Epinephrine translocates GLUT-4 but inhibits insulin-stimulated glucose transport in rat muscle

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Han, Xiao-Xia, and Arend Bonen. Epinephrine translocates GLUT-4 but inhibits insulin-stimulated glucose transport in rat muscle. Am. J. Physiol. 274 (Endocrinol. Metab. 37): E700–E707, 1998.—We examined the effects of epinephrine (25, 50, and 150 nM) on 1) basal and insulin-stimulated 3-O-methylglucose (3-MG) transport in perfused rat muscles and 2) GLUT-4 in skeletal muscle plasma membranes. Insulin increased glucose transport 330–600% in three types of skeletal muscle [white (WG) and red (RG) gastrocnemius and soleus (SOL)]. Glucose transport was also increased by epinephrine (22–48%) in these muscles (P < 0.05). In contrast, the insulin-stimulated 3-MG transport was reduced by epinephrine in all three types of muscles; maximal reductions were observed at 25 nM epinephrine in WG (−25%) and RG (−32.5%). A dose-dependent decrease occurred in SOL (−27% at 25 nM; −55% at 150 nM, P < 0.05). Insulin (20 µM/ml) and epinephrine (150 nM) each translocated GLUT-4 to the plasma membrane, and no differences in translocation were observed between insulin and epinephrine (P > 0.05). In addition, epinephrine did not inhibit insulin-stimulated GLUT-4 translocation, and the combined epinephrine and insulin effects on GLUT-4 translocation were not additive. The increase in surface GLUT-4 was associated with increases in muscle cAMP concentrations, but only when epinephrine alone was present. No relationship was evident between muscle cAMP concentrations and surface GLUT-4 in the combined epinephrine and insulin-stimulated muscles. These studies indicate that epinephrine can translocate GLUT-4 while at the same time increasing glucose transport when insulin is absent, or can inhibit glucose transport when insulin is present.

skeletal muscle: red gastrocnemius; white gastrocnemius; adenosine 3',5'-cyclic monophosphate; perfusion

IT IS GENERALLY BELIEVED that glucose transport is the rate-limiting step in glucose utilization in muscle (29). To increase glucose transport in this tissue, the glucose transporter GLUT-4 is translocated from intracellular pool(s) to the surface of the muscle by insulin and exercise, albeit by different signaling pathways that remain largely unknown (2, 3, 14, 33, 46, 50). Some studies have associated all changes in glucose transport directly with the appearance of GLUT-4 at the surface of the cell (32, 33, 49) or to changes in accessibility of glucose to the cell surface GLUT-4 transporter (44). However, a number of studies have also presented evidence to indicate that glucose transport can be altered as a result of changes in the activity of the surface GLUT-4 (1, 13, 16, 27, 28).

Catecholamines can have profound effects on glucose transport in adipocytes, heart, and skeletal muscle. In adipocytes the β-adrenergic agonist isoproterenol stimulates both GLUT-4 translocation and glucose transport at low concentrations (10–25 nM) and inhibits glucose transport at high concentrations (1 µM) (26, 34) because of an inhibition of the transport activity of surface GLUT-4 (34). cAMP has been implicated in this process, because dibutyryl-cAMP (DBcAMP) increased glucose transport in adipocytes at low concentrations (10 µM) and inhibited glucose transport to below basal transport rates with high concentrations (1,000 µM) (28, 34). In addition, insulin and DBcAMP (1,000 µM) stimulated GLUT-4 translocation additively, but the insulin-stimulated glucose transport was reduced (28).

In contrast, in cardiac myocytes, β-adrenergic stimulation failed to increase glucose transport, whereas α-adrenergic stimulation of glucose transport was increased because of a recruitment of GLUT-4 to the cell surface (13); however, this did not involve a cAMP-dependent mechanism (12). In skeletal muscle, epinephrine (24 nM) increased basal glucose uptake (43), but much higher concentrations of epinephrine (>60 nM) increased surface GLUT-4 while concurrently lowering glucose transport (1). Thus, in skeletal muscles, epinephrine also appears to exhibit a biphasic effect on glucose transport, possibly by altering the activity of surface GLUT-4 transporters.

The effects of epinephrine on glucose transport and GLUT-4 translocation may be muscle fiber specific. Muscles rich in oxidative fibers appear to have a greater density of β2-adrenergic receptors (>90% β2 receptors) than muscles rich in glycolytic fibers (23, 48). Oxidative muscles also have considerably more GLUT-4 transporters than glycolytic muscles (18, 19, 36), resulting in a greater increase in insulin-stimulated glucose transport in oxidative muscles (18, 19, 36). When these observations are considered together, it is possible that the inhibitory effects of epinephrine on glucose transport are greater in oxidative than in glycolytic muscles. Moreover, by examining the independent and combined effects of insulin and epinephrine on skeletal muscle 3-O-methylglucose (3-MG) transport and plasma membrane GLUT-4 content, it is possible to determine whether epinephrine alters 1) the activity of GLUT-4 or 2) the insulin-induced GLUT-4 translocation. Therefore, we examined in three types of rat skeletal muscles [soleus (SOL), red gastrocnemius (RG), and white gastrocnemius (WG)] 1) the effects of epinephrine on basal and insulin-stimulated 3-MG transport and 2) the independent and concomitant effects of epinephrine and insulin on GLUT-4 content in skeletal muscle plasma membranes.

METHODS

Hindquarter perfusion. Male Sprague-Dawley rats weighing about 280 g were used in the present experiments. Ethical approval for this work was obtained from the committee on animal care at the University of Waterloo. Rats were anesthetized with intraperitoneal injection of pentobarbital sodium.
membranes. RG muscles were removed, trimmed in ice-cold glucose transport, we measured GLUT-4 only in the plasma were prepared only from these muscles. Moreover, because responsive to epinephrine and insulin, plasma membranes Glucose transport Because the RG muscles were highly responsive to epinephrine and insulin, a 15-min preperfusion period was used again; during the next 25 min, epinephrine (0, 25, and 150 nM) was present for the first 10 min and for the last 15 min both epinephrine (0, 25, and 150 nM) and insulin (20 µU/ml) were present together. Insulin-stimulated (20 µU/ml) glucose transport was determined while the hormone was present during the last 15 min of the experiments.

Glucose transport was measured using 8 µCi of 3-O-methyl-[3H]glucose (3-[3H]MG), together with unlabeled 3-MG, to attain a final near-saturating concentration of 40 mM (40). Extracellular space determination was determined with 16 µCi of [14C]mannitol. At the end of the perfusion period, perfusate samples and muscle samples from the superficial part of the gastrocnemius muscle [WG; consisting mainly of fast-twitch glycolytic (type IIb) fibers], the deep part of the medial head of the gastrocnemius muscle [RG; consisting mainly of slow-twitch oxidative (type I) fibers] and SOL [consisting mainly of fast-twitch oxidative (type IIa) fibers], and SOL [consisting mainly of slow-twitch oxidative (type I) fibers] (1) were collected. Glucose transport, corrected for extracellular space, was expressed as micromoles per gram of muscle tissue per 5 min, as we have done previously (18, 19).

Membrane preparation. Hindquarters were perfused with epinephrine (25 and 150 nM) in the absence of insulin and in the presence of maximal insulin (20 µU/ml) as described in Glucose transport. Because the RG muscles were highly responsive to epinephrine and insulin, plasma membranes were prepared only from these muscles. Moreover, because the GLUT-4 located on the surface of the muscle cell promotes glucose transport, we measured GLUT-4 only in the plasma membranes. RG muscles were removed, trimmed in ice-cold saline, and freeze-damped in liquid N2 for later membrane isolation. Membranes were prepared using a modification (21) of the procedures initially described by Klip et al. (30). Potassium-stimulated p-nitrophenylphosphatase activity (K+-pNPPase) was assayed in muscle homogenates and plasma membranes as described by Ploug et al. (42). With this measure, the purity of the plasma membrane fractions over the homogenate was increased 17.5 times (P < 0.05) (data not shown). This compares favorably with skeletal muscle plasma membrane preparations published elsewhere (17).

GLUT-4 measurement in plasma membranes. The amount of GLUT-4 protein in muscle plasma membranes of various treatment groups was determined with Western blotting as we have previously reported (25), by use of a commercially available GLUT-4 polyclonal antibody (East Acres Biologicals). An enhanced chemiluminescence detection procedure (ECL, Amersham) was used to visualize the GLUT-4 protein. The quantification of GLUT-4 was carried out by using a scanner (Abaton) and a Macintosh LC computer with appropriate software (Scan Analysis, Biosoft, Cambridge, UK).

cAMP determination. cAMP was determined as described elsewhere (41). Briefly, cAMP was extracted from frozen muscle homogenized in 10 volumes of 7% trichloroacetic acid. The precipitate was removed by centrifugation, and the supernatant was washed five times with 5 volumes of water saturated with deethyl ether. The remaining aqueous extract was lyophilized and dissolved in 50 mM Tris buffer, pH 7.5, containing 4 mM EDTA. Determination of the cAMP concentrations was performed with a [3H]cAMP protein binding assay kit (Amersham, Oakville, ON, Canada).

Statistical analyses. Analyses of variance were used to compare the effects of epinephrine, insulin, and insulin plus epinephrine on glucose transport, GLUT-4 translocation, and cAMP. We also transformed the cAMP data to percentage values of control conditions (i.e., when muscles were perfused without either epinephrine or insulin). This condition was defined as 100%, and then the cAMP data were recalculated as a percentage relative to the mean control. Data in these groups were then analyzed with ANOVA. This then permitted us to examine changes in 3-MG transport and plasma membrane GLUT-4 in relation to cAMP. All data are expressed as means ± SE.

RESULTS

Basal and insulin-stimulated 3-MG glucose transport. The highest basal 3-MG transport rate was observed in the RG, in which transport was slightly (15%) but consistently higher than in SOL (P < 0.05). Basal 3-MG transport was 46% lower in WG than in RG (P < 0.05) (Fig. 1).

![Fig. 1. 3-O-methylglucose transport (means ± SE; n = 5 animals for each condition) in perfused rat hindlimb muscles in the absence (basal) and presence of insulin (20 µU/ml). *P < 0.05, basal red gastrocnemius (RG) vs. basal white gastrocnemius (WG), basal soleus (SOL) vs. basal WG; ††P < 0.05, basal RG vs. basal SOL; *P < 0.05, insulin-stimulated RG vs. basal in each muscle; **P < 0.05, insulin-stimulated RG vs. insulin-stimulated WG, insulin-stimulated SOL vs. insulin-stimulated WG.](http://ajpendo.physiology.org/)

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In the presence of maximally stimulating concentrations of insulin, glucose transport was increased in all three types of muscles (Fig. 1, P < 0.05). The insulin-stimulated transport rates, compared with basal transport rates of each muscle, were 3.3 times greater in WG and 6.0 and 5.0 times greater in SOL and RG, respectively. No differences were observed in the insulin-stimulated glucose transport rates between RG and SOL (P > 0.05; Fig. 1).

Effects of epinephrine on 3-MG glucose transport. Epinephrine increased glucose transport, compared with basal transport rates, in both RG and WG muscles (Fig. 2, P < 0.05). 3-MG transport was maximally stimulated by 25 nM of epinephrine (Fig. 2), because transport rates did not differ among any of the epinephrine concentrations used in this study (P > 0.05). When all epinephrine concentrations used were taken into account, 3-MG transport was increased over basal transport rates by +32% in WG (P < 0.05), by +48% in SOL (P < 0.05), and by +22% in RG (P < 0.05). The absolute increases were greater in the SOL (Δ = +0.28 ± 0.05 μmol·g⁻¹·5 min⁻¹) than in either the RG (Δ = +0.14 ± 0.03 μmol·g⁻¹·5 min⁻¹) (P < 0.05) and WG (Δ = +0.16 ± 0.03 μmol·g⁻¹·5 min⁻¹) (P < 0.05). These slight absolute differences between RG and WG were not significant (P > 0.05).

Effects of epinephrine on insulin-stimulated glucose transport. Epinephrine decreased the insulin-stimulated 3-MG transport in all three muscles (Fig. 3, P < 0.05). In WG and RG the largest decrease in 3-MG transport occurred at an epinephrine concentration of 25 nM (P < 0.05; WG: Δ = −0.41 ± 0.18 μmol·g⁻¹·5 min⁻¹ or −25 ± 11.5%; RG: Δ = −1.05 ± 0.36 μmol·g⁻¹·5 min⁻¹ or −32.5 ± 11.2%). No further decreases in insulin-stimulated 3-MG transport were observed in either WG or RG at 150 nM epinephrine. In contrast, in the SOL there was a marked decrease in insulin-stimulated 3-MG transport at the low epinephrine concentration (25 nM) (P < 0.05; Δ = −0.95 ± 0.10 μmol·g⁻¹·5 min⁻¹ or −26.9 ± 4.5%), and insulin-stimulated 3-MG transport was lowered even further with 150 nM epinephrine (P < 0.05; Δ = −1.99 ± 0.05 μmol·g⁻¹·5 min⁻¹ or −55.1 ± 1.4%). At the highest concentration of epinephrine (150 nM) the absolute 3-MG transport decrements in RG and SOL were greater than in WG (P < 0.05).

Effects of insulin and epinephrine on plasma membrane GLUT-4. For the studies of plasma membrane GLUT-4, we used only RG muscles. These muscles have a high concentration of GLUT-4 (18, 19, 36), and this muscle was highly responsive to epinephrine (Figs. 2 and 3). At the same time RG muscles can also provide sufficient tissue (700 mg) to obtain plasma membranes.

Insulin increased the appearance of GLUT-4 in the plasma membranes (Fig. 4, P < 0.05). Epinephrine also increased GLUT-4 in the plasma membrane at both low (25 nM; P < 0.05) and high epinephrine concentrations (150 nM; P < 0.05) (Fig. 4). The GLUT-4 increase in the plasma membrane induced by 25 nM epinephrine was somewhat smaller (P < 0.05) than that provoked by maximal concentrations of insulin (Fig. 4). Combining insulin and epinephrine (25 nM) resulted in a greater plasma membrane GLUT-4 concentration than when only epinephrine (25 nM) was present (Fig. 4, P < 0.05). At the higher epinephrine concentration (150 nM), there was no difference in the increase in plasma membrane GLUT-4 compared with the GLUT-4 increase provoked by maximally stimulating levels of insulin (20 mU/ml), either alone or in the presence of...
150 nM epinephrine (P > 0.05; Fig. 4). Combining epinephrine (either 25 or 150 nM) and insulin (20 mU/ml) did not increase plasma membrane GLUT-4 in an additive manner (P > 0.05) compared with the increase in GLUT-4 provoked by insulin alone (Fig. 4).

Effects of insulin and epinephrine on muscle cAMP. The cAMP concentrations were increased over basal levels when epinephrine was perfused through the muscles [27% at 25 nM (P < 0.05); 61% at 150 nM (P < 0.05; Fig. 5)]. The increase at 150 nM was greater than at 25 nM (P < 0.05). When insulin alone was administered, the cAMP concentrations decreased sharply (−55%) (P < 0.05, Fig. 5). However, when epinephrine and insulin were both present, large increases in cAMP were observed (P < 0.05). However, the cAMP concentrations in the presence of insulin were somewhat lower at 25 nM epinephrine (−9.5%; P < 0.05) and 150 nM epinephrine (−16%; P < 0.05) than when only epinephrine was present in the perfusate (Fig. 5).

Comparisons of 3-MG transport, plasma membrane GLUT-4, and cAMP. Both insulin and epinephrine increased plasma membrane GLUT-4 compared with GLUT-4 found in the plasma membranes under basal conditions (P < 0.05; Fig. 4). Despite the similar increases in surface GLUT-4 provoked independently by epinephrine (150 nM) and insulin (20 mU/ml) and by epinephrine (150 nM) + insulin (20 mU/ml), there were very marked differences in 3-MG transport among these treatments. Epinephrine alone increased 3-MG transport only slightly (approximately +20%; P > 0.05; Fig. 2), whereas insulin increased 3-MG transport almost 25 times more (+500%). However, in the epinephrine (150 nM) + insulin (20 mU/ml) treatment, the 3-MG transport was sharply reduced (~50%) compared with the insulin-stimulated transport (Fig. 3).

Because the various experimental treatments also resulted in large differences in skeletal muscle cAMP (Fig. 5), it was possible to compare the changes in intramuscular cAMP with changes in plasma membrane GLUT-4 and 3-MG transport. When no insulin was present, the plasma membrane GLUT-4 (P < 0.05) and 3-MG transport were increased (P < 0.05) at the points when cAMP concentrations were also increased (Fig. 6A). Maximal effects on both GLUT-4 and 3-MG transport had already occurred with an ~25% increase in cAMP concentrations, since no further changes were observed thereafter (P > 0.05). In the presence of both insulin and epinephrine, there was a much wider range of cAMP concentrations (Fig. 6B). However, there was no obvious relationship between cAMP and plasma membrane GLUT-4 (Fig. 6B), whereas the insulin-stimulated 3-MG transport was lowered in relation to concurrent reductions in cAMP (P < 0.05; Fig. 6B).

DISCUSSION

In the present studies we have shown that 1) epinephrine increased the GLUT-4 appearance in the plasma membrane, 2) similar increases in surface GLUT-4 could be provoked by insulin or epinephrine, 3) epinephrine increased basal glucose transport in all types of skeletal muscles, and 4) whereas plasma membrane
GLUT-4 content remained elevated, insulin-stimulated glucose transport was greatly reduced by epinephrine. These results suggest that epinephrine reduced the glucose transport capacity of GLUT-4, but only when insulin was present.

Effects of epinephrine alone on 3-MG transport and GLUT-4. In the present studies epinephrine (25–150 nM) alone stimulated 3-MG transport in rat skeletal muscles. The maximal 3-MG transport increase occurred at a high physiological concentration of epinephrine (25 nM), with no further changes at the higher epinephrine concentrations (50 and 150 nM). Others have previously shown that epinephrine stimulated glucose transport in incubated rat skeletal muscles (38) and in perfused rat skeletal muscles (4, 43). In contrast, pharmacological concentrations of epinephrine (1 µM) (24) or high concentrations of isoproterenol (1 µM) (45) inhibit glucose transport in isolated muscles (24, 45). A similar dose-response effect of isoproterenol on glucose transport has also been observed in adipocytes [i.e., low isoproterenol concentrations (1–10 nM) increased glucose transport; high concentrations (1 µM) inhibited glucose transport (26, 34)]. In preliminary studies, high concentrations of epinephrine (>150 nM) caused high perfusion pressures in our preparation, resulting in edema, thereby limiting the epinephrine concentrations that can be used in perfused rat hindquarters.

It is very unlikely that the effects of epinephrine on glucose transport were mediated by GLUT-1. In Chinese hamster ovary (CHO) cells that express only GLUT-1, glucose transport was not altered by very high concentrations of DBcAMP (10 mM). In contrast, in CHO cells that express only GLUT-4, DBcAMP inhibited glucose transport (39). Moreover, GLUT-1 in muscle is found only on the plasma membrane (6, 20); hence glucose transport cannot be increased by translocating this transporter. Collectively, these studies suggest that the epinephrine effects on glucose transport are not due to GLUT-1 but are most likely mediated by the actions of epinephrine on GLUT-4.

From our studies it appears that epinephrine induced the translocation of GLUT-4 from intracellular locations to the plasma membrane. This confirms an earlier study from our laboratory in which a bolus injection of epinephrine increased plasma membrane GLUT-4 and lowered intracellular membrane GLUT-4 in muscle (1). Several studies, using adipocytes, have attributed increases in glucose transport to GLUT-4 translocation by means of cAMP-dependent mechanisms. With low DBcAMP concentrations (10 µM), GLUT-4 was translocated and glucose transport was increased in adipocytes (28, 34). In the present studies the epinephrine-induced increase in cAMP was associated with an increase in plasma membrane GLUT-4 and glucose transport in muscle. Thus the most parsimonious explanation for our observations would seem to be that epinephrine by itself induces GLUT-4 translocation, possibly involving a cAMP-dependent mechanism, and that this increase in the plasma membrane GLUT-4 resulted in a greater 3-MG transport.

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Fig. 6. Comparison between RG muscle cAMP and plasma membrane GLUT-4 and 3-O-methylglucose transport (3-MG; means ± SE; n = 4–5 animals for each condition) in the absence (A) or presence (B) of insulin. Data from Figs. 2–5 were used to obtain comparisons. cAMP in control condition (i.e., nonhormonally stimulated muscles) was designated as 100% (A). Mean values (%) relative to this control were plotted against plasma membrane GLUT-4 and 3-MG transport when muscles were perfused with neither epinephrine nor insulin or with only epinephrine (A) and when muscles were perfused only with insulin, or with insulin + epinephrine (B). A: GLUT-4 and 3-MG transport were increased relative to control condition (i.e., 100% cAMP; *P < 0.05). No differences were observed between conditions in which cAMP was >100% (P > 0.05). B: 3-MG transport was significantly reduced when cAMP concentrations were increased from 45 to >100% (*P < 0.05). Plasma membrane GLUT-4 was not affected by changes in cAMP.
Simultaneous effects of epinephrine and insulin on 3-MG transport and GLUT-4. Very different results occurred in our studies in the presence and in the absence of insulin. The insulin-stimulated glucose transport was markedly inhibited by epinephrine in perfused muscles. This has also been observed in muscle in previous studies (4). In studies with adipocytes (28) and L6 myoblasts (31), insulin-stimulated glucose transport was inhibited by high concentrations of the cAMP derivative DBcAMP (1–2 mM) (28, 31), although low concentrations of DBcAMP (10 µM) did not inhibit insulin-stimulated glucose transport in adipocytes (28). We also observed differences in responses among the muscles. In SOL the decrease occurred in a dose-dependent manner, whereas in RG and WG maximal inhibition was observed at the lowest epinephrine concentration used (25 nM). However, the absolute inhibition, as well as the relative inhibition (%), of 3-MG transport was much greater in RG and SOL than in Wg. The greater β-adrenergic responsiveness of oxidative muscles is presumably associated with the greater number of β-adrenergic receptors in these muscles (23, 47, 48).

To ascertain why insulin-stimulated 3-MG transport was lowered in rat muscles, we measured the GLUT-4 content of sarcolemmal membranes. In our studies the quantitative increase in insulin-stimulated glucose transport and the increase in plasma membrane GLUT-4 were quite dissimilar. This is commonly observed in studies in which the increase in plasma membrane GLUT-4 is compared with whole muscle glucose transport either in the presence of insulin, after exercise, or with epinephrine administration (1, 7–9, 15, 17). The GLUT-4 increase in the plasma membranes in these studies ranges from ~50 to 200%, whereas glucose transport in whole muscle increased much more (200–800%) (1, 7–9, 15, 17). It is thought that the quantitative discrepancies between plasma membrane GLUT-4 and whole muscle glucose transport are due to the isolation of plasma membranes in which the calculated recovery of plasma membranes is quite low. Because the tissues from the various experiments in the present studies were taken through identical procedures, we believe that the relative comparisons of GLUT-4 among the treatments are directly comparable. Such relative comparisons of GLUT-4 translocation have been performed in many previous studies despite the quantitative discordance with the increments in glucose transport (1, 7–9, 15, 17).

As expected, GLUT-4 was increased when muscles were stimulated with insulin. However, the addition of epinephrine to the perfusion medium before addition of insulin did not reduce the translocation of GLUT-4 to the plasma membrane. In adipocytes, low concentrations of isoproterenol (1 µM) (34) or DBcAMP (10 µM) (28) also failed to inhibit the insulin-induced translocation of GLUT-4. Because the insulin- and DBcAMP-induced GLUT-4 translocations were additive in adipocytes, it was proposed that insulin and cAMP recruit GLUT-4 to the surface by different mechanisms, possibly involving an inhibition of endocytosis by DBcAMP (28). However, in the insulin-stimulated skeletal muscles, when they were perfused simultaneously with epinephrine, there was no additive increase in plasma membrane GLUT-4 compared with independent perfusion with each hormone. Moreover, there was no relationship between plasma membrane GLUT-4 and muscle cAMP over a wide range of cAMP concentrations in muscle. Thus we cannot account for the reductions in insulin-stimulated glucose transport by a decrease of surface GLUT-4 by cAMP-dependent mechanisms, as has been proposed for adipocytes (28). Our results also suggest that the postreceptor insulin-signalizing mechanisms involved in activating GLUT-4 translocation are not affected by cAMP-dependent mechanisms. Similarly, in recent studies with cardiac myocytes, no role for cAMP in translocating GLUT-4 was evident (12).

An alternative explanation for the reduction in insulin-stimulated glucose transport in the face of the increased plasma membrane GLUT-4 content, together with increased muscle cAMP concentrations, is a reduction in the intrinsic transport activity of the surface GLUT-4, possibly involving cAMP-dependent mechanisms. It had been thought that GLUT-4 phosphorylation is increased in response to agents that increase intracellular cAMP (22, 31) and that this accounted for the decreased glucose transport in the face of unaltered or an increased GLUT-4 content in the plasma membrane. However, in studies with adipocytes in which the actions of isoproterenol were blocked by adenosine (37) and in studies with chimeric transporters (39), it has been shown that phosphorylation of GLUT-4 did not account for the DBcAMP-induced inhibition of glucose transport (37, 39). Moreover, it appeared that the inhibitory effects did not involve the activation of cAMP-dependent protein kinase (37, 39). On the basis of these studies, it appears that GLUT-4 phosphorylation by cAMP-dependent mechanisms is unlikely to cause the reduction in glucose transport in our studies.

A number of intracellular metabolites may alter glucose transport (11). Epinephrine administration reduces muscle glycogen and increases glucose 6-phosphate in muscle (24, 35). Reductions in muscle glycogen have been linked to increases in basal and insulin-stimulated glucose transport, but there was no association between increases in glucose 6-phosphate and reductions in glucose transport (24). Moreover, the epinephrine-induced inhibition of glucose transport was independent of the muscle glycogen concentrations (24). Thus any changes in muscle glycogen and glucose 6-phosphate induced by epinephrine in our studies are unlikely to cause the observed changes in glucose transport. However, other products of intermediary metabolism (e.g., pyruvate and lactate) have reduced 1) basal, 2) insulin-stimulated, and 3) phenylephrine-stimulated glucose transport in cardiac myocytes because of a reduction in cell surface GLUT-4 (11) rather than an alteration in GLUT-4 activity. Yet we did not observe any reductions in surface GLUT-4. Thus reduced glucose transport in our studies cannot be attrib-
uted to reductions in surface GLUT-4, as measured with Western blotting.

It appears that GLUT-4 can exist in several states in the adipocyte membrane. In one state GLUT-4 is functional and accessible to the substrate, and in the other state it is nonfunctional and unable to bind glucose or the bis-mannose photolabel, while immunodetection remains unaltered (44). These observations suggest that changes in glucose transport are attributable to changes in surface GLUT-4 accessibility rather than a change in the intrinsic activity of the GLUT-4 transporter. The question is then whether bis-mannose photolabeling, in contrast to immunodetection of GLUT-4, provides a more accurate measurement of surface GLUT-4, particularly in muscle. Despite the good concordance between glucose transport and surface GLUT-4, as detected with bis-mannose photolabeling in some studies (10, 33, 44, 49), there are also a considerable number of exceptions in which increments in glucose transport and surface GLUT-4 are not quantitatively related. For example, recent data with cardiac myocytes show very clearly that bis-mannose photolabeling (GLUT-4, a 1.5- to 1.8-fold increase) does not account for the 4- to 5-fold increase in glucose transport observed with phenylerythritol stimulation of glucose transport (13). Similarly, the actions of insulin in adipocytes are not always accounted for by the surface GLUT-4 detected with the bis-mannose photolabel (see Fig. 6 in Ref. 5). Finally, in skeletal muscle, the reported high concordance between surface GLUT-4 and glucose transport (Ref. 10, and references therein) is undermined by the recent observation that the ratio of glucose transport (µmol·g⁻¹·10 min⁻¹) to cell surface GLUT-4 (dpm/mg) is double that in the epitrochlearis (−0.1 µmol·g⁻¹·10 min⁻¹·dpm⁻¹·mg⁻¹) compared with the soleus (−0.05 µmol·g⁻¹·10 min⁻¹·dpm⁻¹·mg⁻¹) (10). We suspect that in some skeletal muscles the bis-mannose photolabeling procedure fails to cross-link all of the GLUT-4. The ultraviolet (UV) irradiation required to induce the cross-linking of bis-mannose with surface GLUT-4 may fail to reach all of the fibers, particularly in a thicker muscle such as the soleus, in which it seems quite unlikely that the UV light would have penetrated to the deeper muscle fibers. Thus, from a number of studies, it seems that measurement of glucose transport cannot always be fully accounted for by the number of surface GLUT-4 transporters, whether for suspected technical reasons (10) or by unknown mechanisms that appear to alter the accessibility or activity of the GLUT-4 transporter (5, 13).

Nevertheless, whether Western blotting or bis-mannose photolabeling of surface GLUT-4 is used, there is normally an increase in GLUT-4 when plasma membrane GLUT-4 is increased and not a decrease in glucose transport in the face of increased surface GLUT-4, as we observed in the muscles perfused simultaneously with epinephrine and insulin. Therefore, the decrease in glucose transport is likely due to a reduced effectiveness of the translocated GLUT-4 transporters. Whether this is because of a reorientation of the transporter to make it less accessible to glucose, as suggested in one study (44), or because of changes in the intrinsic activity of GLUT-4 remains to be determined. Our evidence (increased GLUT-4 but decreased insulin-stimulated 3-MG transport by epinephrine) and recent evidence in cardiac myocytes [a 6.8-fold increase in insulin-induced stimulation of glucose transport in the face of an only 1.8-fold increase in surface GLUT-4 (13)] indicate that the glucose transport capacity of surface GLUT-4 can be altered to either lower or increase insulin-stimulated glucose transport. However, why there are differences in the glucose transport responses when only epinephrine is present compared with when both epinephrine and insulin are present in our studies remains unknown.

In summary, these experiments have shown that epinephrine can translocate GLUT-4 while at the same time increasing glucose transport, when insulin is absent, or inhibiting glucose transport, when insulin is present.

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