Effect of tension on contraction-induced glucose transport in rat skeletal muscle

JACOB IHLEMANN, THORKIL PLOUG, YLVA HELLSTEN, AND HENRIK GALBO
Copenhagen Muscle Research Center, Rigshospitalet, and Department of Medical Physiology,
The Panum Institute, University of Copenhagen, 2200 Copenhagen N, Denmark

IHLEMANN, JACOB, THORKIL PLOUG, YLVA HELLSTEN, AND HENRIK GALBO. Effect of tension on contraction-induced glucose transport in rat skeletal muscle. Am. J. Physiol. 277 (Endocrinol. Metab. 40): E208–E214, 1999.—We questioned the general view that contraction-induced muscle glucose transport only depends on stimulation frequency and not on workload. Incubated soleus muscles were electrically stimulated at a given pattern for 5 min. Resting length was adjusted to achieve either no force (0% P), maximum force (100% P), or 50% of maximum force (50% P). Glucose transport (2-deoxy-D-glucose uptake) increased directly with force development (P < 0.05) [27 ± 2 (basal), 45 ± 2 (0% P), 68 ± 3 (50% P), and 94 ± 3 (100% P) nmol·g⁻¹·5 min⁻¹]. Glycogen decreased at 0% P but did not change further with force development (P > 0.05). Lactate, AMP, and IMP concentrations were higher (P < 0.05) and ATP concentrations lower (P < 0.05) when force was produced than when it was not. 5'-AMP-activated protein kinase (AMPK) activity increased directly with force [20 ± 2 (basal), 60 ± 11 (0% P), 91 ± 12 (50% P), and 109 ± 12 (100% P) pmol·mg⁻¹·min⁻¹]. Passive stretch (–86% P) doubled glucose transport without altering metabolism. In conclusion, contraction-induced muscle glucose transport varies directly with force development and is not solely determined by stimulation frequency. AMPK activity is probably an essential determinant of contraction-induced glucose transport. In contrast, glycogen concentrations per se do not play a major role. Finally, passive stretch per se increases glucose transport in muscle.

2-deoxy-D-glucose; metabolism; exercise; GLUT-4; signal transduction

CONTRACTION AND INSULIN have additive effects on glucose transport in skeletal muscle, suggesting that the two stimuli act via at least partially independent pathways (21, 23). This view is supported by the fact that the effect of contractions may be affected differently from the effect of insulin by, for example, training (15), inactivity (24), and obesity (2). Furthermore, 1–2 mM of wortmannin, a phosphatidylinositol 3-kinase inhibitor, selectively impair insulin-stimulated but not contraction-stimulated glucose transport (16, 18, 33). In addition, we have found that calphostin C, a protein kinase C (PKC) inhibitor, selectively impairs contraction-stimulated but not insulin-stimulated glucose transport (Ihlemann, Galbo, and Ploug, unpublished data). Contractions and insulin exert their effect on glucose transport by translocation of the glucose transporter GLUT-4 to the plasma membrane and T tubules (29). Apparently, contractions result in translocation from a GLUT-4 vesicle pool distinct from that recruited by insulin (7, 25). The effect of contractions on glucose transport does not require the presence of insulin (22).

Two principal mechanisms exist by which contractions, as opposed to insulin, might regulate glucose transport. First, the depolarization of the plasma and T tubule membranes preceding contractions might via second messengers, e.g., Ca²⁺, stimulate GLUT-4 translocation. This would be a feedforward mechanism, glucose transport being enhanced before (“in anticipation of”) development of metabolic needs. Second, glucose transport might be related to the strain put on the muscle or the work done by the muscle and hence regulated by factors coupled to muscle tension or metabolic rate. This would be a feedback mechanism, glucose transport being closely associated with metabolic needs. The first mechanism is in line with a previous study of frog muscle from which it was concluded that only the stimulation frequency, but not the workload, was of importance for the contraction-induced glucose transport (12). This view has been generally accepted for decades. However, it is reasonable to assume that the need for glucose must be closely related to the metabolic rate and, accordingly, to mechanical performance. So, it is difficult to accept that these factors are not directly involved in mediation of glucose transport but only indirectly associated with transport to the extent to which they reflect contraction frequency. In vivo muscle glucose uptake is directly related to workload (1), but this relationship might reflect increase in stimulation frequency and recruitment of more muscle fibers with increasing workload, rather than an effect of increase in mechanical performance and metabolism in individual fibers. However, in line with the view that glucose transport may be intimately coupled to metabolism during contractions at a given frequency, in vitro hypoxia increases glucose transport in skeletal muscle, and the effects of hypoxia and contractions have been found to be nonadditive (3). Furthermore, both hypoxia and contractions may reduce pH and ATP-to-AMP and phosphocreatine (PCr)-to-creatine (Cr) ratios in muscle (6). These changes are compatible with stimulation of AMP-activated protein kinase (AMPK; Ref. 26). Interestingly, a 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR)-induced increase in AMPK activity in skeletal muscle has recently been shown to be accompanied by an increase in glucose transport. Furthermore, contractions were shown to increase AMPK activity (10, 19). Of note is also the fact that it has not been possible to reproduce all results achieved in frog muscle in mammalian skeletal muscle. For example, effects of contractions
and insulin on glucose transport are additive in the latter (21, 23) but not in the former (12). For these reasons, we found it of interest to further examine the relationship between glucose transport, contraction frequency, tension, and metabolism on rat skeletal muscle. In the present study, we have measured glucose transport and AMPK in incubated rat muscle. The muscles were stimulated at a given frequency, whereas tension and, in turn, metabolism were varied.

MATERIALS AND METHODS

Materials. 2-Deoxy-D-glucose (2-DG), glucose, bovine serum albumin fraction V (BSA), pyruvic acid, glutamic acid, trisma base, dithiothreitol (DTT), AMP, ADP, ATP, AMP, and sucrose were from Sigma. 2-deoxy-D-[3H]glucose (2-[3H]DG) and [14C]sucrose were from Du Pont-NEN. Lactate dehydrogenase, lactate, glutamate-pyruvate-transaminase, adenosine-5'-pentaphosphate, hexokinase, glucose-6-phosphate-dehydrogenase, NADP, and NAD+ were from Boehringer. Glycylglycine and MgCl2 were from Merck.

Muscle incubation and stimulation. The experiments were approved by the Animal Research Committee of the Ministry of Justice and were in accordance with the animal experiment guidelines of research of the American Physiology Society.

Fed male Wistar rats (65–75 g body wt) were anesthetized by an intraperitoneal injection of pentobarbital sodium (5 mg/100 g body wt). The hindquarter was perfused through the abdominal aorta for 1 min (flow 20 ml/min) with Krebs-Henseleit buffer containing 2 mM pyruvic acid and 0.2% BSA. Muscles were directly electrically stimulated to calculate the intracellular distribution space for 2-[3H]DG uptake. To calculate 2-[3H]DG uptake, muscles were briefly blotted on filter paper and immediately frozen in liquid nitrogen. Muscles were stored at −80°C until analyzed. Under the applied incubation conditions, 2-DG uptake is linear for at least 20 min (Fig. 1) and, accordingly, phosphorylation of 2-DG by hexokinase is not rate limiting for 2-DG uptake in skeletal muscle, and uptake thus reflects glucose transport. To calculate 2-[3H]DG uptake, muscles were weighed and homogenized in 2 ml of 0.6 M perchloric acid. An aliquot of the incubation medium was mixed with an identical volume of 1.2 M perchloric acid. The samples were centrifuged for 10 min at 1,500 g, and 3H and 14C in the supernatants were counted in a dual-channel liquid scintillation counter (TriCarb 2000, Packard) and corrected for spill-over between the channels. Extracellular space was determined from the [14C]sucrose counts, making it possible to calculate the intracellular distribution space for 2-[3H]DG.
E210

GLUCOSE TRANSPORT IN SKELETAL MUSCLE

stretched, they were freeze-clamped with tongs cooled in liquid nitrogen and stored at 80°C until measurement of metabolite concentrations.

Glycogen was determined by a hexokinase method after hydrolysis with HCl (14). Lactate and PCr were determined by standard enzymatic fluorometric methods (17). Muscle nucleotide concentrations were determined in neutralized perchloric acid extracts by reverse-phase HPLC as described (30). Separation was achieved by a 30-min gradient elution with a Hibar Lichrosphere 100 CH-18/2 (Merck) column (250 × 4 mm). The linear gradient program for the mobile phase was as follows: 0 min, 100% buffer A; 0–4 min, 75% buffer A; 4–17 min, 50% buffer A; 17–22 min, 0–100% buffer A. The composition of buffer A was 150 mM ammonium phosphate (pH 5.80). Buffer B also contained 150 mM ammonium phosphate with 20% methanol and 2% acetonitrile added as organic modifiers (pH 5.45). Separation was achieved at room temperature with a flow of 0.8 ml/min. Detection was at 254 nm, and peaks were identified by comparison of retention times with commercially obtained compounds.

For AMPK activity determination, muscles were homogenized with a Polytron PT 3100 (Kinematica AG, Littau-Luzern, Switzerland) at maximum speed for 10–15 s in ~20 vol of ice-cold buffer (210 mM sucrose, 1 mM EDTA, 5 mM sodium pyrophosphate, 50 mM NaF, 1 mM DTT, 2 mM phenylmethylsulfonyl fluoride, and 50 mM HEPEs, pH 7.4). The homogenate was centrifuged for 45 s at 15,000 g, and the supernatant was stored in aliquots for later determination of protein concentration and AMPK activity. Sample protein was determined by the Coomassie protein assay reagent (Pierce, Rockford, IL) with bovine serum albumin as a standard. AMPK activity was determined as described (31) except that a 50 mM PIPES buffer was used (26). AMPK activity is expressed as picomoles of phosphate incorporated into SAMS peptide per milligram protein in muscle sample supernatant during 1 min (31).

To compare metabolic conditions of incubated muscles with that of muscles at rest in vivo, 65- to 75-g rats were anesthetized by an intraperitoneal injection of pentobarbital sodium (5 mg/100 g body wt) and soleus muscles were dissected free, quickly freeze-clamped with tongs cooled in liquid nitrogen and stored at 80°C until measurement of metabolites. We found that basal values for glycogen, lactate, nucleotides, and AMPK in incubated muscles were identical to values in anesthetized rats (data not shown), and nucleotide values also corresponded to previously published values (28).

Statistics. Groups were compared by one-way ANOVA, and statistically significant differences were localized by Bonferroni’s t-test. P < 0.05 was considered significantly different in two-tailed tests.

RESULTS

In response to repeated tetanic electrical stimulation, developed force always decreased gradually (Fig. 2). Tension-time products were proportional with peak force output during stimulation, being 2,382 ± 52 N/s and 1,205 ± 43 N/s at maximal and 50% of maximal force output conditions, respectively. During passive stretch, integrated tension corresponded to 86% of the force output conditions, respectively. During passive stretch, they were freeze-clamped with tongs cooled in liquid nitrogen and stored at 80°C until measurement of metabolite concentrations.

Glycogen transport in soleus muscle during 5 min of repeated tetanic contractions. Contractions were produced from 0.5 min by electrical stimulation with 25 V in 200-ms trains of 100 Hz and 1 train/s, each impulse being 0.2 ms. Muscle was stimulated at resting length.

with force output being largest at maximal force output, only 60% at 50% of maximal force output, and 30% at no force output.

Metabolites. Glycogen concentrations in muscle decreased ~50% on electrical stimulation (Fig. 4). The decrease tended to be higher when force was developed than when it was not, but the difference was not statistically significant. In contrast, muscle lactate concentrations increased during contractions and varied directly with force development. At maximal force output, lactate concentrations were significantly higher than at the no force output condition. Lactate concentrations at 50% of maximal force output differed significantly from neither the concentrations at no force output nor the concentrations at maximal force output (Fig. 4).

PCr concentrations in muscle always decreased ~70%, and ATP concentrations decreased ~50% on electrical stimulation (Fig. 5). Whereas PCr concentrations did not differ significantly between groups during contractions, ATP concentrations tended to be lower during force production than during contractions without force production. Correspondingly, AMP and IMP concentrations increased during contractions and varied directly with force development.
concentrations were higher \( (P < 0.05) \) during the former than during the latter condition (Fig. 5). No changes in ADP levels could be detected during stimulation (data not shown).

AMPK activity. AMPK activity always increased during electrically stimulated muscle contractions (Fig. 6). The increase varied directly with force output and, accordingly, with 2-DG uptake. At maximal force output, the activity was significantly higher compared with the activity at no force output. The activity at 50% of maximal force output differed significantly from neither the no force output activity nor the activity at maximal force output.

Passive stretch. Uptake of 2-DG was increased two-fold by passive stretch (Fig. 3). In contrast, stretch did not influence metabolite concentrations and AMPK activity (Figs. 4, 5, and 6).

DISCUSSION

The present study aimed at elucidating the mechanisms by which contractions stimulate glucose transport in mammalian skeletal muscle. The major findings are that at a given frequency of contractions glucose transport varies directly with developed force (Fig. 3), probably reflecting AMPK-dependent activation. The general opinion has for decades been that contraction-induced glucose transport is determined only by the

Fig. 4. Influence of force development on substrates in soleus muscle. Muscles were stimulated at resting length to produce maximal force, whereas length was reduced to achieve 50% of maximal force or no force development. Glycogen (top) concentrations are expressed in mmol of glycosyl units. Values are means ± SE; \( n = 5–10 \). § Different from basal value \( (P < 0.05) \); * different from value at maximal force \( (P < 0.05) \). Lactate (bottom) values at 50% of maximal force output differed significantly from neither values at no force output nor values at maximal force output.

Fig. 5. Influence of force development on high-energy phosphate compounds in soleus muscle. Muscles were stimulated at resting length to produce maximal force, whereas length was reduced to achieve 50% of maximal force or no force development. Values are means ± SE; \( n = 5 \). A: ATP; B: AMP; C: IMP; D: phosphocreatine. § Different from basal value \( (P < 0.05) \); * different from value at maximal force \( (P < 0.05) \). ATP concentrations differed at the \( P < 0.1 \) level in 2-tailed testing between maximal and no force conditions.
frequency with which muscle is stimulated and not by mechanical loading (12). The data from the current study suggest that this view should be modified. Regarding the mechanism by which force development enhances glucose transport, it cannot be excluded that development of force in the contractile apparatus is translated directly by mechanical means into GLUT-4 translocation to the plasma membrane and T tubules. However, such a mechanism has yet to be defined. More likely, the coupling between force development and glucose transport is indirect, mediated by metabolic changes associated with force development. As would be expected, we found that metabolic demands varied directly with force development. During contractions, lactate, AMP, and IMP concentrations were significantly higher and ATP concentrations lower when force was produced than when it was not (Figs. 4 and 5). In agreement with the view that energy status of the muscle cell plays a role for contraction-induced glucose transport, hypoxia has in rat epitrochlearis muscle been shown to increase glucose transport, and the effects of hypoxia and contractions were not additive (3). The exact mechanism by which energy status of the cell influences glucose transport has until recently been obscure. However, AMPK has now been proposed as a fuel gauge in skeletal muscle (8). This enzyme is activated by reduction in pH and ATP-to-AMP and PCr-to-Cr ratios (26) and, in turn, initiates energy-restoring mechanisms (8). Among these, according to very recent evidence, there is glucose transport. This has appeared from studies in which rat muscle was treated with AICAR, which is a precursor of the AMP-analog ZMP (10). The treatment resulted in increased intracellular concentrations of ZMP, and these were accompanied by increases in AMPK activity as well as glucose transport (10) and uptake (19). Furthermore, effects of AICAR and contractions on glucose transport were not additive (10). The fact that in the present study the increase in glucose transport with increasing force development was accompanied by reduction in muscle energy status and pH and, furthermore, paralleled by increase in AMPK activity (Fig. 6) strongly supports the view that AMPK activation is an essential determinant of contraction-induced glucose transport.

Glucose transport increased in response to contractions also during zero force conditions (Fig. 3). Metabolic changes and AMPK activation were marked even in the absence of force production (Figs. 4–6) and may have caused the increase in glucose transport. Alternatively, however, signals elicited earlier in the chain of events leading to contraction may have contributed to the stimulation of glucose transport. In favor of a role of the rise in free intracellular Ca\textsuperscript{2+} concentration, it has been shown that even a drug-induced rise in Ca\textsuperscript{2+}, which is too small to elicit contraction, is able to increase glucose transport (34). Furthermore, we have recently found that pharmacological inhibition of Ca\textsuperscript{2+}-dependent calmodulin and PKC activity impairs contraction-induced glucose transport without influencing force development (Ihlemann, Galbo, and Ploug, unpublished data). Another indication that energy status of muscle may not be the sole determinant of contraction-induced glucose transport is the finding that high concentrations (10 mM) of the phosphatidylinositol 3-kinase inhibitor wortmannin reduce the effect of contraction but not the stimulatory effect of hypoxia (32). Correspondingly, we have recently shown that the effects of hypoxia and contractions on glucose transport are additive in fast-twitch oxidative fibers (J. D. Fluckey, T. Ploug, and H. Galbo, unpublished data). The latter finding is in contrast to findings in fast-twitch glycolytic fibers (3). So, a scenario is emerging according to which contractions may enhance glucose transport by a dual mechanism: a feedforward control elicited early during muscle cell stimulation and possibly involving Ca\textsuperscript{2+}, as well as a feedback control elicited by reduced intracellular energy status and involving AMPK. The relative importance of the two signaling pathways probably varies between muscle fiber types, the metabolic feedback mechanism being less important in oxidative fibers, which have a high capacity to regenerate ATP by oxidative phosphorylation and are relatively resistant to fatigue.

The fact that muscle content of glycogen and glucose transport capacity are inversely related (4, 11, 27) is in line with the hypothesis that GLUT-4 transport vesicles are associated with glycogen and released for translocation when glycogen is broken down (5). In the present study, glycogen concentrations decreased in response to contractions but did not differ significantly between contraction-stimulated groups. The latter finding probably reflects that differences in energy demands between these groups were small relative to prevailing glycogen levels and to variation in these levels between rats. During contractions with maximal force development, the increase in glucose transport above basal levels was more than three times the increase seen during contractions with no force development, whereas no difference in glycogen concentrations could be de-
tected between these conditions. This excludes a major role of the glycolgen concentration per se in the mediation of contraction-induced glucose transport, at least regarding the component depending on force development. In line with this view, it has been shown that reversal of contraction-stimulated glucose transport to basal levels can take place in the absence of muscle glycogen depletion (23).

Interestingly, passive stretch caused a considerable increase in muscle glucose transport corresponding to that seen during contraction without force development (Fig. 3). Uptake of 2-DG in muscle cells was calculated with sucrone as extracellular marker. This excludes that damage of the sarcolemma explained the effect of stretch, because such damage would have resulted in similar increases in intracellular accumulation of sucrone and 2-DG. Neither could the effect of passive stretch on glucose transport be ascribed to metabolic events because, as expected, muscle metabolism was not influenced by stretch (Figs. 4 and 5). Although the average tensions in the tendons were comparable during passive stretch and maximal contraction-induced force development, respectively, the intracellular mechanical state differed fundamentally between the two conditions. Accordingly, the fact that passive stretch elicits glucose transport has no bearing on the effect of force-producing contractions. Repetitive stretch-relaxation for more than 4 h has been shown to result in similar increases in intracellular accumula-
tion (Fig. 3). Uptake of 2-DG in muscle cells was comparable during passive stretch and maximal contraction-induced force development, respectively, the component depending on force development. Airly of this view, it has been shown that stretch-relaxation caused a considerable increase in glucose transport in cultured myotubes, an effect requiring ongoing protein synthesis but not electrical activity (9, 20). However, the mechanist for the effect of brief stretching on glucose transport in mature mammalian muscle remains to be elucidated.

In conclusion, at a given stimulation frequency, glucose transport varies directly with force development. Accordingly, stimulation frequency is not the sole determinant of contraction-induced glucose transport. The influence of force development probably reflects effects of enhanced AMPK activity resulting from reduced intracellular energy status and pH. In contrast, glyco-
gen concentrations per se do not play a major role. Finally, passive stretch increases glucose transport in mature muscle.

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mann, Dept. of Medical Physiology, The Panum Institute, Blegda-
vøj 3, 2200 Copenhagen N, Denmark (E-mail: jhielmann@mfi.ku.dk).

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