Dissociation of GLUT4 Translocation and Insulin-stimulated Glucose Transport in Transgenic Mice Overexpressing GLUT1 in Skeletal Muscle*

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Overexpression of the human GLUT1 glucose transporter protein in skeletal muscle of transgenic mice results in large increases in basal glucose transport and metabolism, but impaired stimulation of glucose transport by insulin, contractions, or hypoxia (Gulve, E. A., Ren, J.-M., Marshall, B. A., Gao, J., Hansen, P. A., Holloszy, J. O., and Mueckler, M. (1994) J. Biol. Chem. 269, 18366–18370). This study examined the relationship between glucose transport and cell-surface glucose transporter content in isolated skeletal muscle from wild-type and GLUT1-overexpressing mice using 2-deoxyglucose, 3-O-methylglucose, and the 2-N-[4-(1-aziz,-2,2,2-trifluoroethyl)benzoyl]-1,3-bis(D-mannos-4-ol)-2-propylamine exofacial photolabeling technique. Insulin (2 milliunits/ml) stimulated a 3-fold increase in 2-deoxyglucose uptake in extensor digitorum longus muscles of control mice (0.47 ± 0.07 μmol/ml/20 min) in insulin-stimulated muscle; mean ± S.E.). Insulin failed to increase 2-deoxyglucose uptake above basal rates in muscles overexpressing GLUT1 (4.00 ± 0.40 μmol/ml/20 min in basal muscle versus 3.96 ± 0.37 μmol/ml/20 min in insulin-stimulated muscle). A similar lack of insulin stimulation in muscles overexpressing GLUT1 was observed using 3-O-methylglucose. However, the magnitude of the insulin-stimulated increase in cell-surface GLUT4 photolabeling was nearly identical (3-fold) in wild-type and GLUT1-overexpressing muscles. This apparently normal insulin-stimulated translocation of GLUT4 in GLUT1-overexpressing muscle was confirmed by immunoelectron microscopy. Our findings suggest that GLUT4 activity at the plasma membrane can be dissociated from the plasma membrane content of GLUT4 molecules and thus suggest that the intrinsic activity of GLUT4 is subject to regulation.

Glucose transport into skeletal muscle occurs by a facilitated diffusion process mediated by two isoforms of the glucose transporter protein, GLUT1 (1, 2) and GLUT4 (3–7). GLUT1 is targeted predominantly and constitutively to the sarcolemma and is thought to mediate basal glucose transport (8–10). In the basal state, the GLUT4 isoform is targeted to intracellular membrane compartments in the vicinity of the sarcolemma and T-tubules (11). Stimulation by insulin (12–14), muscle contractions (15–17), or hypoxia (18, 19) results in an increase in the amount of GLUT4 at the cell surface, with a concomitant increase in glucose transport activity.

A line of transgenic mice that overexpress human GLUT1 in skeletal muscle has been described (8–10). These mice exhibit reduced fasting and fed plasma glucose concentrations in the absence of altered levels of pancreatic hormones and a marked increase in the rate of glucose clearance following an oral glucose load (8). The enhanced glucose clearance can be explained by a large increase in basal glucose transport activity that is accompanied by an increase in muscle glucose metabolism (9). In addition, skeletal muscles from these transgenic mice exhibit a loss of insulin-stimulated glucose transport activity (8–10). This defect is not a generalized resistance to insulin action, as stimulation of System A amino acid transport activity by insulin in muscles overexpressing GLUT1 is normal (10). In addition, the impaired activation of glucose transport in GLUT1-overexpressing muscle is not limited to the effects of insulin, as the response to hypoxia and contractions, two stimuli that activate muscle glucose transport by an insulin-independent mechanism (17, 20, 21), is also defective (10). The muscles of the GLUT1 transgenic mice express a normal complement of total cellular GLUT4 protein, but the functional status of the GLUT4 has not been determined. GLUT4 translocation is a common step in the activation of glucose transport by all these stimuli (12–19). It therefore seemed likely that a defect in translocation of GLUT4 could be the mechanism responsible for the impaired stimulation of glucose transport in GLUT1-overexpressing muscle. The purpose of this study was to examine glucose transport and glucose transporter localization under basal and insulin-stimulated conditions in skeletal muscle to determine if GLUT4 translocation is impaired in the GLUT1 transgenic mice. We found that GLUT4 translocates to plasma membrane domains in a normal fashion in muscles of the GLUT1 transgenic mice, despite the absence of any stimulation of glucose transport by insulin. These data indicate that GLUT4 activity at the plasma membrane can be dissociated from the plasma membrane content of GLUT4 molecules and thus suggest that the intrinsic activity of GLUT4 is subject to regulation.

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1 P. A. Hansen, B. A. Marshall, M. Mueckler, and J. O. Holloszy, unpublished observations.
EXPERIMENTAL PROCEDURES

Materials—Purified porcine insulin (Iletin II) was purchased from Lilly. 2-Deoxy-D-[1,2-3H]glucose was purchased from American Radiochemicals. 3-O-[U-14C]Methyl-t-glucose and [U-14C]mannitol were obtained from NEF Life Science Products. Reagents for enhanced chemiluminescence were purchased from Amersham Pharmacia BioTech. The exofacial labeling buffer 2-N-[4-(1-azido-2,2-trifluoroethyl)benzoyl]-1,3-bis[(1-carboxyethyl)amide]EDMA (ATB-[2-3H]BMPA), 2-DG, 2-deoxy-D-glucose; 3-MG, 3-O-methyl-t-glucose; PBS, phosphate-buffered saline.

Glucose transport activity of rat GLUT4 and a rabbit polyclonal antiserum (F350) against a synthetic peptide corresponding to the COOH-terminal 16 amino acids of rat GLUT4 and a rabbit polyclonal antiserum (F350) raised against a synthetic peptide corresponding to the COOH-terminal 16 amino acids of human GLUT1 (22). Both antibodies were purified using immunoaffinity columns (23). The primary antibodies used for the immunoprecipitation of ATB-[2-3H]BMPA-labeled glucose transporters were rabbit polyclonal antisera against synthetic peptides corresponding to the COOH-terminal 16 amino acid residues of rat GLUT4 (G4 829) and human GLUT1 (G1 674A), respectively.

Preparation of Transgenic Mice—The construction of transgenic mice overexpressing the human GLUT1 glucose transporter was described previously (8). The minigenic in this construct contains a 2.47-kilobase polyadenylation signal from the human G6PD gene and a 2.8-kilobase antisense fragment of the human GLUT4 (F350) raising against a synthetic peptide corresponding to the COOH-terminal 16 amino acids of rat GLUT4 and a rabbit polyclonal antiserum (F350) raised against a synthetic peptide corresponding to the COOH-terminal 16 amino acids of human GLUT1 (22). Both antibodies were purified using immunoaffinity columns (23). The primary antibodies used for the immunoprecipitation of ATB-[2-3H]BMPA-labeled glucose transporters were rabbit polyclonal antisera against synthetic peptides corresponding to the COOH-terminal 16 amino acid residues of rat GLUT4 (G4 829) and human GLUT1 (G1 674A), respectively.

Animal Care and Tissue Preparation—Animals were housed in a room maintained at 23 °C with a fixed 12-h light/dark cycle (lights on from 6 a.m. to 6 p.m.) and given free access to Purina chow and water. All sugar transport and photolabeling experiments were performed using fed mice. Animals were anesthetized by an intraperitoneal injection of pentobarbital sodium (5 mg/100 g of body weight). The right soleus muscle was removed (basal) and fixed by incubation in 3% paraformaldehyde and 0.5% glutaraldehyde in PBS for 2 h. Mice were then given glucose (2 g/kg of body weight) and insulin (6 units) by intraperitoneal injection. After 30 min, the left soleus muscle (insulin) was dissected and fixed as described above.

After fixation, the tissues were rinsed with PBS and distilled water and stained en bloc with 0.5% uranyl acetate for 20 min at 4 °C. The tissues were dehydrated through graded ethanol: 30% for 10 min at 4 °C, 50% for 1 h at 20 °C, 70% for 1 h, 95% for 1 h, and 100% for 2 h with three changes, all at –45 °C. The tissues were embedded in Lowicryl HM-20 and polymerized under 360-nm UV light for 1 day at 45 °C, 1 day at –35 °C, and 1 day at room temperature under sunlight.

The cut thin sections were picked on nickel grids coated with polyvinyl formal film. The nonspecific binding sites were blocked by incubating sections with 5% goat serum for 30 min and 2% gelatin for 10 min. Sections were then incubated with PBS and then incubated with colloidal gold-conjugated goat anti-rabbit IgG for 30 min at room temperature. To determine levels of nonspecific labeling, preimmune IgG was substituted for the primary antibody, or the primary antibody was saturated with a 10-fold molar excess of the peptide used to raise the antibody. The grids were then stained with 4% uranyl acetate for 20 min and Reynolds’s lead solution for 50 s and examined in a Zeiss 902 electron microscope.

Immunogold labeling density was determined in micrographs of longitudinal sections, with each sarcomere defined as the basic quantification unit. Quantification of labeling in the region of the sarcomemna was based on the number of gold particles observed per 2-μm length. Nonspecific background labeling either using preimmune IgG or by competition with excess immunpeptide was extremely low under the optimal conditions described above (<1% of the specific labeling for either GLUT1 or GLUT4).

RESULTS

Glucose Transport Activity—Rates of basal and insulin-stimulated 2-DG uptake in EDL muscles isolated from wild-type and GLUT1 transgenic mice are shown in Fig. 1. In agreement with previous findings (8–10), GLUT1 overexpression increased basal glucose transport activity ~8-fold above rates measured in EDL muscles from nontransgenic littermates (0.5 ± 0.1 μmol of 2-DG/ml/20 min in wild-type muscle versus 4.0 ± 0.4 μmol of 2-DG/ml/20 min in GLUT1 transgenic muscle). Insulin caused a 2.7-fold increase in 2-DG uptake in EDL muscles from nontransgenic mice; however, 2-DG uptake was not increased in response to insulin in EDL muscles overexpressing GLUT1 (4.0 ± 0.4 μmol of 2-DG/ml/20 min in basal muscle versus 3.9 ± 0.4 μmol of 2-DG/ml/20 min in insulin-stimulated muscle).

We have previously demonstrated in GLUT1-overexpressing muscle that (a) 2-DG uptake is linear for at least 30 min under the experimental conditions used in this experiment (10), and (b) when the total intracellular concentration of 2-DG is ~20 mM, <2% of the total intracellular 2-DG is present in the

2 The abbreviations used are: ATB-[2-3H]BMPA, 2-N-[4-(1-azido-2,2,2-trifluoroethyl)benzoyl]-1,3-bis[(1-carboxyethyl)amide]EDMA; KHB, Krebs-Henseleit bicarbonate buffer; 2-DG, 2-deoxy-D-glucose; 3-MG, 3-O-methyl-D-glucose; PBS, phosphate-buffered saline.
GLUT1 labeling in the basal state was slight and glucose transport activity using 1 mM 2-[3H]DG as described under "Experimental Procedures." When insulin was present during the 35 °C incubation, it was also included at the same concentration in the wash step and transport assay. Values are means ± S.E. for 9–13 muscles/group, expressed as μmol of 2-DG/ml of intracellular water/20 min. Open bars, basal; closed bars, 2 milliunits/ml insulin.

unphosphorylated form (26). Thus, even in the muscles of the GLUT1 transgenic mice, the rate of 2-DG uptake should accurately reflect glucose transport activity (27). However, to confirm our findings with 2-DG, additional skeletal muscle glucose transport activity measurements were performed using the nonmetabolizable glucose analog 3-MG. As shown in Fig. 2, basal rates of 3-MG transport in the GLUT1 transgenic animals were increased ~7-fold in the epitrochlearis muscle and ~9-fold in EDL muscle. Insulin increased 3-MG transport 2.5–2.6-fold in the epitrochlearis and EDL muscles from nontransgenic mice; the 2.6-fold increase in 3-MG transport in the EDL muscle is in close agreement with the 2.7-fold increase measured using 2-DG in this same muscle (see Fig. 1). Insulin had no effect on the rate of 3-MG transport in either the epitrochlearis muscle or the EDL from the GLUT1 transgenic mice. Intracellular 3-MG concentrations under basal and insulin-stimulated conditions were ~2.0–2.3 μmol/ml of intracellular water in both muscle types following a 12-min incubation with 8 mM 3-MG. Since the rate of 3-MG uptake ordinarily becomes nonlinear when the intracellular concentration of 3-MG attains ~25% of the extracellular concentration (25), it was possible that the linear phase of 3-MG uptake was exceeded during the 12-min assay period. Therefore, we performed an additional experiment in which basal and insulin-stimulated 3-MG transports were measured in GLUT1-overexpressing muscles using a shorter measurement period (4 min). Under these conditions, the intracellular 3-MG concentration remained well below 25% of the equilibrium value. As shown in Fig. 3, initial rates of 3-MG transport following treatment with insulin were not significantly different from basal rates of transport in either the epitrochlearis or EDL muscles overexpressing GLUT1.

Glucose Transporter Translocation—Because insulin-stimulated glucose transport in skeletal muscle is mediated by GLUT4 (26, 28), and there is a normal level of total GLUT4 protein expressed in the muscles of the GLUT1 transgenic mice, we examined the possibility that GLUT4 translocation in insulin-stimulated muscles is defective in the GLUT1 mouse. To test this hypothesis, we assessed basal and insulin-stimulated cell-surface glucose transporter content in EDL muscles from the GLUT1-overexpressing mice and nontransgenic littermates using the exofacial photoaffinity label ATB-[3H]BMPA. GLUT1 labeling in the basal state was ~40-fold higher in EDL muscles from transgenic animals compared with control littermates (Fig. 4, upper panel). GLUT1 labeling was not increased by insulin in muscles from either GLUT1 transgenic mice or nontransgenic controls. Insulin stimulated a 3.2-fold increase in GLUT4 labeling in EDL muscles from the wild-type mice (Fig. 4, lower panel). Surprisingly, an insulin-stimulated increase in labeled GLUT4 was also observed in muscles from the GLUT1-overexpressing mice. Absolute labeling (dpm/g (wet weight)) following insulin treatment was slightly higher in muscles from the transgenic mice compared with the wild-type mice, but because GLUT4 labeling in the basal state was also slightly higher in muscles from these mice, the fold increase in

![Fig. 1. Basal and insulin-stimulated 2-deoxyglucose uptake in isolated EDL muscles from GLUT1 transgenic mice and nontransgenic littermates.](image1)

![Fig. 2. Basal and insulin-stimulated 3-methylglucose uptake in isolated skeletal muscles from GLUT1 transgenic mice and nontransgenic littermates.](image2)

![Fig. 3. Basal and insulin-stimulated 3-methylglucose uptake in isolated skeletal muscles from GLUT1 transgenic mice.](image3)
GLUT4 Translocation in GLUT1 Transgenic Mice

Mice were anesthetized with sodium pentobarbital. The right soleus muscle was removed (basal) and fixed, embedded in Lowicryl HM-20, and processed for immunoelectron microscopy as described under "Experimental Procedures." After resection of the right soleus muscle, mice were given glucose and insulin by intraperitoneal injection. After 30 min, the left soleus muscle (insulin) was removed and treated as described above. Four independent experiments were performed on the GLUT1 transgenic mice and two were performed on the wild-type mice. The numbers represent the mean ± S.E. For quantification of total sarcomere GLUT4, gold particles were counted in longitudinal sections encompassing 1 × 2 μm (roughly equivalent to the size of a sarcomere) and containing two T-tubule profiles. Between 30 and 50 total sarcomeric units were quantitated per experiment. For quantification of GLUT4 on T-tubule membranes, the number of gold particles were counted per 2-μm length of membrane. Note that gold particles appear on both cytoplasmic faces of each T-tubule membrane observed in the longitudinal section. For quantification of GLUT4 on the sarcolemma, the number of gold particles are expressed per 2-μm length of membrane. Between 100 and 120 μm of sarcolemma were quantitated per experiment. For quantification of GLUT4 in vesicles beneath the sarcolemma, gold particles are expressed per 2-μm length of sarcolemma and extending in depth down to the first myofibril. Gold particles within ~15 nm of a bilayer structure were considered to be in the membrane.

**Table I**

| Group                  | No. of animals used | Condition | Sarcolemma (per 2-μm length) | Sarcolemma (1 × 2 μm) |
|------------------------|---------------------|-----------|------------------------------|-----------------------|
|                        |                     | PM        | Space under PM               | SR                    | TC | TT |
| Wild-type              | 2                   | Basal     | 1 ± 0.2                      | 3 ± 0.4               | 8 ± 1.8 | 10 ± 2.3 | 5 ± 0.9 |
|                        |                     | Insulin   | 2 ± 0.3                      | 2 ± 0.5               | 7 ± 1.5 | 14 ± 3.7 | 8 ± 1.7 |
| GLUT1 transgenic       | 4                   | Basal     | 2 ± 0.2                      | 4 ± 1.0               | 10 ± 1.9 | 10 ± 2.0 | 6 ± 0.7 |
|                        |                     | Insulin   | 4 ± 0.8<sup>a</sup>          | 4 ± 0.6               | 10 ± 1.4 | 17 ± 3.2 | 13 ± 2.1<sup>b</sup> |

<sup>a</sup> PM, plasma membrane; SR, sarcoplasmic reticulum; TC, terminal cisternae; TT, T-tubule membrane.
<sup>b</sup> p < 0.05 versus GLUT1 transgenic basal.
<sup>c</sup> p < 0.01 versus GLUT1 transgenic basal.

*Immunoelectron Microscopy*—A second experimental approach was used to assess the subcellular distribution of GLUT4 following insulin treatment of muscle from wild-type and GLUT1-overexpressing mice. Ultrathin sections prepared from soleus muscles isolated prior to (basal) or 30 min following intraperitoneal administration of insulin were stained with an antibody specific for the COOH terminus of GLUT4 followed by a 12-nm colloidal gold-conjugated secondary antibody. The distribution of GLUT4 protein in the muscle of the wild-type mice is nearly identical to that reported previously in skeletal muscle of the GLUT4-overexpressing mice (11). In wild-type muscle, most of the GLUT4 labeling in the basal state was located in the vicinity of the muscle triads, especially in the terminal cisternae of the sarcoplasmic reticulum (Table I). Lesser labeling was observed in vesicles beneath the sarcolemma. Following insulin treatment, the number of gold particles at the cell surface in wild-type muscle was nearly doubled; an increase in GLUT4 labeling was observed both in the sarcolemma (2-fold) and in the T-tubule membranes (~60%) (Table I). The distribution of GLUT4 in unstimulated muscle overexpressing the GLUT1 isof orm did not appear different from that seen in wild-type muscle. Insulin-stimulated changes in the GLUT4 labeling pattern in muscles overexpressing GLUT1 were nearly identical to those seen in wild-type muscle. GLUT4 labeling was increased ~2-fold in both the sarcolemma (Fig. 5 and Table I) and T-tubule membranes (Fig. 6 and Table I). Thus, the results of the immunogold labeling studies are consistent with the findings of the ATB-[3H]BMPA labeling experiments, i.e. the magnitude of the insulin-stimulated increase in GLUT4 at the cell surface in muscle overexpressing the GLUT1 isof orm was not different from that observed in wild-type muscle.

**DISCUSSION**

We have confirmed our previously published observation that insulin does not increase 2-DG uptake above basal rates in muscles overexpressing GLUT1 (8, 10). These measurements were made under experimental conditions where 2-DG uptake normally reflects glucose transport activity accurately (27). Experiments performed with the nonmetabolizable glucose analog 3-MG provide further support for our interpretation that the defect in insulin action in GLUT1-overexpressing muscle lies in the transport step (Ref. 8 and this study) and not in the subsequent phosphorylation of glucose. However, the high rates of glucose transport in the muscles overexpressing GLUT1 complicate the measurement of transport with 3-MG; the measurement period must be sufficiently long to allow equilibration of the glucose analog in the muscle extracellular space, but short enough to ensure that 3-MG efflux is negligible. In this study, 3-MG transport was measured at a relatively low temperature (20 °C) over a short period of time (4 min) to...
ensure that the linear phase of 3-MG uptake was not exceeded. In addition, identical transport measurements were performed using the epitrochlearis muscle, which is an extremely thin muscle of the forelimb. The structure of the epitrochlearis muscle allows very rapid equilibration of the extracellular space with the incubation medium, which, in turn, shortens the lag period before the rate of 3-MG uptake becomes linear.

In both the EDL and epitrochlearis muscles, insulin failed to stimulate 3-MG transport above basal rates in the GLUT1 transgenic mice.

The impaired stimulation of glucose transport in GLUT1-overexpressing muscle is not limited to the effects of insulin, as the responses to hypoxia, contractions, and insulin-like growth factor-1 are also defective (10). As an increase in cell-surface GLUT4 transporters is a common step in the activation of glucose transport by all these stimuli, it seemed reasonable to hypothesize that a defect in GLUT4 translocation is involved in the impaired stimulation of glucose transport in GLUT1-overexpressing muscle. However, using two independent methods, our findings indicate that the inability of insulin to stimulate transport above basal levels in GLUT1-overexpressing muscle is not mediated by decreased GLUT4 translocation. Both the ATB-$^3$H]BMPA labeling technique and the immunoelectron microscopy analysis indicate that the magnitude of the insulin-stimulated increase in cell-surface GLUT4 glucose transporters in muscle overexpressing the GLUT1 transporter is at least as great as that in wild-type muscle.

Skeletal muscle has two distinct plasma membrane domains, the sarcolemma and the T-tubules. Although insulin increases cell-surface GLUT4 in both domains (11, 30, 31), recent evidence suggests that as much as 90% of GLUT4-mediated glucose transport in insulin-stimulated skeletal muscle may take place across the T-tubule membranes (11). To the best of our knowledge, only one published study has examined GLUT4 translocation to T-tubules using the ATB-$^3$H]BMPA labeling technique. Dudek et al. (32) used autoradiography to show that the majority of ATB-$^3$H]BMPA-labeled GLUT4 in insulin-treated rat soleus muscle is associated with T-tubules. Cell-surface labeling of the T-tubules was increased ~30–75% following insulin treatment (32). Although this study demonstrates that skeletal muscle T-tubule transporters can be labeled with ATB-$^3$H]BMPA, it does not speak to the relative efficiency of labeling of transporters in the sarcolemma versus T-tubule membranes with this compound. It is possible that in GLUT1-overexpressing muscle, insulin-stimulated GLUT4 translocation to the sarcolemma is normal, whereas translocation to the T-tubules is impaired. If this were true and if ATB-$^3$H]BMPA labeled T-tubule GLUT4 less efficiently than sarcolemmal GLUT4, then it is possible that a defect in insulin-stimulated GLUT4 translocation to this membrane domain (which would have a large effect on insulin-stimulated transport) could be underestimated or missed altogether using this technique. This possibility seems unlikely, however, in light of the immunogold labeling experiments, which showed changes in plasma membrane and T-tubule GLUT4 labeling in GLUT1-overexpressing muscle that were qualitatively very similar to those seen in wild-type muscle. Thus, both the pattern and the magnitude of the GLUT4 redistribution to the cell surface in response to insulin stimulation appear normal in the GLUT1 transgenic mice.

Although the absolute level of labeling is lower, the distribution of GLUT4 protein in muscle of the wild-type mice was very similar to that reported previously in skeletal muscle of GLUT4-overexpressing mice (11). In both wild-type and GLUT4-overexpressing muscles, most of the GLUT4 labeling in the basal state was located in the vicinity of the muscle triads, with lesser labeling in vesicles beneath the sarcolemma. Following insulin treatment, the number of gold particles at the cell surface in wild-type muscle was increased ~2-fold; an increase in GLUT4 labeling was observed both in the sarcolemma and in the T-tubule membranes. These changes are nearly identical, both qualitatively and quantitatively, to those observed in the GLUT4-overexpressing mice, in which labeling in both the sarcolemma and T-tubules was increased ~2-fold.
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following in vivo insulin administration (11). Thus, the similarities between the previously reported findings in the GLUT4-overexpressing mice (11) and the wild-type mice in this study demonstrate that the targeting and trafficking of GLUT4 in the GLUT4 transgenic mice are normal. This is the expected result, as the muscles of the GLUT4-overexpressing mice exhibit enhanced insulin-stimulated glucose transport and glucose metabolism (24); these functional changes could only occur if the overexpressed GLUT4 transporters underwent normal trafficking in response to insulin.

The most plausible explanation for the loss of insulin-stimulated glucose transport activity despite apparently normal GLUT4 translocation in GLUT1 transgenic muscle is a reduction in the intrinsic activity of GLUT4 at the cell surface. Evidence that it is possible to modulate the intrinsic catalytic activity of individual transporter molecules includes studies of glucose transport activation by inhibitors of protein synthesis in 3T3-L1 adipocytes (33, 34) and the effects of adenosine (stimulatory) and isoproterenol (inhibitory) on insulin-stimulated glucose transport in isolated rat adipocytes (35). These perturbations alter both glucose transport activity and ATB-[3H]BMPA labeling without changing the subcellular distribution of the transporters as assessed by Western blot analysis of plasma membrane fractions (34, 35). The mechanism by which these modulations occur appears to involve a reversible occlusion or disruption of the exofacial sugar-binding sites on the glucose transporter proteins (35). While the results of this study are consistent with a decrease in the intrinsic activity of GLUT4 in skeletal muscle of the transgenic mice, our findings are unique in that this decrease is not associated with reduced ATB-[3H]BMPA labeling, suggesting no disruption of the exofacial sugar-binding site on the GLUT4 protein.

Basal glucose transport activity in EDL muscles isolated from GLUT1 transgenic mice was increased ~7.5–9.0-fold, whereas cell-surface GLUT1 in these muscles was increased ~40-fold. These findings suggest that, like GLUT4, the GLUT1 transporter is present in the plasma membrane in the correct orientation to bind ATB-[3H]BMPA, but is catalytically less active in the muscles of the GLUT1-overexpressing mice. A similar phenomenon has been demonstrated in Xenopus oocytes; the relationship between GLUT1 mRNA injected and the amount of GLUT1 protein in oocyte plasma membranes was linear, but glucose transport activity expressed as a function of GLUT1 protein in the membrane reached a plateau, suggesting that there is a saturation point for the amount of functional transporter that can be accommodated at the cell surface (36). It was proposed that this functional saturation was the result of 1) the accumulation of some totally nonfunctional transporter molecules in the plasma membrane or 2) a reduced intrinsic activity of all of the transporter molecules in the plasma membrane (36). All of these observations are consistent with the hypothesis that the activity of the transporter at the plasma membrane can be modulated under various conditions. The mechanism(s) of this modulation remains unknown.

Increased flux of glucose via the hexosamine biosynthetic pathway has been implicated in glucose-induced insulin resistance of glucose transport (37, 38). The GLUT1-overexpressing muscles are subjected to a chronically increased flux of glucose, and it has been proposed that the insulin resistance of these muscles is due to increased flux of glucose via the hexosamine synthetic pathway. Buse et al. (39) found that, compared with wild-type control littermates, the levels of the major products of the pathway, UDP-N-acetylhexosamines, and the activity of glutamine-fructose-6-phosphate aminotransferase, the rate-limiting enzyme for glucose entry into the pathway, were significantly higher in skeletal muscles isolated from GLUT1-overexpressing mice (the same line of transgenic mice used in this study). The mechanism by which hexosamine products may cause insulin resistance of glucose transport is not known, but two proposed mechanisms include alterations in O- and N-linked glycosylation of glycoproteins and/or glycolipids involved in signaling (39) or a reduction in insulin-stimulated translocation of intracellular GLUT4 to the plasma membrane (40). If, in fact, the hexosamine products are involved in the impaired stimulation of glucose transport observed in this study, our findings would argue against defects in either signaling or GLUT4 translocation as the mechanism responsible, as insulin-stimulated recruitment of GLUT4 to the cell surface was apparently normal in GLUT1 transgenic muscle. Rather, our data suggest that the transporter itself, or some other plasma membrane element that is required for full function of GLUT4 in the membrane, is altered in GLUT1-overexpressing muscle. However, it should be mentioned that the effects of increased glucose flux into muscle in vivo as observed in the GLUT1 transgenic mice may not be identical to the effects induced by incubation of muscle in media containing high concentrations of glucosamine or glucose plus insulin.

An alternative explanation for the loss of stimulated glucose transport activity in skeletal muscles of the GLUT1 transgenic mice is that the high rate of sugar transport generated by the overexpression of GLUT1 at the cell surface creates an unstimulated layer, in which the local concentration of sugar in the compartment just beneath the plasma membrane is higher than the total intracellular concentration. The local accumulation of high sugar concentrations could result in enhanced efflux from the cell, possibly masking the stimulation of transport by insulin. It is currently not possible to test this hypothesis experimentally.

In summary, we have used two independent methods to assess insulin-stimulated GLUT4 translocation in GLUT1-overexpressing skeletal muscles that are resistant to activation of “GLUT4-mediated” glucose transport activity, i.e., transport stimulated by insulin, muscle contractions, or hypoxia. Our findings indicate that the appearance of GLUT4 at the cell surface following insulin treatment is normal in muscles overexpressing GLUT1, suggesting that defective GLUT4 translocation is not the cause of the impaired activation of transport. Instead, our data suggest that the overexpression of GLUT1 in skeletal muscle, which is accompanied by a large (~8-fold) increase in basal glucose transport, results in decreased catalytic activity of GLUT4 transporters in that tissue. Our findings are consistent with previous studies demonstrating that the intrinsic activity of the glucose transporter molecules can be modulated. However, our findings differ from those studies in that there is no disruption of the exofacial sugar-binding site on GLUT4 in transgenic muscle, suggesting that the GLUT1 overexpression per se, or the resulting increase in glucose influx, may bring about a reduction in skeletal muscle GLUT4 transport activity via a novel mechanism.

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