Journal of Cellular Biochemistry 48:51–60 (1992)

Acute and Chronic Signals Controlling Glucose Transport in Skeletal Muscle

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Abstract Glucose transport into muscle cells occurs through facilitated diffusion mediated primarily by the GLUT1 and GLUT4 glucose transporters. These transporter proteins are controlled by acute and chronic exposure to insulin, glucose, muscle contraction, and hypoxia. We propose that acute responses occur through recruitment of pre-formed glucose transporters from an intracellular storage site to the plasma membrane. In contrast, chronic control is achieved by changes in transporter biosynthesis and protein stability. Using subcellular fractionation of rat skeletal muscle, recruitment of GLUT4 glucose transporters to the plasma membrane is demonstrated by acute exposure to insulin in vivo. The intracellular pool appears to arise from a unique organelle depleted of transverse tubule, plasma membrane, or sarcoplasmic reticulum markers. In diabetic rats, GLUT4 content in the plasma membranes and in the intracellular pool is reduced, and incomplete insulin-dependent GLUT4 recruitment is observed, possibly through a defective incorporation of transporters to the plasma membrane. The lower content of GLUT4 transporters in the muscle plasma membranes is reversed by restoration of normoglycemia with phlorizin treatment.

In some muscle cells in culture, GLUT1 is the only transporter expressed yet they respond to insulin, suggesting that this transporter can also be regulated by acute mechanisms. In the L6 muscle cell line, GLUT1 transporter content diminishes during myogenesis and GLUT4 appears after cell fusion, reaching a molar ratio of about 1:1 in the plasma membrane. Prolonged exposure to high glucose diminishes the amount of GLUT1 protein in the plasma membrane by both endocytosis and reduced biosynthesis, and lowers GLUT4 protein content in the absence of changes in GLUT4 mRNA possibly through increased protein degradation. These studies suggest that the relative contribution of each transporter to transport activity, and the mechanisms by which glucose exerts control of the glucose transporters, will be key subjects of future investigations.

Key words: glucose transporters, insulin, diabetes, glucose toxicity

Skeletal muscle is the primary tissue responsible for post-prandial whole body glucose utilization, and glucose transport in this tissue is the rate limiting step of glucose metabolism [see 1]. Glucose transport is mediated by polypeptide members of a family of transporters, which operate by facilitated diffusion [reviewed in 2]. The process is usually unidirectional by virtue of the rapid intracellular metabolism of glucose into glucose-6-phosphate and ensuing glycolysis and/or glycogenesis. Control at the level of glucose transport will result in key regulation of subsequent glucose metabolism. Glucose transport can be regulated by several modes: changes in the number of glucose transporters operating in the plasma membrane, changes in the affinity of the transporters for glucose, and changes in the intrinsic activity of the transporters (intrinsic affinity being defined as the transport capacity or kinetic turnover number divided by the Km). The change in the number of glucose transporters operating in the plasma membrane can in turn be brought about by: a redistribution between the plasma membranes and a pool of latent or inaccessible pre-existing transporters, changes in the biosynthetic rate of the transporters, and changes in the degradation rate of the transporters.

It is believed that these mechanisms of control are called upon under defined metabolic conditions which demand increased or decreased glucose utilization, and further that defects in these mechanisms may result in defective glucose uptake. To date, changes in the subcellular localization, mostly recruitment to the plasma membrane, have been reported in response to *acute* (<1 h) exposure to insulin, exercise, or

Received September 4, 1991; accepted September 25, 1991.

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hypoxia [3–5]. In contrast, changes in the biosynthesis of transporters have been reported in response to prolonged (> 2 h, typically > 16 h) exposure to insulin [6], exercise training [7], and hypoxia [8]. Alterations in the degradation rate of the transporters have not been reported, due to difficulty in measuring transporter half-life. Equally elusive are measurements of changes in either intrinsic activity or affinity for glucose, although a recent study has inferred that an increase in intrinsic activity accompanies exercise and insulin-induced recruitment of transporters [9].

We hypothesize that in order to produce rapid changes in glucose transport, translocation or recruitment of glucose transporters is the preferred mechanism, while responses to more sustained stimuli may involve increases in the net number of glucose transporters. Changes in intrinsic activity may occur in response to both short-term and chronic stimuli; in general, changes in intrinsic activity are invoked when changes in transport activity cannot be explained by alterations in transporter number at the plasma membrane.

DIFFERENT FUNCTIONS FOR THE DIFFERENT GLUCOSE TRANSPORTERS OF MUSCLE?

Skeletal muscle expresses both GLUT1 and GLUT4 transporters. The presence of GLUT4 is unambiguous, and is characteristic of muscle and fat tissues exclusively [see 2]. The presence of GLUT1 intrinsic to muscle tissue has been a matter of recent debate. Whereas GLUT1 mRNA can be detected in RNA extracted from muscle segments, it is possible that it stems from tissues co-existing with the muscle such as endothelial, vascular, and neural cells. Recent studies at the level of immunofluorescence indicate that indeed the largest amount of GLUT1 protein in muscle segments is present in the perineurion (i.e., the cell layer that surrounds the innervating axon), while it is absent from endothelial vascular cells [10]. In the same study it was calculated that in excised, untrimmed muscle, 40% of the GLUT1 immunoreactive transporter protein is inherent to the myocyte, while the rest belongs to accessory tissues. In collaboration with J. Richardson and J.E. Pessin (University of Iowa) we have observed detectable levels of GLUT1 protein by immunofluorescence in the plasma membrane of skeletal muscle.[1] We have also calculated, using quantitative immunoblotting, that the molar ratio of GLUT1 to GLUT4 in red skeletal muscle membranes (total particulate fraction) is about 1:20. However, conditions such as denervation [11] and streptozotocin diabetes [12] augment the amount of GLUT1 protein while diminishing GLUT4, thereby markedly reducing the difference in molar ratio of the two isoforms.

It is the consensus that GLUT1 is responsible for basal transport in muscle and adipocytes, while GLUT4 participates mainly in the rapid response to insulin. The latter involves mostly recruitment of transporters from an intracellular site to the plasma membrane [see 1]. This mechanism has been demonstrated by subcellular fractionation, immunoelectron microscopy, and whole cell labelling of transporters in fat cells [13–15], and by subcellular fractionation in skeletal muscle [3, 16, and see 1] (Fig. 1A). However, at least in adipocytes, GLUT1 is also recruited in response to the hormone, albeit to a lesser extent, and GLUT4 proteins are also detected in the plasma membrane of unstimulated adipocytes [15] and muscle [16].

GLUT1 and GLUT4 transporters differ in their kinetic properties. In conditions of equilibrium exchange using 3-O-methylglucose as the transported sugar, the Km of GLUT1 is about 20 mM [17] while that of GLUT4 is about 2 mM. If these values hold for skeletal muscle, then GLUT1 will be a better sensor of glycemia than GLUT4, since the latter would be practically saturated at euglycemia. Even if GLUT1 transporter content were not significant in skeletal muscle, a transporter capable of responding to glycemia by increasing uptake through mass action is required, since GLUT4 cannot fulfill this function. Recent evidence indicates that a low-affinity transport system (Km about 10 mM) is responsible for non-insulin mediated glucose uptake into muscle in vivo, whereas insulin-mediated glucose uptake has a Km of 5 mM [18]. These values could potentially reflect preferential activity of GLUT1 for basal and GLUT4 for insulin-mediated glucose uptake. Interestingly, Baron et al. [19] have recently reported that the Km of muscle glucose uptake in NIDDM is higher than in control muscle, compatible with the notion that the GLUT1:GLUT4 ratio may increase in human diabetes (as stated above for diabetic rat muscle).

Although several conditions are known to induce transporter redistribution or altered biosynthesis, the signals that mediate such responses

[1]Marette A, Richardson J, Ramlal T, Balon T, Pessin J, Klip A, submitted 1991.

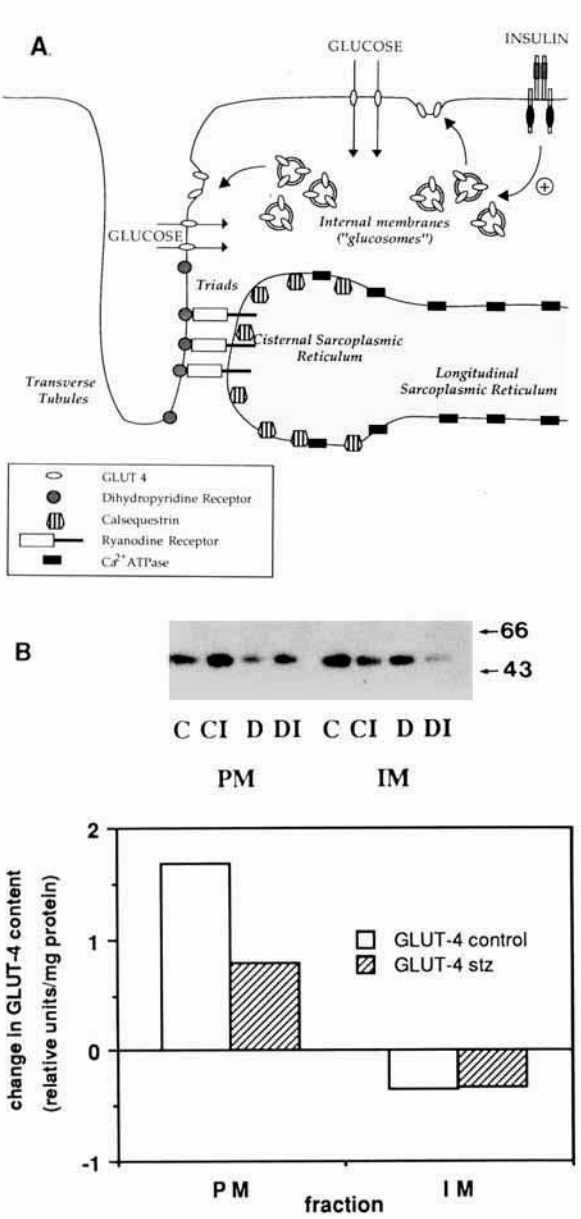

Fig. 1. Recruitment of glucose transporters from an intracellular organelle to the plasma membrane. **A:** Localization of the putative glucosome, a unique intracellular organelle endowed with glucose transporters, distinct from the sarcoplasmic reticulum and the transverse tubules. This uniqueness is based on the biochemical characterization described in the text and in reference 20. **B:** Recruitment of GLUT4 glucose transporters by acute exposure to insulin in control and 7-day diabetic (stz) rat skeletal muscle [see 21 for experimental protocol]. **Top:** Autoradiogram of GLUT4 content in isolated plasma membranes (PM) and glucosomes or intracellular membranes (IM) from control (C) and diabetic (D) rat muscle, without or with insulin (I) treatment. Equal amounts of membrane protein were applied to each gel lane. **Bottom:** GLUT4 transporter gain in the PM and loss in the IM of control (open bars) and diabetic (cross-hatched bars) muscle fractions (expressed as the ratio of transporter units in insulinized:control muscle membranes). The results are from four independent experiments, run on SDS-PAGE as in the representative gel at the top of this figure, laser scanned and averaged.

remain unknown. It is conceivable that transporter recruitment involves phosphorylation of certain cellular elements, attachment to binding or docking proteins, cation-mediated membrane fusion, participation of specific chaperone molecules tightly attached to the transporters, etc. However, these possibilities remain hypothetical since no specific component other than glucose transporters has been identified to be involved in transporter recruitment. Similarly, the regulation of transporter biosynthesis must involve regulation through specific trans-acting factors, but to date no single specific factor has been identified that regulates glucose transporter expression in muscle.

It is clear, therefore, that GLUT1 and GLUT4 gene products are expressed in muscle cells, and that different experimental conditions appear to regulate their levels distinctively. Studies on the regulation of gene expression, subcellular localization, and post-translational modifications of the two isoforms in vivo and in vitro are thus essential for a better understanding of the regulation of glucose transport.

TRANSLOCATION OF GLUCOSE TRANSPORTERS IN MUSCLE: NATURE OF THE INTRACELLULAR ORGANELLE(S) AND DEFECTS ASSOCIATED WITH DIABETES

We have succeeded at establishing a reproducible procedure to subfractionate muscle into plasma membranes, sarcoplasmic reticulum, transverse tubules, and intracellular membrane vesicles containing glucose transporters which we would like to refer as *glucosomes,* that can be distinguished from each other by density separation on sucrose gradients and by specific activity of marker enzymes [3–5,20]. GLUT4 transporters were found in both the plasma membrane and in the glucosomes but not in sarcoplasmic reticulum. GLUT1 transporters were found exclusively in plasma membranes, and were not the result of contamination with endothelial cell membranes [20]. The fractionation procedure has enabled us to detect unambiguously recruitment of GLUT4 glucose transporters from glucosomes to plasma membranes in response to acute (20–30 min) insulin hindquarter perfusion or intravenous injection [16,21]. We have observed a similar increase in transporters in the plasma membrane in response to exercise [4,16], but stemming from a separate intracellular pool of transporters. Short-term (30 min)

hypoxia produced results similar to those of exercise [5].

The subcellular localization of GLUT4 transporters in muscle has lately been the subject of debate. Whereas one electron microscopy study using immunogold labelling with a monoclonal antibody revealed that this protein is localized in the triad region (constituted by the cisternae of the sarcoplasmic reticulum and the transverse tubules) [22], this localization has been disputed and a trans-Golgi network or vesicular organelle near the surface membrane has been proposed [23]. Our biochemical characterization has demonstrated that the isolated glucosomes from rat muscle are devoid of plasma membrane and transverse tubule markers, and are relatively depleted of sarcoplasmic reticulum cisternal markers [20]. This characterization has led us to propose that the glucosomes represent a unique intracellular organelle that is likely specialized in storage and regulates delivery of glucose transporters [24] (Fig. 1A).

The availability of a subcellular fractionation strategy enables one to assess whether defects in recruitment of glucose transporters are associated with specific metabolic conditions characterized by insulin resistance. This strategy is important since severe insulin resistance has been observed in the absence of major changes in total GLUT4 or GLUT1 transporter protein or mRNA [25,26]. In insulinopenic and hyperglycemic rats, two defects were noted 7 days after streptozotocin injection: (i) GLUT4 transporter content diminished in the glucosomes and in the plasma membrane, and (ii) the loss of transporters from the glucosomes in response to insulin was normal, but the gain in the plasma membrane was incomplete, suggesting that the transporters may have been arrested at a prefusion state [21,24] (Fig. 1B). The former defect was found to correlate with a decrease in basal glucose uptake, and the latter with a reduced maximal response of uptake to insulin [24]. The cause for the decrease in the basal amount of GLUT4 transporters in the plasma membrane was likely the hyperglycemia, since the defect was corrected upon normalization of glycemia by phlorizin treatment [12]. Whether the second defect (recruitment defect) was caused by the hyperglycemia or the hypoinsulinemia remains to be determined. Interestingly, restoration of normoglycemia in depancreatized rats did not cause restoration of glucose transporter levels in rat adipocytes in spite of normalization of glucose transport activity [27], highlighting the differential control of glucose transporters in muscle and fat tissues. Moreover, the restoration of GLUT4 transporter number seen in muscle was specific for the plasma membrane, underscoring the importance of studying isolated plasma membranes rather than whole muscle homogenates or crude membranes to investigate glucose transporter regulation. It is hypothesized that the amount of transporters present in the plasma membrane is down-regulated by glucose through endocytosis of transporters, subsequently leading to their degradation.

GLUCOSE TRANSPORT AND TRANSPORTERS IN L6 MUSCLE CELLS: WHY IS IT USEFUL TO STUDY CELL LINES?

Whereas studies in intact animals are essential to assess the impact of the diabetic state on glucose uptake, several metabolic and circulating factors change at once (i.e., insulin, glucose, fatty acids, ketoacids, counter-regulatory hormones), preventing the establishment of absolute cause/effect relationships between a specific parameter and glucose transport. In contrast, muscle cell lines allow the study of separate variables independently of each other. Of course, these cells remain a model and are not mature, innervated muscle cells. Table I summarizes advantages and caveats of muscle cell lines for the study of glucose transport. We believe that a combination of studies at the levels of adult muscle and cell lines will be required to eventually understand fully the regulation of glucose transport and transporters at a molecular level.

L6 muscle cells were derived from leg muscles of day-old rats, and they re-enact in culture the myogenic process observed in vivo during fetal development or muscle regeneration. These cells have been extensively studied in other laboratories and shown to develop the expression of muscle-specific proteins and electrical/contractile properties [see 28]. In preliminary studies we have determined that these cells express the mATPase isozyme typical of oxidative muscles. This is important since oxidative muscles are the most responsive ones to insulin in vivo. We have amply demonstrated that these cells have a glucose transport system kinetically and pharmacologically similar to that of skeletal muscle [29,30]. Basal glucose transport decreases and

TABLE I. Advantages and Caveats Associated With Studies of Muscle Cells in Culture

Skeletal muscle	Muscle cells in culture
	Advantages
Fiber-type heterogeneity	Fiber-type homogeneity
Large inter-cellular space	Small inter-cellular space
Heterogeneous exposure of all fibers to solutes, gases	Homogeneous exposure, cell monolayer
Initial rates of transport compromised	Initial rates of transport easy to measure
Genetic variability (animal to animal)	Genetic constance
Temporal instability in vitro	Temporal stability
Difficult to assess individual variables in vivo	Easy to assess effect of individual variables
	Caveats
Innervated	Not innervated (but co-cultures possible)
Adult stage	Embryonic or regeneration model

TABLE II. Content of GLUT1 and GLUT4 Glucose Transporters in Subcellular Fractions of L6 Muscle Cells and Rat Skeletal Muscle*

Fraction	GLUT1 pmol/mg protein	GLUT4 pmol/mg protein	GLUT1:GLUT4
L6 crude plasma membranes	1.12	1.07	1:1
L6 intracellular membranes	0.67	1.40	0.5:1
Red muscle plasma membranes	0.47	0.77	0.6:1
Red muscle glucosomes	u.d.[a]	2.43	infinite

*Molar content was calculated from quantitative immunoblotting and measurement of equilibrium binding of cytochalasin B (D-glucose-inhibitable component).
[a]u.d., undetectable.

insulin stimulation appears upon fusion of myoblasts into myotubes [28,29]. Concomitantly, GLUT1 transporter levels decrease and GLUT4 transporters appear during differentiation [28]. The molar content of GLUT1 and GLUT4 in L6 cells membrane fractions is given in Table II.

It is clear that GLUT1 expression is higher in L6 muscle cells than in adult rat skeletal muscle, while GLUT4 expression is lower. In other muscle cell lines such as the mouse skeletal muscle derived C_2C_{12} or the mouse smooth muscle like BC_3H-1, GLUT4 protein is immunologically undetectable [31]. Since these cells are responsive to insulin it has been surmised that GLUT1 is the isoform regulated by the hormone. Indeed, recruitment of GLUT1 proteins to the cell surface was demonstrated in these cells by insulin treatment [31].

The rapid stimulation of glucose transport by insulin in L6 myotubes is about twofold, comparable to that in isolated muscle strips (Fig. 2). Importantly, in L6 cells glucose transport is fully accounted for by a gain in transporters in the plasma membrane, stemming from an intracellular membrane pool likely akin to the glucosomes of skeletal muscle [32].

REGULATORY, ADAPTIVE, AND TOXIC EFFECTS OF GLUCOSE IN MUSCLE

In the physiological steady state, 5 mM glucose bathes the exofacial surface of cells while the intracellular milieu is virtually glucose-free. Elevations in blood glucose to about 8–10 mM occur naturally during the post-prandial period. These changes are transient, subsiding when glucose is removed in an insulin-stimulated fashion by the peripheral tissues. However, in addition to the insulin response, it is possible that glucose transporters may react to the sudden elevation in glucose as part of a physiological regulatory response. Clearly, glucose flux increases by the augmented uptake due to increased mass action of glucose, and in response to insulin by the gain in plasma membrane transporters. The question whether glucose itself may regulate glucose transporter activity or number remains unanswered. In L6 muscle cells in culture we have observed a rapid depression of glucose transport activity (within 30–60 min) upon exposure to high glucose concentrations (the exposure is done prior to measurement of glucose transport activity, which is carried out

in the absence of glucose) [33]. This response precedes the diminution in GLUT1 mRNA or protein and occurs in the absence of net changes in GLUT4 protein or mRNA [33]. Hence, post-translational changes appear to underlie the rapid curb of glucose transport activity in response to short-term glucose pre-incubation. The precise mechanisms involved are not yet defined.

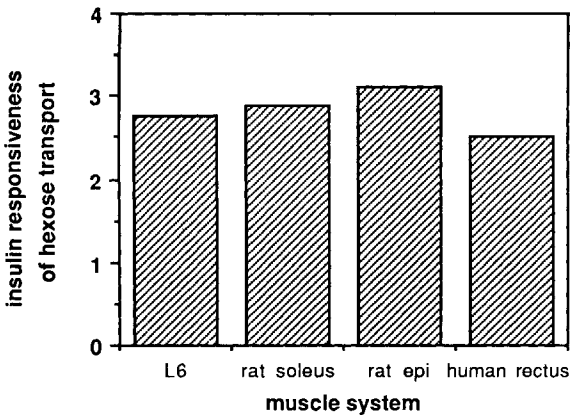

Fig. 2. Comparison of the stimulation of hexose uptake by acute insulin exposure of several in vitro muscle systems. In all cases the hexose was 2-deoxyglucose. Responsiveness is the ratio of transport rates at maximal insulin levels:basal levels. The results in L6 myotubes are adapted from reference 55. The results in the soleus muscle are adapted from reference 56. The results in the epitrochlearis muscle are adapted from reference 57. The results in the rectus abdominis muscle strip are adapted from reference 58.

More sustained elevations in circulating glucose, such as in diabetes (from 8 to 20 mM glucose), may unleash adaptive responses that attempt to prevent glucose overload of the muscle cell. Indeed, after several hours of exposure to high glucose, skeletal muscle shows reduced glucose transport capacity. This phenomenon is also observed in skeletal muscle cells in culture [34,35], and in a variety of experimental muscle systems in which the effect of pre-exposure to glucose on hexose transport capacity was assessed, as summarized in Table III. These adaptive responses may involve decreased transporter synthesis and redistribution of plasma membrane transporters to intracellular sites. These scenarios are supported by observations of reduced GLUT4 mRNA and GLUT4 protein in muscle of diabetic rats [36–38]. We have established a model of hyperglycemic/normoinsulinemic rats (fasting state) in which for the most part of the day the muscles are exposed to normal insulin levels [39]. In these animals, GLUT4 protein in the plasma membrane is reduced, as is the cellular content of GLUT4 mRNA [12], suggesting that glycemia plays an important inhibitory role in muscle GLUT4 biosynthesis.

TABLE III. Effect of Changes in Glucose Availability on Glucose Transport in Muscle

Experimental system	[Glucose]	Effect of high glucose
Whole body diabetic rat[a] with phlorizin	15 mM	Decreased insulin action
	5 mM	Normalized insulin action
Chronic hyperglycemia[b]	9–22 mM	Decreased glucose clearance
Jugular glucose infusion[c]	2.8 M, 72 h	Decreased insulin action
Perfused hindquarter[c,d]	12 mM, 5 h	Decreased insulin action
		Increased muscle basal glucose uptake
Isolated soleus muscle[e]	4–20 mM, 3 h	Reduced glucose transport
Isolated soleus, epitrochlearis[f]	5, 25 mM, 12 h	Increased basal uptake
		Decreased insulin action (soleus)
		Normal insulin action (epitrochelaris)
L8 myocytes[g]	4–20 mM, 3 h	Reduced Vmax of 2dG[j] uptake
L6 myotubes[h]	0–25 mM, 1 h	Reduced 2dG uptake prior to GLUT1 mRNA
		Not mimicked by 2dG, 3OMe
		No effects on GLUT4 mRNA
L6 myotubes[i]	0–25 mM	Reduced 2dG uptake and GLUT1 mRNA
		With insulin:
		Mannose, 2dG,3OMe, glucosamine decreased 2dG transport
		Mannose decreased GLUT1 protein, mRNA

[a]Ref. 39; [b]Ref. 60; [c]Ref. 61; [d]Ref. 62; [e]Refs. 34, 63; [f]Ref. 64; [g]Refs. 63, 65; [h]Refs. 6, 33, 35; [i]Ref. 66.

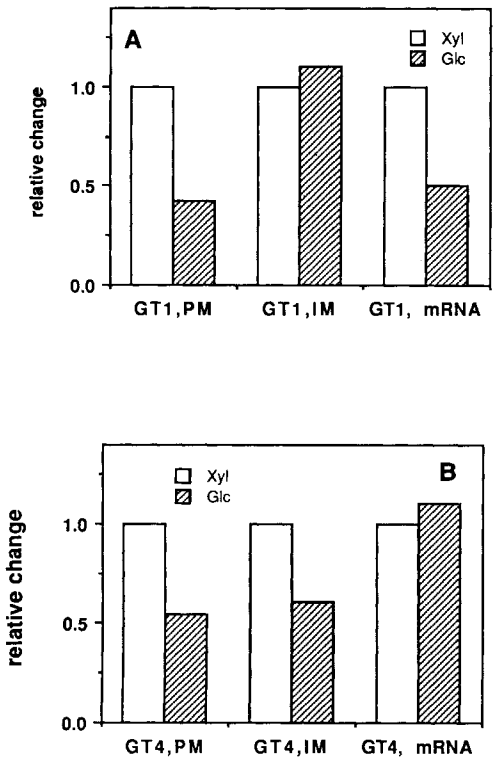

Fig. 3. Effect of chronic (24 h) glucose on glucose transporters and their mRNA in L6 myotubes. **A:** GLUT1. **B:** GLUT4. Open bars are cells incubated in the absence of glucose (25 mM xylose substituted). Cross-hatched bars are cells incubated in the presence of 25 mM glucose. For experimental details see reference 33.

In cells in culture, exposure to high glucose was studied to investigate the independent effects of this variable on glucose transporters. L6 muscle cells exposed to high glucose concentrations showed diminished levels of GLUT1 protein and mRNA, as well as a small increase in transporters in the intracellular membranes [35,40] (Fig. 3A). This has led us to suggest that high glucose induces internalization of GLUT1 transporters, in addition to reducing their synthesis. Glucose also diminishes GLUT4 transporter content in both intracellular membranes and plasma membranes (Fig. 3B), although GLUT4 mRNA is unaffected. We thus propose that glucose may increase GLUT4 degradation [35,40]. These observations suggest that sustained exposure to high glucose causes both biosynthetical and post-translational events leading to reduced transporter number at the cell surface and in total cellular content.

If glucose (or other monosaccharide) overload occurs in spite of the adaptive responses, toxic effects may arise. Indeed, Katz et al. [41] reported very recently that a 2 h glucose infusion in man (with insulin changes prevented by somatostatin) increased markedly the levels of free glucose in skeletal muscle.

PUTATIVE SIGNALS MEDIATING GLUCOSE EFFECTS

Allosteric Control Through Glucose Metabolites

It is likely that the effects of glucose, be they regulatory, adaptive, or toxic, are mediated by the presence of extracellular glucose, or of intracellular sugar metabolites. That extracellular glucose per se, or occupancy of the transporters by the sugar, does not play a crucial role in the regulatory and adaptive responses to glucose is demonstrated by a lack of effect of 3-O-methylglucose on glucose transport activity or transporter levels [33]. A hexose that binds to the glucose transporter, 3-O-methylgucose is transported into the cell, but is not further metabolized. The lack of rapid glucose curb or of delayed down-regulation in glucose transporter number or mRNA suggests that occupancy of the transporter is insufficient to bring about the depression of the glucose transport system caused by glucose.

The possibility that intracellular metabolites of glucose can modulate glucose uptake was strongly suggested by the pioneering work of the groups of Kalckar and Cerasi and later by the work of Marshall and co-workers. Kalckar et al. [42] found that glucose-6-P or metabolites subsequent to its formation contribute to the glucose curb of glucose transport in fibroblasts, since mutants lacking phosphoglucose isomerase do not show glucose curb. Cerasi et al. [43] recently reported that 2-deoxyglucose (a hexose that is transported and phosphorylated but not further metabolized) curbs glucose transport in L8 myocytes (measured after 2–3 h) by altering glucose transporter distribution. This effect was proposed to be mediated by accumulation of 2-deoxyglucose-6-P, mimicking accumulation of glucose-6-P by glucose incubation. Marshall and colleagues [44–46] found that glucose did not curb basal glucose transport in adipocytes, as it does in muscle. However, influx of glucose inhibited markedly the insulin-stimulation of glucose uptake. They recently showed that this desensitization occurs in about 2 h and is likely mediated by accumulation of a glucose metabolite past glucose-6-P, likely glucosamine-6-P or a metabolite thereof. For the regulatory phase ($t_{1/2}$ 2 h) they propose participation of rapid

synthesis of glutamine: fructose-6-P amidotransferase [46], the enzyme synthesizing glucosamine-6-P, and for the adaptive response (24 h) they propose changes in glucose transporter subcellular distribution [44]. Whether these mechanisms also operate in muscle remains to be determined.

The corollary of all these studies is that small glucose metabolites may effect changes in both gene expression of specific proteins and in subcellular distribution of glucose transporters. These actions may participate in insulin desensitization. The concept that glucose or its metabolites have allosteric effects on proteins that ultimately regulate glucose utilization is not altogether new: key enzymes in carbohydrate metabolism are allosterically regulated by glucose metabolites, notably the glycogen synthase complex by glucose-6-P, pyruvate dehydrogenase by acetyl coenzyme A, phosphofructokinase by glucose-1,6-P_2, etc. Similar effects could potentially occur in muscle and participate in the glucose-dependent inhibition of basal glucose transport.

Covalent Non-Enzymatic Reaction of Hexoses With Proteins (Glycation)

Glucose is known to form chemically reversible early glycosylation products with proteins (Schiff bases) at a rate proportional to the glucose concentrations. These Schiff bases then rearrange to form the more stable Amadori-type early glycosylation products (glycated products). The amount of glycated products increases when blood glucose levels are high. Equilibrium levels of the Schiff base and Amadori products are reached in hours and weeks, respectively [47]. We hypothesize that Schiff base formation and glycation of proteins may participate in adaptive and/or toxic responses to glucose exposure. Indeed, the activity of many enzymes is affected upon glycation in vivo or in vitro [see references in 48]. Significant glycation is not circumscribed to proteins that are very long lived, as plasma membrane proteins of liver and kidney from diabetic rats are glycated relative to controls [49]. It is not known whether there is glucose toxicity affecting the glucose transporters directly. However, we have observed that GLUT1 transporters in human erythrocytes are targets for non-enzymatic glycosylation (glycation), and that this reaction depresses their ability to bind the ligand cytochalasin B [48]. In human erythrocytes and cells that gain or equilibrate glucose, glycation can occur at the cell surface as well as on intracellular products (e.g., glycation of hemoglobin to produce hemoglobin A1c). It is not known whether glycation of glucose transporters also occurs in tissues that have higher protein turnover rate than human erythrocytes, but it is possible that in these tissues the elevation in certain monosaccharides that are much more potent glycators than glucose may exert a toxic effect on cellular proteins including glucose transporters. Indeed, glycation can be caused by other sugars such as glucose-6-P [50], fructose [51], mannose [52], and glucosamine [53]. All are more effective glycating agents than glucose itself, both in the extent of glycation achieved and in the velocity of the reaction. Elevations in the concentrations of glucose-6-P, fructose, and glucosamine are detected in tissues of diabetic animals [54], to levels compatible with effective glycation in vivo. Hence, the possibility can be entertained that glycation of glucose transporters, or of proteins regulating the transport system (its intrinsic activity, subcellular localization, or gene expression), may result in adaptive or toxic control of glucose transport.

CONCLUSIONS

Skeletal muscle cells express both GLUT1 and GLUT4 transporters. In rat skeletal muscle, the latter is translocated from a distinct intracellular organelle(s) to the plasma membrane, in response to acute stimulations by insulin, exercise, or hypoxia. Muscle cells in culture are a system amenable to analysis of a single variable over a prolonged period of time. In L6 muscle cells, prolonged exposure to insulin or hypoxia elevates glucose transport through increased synthesis of the GLUT1 isoform. Exposure to elevated glucose in the circulation decreases the amount of plasma membrane GLUT4 transporters in rat muscle, whereas exposure to elevated glucose in the medium reduces plasma membrane levels of GLUT1 transporters in L6 myotubes. The following questions are open for future investigation:

What is the exact intracellular identity of the isolated glucosomes? Are there distinct pools of transporters recruited by insulin and by exercise/hypoxia?

Is the regulation of GLUT1 in L6 cells a reflection of similar changes in adult rat skeletal muscle, or is GLUT1 regulated in a form akin to GLUT4 in muscle, the choice of isoform being

dependent on its relative abundance in each system?

How can glucose affect subcellular distribution and gene expression of transporters? Which are the metabolites that could participate in these pathways?

A combined cellular and molecular approach will be required to answer these questions.

ACKNOWLEDGMENTS

The authors are endebted to all laboratory members and colleagues who participated in the experiments summarized in this article: Dr. Nava Bashan, Philip J. Bilan, Elena Burdett, Dimitrios Dimitrakoudis, Dr. André Douen, Dr. John O. Holloszy, Ulla-M. Koivisto, Loretta Lam, Dr. Yasuhide Mitsumoto, Dr. Jeffrey E. Pessin, Toolsie Ramlal, Vivian Sarabia, and Dr. Mladen Vranic. The work discussed was funded in part by grants to A.K. from the Medical Research Council of Canada and the Muscular Dystrophy Association of Canada.

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