Nocodazole Inhibits Insulin-stimulated Glucose Transport in 3T3-L1 Adipocytes via a Microtubule-independent Mechanism*

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Insulin stimulates glucose transport in adipocytes and muscle cells by triggering redistribution of the GLUT4 glucose transporter from an intracellular perinuclear location to the cell surface. Recent reports have shown that the microtubule-depolymerizing agent nocodazole inhibits insulin-stimulated glucose transport, implicating an important role for microtubules in this process. In the present study we show that 2 μM nocodazole completely depolymerized microtubules in 3T3-L1 adipocytes, as determined morphologically and biochemically, resulting in dispersal of the perinuclear GLUT4 compartment and the Golgi apparatus. However, 2 μM nocodazole did not significantly effect either the kinetics or magnitude of insulin-stimulated glucose transport. Consistent with previous studies, higher concentrations of nocodazole (10–33 μM) significantly inhibited basal and insulin-stimulated glucose uptake in adipocytes. This effect was not likely the result of microtubule depolymerization because in the presence of taxol, which blocked nocodazole-induced depolymerization of microtubules as well as the dispersal of the perinuclear GLUT4 compartment, the inhibitory effect of 10–33 μM nocodazole on insulin-stimulated glucose uptake prevailed. Despite the decrease in insulin-stimulated glucose transport with 33 μM nocodazole we did not observe inhibition of insulin-stimulated GLUT4 translocation to the cell surface under these conditions. Consistent with a direct effect of nocodazole on glucose transporter function we observed a rapid inhibitory effect of nocodazole on glucose transport activity when added to either 3T3-L1 adipocytes or to Chinese hamster ovary cells at 4 °C. These studies reveal a new and unexpected effect of nocodazole in mammalian cells which appears to occur independently of its microtubule-depolymerizing effects.

Insulin stimulates glucose transport in muscle and fat cells by regulated vesicular transport (1–4). In the absence of insulin GLUT4, the major glucose transporter isoform expressed in these cells, is stored in an intracellular tubulovesicular compartment. Insulin stimulates the exocytosis of GLUT4 from this compartment, leading to increased GLUT4 levels at the cell surface. This process occurs with a t1/2 of 2–5 min.

Several recent studies have suggested an important role for microtubules in insulin-regulated GLUT4 trafficking. First, tubulin-α and vimentin have been identified as major components of intracellular GLUT4 vesicles in adipocytes (5). Second, it has been shown that insulin stimulates the long range movement of GLUT4 vesicles along linear tracks in adipocytes (6). Third, the microtubule-depolymerizing drug nocodazole inhibited insulin-stimulated glucose transport in adipocytes (7, 8). Finally, nocodazole caused a dispersal of the perinuclear GLUT4 compartment in adipocytes (7, 9). These studies have led to the notion that microtubules may play a fundamental role in GLUT4 trafficking and insulin action. It has been proposed that insulin may stimulate GLUT4 exocytosis, at least in part, by increasing the rate of association of GLUT4-containing membranes with microtubules. This step likely occurs in the perinuclear region of the cell, implicating an important role for the perinuclear localization of GLUT4 in insulin action (5, 7).

In the present studies we have examined the dose response effects of nocodazole on microtubule integrity and insulin action in 3T3-L1 adipocytes. Using morphological and biochemical methods we observed maximal effects of nocodazole on microtubule depolymerization at a concentration of 2 μM. Functional consequences of microtubule depolymerization were observed at 2 μM nocodazole in that the Golgi apparatus and the perinuclear GLUT4 compartment were dispersed throughout the cytoplasm of the cell. Despite these effects there was no significant inhibition of insulin-stimulated glucose transport or GLUT4 translocation at 2 μM nocodazole. At higher nocodazole concentrations (10–33 μM) we observed inhibition of glucose transport consistent with previous studies (7, 8). We present data to show that the inhibitory effects of high concentrations of nocodazole are the result of an inhibitory effect of the drug on glucose transport activity rather than on the insulin-dependent recruitment of GLUT4 to the cell surface. We conclude that nocodazole does not inhibit insulin-stimulated GLUT4 translocation in 3T3-L1 adipocytes.

**EXPERIMENTAL PROCEDURES**

**Reagents and Antibodies—** All tissue culture media were purchased from Life Technologies, Inc., except fetal calf serum, which was obtained from Trace Biosciences (Clayton, Australia). Insulin, nocodazole, and taxol were obtained from Calbiochem. Bicinchoninic acid reagent, used in protein assays, and ECL Supersignal reagent were obtained from Pierce (Rockford, IL). BSA² was purchased from ICN (Costa Mesa, CA). Polyvinylidene difluoride blotting membranes were obtained from Bio-Rad Laboratories ( Hercules, CA). Antibodies against insulin receptor, GLUT4, actin, tubulin, and vimentin have been identified in previous studies.

1 The abbreviations used are: BSA, bovine serum albumin; HA, hemagglutinin; CHO, Chinese hamster ovary; KRP, Krebs-Ringer phosphate; 2-D0G, 2-deoxyglucose; PBS, phosphate-buffered saline; PIPES, 1,4-piperazinediethanesulfonic acid; PM, plasma membrane.

² BSA was purchased from ICN (Costa Mesa, CA).
Millipore Corp. (Belford, MA). Unless specified, all other reagents were from Sigma. The GLUT4 polyclonal antibody (R017) was raised against a synthetic peptide corresponding to the COOH-terminal 20 amino acids of rat GLUT4. Monoclonal vimentin antibody, V9.5, was a gift from Dr. Robert M. Evans (Department of Pathology, University of Colorado Denver). Monoclonal anti-Tubulin-β antibody (DM1A) was purchased from Sigma, and monoclonal anti-HA (16B12) was purchased from BabCO (Richmond, CA). Phalloidin-fluorescein isothiocyanate and all of the fluorophore-tagged secondary antibodies were obtained from Molecular Probes (Eugene, OR). Peroxidase-coupled secondary antibodies were purchased from Amersham Pharmacia Biotech Inc. (Little Chalfont, U. K.).

Cell Culture and Transfection—3T3-L1 fibroblasts obtained from the American Type Culture Collection (Rockville, MD) were cultured and differentiated into adipocytes as described previously (10). Chinese hamster ovary (CHO) cells were cultured as described previously (11). 116 (HA-GLUT4) 3T3-L1 adipocytes were obtained by transfection of 3T3-L1 fibroblasts with the retroviral expression vector pBabe-puro containing GLUT4 HA-tagged at the extracellular loop, as described by Shewan et al. (12). Briefly, cells were infected with the relevant virus for 3–5 h in the presence of 4 μg/ml Polybrene (Sigma). After a 48-h recovery period, infected cells were selected in Dulbecco’s modified Eagle’s medium containing 5% fetal calf serum supplemented with 2 μg/ml puromycin (Sigma) and then differentiated as described above.

Glucose Uptake Assays—Cells cultured in 6- or 12-well plates were serum starved in KRP buffer (25 mM HEPES, pH 7.4, 120 mM NaCl, 6 mM KCl, 1.2 mM MgSO₄, 1 mM CaCl₂, 0.4 mM Na₂HPO₄, 0.6 mM NaH₂PO₄) containing 0.2% bovine serum albumin for 2 h at 37°C. Cells were incubated in 950 μl of KRP buffer with the corresponding agents at the times and doses indicated in the figure legends. 2-Deoxy-[3H]glucose (2-DOG) uptake was measured as described previously (13). Briefly, the assay was initiated by the addition of 50 μl of 1 mM 2-DOG (20 μCi/ml). After 4 min, the assay was terminated by washing the cells rapidly three times with ice-cold PBS. Cells were subsequently solubilized in 1% Triton X-100, and 3H was quantified by scintillation counting (Packard 1900CA liquid scintillation analyzer, Packard Instrument Co.).

Polymerization and glucose transport were performed.

Indirect Immunofluorescence Microscopy—Cells cultured on coverslips in 6- or 12-well dishes were serum starved in KRP buffer to 5% fetal calf serum supplemented with 2 μg/ml puromycin (Sigma) and then differentiated as described previously (10). Chinese hamster ovary (CHO) cells were cultured as described previously (11). 116 (HA-GLUT4) 3T3-L1 adipocytes were obtained by transfection of 3T3-L1 fibroblasts with the retroviral expression vector pBabe-puro containing GLUT4 HA-tagged at the extracellular loop, as described by Shewan et al. (12). Briefly, cells were infected with the relevant virus for 3–5 h in the presence of 4 μg/ml Polybrene (Sigma). After a 48-h recovery period, infected cells were selected in Dulbecco’s modified Eagle’s medium containing 5% fetal calf serum supplemented with 2 μg/ml puromycin (Sigma) and then differentiated as described above.

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Non-specific 2-DOG uptake was determined in the presence of 50 μM cytochalasin B.

Indirect Immunofluorescence Microscopy—Cells cultured on coverslips in 6- or 12-well dishes were serum starved for 2 h in Dulbecco’s modified Eagle’s medium containing 0.2% BSA or in KRP buffer containing 0.2% BSA buffer and incubated with the appropriate treatments. Cells were fixed with acetone for 5 min, washed with PBS, washed again with PBS containing 0.15 μl in glycine, and incubated with 1% BSA containing PBS for 30 min. Cells were incubated with primary antibodies and diluted in PBS containing 1% BSA for 1 h. Cells were then washed with PBS containing 0.1% BSA and incubated for 30 min with phalloidin-fluorescein isothiocyanate or with the corresponding Alexa 488- or Alexa 594-conjugated secondary antibody diluted in PBS containing 1% BSA. Normal rabbit serum was used as a negative control. Coverslips were washed with PBS, mounted onto glass microscope slides, and viewed using an X63/1.4 Zeiss oil immersion objective on a Zeiss Axioscope microscope, equipped with a Bio-Rad MRC-600 laser confocal imaging system. In experiments designed for staining of cytoskeletal structures, cells were fixed and permeabilized simultaneously in cytoskeleton-stabilizing buffer (10 mM PIPES, pH 6.9, 100 mM NaCl, 300 mM sucrose, 3 mM MgCl₂, 1 mM EGTA) as described by Arcangeli et al. (14).

The preparation of plasma membrane (PM) laws was performed as described by Robinson et al. (15). Briefly, after incubating cells on coverslips with the appropriate treatment, adipocytes were sonicated yielding a lawn of PM fragments attached to the coverslip. Coverslips were then incubated with the relevant antibodies followed by Alexa 594-conjugated secondary antibody. The fluorescence intensity of individual PM fragments (100–200) from five random fields for each experimental condition was quantified using NIH 1.62 software. The assay and quantitation were performed by separate investigators in a double blind fashion. In addition, parallel experiments measuring tubulin depolymerization and glucose transport were performed.

In experiments designed for cell surface staining, 3T3-L1 adipocytes expressing GLUT4 HA-tagged (12) were transfected in 2% paraformaldehyde and in PBS for 20 min but not permeabilized. Cells were incubated with the anti-HA antibody for 1 h, washed, and incubated with the corresponding Alexa 488-conjugated secondary antibody, as described above, and visualized using an X63/1.4 Zeiss oil immersion objective on a Zeiss Axioscope fluorescence microscope, equipped with a Bio-Rad MRC-600 laser confocal imaging system. In some cases we also quantified surface labeling using a colorimetric detection assay as previously described.

Microtubule Integrity Is Not Required for Insulin-stimulated Glucose Transport—Treatment of adipocytes with 0.2 μM nocodazole for 1 h decreased the amount of polymerized tubulin by 65%, and a maximal effect of nocodazole (>95% depolymerization) was observed at a nocodazole concentration of 2 μM (Fig. 1). Time course experiments revealed that these effects were rapid, occurring within 15 min of treatment (data not shown).
Glut4 Trafficking and Cytoskeleton

Zole on microtubules frequently use this drug at concentrations experimental conditions. Studies examining the effects of nocodazole glucose transport or GLUT4 translocation under the same experimental conditions were visualized by indirect immunofluorescence using anti-tubulin antibodies (a and b), anti-vimentin antibodies (c and d), or phalloidin-fluorescein isothiocyanate (e and f) as described under “Experimental Procedures.” Bar, 25 μm.

Fig. 2. Effect of nocodazole on cytoskeleton in 3T3-L1 adipocytes. 3T3-L1 adipocytes cultured on coverslips were brought to basal conditions and then incubated for 1 h with dimethyl sulfoxide (a, c, and e), or 2 μM nocodazole (b, d, and f). Cells were fixed and permeabilized in 2% paraformaldehyde/0.2% Triton X-100 in cytoskeleton-stabilizing buffer, and cytoskeletal elements were visualized by indirect immunofluorescence using anti-tubulin antibodies (a and b), anti-vimentin antibodies (c and d), or phalloidin-fluorescein isothiocyanate (e and f) as described under “Experimental Procedures.” Bar, 25 μm.

Even though we observed a maximal effect of nocodazole on microtubule depolymerization at a concentration of 2 μM we observed no significant inhibition of insulin-stimulated glucose transport or GLUT4 translocation under the same experimental conditions. Studies examining the effects of nocodazole on microtubules frequently use this drug at concentrations >10 μM. At a concentration of 33 μM, nocodazole caused an 80% decrease in insulin-stimulated 2-DOG uptake (Fig. 1), consistent with previous reports (7, 8). Basal 2-DOG uptake was also inhibited significantly in the presence of 33 μM nocodazole (~75%). Basal and insulin-stimulated glucose transport were completely restored after removal of nocodazole from the culture medium (data not shown), ruling out possible cytotoxic effects of the drug.

To confirm the dose response effects of nocodazole on the integrity of microtubules in 3T3-L1 adipocytes we performed immunofluorescence microscopy on 3T3-L1 adipocytes using an anti-tubulin antibody. In the absence of nocodazole we observed an elaborate microtubule network in adipocytes. Microtubules radiated from the microtubule-organizing center to the cell periphery where they formed a tight cortical network (Fig. 2a). After incubation of adipocytes with 2 μM nocodazole microtubules were completely absent (Fig. 2b), corroborating the biochemical data (Fig. 1). Treatment with 2 μM nocodazole also promoted reorganization of vimentin-containing intermediate filaments into thick bundles localized beneath the PM and encapsulating the lipid droplets (Fig. 2, c versus d). The actin-based microfilaments, which presented as a cortical rim underlying the PM, were unaffected by nocodazole treatment (Fig. 2, e and f). Comparable results were achieved when cells were incubated with higher concentrations (10–33 μM) of nocodazole (data not shown).

Dispersal of the Perinuclear GLUT4 Compartment Does Not Inhibit Insulin-stimulated Translocation—We next examined the effects of 2 μM nocodazole on the localization of GLUT4 in basal and insulin-stimulated adipocytes using indirect immunofluorescence microscopy. Insulin induced the translocation of GLUT4 from a perinuclear location to the cell surface (Fig. 3, basal versus ins). Treatment with 2 μM nocodazole resulted in complete dispersal of the GLUT4 perinuclear compartment throughout the cytoplasm (Fig. 3, basal versus noc). Nocodazole (2 μM) had no apparent effect on insulin-stimulated translocation of GLUT4 to the PM (Fig. 3, ins versus noc+ins), consistent with the glucose transport data presented above (Fig. 1). These data suggest that an intact GLUT4 perinuclear compartment is not required for efficient insulin-stimulated GLUT4 translocation. One possibility that has been proposed (7) is that microtubules regulate the movement of GLUT4 from its perinuclear compartment to the cell cortex whereupon they may be escorted to the surface membrane by actin filaments. Microtubule depolymerization may therefore affect the kinetics but not the magnitude of GLUT4 delivery to the cell surface. Extensive time course experiments were performed to look at both GLUT4 translocation and glucose transport in the presence of 2 μM nocodazole. As shown in Fig. 4, we were unable to observe any significant change in the kinetics of either insulin-stimulated glucose transport or GLUT4 translocation to the PM in response to nocodazole.

Taxol Does Not Rescue the Inhibition of Insulin-stimulated Glucose Transport Induced by High Doses of Nocodazole—One possibility to explain the dose response effects of nocodazole on glucose transport (Fig. 1) is that at high doses nocodazole may...
Fig. 4. Nocodazole does not affect the kinetics of insulin-stimulated glucose transport or GLUT4 translocation. 3T3-L1 adipocytes were brought to basal conditions in KRP buffer containing 0.2% BSA buffer and then incubated in the absence (filled circles) or presence (open circles) of 2 µM nocodazole (noc) for 1 h. 100 nM insulin (ins) was added for various periods of time as indicated after which either 2-D0G uptake (A) or GLUT4 translocation (B and C) was measured. 2-D0G uptake was measured at 4 °C to avoid changes in GLUT4 translocation during the actual transport assay. GLUT4 translocation was measured using the PM lawn assay as described under “Experimental Procedures.” In B representative fields of PM lawns from different time points are shown. Fluorescence associated with individual PM fragments was quantified (C). Data shown are the mean ± S.E. of three separate experiments.

Inhibit insulin-stimulated glucose transport via a microtubule-independent effect. To test this hypothesis we employed the anti-tumor agent taxol, which stabilizes microtubules (19). We reasoned that taxol may prevent any effects of nocodazole arising from microtubule depolymerization but not indirect effects of the drug. Treatment of adipocytes with 40 µM taxol for 1 h promoted an increase in the level of polymerized tubulin (Fig. 5A). Moreover, taxol completely prevented the depolymerization of microtubules observed following incubation of cells with 33 µM nocodazole. The presence of an intact microtubule network in cells treated simultaneously with 33 µM nocodazole and 40 µM taxol was confirmed by indirect immunofluorescence microscopy (data not shown). To confirm that taxol restored the function of the microtubule network we examined the localization of GLUT4 as well as two Golgi markers, syntaxin 6 and GS-15. As indicated in Fig. 5B, taxol completely prevented the nocodazole-induced redistribution of GLUT4 from its perinuclear location. Similar results were obtained for both syntaxin 6, a trans-Golgi network marker (20), and GS-15, a protein present in the Golgi apparatus (21) (data not shown).

We next examined the effects of taxol on 2-D0G uptake in adipocytes. Taxol alone had no significant effect on either basal or insulin-stimulated 2-D0G uptake (Fig. 5C) or GLUT4 translocation (data not shown). However, taxol was unable to overcome the inhibitory effect of 33 µM nocodazole on basal or insulin-stimulated 2-D0G uptake (Fig. 5C).

Nocodazole Inhibits Glucose Transport Activity Independently of Effects on Signaling or Trafficking—Even though 33 µM nocodazole inhibited insulin-stimulated glucose transport by 80%, we were unable to detect any significant decrease in insulin-stimulated GLUT4 translocation in the presence of high doses of nocodazole, as measured by the PM lawn assay (Fig. 6, A and B). Moreover, the movement of GLUT4 to the cell surface in the presence of 33 µM nocodazole resulted in its productive fusion with the PM (Fig. 6, C and D), as determined by surface labeling of a GLUT4 construct bearing an HA epitope in one of its exofacial domains (22).

The most likely explanation for these data is that nocodazole somehow impairs the transport properties of GLUT4 after it has fused with the PM. To test this hypothesis we performed two separate experiments. First, we examined the reversal of nocodazole treatment on glucose transport under conditions in which membrane trafficking was inhibited (i.e. at 4 °C). Cells were pretreated with 33 µM nocodazole for 1 h, and insulin was added during the last 15 min. The cells were cooled rapidly to 4 °C to immobilize GLUT4 at the cell surface, by washing with ice-cold KRP/BSA buffer in the presence or absence of 33 µM nocodazole. After a 5-min incubation at 4 °C, 2-D0G uptake was measured. As shown in Fig. 7A, when 2-D0G uptake was measured at 4 °C the stimulatory effect of insulin was maintained. Similarly, the inhibitory effects of 33 µM nocodazole on glucose transport were also maintained when 2-D0G assays were performed at 4 °C. In contrast, when nocodazole was withdrawn during the 5-min incubation at 4 °C there was a dramatic reversal in insulin-dependent glucose transport approaching values observed in control cells. These data provide further evidence that nocodazole does not interfere with trafficking of GLUT4 but somehow impairs the activity properties of the transporter itself. The second experiment that we performed was to determine whether nocodazole could inhibit glucose transport after the translocation process was complete. 3T3-L1 adipocytes were stimulated with insulin for 15 min at 37 °C to promote translocation of GLUT4 to the cell surface. Cells were then cooled rapidly to 4 °C, to prevent further trafficking, and then incubated with nocodazole on ice for 30 min prior to measurement of 2-D0G uptake, which was also performed at 4 °C. The low dose of nocodazole (2 µM) had no significant effect on glucose transport when added at 4 °C, whereas the high dose of nocodazole (33 µM) resulted in >80% inhibition of insulin-stimulated 2-D0G uptake (Fig. 7B). Similar effects of 33 µM nocodazole were also observed after a 2-min incubation with the drug at 4 °C, suggesting that this inhibitory effect was rapid (data not shown). As a control for these experiments we also examined the effects of the phosphatidylinositol 3-kinase inhibitor, wortmannin, at 4 °C. Although this drug inhibits insulin-stimulated glucose transport when added prior to insulin treatment at 37 °C we observed no inhibitory
effect on insulin action when it was added after insulin at 4 °C (Fig. 7B).

To determine whether the inhibition of glucose transport by nocodazole was specific to GLUT4 or common to other facilitative glucose transporters we examined the effects of nocodazole on glucose uptake in CHO cells, which only express the GLUT1 isoform (23). Treatment of CHO cells with 33 μM nocodazole at 4 °C inhibited 2-DOG uptake by 60% (Fig. 7C). These data indicate that nocodazole inhibits glucose uptake by both GLUT4 and GLUT1 transporters in a rapid, dose-dependent manner, which is independent of effects on signaling or trafficking.

**DISCUSSION**

Nocodazole is frequently used by cell biologists to examine the role of microtubules in vesicle transport. Although causing complete fragmentation of the Golgi apparatus, nocodazole has limited effects on vesicle transport between the Golgi and the
In the present study we have examined the effects of nocodazole on GLUT4 trafficking and glucose transport in detail. Initially, we studied the dose response effects of nocodazole on GLUT4 trafficking and glucose transport in detail. At 33°C, we observed a dose-dependent inhibition of nocodazole (7, 8). However, in each of these previous studies concentrations of nocodazole in excess of 10 μM were used to study effects on glucose transport. Consistent with these previous studies, we observed a dose-dependent inhibition of nocodazole on insulin-stimulated glucose transport (Fig. 1). At 33 μM nocodazole we observed >80% inhibition of insulin-stimulated glucose transport. This effect was not likely to be the result of depolymerization of microtubules because at high doses of nocodazole we were able to reverse the microtubule-depolymerizing effects with taxol but not the inhibition of glucose transport (Fig. 5). Strikingly, although nocodazole had a pronounced inhibitory effect on insulin-stimulated glucose transport we were unable to detect any inhibitory effect on GLUT4 translocation to the cell surface (Fig. 6). This was not due to an effect of nocodazole on activation of the GLUT4 vesicles with the PM because we also observed normal GLUT4 translocation using a surface binding assay that only measures transporters that have been incorporated into the PM (Fig. 6, C and D). These results contrast with two other reports in which nocodazole was shown to inhibit GLUT4 translocation, as determined by the PM lawn assay (8, 9). The basis for this discrepancy is not clear.

FIG. 7. Nocodazole inhibits glucose transporter activity in both 3T3-L1 adipocytes and CHO cells. A, 3T3-L1 adipocytes were incubated with 33 μM nocodazole (noc) for 1 h at 37 °C. 100 nM insulin (ins, open bars) was added during the last 15 min of the incubation. Cells were cooled rapidly by washing in HEPES buffer (−/+ nocodazole) preequilibrated to 4 °C and incubated in the same buffer (−/+ nocodazole) for 5 min at 4 °C. 2-DOG uptake measurements were performed at 4 °C. Data shown represent the mean ± S.D. of two experiments. B, 3T3-L1 adipocytes were brought to basal conditions in KRP buffer containing 0.2% BSA and then incubated in the absence (basal) or presence of 100 nM insulin (ins) for 15 min. Where indicated, cells were incubated with 100 nM wortmannin 10 min prior to insulin treatment (ins + wort). C, CHO cells, grown in 12-well plates, were cooled rapidly to ≤ 4 °C by washing in ice-cold buffer and incubated at ≤ 4 °C in buffer with 100 nM wortmannin (ins + wort), 2 μM nocodazole (ins + 2 μM noc), or 33 μM nocodazole (ins + 33 μM noc) for 30 min. 2-DOG uptake was measured for 90 s as described under “Experimental Procedures.” Error bars correspond to the S.D. from two separate experiments. C, CHO cells, grown in 12-well plates, were cooled rapidly to ≤ 4 °C by washing in ice-cold buffer and incubated with 33 μM nocodazole for the indicated times. 2-DOG uptake was measured for 90 s as described under “Experimental Procedures.” Error bars correspond to the S.D. from two separate experiments.

The observation that nocodazole had no effect on insulin-stimulated glucose transport was surprising in light of previous studies (7, 8). However, in each of these previous studies concentrations of nocodazole in excess of 10 μM were used to study effects on glucose transport. Although depolymerization of microtubules may not effect the magnitude of the GLUT4 translocation, it is possible that nocodazole may slow the rate of movement of these vesicles to the cell surface, as proposed previously (9). To investigate this possibility we studied the kinetics of GLUT4 translocation and glucose transport in the presence of nocodazole. However, we could find no evidence to indicate that the kinetics of GLUT4 translocation or glucose transport activation by insulin was impeded in the presence of nocodazole.
In the present study we have used three different approaches to determine the extent of GLUT4 translocation to the cell surface, and all three methods indicate that nocodazole at either low or high doses does not inhibit this process significantly. In parallel, we observed a dramatic effect of nocodazole on the integrity of microtubules and the perinuclear GLUT4 compartment as well as on cellular glucose transport. Hence, it is unlikely that nocodazole was inactive in our assay systems.

The observation that nocodazole inhibited glucose transport independently of translocation of GLUT4 to the cell surface argues strongly in favor of a direct effect of this drug on the transport activity of the protein. In support of this hypothesis we observed that nocodazole inhibited glucose transport in adipocytes even when it was added to cells at 4 °C (Fig. 7B). Under these conditions activation of glucose transport does not rely upon vesicle transport or signal transduction. Furthermore, similar effects were observed in cells that do not express the GLUT4 transporter (Fig. 7C). Moreover, these inhibitory effects were rapid (Fig. 7C). The precise nature of this inhibitory effect of nocodazole remains to be defined. However, nocodazole may either bind directly to the transporter, or it may modify the characteristics of the PM thus impairing the normal function of the transporter.

The present studies do not exclude the possibility that GLUT4 vesicles are transported along microtubules en route to the PM. However, these studies clearly show that the insulin-dependent recruitment of GLUT4 vesicles to the cell surface can occur relatively unimpaired after depolymerization of the microtubule network. Hence, our data suggest that it is unlikely that microtubule-dependent transport is a major rate-limiting step for the insulin-dependent delivery of GLUT4 to the cell surface. Most importantly, these studies reveal an effect of nocodazole, apparently unrelated to its microtubule-depolymerizing effects, to interfere with the transport properties of facilitative glucose transporters. This effect may involve many members of the glucose transport family, and so the use of this drug to study the role of microtubules is probably best confined to low doses where this nonspecific effect does not occur.

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