Differential Regulation of Amino Acid Transporter SNAT3 by Insulin in Hepatocytes

Sumin Gu, Carla J. Villegas, and Jean X. Jiang

From the Department of Biochemistry, University of Texas Health Science Center, San Antonio, Texas 78229-3900

The liver is a metabolite and transfer center of amino acids as well as the prime target organ of insulin. In this report, we characterized the regulation of system N/A transporter 3 (SNAT3) in the liver of dietary-restricted mice and in hepatocytes treated with serum starvation and insulin. The expression of SNAT3 was up-regulated in dietary-restricted mice. The expression of SNAT3 protein was detected on the plasma membrane of hepatocyte-like H2.35 cells with a half-life of 6–8 h. When H2.35 cells were depleted of serum, the expression of SNAT3 was increased. An increased concentration of insulin, however, suppressed SNAT3 expression. Interestingly, the down-regulation of SNAT3 expression by insulin was blocked by the specific phosphoinositide 3-kinase inhibitor LY294002 and mammalian target of rapamycin inhibitor, but not by MAPK inhibitor PD98059, suggesting that insulin exerts its effect on SNAT3 through phosphoinositide 3-kinase-mammalian target of rapamycin signaling. Surface biotinylation assay showed an increased level of SNAT3 on the cell surface after 0.5 h of insulin treatment, although no effect was observed after 24 h of treatment. Consistently, the transport of the substrate L-histidine was increased with short, but not long, treatment by insulin in both H2.35- and SNAT3-transfected COS-7 cells. The L-histidine uptake was inhibited significantly by L-histidine followed by 2-endoamino-bicycloheptane-2-carboxylic acid and L-cysteine and to a lesser extent by L-alanine and aminoisobutyric acid, but was not inhibited by α-(methylamino)isobutyric acid, implying that uptake of L-histidine in H2.35 cells is primarily mediated by system N transporters. In conclusion, differential regulation of SNAT3 by insulin and serum starvation reinforces the functional significance of this transporter in liver physiology.

Amino acid transporters play important roles in a variety of cellular processes in the liver, including uptake of nutrients, energy and chemical metabolism, detoxification, and more importantly, the deamination and urea cycle (1, 2). Mammalian amino acid transporters identified to date belong to a variety of protein transporter families including CAT, A, N, and EAAT protein transporter families including CAT, A, N, and EAAT (6, 7). We have identified and characterized three members of the SNAT family, SNAT1 (formerly mNAT2), SNAT3 (formerly mNAT), and SNAT4 (formerly mNAT3 and hNAT3), which are predominantly expressed in liver, muscle, brain, or retina (8–11). The protein members of SNATs have been identified by our laboratory and others; human G17 and rat SN1 (12, 13) are human and rat orthologs of SNAT3; rat Glut (14) or SA2 (15) is a rat ortholog of SNAT1, and ATA3 (16, 17) and hNAT3 (9) are rat and human orthologs of SNAT4. The members of the SNAT transporter system family mostly convey L-glutamine, L-histidine, L-asparagine, and L-alanine across cell membranes (6, 7).

Transport of glutamine and alanine is essential for control of nitrogen metabolism in the liver and muscle (2, 10). In these organs, glutamine is a central intermediate in the detoxification of ammonia and the production of urea (20). Alanine directly participates in the glucose-alanine cycle between the liver and muscle for glycolysis and in the removal of ammonia from muscle. In addition, we have observed a graded distribution of SNAT3 expression from the central vein to the portal tract in the liver (8).

Previous studies have shown that activities of N/A transport systems are regulated by growth factors and hormones (including insulin in some tissues), substrate availability, cell swelling, starvation, etc. (21–25). Glucagon and glucocorticoid have been reported to stimulate hepatic system N/A transport (26–28). In addition, the activities of system N/A transport are up-regulated in the liver during prolonged fasting and corticosteroid treatment, becoming the rate-determining factor in intrahepatic metabolic processes (22). Although previous studies have shown that activities of system N/A transport appear to be regulated by physiological factors, the precise regulatory mechanism at the molecular level is unknown because of the lack of information regarding the identity of transporter proteins. Recent progress in molecular cloning permits the investigation of the function and regulation of these transporters in close detail. Our recent study showed that the expression and function of SNAT4 are stimulated by insulin-activated phosphoinositide 3 kinase (PI3K) signaling in hepatocyte-like cells (11).

In this study, we report differential regulation by insulin signaling of a member of the system N transport family, SNAT3. SNAT3 was up-regulated in the liver of dietary-restricted mice and serum-starved hepatocytes. The expression of SNAT3 was down-regulated by chronic treatment of insulin in...
hepatocytes. This down-regulation was mediated through insulin-activated PI3K-mTOR signaling pathway. On the other hand, acute treatment by insulin within 0.5 h stimulated the surface expression of SNAT3 with a corresponding increase in substrate uptake. Competitive inhibition analysis suggested that the l-histidine uptake was primarily mediated by system N transporters. The temporal regulation of SNAT3 by insulin in the liver suggests that this transporter plays a key role in liver physiology and function.

**EXPERIMENTAL PROCEDURES**

**Materials—**TRI Reagent® was obtained from Molecular Research Center (Cincinnati, OH). [3H]-Histidine, [a-32P]CTP, and [35S]methionine were purchased from PerkinElmer Life Science. Protease inhibitor mixture tablets were obtained from Roche Applied Sciences. Nitrocellulose membrane was purchased from Schleicher & Schuell, and nylon membrane Hybond-XL was from Amersham Biosciences. Tissue culture medium, protein and RNA standard mixtures, and Lipo-fectamine 2000 were obtained from Invitrogen. FBS was from Hyclone Laboratories (Logan, UT). H2.35 cells were obtained from ATCC (Manassas, VA). EZ-link™ Sulfo-NHS-LC-Biotin, immobilized neutral affinity purified rabbit anti-SNAT3 protein was affinity purified from Pierce. The liver samples of *ad libitum*-fed (AD) and dietary-restricted (DR) mice were generous gifts from Dr. Arlan Richardson at the University of Texas Health Science Center at San Antonio. These mice were originally obtained from NIA, National Institutes of Health. X-OMAT AR films were from Eastman Kodak. Paraformaldehyde (16% stock solution) was from Electron Microscopy Sciences (Fort Washington, PA). Tissue Tek® OCT compound was from Frederick (Maryland, MD). All other chemicals were from either Sigma or Fisher Scientific.

**Cell Cultures—**H2.35 cells derived from mouse primary differentiated hepatocytes were grown in Dulbecco’s modified Eagle’s medium supplemented with 4% FBS and incubated in a 10% CO2 incubator at 37 °C according to the manufacturer’s instructions. COS-7 cells were cultured in Dulbecco’s modified Eagle’s medium with 10% FBS in a 5% CO2 incubator at 39 °C. H2.35 cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 15% FBS at a 37 °C and 5% CO2 incubator at 39 °C according to the manufacturer’s instructions. COS-7 cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% FBS at a 39 °C and 5% CO2 incubator at 39 °C. All experiments were repeated at least three times, and the data collected are presented as mean ± S.D. with more than three determinations. The Student-Newman-Keuls test was used to compare the mean ± S.D. versus experimental groups.

**RESULTS**

**Up-regulation of SNAT3 Expression in the Liver of Diabetic Mice—**We have previously shown that SNAT3 is predominately expressed in the liver (8). The expression of SNAT3 in *ad libitum*-fed (AD) and dietary-restricted (DR) mice was examined by Western blots (Fig. 1, *upper panel*) and densitometric measurements (*lower panel*). There was an increase in the expression of SNAT3 in DR mice of 4–6- and 12-month-old mice (Fig. 1, *lanes 1–4*). The increase became less dramatic in 24-month-old mice (*lanes 5 and 6*). The results imply that increased expression of SNAT3 in DR mice could be caused by the increased levels of certain components or factors in the plasma.

**Expression of SNAT3 in Hepatocyte-like H2.35 Cells—**To understand the molecular mechanism of the regulation and function of SNAT3 in the liver, we utilized mouse hepatocyte-like H2.35 cells as an *in vitro* model. The expression of SNAT3 in H2.35 cells in the form of mRNA and protein was detected (Fig. 2, *A and B*). A 2.4-kb form of mRNA was revealed in H2.35 cells (Fig. 2A, *lane 1*) and in the liver (*lane 2*). A 60-kDa migrating protein band was detected with anti-SNAT3 antibody in H2.35 cells (Fig. 2B, *lane 1*), whereas no expression of SNAT3 protein was observed in neuroblastoma-like N2A cells (Fig. 2B, *lane 2*).
cells were labeled with anti-SNAT3 antibody. Confocal microscopy (Fig. 2) along with an appearance of another chase time (lanes 3–9) and chased for 0 (lanes 1–3 and 9), 1 (lane 4), 2 (lane 5), 4 (lane 6), 8 (lane 7), and 16 h (lane 8). The cells lysates were immunoprecipitated with anti-SNAT3 (lanes 1–9) and preimmune (lane 9) antibodies. The immunoprecipitates were analyzed by SDS/PAGE and fluorography.

**FIG. 2.** Expression of SNAT3 in mouse hepatocyte-like H2.35 cells. A, Northern blots of RNAs from H2.35 cells (lane 1) and mouse liver (lane 2) were hybridized with a [35S]DNA probe of SNAT3. B, immunoblot of crude membranes from H2.35 (lane 1) and N2A (lane 2) cells were labeled with anti-SNAT3 antibody. C, H2.35 cells were immunolabeled with anti-SNAT3 antibody and examined by fluorescence confocal microscopy (b). The corresponding phase-contrast image is shown in panel a.

**FIG. 3.** Metabolic labeling and pulse-chase analysis of the biosynthesis of SNAT3. H2.35 cells were metabolically labeled with [35S]methionine for 0.5 (lane 1), 1.5 (lane 2), and 3 h (lanes 3–9) and chased for 0 (lanes 1–3 and 9), 1 (lane 4), 2 (lane 5), 4 (lane 6), 8 (lane 7), and 16 h (lane 8). The cells lysates were immunoprecipitated with anti-SNAT3 (lanes 1–8) and preimmune (lane 9) antibodies. The immunoprecipitates were analyzed by SDS/PAGE and fluorography.

**FIG. 4.** Up-regulation of the expression of SNAT3 by serum starvation. H2.35 cells were incubated in the presence (lane 1) and absence (lanes 2 and 3) of serum for 48 h. Isolated crude membranes were immunoblotted with anti-SNAT3 (lanes 1 and 2, upper panel), preimmune (lane 3, upper panel), and anti-β-actin antibodies (lanes 1–3, lower panel).

Immunofluorescence confocal microscopy of the cells showed that SNAT3 was localized at the plasma membrane (Fig. 2C, b).

The protein stability and turnover of SNAT3 were determined using pulse-chase and immunoprecipitation analysis (Fig. 3). The cumulative level of SNAT3 protein was observed after 3 h of radioactive labeling (Fig. 3, lanes 1–3). The protein levels of SNAT3 were still visible after 16 h of postlabeling chase time (lanes 4–8) along with an appearance of another protein band (~48 kDa) (lane 8). These results suggest that SNAT3 is a relatively stable protein with a half-life of ~6–8 h. SNAT3 protein was not detected in the preimmune antibody-blotted sample (lane 9).

**Stimulation of SNAT3 Expression in Serum-starved Hepatocytes**—In analogy to dietary restriction in vivo, H2.35 cells were serum-starved for 48 h and the expression of SNAT3 was determined (Fig. 4). In comparison to the expression of the housekeeping protein β-actin (Fig. 4, lower panel), serum starvation dramatically stimulated the expression of SNAT3 (upper panel, lanes 1 and 2). SNAT3 was undetectable using preimmune antibody (upper panel, lane 3). This observation suggests that the expression of SNAT3 is down-regulated in the presence of serum.

**A Decrease in the Expression of SNAT3 Induced by Insulin**—The plasma levels of insulin and insulin-like growth factor in DR mice have been shown to be markedly decreased as compared with AD mice (34, 35). The serum, according to the information provided by the manufacturer, contains 7–13 mcrinits/ml insulin. Treatment with insulin significantly reduced mRNA levels of SNAT3 (Fig. 5A, upper panel) with the most dramatic effect after 24 h of insulin treatment, as shown by the densitometric quantification (lower panel). Similarly, the protein levels of SNAT3 also decreased in insulin-treated H2.35 cells (Fig. 5B). This decrease is directly associated with the concentration of insulin being applied. This effect was initiated at 6.1 ng/ml insulin (Fig. 5B, lane 3) and became more obvious in association with increased concentrations of insulin (lanes 4–7). A similar decrease was also observed in insulin-like growth factor-1 (IGF-1)-treated sample (lane 8). The reduction of SNAT3 expression associated with the increasing concentrations of insulin was confirmed by densitometric measurements (Fig. 5B, lower panel). Moreover, the decrease of SNAT3 expression induced by insulin was dependent upon the treatment period (Fig. 6). In comparison to the expression of the housekeeping protein β-actin, the decrease of SNAT3 ex-
Expression was consistent after 4 h of insulin treatment (Fig. 6A, lanes 5 and 6), becoming more profound starting at 16 h (lanes 9 and 10) and sustaining up to 80 h of treatment (lanes 23–24). This observation was further verified by densitometric quantification (Fig. 6B). The decrease in the amount of SNAT3 between the treatment periods of 16–80 h was stabilized. This could imply that longer periods of the insulin treatment may not inhibit or reduce SNAT3 biosynthesis and that the existing intracellular protein pool of SNAT3 is stabilized. Together, these data show that the expression of SNAT3 is down-regulated by the chronic treatment by insulin and this down-regulation is likely to occur at the mRNA level of SNAT3.

**Regulation of SNAT3 Expression by Insulin-activated PI3K-mTOR Signaling**—The insulin signaling cascade consists of two major downstream pathways, the MAPK pathway and the PI3K pathway (36). To determine which pathway is responsible for the inhibitory effect of insulin, we utilized two well-characterized specific inhibitors, PD98059 for the MAPK pathway and LY294002 for the PI3K pathway (36). The expression of Akt, a downstream effector of PI3K, and MAPK in H2.35 cells was ascertained through the immunoblots with their corresponding antibodies (Fig. 7A). The activation of MAPK and Akt kinase exhibited through the phosphorylation on certain amino acid residues was determined using antibodies against phospho-MAPK (pThr-202/Tyr-204) and phospho-Akt kinase (pThr-308) (Fig. 7A). As compared with the non-treated control (Fig. 7A, lane 1), the activation of both kinases was detected upon treatment with insulin (lane 2). MAPK activation was inhibited with PD98059 (lane 4, blotted with antibodies for MAPK (pThr-202/Tyr-204)). Treatment with the PI3K inhibitor LY294002 (lane 3) attenuated Akt activation (lane 3, blotted with antibodies for Akt (pThr-308)). A similar inhibitory effect was also observed by another Akt inhibitor, Wortmannin (data not shown). Together, these results suggest that insulin activated MAPK and PI3K signaling in H2.35 cells and that the inhibitors PD98059 and LY294002 can efficiently block the activation of each signaling pathway.

These two inhibitors, PD98059 and LY294002, were adopted to determine which downstream pathway(s) of insulin-activated signaling was involved in down-regulation of the expression of SNAT3 in H2.35 cells (Fig. 7B). Consistent with the data shown in Figs. 5 and 6, insulin induced a decrease in SNAT3 expression (Fig. 7B, lanes 1 and 2). This decreased expression was completely blocked by the PI3K inhibitor LY294002 (lane 3), but not by the MAPK inhibitor PD98059 (lane 4). The inhibition of insulin-induced down-regulation of SNAT3 by the PI3K inhibitor Wortmannin was also observed (data not shown). These results suggest that the insulin-induced down-regulation of SNAT3 in hepatocytes is likely to be mediated through the PI3K signaling pathway.

To further confirm the effect of PI3K pathway on the down-regulation of SNAT3 by insulin, H2.35 cells were treated with various concentrations of LY294002 in conjunction with insulin (Fig. 7C). Higher concentrations of LY294002 completely attenuated the inhibitory effect of insulin on SNAT3 expression, implying the involvement of insulin-activated PI3K signaling pathway.

mTOR was recently reported to be activated by insulin-PI3K pathway, and the activated mTOR signaling is involved in metabolic activities and cell proliferation (37–39). To determine
FIG. 6. Time-dependent down-regulation of the expression of SNAT3 by insulin. A, H2.35 cells were treated in the presence (lanes 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, and 23) and absence (lanes 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24) of 0.5 μg/ml insulin for 1 h (lanes 1 and 2), 2 h (lanes 3 and 4), 4 h (lanes 5 and 6), 8 h (lanes 7 and 8), 16 h (lanes 9 and 10), 24 h (lanes 11 and 12), 36 h (lanes 13 and 14), 44 h (lanes 15 and 16), 52 h (lanes 17 and 18), 60 h (lanes 19 and 20), 72 h (lanes 21 and 22), and 80 h (lanes 23 and 24). The immunoblots of membranes from treated cells were labeled with anti-SNAT3 or an- immunoblots of membranes from treated 

the involvement of mTOR, we treated H2.35 cells with various concentrations (10–1000 nM) of mTOR inhibitor, rapamycin, in the absence and presence of 0.5 μg/ml of insulin (Fig. 8). The data showed that the inhibition of SNAT3 expression by insulin was attenuated by increased concentrations of rapamycin (Fig. 8A). To further validate that rapamycin is the regulator for SNAT3 expression, H2.35 cells were treated with various concentrations of rapamycin (10–1000 nM) (Fig. 8B). Rapamycin stimulated the expression of SNAT3 in a dose-dependent manner. Together, the data suggest that insulin-activated downstream PI3K-mTOR pathway is likely to regulate SNAT3 expression.

Acute Treatment by Insulin Stimulated Surface Expression and Transport Activity—The cell surface expression of SNAT3 for under 0.5 h and for 24 h of insulin treatment was determined using the cell surface biotinylation approach. With comparable levels of SNAT3 protein being subjected to precipitation by avidin beads (Fig. 9A, Preload), there was an increase of biotinylated levels of SNAT3 in association with the insulin treatment time of 0.5 h, but this increase was mostly attenuated with 24 h of treatment (Avidin beads precipitated, lanes 1–3). Lysates of the cells without biotin treatment were applied to avidin beads to control for nonspecific binding of SNAT3. Non-biotinylated SNAT3 was not precipitated by the beads (Avidin beads precipitated, lane 4). The flow-through samples from cells treated with 0.5 h of insulin and biotin were reapplied to avidin beads, and none of the SNAT3 was precipitated (Avidin beads precipitated, lane 5), implying that almost all the biotinylated SNAT3 was retained by the avidin beads. The surface expression of SNAT3 corresponds to the activity of L-histidine transport. Consistently, during acute insulin treatment such as the treatment of 0.5 and 1 h, uptake of SNAT3 substrate L-histidine by H2.35 cells was significantly increased (Fig. 9B). There was no significant increase of L-histidine uptake in the extended time periods of insulin treatment. The acute increase of L-histidine uptake was further confirmed by COS-7 cells expressing exogenous SNAT3 (Fig. 9C). A significant increase of substrate uptake was observed after 0.5 h of insulin treatment. However, the acute increase of the transport by insulin was not affected by the inhibitors of PI3K and MAPK (data not shown), implying that both signaling pathways are unlikely to be involved. The results suggest that acute up-regulation of L-histidine uptake by insulin is likely to be mediated by the increased surface expression of SNAT3.

To determine the potential contribution of various amino acid transport systems to L-histidine uptake in H2.35 cells, a competition assay in the presence of 5 mM specific substrates was performed (Fig. 9D). The competitive substrates included α-(methylaminoisobutyric acid and L-alanine for system A transport, AIB for systems A and L, L-histidine for system N,
2-endoamino-bicycloheptane-2-carboxylic acid for system L, and L-cysteine for systems ASC and L (1). The uptake of L-histidine was dramatically inhibited by L-histidine, followed by 2-endoamino-bicycloheptane-2-carboxylic acid and L-cysteine and to a much lesser extent by L-alanine and AIB, but was not inhibited by (methylamino)isobutyric acid. This result suggests that L-histidine uptake was primarily mediated by system N transporters and partially by system L; however, system A transport was unlikely to be involved.

**DISCUSSION**

In this study, we have shown that a member of the system N amino acid transport family, SNAT3, was up-regulated in the liver of dietary-restricted mice and in serum-starved hepatocytes, implying that insulin among other components is involved in the down-regulation of SNAT3. Indeed, the expression of SNAT3 mRNA and protein was down-regulated by chronic treatment by insulin in hepatocytes. This down-regulation was mediated through insulin-activated PI3K-mTOR signaling pathway. Acute treatment by insulin, on the other hand, stimulates the migration and surface expression of SNAT3 with a corresponding increase in substrate uptake. The acute regulation by insulin appears not to be mediated by both PI3K and MAPK signaling pathways. We observed that L-histidine transport was primarily mediated by system N, partially by system L, but not by system A transport. The temporal regulation of SNAT3 by insulin in the liver suggests that this transporter is likely to play a key role in liver physiology and function.

The levels of insulin and insulin-like growth factor in dietary-restricted rodents and primates have been demonstrated to be markedly lower than those of ad libitum-fed controls (34, 35, 40, 41). The up-regulation of SNAT3 observed in DR mice could be caused by depressed levels of plasma insulin or other factors. This in vivo observation is consistent with our result obtained from serum-starved hepatocytes in which SNAT3 is

![Fig. 7 Attenuation of the inhibitory effect of insulin on SNAT3 expression by LY294002.](http://www.jbc.org/)

A) H2.35 cells were incubated in the absence (lanes 1 and 2) and presence of the PI3K inhibitor LY294002 (50 μM) (lane 3) or the MAPK inhibitor PD98059 (50 μM) (lane 4) for 1 h, followed by treatment with (lanes 2–4) and without (lane 1) insulin (0.5 μg/ml) for another hour. The treated cells were immunoblotted with antibodies against phospho-p44/42 MAPK (MAPK(Phe298/Tyr321)) and phospho-Akt kinase (Akt(Phe308)). The membranes were reblotted with antibodies against p44/42 MAPK and Akt. B) H2.35 cells were incubated in the absence (lanes 1 and 2) and presence (lane 3) of PI3K inhibitor LY294002 (50 μM) or MAPK inhibitor PD98059 (50 μM) (lane 4) for 1 h followed by treatment with (lanes 2–4) and without (lane 1) 0.5 μg/ml insulin for 24 h and immunoblotted with anti-SNAT3 and anti-β-actin antibodies.

![Fig. 8 Attenuation of the inhibitory effect of insulin on SNAT3 expression by rapamycin.](http://www.jbc.org/)

A) H2.35 cells were incubated in the absence (lanes 1 and 2) and presence of rapamycin at concentrations of 10 (lane 3), 100 (lane 4), and 1000 nM (lane 5) for 1 h followed by treatment with (lanes 2–5) and without (lane 1) 0.5 μg/ml insulin for 24 h. B) H2.35 cells were incubated in the absence (lane 1) and presence of 10 (lane 2), 100 (lane 3), and 1000 nM (lane 4) rapamycin for 24 h and immunoblotted with anti-SNAT3 and anti-β-actin antibodies.
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A

B

C

D

FIG. 9. An increase in the surface expression of SNAT3 and L-histidine uptake by short-term treatment by insulin, and competitive inhibition by substrates. A, H2.35 cells were treated with 0.5 μg/ml insulin for 0 (lane 1), 0.5 (lanes 2 and 4), and 24 h (lane 3) and then labeled with (lanes 1–3, Bio (biotin)) or without (lane 4, C (control)) sulfo-NHS-LC-biotin. The lysates subjected to avidin beads are shown in the upper panel (Preload) and corresponding avidin bead precipitated samples in the lower panel (Avidin beads precipitated). The avidin bead flow-through samples of 0.5 h of insulin and biotin treatment were precipitated by fresh avidin beads (lane 5, lower panel). The samples were immunoblotted with anti-SNAT3 antibody. B, H2.35 cells were treated with 0.5 μg/ml insulin for 0, 0.5, 1, 6, and 24 h. L-Histidine uptake was performed with 50 μM 3H-labeled L-histidine (n = 3). Control with 0 h of insulin treatment versus insulin treatment: *, p < 0.05, **, p < 0.01. C, COS-7 cells expressing exogenous SNAT3 were treated with or without 0.5 μg/ml insulin for 0.5 h, and the uptake was performed (n = 3). Control versus insulin treatment: *, p < 0.05. D, the competitive inhibition of 50 μM L-histidine uptake in COS-7 cells was conducted in the presence of 5 mM α-methylaminoisobutyric acid, L-alanine, AIB, L-histidine, 2-endoamino-bicycloheptane-2-carboxylic acid, or L-cysteine. The fold inhibition values were derived by dividing the rate of L-histidine uptake without competing substrate with the rate of uptake in the presence of individual substrate.

also similarly up-regulated. Partially consistent with our observation, a recent report shows that dietary restriction increases the expression of SIRT1 deacetylase and this increase is attenuated by insulin and IGF-1, suggesting that the systemic regulation of SIRT1 is mediated, in part, by insulin and IGF-1 (42). This up-regulation could result from the reduced level of insulin. However, SN1 (SNAT3) mRNA is found to be unaffected by streptozotocin-induced diabetic liver (43).

Our results for the first time show that insulin at the molecular level differentially regulates the expression and functions of a member of the SNAT (Slc38a) family. SNAT3 is specifically localized in the basolateral membranes of hepatocytes (8). Mouse H2.35 cells have been characterized to closely mimic the functionality of primary differentiated hepatocytes with the expression of typical marker proteins and enzymes (44, 45), therefore serving as an ideal in vitro model for hepatocytes. In our earlier study, we found that the treatment with insulin up-regulated L-alanine uptake and SNAT4 expression in H2.35 cells but had no effect on cell proliferation. Moreover, this process was mediated by a PI3K-dependent signaling pathway. In contrast to SNAT4, we have shown here that the expression of SNAT3 protein is down-regulated by insulin. This down-regulation is likely to be regulated at the level of gene transcription or/and the stability of mRNA because SNAT3 mRNA levels are also reduced after 24 h of insulin treatment. The decrease of SNAT3 is mediated via PI3K-mTOR signaling pathway. Conversely, acute treatment by insulin promotes surface expression of SNAT3, in conjunction with an increase in the transport activity of L-histidine. Taken together, it appears that SNAT3 and SNAT4, although co-expressed in hepatocytes, play a differential role in response to the stimulation by insulin.

Previous studies have shown that insulin signaling induces the trafficking and transport activities of system A- and N-like transporters (21, 24, 46) and that insulin-induced uptake was facilitated by the activation of PI3K (24). A recent study by Boehmer et al. (46) shows that serum- and glucocorticoid-dependent kinase and protein kinase B, downstream effectors of PI3K signaling, are implicated in the trafficking of SNAT3 in Xenopus oocyte. This different conclusion from our observations could be caused by the different experimental systems used, because their study was conducted using the Xenopus oocyte expression system. In addition, downstream kinases were overexpressed in their study, whereas we examined the activation of endogenously expressed kinases. Another study reveals that the activation of the N-system transport in a burn injury is mediated by PI3K signaling (47). Stimulation of system A transport by IGF-1 depends upon PI3K activation (48). Another study reports that insulin promotes the cell surface recruitment of rat ortholog SNAT2 in skeletal muscle cells (49). In contrast to these reports, stimulation of system A transport by amino acid starvation in cultured fibroblasts requires extracellular signal-regulated kinase 1/2 instead of PI3K activation (50). Previous studies have implied that stimulation of system A-like substrate transport via the MAPK pathway is mediated
through a slow mechanism dependent upon overall protein synthesis (51, 52). However, we found that the expression of SNAT3 in the insulin-treated cells was instead relatively decreased. Another report shows that short treatment by insulin stimulates system N activity in muscle cells by a mechanism that is characterized by its sensitivity to inhibition by cycloheximide, indicating the involvement of specific protein biosynthesis (53). Here, we have shown that short treatment by insulin stimulates surface expression of SNAT3 and transport activity, independent of de novo biosynthesis of SNAT3. It is likely that insulin induces the biosynthesis of certain protein(s), which in turn facilitate migration and assembly of SNAT3 to the membrane. Another insulin-activated downstream signaling pathway, mTOR, is activated by nutrient conditions such as amino acid availability; this pathway is directly involved in the control of cell metabolism, growth, and proliferation (37–39). mTOR signaling has not been elucidated. Regulation of expression and activities of system A and L transport function, and regulatory mechanisms, our results suggest that temporal regulation of SNAT3 is likely to be involved in nitrogen metabolism and other physiological functions in hepatocytes, especially in physiology and pathophysiology in which the processes of gluconeogenesis and glycogen synthesis are prominent. It is our expectation that further studies will shed light on the in vivo physiological significance of differential insulin regulation of SNAT3 in the liver.

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