Thrombin-induced translocation of GLUT3 glucose transporters in human platelets

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Platelets derive most of their energy from anaerobic glycolysis; during activation this requirement rises approx. 3-fold. To accommodate the high glucose flux, platelets express extremely high concentrations (155 ± 18 pmol/mg of membrane protein) of the most active glucose transporter isoform, GLUT3. Thrombin, a potent platelet activator, was found to stimulate 2-deoxyglucose transport activity 3–5-fold within 10 min at 25 °C, with a half-time of 1–2 min. To determine the mechanism underlying the increase in glucose transport activity, an impermeant photolabel, [2-^{3}H]2*N*-4-(1-azi-2,2,2-trifluoethyl)benzoyl-1,3,-bis-(D-mannose-4-ylozy)-2-propylamine, was used to covalently bind glucose transporters accessible to the extracellular milieu. In response to thrombin, the level of transporter labelling increased 2.7-fold with a half-time of 1–2 min. This suggests a translocation of GLUT3 transporters from an intracellular site to the plasma membrane in a manner analogous to that seen for the translocation of GLUT4 in insulin-stimulated rat adipose cells. To investigate whether a similar signalling pathway was involved in both systems, platelets and adipose cells were exposed to staurosporin and wortmannin, two inhibitors of GLUT4 translocation in adipose cells. Thrombin stimulation of glucose transport activity in platelets was more sensitive to staurosporin inhibition than was insulin-stimulated transport activity in adipose cells, but it was totally insensitive to wortmannin. This indicates that the GLUT3 translocation in platelets is mediated by a protein kinase C not by a phosphatidylinositol 3-kinase mechanism. In support of this contention, the phorbol ester PMA, which specifically activates protein kinase C, fully stimulated glucose transport activity in platelets and was equally sensitive to inhibition by staurosporin. This study provides a cellular mechanism by which platelets enhance their capacity to import glucose to fulfil the increased energy demands associated with activation.

INTRODUCTION

The uptake of glucose into most mammalian cells is mediated by a family of facilitative glucose transporters (GLUTs). This family comprises seven genes (numbered *GLUT1–GLUT7* in the order in which they were cloned) encoding six highly homologous proteins; *GLUT6* is a pseudogene. These proteins share a 50–70 % amino acid identity between isoforms and 80–90 % identity across species for a given isoform. The GLUTs have a characteristic structure with 12 membrane-spanning regions, a glycosylation site on the first extracellular loop, a large intracellular loop and cytoplasmic C- and N-termini. Although *GLUT1* is expressed ubiquitously, the other *GLUT* genes are restricted in their tissue expression and exhibit distinct kinetic properties, subcellular localization and modes of regulation (reviewed in [1–4]).

At rest, platelets derive most of their energy (60–75 %) from anaerobic glycolysis, and the remainder from the oxidative metabolism of lactate and fatty acids [5–7]. Platelets have a substantial glycogen store that is not used in the resting state [6]. Thus platelets are heavily dependent on their ability to transport glucose into the cell to meet their metabolic needs. A major energy demand for platelets under these circumstances seems to be for the maintenance of a steady state between the polymerization and depolymerization of actin, which helps to maintain the structural integrity of the cell [7]. On platelet activation induced by agents such as thrombin or adrenaline (epinephrine), a cascade of energy-dependent events is initiated, i.e. actin polymerization, changes in platelet shape, aggregation and secretion of dense, α, and acid-hydrolase vesicles. These processes rapidly triple the expenditure of ATP [8–10] and are all blocked by agents that stimulate adenylate cyclase such as prostaglandin E_1 (PGE_1) (reviewed in [9]). To meet the additional metabolic demands associated with activation, glycogenolysis is immediately stimulated; some early studies report that this increase in glycolytic flux is accompanied by an increase in the uptake and utilization of glucose [11–13]. It was, therefore, of interest to determine which GLUTs are responsible for mediating glucose uptake.

A preliminary study by Craik et al. [14] suggested that GLUT3 might be present in platelets. GLUT3 is generally considered to be the neuronal glucose transporter, although it has been detected in peripheral cells with high metabolic activity, e.g. sperm and placental epithelium [15–20], and is aberrantly expressed in glioblastomas and HIV-infected lymphocytes [21,22]. In neurons, GLUT3 has a high affinity for glucose (K_m 2.8 mM) and the highest V_{max} for transport of all the facilitative glucose transporters [23]. It was generally assumed that in all these cells the GLUT3 transporter resides exclusively in the plasma membrane. This is in contrast with GLUT4, which, in unstimulated heart, muscle, and adipose cells, resides predominantly (95 %) in specialized intracellular vesicles that are recruited to the plasma membrane in response to insulin, resulting in a 10–20-fold

Abbreviations used: ATB-BMPA, [2-^{3}H]2*N*-4-(1-azi-2,2,2-trifluoethyl)benzoyl-1,3,-bis-(D-mannose-4-ylozy)-2-propylamine; CB, cytochalasin B; 2-DG, 2-deoxyglucose; PGE_1, prostaglandin E_1; PKC, protein kinase C.

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increase in glucose transport activity [1–3,24]. A similar GLUT1 response is elicited by growth factors in a variety of cells but the magnitude of the response is smaller, with only 1–2-fold increases in transport activity [25]. In a study by Wilson et al. [26], in L6 muscle cells, GLUTs 1, 3 and 4 were all shown to be translocated from intracellular locations to the plasma membrane in response to insulin, thus constituting the first and only demonstration of the potential intracellular sequestration and translocation of GLUT3.

Here we demonstrate that the transport of glucose into platelets is mediated by an extremely high concentration of the most active of the facilitative glucose transporters, GLUT3. After activation with thrombin, there is a translocation of additional GLUT3 transporters to the platelet plasma membrane, thus enhancing the cell's capacity to transport glucose and meet increased energy demands.

MATERIALS AND METHODS

Materials

Unless otherwise indicated all chemicals and agents were obtained from Sigma.

Platelet isolation

Whole blood (20 ml) was drawn by butterfly needle and syringe from healthy volunteers and placed in polypropylene tubes containing 2.8 ml of citrate phosphate dextrose adenine solution anticoagulant (CPDA-1; USP–Baxter Corp.), supplemented with 10 μM prostaglandin E_1 (PGE_1). The platelets were isolated as described by Horne et al. [27]. Briefly, the CPDA-1/PGE_1-treated whole blood was centrifuged at 550 ***g*** for 5 min at room temperature. The upper two-thirds of the plasma layer (approx. 5 ml) was applied to a 30 ml Sepharose CL-2B column, equilibrated with Mg^{2+}- and Ca^{2+}-free PBS (BioWhittaker) containing 10 μM PGE_1 to prevent activation. Platelets were collected in the void volume and were immediately assayed for glucose transport activity. In experiments involving thrombin or PMA stimulation experiments, the Sepharose column was equilibrated with PBS containing 10 mM EDTA in place of the PGE_1. Mg^{2+}-dependent platelet aggregation occurs in response to activation with thrombin or PMA, and EDTA prevents this aggregation. For studies requiring more cells, i.e. those with cytochalasin B (CB) (Aldrich) binding, 10–20 ml of 'plateletphoresed' cells were obtained from the Department of Transfusion Medicine at the Clinical Center of the NIH. These platelets were treated with 10 μM PGE_1 and centrifuged twice at 110 ***g*** for 1 min to remove residual erythrocytes. The supernatant containing the platelets was then applied to the Sepharose CL-2B column, as described above.

Glucose uptake assay with [^{3}H]2-Deoxyglucose (2-DG)

The purified platelets [100 μl; $(0.5–1.0) \times 10^7$ cells] were aliquoted into small polypropylene tubes and preincubated at 25 °C. The transport reaction was initiated by the addition of 100 μl of 20 or 200 μM 2-DG solution containing tritiated (10 μCi/ml; Dupont–NEN) and unlabelled 2-DG in PBS. At specific intervals the reaction was stopped by the addition of 1 ml of ice-cold PBS containing 0.1 mM CB. The platelets were collected by vacuum filtration on 0.45 μm nitrocellulose filters supported by a Millipore manifold apparatus and washed with 25 ml of ice-cold PBS. The filters were air-dried and the radioactivity was determined by scintillation counting with Filter Count scintillant (Packard).

In experiments with staurosporin or wortmannin to inhibit the action of thrombin (2 i.u./ml) or the phorbol ester PMA (100 nM), platelets were preincubated at 25 °C with the compound for 20 min before initiation of transport. In these experiments, PMA or thrombin was added during the last 5 min of the preincubation period before initiating the 2-DG assay.

To determine platelet numbers, cells were diluted 1:2000 in Isoton II (Coulter) and counted in a Coulter Counter (ZM model) with a 70 μm aperture.

Adipose cells

Adipose cells were isolated from the epididymal fat pads of 180–200 g rats fed *ad libitum* with standard NIH chow. The fat pads were digested with collagenase as described by Weber et al. [28]. All incubations were performed in Krebs–Ringer buffer, pH 7.4, supplemented with 30 mM Hepes (KRBH), 10 mM HCO_3^-, 5% (w/v) BSA and 200 nM adenosine. 3-*O*-Methylglucose transport activity was determined as described previously [28]. The adipose cells were preincubated at 37 °C with either staurosporin or wortmannin for 15 min and subsequently exposed to insulin (0.67 μM; E. Lilly) for a further 15 min before transport activity was assessed.

Western blot analysis

Small aliquots of whole platelets in PBS (approx. 10 μl) were taken to determine protein concentration by the Pierce bicinchoninic acid assay. The remaining samples were solubilized directly in Tris/SDS/urea/dithiothreitol electrophoresis sample application buffer [15 mM/1.5% (w/v)/2.3 M/100 mM, pH 6.8) and separated by SDS/PAGE. Western blot analysis was performed as described previously by Maher et al. [29], with rabbit anti-serum raised against the human GLUT3 C-terminal sequence residues 476–495 (a gift from Hoffman–La Roche). Human brain membranes were run with each gel to serve as an internal control for GLUT3 detection. Antibodies raised against human GLUTs 1, 2, 4, and 5 C-terminal sequences, which are capable of detecting transporter proteins at levels of 1 pmol/mg, were not found to cross-react with platelet membranes (results not shown) [4].

CB binding

Platelets were obtained by plateletphoresis and purified as described above. The platelets were suspended in PBS, supplemented with protease inhibitors [aprotinin, leupeptin and pepstatin at 10 μg/ml each, and 50 μM 4-(2-aminoethyl)benzenesulphonyl fluoride hydrochloride from ICN], and sonicated (three times, for 20 s each) with a Bransom 250 sonifier at setting 2. The membranes were pelleted at 200000 ***g*** for 10 min, resuspended by gentle homogenization with a Teflon homogenizer in Tris (20 mM)/EDTA (1 mM) buffer, and protein concentrations were determined. The membranes (approx. 1 mg/ml) were incubated for 30 min with [^{3}H]CB at room temperature and subsequently pelleted at 220000 ***g*** for 2 h at 4 °C. The radioactivity associated with the pellet and with supernatant was determined by scintillation counting [28].

Incorporation of [^{3}H]ATP-BMPA derivative

Freshly isolated platelets were photolabelled with [^{3}H]ATB-BMPA {[2-^{3}H]2*N*-4-(1-azi-2,2,2-trifluoethyl)benzoyl-1,3,-bis-(D-mannose-4-ylozy)-2-propylamine} as described previously by Maher and Simpson [30]. Briefly, 0.5 ml of cells $[(0.5–1) \times 10^8$ cells], separated in the presence of either PGE_1 or EDTA, were placed in six-well tissue culture plates and incubated at room temperature with PGE_1, EDTA or EDTA plus thrombin, for the

indicated times; 90 s before the end of the incubation period, the tritiated ATB-BMPA (50 μl) was added and the cells were exposed to UV in a Rayonet photochemical reactor for the remainder of the incubation. The platelets were washed three times in PBS and pelleted. The platelet pellets were solubilized in Tris/SDS/urea/dithiothreitol sample application buffer and 90% of each solubilized sample was separated by SDS/PAGE on a large 10% (w/v) polyacrylamide slab gel. The gels were subsequently sliced and solubilized, and the extent of tritium incorporation was analysed by scintillation counting [31]. The remaining 10% of each sample was further divided into two aliquots for duplicate Western blot analysis to normalize for total GLUT3 levels in each sample.

RESULTS

GLUT3 was the only glucose transporter isoform detected by Western blot analysis in human platelets (results not shown) and, as illustrated in Figure 1, it is present in platelets at a 10-fold higher concentration per mg of protein than is found in human brain. There also seems to be considerable variation in levels of GLUT3 between individuals. To obtain a more quantitative estimate of GLUT3 levels, platelets were washed and sonicated, and the D-glucose-inhibitable binding of the transport inhibitor CB to the platelet membrane was determined. The concentration of GLUT3 determined by CB binding ranged from 100 to 200 pmol/mg, with a mean $\pm$ S.E.M. of 154.8 $\pm$ 18.5 pmol/mg. The GLUT3 affinity for CB [K_d 440 $\pm$ 41 nM (mean $\pm$ S.E.M.; $n = 6$)] is significantly less than that observed for GLUT1 or GLUT4, both of which are of the order of 100 nM, but is consistent with that observed for GLUT3 in neurons [23,32].

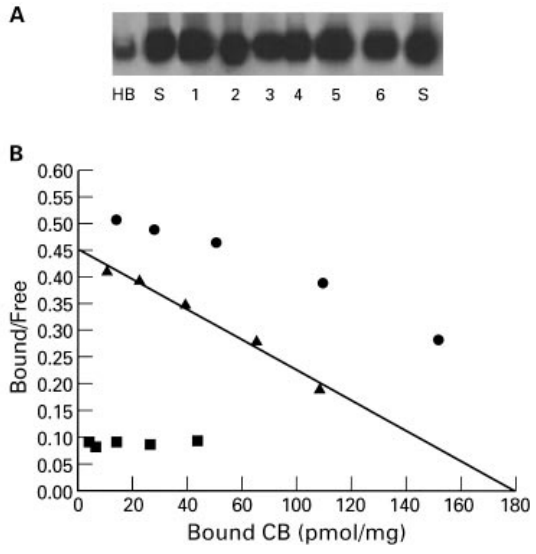

Figure 1 Glucose-transporter quantification

(**A**) Western Blot analysis of GLUT3 in human platelets. Human platelets obtained from six healthy individuals were analysed for GLUT3 content by Western blot analysis (lanes 1–6). Platelet membranes (20 μg), together with a standard platelet sample (S) of known GLUT3 concentration (see Figure 1B) and a human brain (HB) sample, were prepared and separated as described in the Materials and methods section. (**B**) CB binding to platelet membranes. A representative Scatchard plot of ^{3}H-CB binding to platelet membranes, measured in the presence (■) or absence (●) of 500 mM D-glucose as described in the Materials and methods section and by Weber et al. [28]. The derivative curve (▲) obtained by radial subtraction of the two curves reveals a D-glucose-inhibitable binding component of 178 pmol/mg with a K_d of 386 nM.

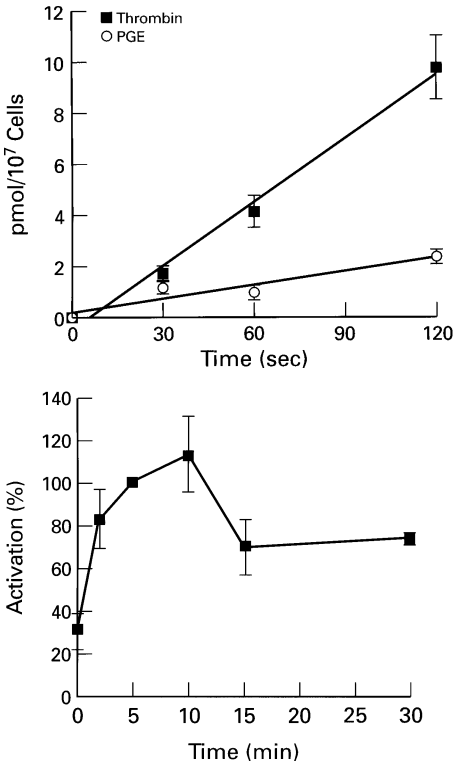

Figure 2 Glucose-transport activation

Upper panel: time course for 2-DG transport in platelets, showing stimulation by thrombin. A representative experiment is shown, illustrating the time course for the uptake of 2-DG measured in the presence of 10 μM PGE_1 (●) or 2 i.u./ml bovine thrombin (■) as described in the Materials and methods section. Lower panel: time course for thrombin stimulation of 2-DG transport activity. Platelets were exposed to 2 i.u./ml bovine thrombin for the indicated times. Uptake of 2-DG was measured over 2 min intervals across the time course. The experiment was performed on platelets obtained from healthy individuals ($n = 6$) and has been normalized by expressing the extent of activation relative to that observed after a 5 min exposure to thrombin.

The individual variation in the level of transporters is also reflected in the range of unstimulated transport rates and in the extent to which thrombin is able to stimulate activity. Figure 2 (upper panel) illustrates a typical 2-DG transport assay. 2-DG uptake was measured in unstimulated cells in the presence of 10 μM PGE_1 to suppress spontaneous activation. To measure 2-DG transport activity in stimulated platelets, the cells were exposed to 2 i.u./ml bovine thrombin 5 min before the addition of the radiolabelled 2-DG (0.2 mM) in the absence of PGE_1. EDTA was included in the assay to prevent platelet aggregation. Uptake of 2-DG was measured over the following 2 min. The rate of 2-DG uptake in the basal, unstimulated state corresponded to 1.3 pmol/min per 10^7 cells, and 6.3 pmol/min per 10^7 cells when fully stimulated by thrombin, indicating nearly 5-fold stimulation. This increase ranged from 2-fold to 6-fold between individuals and might also reflect the partial activation that can occur during platelet handling. These values, at 25 °C, are comparable to earlier measurements by Detwiler and others [13,33].

In an attempt to measure the time course for the stimulation of transport activity by thrombin, platelets were exposed to thrombin for the indicated times and the uptake of 2-DG was assessed at 2 min intervals throughout the time course. The results shown in Figure 2 (lower panel) are a compilation of six experiments in which uptake was measured in platelets obtained

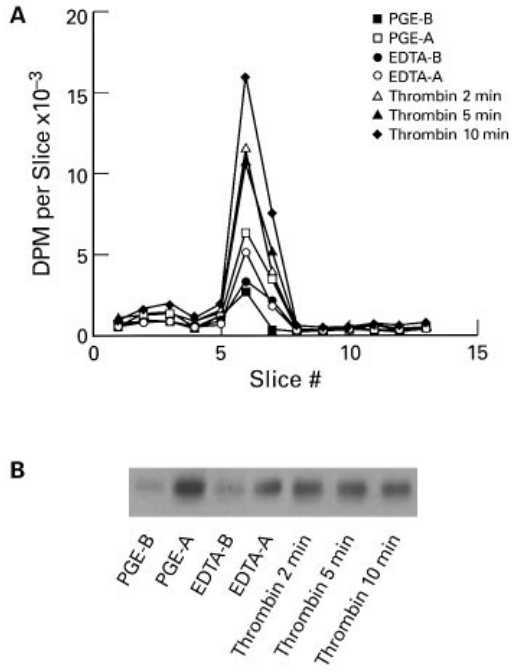

Figure 3 Time course of stimulation of GLUT3 translocation by thrombin

(**A**) SDS/PAGE profile of [^{3}H]ATB-BMPA. Duplicate platelet samples were exposed to thrombin for the indicated times; 90 s before the end of the incubation period, [^{3}H]ATB-BMPA was added and platelet suspensions were continuously exposed to UV as described in the Materials and methods section. Platelets were solubilized and 90% of the total volume was separated by SDS/PAGE; the corresponding profiles of one set of samples are illustrated. PGE_1-B and A and EDTA B and A refer to labelling in control samples of platelets labelled before (B) or after (A) completion of thrombin stimulation. (**B**) Western blot analysis of GLUT3 concentration in photolabelled platelets. The remaining 10% of solubilized, labelled platelets described in (**A**) were analysed by Western blot on two duplicate minigels. The levels of GLUT3 were analysed by PhosphorImager analysis and compared with a human brain standard that was applied to each gel (not shown).

Table 1 Effect of thrombin on [^{3}H]ATB-BMPA incorporation and glucose transport activity in platelets: time course

Platelets were treated as described in the legend to Figure 3. Levels of incorporation of ATB-BMPA measured in Figure 3(A) were normalized for GLUT3 levels measured in Figure 3(B). The [^{3}H]ATB-BMPA incorporation results are means ± range for duplicate photolabel incorporations and corresponding duplicate PhosphorImager analysis of Western blots. 2-DG transport activity was measured in parallel samples at the same time as platelets were photolabelled, and are means ± S.E.M. ($n = 3$). Maximum transport activity was 0.32 pmol/min per 10^7 cells.

Addition	ATB-BMPA incorporation (% of maximum)	2-DG transport activity (% of maximum)
PGE-B	30.2 ± 7.6	39.7 ± 3.4
PGE-A	32.2 ± 8.4	64.6 ± 0.9
EDTA-B	26.0 ± 3.8	60.5 ± 6.1
EDTA-A	40.8 ± 5.1	50.9 ± 2.8
Thrombin (2 min)	74.3 ± 12.2	84.8 ± 8.7
Thrombin (5 min)	72.7 ± 9.8	85.7 ± 7.1
Thrombin (10 min)	100 ± 13.5	100 ± 10.5

Table 2 ATB-BMPA inhibition of 2-deoxyglucose transport activity

Three platelet preparations were incubated in triplicate for 5 min in the presence or absence of thrombin. A 3 min uptake of [^{3}H]2-DG (10 μM) was then initiated in the presence of the indicated ATB-BMPA concentrations in the absence of UV. Results are means ± S.E.M. ($n = 3$).

ATB-BMPA (μM)	2-DG transport activity (%)	
	Basal	Thrombin
0	100	100
10	67.8 ± 9.7	69.7 ± 9.4
30	69.1 ± 7.7	65.7 ± 5.3
100	33.7 ± 4.7	53.4 ± 4.8

from different individuals; uptake thus has been normalized by setting the rate at 5 min to 100%. The apparent half-time for activation was on the order of 1–2 min; full activation was achieved in almost all cases by 5–10 min. The uptake rate declined consistently after 10 min, possibly reflecting a cellular depletion of ATP.

To investigate the mechanism underlying the stimulated rate of transport, we used the impermeant photolabel [^{3}H]ATB-BMPA to determine whether the increase in glucose transport activity induced by thrombin can be accounted for by an increase in the number of accessible transporters in the platelet plasma membrane. Figure 3 and Tables 1 and 2 describe a typical experiment in which platelets have been exposed to PGE_1, EDTA or EDTA plus thrombin for the indicated times: 90 s before the end of each incubation, the [^{3}H]ATB-BMPA was added to each platelet suspension and photolysed. The platelets were washed, and solubilized, then separated by SDS/PAGE as described in the Materials and methods section. In parallel incubations, 2-DG transport activity was determined and the results are presented in Table 2. Figure 3(A) illustrates the ^{3}H-labelling profiles for ATB-BMPA incorporation into one of the duplicate samples, and Figure 3(B) shows corresponding Western blots after exposure to the various conditions. In Table 1 the results from Figure 3(A) for incorporation of ATB-BMPA have been normalized for the total amount of GLUT3 in the respective samples, which was determined by PhosphorImager analysis of the Western blots depicted in Figure 3(B). Both transport and photolabel incorporation was increased approx. 3-fold after a 10 min exposure to thrombin. The mean ± S.E.M. increase in incorporation after a 10 min exposure to thrombin, relative to PGE_1-stimulated cells, for three separate experiments was 2.7 ± 0.6-fold. Consistent with the observations seen in Figure 2 (lower panel) for transport activity, the apparent half-time for enhanced photolabel incorporation is less than 2 min. To confirm that the enhanced glucose transport activity and [^{3}H]ATB-BMPA binding were due to translocation and not an alteration in the affinity of the GLUT3 for 2-DG and ATB-BMPA, we investigated the ability of unlabelled ATB-BMPA to inhibit 2-DG transport in basal and thrombin-stimulated platelets. A change in affinity would result in the thrombin-stimulated activity's being more sensitive to inhibition by ATB-BMPA. It is apparent from Table 2 that in the three samples of platelets the apparent sensitivity to ATB-BMPA inhibition is unchanged between basal and thrombin-stimulated 2-DG transport. This result suggests that thrombin induces the increased glucose transport activity by the translocation of GLUT3 to the platelet plasma membrane in a manner analogous to that seen in insulin-stimulated translocation of GLUT4 in adipose cells.

To determine whether a similar mechanism is involved in both platelets and adipose cells, we compared the abilities of two protein kinase inhibitors, staurosporin and wortmannin, to

Table 3 Effect of stauroporin and wortmannin on insulin-stimulated glucose transport activity in adipocytes and thrombin-stimulated transport activity in platelets

Adipocytes were exposed to staurosporin or wortmannin for 20 min at 37 °C before the addition of insulin (0.67 μM). 3-*O*-methylglucose (3-OMG) uptake was measured after a further 15 min at 37 °C. Thrombin (2 i.u./ml) was added during the last 5 min of the incubation period before measurement of 2-DG transport activity at 20 °C. Values are means ± S.E.M. ($n = 3$).

Addition	Stimulated 3-OMG transport in adipose cells (% of maximum insulin activity)
Basal	4.0 ± 0.3
Insulin	100.0 ± 2.3
Stauroporin (1 μM)	90.6 ± 9.6
Stauroporin (3 μM)	54.2 ± 4.6
Stauroporin (10 μM)	11.1 ± 1.0
Wortmannin (25 nM)	79.0 ± 5.3
Wortmannin (100 nM)	11.5 ± 1.6

Addition	Stimulated 2-DG transport in platelets (% of maximum thrombin activity)
PGE	29.0 ± 6.4
Thrombin	100.0 ± 10.0
Staurosporin (0.3 μM)	92.1 ± 19.6
Staurosporin (1 μM)	33.3 ± 5.5
Staurosporin (3 μM)	20.6 ± 1.7
Wortmannin (25 nM)	104.6 ± 10.0
Wortmannin (300 nM)	141.0 ± 11.6

Table 4 Effect of staurosporin on glucose transport activity in platelets treated with thrombin or PMA

Platelets were incubated in the presence or absence of staurosporin (3 μM) for 15 min. PMA (100 nM) or thrombin (2 i.u./ml) were added for the last 5 min of the incubation period before the determination of 2-DG transport activity. Values are means ± S.E.M. ($n = 3$).

Addition	2-DG transport activity (% of maximum thrombin activity)
PGE_1	31.1 ± 0.4
Thrombin	100.0 ± 3.2
Thrombin + staurosporin	27.5 ± 2.2
PMA	81.9 ± 4.4
PMA + staurosporin	22.6 ± 1.9

inhibit the stimulation of glucose transport activity in both cell types. The results presented in Table 3 reveal that, whereas thrombin stimulation of transport activity in platelets is clearly more sensitive to inhibition by staurosporin than is insulin-stimulated transport activity in adipose cells, it is completely unaffected by the phosphatidylinositol 3-kinase inhibitor wortmannin. In contrast, insulin-stimulated transport activity in adipose cells is completely inhibited by 100 nM wortmannin. We have shown in adipose cells, and others have shown in platelets [34–36], that staurosporin action is not confined to the inhibition of protein kinase C (PKC) and, at the levels used to inhibit insulin-stimulated transport activity in adipose cells, serine/threonine kinases and tyrosine kinases are also inhibited in both cell types [34–36]. Furthermore thrombin is known to initiate a cascade of signalling events, including phospholipase C activation, Ca^{2+} mobilization, PKC activation, and adenylate cyclase inhibition, all of which could potentially be modulated by staurosporin. Thus, to determine whether glucose transport activity is activated through the PKC cascade, we stimulated platelets with the phorbol ester PMA. Table 4 demonstrates that PMA is able to elicit a stimulation of glucose transport comparable to that observed with thrombin and that this stimulation is specifically inhibited by staurosporin.

DISCUSSION

In the course of activation, platelets undergo a series of biochemical alterations, including changes in shape and aggregation and the secretion of different components present in several distinct intracellular vesicles [9]. Activation can be initiated by a variety of agents, including thrombin and adrenaline, and is associated with the stimulation of phospholipase C and PKC, increases in intracellular Ca^{2+} levels, inhibition of adenylate cyclase and release of arachidonate [9]. All of these events are clearly energy-dependent, initiated in a matter of seconds and are complete within 10 min. Metabolically, platelets are constrained by their small size and thus have few mitochondria, i.e. 6–10 per cell [6]. They must therefore derive 60–70 % of their energy at rest from anaerobic glycolysis, with the balance being furnished by the oxidation of lactate and fatty acids [9,10]. On stimulation, ATP consumption is tripled over the first 30 s and remains significantly elevated over the subsequent 30–180 s [9,10]. Accompanying this stimulation is a transient burst of mitochondrial oxygen consumption that lasts 30-45 s and might provide 25 % of the total ATP utilized during the period [9,10,13]. The remaining ATP is provided by accelerated glycolysis and initiation of glycogenolysis, which results in a doubling of the output of lactate [10,13]. Crucial to this accelerated utilization of glucose is the ability of the platelets to increase glucose transport activity acutely. In this study we demonstrate that platelets possess an extremely high concentration of the GLUT3 glucose transporter. Despite significant individual variation, the concentration of GLUT3 transporters in platelets (approx. 150 pmol/mg) is second only to the GLUT1 levels in human erythrocytes [31]. An unexpected outcome of these studies is the demonstration that, in common with GLUT1 and GLUT4, GLUT3 can also be recruited to the plasma membrane in response to a stimulus, supporting the observations of Wilson et al. [26] in L6 cells. However, this study represents the first such demonstration of GLUT3 translocation in a cell in which GLUT3 is the sole transporter. Although not as well characterized as GLUT4, GLUT1 has been shown to be translocated to the plasma membrane in a variety of cells in response to growth factors [25]. In rat adipose cells and 3T3-LI adipocytes, GLUT1 seems to reside in intracellular vesicles distinct from those containing GLUT4 and, in response to insulin, the level in the plasma membrane is approximately doubled [24]. In the same cells, in the absence of insulin, the vast majority (more than 95 %) of GLUT4 glucose transporters reside intracellularly. On stimulation with insulin, these transporters translocate the plasma membrane with a half time of approx. 2 min, and the level in the plasma membrane is increased 10–20-fold [24]. After insertion into the adipocyte plasma membrane, a steady-state recycling of the transporters between the plasma membrane and the intracellular site is established and continues until the insulin is removed [35]. The translocation process has been shown to be sensitive to inhibition by staurosporin; this inhibition is greater when translocation is induced by PMA rather than by insulin [34]. Insulin-stimulated GLUT4 translocation is, however, particularly sensitive to inhibition by wortmannin, a fairly specific inhibitor of phosphatidylinositol 3-kinase, as illustrated in Table 3 [36,37].

In contrast with adipose cells, the mechanism by which translocation is initiated in platelets by thrombin would seem to

involve the activation of PKC and can be readily mimicked by PMA. The actions of both agents are inhibited by staurosporin but not wortmannin. The 3-fold increase in GLUT3 levels in the plasma membrane suggests a distribution between plasma membrane and intracellular sites more comparable to that seen with GLUT1 than with GLUT4. However, the magnitude of the intracellular pool of GLUT3 transporters has yet to be determined. A recent study by Heijnen et al. [38] has demonstrated that GLUT3 can be detected in α vesicles, which translocate to the plasma membrane in response to thrombin.

The observation that GLUT3 in platelets can reside intracellularly and can be recruited to the plasma membrane in response to a stimulus highlights important issues as to GLUT3's action in synaptic terminals. Specifically, the potential for intracellular GLUT3-containing vesicles that can be translocated to synaptic membranes would provide a novel mechanism for the provision of energy during acute increases in neuronal activity.

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