Regulation of the GLUT1 glucose transporter in cultured myocytes: total number and subcellular distribution as determined by photoaffinity labelling

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We have used the impermeant photoaffinity label 2-*N*-4-(1-azido-2,2,2-trifluoroethyl)benzoyl-[2-³H]1,3-bis-(*D*-mannos-4-yl)-2-propylamine (ATB-[2-³H]BMBA) to identify and quantify the glucose transporters on the surface of BC3H-1 cells, a continuously cultured skeletal-muscle cell line lacking the MyoD transcription factor required for cell fusion. ATB-[2-³H]BMBA was used in combination with immunoprecipitation of the GLUT1 glucose transporter, the only isoform expressed in these cells. The total cellular GLUT1 content was also determined by photolabelling and immunoprecipitation after cell permeabilization with digitonin (0.025%). In glucose-starved cells, 85% of the glucose transporters were present at the cell surface in the basal state, with little change in response to insulin (200 nM), correlating with lack of additional 2-deoxyglucose uptake in response to insulin. Feeding the cells with glucose (25 mM) for 24 h resulted in an 80% decrease in the total GLUT1 content

relative to starved cells, of which only 25% were present on the cell surface. This was associated with an 85% decrease in 2-deoxyglucose uptake. In addition, acute stimulation of the fed cells with insulin or phorbol 12-myristate 13-acetate (PMA) led to an increase in GLUT1 at the cell surface, and, in correspondence, an increase in 2-deoxyglucose uptake by approx. 2- and 4-fold respectively. We conclude that exofacial photoaffinity labelling of glucose transporters with ATB-[2-³H]BMBA in the presence and absence of digitonin, followed by specific immunoprecipitation, provides an accurate measure of total and cell-surface glucose transporters in differentiated BC3H-1 muscle cells. This technique demonstrates that glucose pre-feeding (1) decreases the total number of GLUT1 and (2) redistributes the majority of the remaining transporters to an intracellular site, where they can now be translocated to the cell surface in response to insulin and PMA.

INTRODUCTION

Skeletal muscle, the main organ responsible for glucose uptake *in vivo* in the insulin-stimulated state [1], expresses both the GLUT1 and GLUT4 glucose transporter isoforms [2,3]. GLUT1 transporters are thought to reside primarily at the plasma membrane and perineurially [4,5], and are thought to contribute to basal glucose uptake, whereas GLUT4 transporters play the primary role in glucose uptake into the muscle cell, especially in response to stimuli such as insulin and contraction [6–9]. Furthermore, GLUT4 transporters in skeletal muscle are thought to be normally distributed between their site of action on the cell surface and an intracellular pool where they are stored, much as has been established in adipose cells [8–10]. Insulin and exercise have been shown to stimulate glucose uptake in muscle, at least partly through recruitment of GLUT4 from intracellular membrane fractions to the cell surface [6–9]. However, attempts to correlate glucose transport activity in intact skeletal muscle with glucose transporter number in isolated cell-surface membrane fractions, as determined by cytochalasin B binding and Western immunoblotting, have yielded many discrepancies [11,12]. These observations have led investigators to conclude that both GLUT4 subcellular distribution and activity can be regulated, or alternatively, that contamination of the cell-surface membrane fractions with intracellular membranes prevents an accurate assessment of cell-surface GLUT4 by subcellular-fractionation techniques. Therefore, a method has been designed to overcome these problems, which involves the specific labelling of glucose transporters on the surface of intact cells with a radiolabelled

compound to differentiate them from intracellular glucose transporters [13].

2-*N*-4-(1-Azi-2,2,2-trifluoroethyl)benzoyl-1,3-bis-(*D*-mannos-4-yl)-2-propylamine (ATB-BMBA) is an impermeant bis-hexose photoaffinity label that has been used previously to identify cell-surface glucose transporters in isolated rat adipose cells [14,15] and in 3T3-L1 adipocytes [16–18]. The advantage of using this compound is its specificity for facilitative monosaccharide transporters and its impermeance, allowing it to label selectively all cell-surface glucose transporters [19]. The different transporter isoforms can then be separated by selective immunoprecipitation. We therefore used this label to study the translocation process of the glucose transporters in the BC3H-1 myocyte, a non-fusing muscle cell line that expresses skeletal-muscle-specific proteins [20], but lacks the myoD regulatory protein that is necessary for cell fusion [21]. Upon differentiation, BC3H-1 cells develop high-affinity insulin receptors and exhibit insulin-stimulated responses and receptor regulation, both of which are sensitive to physiological hormone concentrations. BC3H-1 myocytes have been extensively used, and thus established as a model for the study of insulin action in muscle [22]. However, the major glucose transporter in this cell line is GLUT-1, although these cells respond to insulin with a 2–3-fold increase in glucose uptake [23]. Exposure of these cells to glucose has been shown to lead to a decrease in glucose uptake, as well as to a decrease in the level of glucose transporter number and gene expression (I. M. El-Kebbi, D. C. Ziemer, C.-I. Pau, S. Roser, L. S. Phillips and R. J. Pollet, unpublished work). We studied the effect of glucose deprivation and glucose exposure on

Abbreviations used: ATB-BMBA, 2-*N*-4-(1-azido-2,2,2-trifluoroethyl)benzoyl-1,3-bis-(*D*-mannos-4-yl)-2-propylamine; PMA, phorbol 12-myristate 13-acetate.

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glucose transporter number, distribution and function, as well as the effects of insulin and phorbol 12-myristate 13-acetate (PMA) treatment on the recruitment of intracellular glucose transporters. Our results show that glucose exposure leads to down-regulation of the basal glucose transport system, manifesting as a decrease in glucose uptake and redistribution of glucose transporters primarily to the intracellular compartment; insulin and PMA treatment of glucose-exposed cells leads to an increase in glucose uptake that corresponds to the number of GLUT1 transporters recruited from within the cell to the surface.

EXPERIMENTAL

Materials

Crystalline zinc pig insulin was purchased from Elanco (Indianapolis, IN, U.S.A.). Sepharose-protein A was purchased from Pharmacia (Piscataway, NJ, U.S.A.). Thesit (polyoxyethylene lauryl ether; $C_{30}H_{62}O_{10}$) was purchased from Boehringer Mannheim (Indianapolis, IN, U.S.A.). Radionuclides were obtained from New England Nuclear (Boston, MA, U.S.A.). Tissue-culture reagents were obtained from Gibco (Grand Island, NY, U.S.A.). Other biochemical reagents were purchased from Sigma (St. Louis, MO, U.S.A.). ATB-BMPA was generously provided by Dr. Geoffrey D. Holman (Department of Biochemistry, University of Bath, U.K.).

Cell culture

BC3H-1 myocytes were cultured in low-glucose (5.5 mM) Dulbecco's modified essential medium supplemented with 15% CPSR-1 serum (purchased from Sigma) and antibiotics (50 units/ml penicillin G sodium, 50 µg/ml streptomycin sulphate), and were grown at 37 °C under 10% CO_2 . The culture medium was not changed during the culture period. The myoblasts reached confluence 3–5 days after plating, at which time they formed a monolayer and started differentiating into myocytes. Glucose level in the culture medium was undetectable by day 8. The fully differentiated myocytes were studied on days 11–13, at which time the cells had acquired insulin receptors and were maximally insulin-responsive [22]. The myocytes were grown in 6-well cluster plates (3.5 cm wells) for both photoaffinity-labelling and 2-deoxyglucose-uptake studies. Starved cells received no glucose supplementation, whereas glucose-exposed cells were supplemented with 25 mM glucose 24 h before use. This concentration was used in order to examine the regulatory mechanisms acting under conditions characterized by a high-glucose state.

2-Deoxyglucose-uptake studies

Well differentiated BC3H1 myocytes were grown in 6-well cluster plates and preincubated with 25 mM glucose for 24 h as indicated. Before the uptake assay, the cells were washed twice with PBS supplemented with 0.1% BSA, after which 1.5 ml of the same buffer was added to each well, and the cells were equilibrated for 20 min at 37 °C. We have previously shown that the addition of a second wash procedure at this point to remove potential effluxed glucose had no effect on the subsequent assay results. Insulin (200 nM), PMA (1 µM) or control buffer was then added to the incubation medium as indicated for an additional 20 min. This brief period of glucose deprivation does not alter the down-regulatory effect of chronic glucose exposure on 2-deoxyglucose uptake (results not shown). 2-Deoxyglucose uptake was measured by the addition of 2-deoxy[3H]glucose (12 µM final concn.) for 6 min, followed by washing three times with ice-cold PBS buffer.

The plates were then dried and the cells incubated for 30 min with 1% SDS to achieve lysis. The amount of protein in each well was then measured by a modified Lowry method [24], and the amount of radioactivity incorporated in the cells was then determined. The 2-deoxyglucose uptake was expressed as c.p.m./µg of protein and refers to transport across the plasma membrane operating in tandem with phosphorylation. Under these conditions, the capacity of the phosphorylating system is not exceeded [25,26].

Photolabelling of BC3H-1 myocyte glucose transporters

Myocytes (10 days old) were pre-fed with or without 25 mM glucose 24 h before use. Immediately before the photolabelling procedure, the cells were washed twice with PBS supplemented with 0.1% BSA, and 1.5 ml of the same buffer was then added to each well. Insulin (200 nM final concn.), PMA (1 µM final concn.) or vehicle buffer was then added as indicated, and the cells were incubated at 37 °C for 20 min. The buffer was then aspirated, and 250 µl of PBS supplemented with the same concentration of insulin, PMA or vehicle buffer was then added for 4 min at room temperature, in the absence or presence of 0.025% digitonin, the cell-membrane permeabilizing agent. ATB-[2- 3H]BMPA (125 µCi) was then added for 1 min, after which the open wells were irradiated for 2 × 1 min, by using an RPR-100 photochemical reactor, and swirling the plates in between irradiations. Then 2% Thesit solution (2% Thesit, 5 mM EDTA, 25 µg/ml phenylmethanesulphonyl fluoride, 1 µg/ml pepstatin, 10 µg/ml aprotinin, 10 µg/ml leupeptin, in PBS, pH 7.4) was added to each well, and the cells were left to solubilize at room temperature for 30 min. The cells from each well were then scraped and individually transferred to polycarbonate tubes. This experimental design retained any cells that may have been lifted off the plate due to digitonin treatment, as well as any cell proteins which may have leaked out of the cells. The samples were then centrifuged in a TL100.3 rotor at 20000 rev./min for 10 min at 4 °C. The pellet, consisting of insoluble cellular debris, was discarded. A small portion (1%) of the supernatant was saved for determination of protein concentration with a protein assay reagent kit (Pearce, Rockford, IL, U.S.A.), and the entire remaining supernatant was immunoprecipitated.

Immunoprecipitation

A 100 µl portion of a 50% Sepharose-Protein A slurry was incubated with 80 µl of specific rabbit anti-GLUT1 antibody and 1 ml of PBS at room temperature for 1.5 h (the antibody was provided by Hoffman-LaRoche to S.W.C.). The Sepharose was then spun down at 10000 rev./min for 2 min and the pellet washed twice with PBS. The supernatant containing solubilized cells in 2% Thesit was added to the Sepharose-Protein A and left at room temperature on a rotator for 1.5 h. At the end of this step, the samples were spun down again and the supernatant was immunoprecipitated a second time as described above. After each immunoprecipitation, the Sepharose-Protein A pellet was washed three times with 0.2% Thesit solution, then three times with PBS. Pellets resulting from both immunoprecipitations were then combined, and the GLUT1-antibody conjugate was released from the gel matrix by using a 20 min incubation with a dithiothreitol-containing buffer (final concns.: urea 4 M, SDS 2.5%, Tris base 25 mM, dithiothreitol 30 mM, Bromophenol Blue, 0.1%, pH 6.8). The samples were then spun (1 min at 12000 g) through a Millipore microfilter (0.45 µm), and the Sepharose-Protein A pellet discarded. The supernatant, con-

taining the solubilized GLUT1 antibody and photolabelled GLUT1 transporter, was then ready for electrophoresis. The double immunoprecipitation described above has been shown to give a 75% yield for GLUT1 in BC3H-1 cells.

Photolabelled-transporter determinations

The entire solubilized sample for each condition was subjected to electrophoresis in 16 cm × 3 mm gels with 7 mm-wide sample wells in the stacking gel. Molecular-mass markers were run along with the samples, overnight. They were then stained with Coomassie Blue and destained. Each lane was cut out separately and then sliced into bands of equal size. The gel slices were then transferred to scintillation vials and dried at 80 °C for 2 h, then dissolved in 500 µl of alkaline H₂O₂ (30% H₂O₂/28% NH₄OH, 100:1, v/v) at 80 °C. The samples were cooled to room temperature, after which Biocount scintillation fluid was added and the amount of radioactivity determined. Aggregated radiolabel at the stacking-gel interface was discarded, since no immunoreactive transporter was detected by Western blotting (results not shown). The level of radioactivity per slice was plotted against the slice number, as shown in Figure 1. The position of each peak was confirmed with the help of concomitantly run molecular-mass markers. The amount of radioactivity incorporated in each peak was calculated by adding the radioactivity of all the slices under the peak and correcting for background radioactivity on either side of the peak, and was expressed per mg of protein for each well to account for cell-number variability between wells.

Statistics

Statistical significance was tested by analysis of variance, followed by *t*-test analysis, and differences were accepted as significant at the *P* ≤ 0.05 level.

RESULTS

Digitonin permeabilization

In order to determine the appropriate concentration of digitonin that would permeabilize the cell to ATB-BMPA, labelling therefore both cell surface and intracellular transporters, we used Trypan Blue internalization as an indicator of cell membrane permeability. Well-differentiated BC3H-1 cells were incubated for 5 min with 0.2% Trypan Blue in PBS and increasing

concentrations of digitonin. At the end of each incubation, the number of Trypan Blue-positive cells was counted, and expressed as percentage of total cells. Dye diffusion inside the cell, reflecting plasma-membrane permeabilization, was dependent on the concentration of digitonin used, and was complete at concentrations of 0.0025% and above (Table 1). This result was confirmed by utilizing efflux of phosphorylated 2-deoxy[³H]glucose as the indicator of plasma-membrane permeabilization (results not shown). For glucose-transporter labelling, digitonin treatment of myocytes for 5 min led to a dose-dependent increase in ATB-BMPA labelling which continued even at concentrations above 0.01% (Table 1), suggesting that additional detergent is required to permeabilize intracellular membrane vesicles and provide access to the inwardly oriented exoface of the intracellular glucose transporters. On the basis of these results, a digitonin concentration of 0.025% was used in subsequent experiments to permeabilize the myocytes.

Glucose-transporter photolabelling

BC3H-1 myocytes were incubated with ³H-labelled ATB-BMPA, and then lysed. The GLUT1 glucose transporter was then isolated by immunoprecipitation and separated by SDS/PAGE. The corresponding lane was subsequently cut and sliced as described in the Experimental section and the amount of radioactivity per slice is shown in Figure 1. The labelled GLUT1 transporter protein ran as a peak at approx. 55 kDa, as compared with molecular-mass markers. Since the intact cell plasma membrane is impermeable to the bis-mannose compound, the amount of labelled GLUT1 represents transporters present at the surface. Also shown in Figure 1 is the total cellular content of glucose transporters as measured by photoaffinity labelling after digitonin permeabilization. In the presence of 200 µM phloretin, an agent that competitively inhibits monosaccharide binding to the glucose transporter, photoaffinity labelling was decreased by 95% (results not shown).

Starvation and feeding studies

Since glucose is a known regulator of glucose transporter number and glucose transport activity in these cells, we studied cells that were glucose-deprived (starved) as well as cells that received 24 h pre-feeding (fed) with 25 mM glucose, in both the absence (black symbols) and the presence (white symbols) of digitonin. Figures 1(a) and 1(b) show representative curves corresponding to each condition studied, and Figure 2(a) gives the average total radioactivity under each peak for three experiments. The results show that in the starved basal state approx. 85% of the glucose transporters are present at the cell surface. Treating these cells with glucose leads to a significant 5-fold decrease in total transporter number, and to a redistribution of glucose transporters, with the majority (75%) being stored inside the cell. Cell-surface GLUT1 transporter number decreased therefore by approx. 95% after glucose feeding. These findings are consistent with the 2-deoxyglucose uptake data shown in Figure 2, where uptake was significantly suppressed by 80% in response to glucose exposure.

Effect of insulin and PMA stimulation

The effect of insulin and the phorbol ester PMA on total and surface glucose transporters is shown in Figure 1, both for the starved (Figure 1a) and the fed (Figure 1b) states. Both insulin and PMA have been previously shown to stimulate glucose uptake in BC3H-1 myocytes, with PMA being more potent than

Table 1 Effect of digitonin exposure on cell membrane permeability

Concentration-dependence of digitonin permeabilization on Trypan Blue exclusion and on GLUT1 photolabelling are shown. For the centre column, well-differentiated myocytes were incubated with 0.2% Trypan Blue and increasing concentrations of digitonin for 5 minutes. At the end of each incubation, the number of Trypan-positive cells was counted and expressed as percentage of total cells. For the right-hand column, differentiated myocytes were incubated with increasing concentrations of digitonin for 5 min, in combination with photolabelling during the last 1 min.

Digitonin concn. (%)	Trypan-Blue-positive cells (%)	Photolabelled GLUT1 (c.p.m./mg of protein)
0	2	1173
0.001	20	1243
0.0025	95	1960
0.01	100	2455
0.025	100	3917

Figure 1 Effects of glucose, insulin and PMA on total and cell-surface glucose-transporter photolabelling

Effect of insulin and PMA on glucose transporter number and distribution in glucose-starved (a) and glucose-fed (b) cells. Differentiated myocytes were treated with control buffer (○, ●), insulin (200 nM; △, ▲) or PMA (1 μ M, □, ■) for 20 min, followed by photolabelling in both the presence and the absence of digitonin, to determine total (○, △, □) and cell-surface (●, ▲, ■) GLUT1 number respectively. Both panels show representative raw data corresponding to each condition studied, and Figure 2(a) gives the average calculated radioactivity under each peak standardized per mg of protein for triplicate determinations.

Figure 2 Effects of glucose, insulin and PMA on glucose transporter number, distribution and function

Differentiated myocytes were preincubated without (starved; S) and with (fed; F) 25 mM glucose for 24 h, then treated with control buffer, insulin or PMA as described in the Experimental section. (a) Bars represent total glucose transporter number as determined by photolabelling in the presence of digitonin. Hatched areas show the glucose transporter number at the cell surface, as determined by photolabelling in the absence of digitonin; white areas represent intracellular transporters. (b) Bars represent the amount of 2-deoxyglucose uptake measured over a period of 6 min. Data points for both panels represent mean values $\pm$ S.D. of triplicate determinations.

insulin in these cells. In the starved state, incubating myocytes with insulin (200 nM) or PMA (1 μ M) for 20 min led to no significant change in cell-surface and total glucose-transporter labelling (Figures 1a and 2a), corresponding to the lack of change in 2-deoxyglucose uptake in response to either agent. The lack of change in total transporter number was confirmed by Western blotting of total GLUT1 protein after each treatment (results not shown).

In the glucose-exposed state, BC3H-1 myocytes treated with insulin and PMA led to a significant 2.2- and 4-fold increase in cell-surface glucose transporter respectively (Figures 1b and 2a),

corresponding to the significant 2.1- and 3.8-fold increase in 2-deoxyglucose uptake observed after treatment with the respective agents (Figure 2b). Again, there was no change in total glucose transporter number as determined both by photolabelling and by Western immunoblotting (results not shown).

DISCUSSION

In this study, we describe a technique for rapidly determining both cell-surface and total glucose transporter number in cultured cells. This technique relies on the use of an impermeant photo-affinity label that binds specifically to the glucose transporter. When used in combination with digitonin, a detergent that permeabilizes cell membranes without disrupting them, the label is able to cross the plasma membrane and penetrate intracellular vesicles, binding specifically to glucose transporter units inside the cell as well as to cell-surface transporters. This technique therefore allows the study of both the total number and distribution of glucose transporters in a given cell system, and obviates the need to separate plasma membranes from the intracellular low-density microsomal fraction, a process that is time-consuming, complex, and involves a significant risk of cross-contamination between fractions [12], especially in muscle tissue with a highly specialized cytoskeletal structure. This technique also makes the correlation between the number of functional surface transporters and glucose transport possible.

Our findings are summarized in Figure 2. In the glucose-deprived state, the total number of GLUT1 glucose transporters in BC3H-1 myocytes is up-regulated, and 85 % of the transporters are present at the cell surface. Glucose exposure for 24 h leads to a decrease in total transporter number, as measured in digitonin-treated cells, in combination with a redistribution of glucose transporters to the intracellular space. These results are consistent with the observed change in 2-deoxyglucose uptake (Figure 2), and may reflect adaptation of the myocytes to the high-glucose

concentration in the medium, providing a way to regulate glucose influx. The mechanism by which glucose suppresses the number of glucose transporters in BC3H-1 cells has been previously investigated (I. M. El-Kebbi, D. C. Ziemer, C.-I. Pao, S. Roser, L. S. Phillips and R. J. Pollet, unpublished work) and appears to involve a decrease in glucose-transporter gene expression as well as post-mRNA suppression of transporter protein number, through decreased transporter synthesis or increased degradation. This combined effect appears to be specific to glucose and is only partially mimicked by other hexoses. The suppressive effect of glucose is also evident in other systems. Walker et al. [27] have shown that glucose exposure leads to a decrease in glucose transport, glucose transporter number and glucose transporter mRNA in cultured L6 myocytes, as compared with xylose exposure. A similar effect has also been described in adipocytes and in murine fibroblasts [28,29].

Both insulin and PMA were shown to stimulate 2-deoxyglucose uptake acutely in BC3H-1 cells largely by increasing glucose transporter number at the surface, with no evidence of an increase in intrinsic glucose transporter activity. Our findings do not indicate, however, whether the increase in cell-surface transporter number is related to translocation from intracellular stores, or to activation of plasma-membrane-associated inactive transporters, incapable of binding the photolabel. The latter possibility has been suggested by Harrison et al. [30] as a mechanism that explains the observed increase in photolabelled cell-surface transporter number in 3T3-L1 adipocytes in response to the protein-synthesis inhibitor anisomycin, in the absence of any change in plasma-membrane transporter content as determined by fractionation. An increase in transporter binding affinity to the photolabel in response to insulin and PMA is a possible, but unlikely, cause of the observed increase in transporter labelling, since treatment of starved cells with these agents led to no significant increase in photolabel binding.

Insulin and PMA had no effect on 2-deoxyglucose uptake in the glucose-deprived state, as expected, since most glucose transporters under these conditions were found to be already located at the cell surface in the basal state. This is consistent with previous studies in Swiss 3T3 cells and rat adipose cells that indicate that the stimulatory action of PMA on glucose transport activity is associated with translocation of transporters from an intracellular pool to the plasma membrane [31,32]. In the glucose-fed state, our results further show that insulin and PMA led to approx. 2- and 4-fold increases in 2-deoxyglucose uptake respectively, indicating that PMA is much more active in recruiting transporters to the cell surface. This agent, however, has been found to be either less than or equally as effective as insulin in stimulating glucose transport in other tissues, including rat diaphragm muscle, rat soleus muscle and rat adipocytes [33–35]. These studies, however, did not examine the differential effect of PMA on the different glucose-transporter isoforms, namely GLUT1 and GLUT4, expressed in these cells. However, Saltis et al. [36] did look at the effect of insulin and PMA on individual glucose-transporter isoforms, and found that insulin stimulated both GLUT1 and GLUT4 transporters in rat adipose cells, whereas PMA stimulated preferentially the GLUT1 transporter. Since these adipocytes have GLUT4 as their major transporter, insulin, which leads to translocation of both GLUT1 and GLUT4 transporters, stimulates 2-deoxyglucose transport in these cells more than PMA does. Similar results were reported by Holman et al. [37], who selectively immunoprecipitated labelled cell-surface GLUT1 and GLUT4 transporters of rat adipocytes. The preferential stimulatory effect of PMA on GLUT1 translocation was also demonstrated in 3T3-L1 adipocytes by Gibbs et al. [38], who studied GLUT1 and GLUT4

Table 2 Comparison of glucose transport activity with glucose transporter number in the glucose-exposed cells

Total and cell-surface transporter number in the basal, insulin- and PMA-stimulated states are expressed relative to the number of GLUT1 transporters on the cell surface in the basal state, which is given an arbitrary value of 1. 2-Deoxyglucose uptake in the basal state is also assigned an arbitrary value of 1.

	Relative amount of total labelled cell transporters	Relative amount of labelled cell surface transporters	Relative amount of 2-deoxyglucose uptake
Basal	3.8	1	1
Insulin-stimulated	4.1	2.2	2.1
PMA-stimulated	4.3	4	3.8

translocation in response to insulin and PMA, by immunoblotting subcellular membrane fractions, and by selective immunoprecipitation of ATB-BMPA-labelled transporters. The fact that BC3H-1 myocytes express predominantly only one transporter simplifies the analysis and demonstrates the close correlation of glucose transport activity with cell surface glucose transporter number, as summarized in Table 2. The superiority of PMA action over that of insulin may be explained by hypothesizing that PMA is more potent in enhancing GLUT1 externalization rate or attenuating its internalization rate as compared with insulin. An alternative explanation is that intracellular transporters may be located in distinct pools that respond differentially to insulin and PMA. The existence of selectively responsive intracellular stores has been previously suggested, as insulin and exercise were found to lead to differential glucose-transporter translocation from inside the cell to the surface [7].

In conclusion, we have described a rapid straightforward method of determining total cellular and cell-surface glucose transporter content through exofacial photoaffinity labelling of GLUT1 glucose transporters in the absence and presence of digitonin. For the differentiated BC3H-1 myocyte, glucose exposure of glucose-starved cells leads to decreased total GLUT1 transporter number coupled with a change in transporter distribution, with the majority located intracellularly, where they can be translocated to their surface site of action in response to various stimuli. PMA was more effective in recruiting GLUT1 transporters to the cell surface compared with insulin, suggesting either the existence of a distinct intracellular PMA-recruitable insulin-unresponsive GLUT1 transporter pool, or that insulin and PMA have differential effects on the kinetics of GLUT1 translocation. The increase in glucose transporter activity in response to either agent was totally accounted for by increased transporter number at the cell surface, with no apparent change in intrinsic transporter activity.

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