factors involved in the regulation of GLUT3 expression have been far less well defined. The question, however, arises as to the distinctions between GLUT1 and GLUT3 which make GLUT3 uniquely suited for neuronal metabolism, as preliminary studies have suggested comparable levels of both transporters in the rodent brain. Studies by Maher and Simpson [1994], in cerebellar granule cells, were the first to reveal that GLUT3 is the predominant glucose transporter in neurons. In addition, although the apparent affinity (K_m) of GLUT3 is comparable to that of GLUT1 (2.6 vs. 1.7 mM, respectively), the turnover number or catalytic center activity for GLUT3 is 7-fold greater than that for GLUT1, when measured for

zero-trans transport [Maher et al., 1996; Vannucci et al., 1997a, b]. As the situation in vivo most closely approximates this form of transport, i.e. interstitial concentrations of glucose range between 1 and 2 mM, this characteristic of GLUT3 affords the neuron the highest capacity to transport glucose. Certainly, the paradigms presented here illustrate regulation of GLUT3 levels in accordance with neuronal activity and rates of glucose utilization. However, the signal(s) whereby this regulation is transduced are not yet known. Three situations in which GLUT3 is aberrantly expressed have been described: human gliomas [Nishioka et al., 1992]; HIV-infected lymphocytes [Sorbara et al., 1996], and IL-1-stimulated granulosa cells [Kol et al., 1997], all of which may shed some light on potential mechanisms of GLUT3 regulation. More recently, it has been demonstrated that GLUT3-mediated glucose transport is also subject to acute regulation. In platelets, stimulation by either thrombin or activators of protein kinase C cause a translocation of α -gran-

ules containing GLUT3 to the plasma membrane with a consequent increase in rates of glucose uptake [Heijnen et al., 1997; Sorbara et al., 1997]. It remains to be determined whether such mechanisms operate in pre- and postsynaptic membranes. Clearly the next level of understanding of glucose transport and utilization in brain during normal and pathological conditions must integrate aspects of cerebral blood flow, metabolic and glucose demand as well as factors producing both acute and chronic regulation of both GLUT1 and GLUT3.

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