Regulation of System A Amino Acid Transport in 3T3-L1 Adipocytes by Insulin*

(Received for publication, June 30, 1997, and in revised form, November 20, 1997)

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The insulin-stimulated uptake of 2-(methylamino)isobutyric acid (MeAIB), a nonmetabolizable substrate for system A, in 3T3-L1 adipocytes was investigated. As cells took on a more adipogenic phenotype, the insulin-stimulated uptake of MeAIB increased by 5-fold. The induced transport activity showed properties characteristic of system A, with a Km value of 190 μM. The half-life of the induced system A activity was independent of de novo protein synthesis and was not accelerated by ambient amino acids, therefore, it was mechanistically distinct from the previously described adaptive and hormonal regulation of system A. Inhibition of mitogen-activated protein kinase kinase by PD98059, Ras farnesylation by PD152440 and B581, p70S6K by rapamycin, and phosphatidylinositol 3-kinase by PD98059 revealed that only wortmannin and LY294002 inhibited the insulin-induced MeAIB uptake with IC50 values close to that previously reported for inhibition of PI 3'-K. These results suggest that the Ras/mitogen-activated protein kinase and pp70S6K insulin signaling pathways are neither required nor sufficient for insulin stimulation of MeAIB uptake, and activation of PI 3'-K or a wortmannin/LY294002-sensitive pathway may play an important role in regulation of system A transport by insulin in 3T3-L1 cells.

Mammalian cells contain multiple systems for uptake of neutral amino acids. System A is an ubiquitous amino acid transport system that mediates the Na+-dependent uptake of a wide range of neutral amino acids (1–3), many of which are gluconeogenic (4). In a number of cell types system A is regulated by a variety of external stimuli and conditions, such as hormones, amino acid starvation (adaptive regulation), cell growth, differentiation, hyperosmotic stress, and trans-inhibition produced by high levels of endogenous amino acid substrates (1, 2, 4, 5). It is generally believed that hormonal and adaptive regulation of system A occurs at the level of transcription (4, 5). Although the molecular mechanisms of the signal transduction in insulin action have been extensively investigated (6), the mechanisms responsible for hormonal regulation of system A transport remains largely unknown. An insulin-insensitive Chinese hamster ovary cell line has been isolated (7). These authors proposed that, when insulin binds to its receptor, it regulates system A activity directly or indirectly by inactivation of a regulatory protein designated r2, but the molecular events leading to neutralizing this protein by insulin have not been resolved (5).

In 3T3-L1 adipocytes, insulin induces the dose- and time-dependent uptake of MeAIB, a nonmetabolizable substrate of system A (8). However, the specificity and kinetics of the insulin-induced amino acid uptake in 3T3-L1 adipocytes and the signaling events potentially involved in insulin stimulation of system A transport in mammalian cells have not previously been explored in great detail. In this report, we investigate the regulation of system A transport by insulin in 3T3-L1 adipocytes.

EXPERIMENTAL PROCEDURES

Materials—Cell culture reagents, epidermal growth factor, PDGF, IGF-I, and IGF-II were purchased from Life Technologies, Inc. [3H]MeAIB was purchased from American Radiolabeled Chemicals, Inc., and [γ-32P]ATP from Amersham Corp., Wortmannin, LY294002, B518, and rapamycin were purchased from BIOMOL Research Laboratories, Inc. (Plymouth Meeting, PA). Anti-p42/p44 MAP kinase antibody was used in Western blotting was prepared from rabbits immunized with a C-terminal peptide (amino acids 425–445) of p44 MAP kinase (p44MAPK) expressed as a glutathione S-transferase fusion protein. PD98059 and PD152440 were synthesized by Parke-Davis. Phosphatidylinositol was purchased from Avanti (Birmingham, AL). The S6 kinase kit and the mouse anti-phosphotyrosine monoclonal antibody were obtained from Upstate Biotechnology, Inc. (Lake Placid, NY). The GH and insulin were obtained from Eli Lilly; IL-11 from R&D Systems (Minneapolis, MN), and all other chemicals from Sigma.

Cell Culture and Differentiation—3T3-L1 fibroblasts were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% calf serum in an atmosphere of 5% CO2, air. Differentiation to adipocytes was induced by incubating confluent monolayers (day 0) for 2 days in DMEM containing 10% fetal bovine serum, 0.5 mM 3-isobutyl-1-methykanethanethiol, and 0.4 μg/ml dexamethasone, followed by incubation for 2 more days in DMEM containing 10% fetal bovine serum and 1 μg/ml insulin. Two days after transfer to the same medium without insulin, greater than 90% of the cells expressed the adipocyte phenotype. Unless otherwise stated, experiments were performed on adipocytes 2–4 days after withdrawal from the differentiation medium (days 6–8).

PI 3'-K Assay—This assay was carried out as described previously (9, 10). In brief, 3T3-L1 adipocytes were serum-starved for about 16 h. After hormone stimulation for 10 min, the cells (10-cm culture dishes) were harvested in cold lysis buffer (1% Nonidet P-40, 150 mM NaCl, 5 mM EDTA, 20 mM Tris, pH 7.5, 1 mM Na3VO4, 100 μM phenylmethylsulfonyl fluoride (PMSF), 20 mM leupeptin, 20 mM pepstatin A) and kept on ice for 30 min. Insoluble materials were removed by centrifugation and the supernatants were incubated with anti-phosphotyrosine anti-

1 The abbreviations used are: MeAIB, 2-(methylamino)isobutyric acid; PDGF, platelet-derived growth factor; AIB, α-aminoisobutyric acid; GH, growth hormone; IGF, insulin-like growth factor; IL-11, interleukin 11; IRS, insulin receptor substrate; MEK, mitogen-activated protein kinase kinase; MAP, mitogen-activated protein; PI 3'-K, phosphatidylinositol 3'-kinase; p70S6K, 70,000 S6 kinase; TPA, 12-O-tetradecanoylphorbol-13-acetate; DME, Dulbecco's modified Eagle's medium; PMSF, phenylmethylsulfonyl fluoride; PBS, phosphate-buffered saline.

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body under agitation at 4 °C for 1 h. Protein A-agarose beads were added and incubated for another hour. The immunoprecipitates were sequentially washed with lysis buffer, washing buffer (100 mM Tris, pH 7.5, 0.5 mM LiCl, 1 mM Na2VO4, 100 mM PMSF, 20 μM leupeptin, 20 μM pepstatin A), and PI 3-kinase buffer (20 mM MgCl2, 10 mM Tris, pH 7.5, 0.2 mM EDTA, 100 mM NaCl, 1 mM Na2VO4, 100 mM PMSF, 50 μM leupeptin, 20 μM pepstatin A). The precipitates were suspended in 50 μl of PI 3-kinase buffer. The reaction was carried out at 25 °C for 20 min in the presence of 0.4 mg/ml phosphatidylinositol and 10 μM ATP (containing 0.2 mM γ32P-ATP). The reaction was terminated by adding to each tube 100 μl of CHCl3:MeOH:H2O:NH4OH (43:38:7:5). The dried plate was visualized by autoradiography on x-ray film.

**Protein Kinase Assays—**For the assay of p70 S6 kinase (p70S6K), 3T3-L1 adipocytes were serum-starved for 16 h. The cells were treated with or without rapamycin (100 ng/ml) for 1 h followed by treatment with insulin for 10 min. The cells were harvested and the S6 kinase activity of cytosolic extract was detected according to the procedure provided by Upstate Biotechnology, Inc. using a peptide substrate. The S6 kinase activity was obtained by subtracting the values for unstimulated cells from the values for the insulin-induced cells. MAP kinase was assayed as described previously (9). In brief, 3T3-L1 adipocytes were serum-starved overnight and then pretreated or untreated with 50 μM PD98059 for 1 h before stimulation by 100 nM insulin for 15 min. Cell lysates were collected in cold buffer (50 mM HEPES, pH 7.5, 150 mM NaCl, 5 mM EDTA, 0.5% deoxycholate, 1% Nonidet P-40, 50 mM NaF, 10 mM Na-pyrophosphate, 1 mM p-nitrophenylphosphate, 25 mM β-glycerophosphate, 1 mM Na2VO4, 10 μg/ml aprotinin, 1 μg/ml leupeptin, 1 mM PMSF, and 1 mM benzamidine). The preclared lysates (10,000 × g, 10 min) containing 10 μg of protein were subjected to 12% SDS-polyacrylamide gel electrophoresis with 75:1 of acrylamide: bisacrylamide (w/w) and 0.5% Tris-HCl. After electrophoresis, the gel was transferred to nitrocellulose and probed with anti-p42/p44 MAP kinase antisera. The immunoreactive proteins were visualized by enhanced chemiluminescence detection.

**Amino Acid Transport—**The sodium-containing buffer for transport assay was phosphate-buffered saline (PBS) consisting of 137 mM NaCl, 2.7 mM KCl, 10.6 mM Na2HPO4, and 1.5 mM KH2PO4. Prior to use, PBS buffer (pH 7.4) was supplemented with 20 mM t-glucose, 0.49 mM MgCl2, 0.9 mM CaCl2, and 0.2% bovine serum albumin. Before stimulation, the cells were incubated in serum-free DMEM for 3–4 h and then switched to the same medium containing stimuli and incubated for 5–6 h.

The cluster transport assay was used as described previously (11). To eliminate trans-inhibition, the intracellular pool of amino acids was depleted by incubation in PBS for 40 min, with a change to fresh PBS at 20 min in the presence or absence of stimuli. An appropriate amount of choline chloride was added to each reaction mixture to keep all solutions at equal osmolality. Since uptake of MeAIB was linear at 37 °C for at least 15 min, 10-min uptake was used for determining initial uptake rates. Unless otherwise noted, the MeAIB concentration for initial rate of transport measurements was 50 μM, the concentration of insulin was 100 nM, and all transport rates were referred to as saturable uptake rates. The saturable uptake rates were calculated by subtracting the MeAIB uptake rates in the presence of 10 mM excess unlabeled MeAIB from the total uptake rates. The time course of MeAIB uptake rate ratios between insulin-stimulated and unstimulated cells shown in panel B is deduced from the same data in panel A. Values are mean ± S.D. (n = 3). DM, differentiation medium; INS, insulin-containing DMEM.

**RESULTS**

**Differentiation-dependent Stimulation of MeAIB uptake by insulin in 3T3-L1 Cells—**The differentiation of 3T3-L1 fibroblasts to adipocytes is accompanied by a dramatic increase in insulin sensitivity (12). To evaluate the regulation of amino acid transport in these cells, the uptake of 50 μM MeAIB was determined for a period of 14 days after induction of differentiation. In preadipocytes insulin produced only about a 2-fold increase over the saturable basal uptake of MeAIB (Fig. 1). The maximal insulin response occurred 3 days after induction of differentiation. The maximal insulin-stimulated transport decreased from days 4 to 5, reaching levels about 2-fold higher than that of preadipocytes. Basal MeAIB uptake was increased 3–4 days after initiation of differentiation. However, the basal uptake rates at these time points were determined 8 h after switching from insulin-containing to insulin-free medium, suggesting that the increased basal uptake may represent residual activity resulting from previous stimulation (see below). As cells took on a more adipogenic phenotype, basal activity was reduced to about 20% of that seen in fibroblasts. Thus the effects of insulin to stimulate amino acid transport increased to 10-fold. This pattern of reduced basal and increased insulin-dependent activity in fully differentiated adipocytes is similar to that observed for glycogen synthesis in these cells (13).

Protein synthesis in 3T3-L1 cells was also significantly enhanced during the incubation with insulin for 2 days (days 3 to 4) and reached a steady state level after switching to insulin-free medium (Fig. 1). An increase in adipocyte protein content (about 20%) was consistently observed during 5–6 h of stimulation of amino acid transport by insulin. Thus the transport velocity when expressed as the amount of substrate incorporated per unit surface area of cell monolayer (8) may not be representative of the insulin action specific for system A transport (14).

To evaluate the kinetics of stimulation of amino acid transport, MeAIB uptake was assayed after exposure to insulin for different times (Fig. 2A). The effect of insulin was maximal by 5–6 h. Evaluation of the dose response to insulin revealed an EC50 of 5.2 nM (Fig. 2B). To determine the initial uptake rate kinetics in differentiated adipocytes, the concentration dependence of the process was examined. The basal MeAIB uptake increased in direct proportion to concentration (Fig. 2C), suggesting that the unstimulated or basal entry of MeAIB in adipocytes was predominantly mediated by a nonsaturable process (60–70%). In contrast, the insulin-stimulated MeAIB uptake
uptake was saturable, exhibiting typical Michaelis-Menten kinetics with a $K_m$ value of 190 $\mu$M and $V_{\text{max}}$ of 390 pmol/min/mg of protein.

**Insulin-stimulated Amino Acid Transport Is Mediated by System A**—Saturable uptake of MeAIB has been regarded as the simplest indication for mediation by system A (2). To determine that system A mediates the insulin-stimulated amino acid transport in 3T3-L1 adipocytes, the MeAIB transport was characterized. Inhibition of MeAIB uptake by various amino acids produced a typical system A transport pattern (Fig. 3, left). Alanine, serine, cysteine, proline, and histidine inhibited MeAIB uptake by more than 90%, whereas the cationic amino acids, arginine and lysine, had no effect. It is noteworthy that the anionic amino acids, glutamate and aspartate, showed moderate inhibition of MeAIB uptake (30–40%). Inhibition by glutamate was inversely proportional to pH (data not shown), indicating that the anionic amino acids could be interacting with system A as uncharged zwitterions (2). Consistent with the major properties of system A transport, uptake of MeAIB in 3T3-L1 adipocytes was strictly Na$^+$-dependent. More than 90% of the MeAIB entry was eliminated when sodium was replaced with choline. In addition, system A activity decreased by 35% in cells preloaded with 50 $\mu$M MeAIB for 40 min (trans-inhibition), and by 65% at pH 5.0 as compared with pH 7.4 (Fig. 3, right). The substrate specificity, hormonal stimulation, Na$^+$ dependence, decreased transport activity at lower pH, and trans-inhibition indicate that the insulin-stimulated MeAIB uptake in 3T3-L1 adipocytes is mediated by a typical A-type transport system (1). In contrast to the insulin-stimulated MeAIB uptake, the basal or unstimulated transport was resistant to inhibition by histidine, leaving open the possibility that the constitutively expressed low transport activity is mediated by a different subtype of system A.

**Decay of the Insulin-stimulated MeAIB Uptake Is Independent of Protein Synthesis and Ambient Amino Acids**—Instability is a mechanistically important feature of the regulation of system A transport (15–17). To determine the half-life of the stimulated MeAIB transport in 3T3-L1 adipocytes, the time course of its activity was followed after removal of insulin from the adipocyte growth medium. At the onset of adipocyte differentiation (day 2), the insulin-stimulated uptake decayed rapidly with an estimated half-life of 1.5 h (Fig. 4, PBS), similar to what is observed in hepatocytes (15). In fully differentiated adipocytes, the insulin-induced transport activity decayed at much slower rates, with a $t_{1/2}$ of 4.3 h. To determine if the decay of system A activity was associated with a newly synthesized repressor-type protein or repression by ambient amino acids (15, 16), cycloheximide and actinomycin D (1 $\mu$g/ml) were added 1 h before removal of insulin, or the cells were incubated in insulin-free medium in the presence or absence of ambient amino acids. As shown in Fig. 4, these agents had no effect on the half-life. Moreover, the similarity in half-life between amino acid-free and amino acid-containing media (PBS versus DMEM) suggests that the decay is not caused by ambient amino acids. Following decay of the insulin-stimulated uptake, the MeAIB transport activity could be re-established by switching to insulin-containing medium. As with the stimulated transport activity before the decay, most of the re-established MeAIB uptake was also substantially inhibited by cycloheximide (81 ± 2%) and actinomycin D (95 ± 2%) (Fig. 3, right), suggesting that the insulin-induced or the decayed MeAIB uptake is associated, respectively, with de novo synthesis or degradation of protein(s) essential for system A function.

**Activation of MAP Kinase and p70 S6K Is Neither Sufficient Nor Required for the Insulin-stimulated System A Transport**—The regulation of protein phosphorylation is believed to play a central role in insulin action (6). The two best characterized pathways leading to insulin-dependent serine phosphorylation involve either MAP kinase or p70 S6K (18). To determine if Ras/MAP kinase pathway is required for the stimulation of system A transport by insulin, PD98059, a specific inhibitor of MEK, was examined. At 10 $\mu$M concentration, PD98059 is sufficient to block the activation of MEK, MAP kinase, and pp90 S6K and the induction of c-fos transcription (9, 10, 19, 20). However, this agent did not significantly affect the insulin-stimulated MeAIB uptake even at concentrations up to 50 $\mu$M, while the same concentration of PD98059 caused nearly complete inhibition of the insulin-induced activation of MAP kinases (Fig. 5). To further exclude involvement of this signaling pathway, inhibition of the farnesylation of Ras, the upstream mediator for activation of the MAP kinase pathway, by specific...
Fig. 3. Substrate specificity and selected properties of the insulin-stimulated MeAIB uptake in 3T3-L1 adipocytes. The adipocytes at days 6–8 after induction of differentiation were used for transport assay. The MeAIB uptake was carried out as described in Fig. 1. Transport rates are expressed as percentage of the uptake rates in the corresponding untreated cells. To determine substrate specificity, uptake of 50 μM of [3H]MeAIB was measured in the presence of 10 μM various amino acids. To inhibit transcription and translation, 1 μg/ml of cycloheximide or actinomycin D (marked as CHX and AcD, respectively) was added 1 h before the first or second insulin treatment (marked as 1st and 2nd, respectively). The second insulin treatment was initiated after a large part of the stimulated transport activity by the first insulin treatment was lost by incubation of the cells in the insulin-free PBS medium for 4 h (see Fig. 4). To examine pH dependence of the insulin-induced MeAIB uptake, the cells were incubated in buffer containing 25 mM HEPES (pH 5–7.4), 140 mM NaCl, 20 mM d-glucose, 0.49 mM MgCl₂, 0.9 mM CaCl₂, and 0.2% bovine serum albumin (marked as pH 5). The Na⁺-independent uptake was carried out in medium with choline in place of sodium (marked as NR-free) as described previously (11). Values are mean ± S.D. (n = 3). AcD, actinomycin D; ala: alanine; asp, aspartate; arg, arginine; BCH, 2-aminobicyclo(2,2,1)heptane-2-carboxylic acid; CHX, cycloheximide; cys, cysteine; glu, glutamate; his, histidine; leu, leucine; lys, lysine; phe, phenylalanine; pro, proline; ser, serine; trp, tryptophan.

Fig. 4. Decay of the insulin-stimulated system A activity in 3T3-L1 adipocytes at days 2 and 10 after induction of differentiation. The cells were stimulated by 100 nM insulin for 24 h. Following sufficient wash with PBS, the cells were switched to insulin- and serum-free DMEM and PBS. The concentration of cycloheximide (CHX) and actinomycin D (AcD) were 1 μg/ml, and they were added 1 h before terminating insulin treatment. The data were expressed as percentage of the MeAIB uptake rates at the end of insulin treatment. Values are mean ± S.D. (n = 3).

Inhibitors PD152440 (20) and B581 (21) was tested. At concentrations of 10 and 50 μM, respectively, these two compounds have been shown to effectively block activation of MAP kinase (20), but at the same concentrations these two reagents had no effect on the insulin-stimulated transport activity, suggesting further that activation of MAP kinase is not responsible for this action of insulin.

Rapamycin, a macrolide immunosuppressant, has been widely used to block activation of p70S6K (18, 22). This drug completely eliminates activation of p70S6K at 10–20 ng/ml (22, 23). Fig. 5 shows that rapamycin at concentrations up to 200 ng/ml did not produce significant inhibition of the insulin-stimulated MeAIB uptake, while 100 ng/ml of rapamycin eliminated activation of p70S6K by 90%. The same results were obtained when rapamycin and PD98059 were added together, suggesting that the p70S6K signaling pathway is not likely involved in the insulin stimulation of system A transport in 3T3-L1 adipocytes.

Insulin-Stimulated System A Transport Is Highly Sensitive to Wortmannin and LY294002—To explore the role of PI 3'-K in the regulation of system A transport, the two structurally distinct inhibitors of PI 3'-K, wortmannin and LY294002, were employed (24, 25). As seen in Fig. 5, both compounds were potent inhibitors of the insulin-stimulated system A activity with IC₅₀ values of 17 nM and 9.8 μM, respectively. These IC₅₀ values are similar to those reported for inhibition of insulin-activation of PI 3'-K (20, 26, 27) and phospholipase A₂ (28), but are significantly lower than that for inhibition of phospholipase D and phospholipase C (29), myosin light chain kinase (30), mammalian target of rapamycin (31), and phosphatidylinositol 4'-kinase (32).

To explore the link between activation of PI 3'-K and stimulation of system A transport by insulin, we examined the effects of a series of hormones and growth factors that differentially regulate these processes. As seen in Fig. 6, epidermal growth factor, glucagon, and TPA were ineffective in activation of PI 3'-K; however, GH, PDGF, and IL-11 produced a marked elevation of PI 3'-K as detected in anti-phosphotyrosine immunoprecipitates. However, capability for activation of PI 3'-K did not correlate well with stimulation of MeAIB uptake. GH, PDGF, and IL-11 did not produce substantial stimulation of system A activity.
Regulation of System A Transport by Insulin

DISCUSSION

Several models have been proposed to interpret the kinetic and genetic bases for the regulation of system A amino acid transport (5, 15–17). According to these models, a “transport inactivation protein” or an active form of “repressor/inactivator” is thought to be responsible for the decay or adaptive regulation of system A activity. Evidence suggests that the histone-like nuclear proteins (16, 17) or heat shock protein P1-related proteins (33) may serve to inactivate the system A transporter. However, data presented here indicate that the decay of system A transport in adipocytes is not caused by synthesis of a repressor-type protein. Moreover, it also appears to be independent of ambient amino acids. Taken together, these data suggest that the insulin-stimulated system A transport in adipocytes is mediated by a mechanism different from that reported in hepatocytes (15–17). The lack of repressor-type control has been reported for regulation of system A transport by other factors such as osmotic stress (5, 34).

The precise molecular events involved in regulation of system A amino acid transport remain uncertain. The time course of the insulin-stimulated MeAIB uptake and its sensitivity to cycloheximide and actinomycin D suggest that the hormone probably regulates expression of the genes encoding proteins involved in system A transport. We demonstrate here that the Ras/MAP kinase and pp70S6K pathways are not necessary for activation of this transport in 3T3-L1 adipocytes. This finding is consistent with dissociation of Ras/MAP kinase pathway from most of the metabolic responses to insulin, including glucose transport (9, 10, 35), glycogen synthesis (9, 10, 36, 37), and lipogenesis (9, 10). Regulation of gene expression by insulin appears to be differentially sensitive to blockade of the MAP kinase pathway. While c-fos induction clearly requires MAP kinase activation (9), the regulated expression of phosphoenolpyruvate carboxykinase is not MAP kinase-dependent (20). Moreover, dissociation of pp70S6K pathway from stimulation of amino acid transport by insulin is similar to that observed in insulin-induced translocation of GLUT4 (38) and activation of glycogen synthase (23).

In contrast to the MAP kinase and pp70S6K pathway inhibitors, the two PI 3'-K inhibitors, wortmannin and LY294002, strongly attenuate the insulin-stimulated MeAIB uptake with the IC50 values close to that for inhibition of PI 3'-K (20, 26, 27). Inhibition by these two agents has been widely applied in investigations of the importance of PI 3'-K in a number of insulin-mediated metabolic responses, including glucose transport (18, 39–42), antilipolysis (39, 43), glycogen synthesis (36, 37, 44), and phosphoenolpyruvate carboxykinase gene expression (45). Wortmannin has also been used in the studies of insulin-induced α-aminoisobutyric acid (AIB) uptake in muscle cells (46). In this previous study, AIB uptake was taken as system A transport. However, AIB is a nonspecific probe for system A, and in muscle cells, system A is not the only insulin-inducible amino acid transport system (47). Because the insulin-induced AIB uptake was totally insensitive to actinomycin D, its regulatory mechanism could be quite different from the commonly observed transcriptional regulation of system A.

It should be noted that the specificity of wortmannin and LY294002 for PI 3'-K has been challenged by several recent studies (28, 31, 48). In addition to inhibition of PI 3'-K, wortmannin also inhibits a number of other signaling mediators, although the inhibition may occur at relatively higher concentrations. These wortmannin-sensitive enzymes include mammalian target of rapamycin (31), phospholipase A2 (28), phosphatidylinositol 4'-kinase (32), myosin light chain kinase (30), phospholipase C, and phospholipase D (29). Even though the effective doses of wortmannin for inhibition of the insulin-induced amino acid transport occurred at low nanomolar concentrations, the present study does not exclude PI 3'-K-independent signaling pathways involved in the regulation of system A. It has been shown that activation of glycogen synthase by insulin and activation of MAP kinase by platelet-activating factor are unaffected by introducing overexpressed dominant-negative Δ85, but the induced activities remain wortmannin-sensitive (37, 49). These latter results suggest
that there might be an additional wortmannin/LY294002-sensitive mediator(s) involved (36).

Activation of PI 3'-K represents an earlier step in transducing signals from many receptor tyrosine kinases (50). In addition to insulin, mitogens, such as PDGF, IL-4, IL-11, and GH, also activate PI 3'-K (10, 49) (this study). In the present report, we demonstrate that activation of PI 3'-K by GH, PDGF, and IL-11 is not accompanied by coordinate stimulation of system A transport. This dissociation of increase in MeAIB uptake from activation of PI 3'-K is consistent with a recent study showing that PDGF and IL-4 activate PI 3'-K, but fail to induce glucose transport (51). It has been suggested that activation of PI 3'-K may not be sufficient for some of the metabolic responses to insulin (51, 52). Alternatively, insulin may activate PI 3'-K in a way distinct from other growth factors and hematopoietic cytokines. It has recently been shown that insulin and PDGF trigger compartment-specific regulation of PI 3'-K (53). In contrast to insulin stimulation, the phosphatidylinositol-(3,4,5)P₃ synthesis induced by PDGF has been reported to be barely detectable in 3T3-L1 adipocytes (54). These differences may account for the failure to stimulate amino acid transport by PDGF observed here. The signaling pathway for activation of PI 3'-K by IL-11 is unknown. Since Western blots did not show coimmunoprecipitation of p85 with IRS-1 in IL-11-stimulated 3T3-L1 adipocytes, IRS-1 might not be involved in activation of PI 3'-K by IL-11. Therefore, the IRS-1-independent activation by PDGF and possibly by IL-11 may trigger downstream signaling pathways distinct from that by insulin. Although GH activates PI 3'-K via phosphorylation of IRS-1 (55), GH is ineffective for stimulation of MeAIB uptake (this study) and glucose transport (55). For the latter results it was hypothesized that GH and insulin may not induce phosphorylation of the same subset of tyrosine residues of IRS-1 or produce the same type of interaction among the signal mediators that are bound to the phosphorylated IRS-1 (55). Furthermore, it is possible that the insulin-stimulated system A transport is mediated by an unique form of PI 3'-K (56, 57). Various subtypes of PI 3'-K may be differentially regulated. The precise role of PI 3'-K in insulin action is not fully understood. Recent studies suggest that phosphoinositide-dependent protein kinase and protein kinase B may be the two sequential downstream mediators after activation of PI 3'-K by insulin (58). Interestingly, these authors showed that activation of protein kinase B was not inhibited by rapamycin and PD98059, but was prevented by wortmannin. It is unclear whether these two kinases par-

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2 T.-Z. Su, M. Wang, L.-J. Syu, A. R. Saltiel, and D. L. Oxender, unpublished data.
