Electrical stimulation induces fiber type-specific translocation of GLUT-4 to T tubules in skeletal muscle

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Department of Physiology and Lipid Research Unit, Laval University Hospital Research Center, Ste-Foy, Québec G1V 4G2; Department of Kinesiology, University of Waterloo, Ontario, Canada N2L 3G1; and Department of Anatomy, Institute of Basic Medical Sciences, University of Oslo, N-0317 Oslo, Norway

Roy, Denis, Erlingur Jónnsson, Arend Bonen, and André Marette. Electrical stimulation induces fiber type-specific translocation of GLUT-4 to T tubules in skeletal muscle. Am. J. Physiol. 273 (Endocrinol. Metab. 36): E688–E694, 1997.—Insulin and contraction independently stimulate glucose transport in skeletal muscle. Whereas insulin activates glucose transport more in muscles composed of type I and IIa fibers, electrical stimulation increases glucose transport at least as much in type IIb fiber-enriched muscles despite the fact that the latter fiber type contains less GLUT-4 glucose transporters. The aim of the present study was to test the hypothesis that a greater GLUT-4 translocation to the cell surface may underlie the higher contraction-stimulated glucose transport in type IIb myofibers. Leg muscles from rats were stimulated in situ at 100 Hz (200 ms) each 2 s via the sciatic nerve over a period of 20 min while the contralateral leg was kept at rest. Muscle 2-[3H]deoxy-o-glucose uptake (2-DG) was measured in separated red gastrocnemius (RG, type I and IIa fibers) and white gastrocnemius (WG, type IIb fibers) muscles. Resting 2-DG uptake was greater in RG than WG. Electrical stimulation increased 2-DG uptake over resting values similarly in WG and RG. Fractions enriched with either plasma membranes, transverse (T) tubules, triads, or GLUT-4-enriched intracellular membranes were isolated from RG and WG using a recently developed subcellular fractionation procedure. Electrical stimulation similarly increased GLUT-4 protein content in plasma membranes of RG and WG, whereas it stimulated GLUT-4 translocation more (~50%) in T tubules of WG than in RG. GLUT-4 content was not changed in triads of both muscle types. The increments in cell surface GLUT-4 protein levels were paralleled by significant reductions in the amount of the transporter in the intracellular membrane fractions of both muscle types (by 60% in RG and 56% in WG). It is concluded that electrically induced contraction stimulates GLUT-4 translocation more in T tubules of WG than RG. The physiological implications of this finding for glucose uptake by contracting RG and WG muscles is discussed.

IT IS WELL ESTABLISHED that the maximal glucose transport capacity of skeletal muscle is strongly correlated to the fiber type composition (1, 17, 20, 30, 34). Thus the stimulatory effects of both insulin and exercise on glucose transport are higher in more oxidative type I and IIa fibers [i.e., soleus, red gastrocnemius (RG) muscles] than in glycolytic type IIb fibers [i.e., white gastrocnemius (WG) muscles] (20, 21, 29, 30, 34). However, a very different picture emerges when the relationship between glucose transport and muscle fiber composition is examined in electrically induced contracting muscles. Indeed, maximal glucose transport rates have been reported to be similar or greater in type IIb fibers than type I or IIa fibers during electrical stimulation (3, 17, 22). This paradox may be explained by a differential recruitment of muscle fibers in exercised vs. electrically stimulated muscles. Thus type I fibers are more active than type IIb fibers during voluntary contraction of moderate intensity (16). It has been shown that the motoneurons innervating fiber types have distinct firing frequencies and impulse patterns in vivo (16). Whereas type I fibers are activated by long trains with low impulse frequency that occupy about 25–35% of the total stimulation time, type II fibers are characterized by shorter trains of a higher frequency that take place for about 5% of the time. In contrast, supramaximal electrical stimulation recruits all fiber types to the same level for any given muscle.

The relationship between skeletal muscle glucose transport and fiber type composition during electrical activation has been recently investigated in more detail (22). It was noted that the fraction of time occupied by stimulation affects glucose transport very differently in muscle composed of various fiber types. Thus glucose transport rates were found to be similar or greater in type IIb-enriched WG muscles compared with muscles mostly composed of type I and IIa fibers (soleus and RG) when the net stimulation time (NST) was less than or equal to 10%. At longer NST (20–50%), however, the type I fiber-enriched muscles showed higher glucose uptake than the WG. Thus these results are in agreement with the postulate that muscle glucose transport is influenced by the NTS, which is known to vary considerably in different muscles in vivo.

Glucose transport is mediated by the GLUT-1 and GLUT-4 glucose transporters in skeletal muscle. The expression of the GLUT-4 isoform is much greater (~15–20 times) than that of GLUT-1 in skeletal muscle (29). Muscle contraction, like insulin, stimulates glucose transport into muscle cells mainly by inducing the translocation of GLUT-4 from an occluded intracellular compartment to the plasma membrane (4, 9, 12, 13) and the transverse tubules (33). On the other hand, GLUT-1 is strictly localized to the plasma membrane, and its concentration is not affected by either insulin or exercise (8, 28, 33). It has been shown that both resting and insulin-stimulated glucose transport are correlated with total GLUT-4 protein content in rat skeletal muscle (29, 30). The expression of GLUT-4 appears to be related to the metabolic nature of the myofiber (oxidative > glycolytic) rather than to their contractile properties (23, 29). However, the correlation between glucose transport and GLUT-4 protein content was not...
apparent in electrically stimulated muscles because the highly responsive WG muscle contains only ~25–50% of the amount of GLUT-4 present in RG (3, 22). In fact, electrical stimulation of glucose transport was even found to vary substantially between red muscles with comparable GLUT-4 content (22). It is, therefore, possible that a differential translocation of GLUT-4 transporters to the cell surface underlies the greater responsiveness of type IIb fibers toward electrical stimulation. This hypothesis was directly addressed in the present study using a recently developed membrane fractionation procedure that allows the isolation of distinct membrane fractions enriched with either cell surface compartments (plasma membranes or transverse tubules) or with GLUT-4-enriched intracellular membranes from rat skeletal muscle.

MATERIALS AND METHODS

Animals and surgery. Overnight-fasted male Wistar rats (250–300 g body wt) were used in these studies. All animals were anesthetized by an intraperitoneal injection of pentobarbital sodium (50 mg/kg body wt). The muscles of the right leg were exposed, and the sciatic nerve was dissected free and cut in the thigh. A small bipolar electrode was placed around the cut nerve that remained connected to the muscle. A catheter (polyethylene tubing, 0.40 mm ID, 0.80 mm OD) for blood sampling was inserted into the right carotid artery, and another catheter was inserted into the right jugular vein for injection of radiolabeled 2-deoxy-o-glucose (2-[^1,2-3H(N)]DG) and mannitol (0-[^1-14C]mannitol; Du Pont-NEN, Boston, MA). Surgery time for these procedures was ~30 min for each animal.

Electrical stimulation protocol. The muscles were stimulated in situ through the sciatic nerve at three times the threshold value, usually a current strength of 0.3–0.4 mA, to ensure that a supramaximal stimulation was obtained. The stimulator (Pulsar 6 bp Stimulator, Frederick Haer, Brunswick, GA) produced bipolar pulses at 100 Hz stimulation frequency. Impulse trains of 200 ms were given to one leg of a rat every 2 s for a period of 20 min while the contralateral leg was kept at rest. This condition could be repeated for contraction duration exceeding 10% of the total experimental time, termed NST. In previous studies it has been shown that this NST causes maximal 2-DG transport rates in both RG and WG muscles (22).

2-DG transport assay. A bolus injection of 15 µCi 2-DG and 50 µCi mannitol in 0.2 ml saline solution was injected 5 min before the start of the electrical stimulation. Blood samples were taken at regular intervals (5, 15, and 25 min) after the bolus injection for determination of plasma glucose and radiolabeled 2-DG and mannitol. Plasma glucose concentration was constant under all experiments. After 20 min of electrical stimulation RG and WG muscles from both the stimulated and unstimulated limbs were removed and immediately frozen in liquid nitrogen and stored at −80°C. Muscle samples (10–20 mg) were dissolved in 1 ml of 0.5 M ammonium hydroxide (Solusen-350, Packard) at room temperature and left to dissolve for 16–18 h. Thereafter, 5 ml of scintillation fluid were added, and the samples were counted in a liquid scintillation counter (Packard). Plasma samples (15 µl) were added to scintillation fluid (5 ml) and counted in the same way. By using the accumulation of 2-DG in muscle and correcting for the extracellular space we calculated an index of the transport rates of 2-[^3H]DG as previously described (22).

Muscle glycogen and lactate determinations. Muscle samples were used for glycogen and lactate determination were freeze-dried and weighed at −25°C. Glycogen was hydrolyzed to glucose in 1 M HCl for 2.5 h at 100°C and then analyzed as glycosyl units according to the method of Lowry and Pasonneau (26). Lactate concentration was measured after extraction in perchloric acid as previously described (26).

Subcellular membrane fractionation. Plasma membranes, transverse tubules, triads, and intracellular membranes were isolated from 5 to 6 g of RG or WG muscles using a new procedure recently developed in our laboratory (8) with minor modifications (7). This subcellular fractionation protocol has been extensively characterized with immunological and enzymatic markers (7, 8, 33). In brief, this technique allows the simultaneous and separated isolation of plasma membrane and transverse tubule vesicles from the same muscle homogenate. In addition, the use of a strong salt treatment (lithium bromide) yields a fraction containing triad membranes (where the transverse tubules appose the sarcoplasmic reticulum cisternae) as well as an intracellular fraction that is greatly enriched with GLUT-4 but totally devoid of plasma membranes and transverse tubule markers. With the use of this fractional protocol, we have previously reported that both insulin and treadmill exercise rapidly activate the translocation of GLUT-4 from the GLUT-4-containing intracellular pool to the plasma membrane and transverse tubules (8, 33).

Membrane protein concentrations were determined by the biocinonic acid assay (Pierce) using bovine serum albumin (BSA) as standard. 5'-Nucleotidase and K^+-stimulated p-nitrophenol phosphatase (KpNPPase) activities were determined as previously described (8).

Western blot analysis. Membranes (10 µg) were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis on 7.5% polyacrylamide gels as described by Laemmli (24) and electrophoretically transferred to polyvinylidene difluoride (PVDF) filter membranes for 2 h. PVDF membranes were then incubated for 1 h at room temperature with buffer 1 (50 mM tris(hydroxymethyl)aminomethane-HCl, pH 7.4, 150 mM NaCl) containing 0.04% NP-40 and 0.02% Tween 20 and 3% BSA (fatty acid-free BSA, Sigma Chemical, St. Louis, MO), followed by overnight incubation at 4°C with primary antibodies as described in the legends to Figs. 1 and 2. The PVDF membranes were then washed for 30 min by following a 1-h incubation with either anti-mouse or anti-rabbit immunoglobulin G conjugated to horseradish peroxidase (Amersham, Oakville, Ontario) in buffer 1 containing 1% BSA. The PVDF membranes were washed for 30 min in buffer 1, and the immunoreactive bands were detected by the enhanced chemiluminescence method. Autoradiographs were analyzed by laser scanning densitometry using the a tabletop Agfa scanner (Arcus II) and quantitated with the National Institutes of Health Image program. Muscle standards were run on every gel for comparison of samples from different immunoblots.

Antibodies. Polyclonal antibodies against the GLUT-1 and GLUT-4 glucose transporters were purchased from East Acres Biologicals (Southbridge, MA). A monoclonal (IIF7) antibody generated against the α2- subunit of the dihydropyridine receptor (DHPr) was kindly provided by Dr. K. Campbell (University of Iowa). The monoclonal antibody Mck-1 against the α2- subunit of the Na-K-adenosine triphosphatase (ATPase) was a kind gift from Dr. K. Sweadner (Massachusetts General Hospital).

Statistical analysis. Values are means ± SE. The effect of electrical stimulation between RG and WG muscles was compared by a two-way analysis of variance (ANOVA). The effect of electrical stimulation on 2-DG uptake, glycogen, and lactate concentrations in RG and WG muscles were compared by repeated measures two-way ANOVA.
Table 1. Physiological parameters of control and stimulated RG and WG muscles

<table>
<thead>
<tr>
<th>Muscle Condition</th>
<th>RG</th>
<th></th>
<th>WG</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>2-DG uptake, µmol·g⁻¹·20 min⁻¹</td>
<td>0.43 ± 0.04</td>
<td>3.18 ± 0.21*</td>
<td>0.30 ± 0.03†</td>
<td>3.06 ± 0.23*</td>
</tr>
<tr>
<td>Lactate, mmol/kg</td>
<td>4.8 ± 0.5</td>
<td>13.1 ± 1.3*</td>
<td>5.3 ± 0.9</td>
<td>15.5 ± 1.1*</td>
</tr>
<tr>
<td>Glycogen, µmol/g</td>
<td>137.4 ± 8.4</td>
<td>45.7 ± 6.2*</td>
<td>152.7 ± 6.7</td>
<td>36.3 ± 5.7*</td>
</tr>
</tbody>
</table>

Values are means ± SE for 6 different experiments. Muscle 2-[³H]deoxy-D-glucose (2-DG) uptake, lactate, and glycogen concentrations in control (C) and stimulated (S) red gastrocnemius (RG) and white gastrocnemius (WG) muscles. Significant difference at *P < 0.01 and †P < 0.05 compared with control values.

RESULTS

Resting and electrically stimulated 2-DG uptake is shown in Table 1. Resting glucose uptake was significantly higher in RG than in WG (+30%, P < 0.05). Compared with unstimulated muscle values, contraction markedly and similarly increased 2-DG uptake by 2.76 and 2.75 µmol·g⁻¹·20 min⁻¹ in RG and WG, reaching comparable maximal 2-DG transport rates in both muscle types (3.18 ± 0.21 vs. 3.06 ± 0.23 µmol·g⁻¹·20 min⁻¹, respectively) (Table 1). Muscle glycogen concentrations were significantly reduced by contraction in both types of muscle (−91.7 µmol/g in RG and −116.4 µmol/g in WG, P < 0.01), whereas lactate concentrations were increased (8.3 mmol/kg in RG and 10.2 mmol/kg in WG, P < 0.001; Table 1).

We next investigated the effect of electrical stimulation on GLUT-4 subcellular distribution in RG and WG. Surface and intracellular membrane compartments of resting and stimulated muscles were isolated from several rats, and the identity of the fractions were confirmed using specific enzymatic and immunological markers (Fig. 1, and Tables 2 and 3). As expected, the enzymatic activities of 5'-nucleotidase and KpNPPase were markedly enriched in plasma membrane fractions (Table 2). Significant KpNPPase activity was also detected in the transverse tubule fraction because the α-subunits (particularly the α₁-isofrom) of this enzyme is also localized to these structures (Ref. 25 and Table 3). These enzymes were barely detectable in triads and intracellular membranes. Immunological markers of plasma membranes such as the GLUT-1 glucose transporter and the α₁-subunit of the Na-K-ATPase were also predominantly enriched in the plasma membrane fraction in both RG and WG (Fig. 1 and Table 3). In contrast, the DHPr was mainly detected in transverse tubule and triad fractions, in accordance with the specific localization of this protein to the tubular membranes in skeletal muscle. Importantly, the LiBr-released intracellular membranes were depleted of these muscle cell surface markers. The subcellular distribution of enzymatic and immunological markers is in good agreement with our previous observations (7, 8, 33).

Electrical stimulation failed to change the distribution or the content of the above markers in either RG or WG (Tables 2 and 3 and Fig. 1). This indicates that the recovery and distribution of plasma membranes and transverse tubules were comparable between the experimental groups. Furthermore, no difference in membrane protein recoveries was observed between control or stimulated RG and WG (Table 2). As expected, GLUT-1 protein content in the plasma membrane fraction was not changed by electrical stimulation in both muscle types (see Table 3).

The subcellular distribution of GLUT-4 and the effects of contraction on the content of the transporter protein in surface and internal fractions isolated from RG and WG are shown in Figs. 1 and 2. A representative Western blot of immunoreactive GLUT-4 content is shown in Fig. 1, whereas scanning data of relative concentrations of GLUT-4 in the different fractions from control and stimulated muscles are presented in Fig. 2. In resting muscle, transverse tubule and triad fractions isolated from RG contained greater amounts of the GLUT-4 isoform.
of GLUT-4 than WG muscles (transverse tubule 253.6 ± 23.0 vs. 190.5 ± 17.0, P < 0.05, and triads 405.5 ± 28.7 vs. 187.5 ± 26.7, P < 0.001). No significant differences in GLUT-4 protein abundance was observed in the plasma membrane and intracellular membrane fractions between muscle types.

Electrical stimulation significantly increased GLUT-4 content in plasma membranes of both muscle types (in relative densitometric units, RG 188.2 ± 19.4 vs. 326.7 ± 45.5 and WG 219.1 ± 18.7 vs. 370.5 ± 40.0, P < 0.01). Although contraction also significantly increased GLUT-4 content in transverse tubules of both muscles (RG 253.6 ± 23.0 vs. 340.37 ± 28.9 and WG 190.5 ± 17.0 vs. 340.8 ± 33.4, P < 0.01), this effect was greater in WG than in RG (150 vs. 84 increase in relative densitometric units, respectively, P < 0.05). Contraction also tended to enhance GLUT-4 content in triad fractions from both muscle types, but the effect was much smaller and failed to reach the level of significance (RG 405.5 ± 28.7 vs. 464.4 ± 23.8, P = 0.23, and WG 187.5 ± 26.7 vs. 235.2 ± 29.5, P = 0.20). Furthermore, contraction concomitantly reduced GLUT-4 content in the GLUT-4-enriched intracellular membrane fraction in both RG and WG muscles (RG 1,434.6 ± 186.7 vs. 578.7 ± 164, P < 0.05, and WG 253.8 ± 98.8 vs. 521.9 ± 141.9, P < 0.01). When the total amount of GLUT-4 in the intracellular membrane fraction (fraction protein yield multiplied by its relative GLUT-4 content) and in the plasma membrane and transverse tubule fractions is considered, it can be calculated that the amounts of GLUT-4 appearing in the surface membrane compartments of RG can be fully accounted for by the amounts of the transporter protein leaving the internal pool. However, the GLUT-4 increments in the surface membranes of WG can be mostly (−80%), but

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### Table 2. Protein recoveries, 5'-nucleotidase, and KpNPPase enzymatic activities in freshly isolated muscle membrane fractions for RG and WG muscles

<table>
<thead>
<tr>
<th>Fraction</th>
<th>State</th>
<th>Protein Recoveries</th>
<th>5'-Nucleotidase</th>
<th>KpNPPase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>µg/g muscle wt.</td>
<td>± SE in relative units</td>
<td>NG/µg protein/g muscle wt.</td>
</tr>
<tr>
<td>PM</td>
<td>C</td>
<td>15.9 ± 2.1</td>
<td>315.2 ± 51.8</td>
<td>2,896.6 ± 319.2</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>24.0 ± 6.9</td>
<td>268.8 ± 51.5</td>
<td>2,635.7 ± 430.9</td>
</tr>
<tr>
<td>TT</td>
<td>C</td>
<td>215.4 ± 28.2</td>
<td>59.7 ± 12.6</td>
<td>635.9 ± 51.3</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>235.4 ± 22.6</td>
<td>55.2 ± 10.7</td>
<td>651.9 ± 63.4</td>
</tr>
<tr>
<td>TR</td>
<td>C</td>
<td>143.5 ± 57.9</td>
<td>23.6 ± 5.3</td>
<td>42.7 ± 17.7</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>122.5 ± 14.5</td>
<td>40.9 ± 21.3</td>
<td>38.7 ± 15.3</td>
</tr>
<tr>
<td>L-IM</td>
<td>C</td>
<td>35.4 ± 7.9</td>
<td>ND</td>
<td>21.6 ± 6.9</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>36.0 ± 2.2</td>
<td>ND</td>
<td>24.3 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE of 6 individual membrane preparation for RG and 8 for WG. 5'-Nucleotidase activities are in nmol·mg⁻¹·min⁻¹; KpNPPase activities are in nmol·mg⁻¹·h⁻¹; and protein yields are in µg protein/g muscle wt. PM, plasma membranes; TT, transverse tubules; TR, triads; L-IM, LiBr-released intracellular membranes; ND, not detectable. No significant differences were observed between control and stimulated samples both in RG and WG. Muscle weight of RG and WG used for membrane preparation was not different between control and stimulated animals (5.61 ± 0.05, and WG 2.1 vs. 5.73 ± 0.22 g, respectively).

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### Table 3. Scanning data of GLUT-1, α3-Na-K-ATPase, and DHRp protein contents in isolated membrane fractions from control and stimulated RG and WG muscles

<table>
<thead>
<tr>
<th>Fraction</th>
<th>State</th>
<th>GLUT-1</th>
<th>α3-Na-K</th>
<th>DHRp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>µg/g muscle wt.</td>
<td>± SE in relative units</td>
<td>ng/g muscle wt.</td>
</tr>
<tr>
<td>PM</td>
<td>C</td>
<td>124.0 ± 18.7</td>
<td>1,544.9 ± 130.7</td>
<td>69.4 ± 4.5</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>121.2 ± 6.8</td>
<td>1,574.0 ± 169.9</td>
<td>77.8 ± 3.9</td>
</tr>
<tr>
<td>TT</td>
<td>C</td>
<td>165.4 ± 46.6</td>
<td>762.9 ± 23.8</td>
<td>133.7 ± 43.0</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>133.7 ± 39.0</td>
<td>794.6 ± 35.0</td>
<td>1,321.0 ± 93.3</td>
</tr>
<tr>
<td>TR</td>
<td>C</td>
<td>ND</td>
<td>1,257.3 ± 69.6</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>L-IM</td>
<td>C</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>ND</td>
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</table>

Values are means ± SE in relative units of 4 individual membrane preparations. No significant differences were observed between control and stimulated samples both in RG and WG.
not totally, explained by recruitment from the intracellular pool.

In other experiments we confirmed that homogenates of RG contained twice as much GLUT-4 as that of WG (data not shown). However, this difference is less important in fractionated muscles possibly because several fractions are not kept for analysis, since they contain mixed amounts of plasma membranes, transverse tubules, or other intracellular compartments (e.g., sarcoplasmic reticulum; Ref. 8). Nevertheless, when the amounts of GLUT-4 recovered in the four fractions (i.e., GLUT-4 relative densitometric units × yields of fractions) kept in the present study were added, the content of GLUT-4 in RG (resting state) is about 1.6-fold greater than in WG (165,659 vs. 102,175 relative densitometric units, respectively).

**DISCUSSION**

The results of the present study show that electrically induced contraction stimulates GLUT-4 translocation to both the plasma membrane and transverse tubules of RG and WG. The novel observation that GLUT-4 is also translocated to muscle transverse tubules on electrical stimulation is of physiological significance because the tubular structures represent >60% of the total muscle cell surface area (11) and thus are expected to contribute very significantly to glucose disposal by muscle cells (6–8, 10, 28, 33). In both muscle types the effect of electrical stimulation on 2-DG transport (7- to 10-fold) was much greater than its effect on GLUT-4 translocation to the plasma membrane or transverse tubules (~1.5- to 2-fold). This was also observed by others groups using different fractionation procedures (2, 15). It is possible that the actual magnitude of GLUT-4 translocation is underestimated because of cross-contamination of surface membranes with GLUT-4-enriched intracellular membranes during the fractionation procedure. However, preliminary experiments (L. Dombrowski, K. Kandror, P. Pilch, and A. Marette, unpublished observations) suggest that both the plasma membrane and transverse tubule fractions isolated by the present technique are devoid of the insulin responsive amino peptidase (IRAP)-gp160, a protein that has been recently shown to be enriched in the GLUT-4-containing intracellular pool of adipocytes and skeletal muscle (5). This suggests that cross-contamination of surface membranes with intracellular membranes is unlikely to be the only factor responsible for the observed difference between the functional activation of glucose transport and GLUT-4 translocation in the present study.

The lack of concordance between the stimulation of glucose transport and GLUT-4 translocation may be related to the stimulation of GLUT-4 or GLUT-1 intrinsic activities in the plasma membrane or GLUT-4 activity in the transverse tubules. However, exercise-contraction at most doubles the intrinsic activity of plasma membrane glucose transporters (14, 32), and therefore this effect is not sufficient to account for the marked difference between stimulation of glucose transport and GLUT-4 translocation. It remains to be tested whether contraction increases the intrinsic activity of transverse tubule GLUT-4 transporters to a level that could account for the above difference.

Another important factor to bear in mind is the effect of contraction on muscle blood flow. Indeed, electrical stimulation of contraction was performed in situ, and the increase in blood flow is expected to have significantly contributed to the uptake of 2-DG in contracting muscles. The important role of muscle perfusion in glucose uptake has recently been demonstrated by Hespel et al. (18). They showed that the increase in blood flow plays a major role in exercise-contraction-induced glucose uptake during hindlimb perfusion. Contraction stimulates blood flow up to 20 times, whereas arteriovenous glucose differences only increase by two- to fourfold at a constant arterial glucose concentration (31). Moreover, contraction stimulates glucose uptake by approximately two- to fivefold in isolated muscle preparation, an extent that is closer to the change in cell surface GLUT-4 transporters measured in this study by subcellular fractionation.

Previous studies have shown that the stimulation of glucose transport by both insulin and treadmill exercise is greater in muscles enriched with type I and type IIa fibers than in muscles mainly composed of type IIb fibers (1, 17, 20, 21). Thus the muscle fiber composition generally believed to be a good predictor of glucose transport capacity. However, this relationship is not apparent when glucose transport is activated by supramaximal electrical stimulation of the sciatic nerve. Indeed, this and previous studies (3, 22) showed that muscles enriched with type IIb myofibers have similar or even greater maximal rates of glucose transport than muscles composed mainly of type IIa and type I fibers during electrically induced contraction, a stimulation process that recruits all fiber types to the same extent. In the present study we have used a stimulation protocol in which the fraction of the time occupied by contraction is 10% because it has been shown to induce maximal glucose transport in both RG and WG muscles (22). This stimulation protocol increased 2-DG transport rates to similar levels in RG and WG muscles (net gains of 2.75 vs. 2.76 μmol·g⁻¹·20 min⁻¹, respectively). GLUT-4 protein levels of RG is two to four times that of WG muscles (22, 29). Whereas this may explain the greater insulin responsiveness of RG vs. WG muscles, it cannot account for the comparable responsiveness of both muscle types toward electrical stimulation. It is also noteworthy that in the present study, 2-DG uptake by electrically stimulated WG was similar to that of RG despite the fact that an identical pattern of stimulation was previously reported to increase blood flow approximately three times more in the latter muscle type (27). Thus it is possible that the marked increase in RG perfusion during electrical stimulation significantly contributed to glucose uptake by this muscle. Assuming that the contribution of muscle perfusion to glucose disposal is equally important in RG and WG, the similar 2-DG uptake in contracting WG in the face of a much smaller increase in blood flow compared with RG strongly suggests that the extraction rate of the hexose was more increased in the former muscle. Clearly, this assumption remains to be confirmed.
The present data suggest that a greater translocation of GLUT-4 to transverse tubules of WG myofibers may provide a cellular basis for the similar action of electrically induced contraction on glucose transport in this muscle type. GLUT-4 was found to be translocated to both the plasma membrane and the transverse tubules isolated from either RG or WG on electrical activation of contraction. However, whereas the effects of contraction on GLUT-4 translocation to the plasma membrane were comparable between RG and WG, electrical stimulation induced a significantly greater recruitment of the transporter to the transverse tubules. Thus, whereas GLUT-4 content in the transverse tubules was higher in resting RG than WG, the relative concentrations of the transporter in the tubular structures (and the plasma membrane) were comparable in maximally contracting RG and WG muscles (Fig. 2), thus allowing maximal glucose transport to be similar in both muscle types. Whether a greater activation of transverse tubule GLUT-4 is also involved in the enhanced 2-DG transport by electrically stimulated WG remains to be tested.

GLUT-4 levels in the triad fraction were not related to glucose transport in the present study. Indeed, triad GLUT-4 content was much greater in resting RG than WG and this difference persisted in contracted muscles (Fig. 2) despite the fact that 2-DG uptake rates were similar in both muscle types. This suggests that GLUT-4 proteins localized to the triads are not significantly contributing to glucose disposal by skeletal muscle cells. The physiological relevance for a greater abundance of GLUT-4 in the triads of RG compared with WG is not known at present. One possibility is that the triad fraction contains a pool of recently synthesized GLUT-4 molecules that are segregated to the trans-Golgi network. It has been shown that this intracellular pool of GLUT-4 is not involved in the translocation of GLUT-4 to the cell surface of skeletal muscle cells (19).

Another finding of the present study is that we observed a significant contraction-induced decrease in GLUT-4 content in an intracellular fraction that is greatly enriched in GLUT-4 transporter proteins but devoid of plasma membranes and transverse tubules in both RG and WG muscles. Although contraction-induced GLUT-4 translocation to transverse tubules was found to be more important in WG than RG muscle, similar decrements in the transporter concentrations were observed in the intracellular fractions of both muscle types. Accordingly, whereas the total amount of GLUT-4 appearing in the plasma membranes and the transverse tubules of RG could be fully accounted for by the amount of GLUT-4 leaving the intracellular membrane fraction, this was not the case in WG muscle because ∼20% of the transporters recruited to the muscle cell surface could not be explained by a reduction in intracellular GLUT-4. It is therefore possible that a small portion of the contraction-responsive intracellular GLUT-4 pool in WG is not isolated by our fractionation procedure. It is important to note that we have recently shown, using the same fractionation procedure, that both insulin and treadmill exercise also induce the translocation of GLUT-4 to the plasma membrane and transverse tubules from the same intracellular fraction of mixed hindlimb muscles (8, 33). These results suggest that insulin and contraction activate GLUT-4 recruitment from the same intracellular pool in skeletal muscle. However, it cannot be excluded that the intracellular membrane fraction isolated in the present study contains not only one but a mixture of two distinct, but biochemically similar, GLUT-4-containing pools that respond to either insulin or contraction. Indeed, it has been previously suggested that insulin and contraction stimulate GLUT-4 translocation from different intracellular compartments (5, 9). An extensive and detailed biochemical analysis of the intracellular fraction will be necessary to clarify this important question, but this was not the goal of the present study.

In summary, the present study shows that electrical stimulation induces the translocation of GLUT-4 from an intracellular pool to the transverse tubules in addition to the plasma membrane in both RG and WG. Our results also suggest that a greater translocation of GLUT-4 to transverse tubules of WG compared with RG may explain the comparable responsiveness of both muscle types toward supramaximal electrical stimulation.

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