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Glucose transporter expression in the central nervous system: relationship to synaptic function

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Abstract

The family of facilitative glucose transporter (GLUT) proteins is responsible for the entry of glucose into cells throughout the periphery and the brain. The expression, regulation and activity of GLUTs play an essential role in neuronal homeostasis, since glucose represents the primary energy source for the brain. Brain GLUTs exhibit both cell type and region specific localizations suggesting that the transport of glucose across the blood–brain barrier is tightly regulated and compartmentalized. As seen in the periphery, insulin-sensitive GLUTs are expressed in the brain and therefore may participate in the central actions of insulin. The aim of this review will be to discuss the localization of GLUTs expressed in the central nervous system (CNS), with a special emphasis upon the recently identified GLUT isoforms. In addition, we will discuss the regulation, activity and insulin-stimulated trafficking of GLUTs in the CNS, especially in relation to the centrally mediated actions of insulin and glucose.

Keywords: GLUT; Glucose; Insulin; Cognition; Hippocampus; Diabetes

1. Introduction

The family of facilitative glucose transporter (GLUT) proteins is responsible for the entry of glucose into cells throughout the periphery and the brain (Maher et al., 1994; Shepherd and Kahn, 1999; Vannucci et al., 1997b). The expression, regulation and activity of glucose transporters play an essential role in neuronal homeostasis, since glucose represents the primary energy source for the brain (Lund-Anderen, 1979; Pardridge, 1983). While many GLUT isoforms have been identified and characterized, a minority of these transporters is expressed in central nervous system (CNS). The expanding GLUT gene family has recently added several novel members that are currently being vigorously investigated, some of which are expressed in the CNS. Since these novel GLUTs were isolated and characterized by several different investigative teams, there has been some confusion regarding the nomenclature of the glucose transporter gene family. The nomenclature of the GLUT family has recently been clarified and arranged into three different classes based upon their sequence similarities (Joost et al., 2002). The brain GLUTs are represented in Class I (GLUTs 1–4) and Class II (GLUT5). The novel GLUT isoforms are grouped together in Class III and three of these isoforms are expressed in the brain, namely GLUT6, GLUT8 and GLUT10. Brain GLUTs exhibit both cell type and region specific localizations suggesting that the transport of glucose across the blood–brain barrier is tightly regulated and compartmentalized. For example, some GLUT isoforms are widely expressed in the CNS (the 45 and 55 kDa isoforms of GLUT1; GLUT3) and appear to be responsible for the majority of glucose uptake and utilization in the brain (Duelli and Kuschinsky, 2001). Other GLUTs exhibit more discrete localizations (GLUT2; GLUT4) indicating that these GLUTs may be involved in highly specialized activities in the CNS (Fig. 1; Table 1). GLUT isoforms exhibit unique subcellular localizations (GLUT8), also suggestive of specialized functional activities. As seen in peripheral tissues, insulin sensitive GLUTs are also expressed in the brain (GLUT4; GLUT8) signifying that GLUTs may participate in the central actions of insulin. Other recently identified GLUTs (GLUT6; GLUT10) await

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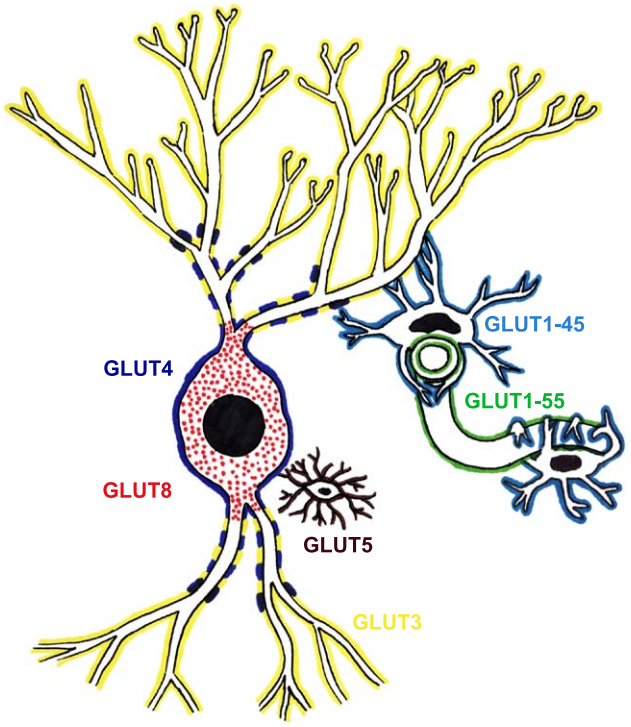

Fig. 1. Localization of GLUT isoforms in the CNS. Shown in green is the 55 kDa isoform of GLUT1 expressed in endothelial cells. Shown in light blue is the 45 kDa isoform of GLUT1 expressed in astrocytes. Neuropil expression of GLUT3 is depicted in yellow. Illustrated in dark blue is the somatodendritic labeling of GLUT4. Potential overlap of GLUT3 and GLUT4 distribution in some neurons of the brain is shown as yellow and dark blue-hatched areas. Shown in brown is GLUT5 expressed in microglia. Illustrated as red dots is the intracellular and somatodendritic localization of GLUT8. Not shown is the astrocytic localization of GLUT2. See text for a more detailed description of GLUT localizations.

further characterization. The aim of this review will be to discuss the localization of GLUTs expressed in the CNS, with a special emphasis upon the recently identified GLUT isoforms. In addition, we will discuss the regulation, activity and insulin-stimulated trafficking of GLUTs in the CNS in relation to the centrally mediated actions of insulin and glucose.

2. Expression and localization of brain glucose transporters

2.1. GLUT1

GLUT1 was the first glucose transporter identified in the brain and is expressed as two protein species that originate from the same gene that differ in the degree of their post-translational modification. These GLUT1 isoforms represent the passageway for glucose into the brain and regulate the availability of metabolic fuels to neurons (Giaume et al., 1997). The 55 kDa isoform of GLUT1 was originally localized to blood–brain barrier microvessels by saturable cytochalasin B binding, as well as by immunodetection using antisera raised against the human erythrocyte glucose transporter (Dick et al., 1984). Molecular cloning of GLUT1 from a rat brain cDNA library confirmed these biochemical and immunological observations and also suggested the presence of a 45 kDa isoform of GLUT1 (Birnbaum et al., 1986). Subsequent studies determined that the 55 kDa species of GLUT1 was specifically localized to cerebral microvessel endothelial cells (Boado and Pardridge, 1990; Kasanicki et al., 1987) and exhibited a widespread distribution in the rat brain (Brant et al., 1993; Rayner et al., 1994). Immunohistochemistry performed at the light and electron microscopic levels localized GLUT1 to the luminal and abluminal surfaces of cerebral microvessels (Farrell and Pardridge, 1991; Gerhart et al., 1989; Kasanicki et al., 1989). When combined with additional observations (Boado and Pardridge, 1990; Farrell et al., 1992; Pardridge et al., 1990a), these results suggested that GLUT1 was exclusively expressed in endothelial cells and was not expressed in neurons or non-neuronal cells in the brain. However, analysis of microvessel-free brain preparations revealed the presence of a 45 kDa isoform of GLUT1 (Maher et al., 1994; Vannucci, 1994); similar observations were made in rat brain synaptosomes (Bhattacharyya and Brodsky, 1988) and in crude brain homogenates (Morgello et al., 1995). Additional evidence for the existence of a non-vascular isoform of GLUT1 was provided by co-localization of GLUT1 mRNA with glial fibrillary acidic protein (Lee and Bondy, 1993). Immunohistochemical studies confirmed the expression of GLUT1 in astrocytes surrounding cerebral microvessels, as well as astrocytic cell bodies and processes (Leino et al., 1997; Morgello et al., 1995). Collectively, these studies demonstrate that GLUT1 exists as two isoforms in the brain: a more heavily glycosylated 55 kDa isoform expressed in endothelial cells and a less-glycosylated 45 kDa isoform localized to astrocytes.

2.2. GLUT2

GLUT2 is expressed in several peripheral tissues, in particular the pancreas and liver, where it serves to detect plasma glucose levels. This ‘glucose sensor’ function of GLUT2 is most evident in the pancreas, where glucose transport mediated by GLUT2 serves as the signal for

Table 1
Glucose transporter expression in the central nervous system

GLUT1–45	Astrocytes; neurons (?)	Widespread
GLUT1–55	Endothelial cells	Widespread
GLUT2	Astrocytes; tanycytes (?)	PVN; LH, VMH; Arcuate
GLUT3	Neurons; neuropil	Widespread
GLUT4	Neurons; somatodendritic	Cb, CTX, Hp, Hypo
GLUT5	Microglia	Widespread
GLUT8	Neurons; somatodendritic	Widespread
GLUT6; GLUT10	?	?

Abbreviations: Cb: cerebellum; CTX: cortex; Hp: hippocampus; Hypo: hypothalamus; LH: lateral hypothalamus; PVN: paraventricular hypothalamic nucleus; VMH: ventromedial hypothalamus.

insulin release from pancreatic β cells. In the CNS, several neuronal populations have been identified that possess glucose sensing capabilities, including specific neurons in the hypothalamus (Levin et al., 2001). The phenotype of these glucose sensing neurons is similar to pancreatic β cells in that these specialized neurons express sulfonylurea receptors, the pore forming subunit of the ATP-sensitive K^+ channel (KiR 6.2) and glucokinase. It is therefore tempting to speculate that GLUT2 may serve a similar role in glucose sensing neurons (Levin et al., 2001; Miki et al., 2001). A variety of studies to date provide some provocative evidence to support this hypothesis. For example, GLUT2 mRNA shows a highly selective expression in the paraventricular hypothalamic nucleus, the arcuate nucleus and the lateral hypothalamic region, brain regions that are proposed to be particularly responsive to changes in glucose levels (LeLoup et al., 1994). These same studies revealed that GLUT2 expression is limited to astrocytes, suggesting that non-neuronal cells may participate in the glucose sensing functions of these highly specialized neurons. Real time reverse transcription polymerase chain reaction (RT-PCR) studies have revealed that GLUT2 mRNA is co-expressed with glucokinase and sulfonylurea receptor-1 (SUR1) in several hypothalamic regions, including the arcuate nucleus, the ventromedial hypothalamic nucleus, the paraventricular hypothalamic nucleus and the lateral hypothalamus (Li et al., 2003). GLUT2 mRNA and protein have also been localized to highly specialized hypothalamic glial cells referred to as tanycytes (Garcia et al., 2003), which also express KiR 6.2. Collectively, these anatomical studies suggest a functional role for GLUT2 in monitoring changes in brain glucose levels. In this regard, GLUT2 may be involved in homeostatic processes since central administration of GLUT2 antisense oligonucleotides decreases cumulative food intake and body weight, and also attenuate glucoprivic feeding in rats (LeLoup et al., 1998; Wan et al., 1998). However, some studies have failed to detect GLUT2 protein in the brain (Rayner et al., 1994), while others have reported a more widespread distribution, albeit at very low levels compared to other brain GLUT isoforms such as GLUT1 and GLUT3 (Brant et al., 1993). As a result, the expression and functional role of GLUT2 in the brain remains equivocal and will require additional investigations.

2.3. GLUT3

GLUT3, referred to as the neuron specific glucose transporter, was originally cloned from a human fetal muscle cDNA library and was found to be expressed in a variety of human peripheral tissues, as well as the brain (Kayano et al., 1988). Subsequent reports suggested a more restricted expression of GLUT3 in human tissues, including the brain, testis and spermatozoa (Haber et al., 1993). In rodents, GLUT3 mRNA and protein exhibit an even more restricted expression profile, being observed only in the brain (Maher et al., 1992; Nagamatsu et al., 1992; Yano et al., 1991). The expression of GLUT3 mRNA was established by in situ hybridization histochemistry, which revealed that GLUT3 mRNA is widely expressed in neurons in the rat brain, including the cerebellum, striatum, cortex and hippocampus (Nagamatsu et al., 1993). Similarly, immunoblot analyses determined that GLUT3 protein existed as a 45 kDa protein that was expressed throughout the rat CNS (Maher et al., 1994; Nagamatsu et al., 1993). Immunohistochemistry provided a more detailed description of GLUT3 expression in the brain, illustrating that GLUT3 was localized to the neuropil and was largely absent from neuronal cell bodies (Gerhart et al., 1995; McCall et al., 1994). This expression pattern was particularly evident in the hippocampus, where mossy fibers showed a strong immunopositive signal for GLUT3, while pyramidal cell and granule cell bodies displayed little GLUT3-positive labeling. Radioimmunocytochemical approaches using specific GLUT3 antisera and radiolabeled secondary antisera provided similar immunological profiles for GLUT3 protein expression (Reagan et al., 1999; Zeller et al., 1995). Additional support for a neuropil localization of GLUT3 was provided by electron microscopic studies, which also demonstrated that GLUT3 immunoreactivity was not associated with non-neuronal cell types (Leino et al., 1997). It is interesting to note that in spite of the absence of GLUT3 in cell bodies, local cerebral glucose utilization is relatively similar for neuronal cell body layers when compared with the neuropil (Duelli et al., 1999; Zeller et al., 1995). Such results suggest that additional GLUT isoforms exhibit somatodendritic expression in the brain and fulfill the metabolic demands of neuronal cell bodies (see below).

2.4. GLUT4

GLUT4, the insulin-sensitive glucose transporter, was originally identified and characterized in peripheral tissues such as muscle, adipose and heart (Birnbaum, 1989; Charron et al., 1989). In peripheral tissues such as muscle and adipose cells, insulin receptor activation stimulates GLUT4 translocation to the plasma membrane to increase glucose uptake and utilization (Saltiel and Pessin, 2002). The insulin receptor is also expressed in discrete neuronal populations in the CNS, including the cerebellum, hypothalamus and the hippocampus (Doré et al., 1997; Kar et al., 1993b; Marks et al., 1991). Interestingly, the insulin receptor and GLUT4 often exhibit overlapping distributions in the rat brain. In this regard, GLUT4 mRNA and protein were localized to neurons in the hypothalamus (LeLoup et al., 1996) and cerebellum (Brant et al., 1993; Kobayashi et al., 1996), neuronal populations that also express the insulin receptor. Subsequent studies confirmed and extended these observations and demonstrated that GLUT4 was also expressed in other regions in the brain, including the cortex and hippocampus (Messari et al., 1998). Studies from our laboratory revealed that GLUT4 was expressed in neuronal cell bodies and neuronal processes in the rat hippocampus (Fig. 2), in

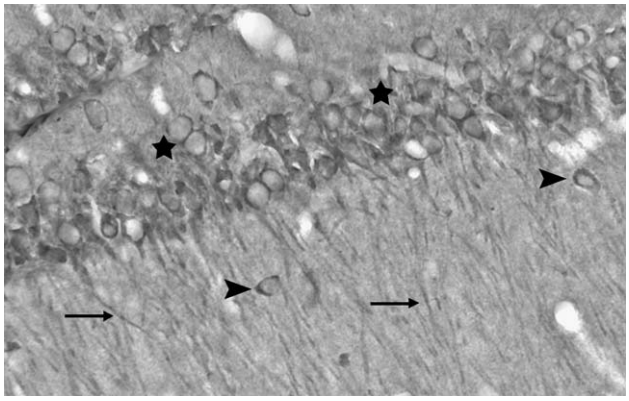

Fig. 2. Representative bright field photomicrograph of GLUT4 immunoreactivity in the rat hippocampus. GLUT4 immunoreactivity is observed in principal cells (stars) and nonprincipal cells (arrowheads), as well as neuronal processes (thin arrows) in the rat hippocampus, as shown in the CA1 region.

agreement with previous immunohistochemical approaches at the light and electron microscopic levels that demonstrated that GLUT4 exhibited a somatodendritic labeling and was absent from non-neuronal cells in rat brain (Messari et al., 1998). GLUT4 was associated with the plasma membrane, but was also observed in the cytoplasm, suggesting that a readily mobilizable pool was available for translocation to the plasma membrane (Messari et al., 1998). More recent studies have characterized GLUT4-positive neurons in the brain and based upon their neuronal phenotypes, it is not unexpected that GLUT4 was co-localized with GLUT3-positive neurons in the rat brain (Aplet et al., 1999). Collectively, these studies have demonstrated that GLUT4 expression in the brain is limited to neuronal cell bodies and dendrites and often exhibits overlapping distribution with the insulin receptor, suggesting that insulin stimulates the translocation of GLUT4 to the plasma membrane in the brain. Indeed, plasma membrane association of GLUT4 is modulated in the cortex and cerebellum in experimental models of type 1 and type 2 diabetes (Vannucci et al., 1998b). GLUT4 trafficking in the rat hippocampus is also impaired in experimental models of type 1 diabetes (Reagan, 2002). These results indicate that neuronal GLUT4 trafficking is sensitive to changes in insulin levels, as described for peripheral tissues such as muscle and fat (Watson and Pessin, 2001) and provide evidence that insulin-stimulated trafficking of GLUTs may participate in the centrally mediated actions of insulin and glucose (see below).

2.5. GLUT5

A glucose transporter isolated from a human small intestine cDNA library that exhibited cytochalasin B-sensitive glucose transport is expressed in a variety of tissues, including intestine, skeletal muscle, fat, testis and spermatozoa (Burant et al., 1992; Kayano et al., 1990). This GLUT isoform, referred to as GLUT5, was later shown to also be expressed in human brain (Shepherd et al., 1992). Additional biochemical characterizations of GLUT5 revealed that this GLUT isoform functionally serves as a high affinity fructose transport, and possesses lower transporter activity for glucose (Burant et al., 1992). Rat GLUT5 was also isolated from a small intestine cDNA library and exhibited similar functional characteristics and distribution as human GLUT5 (Rand et al., 1993). Immunoblot analysis of human brain determined that GLUT5 antisera recognized a protein of similar molecular weights in human brain and cultured peripheral macrophages; immunohistochemistry more specifically localized GLUT5 to microglial cells in human and rodent brain (Maher et al., 1994; Payne et al., 1997). A recent study revealed that GLUT5 may also serve as a marker to differentiate between monocyte and microglia derived macrophages (Sasaki et al., 2003). In spite of these advances, the physiological role of this brain fructose/glucose transporter isoform remains to be determined.

3. Novel glucose transporter isoforms

As described above, the GLUT gene family has recently been grouped into several classes, including three novel isoforms that are members of Class III: GLUT6, GLUT8 and GLUT10. Northern blot analysis has demonstrated that human GLUT6 mRNA (previously referred to as GLUT9) is expressed in a variety of cell types, including the brain (Doege et al., 2000a). Human and mouse GLUT10 mRNA have also been shown to be expressed in the brain by Northern blot analysis (Dawson et al., 2001). Glucose transport mediated by these novel GLUTs is cytochalasin B and phloretin sensitive. Nonetheless, the exact functional role, as well as a more detailed description of the localization these novel GLUTs awaits elucidation.

3.1. Novel insulin-sensitive GLUT: GLUT8

Cloning of GLUT8 was stimulated by the unexpected phenotypes of GLUT2 and GLUT4 knockout mice (Guillam et al., 1997; Katz et al., 1995) and by the ability to search databases for sequence similarities with GLUTs 1–5 (Ibberson et al., 2000). GLUT8, previously referred to as GLUTx1, exhibits between 29% and 32% identity with rat GLUTs 1–5 and transport activity of GLUT8 expressed in *Xenopus* oocytes was inhibited by cytochalasin B (Doege et al., 2000b; Ibberson et al., 2000). Northern blot analysis demonstrated that GLUT8 mRNA was abundantly expressed in testis, as well as the brain (Doege et al., 2000b; Ibberson et al., 2000). Our laboratory provided the first description of the localization of GLUT8 in the rat brain. Our studies revealed that GLUT8 mRNA was expressed in the hippocampus and also appeared to exhibit a more widespread distribution in the rat brain (Reagan et al., 2001). Similar to GLUT3, GLUT8 protein appears to be

ubiquitously expressed in the rat brain, as shown by immunofluorescence in the cortex, cerebellum, the paraventricular hypothalamic nucleus, the amygdala and the supraoptic nucleus (Fig. 3). Unlike GLUT3 that is localized to the neuropil, GLUT8 is expressed primarily in neuronal cell bodies and the most proximal apical dendrites of neurons (Fig. 3F) (Reagan et al., 2001, 2002). Subsequent immunohistochemical studies from other investigators have provided similar findings regarding the localization of GLUT8 (Ibberson et al., 2002; Sankar et al., 2002). The absence of colocalization with the astroglia marker glial fibrillary acidic protein (GFAP) and the microglia marker OX-42 (Reagan et al., 2002) suggest that GLUT8 is expressed exclusively in neurons, an observation supported by our immunogold electron microscopic studies (Piroli et al., 2002). Collectively, these studies demonstrate that GLUT8 is a somatodendritic glucose transporter that is ubiquitously and exclusively expressed in rat brain neurons.

One of the more interesting aspects of GLUT8 expression is that this GLUT isoform is not associated with the plasma membrane in the CNS under physiological settings or in experimental models of type 1 diabetes (Reagan et al., 2001). In the brain, Western blot analysis of hippocampal membrane fractions determined that GLUT8 was immunodetected in high-density microsomal and low-density microsomal fractions, but not in plasma

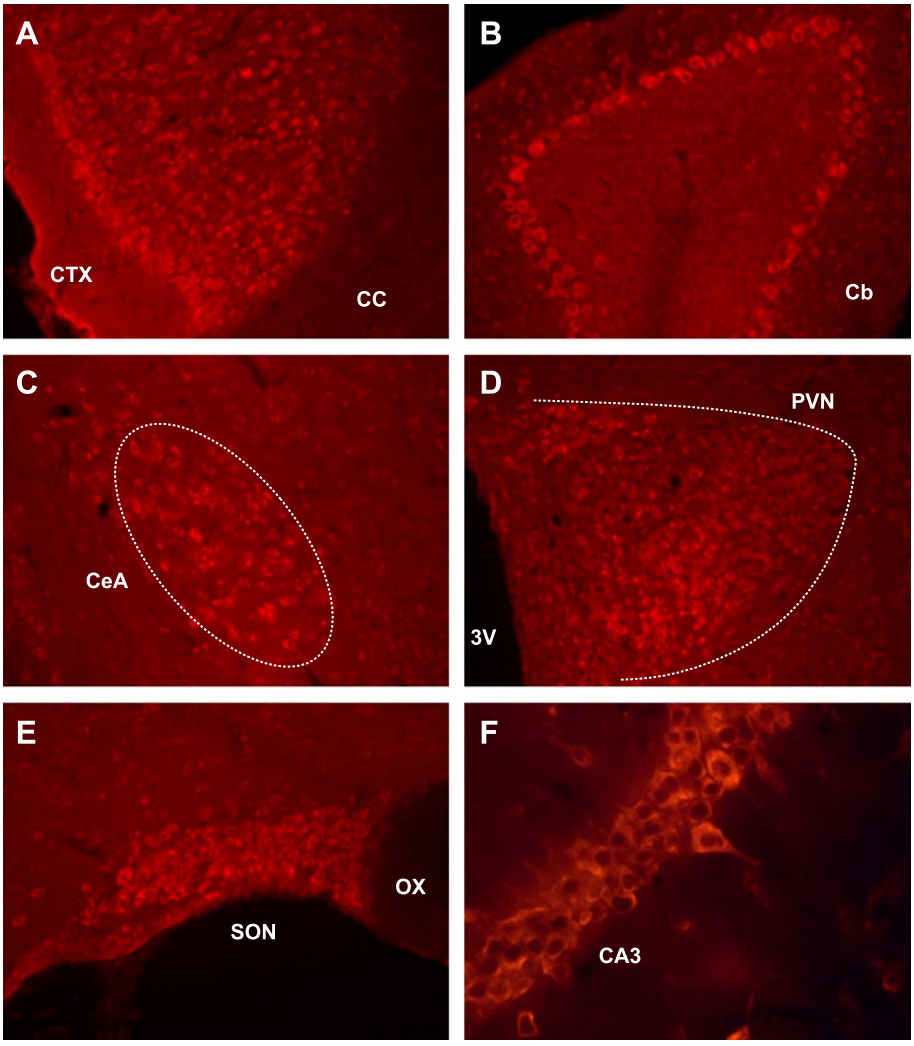

Fig. 3. Representative dark field photomicrographs of GLUT8 immunofluorescence demonstrating that GLUT8 is exclusively expressed in neuronal cell bodies in the rat brain. Panel A: GLUT8 labeling associated with neuronal cell bodies in the rat cortex (CTX). Panel B: GLUT8 immunofluorescence associated with Purkinje cells in the rat cerebellum (Cb). Panel C: GLUT8 positive cell bodies in the central nucleus of the amygdala (CeA). Panel D: GLUT8 immunofluorescence in the paraventricular hypothalamic nucleus (PVN). Panel E: GLUT8 positive neuronal cell bodies in the supraoptic nucleus (SON). Panel F. Higher power magnification of GLUT8 immunolabeling in principal and nonprincipal neurons in the CA3 region of the rat hippocampus. Note that GLUT8 immunofluorescence is limited to neuronal cell bodies and the most proximal apical dendrites. The distribution and characterization of GLUT8 expression was examined as described previously (Reagan et al., 2002). Primary antisera selective for GLUT8 were used at a dilution of 1:1500. Primary antisera were detected using anti-rabbit IgG secondary antisera coupled to Alexa 546 (red, 1:800) (Molecular Probes, Eugene, OR). Abbreviations: cc: corpus callosum; 3V: third ventricle; ox: optic chiasm.

membrane fractions. In support of these findings, our electron microscopic analyses revealed that GLUT8 was associated with the endoplasmic reticulum and the cytosol, but not the plasma membrane (Piroli et al., 2002). Studies that have examined the functional characteristics of GLUT8 in transfected cells revealed that mutagenesis or disruption of intracellular trafficking mechanisms were required to promote GLUT8 association with the plasma membrane (Ibberson et al., 2000; Lisinski et al., 2001). When GLUT8 was expressed at the plasma membrane under these experimental conditions, 2-deoxy-D-glucose uptake was eliminated by D-glucose and was significantly reduced by D-galactose, D-fructose and the GLUT inhibitor cytochalasin B (Ibberson et al., 2000). These results illustrate that GLUT8 possesses transport activity, although these functional activities do not normally occur at the plasma membrane. Alternatively, our findings suggest that GLUT8 transport activity resides in the rough endoplasmic reticulum (Piroli et al., 2002) and provides insight into the possible functional role of this glucose transporter in the CNS. For example, since glucose is liberated from oligosaccharides during protein glycosylation events that occur in the rough endoplasmic reticulum, GLUT8 may transport glucose out of the rough endoplasmic reticulum into the cytosol. As such, we propose that GLUT8 transports the glucose molecules removed from glycoproteins out of the rough endoplasmic reticulum lumen into the cytoplasm and thereby plays a key role in maintaining neuronal glucose homeostasis. Indeed, since glucose utilization of neuronal cell bodies is similar to the those of the neuropil in the hippocampus (Duelli et al., 1999; Zeller et al., 1995), our results suggest that the somatodendritic expression of GLUT8, as well as GLUT4, supports the metabolic requirements of neuronal cell bodies. Interestingly, evidence continues to accumulate to support the hypothesis that the trafficking of these somatodendritic GLUT isoforms is insulin sensitive.

4. Insulin-sensitive translocation of GLUT4 and GLUT8 in the rat brain

As described above, neuronal GLUT4 trafficking is modulated in the cerebellum, cortex and hippocampus by changes in insulin levels observed in experimental models of diabetes (Reagan, 2002; Vannucci et al., 1998b). Similarly, while total GLUT8 protein expression is not modulated by streptozotocin diabetes, the subcellular trafficking of GLUT8 to the high-density microsomal fraction is impaired by hyperglycemic/insulinopenic conditions (Piroli et al., 2002). Conversely, under conditions that increase plasma insulin levels, such as following peripheral glucose administration, the trafficking of GLUT4 to the plasma membrane is increased in the rat hippocampus (Fig. 4). Glucose administration also stimulates GLUT8 trafficking from the cytosol to the rough endoplasmic reticulum, but does result in GLUT8 association with the plasma membrane (Piroli et al., 2002). These dramatically divergent outcomes of GLUT4 and GLUT8 translocation events in response to changes in plasma insulin levels strengthen the hypothesis that insulin stimulates the trafficking of GLUT4 and GLUT8 in the CNS. Indeed, ultrastructural analyses have localized both GLUT4 and GLU8 to the cytoplasm (Messari et al., 1998; Piroli et al., 2002), indicative of the availability of a readily mobilizable, insulin-sensitive pool of GLUTs. Our current understanding of the stimuli involved in the trafficking of the insulin-sensitive GLUTs in the rat brain is shown in Fig. 5. Glucose administration increases plasma insulin levels, which in turn stimulates the translocation of GLUT4 to the plasma membrane and GLUT8 to the endoplasmic reticulum. In insulin deficient states, such as streptozotocin-induced diabetes, trafficking of GLUT4 and GLUT8 is impaired. Since both of these conditions are associated with increased plasma glucose levels, the presence or absence of insulin appears to be the stimulus for these translocation events. Insulin also stimulates GLUT8 translocation in mouse blastocysts (Carayannopoulos et al., 2000; Pinto et al., 2002) suggesting that insulin stimulates GLUT8 trafficking in both the periphery and the CNS. Nonetheless, it is important to note that unlike GLUT4, which is often colocalized with the insulin receptor, GLUT8 exhibits a more widespread distribution, suggesting that additional peptide or

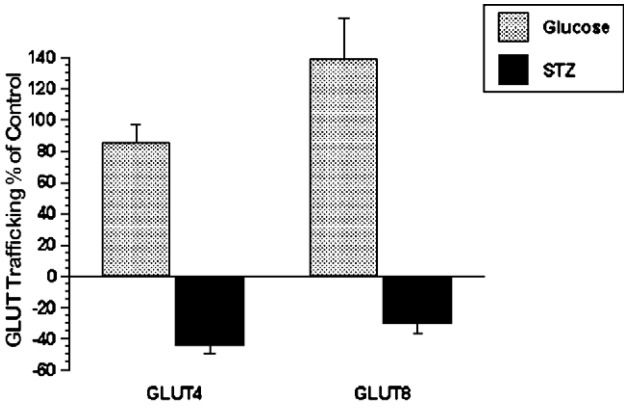

Fig. 4. Immunoblot analysis of insulin-sensitive GLUT expression in hippocampal membrane containing fractions following glucose administration or in streptozotocin diabetes. Increases in plasma insulin levels that occur following peripheral glucose administration stimulate the rapid and robust translocation of GLUT4 to the plasma membrane, as well as the translocation of GLUT8 to the high-density microsomal fraction (Piroli et al., 2002), in the hippocampus relative to vehicle treated control rats. Conversely, in hyperglycemic/insulinopenic conditions produced by streptozotocin treatment, GLUT4 association with the plasma membrane (Reagan, 2002) and GLUT8 association with the high-density microsomal fraction (Piroli et al., 2002) is significantly reduced compared with euglycemic controls. See text for details. Data are expressed as percent change of GLUT4 or GLUT8 immunoreactive bands observed in hippocampal membrane-containing fractions isolated from control rats.

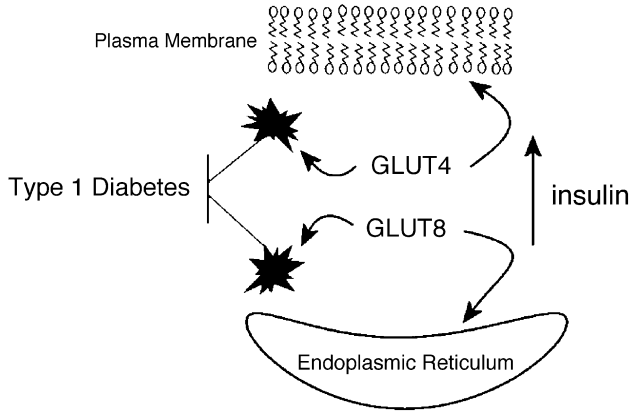

Fig. 5. Insulin stimulated trafficking of GLUT4 and GLUT8 in the rat hippocampus. Increases in plasma insulin levels, as occurs following peripheral glucose administration (Piroli et al., 2002), stimulates the translocation of GLUT8 from the cytosol to the endoplasmic reticulum and the translocation of GLUT4 from the cytosol to the plasma membrane. Conversely, in insulin deficient states, such as in streptozotocin diabetes, GLUT8 trafficking to the endoplasmic reticulum (Piroli et al., 2002) and GLUT4 trafficking to the plasma membrane (Reagan, 2002) are reduced. Since the insulin receptor is expressed in the rat hippocampus, these results suggest that insulin serves as the stimulus for these trafficking events. See text for details.

neurotransmitter systems may be involved in the trafficking of GLUT8 in the CNS.

5. Insulin-sensitive GLUTs in the CNS: functional implications

An emerging relationship between insulin, glucose utilization, GLUTs and cognition in physiological and pathophysiological settings is taking shape based upon clinical and experimental studies (Park, 2001). Indeed, numerous studies have demonstrated a positive correlation between GLUT expression and cerebral glucose utilization (Duelli and Kuschinsky, 2001; Vannucci et al., 1998a). The deleterious consequences of diabetes phenotypes (McCall, 1992; Mooradian and Morin, 1991) and the cognitive enhancing properties of insulin and glucose (Gold, 1995; Greenwood, 2003; Park, 2001; Watson and Craft, 2003) in relation to GLUT expression and glucose utilization has been detailed in previous reviews and also will be discussed in other sections of this special issue. Accordingly, we will briefly review our current understanding of the regulation of GLUTs and glucose utilization in these clinical and experimental settings with a special emphasis upon the potential role of the insulin sensitive GLUTs.

5.1. Diabetes, GLUTs and cognitive performance

One of the earliest recognized neurological complications associated with diabetes was cognitive impairments (Dashiell, 1930; Miles and Root, 1922). The age of onset, as well as the degree of metabolic control, have been identified as risk factors in the development of cognitive impairments in type 1 diabetes patients (Holmes, 1986; Meuter et al., 1980; Rovet et al., 1987; Ryan et al., 1984, 1985; Ryan, 1988; Skenazy and Bigler, 1984). In experimental models of diabetes, diabetic rats also exhibit behavioral deficits, as well as decreases in hippocampal synaptic plasticity (Biessels et al., 1996, 1998; Magariños and McEwen, 2000). Investigations of the utilization and transport of glucose in the brain during diabetes have produced equivocal results (McCall, 1992; Mooradian and Morin, 1991). A comprehensive study demonstrated that glucose utilization is decreased in response to hyperglycemia in many brain regions, including the hippocampus (Duelli et al., 1994). Interestingly, some regions exhibited little or no change in glucose utilization while other regions exhibited robust increases in glucose utilization in chronic diabetes. The heterogeneity of responses observed in different neuronal populations in this study may help to explain the disparate results in studies examining glucose utilization and glucose uptake in experimentally induced diabetes.

The regulation of GLUTs in the brain during diabetes also remains controversial. Some investigators have reported that GLUT1 expression is increased in experimental models of diabetes (Choi et al., 1989; Lutz and Pardridge, 1993; Vannucci et al., 1997a), while other studies reported that GLUT1 expression is not altered following chronic hyperglycemia (Duelli et al., 2000; Kainulainen et al., 1993; Nagamatsu et al., 1994; Pardridge et al., 1990b). Investigations into the regulation of GLUT3 by diabetes have also failed to reach a consensus (Kainulainen et al., 1993; Nagamatsu et al., 1994; Vannucci et al., 1997a). Our studies demonstrated that GLUT3 mRNA is increased in the hippocampus, but not the cortex of diabetic rats, while immunoreactive GLUT3 is not modulated by diabetes (Reagan et al., 1999). As described above, trafficking of the insulin sensitive GLUTs is impaired in the cerebellum, cortex and hippocampus of diabetes phenotypes (Piroli et al., 2002; Reagan et al., 1999; Vannucci et al., 1998b). Collectively, these results further demonstrate that the regulation of glucose transporter expression in the brain in response to diabetes remain equivocal and suggest that region specific changes in GLUT expression may account for these disparities.

5.2. Insulin, glucose and cognition: role for insulin-sensitive GLUTS?

The insulin receptor is proposed to participate in a variety of functional activities of the CNS, including feeding, energy metabolism, reproduction and cognition (Park, 2001; Schwartz et al., 1992). The cognitive enhancing properties of insulin may be mediated through insulin receptors expressed in the hippocampus (Adamo et al., 1989; Kar et al., 1993a), a region that is recognized as an important integration center for learning and memory (McE-

wen and Sapolsky, 1995). In support of this hypothesis, spatial learning in the Morris water maze in non-diabetic control rats was associated with increases in hippocampal insulin receptor expression and signaling (Zhao et al., 1999). In streptozotocin-treated rats, impairments in cognitive function and hippocampal synaptic plasticity were reversed by insulin replacement (Biessels et al., 1998; Magariños et al., 2001), further supporting a role for insulin in cognitive function. Therefore, insulin receptor activation and subsequent stimulation of insulin receptor second messenger cascades, including the translocation of insulin-sensitive GLUTs, may participate in the cognitive enhancing properties of insulin. Some studies would argue against a significant role for the insulin-sensitive GLUTs since the majority of glucose utilization in the CNS appears to be mediated through GLUT1 and GLUT3 (Duelli and Kuschinsky, 2001). However, these studies have also demonstrated that GLUT1 and GLUT3 cannot account for all cerebral glucose utilization, implicating an important functional contribution of other GLUT isoforms. Moreover, such autoradiographic approaches do not posses the temporal or spatial resolution that may be required to evaluate the rapid and transient changes in cerebral glucose utilization that may occur following insulin receptor mediated translocation of GLUT4 and GLUT8.

Brain glucose transport and utilization are impaired in elderly patients with mild cognitive impairment (De Santi et al., 2001), as well as in Alzheimer's disease (Benson et al., 1983; De Santi et al., 2001; Duara et al., 1986; Friedland et al., 1989; Harr et al., 1995; Piert et al., 1996). Decreases in glucose transporter expression may contribute to impairments in glucose utilization in mild cognitive impairment and Alzheimer's disease. For example, GLUT1 levels are decreased at the blood–brain barrier and in a variety of brain regions, including the hippocampus in Alzheimer's disease subjects (Kalaria and Harik, 1989; Mooradian et al., 1997; Simpson et al., 1994; Vannucci et al., 1998a). GLUT3 protein levels are also decreased in the hippocampus of Alzheimer's disease patients (Harr et al., 1995), even when accounting for hippocampal neuronal loss (Simpson et al., 1994; Vannucci et al., 1998a). Moreover, cerebrospinal fluid (CSF) to plasma insulin ratios are reduced in Alzheimer's disease patients (Craft et al., 2000), suggesting that insulin stimulated GLUT trafficking may also be adversely affected in mild cognitive impairment and Alzheimer's disease. Such results suggest that decreases in cerebral glucose utilization in Alzheimer's disease patients may result from impairment in glucoregulatory activities and decreases in glucose transporter expression and trafficking. In support of this hypothesis, cognitive performance was improved by glucose ingestion or by hyperglycemic/hyperinsulinemic clamp conditions in patients with dementia of the Alzheimer's type (Craft et al., 1996; Manning et al., 1993). Subsequent studies have more selectively implicated the cognitive-enhancing properties of insulin in this patient population (Craft et al., 1999). It is tempting to speculate that increased trafficking of a readily mobilizable pool of GLUT4 and GLUT8 contribute to the insulin-mediated enhancement of cognitive function in dementia of the Alzheimer's type patients, especially since cognitive measures were normally evaluated in time periods that preclude the de novo synthesis of new GLUTs.

Cognitive performance is also increased in healthy elderly subjects following glucose ingestion (Gold, 1995; Greenwood, 2003; Manning et al., 1998). Interestingly, cognitive status following glucose ingestion was related to glycemic control in that subjects with greater glucoregulation performed better than those with poor glycemic control (Allen et al., 1996; Messier et al., 1997), further establishing the important relationship between glucoregulatory activities and cognitive performance. Glucose enhancement of behavioral performance has also been observed in aged rodents (Gold, 1995). For example, aged rats given a glucose bolus improved their performance of several hippocampally dependent tasks, often reaching performance levels observed in young rats (Winocur and Gagnon, 1998). As seen in elderly human subjects, performance was correlated with glucose metabolism in aged rats. In summary, cognitive performance may be enhanced in otherwise healthy elderly subjects by increasing plasma glucose levels, an effect that is dependent upon the glycemic control of the individual.

Interestingly, several studies have illustrated that dietary intake of carbohydrates, fat or protein can enhance memory recall in healthy elderly subjects independent of increases in plasma glucose levels (Greenwood, 2003; Kaplan et al., 2001). This raises the important question of whether insulin of peripheral or central origin, or a combination thereof, contributes to the cognitive enhancing properties of insulin. This is especially relevant since cognitive performance may be evaluated over periods that do not provide sufficient time for CNS insulin levels to be significantly increased. Gastrointestinal signals can influence behavioral performance in rats, which suggests that the rapid effects of some dietary intake paradigms that do not modulate plasma glucose levels may activate a brain–gut axis to stimulate memory consolidation and recall (Greenwood, 2003). As such, the role of peripheral versus central insulin in relation to cognitive function remains to be clarified, as does the role of glucose metabolism and GLUTs, in particular the insulin-sensitive GLUTs.

6. Future perspectives

As described above, impairments in glucose metabolism, glucoregulatory activities and the expression and trafficking of GLUTs have a negative impact upon hippocampal-dependent function. Beyond GLUTs and glucose metabolism, it is important to note that a variety of factors may contribute to cognitive deficits in humans and behavioral impairments in animals (Gispen and Bies-

sels, 2000; Reagan, 2002). Neuroendocrine changes, reversible and irreversible morphological changes, neurochemical changes, production and accumulation of reactive oxygen species, protein glycosylation, among other alterations, undoubtedly contribute to the development of cognitive impairments associated with neurodegenerative disorders, diabetes or aging. While these deleterious neurochemical and neuroanatomical alterations may have unique and independent etiologies, they are also intimately woven together and may act in an additive or synergistic manner. As such, it would be difficult to establish a hierarchy that designates the relative importance of these changes in relation to the development of cognitive deficits. Nonetheless, such observations provide the framework for exciting avenues of research to investigate the functional roles of brain GLUTs and glucose metabolism in the CNS, as well as how impairments in GLUT function may be restored in pathophysiological settings such as mild cognitive impairment, Alzheimer's disease and diabetes.

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