V-type ATPase is involved in biogenesis of GLUT4 vesicles

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Malikova, Marina, Jun Shi, and Konstantin V. Kandror. V-type ATPase is involved in biogenesis of GLUT4 vesicles. Am J Physiol Endocrinol Metab 287: E547–E552, 2004. First published May 27, 2004; 10.1152/ajpendo.00571.2003.—Proton pumps participate in several aspects of endocytic protein trafficking. However, their involvement specifically in the GLUT4 pathway has been a matter of great controversy. Here, we report that incubation of 3T3-L1 adipocytes with specific inhibitors of V-type ATPase, concanamycin A and bafilomycin A1, inhibits insulin-regulated glucose transport and results in accumulation of GLUT4 in heavy, rapidly sedimenting intracellular membranes. Correspondingly, the amount of small responsive GLUT4 vesicles in concanamycin A- and bafilomycin A1-treated cells is decreased. We conclude that these drugs block translocation of GLUT4 in adipose cells by inhibiting formation of small insulin-responsive vesicles on donor intracellular membranes. At the same time, proton pump inhibitors do not affect insulin-dependent translocation of preexisting vesicles or GLUT4 sorting in recycling endosomes. On the contrary, wortmannin acutely inhibits insulin-dependent translocation of the preexisting vesicles but has no effect on vesicle formation.

IN ADIPOSE AND SKELETAL MUSCLE CELLS, insulin causes redistribution of glucose transporter isoform 4 (GLUT4) from its intracellular compartments to the cell surface (2, 24). The nature of the “GLUT4 pathway” in insulin-sensitive cells is not completely understood. It has been shown, however, that internalized GLUT4 passes from early endosomes (31) to recycling endosomes (17, 37, 40) and then to specialized insulin-responsive storage vesicles, or IRVs, that represent the final target of insulin regulation (19, 22, 36). Transport of GLUT4 from early to recycling endosomes may proceed via a distinct population of vesicles that are marked by the presence of cellugyrin, which is absent from the IRVs (14, 15). In basal adipose cells, IRVs accommodate 70–75% of the total GLUT4 pool with the rest of the transporter residing in endosomes and interendosomal cellugyrin-positive vesicles (15, 18, 38, and this paper). GLUT4 continuously traffics between its intracellular compartments and the plasma membrane both in the absence and in the presence of insulin (6, 12, 29), indicating that IRVs and cellugyrin-positive vesicles are maintained in a dynamic equilibrium with their donor and target membranes. The mechanism of how small GLUT4 vesicles are formed on large donor membranes is not known. It is thought, however, that cargo molecules, such as GLUT4, must be somehow recognized by adaptors and protein coats and be recruited into coated buds. Weak binding to cytoplasmic tails of the cargo proteins may not be sufficient to recruit adaptor complexes and protein coats to the donor membranes. This process requires additional interactions between adaptors and small GTPases of the ADP ribosylation factor (ARF) family (3, 7). Because recruitment of ARF protein(s) onto endosomes may depend on the acidic luminal pH (10, 21), it may be mediated by a putative transmembrane pH sensor (1). Thus, to better understand the mechanisms of IRV formation, it is essential to determine whether or not this process involves a pH-sensitive step.

In the past, several research groups studied the effect of acidotropic agents, such as chloroquine, on GLUT4 traffic in adipose cells. The results of those studies were inconsistent. In particular, Oka et al. (23) reported that GLUT4 recycles in a chloroquine-independent fashion. On the contrary, Romanek et al. (28) established that chloroquine inhibits insulin-stimulated GLUT4 translocation, but independently of its action on luminal pH. More recently, Chinni and Shisheva (4) unexpectedly found that the specific inhibitor of V-type ATPase bafilomycin A1 induces translocation of GLUT4 to the plasma membrane equivalent to 50–65% of the acute insulin effect. Given this controversy, we decided to reexamine the effect of bafilomycin A1 as well as another macrolide inhibitor, concanamycin A, on the GLUT4 pathway in cultured and primary adipose cells. These compounds demonstrate a high degree of specificity toward V-type ATPases so that a 10,000-fold increase in concentration is required before inhibition of other ion-translocating ATPases is observed (8).

We found that both bafilomycin A1 and concanamycin A reduce insulin-stimulated glucose transport by blocking the formation of small GLUT4 vesicles and “locking” GLUT4 in large intracellular membrane structures that are not responsive to insulin. Along with this, inhibitors may not acutely inhibit translocation of the preformed IRVs. On the contrary, the widely used phosphatidylinositol 3-kinase inhibitor wortmannin completely blocks translocation of the IRVs but has no visible effect on the formation of this compartment.

MATERIALS AND METHODS

Materials. In the present study, we used monoclonal anti-GLUT4 antibody IF8 (11); rabbit polyclonal antibody against cellugyrin (Ac-CQNVETTEGYPPPPYY-OH) that was raised and affinity purified by BioSource International (Camarillo, CA); rabbit polyclonal antibody against insulin-responsive aminopeptidase (IRAP; kindly provided by Dr. Paul Pilch, Boston University School of Medicine) and rabbit polyclonal antibody against GLUT1 (kindly provided by Dr. Ian Simpson, Penn State University School of Medicine). Mouse monoclonal antibodies against phospho-(P)-Ser473-Akt and P-Thr202/Tyr204 p44/42 MAPK and rabbit polyclonal antibody against Akt were from Cell Signaling (Beverly, MA). Rabbit polyclonal antibody against p44/42 MAPK was from Santa Cruz Biotechnology (Santa Cruz, CA). Reagents, including bafilomycin A1, concanamycin A, and wortmannin were from Cell Signaling (Beverly, MA). Rabbit polyclonal antibody against cellugyrin, adipocytes; bafilomycin; concanamycin; wortmannin

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and wortmannin were purchased from Sigma (St. Louis, MO). Aprotinin, leupeptin, pepstatin A, and PMSF were obtained from American Bioanalytical (Natick, MA). Calf serum and Dulbecco’s modified Eagle’s medium (DMEM) were purchased from Gibco (Grand Island, NY).

Cell culture. Murine 3T3-L1 preadipocytes were cultured, differentiated, and maintained as described previously (33). Briefly, cells were grown in DMEM supplemented with 10% calf serum until confluence. Two days later, the cells were transferred to differentiation medium (DMEM containing 10% serum, 0.5 mM 3-isobutyl-1-methylxanthine, 1 mM dexamethasone, and 1.7 mM insulin). After 48 h, the differentiation medium was replaced with maintenance medium (DMEM supplemented with 10% serum). The maintenance medium was changed every 48 h. The cells were used after 8 days of differentiation.

Stable transfection of 3T3-L1 cells with myc-tagged GLUT4 was performed with the help of pBabe-puro retroviral expression vector (kind gift of Dr. S. Farmer, Boston University School of Medicine). First, the pLNCX2 Vector was modified by substituting the fragment between Xhol and NotI sites for the annealed synthetic TCGAGG-GATCCGTTAAACGCC and GCGCCGCTTAAACGATCC oligonucleotides, which contain the BamHI and Pmel sites. The modified pLNCX2 vector was cut by BamHI and NotI, and the corresponding fragment from pcDNA3.1(-)myc-c-LGLUT4 (human GLUT4 with 7 myc epitopes in the first extracellular loop, a kind gift of Dr. Jonathan Bogan, Yale Medical School) was ligated back, producing the pBabe-myc-c-LGLUT4 construct. The pBabe-myc-c-LGLUT4 construct was constructed by subcloning the BamHI and SalI fragment from mLNCX2-myc-GLUT4 into the corresponding sites of the pBabe-puro vector. Transfection of 3T3-L1 cells was performed as described previously (34). Infected 3T3-L1 cells were selected for 7–10 days in medium containing 3 μg/ml puromycin.

2-[3H]deoxyglucose uptake. This assay was performed as previously described (9). Briefly, cells were grown and differentiated in 12-well culture plates and deprived of serum for 1.5–2 h. Cells were then washed twice with 1 ml of Krebs-Ringer-HEPES (KRH) buffer (121 mM NaCl, 4.9 mM KCl, 1.2 mM MgSO4, 0.33 mM CaCl2, 12 mM HEPES, pH 7.4) and incubated in KRH with or without inhibitors (dissolved in DMSO) at 37°C in a total volume of 1 ml. Control cells were incubated with an equivalent volume of DMSO alone. At the end of the incubation, insulin (100 nM) was added to some cells for 20 min followed by 2-[3H]deoxyglucose (1 μCi) to a final concentration of 0.1 mM. After 3 min of incubation at 37°C, the radioactive mixture was aspirated, and cells were washed twice with ice-cold KRH buffer with 25 mM of cold d-glucose. Cells were then solubilized in 0.4 ml of 0.1% SDS, mixed with 4 ml of EcoLume Scintillation Liquid (ICN Biomedical, Costa Mesa, CA), and counted. Measurements were made in triplicate and corrected for specific activity and nonspecific diffusion (as determined in the presence of 5 μM cytochalasin B), which was <10% of the total glucose uptake.

Isolation of primary rat adipocytes. Adipocytes were isolated from epididymal fat pads of male Sprague-Dawley rats (Taconic, 150–175 g) by collagenase digestion (27). All animals had continuous unrestricted access to chow (TAC no. 31) and water. Fat pads were immersed in Krebs-Ringer phosphate (KRP) buffer (12.5 mM HEPES, 120 mM NaCl, 6 mM KCl, 1.2 mM MgSO4, 1 mM CaCl2, 0.6 mM Na2HPO4, 0.4 mM Na2PO4, 2.5 mM d-glucose, and 2% bovine serum albumin, pH 7.4), minced, and subjected to collagenase digestion for 35 min at 37°C with constant shaking at 125 cycles/min. Adipocytes were then filtered through a 300-μm nylon mesh (Safar America, Depew, NY), washed three times with KRP, diluted 1:4 (vol/vol) with KRP, and equilibrated for 20 min at 37°C with constant shaking at 25 cycles/min.

Fractionation of 3T3-L1 adipocytes. Cultured 3T3-L1 adipocytes were washed twice with HES buffer cooled to 14–16°C (20 mM HEPES, 250 mM sucrose, 1 mM EDTA, 5 mM benzamidine, 1 mM PMSF, 1 μM pepstatin, 1 μM aprotinin, 1 μM leupeptin, pH 7.4), and homogenized by 10 strokes in a ball-bearing homogenizer (Isotomi, Heidelberg, Germany) with a 12 μM clearance. Homogenates were centrifuged at 16,000 g for 20 min at 4°C. The fat layer was removed, and light microsomes and the plasma membrane were purified by differential centrifugation as previously described (13, 30), with minor modifications. Specifically, the plasma membrane was purified in the TST60.4 Sorvall rotor and then pelleted in the same rotor at 35,000 rpm for 20 min at 4°C. Primary rat adipocytes were transferred to HES at a 80% suspension and centrifuged at 16,000 g for 20 min at 4°C without homogenization (15).

Sucrose gradient centrifugation. Samples (0.2 ml) were loaded onto a 4.6-ml continuous 10–30% sucrose gradient and centrifuged for 55 min in a Beckman SW-55 rotor at 48,000 rpm. Each gradient was collected into 20–21 fractions starting from the bottom of the tube.

Immunofluorescence staining. 3T3-L1 adipocytes were serum starved for 4 h in DMEM with 0.2% BSA and fixed at room temperature with 4% paraformaldehyde in PBS, pH 7.4, for 15 min with mild shaking. Cells were then washed twice with PBS, incubated in 0.2% Triton X-100 for 5 min, and washed again three times with PBS. For cell surface staining, the permeabilization step was omitted. Permeabilized and nonpermeabilized cells were blocked with 5% donkey serum and 5% BSA in PBS overnight on a rocking platform. Cells were then incubated with monoclonal anti-myc antibodies (Cell Signaling Technology, Beverly, MA) diluted 1:1,000 in blocking solution for 1 h on a rocking platform. Cells were washed six times with PBS and incubated with Cy3-conjugated donkey anti-mouse IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) diluted 1:250 in blocking solution for 1 h. Cells were then washed six times with PBS. Staining was examined with the help of a fluorescent microscope (Axiovert 200; Zeiss, Jena, Germany).

Gel electrophoresis, immunoblotting, and protein content. Proteins were separated in SDS-polyacrylamide gels according to Laemmli (16) but without reducing agents and were transferred to Immobilon-P membrane (Millipore) in 25 mM Tris and 192 mM glycine. After transfer, the membrane was blocked with 10% nonfat dry milk in PBST for 1 h at 37°C. Proteins were visualized with specific antibodies, horseradish peroxidase-conjugated secondary antibodies (Sigma), and an enhanced chemiluminescence substrate kit (NEN) by use of a Kodak ImageStation 440. Protein content was determined with the bicinchoninic acid kit (Pierce) according to manufacturer’s instructions.

RESULTS AND DISCUSSION

Figure 1 shows that administration of the specific inhibitor of V-type ATPase concanamycin A to adipocytes inhibits insulin-stimulated glucose uptake in a concentration- and time-dependent fashion. We have performed analogous experiments with a related inhibitor, bafilomycin A1, and obtained similar results (not shown). We have found, however, that concanamycin A inhibits insulin-stimulated glucose uptake faster and at a lower concentration than bafilomycin A1. Therefore, here, we will show only data obtained with the help of concanamycin A. Control experiments demonstrate that concanamycin A (and bafilomycin A1, not shown) does not decrease expression levels of GLUT4 and GLUT1 (Fig. 2A) or interfere with early insulin signaling (Fig. 2B) in 3T3-L1 adipocytes.

Next, we carried out subcellular fractionation of 3T3-L1 adipocytes treated and not treated with insulin and concanamycin A. In these experiments, we employed the classical fractionation protocol of Simpson et al. (30). In agreement with numerous previous studies, Fig. 3 demonstrates that insulin causes translocation of both GLUT4 and, to a lesser extent,
GLUT1 from intracellular light microsomes (LDM in Ref. 30) to the plasma membrane. Unexpectedly, we found that concanamycin A also induces significant accumulation of both transporters in the plasma membrane fraction. We believe that this same effect was previously observed by Chinni and Shisheva (4), who concluded that arrest of endosomal acidification by bafilomycin A1 mimicked insulin action on GLUT4 translocation in 3T3-L1 adipocytes. Those authors, however, limited their study to subcellular fractionation experiments and did not measure insulin-stimulated glucose uptake in bafilomycin A1-treated and nontreated cells.

To determine the reason for the controversy between glucose uptake (Fig. 1) and cell fractionation (Fig. 3) results, we used 3T3-L1 cells stably transfected with GLUT4 tagged with seven myc epitopes in the first extracellular loop. In the control experiment, we found that, upon sucrose gradient centrifugation, the distribution of myc7-GLUT4 completely overlaps with the distribution of endogenous GLUT4 and IRAP, another major component protein of GLUT4 vesicles, suggesting that the marker protein is faithfully targeted to GLUT4 vesicles (not shown). Incubation of these cells with concanamycin A (and bafilomycin A1, not shown) does not visibly interfere with the intracellular localization of myc7-GLUT4 (Fig. 4A) but dramatically inhibits translocation of the tagged transporter to the cell surface upon insulin stimulation (Fig. 4B).

The kinetics of bafilomycin A1 and concanamycin A action on insulin-stimulated glucose transport (Fig. 1) suggest that alkalization of intramembrane space may not acutely block translocation of the preexisting IRVs but may interfere with the formation of this compartment. Such a hypothesis is consistent with several earlier reports showing that bafilomycin A1 inhibits budding of small vesicles from donor endosomal membranes (1, 5, 25).

In adipose cells, the major pool of GLUT4 is localized in small vesicles with the rest of the transporter residing in much larger membrane structures that may represent endosomes and/or trans-Golgi network (20, 26, 31, 32). We have previ-
Importantly, the pellet of the 16,000-g centrifugation also contains virtually all of the plasma membrane (30) that, however, has a nearly undetectable amount of GLUT4 (~1–2% of the total pool) under basal conditions (2). Special control experiments, including in vitro reconstitution assay (15, 38), demonstrate that small GLUT4-containing vesicles do not represent an artifact of cell homogenization and most likely correspond to the small GLUT4-containing tubulovesicular compartment detected by immunoelectron microscopy (20, 26, 31, 32).

To study the effect of concanamycin A on the distribution of GLUT4 between large donor membranes and small vesicles, we incubated 3T3-L1 adipocytes in the absence and in the presence of concanamycin A and separated vesicular and endosomal pools of IRAP, GLUT4, and cellugyrin by centrifugation at 16,000 g. We fractionated 16,000-g supernatants from 3T3-L1 cells treated and not treated with concanamycin A in sucrose velocity gradients (Fig. 5A) and found that both inhibitors significantly decrease the amount of IRAP, GLUT4, and cellugyrin in the vesicular fraction. Correspondingly, Fig. 5B shows that, in concanamycin A-treated cells, vesicular proteins are accumulated in the donor membranes recovered in the pellet of the 16,000-g centrifugation. Similar results were obtained upon treatment of cells with bafilomycin A at 375 nM for 4 h (not shown). On the basis of these results, we suggest that concanamycin A and bafilomycin A inhibit formation of small GLUT4 vesicles and cause accumulation of GLUT4 in large intracellular donor membranes that copurify with the plasma membrane upon differential centrifugation. This effect of concanamycin A and bafilomycin A1 is consistent with earlier results of Romanek et al. (28), who found that the membrane-permeable weak base chloroquine blocks insulin-stimulated GLUT4 recruitment. Those authors, however, failed to detect V-type ATPase in small GLUT4 vesicles and concluded that the inhibitory effect of chloroquine on GLUT4 traffic is independent from its action on endomembrane pH. Similar to Romanek et al., we could not detect V-type ATPase in immunopurified IRVs (results not shown). We believe, however, that, to perform its biological functions, V-type ATPase should be localized not in the IRVs but rather in the donor endosomal membranes that are separated from the donor endosomal membranes that are separated from the plasma membrane upon differential centrifugation.

![Image](http://ajpendo.physiology.org/)

**Fig. 5.** Concanamycin A decreases content of small GLUT4 vesicles in 3T3-L1 adipocytes. Cells were incubated in the absence and presence of concanamycin A (50 nM) for 1 h, homogenized, and centrifuged at 16,000 g for 20 min. Supernatants (430 μg total protein) were fractionated in 10–30% sucrose gradients for 55 min in a Beckman SW-55 rotor at 48,000 rpm. Arrow indicates direction of sedimentation. A: gradients were separated into 20 fractions starting from the bottom of the tube and analyzed by Western blotting with specific antibodies. B: pellets of the 16,000-g centrifugation (100 μg total protein/lane) were analyzed by Western blotting. IRAP, insulin-responsive aminopeptidase; GLUT4, glucose transporter 4; cellugyrin, aminopeptidase.

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**Fig. 4.** Concanamycin A inhibits insulin-stimulated GLUT4 translocation in 3T3-L1 adipocytes. Serum-starved 3T3-L1 adipocytes stably transfected with myc-tagged GLUT4 were incubated in the absence and presence of concanamycin A (50 nM) for 1 h followed by treatment with insulin (100 nM) for 20 min. Cells were then fixed and stained with monoclonal anti-myc antibody and Cy3-conjugated donkey anti-mouse IgG. A: Triton-permeabilized cells at ×40 magnification. Bar, 5 μm. B: nonpermeabilized cells at ×40 magnification. Bar, 15 μm. Incubation of fixed cells with nonspecific mouse IgG and Cy3-conjugated donkey anti-mouse IgG shows no staining.

Importantly, the pellet of the 16,000-g centrifugation also contains virtually all of the plasma membrane (30) that, however, has a nearly undetectable amount of GLUT4 (~1–2% of the total pool) under basal conditions (2). Special control experiments, including in vitro reconstitution assay (15, 38), demonstrate that small GLUT4-containing vesicles do not represent an artifact of cell homogenization and most likely correspond to the small GLUT4-containing tubulovesicular compartment detected by immunoelectron microscopy (20, 26, 31, 32).

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**Fig. 4.** Concanamycin A inhibits insulin-stimulated GLUT4 translocation in 3T3-L1 adipocytes. Serum-starved 3T3-L1 adipocytes stably transfected with myc-tagged GLUT4 were incubated in the absence and presence of concanamycin A (50 nM) for 1 h followed by treatment with insulin (100 nM) for 20 min. Cells were then fixed and stained with monoclonal anti-myc antibody and Cy3-conjugated donkey anti-mouse IgG. A: Triton-permeabilized cells at ×100 magnification. Bar, 5 μm. B: nonpermeabilized cells at ×40 magnification. Bar, 15 μm. Incubation of fixed cells with nonspecific mouse IgG and Cy3-conjugated donkey anti-mouse IgG shows no staining.
IRVs by the 16,000-g centrifugation. Therefore, the absence of V-type ATPase from the IRVs cannot serve as an argument in favor of GLUT4 traffic being independent of the endosomal membrane pH.

The decreased amount of GLUT4 in the IRVs from concanamycin A-treated cells may have two explanations. First, concanamycin A may inhibit the formation of IRVs from recycling endosomes. Alternatively, concanamycin A may inhibit protein sorting in recycling endosomes, so that the total number of IRVs is the same, but each vesicle may have fewer copies of the GLUT4 protein. To discriminate between these two possibilities, we used an antibody decoration technique described previously (15). Briefly, adipocyte extracts are incubated with nonspecific IgG or with an excess of monoclonal antibody 1F8 that recognizes the cytoplasmic COOH terminus of the transporter. Binding of antibody to IRVs makes them heavier. By measuring the shift in the sedimentation distribution of the IRVs and knowing that each antibody molecule has the sedimentation coefficient of 4 S, we can estimate the number of GLUT4 molecules per “average” vesicle (Fig. 6A). Importantly, the shift in the sedimentational distribution of GLUT4 vesicles caused by binding to 1F8 does not depend on experimental conditions, such as the concentration of 1F8 antibody (as long as it is used in excess) and the incubation time (in the range 1–5 h) (15).

We decided to perform these experiments using primary rat adipocytes, which can be broken open without homogenization, by centrifugation only (15), since this mild procedure minimizes the risk of artificial shattering of endosomal membranes by cell homogenization.

As previously shown, incubation of the 16,000-g extract of primary adipocytes with monoclonal anti-GLUT4 antibody 1F8 results in a 20- to 22-S increase in the sedimentation coefficient of the IRVs (15). We now reproduce these data and show that incubation of the adipocyte extract with 1F8 antibody (but not with nonspecific IgG) shifts the peak of the IRV distribution from fraction 14 to fraction 10 (Fig. 6B, top, left and right). On the average, this shift (~20–22 S) corresponds to 5–6 1F8 molecules bound to the vesicle. At the same time, 1F8 does not shift the sedimentational distribution of cellugyrin-positive vesicles that have low specific content of GLUT4 (see also Ref. 15).

As is the case with 3T3-L1 cells, incubation of primary adipocytes with concanamycin A (and bafilomycin A1; not shown) decreases the amount of GLUT4 and cellugyrin in the vesicle zone of the gradient (fractions 6–14 for GLUT4-enriched IRVs, fractions 12–20 for cellugyrin-positive vesicles). However, the shift in the sedimentation coefficient of the IRVs caused by binding of 1F8 antibody to GLUT4 remains the same in the absence and in the presence of the inhibitors (Fig. 6B). This suggests that, in the presence of concanamycin A, IRVs still have the same specific GLUT4 content, namely, 5–6 molecules per average vesicle. In other words, inhibitors of V-type ATPase block the formation of the IRVs but may not interfere with GLUT4 sorting in recycling endosomes. These results are consistent with the earlier report by Van Weert et al. (35), who found that sorting of integral membrane proteins in endosomes is not affected by bafilomycin A1.

In agreement with our results, Yang et al. (39) recently reported that bafilomycin A1 inhibits insulin-stimulated glucose uptake and cell surface biotinylation of GLUT4 in cardiomyocytes. At the same time, when the drug was administered to cells simultaneously with insulin, it did not interfere with translocation of GLUT4 to the sarcolemma. The authors concluded that bafilomycin A1 inhibits incorporation of GLUT4 into the plasma membrane. Such an interpretation does not contradict our results, although the short-term effect of bafilomycin A1 on glucose uptake in cardiomyocytes seems to be more pronounced than in 3T3-L1 adipocytes. In any case, it is possible that inhibitors of V-type ATPase may block both formation of the IRVs and their fusion with the target membrane.

Finally, we decided to compare the mechanisms of action of concanamycin A and wortmannin, a widely used inhibitor of insulin-stimulated glucose transport. As expected, wortmannin acutely and completely suppressed the effect of insulin on glucose uptake (not shown). However, wortmannin had no effect on the ratio between small GLUT4 vesicles and large donor membranes recovered in the 16,000-g pellet and on the sedimentation coefficient of the IRVs in the 16,000-g supernatant (results not shown). Thus inhibitors of V-type ATPase and wortmannin block different steps in the GLUT4 pathway. The former block formation of small vesicles from the donor membranes, whereas the latter inhibit translocation of the...
performed IRVs and/or their fusion with the plasma membrane.

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