The facilitative glucose transporter GLUT3: 20 years of distinction

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GLUCOSE METABOLISM IS VITAL to most mammalian cells, and the passage of glucose across cell membranes is facilitated by a family of integral membrane transporter proteins, the GLUTs. There are currently 14 members of the SLC2 family of GLUTs, several of which have been the focus of this series of reviews. The subject of the present review is GLUT3, which, as implied by its name, was the third glucose transporter to be cloned (Kayano T, Fukumoto H, Eddy RL, Fan YS, Byers MG, Shows TB, Bell GI. J Biol Chem 263: 15245–15248, 1988) and was originally designated as the neuronal GLUT. The overriding question that drove the early work on GLUT3 was why would neurons need a separate glucose transporter isoform? What is it about GLUT3 that specifically suits the needs of the highly metabolic and oxidative neuron with its high glucose demand? More recently, GLUT3 has been studied in other cell types with quite specific requirements for glucose, including sperm, preimplantation embryos, circulating white blood cells, and an array of carcinoma cell lines. The last are sufficiently varied and numerous to warrant a review of their own and will not be discussed here. However, for each of these cases, the same questions apply. Thus, the objective of this review is to discuss the properties and tissue and cellular localization of GLUT3 as well as the features of expression, function, and regulation that distinguish it from the rest of its family and make it uniquely suited as the mediator of glucose delivery to these specific cells.

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facilitates insulin-stimulated glucose uptake in insulin-sensitive cells: muscle and fat. The overriding question that drove the early work in GLUT3 in the brain was: why would neurons need a separate glucose transporter isoform; what is it about GLUT3 that specifically suits the needs of the highly metabolic and oxidative neuron with its high glucose demand? More recently, GLUT3 has been studied in other cell types with quite specific requirements for glucose, including sperm, preimplantation embryos, circulating white blood cells, and an array of carcinoma cell lines. The latter are sufficiently varied and numerous to warrant a review of their own and will not be discussed here. However, for each of these cases, the same questions apply. Thus, the objective of this review is to discuss the properties and tissue and cellular localization of GLUT3 as well as the features of expression, function, and regulation that distinguish it from the rest of its family and make it uniquely suited as the mediator of glucose delivery to these specific cells.

History

Human GLUT3 was initially cloned by low-stringency hybridization from a fetal skeletal muscle cell line, using a GLUT1 cDNA probe, and was found to share 64.4, 51.6, and
subsequently 57.5% identity with GLUT1, -2, and -4, respectively (62). The original Northern blots suggested that, while GLUT3 was highly expressed in brain, it was also detected in various carcinoma cell lines in kidney, colon, and placenta, and to a lesser extent in small intestine, stomach, and subcutaneous fat. It was barely detectable in adult skeletal muscle and thus was not considered to be the then-elusive insulin-regulated glucose transporter GLUT4, which was cloned by a variety of groups shortly thereafter (17, 23, 39, 55, 56, 58). Subsequent studies using Western blot analysis have supported a far less ubiquitous distribution of GLUT3 protein (48, 107). GLUT3 received its commonly used title as the “neuronal glucose transporter” when it was cloned from a mouse library by Nagamatsu et al. (92). These studies revealed that the distribution of GLUT3 mRNA was much more restricted in mouse than in human tissues, being essentially confined to brain; GLUT3 was later found in other murine cells, as discussed below. The same study demonstrated GLUT3 expression by in situ hybridization exclusively in neurons, thus the designation neuronal glucose transporter. Having defined GLUT3 as the neuronal transporter, the question still arose as to its localization within the neuron. Currently, the consensus opinion is that GLUT3 is found predominantly in the cell processes, i.e., axons and dendrites, with less labeling in the cell body. However, as is evident in Fig. 1, which depicts rat cerebellar granule neurons in culture and pyramidal neurons in human hippocampus, labeling is evident in axons and dendrites as well as in the neuronal cell bodies (44, 71, 75, 78, 82, 84, 128).

Structure

Although X-ray crystal structures of GLUT proteins are currently unavailable, a molecular model has been created for GLUT3 (32). High affinity glucose transporter proteins may be characterized by several structural features: the first intracellular loop; the N-terminal (membrane proximal) segment of intracellular loop 6; and central residues of TM9 and TM11 (depicted in the model of GLUT3 in Fig. 2). Class I (GLUT3) and II glucose transporter proteins bear an N-linked glycosylation site in loop 1, whereas Class III glucose transporters are glycosylated in extracellular loop 9. N-glycosylation of the GLUT is required to maintain high-affinity transport of glucose (9). The significance of the first extracellular loop is further highlighted by the fact that the length of this loop is inversely correlated \( r = 0.8–0.9 \) with the \( K_m \) of transport for GLUT1–4, GLUT8, and GLUT10 (D. Dwyer, unpublished observation). A second region of interest is intracellular loop 6. The membrane proximal segment just COOH-terminal to TM6 is in close proximity to the exit site of the transporter pore. The amino acid sequences of the GLUT family diverge at this point, which may determine the disposition of this segment relative to the pore or its flexibility and thus transport kinetics. Residues near the constriction of the pore in TM9 and TM11 represent a third area where sequence differences may explain function (Fig. 2). In human GLUT3, these segments include a number of small amino acids (particularly glycine) and residues capable of hydrogen bonding to glucose (e.g., serine and asparagine). In lower-affinity transporters such as GLUT1 and GLUT2, bulkier amino acids such as alanine, isoleucine, and phenylalanine replace glycine, threonine, and leucine/cysteine, respectively, in TM9 and TM11. The bulkier substitutions may alter local properties of the pore and increase the \( K_m \). Of course, changes at other sites may also contribute to the differences in transport kinetics.

Kinetics

The determination of substrate specificity and kinetic parameters of the individual facilitative glucose transporter proteins as expressed in mammalian cells is frequently complicated by the simultaneous expression of multiple isoforms in a given cell. In addition, it is difficult to accurately determine the number of transporters on the cell surface. To circumvent the specificity problem, all of the family members have now been expressed in Xenopus oocytes (see Ref. 81 for review). In the original studies, GLUT3 was consistently found to exhibit a lower \( K_m \) than its other Class 1 counterparts, GLUT1, -2, and -4, with \( K_m \) values for 2-deoxyglucose uptake of 1.4 mM compared with GLUT1, 6.9 mM, GLUT2, 11.2 mM, and GLUT4, 4.6 mM; \( K_m \) values for 3-O-methylglucose equilibrium exchange of 10.6 compared with \( K_m \) for GLUT1, -2, and -4 of 21, 42, and 4.5 mM, respectively (6, 46, 47, 63, 81, 96). GLUT3 was also shown to transport mannose, galactose, and xylose but is unable to transport fructose. More recently, the Class II and III members of the transport family, particularly GLUT7, -9, -10, and -11 have been shown to have particularly high affinities for glucose with \( K_m \)s on the order of 0.1–0.3 mM. With the exception of the study by Nishimura et al. (96), these studies were unable to shed any light on the transport capacity, i.e., \( K_{cat} \), or turnover number for the respective

![GLUT3 distribution in rat cerebellar granule cells and human hippocampal neurons.](http://ajpendo.physiology.org/)

**Fig. 1.** GLUT3 distribution in rat cerebellar granule cells and human hippocampal neurons. **Top left:** immunohistochemical distribution of GLUT3 in cerebellar granule cells derived from the cerebellum of 8-day-old rats having undergone differentiation over a subsequent 6 days in culture. **Bottom left:** corresponding phase micrograph. **Right:** distribution of GLUT3 in human hippocampal pyramidal neurons. In both human and rat neurons, it is evident that GLUT3 is in axons, dendrites, and cell bodies. Micrographs were previously published (78, 128) and are reprinted here with permission.
GLUT1 and GLUT3 have been shown to be expressed in the mammalian brain, and their expression is regulated by various factors such as glucose levels, dehydration, and disease states. GLUT1 is expressed in higher numbers compared to GLUT3, which is more prevalent in the cerebellar granule neurons. The GLUT3 mRNA content was found to be significantly increased in the brain tissue of rats with diabetes, whereas GLUT1 expression was not significantly affected.

GLUT3 expression is upregulated in response to dehydration, which results in increased cerebral glucose utilization. This increase is likely due to the preferential use of GLUT3 in neurons that are sensitive to glucose levels, allowing them to access glucose from the extracellular space more efficiently. The increase in GLUT3 expression is also associated with changes in the synaptic connectivity and communication, which drive the large energy demand of the brain.

In conclusion, GLUT3 plays a significant role in the regulation of cerebral glucose utilization and synaptic connectivity, and its expression is regulated by various factors such as glucose levels, dehydration, and disease states. Further studies are necessary to understand the mechanisms underlying the regulation of GLUT3 expression and its role in brain function.
expression. This might be explained by the dramatic increase in GLUT3 mRNA in the axon terminals that make up the neurohypophysis. It seems unlikely that the mRNA is translated within the axon, as there is no apparent rough endoplasmic reticulum (ER) and Golgi that would be necessary to synthesize a transporter protein (40). However, together with mRNAs for oxytocin and vasopressin, GLUT3 mRNA may be associated with a ribonucleoprotein complex that serves as a potential docking, storage, and posttranscriptional regulation site (114, 120). Within 3 days of rehydration, rates of rCGU and GLUT3 mRNA return to control levels, whereas restoration of GLUT3 protein levels within the neural lobe of the pituitary requires between 3 and 7 days (68).

These studies examined both normal and experimental increases in cerebral glucose utilization and the accompanying increase in GLUT3 expression. Marked declines in cerebral glucose metabolism are associated with Alzheimer’s disease, especially in the parietal and temporal cortices (28, 29, 37, 54). Initial studies by Kalaria and Harik (59) demonstrated a reduction in the 55-kDa form of GLUT1 in cerebral microvessels prepared from Alzheimer’s patients compared with normal age-matched controls (60). These observations were confirmed and extended in studies by Simpson et al., who also demonstrated a loss of the 45-kDa glial form of GLUT1 and a striking loss of GLUT3 protein expression in parietal, occipital, and temporal cortex, together with caudate nucleus and hippocampus; only the frontal cortex did not show any significant loss (109). Importantly, the decreases in GLUT3 in the parietal and temporal cortices, hippocampus, and caudate were still significant even after correction for overall neuronal loss using the synaptic protein SNAP25 as a marker.

Over the past decade, the central role of GLUT3 in cerebral metabolism has been questioned by the studies of Magistretti and colleagues who have proposed that astrocytes play the key role in the coupling of neuronal activity and cerebral glucose utilization, according to what has come to be known as the astrocyte-neuron lactate shuttle (ANLS) hypothesis (74, 103). According to this hypothesis, the astrocyte, which relies on the ANLS has become widely interpreted as suggesting that the principle source of energy consumed by the neuron is lactate, thus obviating the need for neuronal glucose uptake via GLUT3. This has recently been challenged in a publication by Simpson et al. (108) who support the more traditional concept of cerebral metabolism that glucose is taken up and metabolized primarily in the neuron (also Ref. 111). By modeling the kinetic characteristics and respective cellular concentrations of the neuronal and glial glucose and lactate transporters, it was concluded that the glucose transport capacity of the neuron via GLUT3 far exceeds that of the astrocyte (GLUT1). Consequently, the interstitial lactate results from excessive glycolysis in the neuron and not the astrocyte (108), thus reestablishing the uniqueness of GLUT3’s role in neuronal metabolism. Certainly, during periods of activation and high neuronal energy demand, the neuron will likely utilize any available fuel; however, the demonstration of an increase in GLUT3 expression during all in vivo conditions associated with increased CGU described above provides further confirmation for the central role of GLUT3.

Although GLUT3 is clearly the ideal teleological choice for neuronal transporter, substantial evidence indicates that it is not the exclusive neuronal glucose transporter. GLUT1 can be readily detected in cultured neurons and is detected in vivo under conditions of stress such as following a hypoxic-ischemic insult (45, 70, 78, 129, 131). The presence of GLUT2 has been reported in neurons in the hypothalamus (7, 8), and GLUT4 has been found in several subsets of neurons, including Purkinje and granule cells in cerebellum, principle and nonprinciple cells in the hippocampus, and isolated cells in the cortex and hypothalamus (5, 7, 12, 67, 72, 106, 126). Both GLUT6 and -8 have been reported to be present in neurons; however, as with GLUT4, both proteins contain a dileucine motif in their COOH termini and would appear to reside in intracellular membranes; GLUT8 may be recruited to the ER in the presence of insulin (see Ref. 85 for review).

GLUT3 in Murine Sperm

Glucose metabolism has been shown to be critical for sperm function, through both glycolysis and the pentose phosphate pathway. As with other cell types that express GLUT3, murine sperm experience states of “activation” with heightened energy demand. Sperm are stored in a quiescent state in the cauda epididymides of most mammalian species. Upon ejaculation, they mix with seminal plasma and acquire an “activated” pattern of motility. However, sperm are not competent to fertilize an egg until they mature functionally in response to stimuli within the female tract. This process is known as “capacitation” and involves changes in both the head and flagellum of the sperm (117). The head acquires the ability to undergo acrosomal exocytosis, and the flagellum acquires a “hyperactivated” pattern of motility. Both changes are essential for fertilization to occur.

To achieve maximal reproductive success, males have evolved a strategy of producing numerous sperm while investing as little as possible in each one. To supply the highly polarized sperm with the metabolic products it needs, precisely where it needs them, sperm have compartmentalized these pathways to the various regions of the cell. For example, the pentose phosphate pathway is found in the head and midpiece, and glycolysis is found down the length of the flagellar principal piece (Fig. 3). Remarkably, although the restriction of mitochondria to the sperm midpiece is highly conserved across species, at least in the mouse these mitochondria do not need to produce energy for sperm to have a normal activated pattern of motility (91). However, the ATP produced from glycolysis is essential for normal flagellar motility even in the presence of otherwise functional mitochondria (86, 91). In addition, ATP produced by glycolysis is essential for the protein tyrosine phosphorylation events associated with capacitation (116). Thus the glycolytic machinery compartmentalized to the principal piece of the flagellum powers both the dynein ATPases that provide motility, as well as the kinases that regulate it.

Of note, glucose concentrations as low as 10–100 μM are all that are necessary to support the tyrosine phosphorylation signaling in murine sperm (116). This low concentration is consistent with a primary role for GLUT3, which is the predominant family member in murine sperm (122). In this
GLUT3 in the Embryo

Several studies have demonstrated that GLUT3 expression is crucial for optimal preimplantation embryo development and survival. Pantaleon et al. (99) first described the expression of this transporter in murine embryos in 1997. GLUT1 and GLUT2 had been identified six years earlier in the murine preimplantation embryo (1, 51). GLUT1 was expressed through this period, from one-cell zygote to blastocyst stage. GLUT2 expression was first detected at an eight-cell stage. At the blastocyst stage, GLUT2 protein expression was restricted to basal trophectoderm in direct contact with the blastocele cavity, whereas GLUT1 was localized to apical, basolateral, and intercellular junctions between cells. In that report, GLUT1 was purported to be the main glucose transporter, responsible for uptake of maternal glucose. Pantaleon et al. (99) noted, however, that this would be unlikely given that the $K_m$ of glucose transport was much lower than that of GLUT1. Gardner and Kaye (43) had demonstrated that the mouse blastocyst had a $K_m$ of 6.3 ± 0.5 mM for 3-O-methylglucose (3-OMG) whereas GLUT1’s $K_m$ for 3-OMG is 20.1 ± 2.9 mM. In contrast, GLUT3 has a $K_m$ of 10.6 ± 1.3 mM for 3-OMG and is normally expressed on apical surfaces where it typically functions as a high-capacity glucose transporter. Pantaleon et al. (101) went on to reveal that GLUT3 was expressed at an mRNA level at a late four-cell stage and at a protein level first at late eight-cell early morula stage and most clearly seen at a late morula stage on the apical surface of the polarized outer cells and finally in the blastocyst stage in the apical surface of the trophectoderm. They went on to show that downregulation of expression of GLUT3 with antisense oligonucleotides resulted in a significant drop (>40%) in glucose uptake and a 50% decrease in the number of embryos progressing to a blastocyst stage compared with blastocysts treated with sense oligonucleotides. Inhibition of GLUT1 expression with antisense oligonucleotides did decrease glucose uptake, albeit to a much lesser extent than GLUT3. They concluded that GLUT3 functions as the high-affinity glucose transporter and that its expression correlates with compaction and the switch to a metabolic preference for glucose vs pyruvate (99).

Subsequently, Moley et al. (88) described a pathological condition, maternal diabetes mellitus induced in the mouse by streptozotocin injection, in which GLUT3 expression was physiologically downregulated at both the mRNA and protein levels of expression in the blastocyst. These blastocyst stage embryos displayed decreased glucose uptake by measurements using nonradioactive microanalytic cycling reactions on individual blastocysts. These findings were consistent with those of Pantaleon et al. (99), in which downregulation with antisense oligonucleotides induced a similar decrease in glucose uptake. In addition, Moley et al. demonstrated increased apoptosis and an abnormal metabolic profile (25, 88). In vitro culture in high glucose concentrations induced the same decrease in GLUT3 expression and when transferred into recipient mice, these embryos displayed intrauterine growth retardation and a high incidence of fetal resorptions (133). This group concluded that decreased expression of GLUT3 in these embryos exposed to

become able to fertilize (102). Species that rely on sperm GLUT3 would be predicted to have reliable, but low glucose intermediates in oviductal fluid.
high maternal glucose levels was in response to unfavorable maternal milieu but that a threshold of glucose was required for embryonic growth and further development. They proposed that, if this threshold was crossed, an apoptotic pathway was triggered leading to loss of key cells required for optimal survival (88, 89). These findings all point to GLUT3 being a critical transporter protein required for this stage of development.

These findings were substantiated by the creation of a GLUT3-null (GLUT3−/−) mouse, as described by Devaskar’s group [Ganguly et al. (42)]. In this model, the knockout construct globally removed exons 7–9 and coding region 10 of GLUT3. At a blastocyst stage, the GLUT3−/− embryo was detected but displayed increased apoptosis and delayed development. Despite the high incidence of apoptosis, the blastocysts implanted and GLUT3−/− embryos were detected at embryonic day 6.5; however, further investigation revealed a loss at embryonic day 8.5. Interestingly, the heterozygous blastocysts displayed an atypical localization of GLUT3 protein (Fig. 4). As opposed to the wild-type blastocyst, in which GLUT3 localized consistently to the apical trophectoderm, the heterozygote blastocysts expressed GLUT3 both apically and basolaterally in the trophectoderm and inner cell mass cells. These heterozygotes also displayed an increase in TUNEL-positive nuclei or apoptosis. The rate of apoptosis was intermediate between the GLUT3−/− and wild-type embryos. In addition, GLUT1 localization was also abnormal in both the GLUT3−/− and GLUT3−/+ blastocysts; GLUT1 was detected on both apical and basolateral surfaces of the trophectoderm in the heterozygote, whereas in the GLUT3−/− blastocyst GLUT1 resided in punctate cytoplasmic vesicles. This distribution pattern is identical to that seen in precompaction blas-tomeres prior to the establishment of polarity and the differentiation into trophectodermal epithelium (100). GLUT1 localiza-tion in the wild-type blastocyst was in the expected basolateral plasma membrane. Those authors concluded that some degree of GLUT3 expression was required to trigger polarization and successful establishment of a healthy blasto-cyst (42). The authors went further to postulate that GLUT1 and GLUT3 might need to be expressed differentially in timing and location to permit adequate glucose uptake and development.

The GLUT3−/− mouse model was similar to the diabetic model in another aspect. The blastocysts exposed to diabetic conditions by downregulation of GLUT3 displayed increased fetal resorptions and significant growth retardation (133). Similarly, the GLUT3−/− embryos implanted but failed to progress past embryonic day 6.5 (42) (Fig. 5). The GLUT3−/− fetuses demonstrated an intermediate expression of GLUT3 at both blastocyst stage and postimplantation and survived but were growth retarded with a 20% peak decline in body weight compared with controls. It is unclear whether these growth effects are due to decreased expression of placental or fetal GLUT3; however, due to the lack of significant growth retardation in the GLUT1-null mouse (130), the conclusion appears to be that GLUT3’s critical function is the trophectoderm/placenta, which indirectly affects fetal growth, whereas GLUT1 functions to provide glucose for embryonic organogenesis (Fig. 6). Transplacental uptake studies in Devaskar’s group demonstrated that, in the murine hemochorial placenta, GLUT3 deficiency led to a decrease in transplacental glucose transport and fetal uptake that was more than a slight decrease in intraplacental glucose uptake (42).

Several studies using different technologies have shown that GLUT3 is expressed at high levels in the extra-embryonic membranes (amnion, chorion, and yolk sac) and goes on to be expressed in the rapidly dividing, poorly differentiated placental extravillous trophoblast and villous cytotrophoblast subpopulations (49, 110). This finding is consistent in both human and mouse early placental development, despite the fact that placental structures differ significantly between the species. Human placenta is hemomonochorial and villous in structure, whereas mouse placenta is hemotrophichorial and labyrinthine in structure. GLUT3 protein has been detected in first trimester mouse and human trophoblast cells as well as in choriocarcinoma cell lines (52, 97), suggesting that GLUT3 protein may be most critical early in postimplantation gestation, when a high-affinity glucose transporter like GLUT3 would allow constant uptake of this energy substrate under conditions of decreased substrate concentration. These trophoblast cells invade and erode the uterine epithelium upon implantation and eventually reach the maternal blood supply where they bathe in these islands. Expression of GLUT3 on the extra-embryonic membrane surfaces would permit maternally produced glucose to travel to the rapidly growing and energetically demanding embryo.

Human term placenta expresses no GLUT3 protein despite the fact that GLUT3 mRNA is localized to the same placental membranes as GLUT1 mRNA in both syncytiotrophoblasts and cytotrophoblasts throughout gestation. This developmental differential expression of GLUT3 protein and constant expression of GLUT3 mRNA at term suggests some form of active regulation that suppresses expression of the protein. Studies have demonstrated that this level of mRNA in term human trophoblast is regulated by hypoxia, suggesting that a constitutive block in translation of the mRNA does exist (33).

The postimplantation embryonic expression of GLUT3 is transient. Although expressed in the 8.5-dpc nonneuronal surface ectoderm of the embryo proper, GLUT3 is downregulated significantly by dpc 10.5 (110) and is no longer detectable by dpc 8.5 in fetal brain (64). The consensus of all these studies is

Fig. 4. Preimplantation embryos demonstrate dual immunohistochemical staining for GLUT3 and TUNEL. A: representative wild type (WT); B: heterozygous (Het); C: homozygous knockout (KO) embryo demonstrating GLUT3 (green) and TUNEL (red) along with nuclear ToPro-3 (blue) staining. WT demonstrates apical distribution (arrow); heterozygous embryo demonstrates punctate distribution of GLUT3 on apical and basolateral surfaces of the trophectoderm (arrowhead); homozygous embryos demonstrate no GLUT3. TUNEL staining progressively increases from WT to homozygous embryos.
that blastocyst expression of GLUT3 is confined to trophectoderm and that postimplantation expression is predominantly extra-embryonic, supporting GLUT3 as a critical mediator of transplacental glucose transporter necessary for fueling fetal growth.

Finally, in a recent study, Kaye’s group [Pantaleon et al. (101)] has suggested an interesting role for glucose in the metabolic differentiation of the murine blastocyst and in the induced expression of GLUT3. It has been known for over a decade that, although murine precompaction stage embryos are unable to metabolize glucose, they require a brief exposure to glucose prior to the morul stage (24). Without this glucose exposure, the embryos undergo increased apoptosis, decreased proliferation, and reduced glucose transport, and their progression to a blastocyst stage is impaired. This phenotype is similar to the GLUT3-null blastocyst; thus, this group hypothesized that perhaps GLUT3 expression was blocked. They confirmed this and went on to demonstrate that a pulse of glucose (27 mM for 1–3 h prior to 67 h post-hCG) is necessary to activate transcriptional and translational expression of GLUT3. In addition, glucosamine at the same concentration and timing is also sufficient to prime the embryo, suggesting that the hexosamine pathway may be involved. They concluded by postulating that the downstream component of this pathway, UDP-Glc-NAC (uridine diphosphate N-acetylglucosamine), may be the effector which triggers metabolic differentiation via O-linked glycosylation of the transcription factors regulating GLUT3 and possibly other key players in this process.

GLUT3 in Human White Blood Cells

The first observation of GLUT3 in white cells occurred when its localization in human brain was being studied (82). In addition to the expected pronounced neuronal localization, GLUT3 displayed both a vascular and an intravascular expression, and the latter was not associated with erythrocytes. GLUT3 has subsequently been shown to be present in human lymphocytes, monocytes/macrophages, neutrophils, and platelets. What is particularly significant about the presence of GLUT3 in white cells is that it is present in an intracellular pool and may be recruited to the plasma membrane upon activation in a manner entirely analogous to that of GLUT4 translocation in muscle and adipose tissue. Indeed, in B-lymphocytes and monocytes, insulin is able induce a translocation of GLUT3 but is without effect on neutrophils or T-lymphocytes (27, 34, 38, 83). It should be noted that an earlier study by Bilan et al. (16) demonstrated the translocation of GLUT3 in response to insulin and IGF-I in the fetal L6 rat muscle cell line.

Activation of the white cells with either PMA or the appropriate activator, e.g., lipopolysaccharide (LPS) and formylmethionyl-leucyl-phenylalanine (fMLP) for neutrophils and monocytes/macrophages, phytohemagglutinin (PHA) for lymphocytes, and thrombin for platelets also results in the recruitment of GLUT3 to the plasma membrane (26, 35, 38, 50, 53, 83, 112). Figure 7 depicts the activation of neutrophils and platelets and the recruitment of GLUT3 to the respective plasma membranes. This figure also illustrates the subcellular localization of GLUT3 in quiescent neutrophils and platelets (Fig. 7, A and B) and their subsequent activation by PMA (Fig. 7, C and D) and, in the case of neutrophils, by bacteria (Fig. 7, E–H). In the case of the platelets, GLUT3 is clearly recruited to the membrane from an intracellular location, namely the α-granules (50). A similar recruitment is seen in the neutrophils when activated with PMA. However, when activated with...
bacteria, not only is GLUT3 recruited to the cell surface and spike-like projections of the plasma membrane (Fig. 7F), but it also appears highly concentrated intracellularly in phase-dense (phagosome-like) structures (Fig. 7H). In some of these structures, the intensity of the fluorescence is higher or at least comparable to that observed over platelets (Fig. 7H). Very little is known of the nature of the intracellular membranes containing the GLUT3 or the signal transduction mechanism(s) pro-

Fig. 7. Immunolocalization of GLUT3 in resting and activated neutrophils by confocal microscopy. Phase contrast micrographs (A), corresponding to fluorescence micrographs (B), show the morphology of unstimulated (control) cells: characteristic multinucleated cells with thin cytoplasm. In these cells, GLUT3 (B) exhibits punctate intracellular staining, which appears dispersed throughout the cytoplasm. Phase contrast (C) and corresponding fluorescent images (D) are presented from cells incubated with 100 nM PMA for 10 min. In response to PMA, GLUT3 displays clear redistribution to the cell surface from intracellular locations (D). Intensity of fluorescence at the cell surface remains below that observed over the platelet in the same field (arrow in D). Phase contrast micrographs (E and G) and corresponding fluorescence images (F and H) are presented for cells incubated with bacteria for 20 and 30 min, respectively. Striking morphological changes can be observed during cell activation: cells are larger and have more cytoplasm and large expansions of their plasma membrane; phagocytosed bacteria are seen inside most cells. These morphological changes are accompanied by marked redistribution of GLUT3 immunofluorescence from intracellular locations toward the cell surface, outlining the cell periphery and spike-like plasma membrane projections (E and F). Very bright intracellular spots appear to correspond to phase-dense membrane compartments (arrows in G and H). Platelets (small arrows in G and H) have much lower intensity of fluorescence than observed when activated in D. Bars, 5 μm (D. Malide, I. A. Simpson, and M. Levine, unpublished observations).
motoring recruitment in each of these cells. In the case of platelets, EM studies of Heijnen et al. (50) demonstrated that GLUT3-containing vesicles are the α-granules that contain the adhesion factor P-selectin, and various growth factors including platelet-derived growth factor (PDGF), IGF-1, and transforming growth factor (TGF)-β. Analogous to the action of insulin in adipose cells, thrombin appears to mediate the recruitment of the α-granules through a mechanism that clearly involves PI-3 kinase phosphorylation of Akt (35) (69). However, the mechanism appears to be augmented by a Ca2+-PKC phosphorylation of Akt. This can be mimicked by actions of PMA, which is able to stimulate transport activity in lymphocytes and monocytes, as well as neutrophils and platelets, illustrated in Fig. 7 (38, 79, 83, 112). The α-granules would also appear to share the same SNARE molecules as GLUT4-containing vesicles (22, 36).

In each of the cell types the translocation of GLUT3 is in response to a substantial increase in energy need associated with cell activation. In the case of platelets, the action of thrombin induces a cascade of energy-dependent events: actin polymerization, changes in platelet shape, aggregation and secretion of α- and dense vesicles, and ultimately aggregation and clot formation. These processes rapidly triple the expenditure of ATP, which, due to the relative paucity of mitochondria, must be replenished by glycolysis. In addition, the absence of blood flow and thus low prevailing glucose clearly favors the kinetic characteristics of GLUT3 to mediate glucose uptake (2, 3). Neutrophils upon activation, and monocytes upon transformation to macrophages, also undergo dramatic shape and size changes to facilitate phagocytosis of bacteria and senescent cells. The elimination of the bacteria involves the phagocytosis of the organism, the generation of prodigious numbers of superoxide molecules to kill the organism, and an array of lysosomal hydrolases to digest the phagocytosed body. Upon activation, lymphocytes also undergo dramatic changes in size, shape, and protein content. In the case of B-cells, they become enlarged immunoglobulin-synthesizing factories, whereas T-cells undergo conformational changes to accommodate antigen and polarized release of cytokines or perforins. Both cell types also undergo extremely rapid proliferation (18, 61, 124).

GLUT3: What Is in the Future

We have clearly moved beyond thinking of GLUT3 as solely a neuronal glucose transporter to appreciate that it is expressed in a variety of cell types with very specific, and high, energy needs. We know that in the neuron, which is not a rapidly turning-over cell, both acute and chronic changes in energy demand and glucose utilization result in changes in GLUT3 expression. In cells with short half-lives, such as circulating human white cells, changes in energy demand seem to be met by translocation of GLUT3 rather than changes in expression/concentration. Although the signal transduction and translocation mechanism(s) appear similar to those employed by GLUT4, they are clearly distinct and warrant further investigation. In the case of neurons and sperm, where pronounced intracellular pools of GLUT3 are not apparent such that translocation is unlikely, the question arises as to whether there are other mechanisms of GLUT3 activation to account for the rapid changes in transport activity occurring during seizures (neuron) and capacitation (sperm), such as movement in and out of lipid rafts as seen for GLUT1 (13, 105). In the mouse blastocyst, the level of GLUT3 expression appears to direct proper trafficking of GLUT1 to the plasma membrane (42), implying a role for GLUT3 in the trafficking of other GLUTs in the same cell.

There is still much to be learned about the regulation of this vital transporter isoform. For example, it remains to be determined which factors actually regulate expression in response to metabolic demand in the neurons at both the transcriptional and translational levels. We know that GLUT3 responds to hypoxia through Hif in a variety of cells, including neurons, carcinomas and monocytes, but what other factors regulate expression in response to metabolic demand remain to be determined (11, 14, 87, 129). Studies have also demonstrated that transcriptional regulation of GLUT3 in neurons depends on both the stage of differentiation and the function of the cell. This work suggests that nuclear factors Sp1/Sp3 may mediate the transcriptional activation of GLUT3 along with MSY-1 during neurodevelopment; whereas phosphorylated cAMP regulatory element-binding (pCREB) protein may regulate transactivation of GLUT3 expression during neurotransmission under conditions of substrate deficiency (104). Furthermore, posttranscriptional regulation of GLUT3 protein expression occurs in term placenta, suggesting a tight regulation of expression dependent on environmental conditions in the developing placenta (33). In the preimplantation embryo, a pulse of glucose or glucosamine for 1–2 h prior to compaction is necessary to induce expression of GLUT3 and thus to progress through normal development (101).

Furthermore, there are in vivo situations in which acute activation is associated with an increase in mRNA expression, which may or may not be translated into protein (95). The physiological value of this response of GLUT3, as well as potential regulators of both transcription and translation (65), are targets for future research.

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