Glucose Transport and Glucose Transporters in Muscle and Their Metabolic Regulation

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Skeletal muscle is the primary tissue responsible for insulin-dependent glucose uptake in vivo; therefore, glucose uptake by this tissue plays an important role in determining glycemia. Glucose uptake in muscle occurs by a system of facilitated diffusion involving at least two distinct glucose transporters, GLUT-1 and GLUT-4. Both bind the fungal metabolite and inhibitor of glucose transport cytochalasin B. In human skeletal muscle, both types of transporters are detected immunologically, and corresponding mRNA transcripts of both transporter forms are detected. In human skeletal muscle cells in culture, in which contamination by other tissues is ruled out, a 50,000-*M*, polypeptide is photolabeled with cytochalasin B. In rat skeletal muscle, acute treatment with insulin in vivo increases glucose-transport activity and the number of specific cytochalasin B–binding sites at the plasma membrane. In mildly diabetic (streptozocin-induced) rats, the number of cytochalasin B–binding sites is decreased in total membranes, and preferentially in the plasma membrane. In response to acute insulin treatment, however, there is still recruitment of glucose transporters to the plasma membrane from an intracellular membrane store. Hence, migration of transporters does occur in this form of diabetes. In L6 muscle cells in culture, acute treatment (1 h) with insulin causes recruitment of glucose transporters to the plasma membrane, and prolonged exposure to insulin or to glucose-deprived medium causes increased expression of GLUT-1 mRNA and GLUT-1 protein. Prolonged exposure (24 h) to high glucose in the medium causes a decrease in the number of glucose transporters in the plasma membrane. Hence, in those cells the expression of the GLUT-1 glucose transporter is modulated by insulin. *Diabetes Care* 13:228–43, 1990

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The studies summarized in this review represent 7 yr of investigation of the regulation of glucose transport in skeletal muscle. This research was initiated on the premise that glucose homeostasis in muscle need not be dictated by the same factors or occur through the same mechanisms as in adipocytes, a more widely studied cell type. An important difference between these tissues is that muscle contributes greatly to the regulation of glycemia. Moreover, in skeletal muscle, glucose metabolism is regulated by acute exercise and prolonged exercise training and in vitro by electrically induced muscle contraction, clearly an activity inherent to this tissue. Whereas classic physiology studies have amply documented the stimulation of muscle glucose metabolism by insulin, exercise, and training, there has been surprisingly less information on the molecular mechanisms underlying these effects. Molecular knowledge of glucose transport in this tissue was falling behind the knowledge of other cells. In fact, the muscle glucose transporter in skeletal muscle had not been identified when that of human erythrocytes had been purified, its activity reconstituted, and a cDNA cloned and sequenced.

MUSCLE GLUCOSE UPTAKE IN VIVO

In the fed state, skeletal muscle is the primary tissue responsible for the insulin-dependent fraction of R_d, the overall body glucose uptake (1–3). This conclusion stems

from measurements of leg glucose balance (arteriovenous catheter technique) coupled to euglycemic-hyperglycemic clamps. In the basal state (i.e., in the presence of basal circulating insulin levels), skeletal muscle is responsible for only 20% of R_d. Under these conditions, non–insulin-mediated glucose uptake (NIMGU) and insulin-mediated glucose uptake (IMGU) caused by circulating basal levels of insulin each represent ~50% of the total glucose uptake so that basal IMGU into skeletal muscle is 10% of whole-body R_d (the rest of the IMGU under basal conditions occurs in fat, cardiac muscle, and splanchnic tissues; the central nervous system is responsible for most of the basal NIMGU). However, skeletal muscle becomes the determining organ when the circulating concentration of glucose rises. A detailed study showed that during hyperglycemia, virtually all the increment in human R_d occurs in skeletal muscle, and this tissue determines the overall glucose uptake after a glucose load (3). These results confirmed earlier studies indicating that skeletal muscle is a major factor in the regulation of blood glucose in vivo (4). The latter study showed that, in insulin-treated animals, ~25% of an intravenous dose of glucose enters the muscle cells within 1 min, thus illustrating the quantitatively important regulatory role that skeletal muscle plays in limiting the extent of a rise in circulating glucose.

As stated above, skeletal muscle is the primary tissue involved in IMGU during elevation of the circulating insulin levels. Baron et al. (3) showed that during hyperinsulinemia, IMGU into skeletal muscle represents 75% of R_d at euglycemia and 95% of R_d at hyperglycemia, making this tissue the predominant glucose-uptake site in response to elevations in insulin levels. In contrast, IMGU into fat tissue at either euglycemia or hyperglycemia is much less significant (5,6).

Despite the high glucose uptake that occurs in human muscle during euglycemic hyperinsulinemia, the intracellular concentration of glucose does not change (7). This indicates that glucose is quickly metabolized by human muscle and that glucose transport across the cell membrane is the rate-limiting step in glucose utilization not only in the basal state but also in the presence of insulin (8). Thus, the rate of glucose transport across the muscle membrane is a key determinant in glucose metabolism in this tissue and in whole-body glucose utilization in the basal and insulin-stimulated states.

GLUCOSE-TRANSPORT ACTIVITY IN SKELETAL MUSCLE

Investigations of glucose uptake into skeletal muscle began in 1939 with the suggestion by Lundsgaard that insulin increased the rate of entry of glucose into muscle by stimulating what he called an active factor on the cell surface (8). This was followed by the work of Park et al. (9), Narahara et al. (10), and Morgan et al. (11), who showed that glucose uptake in the diaphragm was saturable and increased by insulin. Further work established that uptake was carrier mediated insofar as it was susceptible to inhibition by cytochalasin B and displayed counterflux (12). The effect of insulin was ascribed entirely to an increase in V_{max} without changes in K_m (13). These studies have been reviewed previously and are not discussed further here (14).

Measurements of glucose transport into isolated skeletal muscles are complicated by the complex architecture of this tissue and by its heterogeneous fiber composition. The first complication arises from the fact that not all fibers are equally exposed to glucose in the medium, and thus intercellular space has to equilibrate with glucose before uptake measurements can be successfully performed. However, equilibration of the intercellular space is achieved only after the outermost fibers have equilibrated intracellularly with glucose. Hence, initial rates of hexose uptake cannot be measured except in cases of very thin muscles with small intercellular space (e.g., rat epitrochlearis and soleus and frog sartorius muscles; 13,15), and the measurements are not simple even in these muscles, 3-*O*-methylglucose efflux being the preferred measurement in some cases (16).

The second complication arises from the heterogeneous fiber composition of each muscle so that eventually glucose uptake and its regulation will have to be defined for each individual muscle. In rats and humans, there are no muscles with pure individual fiber composition, and hexose uptake and its stimulation by insulin or exercise have been shown to differ in the red and white gastrocnemius, the soleus, and the epitrochlearis muscles, to name a few (15,17–19). As an oversimplified rule, glucose uptake in slow muscles has a lower K_m than in fast ones, and the V_{max} is higher and stimulated more by insulin in red than in white muscles. This conclusion is based on comparisons of measurements performed in soleus, red and white gastrocnemius, quadriceps, plantaris, and extensor digitorum longus muscles. Depending on the fiber composition, insulin stimulates glucose uptake between 2- and 20-fold in rat muscle. In contrast, the hormone increases glucose uptake only 2-fold in human adipocytes and up to 30-fold in rat adipocytes. The particularly high response observed in rat adipocytes is the subject of analysis in other sections of this issue.

CHARACTERISTICS OF GLUCOSE TRANSPORTERS

Strategies for glucose-transporter identification. Glucose is a hydrophilic molecule and cannot cross the plasma membrane freely; therefore, a carrier system is required to promote its transport. In most cell types, phosphorylation of glucose inside the cell by hexokinase is much faster than the rate of uptake from the extracellular milieu. Consequently, the actual concentration of glucose inside the cell is negligible compared with that outside the cell (normally ~5 mM). Because of this significant concentration gradient between the external

milieu and the inside of the cell, a carrier protein allows specific glucose influx into the cell by facilitated diffusion. A glucose-specific membrane-bound carrier, the glucose transporter, has been identified in various cell types.

The kinetics of facilitated glucose transport were first characterized in human erythrocytes as stereospecific and saturable, with a K_m of ~5–10 mM for D-glucose influx (20–26). D-Glucose uptake in erythrocytes was shown to be specifically inhibited by the fungal metabolite cytochalasin B (27). The glucose transporter was first purified by anion-exchange chromatography from human erythrocyte ghosts solubilized in detergent. During purification, the activity of the transporter was followed by reconstitution in artificial lipid vesicles and measurement of the stereospecific and cytochalasin B–inhibitable uptake of [^{3}H]-D-glucose (28–33). The purified protein is a glycoprotein of ~55,000 M_r (34). The human erythrocyte glucose transporter is heterogeneously glycosylated, causing a distinctive diffusely migrating band when subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) (34,35).

In addition to its inhibitory effect on glucose transport, cytochalasin B can be used as a photoaffinity label of the transporter and as a probe to quantitate the number of transporters in various subcellular fractions. When membranes containing glucose transporters are incubated in the presence of radioactive cytochalasin B and irradiated with UV light, cytochalasin B is covalently linked to the transporter (36–38). Labeled glucose transporters are detected by fluorography or liquid scintillation counting of gel slices after SDS-PAGE. The specificity of the photoaffinity labeling with cytochalasin B is confirmed because it can be inhibited by D-glucose but not L-glucose (39). Glucose transporters can be quantitated in membrane fractions by the ability of the transporters to bind cytochalasin B in a D-glucose–protectable manner at equilibrium. These approaches, initially developed for the glucose transporter of human erythrocytes, have been amply used with several other tissues and have been invaluable in the identification and determination of the number of glucose transporters on fat and muscle cell membranes derived from cells exposed to diverse metabolic conditions (40). However, cytochalasin B binding and photolabeling do not differentiate between different types of glucose transporters (see below for description of different types of glucose transporters).

Glucose transporters can also be detected immunologically. Polyclonal and monoclonal antibodies have been raised against the purified human erythrocyte glucose transporter (41–46). Both cytochalasin B photoaffinity labeling and immunoblotting with these antibodies have been used to identify the glucose transporters in different tissues and species (47–52) and to clone the cDNA coding for the erythrocyte-type glucose transporter. An antiserum prepared against the human erythrocyte glucose transporter was used to screen a HepG2 cDNA library in the expression vector λ-gt11 for phages expressing glucose-transporter antigenic determinants (53). The cDNA coding for the HepG2 glucose transporter is designated GLUT-1 throughout this article. It was demonstrated that GLUT-1 cDNA indeed coded for a glucose-transport protein when it was functionally expressed in *Escherichia coli* (54). The protein coded for by GLUT-1 cDNA can be immunoprecipitated with an antiserum prepared against the human erythrocyte glucose transporter (55). Northern blot analysis with labeled GLUT-1 cDNA as a probe detected the presence of the message in several tissues, including brain, fat, and muscle (53,55,56).

Subcellular distribution of glucose transporters. Cushman and Wardzala (57), Wardzala et al. (58), Suzuki and Kono (59), and Kono et al. (60) simultaneously provided evidence for the existence of an intracellular pool of glucose transporters in rat adipocytes.* They quantitated the glucose transporters by cytochalasin B–binding assays on subcellular membrane fractions (57,58) and reconstitution of glucose-transport activity in lipid vesicles, respectively (59,60). Moreover, they showed that glucose transporters were recruited from the intracellular pool to the plasma membrane after insulin stimulation (57–60). These observations were subsequently confirmed when the transporter was detected by immunoblotting with an antiserum raised against the human erythrocyte glucose transporter (47,48). This recruitment of glucose transporters results in a net increase in the number of glucose transporters in the plasma membrane and appears to be partly responsible for the increased rate of glucose transport after insulin stimulation. Insulin-stimulated translocation of glucose transporters to the plasma membrane was also observed in human fat cells (61,62) and rat diaphragm (63), heart (64), and hind limb muscles (65).

Family of related glucose transporters. The first evidence for the existence of different facilitative glucose transporters was provided by the production of a monoclonal antibody prepared against low-density microsomes from rat adipocytes (66). This monoclonal antibody (1F8) reacted strongly with a 43,000-M_r protein in low-density microsomes prepared from unstimulated adipocytes, but no reactivity could be detected in a plasma membrane fraction prepared from the same adipocytes. However, when the adipocytes were stimulated with insulin before the preparation of the subcellular fractions, the 43,000-M_r protein could be detected in the plasma membrane fraction, and its intensity was proportionally decreased in the low-density microsome fraction (66). The protein immunoprecipitated with the 1F8 monoclonal antibody could be photolabeled with cytochalasin B, lending support to the identification of the rat adipocyte 43,000-M_r protein as a glucose transporter. Hence, it was proposed that the 43,000-M_r pro-

*The intracellular pool of glucose transporters is localized in a membrane fraction obtained by differential centrifugation. Because of its migration in sucrose density gradients, compared with other subcellular compartments, this membrane fraction is conventionally called low-density microsomes.

tein was a glucose transporter that responded to acute insulin stimulation.

The availability of the GLUT-1 sequence permitted the cloning of the GLUT-1 cDNA from different species and tissues (when screened at high stringency) and of distinct but related glucose-transporter cDNAs (when screened at low stringency). For example, cDNAs coding for erythrocyte-type glucose transporters (GLUT-1) were cloned and sequenced from rat (55) and rabbit (67) brain. These two cDNAs were highly homologous (>95% identical at the protein level) to the cDNA originally cloned from the HepG2 cells, the differences being attributed to interspecies variations. Distinct but related cDNAs were also cloned from liver (GLUT-2; 68,69) and fetal muscle (GLUT-3; 70).

The cDNA coding for the protein recognized by the 1F8 monoclonal antibody was recently cloned and sequenced from rat adipocytes (71). Virtually identical cDNAs were also cloned from libraries of rat skeletal muscle (72,73), mouse 3T3-L1 adipocytes (74), and human skeletal muscle (75). This cDNA (and its protein product) is designated GLUT-4 herein.

Although the different members of the glucose-transporter family are significantly (65%) homologous, most of the homology lies in the proposed transmembrane segments (deduced from hydropathy plots), whereas the more hydrophilic and exposed domains, most likely recognized by antibodies, differ significantly. This may explain the poor cross-reactivity of antibodies between the different transporters. For example, the 1F8 monoclonal antibody does not recognize the glucose transporter purified from human erythrocytes (66), whereas anti-human erythrocyte glucose-transporter polyclonal antibodies recognize a protein in rat adipocyte internal membranes of a somewhat higher molecular weight than that recognized by 1F8 (A.G. Douen and M.R.P., unpublished observations). The specificity of these antibodies can prove invaluable in assessing the proportion of different transporters in a given tissue and in determining whether a unique type or multiple types of glucose transporters are recruited to the plasma membrane on insulin stimulation. The ability to move between the intracellular storage compartment and the plasma membrane could be either an intrinsic property of the insulin-responsive tissue or a built-in characteristic of different glucose transporters.

Skeletal muscle glucose transporters. Adult mammalian tissues can express either one or both transporters. GLUT-1 mRNAs are more abundant in brain and the basolateral membrane of kidney epithelial cells than in other tissues (53,55,67). However, detectable levels of GLUT-1 mRNA are present in human skeletal muscle (56). GLUT-4 mRNA is found almost exclusively in tissues that typically respond to acute insulin stimulation, e.g., fat and muscle (71–75). It has been hypothesized that the two transporter proteins coexist in insulin-responsive tissues, GLUT-1 being mostly in the plasma membrane and responsible for the basal rate of glucose transport in the unstimulated state and GLUT-4 (predominantly stored intracellularly) being responsible for the increased rate of glucose transport after the insulin-stimulated recruitment to the plasma membrane (66).

The GLUT-4 transporter protein has been detected in rat skeletal muscle homogenates by immunoblot analysis with the 1F8 monoclonal antibody (66) and with a rabbit antiserum prepared against a synthetic peptide corresponding to the last 12 amino acids at the COOH-terminal end of GLUT-4 (71). Both antibodies identified the same protein of ~43,000 M_r in rat skeletal muscle. This leaves little doubt that the GLUT-4 transporter is present in muscle.

By use of cytochalasin B photoaffinity labeling, a glucose transporter was detected in the plasma membrane of unstimulated rat muscle (52). The photoaffinity-labeled protein had a 45,000–50,000 M_r. Because cytochalasin B photolabeling does not differentiate between the different members of the glucose-transporter family, these results alone are not sufficient to determine which transporter is present in the muscle plasma membrane. However, an antiserum prepared against purified human erythrocyte glucose transporter also detected a protein of 45,000–50,000 M_r in rat skeletal muscle plasma membrane by immunoblotting (52). Given the low cross-reactivity of this antiserum for different transporters, these results confirm that rat skeletal muscle expresses not only GLUT-4 but also the GLUT-1 protein.

In a separate study focusing on nerve tissue and the blood-nerve barrier, it was reported that antibodies prepared against the purified human erythrocyte glucose transporter or against a synthetic peptide corresponding to the last 16 amino acids at the COOH-terminal end of GLUT-1 detected the GLUT-1 protein immunocytochemically in the perineurial cells surrounding diaphragm muscle cells (51). In contrast, muscle cells in that study did not exhibit significant reactivity (51). The authors concluded that the density of GLUT-1 transporter in muscle cells, if present, was much lower than that in the blood-nerve barrier.

It cannot be formally ruled out that the immunoreactivity observed in the study by Klip et al. (52) with the anti-GLUT-1 antiserum in muscle plasma membranes was from contaminating nerve tissue as opposed to being intrinsic to muscle cells, although care was taken to extract as much nerve tissue as possible from the muscle before homogenization and isolation of the membrane fraction. However, if all the immunoreactivity observed in the muscle plasma membrane fraction were from nerve tissue, the degree of nerve tissue contamination in the muscle extract would need to be far greater than is conceivable.

Based on the immunological studies and the detection of GLUT-1 mRNA in skeletal muscle described above, we conclude that the GLUT-1 transporter is present in rat skeletal muscle, in addition to the GLUT-4 transporter. The GLUT-1 transporter has also been detected immunologically in rat L6 muscle cells (76) and in human muscle cells maintained in culture (V. Sarabia and

A.K., unpublished observations). In this case, immunoreactivity cannot be attributed to any contaminating material and consequently confirms the presence of the GLUT-1 protein in cells of muscle origin.

Glucose transporters in human muscle. Partly because of the difficulty in obtaining biopsy material, few studies have been performed on human muscle. The GLUT-4 transporter has recently been cloned from a human muscle cDNA library (75). Northern blot analysis confirmed the presence of the GLUT-4 mRNA in human skeletal muscle (75). In addition, human muscle was shown to contain GLUT-1 mRNA (56).

Although the message for the two transporters was detected, the transporter proteins that are expressed in human skeletal muscle have not been quantified. In preliminary experiments performed on human muscle cells maintained in culture, we have detected a protein of ~45,000 M_r that is photolabeled with cytochalasin B and immunoreactive GLUT-1, confirming the presence of at least one type of glucose transporter in human muscle (V. Sarabia, M.R.P., and A.K., unpublished observations). In addition, we have detected positive reactivity on immunoblotting a crude membrane fraction isolated from human skeletal muscle with either an antiserum prepared against human erythrocyte glucose transporter (specific for GLUT-1) or an antiserum prepared against a synthetic peptide corresponding to the last 12 amino acids at the COOH-terminal end of GLUT-4 (M.R.P., unpublished observations). These preliminary results suggest that both types of proteins, GLUT-1 and GLUT-4, are present in human muscle.

Distribution of glucose transporters on muscle surface: plasma membrane and transverse tubules. The architecture of the skeletal muscle surface is complex, comprising the plasma membrane proper and the transverse tubules. With the equilibrium cytochalasin B–binding approach and subcellular fractionation that separated plasma membrane and transverse tubules, we detected the presence of glucose transporters in both membranes. The transporters were enriched severalfold in the transverse tubules relative to the plasma membrane proper in membrane fractions isolated from either rat or rabbit skeletal muscle (77). The potential role of each source of surface glucose transporters in glucose-transport activity has not been defined. However, glucose transporters in the transverse tubules may contribute less to glucose transport than those in the plasma membrane when glycemia rises, because diffusion of small molecules into the tubule lumen is highly restricted (78). Under metabolic conditions requiring increased glucose uptake, glucose transporters present in transverse tubules may either migrate to more exposed regions of the plasma membrane, or the volume of the transverse tubule lumen may increase to allow improved access to glucose. Indeed, an increase in the transverse tubule luminal space has been observed in response to chronic exposure to thyroid hormone (79). It will be important to study whether such regulation also occurs in insulin-treated or exercised skeletal muscle.

MECHANISM OF STIMULATION OF GLUCOSE TRANSPORT BY INSULIN IN MUSCLE

Because of the difficulty in studying glucose transport in intact tissue, efforts have been made to isolate transport-competent membrane vesicles from skeletal muscle. Several preparations of skeletal muscle membranes have been described in the past two decades (14), and three such preparations have been used for measurements of glucose-transport activity (80–82). The difficulties and advantages of these systems have been reviewed previously (14) and will not be discussed here except to indicate that vesicles can be prepared that are osmotically active and take up D-glucose stereospecifically, and this uptake is inhibited by cytochalasin B. Although the vesicles contain both insulin receptors (82) and glucose transporters (81), addition of insulin to the vesicles in vitro does not result in stimulation of glucose uptake (14,80,83), suggesting that intracellular elements participate in the hormonal response.

In recent years, a cellular redistribution of glucose transporters has been detected in response to acute exposure of muscle to insulin. In these studies, the number of glucose transporters was considered equivalent to the number of D-glucose–protectable cytochalasin B–binding sites. As stated above, the cytochalasin B–binding assay does not distinguish between subgroups of glucose transporters but is thought to reflect closely the total number of GLUT-1 and GLUT-4 transporters.

Wardzala and Jeanrenaud (84) demonstrated that incubation of diaphragm muscles in the presence of insulin, followed by subcellular fractionation, yielded a plasma membrane fraction with increased cytochalasin B binding compared with membranes from untreated muscles. The increase was roughly twofold when maximum binding was calculated from Scatchard binding plots. A microsomal fraction isolated concomitantly showed a twofold reduction in cytochalasin B binding relative to its corresponding untreated control. Similarly, work from our laboratory showed a doubling in cytochalasin B binding to plasma membranes derived from insulin-perfused (1000 μU/ml for 20 min) hindquarter muscles relative to control (Ringer-perfused) muscles and a concomitant twofold decrease in an intracellular membrane fraction (Fig. 1*A*; 65). Similar observations were made by Sternlicht et al. (83) with isolated plasma membranes from combined gastrocnemius and quadriceps muscles (although an intracellular fraction was not obtained) and by Zaninetti et al. (85) with membranes derived from heart muscle. It is encouraging that all of these studies used different muscles and different membrane isolation procedures, yet all detected a doubling in the number of cytochalasin B–binding sites in the plasma membrane. A recent report by Fushiki et al. (86) compared the preparations of Grimditch et al. (82) and Klip et al. (65) with the quadriceps and gastrocnemius muscles from insulin-perfused hindquarters in both cases and essentially confirmed the original observations.

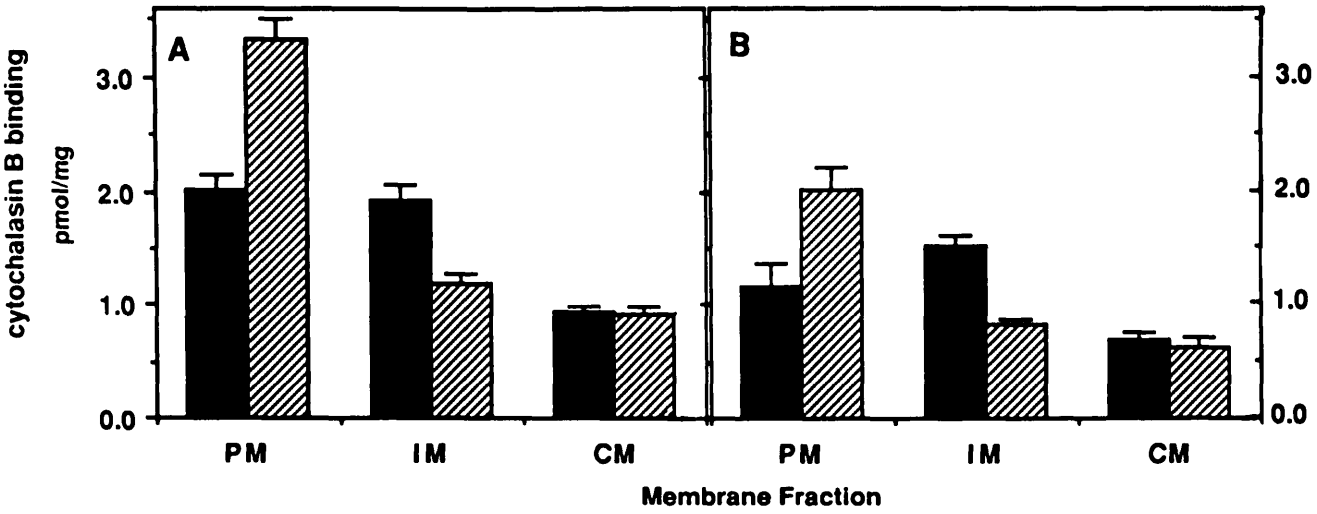

FIG. 1. Effect of insulin on subcellular distribution of glucose transporters. *A*: hindquarter muscles from overnight-fasted rats were perfused for 20 min with maximal insulin as described earlier (65). Hindquarter muscles were immediately isolated, trimmed of all visible fat, connective, and nervous tissue, and used for subcellular fractionation (65,104). Glucose transporters were assessed by D-glucose–protectable binding of cytochalasin B. *B*: rats were made diabetic by intravenous injection of streptozocin (65 mg/kg) and studied 7 days later, as in *A*. Results presented in *A* and *B* are from parallel determinations made simultaneously in control and diabetic rats. PM, plasma membrane fraction; IM, intracellular membrane fraction; CM, crude membrane (total-membrane) fraction. Values are means ± SE of at least 4 independent experiments, each performed in triplicate. Basal rats, *solid bars*; insulin-treated rats, *hatched bars*.

We have recently observed that the number of glucose transporters is increased twofold in the plasma membrane and decreased in the internal membranes from rat muscles isolated 30 min after injection of insulin (1.5 U/350 g i.v.) (S. Rastogi, T. Ramlal, M. Vranic, and A.K., unpublished observations). This confirms that insulin perfusion of the hindquarter reproduces the effects of the hormone in vivo. Acute insulin treatment does not change the number of glucose transporters in the total membrane fraction (Fig. 1*A*), suggesting there is no net synthesis of transporters in response to a 20-min exposure to the hormone. This is consistent with observations made in rat adipocytes.

The above results, determined by analogy to observations made earlier in fat cells (57,87), suggest that the muscle glucose transporters migrate from an intracellular membrane organelle to the cell membrane in response to insulin. There are, however, significant differences in the subcellular localization of glucose transporters in muscle and fat cells. In the latter, the intracellular pool is enriched in glucose transporters compared with the plasma membrane by at least 4-fold when cytochalasin B binding is expressed per milligram of membrane protein. By contrast, in the gastrocnemius and quadriceps (86) and in the hindquarter muscle (65) preparations, the density of glucose transporters in both membrane fractions is comparable. A disadvantage of muscle relative to fat is the more complicated membrane composition of muscle, which precludes the comparison of increases in glucose-transport activity in the intact muscle with increases in glucose-transporter number in subcellular fractions. There are two reasons for this: the yield of muscle membranes is small, and the determinations of marker enzymes in muscle homogenates are unreliable because of the abundance of other proteins. Thus, it is difficult to calculate the total amount of muscle plasma membrane protein, which is the parameter needed to relate transport to transporter number in the intact muscle. However, the increase in glucose-transporter number in the plasma membrane is a mere 2-fold, whereas the increase in glucose uptake has been reported to be 5-fold in the overall hindquarter (65), 3-fold in white gastrocnemius (19), and 8-fold in red gastrocnemius (19) muscles. This suggests that, if the isolated plasma membranes are representative of the totality of plasma membranes, recruitment of glucose transporters to the cell surface is insufficient to account for the full stimulation of transport caused by insulin. In plasma membrane vesicles isolated from insulin-perfused rat hearts, there is an increase in the transport activity (64), but unfortunately the number of glucose transporters was not directly determined in the same preparation. However, both parameters were measured simultaneously by Sternlicht et al. (83) in plasma membrane vesicles isolated from skeletal muscle. Transport activity was increased by 3.2-fold in this in vitro system, whereas the number of cytochalasin B–binding sites only doubled. Thus, in rat skeletal muscle, mechanisms other than recruitment may participate in elevating transport.

Whether a potential elevation in intrinsic activity of the glucose transporters occurs as a posttranslational modification or is accounted for by differences in intrinsic activity of the recruited transporters remains to be determined. Experiments are needed to investigate the

TABLE 1
Cytochalasin B binding to isolated membranes from insulin-treated and exercised muscles

Fraction	Basal	Insulin	Exercise or work load	Refs.
Plasma membrane				
B_{max} (pmol/mg)				
Hindquarter	1.5	2.8	2.6	65
Gastrocnemius	4.1	ND	8.7	103
Gastrocnemius and quadriceps	5.6	9.4	5.1	83
Gastrocnemius and quadriceps	17,20*	31	30	86
Diaphragm	13	24	ND	84
Heart	10	40	56	85,105
K_d (nM)				
Hindquarter	50	160	70	65
Gastrocnemius	81	ND	89	103
Gastrocnemius and quadriceps	47	51	48	83
Gastrocnemius and quadriceps	41,82*	54	56	86
Diaphragm	145	139	ND	84
Heart	230	136	192	85,105
Internal membranes				
B_{max} (pmol/mg)				
Hindquarter	1.0	0.25	1.1	65
Gastrocnemius	ND	ND	ND	103
Gastrocnemius and quadriceps	ND	ND	ND	83
Gastrocnemius and quadriceps	30,21*	17	10	86
Diaphragm	22	10	ND	84
Heart	22	13	10	85,105
K_d (nM)				
Hindquarter	40	10	60	65
Gastrocnemius	ND	ND	ND	103
Gastrocnemius and quadriceps	ND	ND	ND	83
Gastrocnemius and quadriceps	41,81*	54	55	86
Diaphragm	142	105	ND	84
Heart	187	165	267	85,105

In the studies cited, the size of the animals, feeding program, insulin treatment, and exercise or work-load program varied, as did the preparations used to isolate subcellular fractions. Some important differences are as follows. In hindquarter muscle, 1 mU/ml insulin was administered in a 20-min perfusion. Rats exercised on the treadmill for 5 min at 20 m/min and for 40 min at 30 m/min with a 15% grade (65). In gastrocnemius muscle, rats exercised on the treadmill for 60 min at 20 m/min with a 10% grade (103). In the study by Wardzala and Jeanrenaud (84) with gastrocnemius and quadriceps muscles, 1-U/kg i.v. injection of insulin was administered for 10 min. Rats exercised on the treadmill for 15 min each at 22 m/min with a 15% grade, 26 m/min with a 20% grade, and 32 m/min with a 25% grade. In the other gastrocnemius and quadriceps study (86), 14 mU/ml of insulin was administered in a 50-min perfusion. Rats exercised on the treadmill for 2 h at 25 m/min with a 0% grade. In the diaphragm, 39 mU/ml insulin was administered in a 30-min in vitro incubation (84). In the heart, 10 mU/ml insulin was administered in a 15-min perfusion. The work-load program was 15 min, raising perfusion pressure from 50 to 100 mmHg (85,105). ND, not determined.

*When 2 values are quoted for the same parameter, they refer to the control values for the insulin and exercised rat preparations, respectively.

kinetic properties of glucose-transport activity in isolated vesicles derived from plasma membranes and from intracellular membranes of control and insulin-treated muscles. Such studies would require precise estimations of initial rates of transport. Interestingly, the K_d of cytochalasin B binding appears to differ in both membranes, but there is no consensus on these differences. Table 1 shows the K_d values calculated for each membrane fraction derived from control and insulin-treated muscles. In addition, Zaninetti et al. (85) measured the Hill coefficient of cytochalasin B binding and reported an increase in this parameter in plasma membranes from insulin-perfused heart muscle. They suggested that the change in the Hill coefficient of inhibitor binding may reflect differences in the interaction of glucose and the plasma membrane transporters in the basal and insulin-stimulated states. There is, however, no experimental confirmation of this conclusion.

In rat adipocytes, both GLUT-1 and GLUT-4 are recruited to the plasma membrane in response to insulin stimulation, albeit to different extents (49). Also, the two transporters are present in separate intracellular compartments and could thus be recruited independently (88). Although the participation of each transporter in insulin-stimulated recruitment has not been precisely determined, it is widely accepted that in rat adipocytes, GLUT-4 is the major species mobilized. After the transfection and expression of the GLUT-1 cDNA in Chinese

hamster ovary cells (89) and 3T3-L1 adipocytes (90), there is successful recruitment of GLUT-1 to the plasma membrane in response to insulin. These results suggest that the ability to move from an intracellular storage compartment to the plasma membrane depends on a tissue-specific signaling mechanism as opposed to an intrinsic property of the GLUT-4 transporter.

In muscle, recruitment was assessed by cytochalasin B binding in different membrane fractions, and it was therefore not possible to determine which of the two transporters is recruited (65). No information is available concerning the respective intracellular locations of GLUT-1 and GLUT-4 in muscle. Recent results from our laboratory indicate that the GLUT-1 transporter is primarily localized to the plasma membrane, whereas the GLUT-4 transporter is more abundant in the intracellular membranes. Insulin increases the GLUT-4 but not the GLUT-1 transporter content of the plasma membrane (A.G. Douen, T. Ramlal, S. Rastogi, P.J. Bilan, G.D. Cartee, M. Vranic, J.O. Holloszy, and A.K., unpublished observations).

GLUCOSE TRANSPORTERS IN DIABETIC RAT MUSCLE

Whole-body glucose uptake is decreased in streptozocin-induced diabetic (STZ-D) animals (91) and in type II (non-insulin-dependent) diabetic individuals (92). Glucose uptake into muscle is decreased in STZ-D rats under basal and insulin-stimulated conditions (93). We recently investigated the molecular basis for this decrease in transport function.

In membranes isolated from hindquarter muscles of mild STZ-D rats, the number of glucose transporters, detected as cytochalasin B–binding sites, was found to be diminished (94). A decrease of 34% was observed in total membranes, suggesting that fewer glucose transporters are synthesized or that more transporters are degraded in the diabetic muscle relative to control tissue. Internal membranes from the insulin-responsive pool and plasma membranes were each partly depleted of glucose transporters relative to their counterparts in control animals (compare Fig. 1*B* with *A*). The reduction in glucose transporters in plasma membranes (68%) was larger than in either internal membranes (31%) or total membranes (34%), suggesting a specific loss of transporters from the cell surface. These observations in skeletal muscle differ from those in adipocytes of STZ-D rats in which neither basal glucose transport nor the number of cytochalasin B–binding sites in the plasma membrane are different from controls (95). This difference suggests that skeletal muscle is the main tissue responsible for the drop in basal whole-body glucose uptake observed in diabetes.

Because membrane-marker enzymes were not appreciably decreased in the membrane fractions obtained from skeletal muscle of STZ-D rats, it can be concluded that the reduction in glucose transporters is a specific response to the diabetic state. The animals in this study were only mildly diabetic; 1 wk after a 65-mg STZ/kg injection, glycemia was elevated about threefold, but the circulating insulin level in the fasting state was not significantly different from that in control rats (94). Thus, although the insulin levels were low relative to the prevailing hyperglycemia, the hormone concentrations bathing the muscles in vivo were probably not largely different in control and diabetic animals, suggesting that hyperglycemia, rather than hypoinsulinemia, may be the factor regulating glucose-transporter number in skeletal muscle plasma membrane. The nature of this regulation is under investigation.

Preliminary results indicate that, in contrast to the decrease in the number of cytochalasin B–binding sites, the amounts of GLUT-1 mRNA or GLUT-4 mRNA did not appreciably decrease in muscle from these mildly diabetic rats (S. Rastogi, U. Koivisto, M. Vranic, and A.K., unpublished observations). This suggests that in skeletal muscle of mildly diabetic rats, the decrease in glucose transporters detected by cytochalasin B binding may be because of decreased translation of the transporter mRNA and/or of increased degradation of the transporter protein. Which type of transporter decreases in parallel to the decrease in cytochalasin B binding has yet to be determined. In contrast to these results, it was recently reported that the amount of GLUT-4 mRNA decreased markedly in rat adipocytes (96–98) and modestly in skeletal muscle (98) from hypoinsulinemic diabetic rats. In these adipocytes, the amount of GLUT-4 but not of GLUT-1 protein (estimated immunologically with specific antibodies) was found decreased relative to controls (96–98).

The lower number of glucose transporters on the cell surface may be responsible for the poor basal glucose uptake displayed by diabetic muscle. In addition, the cause for the reduced response of glucose transport to acute insulin in STZ-D rats was recently investigated. The reduced size of the intracellular pool of glucose transporters suggested the possibility that there may be fewer glucose transporters available for translocation to the plasma membrane in response to the hormone. Interestingly, in STZ-D rats treated with supramaximal concentrations of insulin for 20 min, there was a redistribution of glucose transporters such that their number in the plasma membrane increased by almost twofold (A.K., T. Ramlal, G. Cartee, E. Gulve, and J.O. Holloszy, unpublished observations) (Fig. 1*B*). The hormone caused a somewhat smaller increase in plasma membranes from diabetic and control animals. In addition, because membranes from diabetic rats had a decreased amount of glucose transporters in the basal state, the number of glucose transporters in the insulin-stimulated state remained lower than that in control muscle (by ~30%). Concomitantly, insulin stimulation caused a decrease in the number of glucose transporters in the intracellular pool of STZ-D rats (Fig. 1*B*). This suggests that the decrease in insulin-dependent glucose-transport activity typical of diabetic muscle is not related to an impairment in the mechanism of recruiting of glucose

transporters. Moreover, it suggests that the decrease in basal glucose-transporter number in the plasma membrane may be indirectly responsible for insulin resistance. This possibility is only speculative, because an estimation of the recovery of membranes from the muscle preparation is required. A complete account of all the transporters that leave the intracellular pool and appear in the plasma membrane is not methodologically possible. In contrast, in the diabetic rat adipocyte, it is the decrease in the number of glucose transporters in the intracellular pool that has been related to insulin resistance (96).

MECHANISM OF REGULATION OF GLUCOSE TRANSPORT BY ACUTE EXERCISE IN MUSCLE

It has long been recognized that muscle contraction increases glucose uptake in this tissue (99). A short period of in vivo exercise, typically a 30-min bout of treadmill running or swimming, brings about a three- to fivefold increase in glucose uptake in the hindquarter muscles or isolated epitrochlearis muscles (100). Similarly, 10 min of electrically induced contraction, whether in vivo by stimulation of the sciatic nerve or in vitro by direct stimulation of isolated epitrochlearis muscles, results in substantial elevation in glucose uptake (100). This elevation persists for several hours after exercise (101), and its duration partly depends on the feeding status of the animals (102). The biochemical basis for the increase was investigated simultaneously by three groups. Hirshman et al. (103), using a modification of the preparation of Grimditch et al. (82), isolated plasma membranes from gastrocnemius muscles of treadmill-exercised rats and detected a near-twofold increase in the number of cytochalasin B–binding sites relative to membranes from unexercised animals. Douen et al. (104), using the preparation of Klip et al. (65), demonstrated a near-twofold increase in the number of glucose transporters in plasma membranes isolated from hindquarter muscles of treadmill-exercised rats (Fig. 2). The K_d of cytochalasin B binding was not affected by exercise (Table 1). Interestingly, the intracellular membranes prepared from hindquarter muscles that were insulin sensitive (Fig. 1*A*) did not show a decrease in the number of cytochalasin B–binding sites in response to acute exercise (Fig. 2; 104). In contrast, Fushiki et al. (86) confirmed the increase in glucose transporters in the plasma membrane and further detected a decrease in glucose transporters in the internal membranes from quadriceps and gastrocnemius muscles after a 2-h treadmill run. Similarly, an increase in work load in isolated rat hearts caused an increase in cytochalasin B binding in the plasma membrane fraction and a decrease in the internal membrane fraction (105).

Hence, these four studies with different muscle preparations concluded that at least part of the increase in glucose uptake that occurs during exercise is the result of a redistribution of glucose transporters to the plasma membrane. An exception to this conclusion is a study by Sternlicht et al. (106) that failed to find an increase in cytochalasin B–binding sites in plasma membranes from treadmill-exercised rats. The reason for this discrepancy is not known, especially because the study by Fushiki et al. (86) used the same membrane preparation protocol as the Sternlicht study (Table 1).

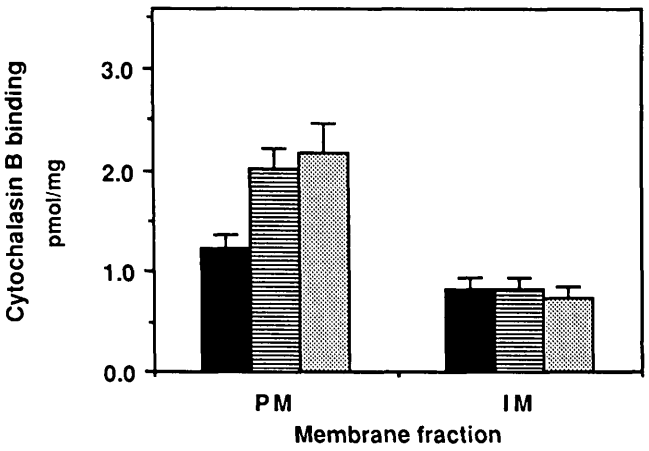

FIG. 2. Effects of exercise and of insulin following exercise on glucose transporters in rat skeletal muscle. Rats were exercised for 45 min by treadmill run as described earlier (104), and, where indicated, followed by 20 min of supramaximal insulin perfusion. Hindquarter muscles were immediately isolated, trimmed of all visible fat, connective, and nervous tissue, and used for subcellular fractionation (104). Glucose transporters were assessed by D-glucose–protectable binding of cytochalasin B. PM, plasma membrane fraction; IM, intracellular membrane fraction. Values are means ± SE of at least 4 independent experiments, each performed in triplicate. Basal rats, *solid bars*; exercise-treated rats, *striped bars*; exercise- and insulin-treated rats, *shaded bars*.

The above experiments were performed in normal overnight-fasted (104) or fed rats in which basal levels of insulin may play a role in the effect of exercise. It was previously reported, however, that an increase in glucose uptake is observed in response to exercise in diabetic rats in the absence of detectable circulating insulin (93). This, coupled to the observation of stimulation of glucose transport by electrically induced contraction in isolated muscles, strongly suggests that the exercise-mediated stimulation of glucose transport is not mediated by the hormone. However, in addition to increasing glucose transport independently of insulin, exercise also potentiates insulin action by increasing both insulin responsiveness and sensitivity. These effects outlast the stimulation of glucose transport by exercise alone in the absence of the hormone (107). Recent work suggests that the basis for the effects of exercise on insulin responsiveness and sensitivity is different, and only the recovery of resting-level sensitivity is coupled to overcompensation of the replenishment of glycogen stores in the muscle (107).

The molecular basis for the modulation of insulin ac-

tion by exercise has not been determined. Sternlicht et al. (106) failed to see additive recruitment of glucose transporters by insulin and exercise when the hormone was added immediately after the treadmill run and in fact observed a decrease in glucose-transporter number in the plasma membrane relative to that of unexercised insulin-treated animals. In contrast, work from our laboratory (A. Douen, G. Cartee, T. Ramlal, and A.K., unpublished observations) detected the same increase in glucose-transporter number caused by either exercise or exercise followed by insulin (Fig. 2). Thus, the additivity in glucose uptake that is seen in the exercised and insulin-treated hindquarter muscles relative to the two independent stimuli is not reflected in a further increase in the number of glucose transporters in the plasma membrane. The additive nature of the response of glucose uptake to insulin after recruitment of transporters by exercise may result from an increase in the intrinsic activity of the exercise-recruited transporters. This possibility has not been experimentally tested.

REGULATION OF GLUCOSE TRANSPORT IN MUSCLE CELL CULTURES

Advantages of studies in muscle cell cultures. The study of glucose-transport regulation demands the elucidation of the signals that communicate between occupied insulin receptors and intracellular glucose transporters. Such studies require an intact cell system because in vitro reconstitution of the signal-transduction pathway has not been achieved.

Whereas isolated skeletal muscles have been widely used to study the regulation of carbohydrate metabolism by insulin, the inability to determine initial rates of hexose transport, the fiber heterogeneity of the individual muscles, and the large extracellular space compromise an in-depth study of the mechanism of signal transduction. In contrast, muscle cells in culture offer the possibility of measuring the kinetic parameters of glucose transport in a system of homogeneous cell types and with minimal extracellular space. These and other advantages of cell culture over isolated muscles have previously been reviewed (14). Clearly, muscle cell lines should only be considered as a model of insulin-responsive cells, not necessarily as a paradigm of adult skeletal muscle.

Characteristics of glucose-transport regulation in muscle cell lines. Studies to characterize glucose-transport regulation in muscle cell lines have been carried out in our laboratory with the rat L6 cell line of skeletal muscle (108) and in the laboratory of Pollet et al. with the mouse BC_3H1 cell line of smooth muscle. The basal rate of hexose (2-deoxyglucose) transport decreases in L6 cells on fusion into myotubes (108) and in the nonfusing BC_3H1 cells on differentiation from myoblasts to myocytes (109). Concomitantly, the number of insulin receptors decreases by ~50% in both cell types (109, 110). Insulin stimulates hexose transport by ~100% in L6 cells (108) and by ~200% in BC_3H1 cells (109), and this response to the hormone is significant only after cell differentiation and/or fusion. The hexose-transport response to the hormone is fast (detectable within 10 min) and peaks at ~60 min. The concentration dependence of insulin action in these cells is shifted to the right relative to adult skeletal muscle, with half-maximal stimulation occurring at $\sim 5 \times 10^{-8}$ M insulin. The response to the hormone within the first 60 min is independent of protein synthesis insofar as it is insensitive to inhibition by cycloheximide (108,111).

We have demonstrated that, as in skeletal muscle, acute (up to 60 min) exposure to insulin causes translocation of glucose transporters from intracellular membranes to the cell membrane in L6 muscle cells (112), confirming that the cell line resembles the adult tissue in this aspect of the mechanism of stimulation of glucose transport. The absolute number of picomoles of cytochalasin B–binding sites disappearing from intracellular membranes matched the number recovered in the plasma membranes. Moreover, the increase in glucose-transport activity caused by insulin (70%) was matched by the increase in glucose-transporter number. Thus, in L6 muscle cells in culture, recruitment of glucose transporters suffices to explain the acute stimulation of glucose transport caused by insulin. The type of glucose transporter that is recruited remains to be determined. L6 cells express proteins that cross-react with antibodies to GLUT-1 and GLUT-4 (U. Koivisto, H. Martinez, and A.K., unpublished observations). Interestingly, studies with forskolin, a recently identified inhibitor of glucose transport, revealed differences between the basal and the insulin-stimulated transport (113). Whereas inhibition by forskolin of basal transport was readily reversible, the insulin-stimulated fraction of transport (measured after full activation by the hormone) was obliterated by subsequent treatment with this agent. A plausible explanation for this observation is that the glucose transporters recruited to the plasma membrane by insulin differ pharmacologically from those in the basal state or that signals necessary to maintain elevated transport are suppressed by forskolin.

Potential signaling mechanisms of stimulation of hexose transport by insulin. Studies of the potential messenger signals responsible for stimulation of glucose transport by insulin have met with relatively little success. The search for potential mediators has included the evaluation of the roles of changes in cytoplasmic Ca^{2+}, the membrane potential, cytoplasmic pH, protein kinase C activity, glycerolipids, and inositol phosphoglycans.

Insulin failed to change the levels of cytoplasmic Ca^{2+} in either L6 muscle cells (114) or heart myocytes (115). Similar observations have been made in 3T3-L1 fibroblasts and 3T3-L1 adipocytes (116) and in Swiss fibroblasts (117). In contrast, insulin caused a modest increase in cytoplasmic Ca^{2+} in rat adipocytes (118). However, this increase occurred after stimulation of hexose transport was complete. Conversely, chelation

of intracellular Ca^{2+} coupled to removal of extracellular Ca^{2+} failed to block stimulation of glucose uptake by the hormone in L6 muscle cells (114), suggesting that changes in the cytoplasmic concentration of the cation are not necessary for insulin activation of this function. Hence, recruitment of glucose transporters occurs in the absence of generalized changes in the levels of cytoplasmic Ca^{2+}.

Similarly, insulin failed to change the membrane potential in L6 muscle cells (119). Moreover, neither membrane hyperpolarization through the opening of Ca^{2+}-dependent K^+ channels nor membrane depolarization induced with the monovalent cation ionophore gramicidin caused stimulation of glucose transport. These conditions also did not prevent stimulation of hexose transport by insulin, suggesting that changes in membrane potential do not participate in insulin signaling.

In contrast to the lack of effects of insulin on cytoplasmic Ca^{2+} or membrane potential, the hormone rapidly increased the cytoplasmic pH by ~0.10 pH units in L6 muscle cells (120). This increase was mediated by activation of Na^+-H^+ exchange and cytoplasmic alkalinization has been claimed to participate in insulin stimulation of phosphofructokinase and thus of glycolysis (121). In contrast, prevention of the alkalinization by inhibition of Na^+-H^+ exchange did not prevent insulin stimulation of hexose transport (120,122), suggesting that different signals mediate activation of this function and glycolysis.

Activation of protein kinase C has been proposed to participate in stimulation of hexose transport by insulin in BC_3H1 myocytes (123) but not in L6 myotubes (124). A thorough evaluation of the experimental evidence for and against participation of this kinase in stimulation of glucose transport is beyond the scope of this review but has been reviewed elsewhere (125). The most compelling evidence against a role for the kinase in L6 cells is that downregulation of the kinase by prolonged exposure to phorbol esters did not prevent stimulation of glucose transport by insulin yet prevented further stimulation of glucose transport by reexposure to phorbol dibutyrate (124).

Regulation of glucose transport, transporters, and mRNA by chronic insulin administration. As stated above, L6 muscle cells express both the GLUT-1 and GLUT-4 transporters based on specific immunological detection in whole-cell extracts and on detection of the corresponding mRNAs (U. Koivisto, H. Martinez, and A.K., unpublished observations). The level of GLUT-1 mRNA is regulated by environmental factors in these cells. Thus, prolonged incubation (>2 h) with insulin leads to an increase in the levels of GLUT-1 mRNA. Under basal conditions, cells have barely detectable levels of GLUT-1 mRNA; on addition of insulin, there is a marked increase in GLUT-1 mRNA. The increase is detectable 2 h after insulin addition, peaks after 6 h, and then declines somewhat but remains elevated for up to 24 h (111). Concomitantly, there is an increase in glucose-transport activity (up to 4-fold stimulation after 24 h) above that observed with acute exposure to insulin (just under 2-fold stimulation after 1 h). The effect on transport activity of prolonged exposure to insulin is accompanied by an increase in immunodetectable GLUT-1 glucose transporter and is blocked by cycloheximide (111), suggesting that net transporter synthesis underlies the physiological response. This characteristic differentiates the acute and chronic effects of the hormone on glucose transport. Thus, insulin regulates the expression of GLUT-1 mRNA and GLUT-1 transporter protein. In contrast, the levels of GLUT-4 mRNA are not increased by prolonged exposure to insulin (U. Koivisto, H. Martinez, and A.K., unpublished observations).

Downregulation of glucose transport and transporters by hyperglycemia. Glucose-transport activity in L6 muscle cells is highly sensitive to the glucose concentration in the medium. The rate of hexose uptake decreases severalfold on introduction of cells previously incubated in zero-glucose medium (supplemented with xylose as a carbon source) to high-glucose (25 mM) medium. The decrease in transport activity correlates inversely with the concentration of glucose in the medium (Fig. 3). Transport is inhibited by >30% on increasing the extracellular glucose concentration from 5 to 15 mM, concentrations that mimic those circulating in plasma of normal and diabetic patients, respectively. This reflects an adaptive response of muscle to changes in glycemia that is fast (i.e., it is observed as early as 30 min after the change of medium) and is complete after 4 h (U. Koivisto, H. Martinez, and A.K., unpublished observations). A comparison of the number of glucose transporters in the plasma membrane of cells grown for 24 h in either zero- or high-glucose medium shows a decrease in the number of transporters in the plasma

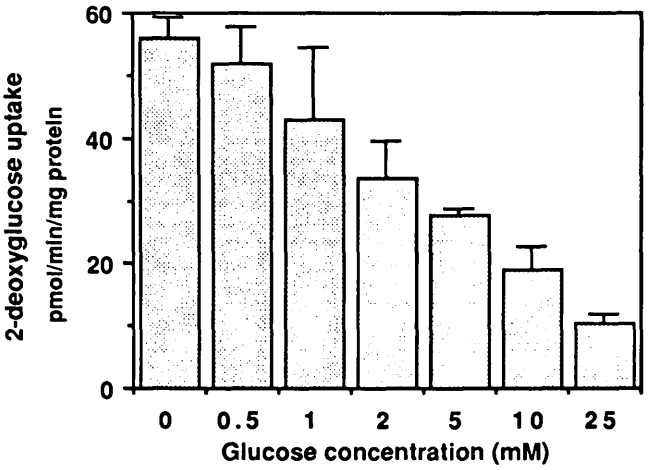

FIG. 3. Downregulation of glucose transport activity in L6 muscle cells by hyperglycemic medium. L6 muscle cells were incubated for 24 h with indicated concentrations of D-glucose. Medium was changed to glucose free, and 2-deoxyglucose (hexose) transport was immediately determined as described earlier (111). Values are means ± SE of 4 independent experiments, each performed in triplicate.

membrane and a concomitant increase in the intracellular membrane pool (Fig. 4; 76). Thus, it can be proposed that hyperglycemia regulates glucose transport and that glucotoxicity occurs in muscle cells in culture.

The downregulation of glucose transport by glucose is a reversible process. Cells grown in high-glucose medium show an increase in glucose-transport activity on incubation in zero-glucose medium. This increase in transport is evident as early as 30 min after the medium change and continues to increase for up to 24 h (111). Neither early nor late phases are inhibited by addition of cycloheximide (76), although the levels of GLUT-1 mRNA and of immunologically detected GLUT-1 transporter protein are elevated after 12 h. This suggests that the concentration of glucose in the medium can regulate GLUT-1 mRNA; furthermore, stimulation of glucose-transport activity can precede the increase in this mRNA. The newly formed GLUT-1 mRNA and GLUT-1 transporters may be synthesized as a preventive measure for further glucose deprivation, but this possibility remains to be analyzed by measuring the cycloheximide sensitivity of glucose transport after longer periods (>24 h) in zero-glucose medium. In contrast, glucose deprivation does not increase the level of GLUT-4 mRNA (U. Koivisto, H. Martinez, and A.K., unpublished observations).

Glucose transport in human muscle cell cultures. We have developed muscle cell cultures derived from satellite cells present in biopsies of human skeletal muscle (126). These cells, after clonal selection for fusion potential, differentiate in culture to form multinucleated myotubes. Glucose transport in fused cells is inhibited by cytochalasin B. Glucose transport in human muscle cell cultures is regulated in a qualitatively similar fashion to that in muscle cell lines, because it is stimulated by insulin (126) and insulinlike growth factor I, and downregulation of glucose transport occurs in response to high glucose in the medium (unpublished observations).

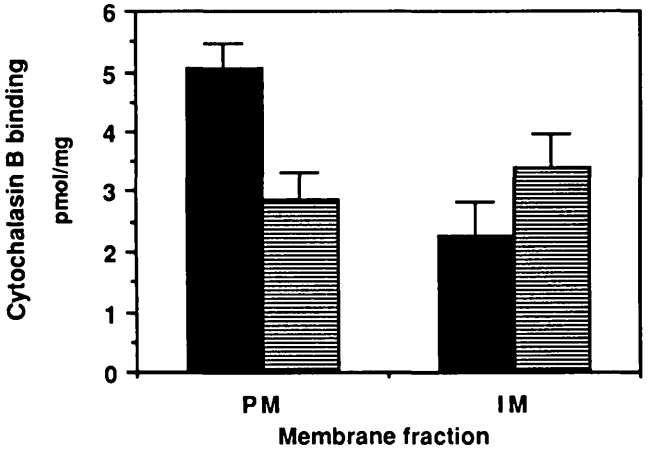

FIG. 4. Downregulation of glucose transporters by hyperglycemic medium in L6 muscle cells. Cells were incubated for 24 h in presence (*shaded bars*) or absence (*solid bars*) (xylose-substituted medium) of 25 mM glucose. Medium was changed, and subcellular membrane fractions were immediately prepared as described earlier (112). Number of glucose transporters determined by D-glucose–protectable binding of cytochalasin B was determined in plasma membranes (PM) and intracellular membranes (IM). Values are means ± SE of 4 independent experiments, each performed in triplicate.

DISCUSSION

Two glucose transporters coexist in adult skeletal muscle and in muscle cells in culture, GLUT-1 and GLUT-4. In adult skeletal muscle, there is translocation of glucose transporters to the plasma membrane in response to acute doses of insulin or acute periods of exercise, as detected by D-glucose–protectable binding of cytochalasin B. Because this compound binds to the two types of glucose transporters, it is unclear whether both are translocated in response to acute insulin or exercise. If so, their relative participation to the increase in transport activity should be defined.

In STZ-D, there is an overall decrease in muscle glucose-transporter number detected by binding of cytochalasin B. This may explain the decrease in basal glucose uptake observed in diabetic muscle. The translocation mechanism appears intact in that approximately the same increase in number of glucose transporters in the plasma membrane is observed in both control and diabetic rat muscle in response to a supramaximal acute dose of insulin. However, the final amount of glucose transporters at the cell surface after insulin stimulation is less in diabetic than in control muscle, thus correlating with the observed insulin resistance (reduced responsiveness).

In L6 muscle cells in culture, translocation of transporters occurs in response to acute exposure to insulin, and downregulation of transporters occurs in response to an increase in the concentration of glucose in the medium. The signals that mediate communication between the insulin receptor and the different glucose transporters remain unknown, but changes in cytoplasmic Ca^{2+}, pH, or membrane potential have been ruled out.

In L6 muscle cells in culture, increased levels of the GLUT-1 mRNA and GLUT-1 transporter protein are observed in response to prolonged hypoglycemia or prolonged hyperinsulinemia. Elevations in the glucose concentration in the medium depress the levels of GLUT-1 mRNA, of GLUT-1 protein, and of transporter present in the plasma membrane (detected by cytochalasin B binding). Thus, the glucotoxicity theory of DeFronzo (1) is confirmed in muscle cells in culture.

Elucidation of the type of transporter operating in each of the above conditions and its regulation at the levels of transport activity, subcellular distribution, and protein synthesis will help in understanding the complex nature of glucose homeostasis in skeletal muscle. The main conclusion is that muscle glucose transporters redistrib-

ute between cellular compartments in response to metabolic conditions such as hyperglycemia, acute insulin administration, and exercise.

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