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Effects of cadmium on glucose transport in rat adipocytes and human erythrocytes. Stimulation of GLUT1 catalytic activity

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Abbreviations: TPA, 12-*O*-tetradecanoyl phorbol-13-acetate; 3OMG, 3-*O*-methyl-D-glucose

Abstract

The effects of cadmium on facilitative glucose transporter function in rat adipocytes were studied. A short (30 min) incubation with cadmium resulted in stimulation of 3-*O*-methyl-D-glucose (3OMG) equilibrium exchange in rat adipocytes as much as four-fold. The stimulation was a saturable function of cadmium concentation with the half-maximal effect at approximately 0.5 mM $CdSO_4$. The stimulation was due to an increase in V_{max} with no significant changes in K_M. No further stimulation of 3OMG flux was observed once adipocytes were maximally stimulated by insulin. Semiquantitative immunoblot analysis of subcellular fractions revealed that the stimulation was accompanied by an insignificant and only a modest (less than 50%) increase in plasma membrane GLUT4 and GLUT1 levels, respectively, suggesting that the stimulation involves largely an increased catalytic activity of either or both of GLUT1 and GLUT4. We next studied effect of cadmium on GLUT1 selectively using human erythrocytes and purified GLUT1 reconstituted in liposomes. With purified GLUT1, cadmium inhibited cytochalasin B binding and stimulated 3OMG flux, indicating that cadmium directly interacts with GLUT1. Cadmium, however, did not affect the 3OMG flux in intact human erythrocytes or their resealed ghosts. These findings strongly suggest that cadmium stimulates the catalytic activity of GLUT1 and GLUT4 in adipocytes, and this effect is suppressed by a cell-specific factor or factors in human erythrocytes.

Keywords: adipocytes, cadmium, erythrocytes, glucose transporter

Introduction

Glucose metabolism in muscle and adipose tissues are regulated at the step of cellular uptake of glucose (Carruthers, 1990; Kasanicki and Pilch, 1990), the process catalyzed by a family of intrinsic membrane proteins (facilitative glucose transporters) and further subjected to hormonal and metabolic regulation (Simpson and Cushman, 1986). In rat epididymal adipocytes, for example, the glucose uptake is catalyzed primarily by GLUT4, a specific isoform of this family expressed in fat and muscle cells, and in a small extent by GLUT1, a more ubiquitous isoform (Zorzano *et al.*, 1989). Constitutively (in the absence of insulin), most of these transporters in adipocytes reside in intracellular microsomes rather than at the cell surface, and insulin induces a net movement of these transporters from the storage pool to the cell surface, thus increasing their plasma membrane levels (Cushman and Wardzala, 1980; Suzuki and Kono, 1980). It is now widely accepted that this apparent transporter recruitment is the major machanism for the regulation of glucose metabolism by insulin. The insulin-induced stimulation of glucose transport, however, significantly exceeds the increases in plasma membrane glucose transporter levels in adipocytes, suggesting that mechanisms other than transporter recruitment are also oparative for stimulation of GLUT4 and/or GLUT1 function.

A number of recent observations demonstrate that the intrinsic activity of glucose transporters is indeed modulated (Baly and Horuk, 1987; Matthaei *et al.*, 1988). A phorbol ester, 12-*O*-tetradecanoyl phorbol-13-acetate (TPA) and insulin cause glucose transporter recruitment to a similar extent in adipocytes, yet the glucose transport stimulation by TPA is only less than 50% of that by insulin (Muhlbacher *et al.*, 1988). Catecholamine inhibits insulin-stimulated glucose transport in rat adipocytes by 50-75% without affecting the glucose transporter level in the plasma membrane (Joost *et al.*, 1986). Cholera toxin or dibutyryl cAMP causes a 10-fold stimulation of 2-deoxy D-glucose uptake in 3T3-L1 adipocytes with a minimal increase in GLUT4 (1.3-fold) and GLUT1 (1.6-fold) levels at the cell surface (Clancy and Czech, 1990), indicating that transporter intrinsic activity was stimulated at the plasma membrane.

More recently, Harrison, *et. al.* (I991) have shown that an incubation of 3T3-L1 adipocytes with cadmium [Cd(II)] results in a large (7-11 fold) stimulation of 3-*O*-methyl-D-glucose (3OMG) flux with little or no increase

in the plasma membrane GLUT4 and GLUT1 levels, and concluded that cadmium increases the intrinsic activity of glucose transporters in 3T3-LI adipocytes. This conclusion, however, contradicts the observation made with isolated rat adipocytes by Ezaki (1989) where cadmium was shown to stimulate glucose transport more than 10-fold, and this stimulation was accompanied by an equally large, recruitment of GLUT1 immunoreactivity from the intracellular pool to the plasma membrane. This apparent contradiction, and the fact that Ezaki (1989) did not measure the Cd(II) effect on the plasma membrane GLUT4 level in adipocytes, prompted us to reexamine the effects of Cd(II) on GLUT1 and GLUT4 function in rat adipocytes in the present study.

In the present study, we demonstrate that cadmium stimulates glucose transport in rat adipocytes up to 3.5-fold where insulin stimulated as much as 7-fold, and the stimulation is due to an increase in transport V_{max} with little change in K_M. We also demonstrate that the stimulation is accompanied by only a less than 50% increase in plasma membrane GLUT1 level with little change in GLUT4 level and no detectable changes in their total cellular contents. These findings indicate that Cd(II) stimulates glucose transport in rat adipocytes mostly by a mechanism other than recruitment of GLUT1 and GLUT4. Using purified GLUT1 reconstituted in liposomes, we demonstrate that Cd(II) stimulates 3OMG flux and inhibits cytochalasin B binding activity. GLUT1 transport activity in intact human erythrocytes, however, was not affected significantly by Cd(II). These and other published findings strongly suggest that the Cd(II) may stimulate GLUT1 intrinsic activity *in vitro* and in rat adipocytes, but this effect is suppressed by a cell-specific factor in human erythrocytes.

Materials and Methods

Materials

$CdSO_4$ and cytochalasin B were obtained from Sigma(St. Louis, U.S.A.). [^{3}H]Cytochalasin B was purchased from Amersham (Buckinghamshire, U.K.) and 3OMG from ARC. BSA was from Boeringer Mannheim (Mannheim, Germany) and collagenase from Worthington (Freehold, U.S.A.). [^{14}C]OMG was purchased from ICN (Costa Mesa, U.S.A.). Fresh blood was obtained from healthy volunteers. Outdated human whole blood was supplied by the American Red Cross, Buffalo, N.Y., U.S.A.

Isolation of adipocytes

Epididymal fat pads were obtained from Sprague-Dawley rats (weighing 150-175 g) sacrificed by cervical dislocation. The fat pads were removed through a surgical incision, then put immediately in KRH buffer (Martz *et al.*, 1989). Adipocytes were isolated as described. The cells were stabilized for 45 min at 37°C by incubating in KRH buffer containing 5 mM D-glucose and 3OMG at a specified concen-tration specified in each experiment.

Subcellular membrane fractionation

Isolated adipocytes were washed three times with STEP buffer (250 mM sucrose, 10 mM Tris-HCL, 2 mM EGTA, 10 μM PMSF) at 20°C. Subcellular membrane fractionation was performed exactly as described (Martz *et al.*, 1989) to obtain crude plasma membrane (PM/NM), and the high and low density microsomes (HDM and LDM, respectively).

Measurement of 3OMG transport in adipocytes

Cells were first chemically equilibrated by incubating for 45 min at 37°C in the presence of 3OMG at varying concentrations (0.3-30 mM) and 5 mM D-glucose. When applicable, insulin (10 nM) and varying concentrations of $CdSO_4$ were present at this stage. To run the equilibrium exchange flux, different sets of 8 tubes containing 60 μl of adipocytes (50% cytocrit) were arranged in a warm bath maintained at 37°C, and the exchange was initiated by adding 6 μl of [^{3}H]30MG stock solution to get a final concentration of 1 μCi/ml. The cells were mixed gently and allowed to equilibrate for 3-60 sec. Flux was terminated by adding 200 μl of ice-cold stopping solution that contains 0.2 mM phlorentin, varying concentrations of 3OMG and 5 mM D-glucose in KRH buffer, pH 7.4. The cell suspensions were immediatly centrifuged in microfuge tubes containing silicon oil, then the supranatants and pellets were separated. The final equilibration was approximated at 5 min for insulin stimulated flux and 15 min for the basal flux. Flux data were analyzed as described (Martz *et al.*, 1986).

Cytochalasin B binding assay

Equilibrium binding of cytochalasin B in the presence and absence of an excess (500 mM) glucose were quantitated as described (Jacobs and Jung, 1985) using six different cytochalasin B concentrations (10^{-8} to 10^{-5} M) and a fixed amount of [^{3}H]cytochalasin B as a radioactive tracer. Radioactivities were measured in an LKB 1209 Rackbeta liquid scintillation counter.

Transport assay in human erythrocytes, resealed ghosts, and in liposomes containing purified GLUT1

Human erythrocytes were prepared using freshly drawn blood obtained by venipuncture from healthy volunteers as described (Jung and Rampal, 1977). Hemoglobin-free, human erythrocyte ghosts were prepared and resealed as described (Jung *et al.*, 1971). GLUT1 was

purified from erythrocyte ghosts as proteoliposomes as described (Rampal *et al.*, 1986). The glucose transport activities of intact cells (Jung and Rampal, 1977), resealed ghosts (Jung *et al.*, 1971), and purified GLUT1 proteoliposomes (Lachaal and Jung, 1993) were assayed by measuring the mercuric chloride-arrestable, equilibrium exchange or influx of 3OMG using [^{14}C]3OMG as a tracer.

Semiquantitative immunoblotting analysis of GLUT1 and GLUT4

Subcellular fractions were subjected to 10% SDS-PAGE (Laemmli, 1970), and proteins were transferred to nitrocellulose paper (Towbin *et al.*, 1979). The transfer of proteins of all lanes was verified by Ponceaus red staining. After 3-4 washes with TBS (150 mM NaCl, 10 mM Tris-HCl, pH 7.4), 5% nonfat milk was used to block nonspecific protein adsorption to the paper. After one hour rinse with TTBS (TBS containing 0.05% Tween 20), antibodies were added and incubated overnight while shaking. The nitrocellulose filters were again washed with TTBS three times, then treated with [^{125}I]protein A. Film was developed after film impression in ultra-freezer.

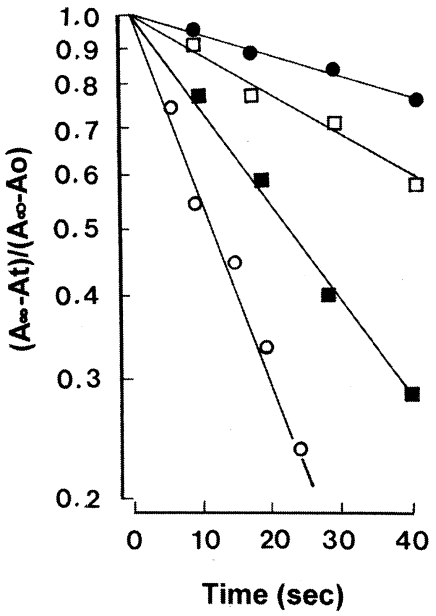

Figure 1. Time course of the tracer equi1ibrium exchange of 3OMG in adipocytes in the presence and the absence of Cd(II). Isolated rat adipocytes were incubated with 10 mM 3OMG for 20 min at 37°C, $CdSO_4$ was added when used, then further incubated for 30 min. The flux measurement was initiated by quickly adding a tracer amount of [^{14}C]3OMG at t = 0, and tracer equilibration time course was followed by arresting flux at a specified time t. Results were plotted according to the kinetic model of equilibrium tracer exchange in a closed, two compartment system of $(S_{00}-S_t)/(S_{00}-S_0) = \exp(-k_t)$, where S_t, S_0 and S_{00} denote the cell-associated radioactivities at a given time, t (in sec), at t = 0, and at complete tracer equilibrium (measured at 10 min), respectively, t is the time of equilibration in sec, and *k* is the first order rate constant. Time courses were measured in the absence (solid circles) or in the presence of Cd(II) at 0.5 (open squares) and 5 (solid squares) mM. Also shown is the 10 nM insulin-stimulated 3OMG equilibrium time course (open circles) measured in parallel for comparison.

Results

Cd(II) effects on GLUT1 and GLUT4 in rat adipocytes

Incubation of rat adipocytes with millimolar concentrations of $CdSO_4$ for 30 min resulted in a dose-dependent increase in the rate of 3OMG equilibrium exchange (Figure 1). The exchange rate constant (*k*, in per sec) calculated from the exchange time course (Figure 1) was found to be a saturable function of Cd(II) concentration (Figure 2). Cd(II) increased the rate constant 3.5-3.8 fold at maximum, and 50% of the maximum stimulation was achieved at around 0.5 mM Cd(II). Kinetic analysis of the 3OMG flux as a function of 3OMG concentration measured in the absence and in the presence of two different concentrations of Cd(II) revealed that the stimulation was exclusively due to an increase in transport V_{max}; the K_M was 9 mM regardless of the presence and the absence of Cd(II) (Figure 3). An incubation of adipocytes with 10 nM insulin for 20 min stimulated 3OMG flux 7-8 fold (Figures 1 and 4).

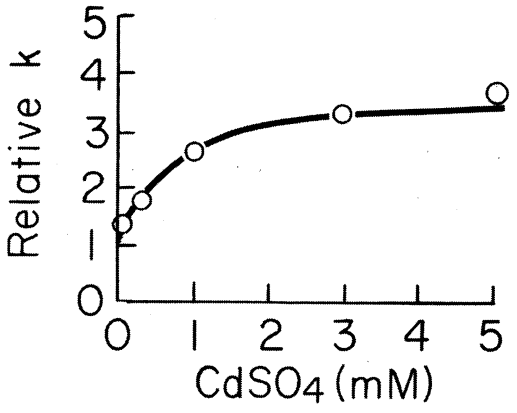

Figure 2. The stimulation of 3OMG (10 mM) equilibrium exchange by Cd(II) in rat adipocytes as a function of $CdSO_4$ concentration. The exchange time course was measured in the absence and in the presence of an increasing concentration (0.1-5 mM) of $CdSO_4$, and the first order rate constant (*k*) was calculated from equilibration time course as in Figure 1. The *k* values were then normalized against that measured in the absence of $CdSO_4$, and plotted against $CdSO_4$ concentration.

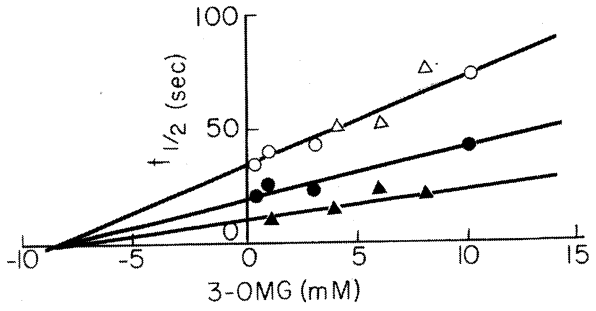

Figure 3. Kinetic analysis of the equilibrium exchange of 3OMG in rat adipocytes in the absence (open circles and triangles) and in the presence of 1 (solid circles) and 3 (solid triangles) mM $CdSO_4$ as a function of 3OMG concentration. Each data point represents a 40-60 sec, 3OMG equilibrium exchange time course similar to those illustrated in Figure 1, from which the half-equilibration time ($t_{1/2}$) was calculated, and plotted against 3OMG concentration. The plot is based on the relationships; $[S]/v = K_M/V_{max} + [S]/V_{max}$, and $t_{1/2} = 0.693[S]/v$, where v is the rate of 3OMG exchange measured at the 3OMG concentration [S], and K_M and V_{max} are the Michaelis-Menten constant and the maximum exchange rate, respectively.

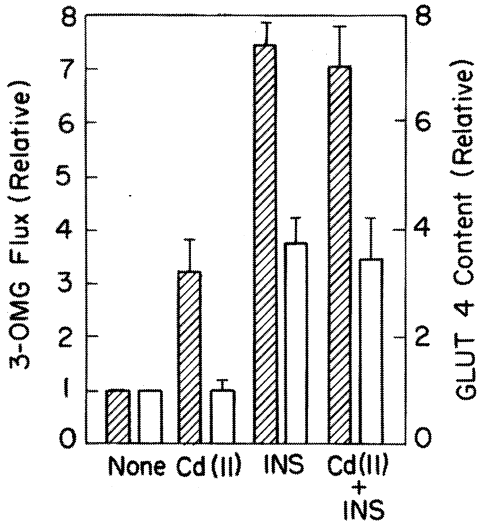

Figure 4. Comparison of $CdSO_4$ and insulin effects on 3OMG equilibrium exchange flux and plasma membrane GLUT4 content in adipocytes. Flux (shaded bars) and GLUT4 content (open bars) were measured prior to (None), and after a 20 min-treatment with 3 mM $CdSO_4$ (Cd(II)), 10 nM insulin (INS), or 3 mM $CdSO_4$ plus 10 nM insulin (Cd(II)+INS). The flux was measured following equilibrium exchange time course as illustrated in Figure 1. GLUT4 content was measured by immunoblotting crude plasma membrane (PM/NM) fraction. Blot intensities were quantitated by optical densitometry, and expressed in quantities relative to those of control (None). Data represent the means of four independent determinations with S.E.M. in vertical bars.

Cd(II) did not further increase this insulin-stimulated 3OMG flux (Figure 4).

Semiquantitative immunoblotting analysis (Figure 5) revealed that this Cd(II)-induced, glucose transport stimulation in adipocytes seen above was accompanied by a small but detectable increase in the steady state GLUT1 content in the plasma membrane fraction (Figure 5A). This increase, however, was modest (less than 1.6-fold or less) compared to the increase in transport rate observed (3.5-fold). Cd(II) also caused apparent increase in GLUT4 content in the plasma membrane in certain experiments (Figure 5B). This effect, however, was not statistically significant as it was not reproduced in other (three out of four) experiments (Figure 4). In parallel experiments, insulin was shown to increase plasma membrane GLUT4 level by 3-4 fold (Figures 4 and 5). It is thus apparent that the Cd(II) effects on transporter recruitment alone can not account for the stimulation of glucose transport in adipocytes by Cd(II). This strongly suggests that Cd(II) increases intrinsic activity of individual glucose transporters in rat adipocytes. Results of immunoblots also revealed that the Cd(II) treatment does not further affect the insulin-induced GLUT4 (Figure 4) and GLUTI (not illustrated) redistribution to any detectable extent.

Cd(II) effects on GLUT1 in human erythrocytes and purified GLUT1 in liposomes

The modulation by Cd(II) of glucose transporter intrinsic activity suggested above may be isoform-specific and/or cell-specific. To examine these possibilities, we studied here the effects of Cd(II) on GLUT1 selectively using intact human erythrocytes and proteoliposomes containing purified GLUT1 from human erythrocytes. Cd(II) stimulated 3OMG equilibrium exchange flux only slightly (less than 50%) in human erythrocytes, and inhibited 3OMG flux significantly in resealed ghosts (Figure 6).

Cd(II), however, inhibited the cytochalasin B binding activity of purified GLUTI reconstituted in liposomes (Figure 7), indicating that Cd(II) interacts with GLUT1 *in vitro*. This inhibition was dose-dependent with respect to Cd(II), with the 50% maximum inhibition effected at about 0.25 mM Cd(II) (Figure 8). Interestingly, on the other hand, Cd(II) did not appear to interfere with the displacement of cytochalasin B bound to GLUT1 (Figure 9). In fact, it was suggested that glucose displaces cytochalasin B more effectively in the presence of Cd(II) than its absence (Figure 9).

Quantitation of the transport function of purified GLUT1 reconstituted in liposomes is extremely difficult if not impossible (Lachaal and Jung, 1993). Data illustrated in Figure 10 nevertheless indicate that the rate of 3OMG uptake by liposomes containing purified GLUT1 is significantly faster in the presence of 1 mM Cd(II) than in the absence of Cd(II), suggesting that Cd

(II) enhances GLUT1 transport function. The GLUT1 proteoliposome preparation used here had protein-to-lipid mass ratio of approximately 10, made of endogenous erythrocyte lipids and exogenously added phosphatidyl choline in roughly equal proportions.

Discussion

Ezaki (1989) was the first to show the acute effect of Cd(II) causing stimulation of glucose transport in rat adipocytes. This Cd(II) effect is essentially reproduced in the present study (Figures 1 and 2), but with a notable quantitative difference; the magnitude of the stimulation was significantly less in our study (3-4 fold) compared with that (more than 15-fold) reported by Ezaki (1989). Different transport assay methods used, namely, equilibrium exchange measurement in our study versus zero-trans initial velocity determination by Ezaki (1989), may in part be responsible for some of this discrepancy. The effective concentration range of Cd(II) was similar in both studies, although 10 mM Cd(II) caused a significant cell damage in our study. The transport stimulation by Cd(II) and that by insulin are not additive in our experiments (Figure 4) as in Ezaki's (1989).

Subcellular redistribution of GLUT1 and particularly that of GLUT4 to the cell surface (recruitment) is a major mechanism by which insulin stimulates glucose transport in adipocytes (Cushman and Wardzala, 1980; Suzuki and Kono, 1980). Ezaki (1989) has studied the effect of Cd(II) on the glucose transporter recruitment in rat adipocytes using GLUT1-specific antibodies, and shown that Cd(II) induces a massive GLUT1 recruitment. This Cd(II)-induced GLUT1 recruitment in his study was comparable in extent to the insulin-induced GLUT1 recruitment. We also observed GLUT1 recruitment after Cd(II) treatment, but the effect was much modest (less than 50% increase) (Figures 4 and 5). In our present study, the Cd(II)-induced GLUT1 recruitment is much less than the insulin-induced GLUT1 recruitment. The nature of this discrepancy is not immediately clear. More importantly, we studied for the first time the Cd(II) effect on GLUT4 recruitment in rat adipocytes, and demonstrate (Figures 4 and 5) that the acute stimulation of glucose transport in adipocytes by Cd(II) is not accompanied by any significant recruitment of GLUT4, the major isoform in this cell

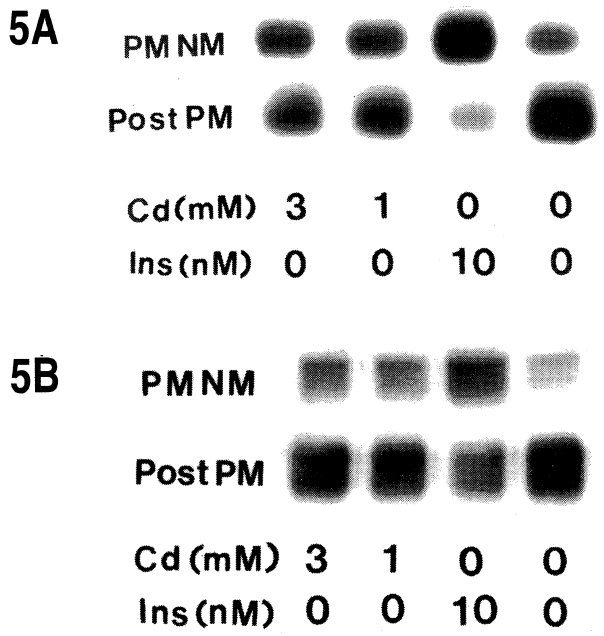

Figure 5. Semiquantitative immunoblotting analysis of GLUT4 and GLUTl contents in crude plasma membrane (PMNM) and microsomal (PostPM) fractions of adipocytes before and after insulin (INS) or $CdSO_4$ (Cd) treatment at a given concentration. Data similar to these were observed in three independent analyses, with an exception that a slightly increased GLUT4 blot in PMNM after cadmium seen here was not reproduced.

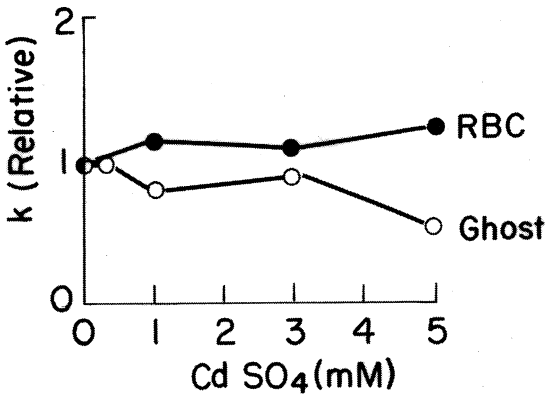

Figure 6. Effects of Cd(II) on 3OMG equilibrium exchange flux in human erythrocytes and resealed ghosts. (solid circles) and resealed ghosts (open circles) were equilibrated with 10 mM 3OMG for 30 min. [^{14}C]3OMG was added at t = 0, and a 12-15 sec tracer exchange time course was followed at 37°C, arresting flux at every 2.0-2.5 sec interval as described in Materials and Mehthods. The first order rate constants (k) were calculated from observed time courses and plotted as a function of $CdSO_4$ concentration as illustrated in Figures 1 and 2.

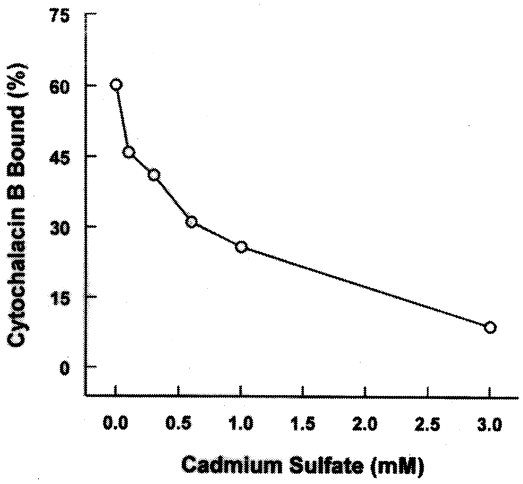

Figure 7. Effects of Cd(II) on the cytochalasin B binding activity of purified GLUT1 reconstituted in liposomes as a function of Cd(II) concentration. Aliquots of purified GLUT1 proteoliposome suspension (25 μg protein in 1 ml) were incubated with 10^{-7} M cytochalasin B with its radioactive tracer, and in the absence and presence of an increasing concentration of $CdSO_4$ for 30 min. The proteoliposomes were then separated free of suspension medium by centrifugation (200,000 *g* for 45 min), and radioactivities of both pellets and supernatants were measured by a liquid scintilation counter, from which the amount of bound cytochalasin B (in % of total) was calculated for each Cd(II) cencentration.

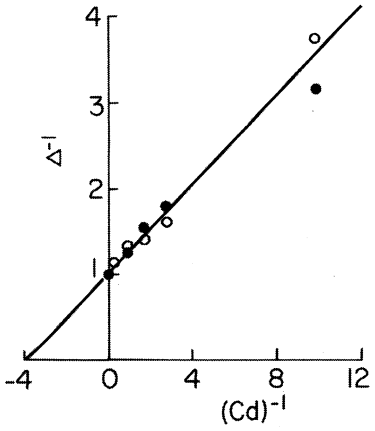

Figure 8. Double reciprocal analysis of the Cd(II)-induced inhibition of cytochalasin B binding to purified GLUT1 proteoliposomes. The inhibition was calculated the displacement (Δ) by a given concentration of $CdSO_4$ (Cd). The straight line represents the least squares regression fit to two sets of measurements shown in different symbols.

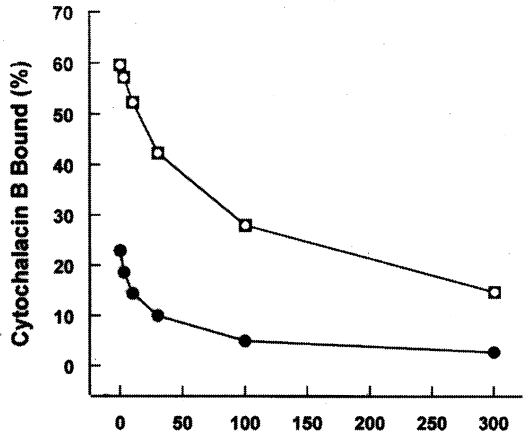

Figure 9. Effects of Cd(II) on the displacement of cytochalasin B from purified GLUT1 proteoliposomes by an increasing concentration of D glucose. Data are without (open circles) and with (solid circles) 3 mM $CdSO_4$ using 10^{-7} M cytochalasin B with its radioactive tracer, and 25 ug of GLUT1 in liposomes. Double reciprocal analysis of these data similar to Figure 8 above revealed apparent K_i values of approximately 20 and 8 mM for the data without and with 3 mM $CdSO_4$, respectively.

type. It is thus clear in our data that the extent of Cd(II)-induced recruitment for either isoform is not great enough to account for the extent (3.5-fold) of the Cd(II)-induced transport stimulation in rat adipocytes.

We have also demonstrated for the first time that the stimulation of glucose transport in rat adipocytes by Cd(II) is due to an increase in transport V_{max} with no significant change in K_M (Figure 3). It has been well documented (Whitesell and Glieman, 1979; Martz *et al.*, 1986) that the adipocyte glucose transport system follows the simple, Michaelis-Menten kinetics with the relationship; $V_{max} = k_{cat} \times E_T$, where E_T denotes the amount of glucose transporter available at the cell surface, and k_{cat} is a constant inherent to the transporter. Since E_T was increased by less than 60% for GLUT1 and not significantly for GLUT4 by Cd(II) (Figure 5), the 3.5-fold increase in V_{max} by Cd(II) must be in large part due to an increased k_{cat} of either or both of GLUT1 and GLUT4.

The molecular basis of how Cd(II) stimulates the catalytic turnover number (k_{cat}) of glucose transporter protein is yet to be elucidated. The stimulation could be due to a direct interaction of Cd(II) with glucose transporter, or indirectly via interaction of Cd(II) with a putative, glucose transporter regulatory protein. The fact that Cd(II) inhibits cytochalasin B binding activity of purified GLUTI *in vitro* in reconstituted vesicles (Figures 7, 8 and 9) demonstrates that there is a direct interaction of Cd(II) to GLUT1. This interaction occurs at the Cd(II) concentration range (with the half-maximum effect at 0.25 mM) that is effective for glucose transport stimulation in adipocytes (with the half-maximual effect at 0.5 mM). Cd(II) is only 40-50% as effective compared to insulin in the transport stimulation (Figure 4). Evidence suggests that insulin

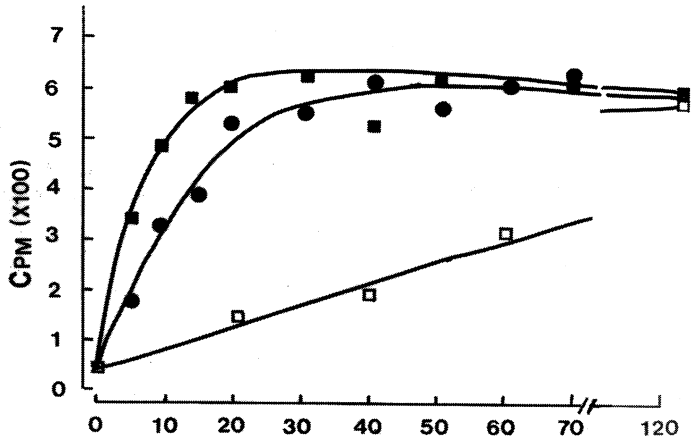

Figure 10. Effects of Cd(II) on the 5 mM 3OMG uptake time course by liposomes containing purified GLUT1. The lipid-to-protein mass ratio of the purified GLUT1 proteoliposomes used here was increased by aproximately two-fold to increase intravesicular volume and to reduce the speed of uptake. This was achieved by reconstitution after addition of an equal amount of exogenous phosphatidyl choline to regular purified GLUT1 preparation (the final lipid-to-protein mass ratio of 9-10). Data are with no (solid circles) and with 3 mM $CdSO_4$ (solid squares). Also shown (open squares) is a leak flux of 3OMG as measured in the presence of 2% $HgCl_2$.

stimulates transporter translocation and transporter's intrinsic activity in a two-step mechanism (Muhlbacher *et al.*, 1988). It is an interesting possibility that Cd(II) and insulin may stimulate intrinsic activity of glucose tranpsorter by a common mechanism. The findings that Cd(II) does not stimulate insulin-stimulated glucose flux further, nor affect insulin-induced transporter redistribution (Figure 4) are consistent with this possibility.

It was not possible in the present study to measure the effect of Cd(II) on the intrinsic activity of GLUT1 and GLUT4 separately in adipocytes. The observation that Cd(II) had very little effect on the 3OMG exchange in human erythrocytes and their resealed ghosts (Figure 6) would suggest that Cd(II) has no effect on GLUTI transport activity. Our data with purified GLUT1 in liposomes (Figures 7-10), on the other hand, clearly demonstrate that GLUT1 function is affected *in vitro* by Cd(II). Cd(II) inhibits cytochalasin B binding activity of purified GLUT1 (Figures 7 and 8), and may stimulate GLUT1 substrate binding activity (Figure 9). 3OMG uptake by liposomes containing GLUT1 appears to be significantly faster in the presence of Cd(II) than its absence (Figure 10). Together, these findings strongly suggest that Cd(II) stimulates GLUTI intrinsic transport activity in adipocytes as in purified GLUT1 in liposomes, and that this effect is blocked in erythrocytes and ghosts by a mechanism that is specific to the erythrocyte. In fact, an increase in GLUT1 intrinsic activity by Cd(II) has been demonstrated by Harrison *et al.* (1991); they have shown that Cd(II) at micromolar concentrations stimulates glucose transport in 3T3-LI fibroblast without increasing glucose transporter number in the plasma membrane, and these cells express GLUT1, but not GLUT4. Harrison *et al.* (1991) have also observed a large stimulation of glucose flux in 3T3-L1 adipocytes, and this occurred without any significant increase in the plasma membrane GLUT4 or GLUT1 content. It should be noted here, however, that the effects in 3T3-L1 adipocytes were observed only after a long (18 h) incubation with micromolar concentrations of Cd(II), and may be unrelated to the acute effect of Cd(II) at millimlar concentrations studied here with rat adipocytes.

One unexpected finding in the present study is that Cd(II) inhibits the cytochalasin B binding activity of GLUTI and stimulates its transport activity and glucose binding activity (Figures 7-10). This is the first demonstration where the cytochalasin B binding activity of a glucose transporter and its transport activity or glucose binding activity dissociate. This cadmium-induced dissociation between these two parameters may provide a tool in selectively probing the roles of the exposed cysteine side chains in GLUT1 conformational dynamics induced by substrate and the inhibitor.

Acknowledgements

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