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Cell surface accessibility of GLUT4 glucose transporters in insulin-stimulated rat adipose cells

Modulation by isoprenaline and adenosine

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Insulin-stimulated glucose transport activity in rat adipocytes is inhibited by isoprenaline and enhanced by adenosine. Both of these effects occur without corresponding changes in the subcellular distribution of the GLUT4 glucose transporter isoform. In this paper, we have utilized the impermeant, exofacial bis-mannose glucose transporter-specific photolabel, 2-*N*-4-(1-azi-2,2,2-trifluoroethyl)benzoyl-1,3-bis-(D-mannos-4-yloxy)-2-propylamine (ATB-BMPA) [Clark & Holman (1990) Biochem. J. **269**, 615–622], to examine the cell surface accessibility of GLUT4 glucose transporters under these conditions. Compared with cells treated with insulin alone, adenosine in the presence of insulin increased the accessibility of GLUT4 to the extracellular photolabel by ~ 25%, consistent with its enhancement of insulin-stimulated glucose transport activity; the plasma membrane concentration of GLUT4 as assessed by Western blotting was unchanged. Conversely, isoprenaline, in the absence of adenosine, promoted a time-dependent ($t_{\frac{1}{2}}$ ~ 2 min) decrease in the accessibility of insulin-stimulated cell surface GLUT4 of > 50%, which directly correlated with the observed inhibition of transport activity; the plasma membrane concentration of GLUT4 decreased by 0–15%. Photolabelling the corresponding plasma membranes revealed that these alterations in the ability of the photolabel to bind to GLUT4 are transient, as the levels of both photolabel incorporation and plasma membrane glucose transport activity were consistent with the observed GLUT4 concentration. These data suggest that insulin-stimulated GLUT4 glucose transporters can exist in two distinct states within the adipocyte plasma membrane, one which is functional and accessible to extracellular substrate, and one which is non-functional and unable to bind extracellular substrate. These effects are only observed in the intact adipocyte and are not retained in plasma membranes isolated from these cells when analysed for their ability to transport glucose or bind photolabel.

INTRODUCTION

Insulin stimulates glucose transport activity in rat adipose cells primarily by promoting the translocation of the two glucose transporter isoforms, GLUT1 and GLUT4, from intracellular membranous compartments to the plasma membrane [1,2]. However, the extent of this translocation, as determined by either cytochalasin B binding to, or Western blotting of, the plasma membranes, does not fully account for the increase in glucose transport activity measured in the intact cells [3]. The discrepancy between glucose transporter number and activity is even more pronounced when the actions of hormones such as catecholamines, adrenocorticotrophin (ACTH) and glucagon, which are mediated by G_s-coupled receptors (R_s), and adenosine, prostaglandins and nicotinic acid, agents whose actions are mediated by G_i-coupled receptors (R_i), are superimposed upon insulin stimulation [4,5]. R_sG_s hormones inhibit, and R_iG_i agents augment, insulin-stimulated glucose transport activity by mechanisms that are rapid ($t_{\frac{1}{2}}$ = 2 min) and involve a change in the $V_{max.}$ for transport but not the apparent affinity (K_m) of the transporter for 3-*O*-methylglucose [6]. These effects are also distinct from the established alterations in insulin sensitivity induced by these agents (for review see ref. [7]), since they are observed at supramaximal concentrations of insulin. The observation that these changes in glucose transport activity occur without a major change in the subcellular distribution of glucose transporters led to the initial hypothesis that these hormones alter the intrinsic activity of the glucose transporter in the plasma membrane [4].

GLUT4 is now known to be the predominant glucose transporter isoform present in the rat adipocyte and is responsible for most, if not all, of the insulin-stimulated increase in glucose transport activity [8,9]. It is therefore probable that GLUT4 is also the isoform that is acutely modulated by the R_sG_s and R_iG_i hormones. The phosphorylation state of GLUT4 has been shown to be increased in adipocytes, primarily intracellularly, in response to isoprenaline and cyclic AMP (cAMP) derivatives, and it has been proposed that cAMP-dependent phosphorylation of this protein is the mechanism of the catecholamine-induced inhibition of glucose transport activity [10,11]. However, earlier studies suggested that these effects on glucose transport activity can be elicited independently of changes in cAMP-dependent protein kinase activity [4]. Furthermore, recent studies from our laboratory confirm the isoprenaline-mediated increase in the phosphorylation of intracellular GLUT4, but indicate that changes in the level of phosphorylation of GLUT4 in the

Abbreviations used: ACTH, adrenocorticotropin; ATB-BMPA, 2-*N*-4-(1-azi-2,2,2-trifluoroethyl)benzoyl-1,3-bis-(D-mannos-4-yloxy)-2-propylamine; TES, Tris/EDTA/sucrose (20/1/255 mM); ADA, adenosine deaminase; IGF-II, insulin-like growth factor II; cAMP, cyclic AMP; R_s and R_i, G_s- and G_i-coupled receptors respectively.

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adipocyte plasma membrane are minimal and can be dissociated from the observed inhibition of glucose transport activity in these cells [12].

A recently described technique for studying glucose transporters in the plasma membrane of the intact adipose cell involves surface labelling of the glucose transporter with the impermeant bis-mannose photolabel, 2-*N*-4-(1-azi-2,2,2-trifluoroethyl)benzoyl-2,3-bis-(D-mannos-4-yloxy)-2-propylamine (ATB-BMPA). By using this ligand, which only binds to those transporters (GLUT1 and GLUT4) which are appropriately exposed to the extracellular fluid [13], a more accurate correlation between transport activity and transporter number can be obtained. ATB-BMPA has been used to study the effects of insulin and the phorbol ester phorbol 12-myristate 13-acetate on the translocation and activity of both GLUT1 and GLUT4 in the intact adipocyte [8]. This approach has confirmed GLUT4 as the major glucose transporter isoform in these cells, as it is approximately ten times more abundant than GLUT1. Furthermore, in response to insulin, the increase in the level of photolabelled GLUT4 on the cell surface more closely paralleled the increase in glucose transport activity, i.e. 15–20-fold [8,13] as compared with the previously reported 3–6-fold increase in GLUT4 detected by Western blotting. Similar studies have been performed in 3T3-L1 cells, where anisomycin appears to enhance the labelling of GLUT1 glucose transporters [14]. A comparison of the extent of ATB-BMPA binding with glucose transport activity thus provides (assuming no changes in ATB-BMPA affinity) a direct measure of alterations in the rate of transport per labelled transporter. Using such an approach, we have investigated the mechanism(s) of glucose transport modulation induced by isoprenaline and adenosine in the intact rat adipocyte and conclude that the changes in transport activity correlate closely with the accessibility of GLUT4. Isoprenaline appears to induce an alteration, but not a loss, of GLUT4 glucose transporters in the plasma membrane of the intact cell such that they can neither bind photolabel nor transport glucose. Adenosine prevents this catecholamine effect and renders the transporters more readily accessible.

EXPERIMENTAL

Animals and cell preparation

Adipose cells were isolated from the epididymal fat pads of male rats (170–200 g; CD Strain, Charles River Breeding Laboratories) as previously described [15]. All incubations were carried out at a 15% cytocrit in Krebs–Ringer bicarbonate/Hepes buffer, pH 7.4, supplemented with 200 nM-adenosine, 5% (w/v) BSA and 2.5 mM-glucose. All cells were incubated with supramaximal concentrations of insulin (670 nM) for 20 min prior to removal of adenosine and/or addition of isoprenaline as described in the Figure legends. All incubations were carried out at 37 °C.

Glucose transport measurements

Glucose transport activity was determined in intact adipocytes by the 3-*O*-methylglucose uptake technique, as described by Karnieli *et al.* [15]. Glucose transport activity was determined in plasma membranes under equilibrium exchange conditions as previously described [16].

Photolabelling of adipocytes and preparation of membrane fractions

At the end of the experimental incubation, 3 ml of each 15% (v/v) cell incubation was mixed with 500 μCi of ATB-[2-^{3}H]BMPA (10 Ci/mmol) in 9 cm polystyrene dishes. The samples were immediately irradiated for 2 × 30 s in a Rayonet RPR photochemical reactor (RPR 3000 lamps), with gentle mixing after the initial 30 s. Glucose transport activity was determined simultaneously on the remaining unlabelled cells. Following irradiation, cells were transferred into 17 ml test tubes with 2 × 3 ml washes in TES buffer (20 mM-Tris/HCl, 1.0 mM-EDTA, 255 mM-sucrose, pH 7.4) at 18 °C and spun up through 5 ml of dinonyl phthalate oil (2 min, 1000 *g*). The infranatant was removed and the labelled cells (0.5 ml) were homogenized in 3.5 ml of TES. The homogenates were subjected to one short (30 s) low-speed (8000 *g*) centrifugation to remove the nuclei [17] and the supernatant was spun at 100000 *g* for 60 min to pellet all membranes. The pellets were resuspended in TES and an aliquot was removed for protein determination. The remaining sample was then solubilized in 2% Thesit (Boehringer Mannheim) for immunoprecipitation [8]. This method of total membrane preparation provided better recoveries than obtained with previous methods involving subcellular fractionation, particularly with the very small number of labelled cells. In a separate experiment, in which the standard subcellular fractionation technique was employed [16], we determined that none of the photolabel appeared in the intracellular membranes during the incubation times of these experiments (results not shown). The remaining unlabelled cells were simultaneously spun through dinonyl phthalate oil and plasma membranes, and intracellular membranes were prepared for electrophoresis and Western blotting by standard procedures [16].

Plasma membranes were photolabelled by incubating 300 μg of purified plasma membranes with 200 μCi of ATB-[2-^{3}H]BMPA in a final volume of 0.5 ml. The samples were irradiated and solubilized for immunoprecipitation as described above.

Quantification of ATB-[2-^{3}H]BMPA-labelled GLUT4 glucose transporters

Labelled membrane samples were solubilized, immunoprecipitated and separated electrophoretically as previously described [8]. The levels of radioactivity associated with the single peak of GLUT4 were obtained by summing the radioactivity in all of the slices included in the peak and subtracting a background radioactivity determined from the average level of radioactivity in the slices on either side of the peak. Immunoprecipitation and Western blot analysis were carried out using a rabbit antibody raised against the 20-amino-acid *C*-terminal sequence of GLUT4 (kindly provided by Hoffman–LaRoche). For comparison with the total amount of GLUT4 glucose transporter present in the plasma membrane and intracellular membrane fractions, the membranes from the corresponding unlabelled cells were Western-blotted using the same anti-GLUT4 antiserum and ^{125}I-protein A. Quantification was obtained by excision of the appropriate bands from the nitrocellulose paper and counting of γ-radiation.

Calculations

For each of the experiments, the response obtained in the presence of insulin plus 200 nM-adenosine was taken to be 100% and the other values were normalized to a percentage of that maximal value. Statistical significance was tested with a one-way analysis of variance (ANOVA) and differences between the maximal and experimental values were accepted as significant at the $P < 0.05$ level.

RESULTS

The initial experiments of this study were conducted to determine the correlation between the hormonal modulation of glucose transport activity and the extent of binding to the

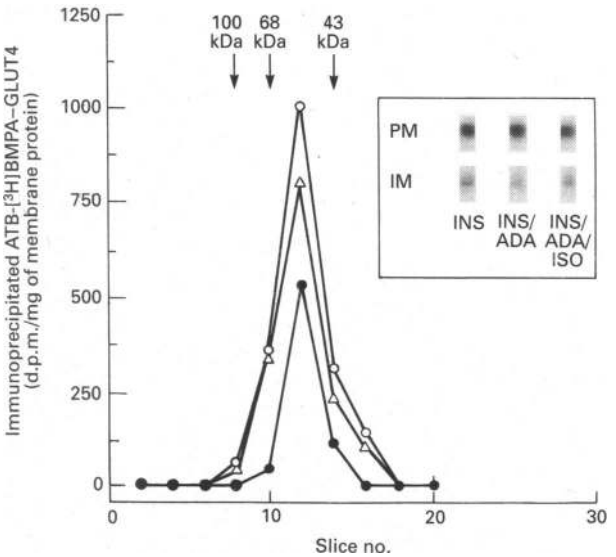

Fig. 1. Effects of adenosine and isoprenaline on insulin-stimulated translocation of GLUT4 glucose transporters: accessibility to exofacial photolabel

Isolated adipocytes were incubated at a 15% cytocrit in the presence of insulin plus adenosine (○), insulin alone (△) or insulin plus isoprenaline (●). At the end of the incubation, 3 ml portions were removed, incubated with 500 μCi of ATB-[2-^{3}H]BMPA and irradiated for 2×30 s. Total membranes were prepared and solubilized, and photolabelled GLUT4 was immunoprecipitated and analysed as described in the Experimental section. The remaining unlabelled cells were analysed for 3-*O*-methylglucose transport activity and then subjected to subcellular fractionation and Western blot analysis of the membrane fractions for GLUT4 (inset): INS, insulin; ISO, isoprenaline; PM, plasma membranes; IM, intracellular membranes.

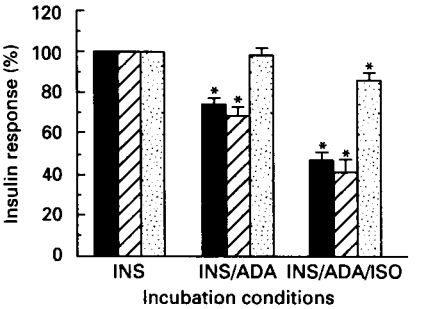

Fig. 2. Insulin stimulation of glucose transport: effects of adenosine and isoprenaline (ISO)

The experiment depicted in Fig. 1 was repeated four times and the results presented are means ± S.E.M. for 3-*O*-methylglucose transport (□), incorporation of photoligand (▨) and Western blot analysis of the plasma membranes (⊡). For each experiment the response obtained with insulin plus adenosine (INS) was taken as 100% and the results obtained with the other conditions are expressed as percentages of that maximal value. * denotes significant decrease compared to insulin-stimulated condition ($P < 0.05$ as determined by ANOVA).

photolabel. The enhancement of insulin-stimulated glucose transport activity in isolated adipocytes by R_iG_i agents (e.g. adenosine), as well as the inhibition of this activity by R_sG_s hormones (e.g. isoprenaline), is well established. In order to avoid the known effects of these agents on the sensitivity to insulin [7], all of the experiments employed a supramaximal concentration of insulin. The results of a representative experiment using the [^{3}H]-bis-mannose ligand ATB-BMPA to photolabel the cell surface GLUT4 glucose transporters under the above conditions are presented in Fig. 1. The curves represent SDS/PAGE profiles of immunoprecipitated ^{3}H-photolabelled GLUT4 glucose transporter, which runs as a discrete peak at 45 kDa. Maximal insulin-stimulated glucose transport activity (3.69 ± 0.61 fmol/min per cell; mean ± S.D.) and maximal incorporation of the photolabel into immunoprecipitable GLUT4 was observed in the presence of 200 nM-adenosine. Removal of adenosine by the addition of adenosine deaminase (ADA) produced a 20% reduction in the ability of these cells to bind ATB-BMPA, which correlated well with an 18.5% inhibition of glucose transport activity (3.01 ± 0.87 fmol/min per cell). The addition of isoprenaline in combination with ADA resulted in a marked 50% decrease in the incorporation of the photolabel and a corresponding 43% inhibition of glucose transport activity (2.16 ± 0.43 fmol/min per cell). These results are in sharp contrast with the data presented in the inset to Fig. 1, which shows the autoradiograph obtained following analysis of these membranes for GLUT4 by Western blotting. The removal of adenosine from the insulin-stimulated cells did not produce any detectable loss of GLUT4 glucose transporter in the plasma membrane fraction isolated from these cells. Isoprenaline did produce a change in the subcellular redistribution of GLUT4 which resulted in a 15% loss of GLUT4 from the plasma membrane fraction and a corresponding increase in this glucose transporter isoform in the intracellular membranes. This finding is in agreement with previous results obtained by both Western blotting and cytochalasin B binding: lipolytic hormones produce a decrease in the translocation of glucose transporters which is substantially less than the magnitude of the inhibitory effect on insulin-stimulated glucose transport activity as measured in the intact adipocytes [4–6,11,12].

This experiment was repeated four times and the compiled results are presented in Fig. 2. In these experiments the magnitude of the response to insulin in the presence of 200 nM adenosine (INS in Fig. 2) was equated to 100% and the results obtained with the other conditions were expressed as a percentage of that value. The removal of adenosine with ADA produced 14% and 20% inhibition of insulin-stimulated glucose transport activity and incorporation of GLUT4 immunoprecipitable photolabel ($P < 0.05$) respectively, without a detectable loss of GLUT4 from the plasma membrane fraction (INS/ADA). The combination of the removal of adenosine and the addition of isoprenaline inhibited both glucose transport activity and incorporation of photolabel to approx. 50% (47.3 ± 4.5% and 43.7 ± 4.2% respectively) (INS/ADA/ISO). However, the corresponding decrease in GLUT4 detected by Western blot analysis of these membranes was only ~ 15%. The magnitude of this decrease varied between 0%, i.e. no change, and 15%. The mean decrease (13.9 ± 2.3%) obtained in an extensive series of experiments from our laboratory was statistically significant [12]. However, the decrease in GLUT4 content in the plasma membranes was always significantly less than the decrease in glucose transport activity. The observation that a substantial proportion of the GLUT4 glucose transporters is present in the plasma membrane, but not in the correct orientation to bind ATB-BMPA, implies that the previously described change in intrinsic activity may well result from an altered accessibility of the transporter protein within the plasma membrane [5].

We have previously shown that the inhibitory effects of isoprenaline on glucose transport activity are not retained when plasma membranes are isolated as described here [18]. The data presented in Fig. 3 support the transitory nature of the iso-

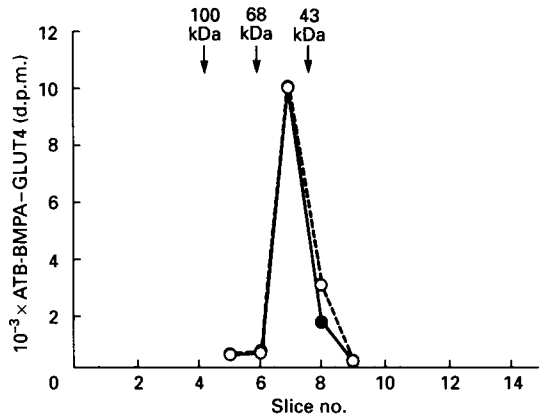

Fig. 3. Transience of isoprenaline effects on glucose transport

In this representative experiment plasma membranes (300 μg) prepared from cells that had been incubated with insulin in the absence (○) or presence (●) of isoprenaline as described in Fig. 1 were incubated with 200 μCi of ATB-BMPA in a final volume of 400 μl. Membranes were irradiated for 3 × 1 min and GLUT4 glucose transporters were processed as described in the Experimental section. Plasma membrane glucose transport activity in the corresponding membranes was reduced by 16% (25.7 pmol/mm per mg cf. 21.6 pmol/min per mg) and a 12% decrease in GLUT4 was observed on Western blotting of these membranes.

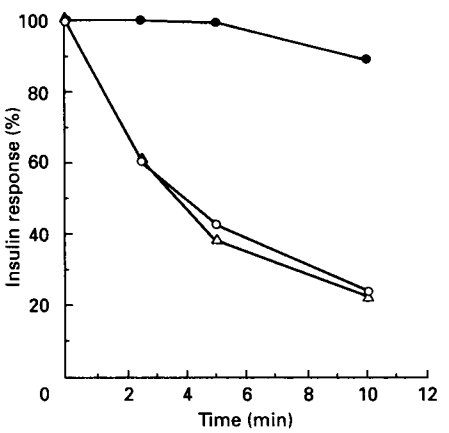

Fig. 4. Time course of isoprenaline effects on insulin-stimulated glucose transport

Isolated adipocytes were incubated at 15% cytocrit in the presence of insulin plus adenosine for 20 min (0 time, 100% value). Following the addition of ADA (0.5 unit/ml) and isoprenaline (200 nM), 3 ml aliquots were removed for incubation with ATB-[2-^{3}H]BMPA (500 μCi), irradiation and immunoprecipitation of labelled GLUT4 as described in the Experimental section. The time course for the decrease in incorporation of the photolabel is shown by ○. 3-*O*-Methylglucose transport activity (△) was determined for the remaining unlabelled cells at the same time as the photoincorporation. The total content of GLUT4 in the plasma membrane fractions prepared from the unlabelled cells (●) was determined by Western blot analysis as described. Shown is a single experiment representative of three such experiments. Each time point was determined in duplicate.

prenaline effects. The 12% reduction in the level of binding of ATB-BMPA to the corresponding plasma membranes is entirely consistent with the decreases in GLUT4 content (13%) and plasma membrane transport activity (16%), but not with the 45% reduction in glucose transport activity observed in the intact cell. Thus the isoprenaline-induced effect is restricted to the intact adipocyte and is not retained in the isolated membrane preparation.

The results thus far show that isoprenaline, in the absence of adenosine, produces both an inhibition of insulin-stimulated glucose transport activity and an alteration in the accessibility of a portion of those GLUT4 glucose transporters translocated to the plasma membrane in response to insulin, such that they are no longer accessible to the exofacial ligand ATB-BMPA. The time course of the isoprenaline-mediated inhibition of transport activity is known to be rapid [4]. In order to determine whether the altered state of the transporter is the primary mechanism responsible for mediating this inhibition, the time course for the decrease in incorporation of the photolabel was compared with the inhibition of 3-*O*-methylglucose transport. The experiment depicted in Fig. 4 shows that these two events occur simultaneously following treatment of the insulin-stimulated adipocytes with isoprenaline in the absence of adenosine. In this experiment, isoprenaline produced a greater than 70% inhibition of both glucose transport activity and incorporation of the photolabel into GLUT4. The time courses for these effects were superimposable, with $t_{\frac{1}{2}}$ values of ~ 2.5 min for both. However, as has been apparent throughout these studies, isoprenaline had little effect on the amount of GLUT4 detectable in the plasma membrane by Western blotting. These results indicate that both isoprenaline and adenosine produce changes in the state of GLUT4 within the plasma membrane which are coincident with, and probably responsible for, the modulations of insulin-stimulated glucose transport activity observed.

DISCUSSION

A modulation of glucose transporter intrinsic activity has been invoked to explain those alterations in glucose transport activity which appear to occur without equivalent alterations in the subcellular distribution of the glucose transporter proteins, especially GLUT4 [4]. Such changes in glucose transporter intrinsic activity were first proposed to explain the effects of the R_sG_s and R_iG_i agents on insulin-stimulated glucose transport activity in rat adipose cells [4,6]. In the present study we have used the impermeant cell surface glucose-transporter-specific photolabel ATB-BMPA to monitor changes in the accessibility of GLUT4 induced by isoprenaline and adenosine in rat adipocytes in the insulin-stimulated state.

The scheme presented in Fig. 5 was designed to incorporate and pictorially highlight the actions of adenosine and isoprenaline into the original translocation model of Karnieli *et al.* [15]. In this representation, the GLUT4 glucose transporters traverse an exocytotic/endocytotic route between the intracellular compartment and the plasma membrane. According to the model as originally described [15], insulin stimulates glucose transport activity by promoting the redistribution of glucose transporters from an intracellular pool of glucose-transporter-containing vesicles to the plasma membrane, although the precise locus of insulin action, i.e. steps k_1, k_2 or k_4, was not known. Recently, Satoh *et al.* [19] have shown that the primary site of insulin action appears to be the translocation step (k_1 and k_2), with apparently no effect on the internalization of GLUT4 (k_4). Furthermore, the glucose transporters are now known to continuously recycle in the presence of insulin [19,20].

The primary focus of the present investigation, however, is the step depicted as k_3. We propose that isoprenaline, in the absence of adenosine, induces a change in the conformation of the transporter such that it is unable to either bind the photolabel or transport glucose. Adenosine can totally prevent this effect and appears to stabilize the fully activated form. We have chosen to depict the isoprenaline-inhibited state as the formation of an

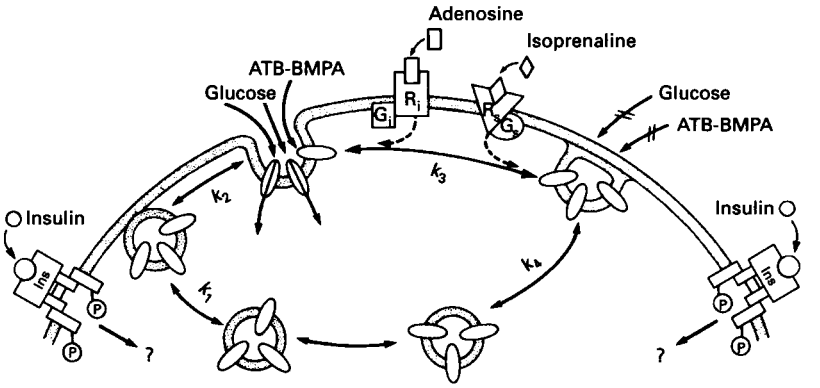

Fig. 5. Proposed actions of adenosine and isoprenaline on glucose transport

This represents a schematic interpretation of the data presented in this paper and in previous publications [12,13,15] from our laboratory.

occluded vesicle analogous to, and indistinguishable from, the form immediately preceding k_2. Such a depiction would be consistent with the observations of Smith *et al.* [6] that isoprenaline and adenosine induce changes in the $V_{max.}$ of the glucose transporter without altering its apparent affinity for 3-*O*-methylglucose (K_m). However, we cannot exclude the possibility that, in response to isoprenaline, there is a conformational change in the glucose transporter itself such that it is unable to bind either substrate or photolabel. This could be similar to the report of Oka *et al.* [21], where a mutation in GLUT1 appears to lock the transporter in an inward-facing conformation. A more analogous observation has been reported by Harrison *et al.* [14], who demonstrated an enhancement of photolabelling of GLUT1 induced by anisomycin under circumstances in which the total GLUT1 in the plasma membrane was unaltered.

Previously we have contrasted the actions of adenosine and isoprenaline on insulin-stimulated glucose transport activity with the effects of these agents on the insulin-mediated translocation of insulin-like growth factor II (IGF-II)/mannose-6-phosphate receptors [7,22]. Such apparent contrasts were mainly based on the absence of a change in glucose transporter concentration in the isolated plasma membranes as compared with changes in the binding of IGF-II to intact rat adipose cells. However, such changes in binding are entirely analogous to the alterations in accessibility of GLUT4 described here. In addition, as with GLUT4, adenosine enhances and isoprenaline inhibits the capacity of IGF-II to bind to its receptor, and by similar proportions [22]. A further important similarity relates to the mechanism of recycling of the IGF-II/mannose-6-phosphate receptor and the GLUT4 transporter. It has long been appreciated that the IGF-II receptor recycles via a coated pit/endosome mechanism [23,24]. Recently, Slot *et al.* have observed GLUT4 in coated pits in brown adipocytes [25] and cardiac myocytes [26], suggesting that these transporters may recycle by a similar mechanism. In addition, it is reasonable to presume that if the proposed changes in accessibility of both proteins are due to membrane conformational changes, they would depend on adequate membrane fluidity and thus be inhibited at low temperature. This is indeed the case, as temperatures below 22 °C block isoprenaline-mediated inhibition of glucose transport activity and IGF-II binding in intact adipocytes [18,22].

The inability to retain the alterations in glucose transport activity and ATB-BMPA binding in the isolated plasma membranes (Fig. 3) suggests that the mechanism does not involve covalent modification. Although it was known that isoprenaline-induced inhibition of glucose transport activity is not retained in the plasma membrane vesicles isolated as described here [18], the data presented in Fig. 3 provide additional evidence that the ability of GLUT4 to bind photolabel in the isolated vesicles is not altered. These plasma membrane transport data contrast with previous observations [18] in which altered transport activity was retained when plasma membranes were prepared in the presence of KCN. We now attribute these observations to a KCN-induced loss of GLUT4 transporters that occurs specifically in isoprenaline-treated cells. The mechanism for this loss is not yet understood. The notion that modulations in glucose transport activity are not mediated by covalent modification is supported by recent studies from our laboratory investigating the phosphorylation state of GLUT4 glucose transporters. In these studies isoprenaline, either in the presence of adenosine (where no alteration of transport activity was observed) or in the absence of adenosine (where full inhibition was observed) produced a substantial activation of cAMP-dependent protein kinase activity and a 1.4-fold increase in the phosphorylation state of intracellular GLUT4, but no significant change in GLUT4 phosphorylation in the plasma membrane [12].

The mechanism(s) by which the R_iG_i agents maintain an accessible transporter configuration and in the absence of these agents isoprenaline promotes an occlusion, has yet to be defined. The potential for the direct involvement of G_s and G_i, as depicted in the model of Fig. 5, has been implicated from the profile of ligand effects [4] as well as the actions of cholera and pertussis toxins in this system [27]. Following treatment of the adipocytes with pertussis toxin, adenosine is no longer able to prevent the catecholamine-mediated inhibition of insulin-stimulated glucose transport activity, implying that adenosine exerts these effects via the A_1 adenosine receptor–G_i complex. Cholera toxin treatment results in a partial inhibition of insulin-stimulated glucose transport activity but, more importantly, prevents any further inhibition by those hormones which mediate their actions through receptors coupled to the G_s protein. These G-protein-mediated effects observed at supramaximal concentrations of insulin are distinct from those previously described which alter the cell's sensitivity to insulin at lower concentrations [7]. Although the involvement of the G-proteins G_s and G_i in insulin-stimulated glucose transport activity has also been suggested to occur in cardiac myocytes [28], no direct interaction between the G-proteins and GLUT4 has yet been demonstrated in either cell type, and this clearly requires further study.

In conclusion, we have shown that cell surface labelling of the GLUT4 glucose transporter with the exofacial ligand ATB-BMPA correlates well with the level of glucose transport activity measured in the intact adipocyte, but not necessarily with the amount of GLUT4 present in the plasma membrane fraction

following subcellular fractionation. We have utilized this technique to demonstrate that R_sG_s and R_iG_i ligands exert their effects on insulin-stimulated glucose transport by promoting either an open (R_iG_i) or an occluded (R_sG_s) configuration of the GLUT4 glucose transporter within the plasma membrane. This cycle is envisaged as part of the normal cycling observed with insulin treatment, but we cannot at present totally rule out the possibility that isoprenaline induces an independent cycle of events involving direct or indirect intervention of the G-proteins. The continued utilization of the ATB-BMP photolabel should provide important information relative to these aspects of GLUT4 translocation and activity.

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