Stimulation of Glucose Uptake in Muscle Cells by Prolonged Treatment with Scriptide, a Histone Deacetylase Inhibitor

Hisako TAKIGAWA-IMAMURA, Takumi SEKINE, Mitsuo MURATA, Kiyoshi TAKAYAMA, Kiyoshi NAKAZAWA, and Junichi NAKAGAWA†

Medicinal Research Laboratories, Taisho Pharmaceutical Co., Ltd., 1-403 Yoshino-cho, Kita-ku, Saitama-shi, Saitama 331-9530, Japan

Received January 21, 2003; Accepted April 10, 2003

Glucose incorporation is regulated mainly by GLUT4 in skeletal muscles. Here we report that treatment of L6 myotubes with scriptide, a hydroxamic acid-based histone deacetylase (HDAC) inhibitor, stimulated 2-deoxyglucose uptake. The effect appeared only after 24 hr, resulting in 2.4-fold glucose uptake at treatment day 6. Scriptide acted synergistically with insulin, indicating it stimulated a distinct pathway from the insulin signaling pathway. It was not observed in undifferentiated myoblasts or 3T3-L1 adipocytes, suggesting a muscle-specific effect of scriptide. A five-carbon chain and hydroxamic acid, essential for histone deacetylase inhibition, were indispensable for this effect, and trichostatin A stimulated glucose uptake as well. Scriptide increased the cellular content of GLUT4, and induced GLUT4 translocation, but GLUT4 mRNA level did not change, indicating scriptide functions posttranslationally. Our results indicated a novel function for HDAC inhibitors of increasing GLUT4 content and its translocation in muscle cells, resulting in stimulation of glucose uptake.

Key words: glucose transport; diabetes mellitus; muscle; histone deacetylase; scriptide

Skeletal muscle is the primary site responsible for postprandial glucose use. Furthermore, it is the most abundant tissue in the whole body, and thus, proper function of skeletal muscle is important to maintain normal blood glucose levels. Defects in insulin-stimulated skeletal muscle glucose uptake are common pathological states in non-insulin-dependent diabetes mellitus (NIDDM). GLUT4 is the major glucose transporter expressed in insulin-responsive tissues such as skeletal muscle and adipose tissue, where they respond to an acute insulin challenge by translocating GLUT4 rapidly from an intracellular-membrane storage site to the plasma membrane.

We have isolated XCB5667 (6-(1,3-dioxo-1H,3H-benzo[de]isoquinolin-2-yl)-hexanoic acid hydroxamide) as a compound that induces the expression of uncoupling protein 2 (UCP2) in L6 myotubes. UCP family proteins, important regulators of mitochondrial energy consumption, may become a new therapeutic target for diabetes or obesity, considering that induction of UCP expression has antidiabetic or antiobesity effects both in vitro and in vivo. Subsequent studies reported this compound as scriptide, a novel histone deacetylase (HDAC) inhibitor with a structural similarity to trichostatin A (TSA). Both TSA and scriptide have a five-carbon linker between a bulky end-group and hydroxamic acid. This structural moiety is apparently essential for the inhibitory function of HDAC according to the crystallographic study of TSA-HDAC interaction. Histone deacetylase, along with histone acetyl transferase, determines the levels of the reversible acetylation of histone, and thereby plays key roles in the regulation of gene transcription. Inhibition of HDAC was reported to induce cell cycle arrest, cell differentiation, and apoptotic cell death of transformed cells in vitro and in vivo, which renders HDAC inhibitors as candidate pharmaceuticals in cancer treatment.

Considering that scriptide has the capacity to induce UCPs transcription, it is of interest to explore whether scriptide can modulate glucose metabolism as well. In this report, we show that scriptide potently increases glucose uptake in muscle cells via translocation of GLUT4, implying a new role for hydroxamic acid-based HDAC inhibitors in glucose metabolism.

Materials and Methods

Materials. The following materials were purchased: α-minimal essential medium (α-MEM), fetal...
bovine serum (FBS), antibiotic solution, TRIzol, deoxyribonuclease I, Amplification Grade (DNase I), and SuperScript Preaplication System for First Strand cDNA Synthesis, from Life Technologies, Inc. (Gaithersburg, MD), human insulin from WAKO (Shiga, Japan), scriptide, nullscript, XCB5717, and XCB5647 from ChemBridge (San Diego, CA), trichostatin A (TSA) from Sigma (St. Louis, MO), XAS1218 from AsInEx (Moscow, Russia), LA-taq with GC Buffer from TaKaRa (Shiga, Japan), 2-deoxy-[U-14C]glucose, Horseradish peroxidase-conjugated anti-rabbit IgG antibody, anti-mouse IgG and ECL from Amershams Pharmacia Biotech (Buckinghamshire, UK), M-PER from Pierce (Rockford, USA), BlockAce from Snow Brand (Tokyo, Japan), anti-GLUT4 antibody from Chemicon International, Inc. (Temecula, CA), anti-c-myc (9E10) antibody from Santa Cruz Biotechnology (Santa Cruz, CA). L6 rat skeletal myoblasts and L6 expressing c-myc epitope-tagged GLUT4 (GLUT4myc) was kindly provided by Dr. Amira Klip (Programme in Cell Biology, Hospital for Sick Children, Toronto, Canada).

Cell culture and scriptide treatment. Rat L6 skeletal myoblasts and GLUT4myc were maintained in α-MEM supplemented with 10% (v/v) FBS and antibiotics at 37°C in an atmosphere of 5% CO₂—95% air. For differentiation, myoblasts were plated at 5 × 10⁴ cells/cm² in α-MEM containing 2% (v/v) FBS and antibiotics. The differentiation medium was changed every other day. Scriptide was dissolved in DMSO and freshly added to the differentiation medium as indicated in the figure legends. The DMSO concentration in cultured medium was <0.007%. The medium containing scriptide was changed every other day. The purity of synthesized scriptide was found by LC-MS to be virtually 100% pure (data not shown), and scriptide purchased from Ambinter (Paris) is also active in 2-DG uptake stimulation though its purity was not measured.

RT-PCR. Total RNA was extracted using TRIzol reagent and incubated with DNase I. Two µg of total RNA was used as a template to generate the cDNA by reverse transcription (RT) with the SuperScript Preaplimination System. The RT products were amplified by the polymerase chain reaction (PCR) using the following primers described as sense (f) and antisense (r) primers, respectively: GLUT4 (for L6 cells), (f) 5'-TACCTCAGAGGTTAAGAAACACGCAG-3' and (r) 5'-AGATTCTATGGGCAAGAGTTGATTG-3'; GLUT4 (for 3T3-L1 cells), (f) 5'-TCAAGACAGAGATGGGATGGTCC-3' and (r) 5'-CTACTCGTCTCTATCTGG-3'; UC-P2 (f) 5'-CACTACAGATGTTAAGGCCCTC-3' and (r) 5'-AGCCATTAGGCTCTTTTGGAG-3'; MyoD (f) 5'-ATGACTTCTATGATCCGGTGGTTG-3' and (r) 5'-CAGACCTTCATGAGGATGGCGGCG-3'; GLUT1 (f) 5'-CAGACGCTCGCTCATGAGG-3' and (r) 5'-CGACTCAGACTGGGAAATC-3'; rat S26 (f) 5'-AAAGAGAAACACGGCTCTGATG-3' and (r) 5'-ATCACCTTTTATCATGGGAGTG-3'; mouse S26 (f) 5'-AAAGAAAGAACACGGCTCGGAGCC-3' and (r) 5'-ACGCTCTTTATCATGGGAGTG-3'; IR (f) 5'-CTAACGGATCTAGGAGCAGTGCTTT-3' and (r) 5'-GCCAAGCTATGGATTTCCTAAAGG-3'; PPARγ (f) 5'-AAGCCCTGATGAGCCCTGAC-3' and (r) 5'-AACTTTTGGAATGACGCTCC-3'; lipoprotein lipase (f) 5'-TGCAACTCCAAGAGAGGAGCTT-3' and (r) 5'-AGTGTCAGCCAGACTTCTC-3'; HMG-CoA reductase (f) 5'-AGCGTGCTCTGCAATTTGGA-3' and (r) 5'-GCATGTTAGTCTTTGAGAACC-3'; acetyl CoA carboxylase (f) 5'-CTGATGACATGACTTCTGAGGAGG-3' and (r) 5'-CACGCTTCTCAGGAAAATCTCTG-3'; MCAD (f) 5'-CTGCACACAGAGAATTGCCTTGCG-3' and (r) 5'-CAATGATGGCTGTTAGGTTGAC-3'. PCR amplification was done using LA-taq with the following cycle profile: denaturing at 94°C for 2 min, followed by 32 cycles at 94°C for 30 sec, 60°C for 2 min, and 72°C for 1 min for GLUT4, and 32 cycles for GLUT1, respectively. S26 was used for an internal control. The PCR products were electrophoresed and stained with ethidium bromide.

Glucose uptake measurement. L6 myotubes were washed and left unfed for 3 hr in Krebs-Ringer/Hepes buffer (KRH; 20 mM Hepes-Na (pH 7.4), 140 mM NaCl, 2.5 mM MgSO₄, 5 mM KCl, and 1 mM CaCl₂). The cells were then incubated with KRH with or without 100 nM insulin for 30 min at 37°C, 2-deoxy-[U-14C]glucose was added to the solution to a final concentration of 50 µM (1 µCi/ml), and the incubation was continued for 1 hr at 37°C. Cells were then washed three times with ice-cold PBS before lysis in 300 µl of 0.1% SDS. Cell-associated radioactivity was measured by liquid scintillation counting.

Immunoblotting. Cells were washed and harvested immediately in M-PER lysis buffer. The lysate was cleared by centrifugation (16,000 × g) and portions containing 20 µg of protein were put through SDS-PAGE (12.5%). Separated proteins were transferred onto PVDF membranes and blocked with Block Ace. Membranes were probed with antibodies against GLUT4 at dilution of 1:1,000. Following primary antibody incubation, membranes were incubated with anti-rabbit IgG at dilution of 1:5,000. Immunoreactive protein bands were made visible by ECL.
Measurement of cell-surface GLUT4myc. GLUT4myc levels at the cell surface were measured by an antibody-coupled optical assay developed and described by Wang et al. Following treatment, L6 myotubes expressing GLUT4myc were incubated in KRH for 20 min at 37°C, and then treated with 1 μM insulin for 30 min at 37°C. The cells were fixed in 2% paraformaldehyde (v/v) for 5 min at room temperature and washed once with PBS. Following fixation and blocking, primary antibody (anti-c-myc, 9E10) was added at a dilution of 1:1,500 and maintained for 2 hr. The cells were washed with phosphate-buffered saline (PBS) before introducing peroxidase-conjugated anti-mouse IgG (1:2,000). One hr later, the cells were washed 5 times with PBS, and ECL reagent was added to each well. Luminescence was measured at 450 nm. Standard curves were generated using either peroxidase-conjugated anti-mouse IgG alone or myc-tag peptide, as indicated.

Statistical analysis. All data were expressed as mean ± SEM. Differences between groups were examined for statistical significance using Student’s t-test. A P value less than 0.05 denoted the presence of a statistically significant difference.

Results

Scriptide stimulates 2-deoxyglucose Uptake by L6 myotubes

Figure 1A (upper panel) shows 2-deoxyglucose (2-DG) uptake in L6 myotubes. Pretreatment with 1.0 μM scriptide increased the basal 2-DG uptake by 156%, 196%, and 243% for 1, 3, and 6 days, respectively, thereby exceeding maximal insulin-stimulated levels (149%), although 3 μM scriptide was toxic to the cells (data not shown). When myotubes were stimulated with insulin together with scriptide, 2-DG uptake was further increased to 153%, 213%, and 255% for 1, 3, and 6 days, respectively, compared to insulin alone. These results also suggest a time-dependent effect of scriptide on glucose uptake, which is independent of insulin. In contrast, when the effect of scriptide was tested at shorter intervals, i.e., for 20 min to 6 h, no influence on basal or insulin-stimulated 2-DG uptake was observed (data not shown). The dose dependency of this effect was demonstrated using 0.04, 0.2, and 1 μM scriptide (Fig. 1A, lower panel). In contrast to differentiated L6, undifferentiated myoblasts and differentiated 3T3-L1 adipocytes did not respond to scriptide (data not shown).

Previous studies reported that TSA treatment markedly changes the morphology of T24 and HeLa cells to an elongated filamentous shape and results in the reappearance of actin stress fibers. We observed that scriptide-treated myotubes also had elongated morphology and grew to a high density. The cell number remained unchanged at day 1, but it increased to 175% of the basal level at day 6. The stimulation ranges of proliferation were substantially smaller than that of 2-DG uptake, suggesting that increased 2-DG uptake in myotubes was not due to proliferation.

Structure-activity relationship

In order to identify which structural moiety of scriptide affects glucose uptake in myotubes, we did a preliminary structure-activity-relationship study. We treated the cells with 1 μM of each of 19 analogues of scriptide for 6 days, of which representative results are shown in Fig. 1B. The scriptide analog, nullscript, showed no activity. This compound is structurally identical to scriptide except for the three-carbon chain replacing the five-carbon chain at the corresponding portion of scriptide (Fig. 1C). Likewise, a compound with a shorter carbon chain, XCB5717 (Fig. 1C) did not show activity. Two other compounds with minor substitutions in the hydroxamic acid moiety of scriptide, namely, XCB5647 and XAS1218 (Fig. 1C), were not active either in stimulation of glucose uptake, and it was also the case for the insulin-stimulated glucose uptake (Fig. 1B).

On the other hand, TSA, the only HDAC inhibitor among these scriptide analogues, increased 2-DG uptake, with a maximum increase of 169% over the basal uptake by treatment with 200 nM, demonstrating that TSA has also a potent effect on glucose uptake (Fig. 1A upper panel). Moreover, TSA seemed to induce 2-DG uptake in a dose-dependent manner, although 1 μM of TSA was toxic to the cells (data not shown). In contrast, other scriptide analogues, which did not affect glucose uptake, did not have hydroxamic acid or have shorter carbon linkers. Therefore, the essential structure for induction of glucose uptake by scriptide seems to be nearly the same, if not identical, as that in TSA for HDAC inhibition, strongly implying the stimulation of glucose uptake by scriptide may be mediated via its HDAC inhibitory activity.

Scriptide increases GLUT4 in L6 myotubes

We also investigated the mechanism of scriptide-stimulated glucose transport in L6 myotubes. Glucose uptake was only induced when the cells were treated with scriptide for periods exceeding 24 hr, and the extent of induction increased with longer treatment periods. These findings implied that scriptide changed the level of proteins that contribute strongly to glucose uptake. This notion prompted us to examine the protein level of GLUT4, one of the most important molecules in glucose uptake. Figure 2 shows RT-PCR and western blotting of GLUT4 in whole cell lysates of myotubes treated with scriptide for indicated periods. Noticeably, scriptide treatment led to elevation of UCP-2 mRNA levels with con-
Fig. 1. Stimulation of Glucose Uptake in L6 Myotubes by Scrip
tide and Its Structure-activity Relationship.

Incorporation of 2-deoxyglucose in L6 myotubes treated with
scriptide and TSA (A: upper panel). Scriptide and TSA were
added to the differentiated myotubes at differentiation day 4 (for
6-day treatment), day 7 (3-day treatment), and day 9 (1-day
treatment), and used for the assay at day 10. Before uptake
determination, cells were starved in a serum-free buffer for 3 hr
without scriptide or TSA. Insulin (100 nM) was added to the
buffer 30 min before the determination. Glucose uptake in
untreated cells (without insulin, period = 0 day) was set to 100%,
and all other values are expressed in relative units (z). ■:
1.0 μM scriptide pretreatment and insulin stimulation, ○:
1.0 μM scriptide pretreatment, ○: 0.2 μM trichostatin A
pretreatment. These are representative data of multiple experi-
ments. Dose dependency (A: lower panel) was measured in the
absence of insulin at differentiation day 4 following treatment
with scriptide at the indicated concentration for 6 days. Struc-
ture-activity relationship of scriptide analogs (B): Effects of
scriptide analogs on glucose uptake were measured as in A (low-
er panel) with each compound at 1 μM, in the absence (open bar)
or presence (filled bar) of insulin. The chemical structure of each
compound is illustrated in C. Note that nullscript and XCB5717
have substituted carbon-chains, and XCB5647 and XAS1218
have alteration at hydroxamic acid moiety.

comitant decreases in the MyoD mRNA levels, while,
scriptide did not change GLUT4 (nor GLUT1) tran-
scription when it induced glucose uptake in myotubes
(Fig. 2A). However, treatment with 1 μM scriptide
for 1, 3, and 6 days changed GLUT4 protein level to
72%, 141%, and 190%, respectively. Treatment for
6 days, even at lower doses (0.04 μM and 0.2 μM)
resulted in 159% and 166% increases in GLUT4 con-
tents, respectively, demonstrating its dose-dependen-
cy (Fig. 2B). It should be noted that the time- and
dose-dependent effect of scriptide on GLUT4 protein
level was consistent with the increased glucose uptake
shown in Fig. 1, though treatment for 1 day slightly
decreased GLUT4. In contrast, scriptide did not
change transcription of GLUT4, suggesting that
scriptide might regulate translation or stabilization of
GLUT4, but not via transcription.

Scriptide translocates GLUT4myc to plasma mem-
brane

The GLUT4 translocation is the rate-limiting step
for glucose incorporation, and thus it was of interest
to test whether scriptide translocated GLUT4 in addi-
tion to increasing its cellular content. In order to
measure it, we measured the translocation of GLUT4
with the c-myc tag on the outer surface of the molec-
ule, previously reported by Wang et al.11) This c-myc-
tagged GLUT4 was designed to allow measurement
of properly translocated GLUT4 molecules facing
outside of the cells on the plasma membrane,11) thus
allowed us to measure the translocation reflecting
functional relevance to glucose uptake. In Fig. 3, we
measured GLUT4 translocation in L6 myotubes
Fig. 2. Effects of Scriptide on Gene Transcription (A) and GLUT4 Protein (B).
Scriptide was added to the differentiation medium as described in the legend to Fig. 1. C: control untreated myotubes.

Fig. 3. Effects of Scriptide on GLUT4myc Subcellular Distribution.
L6 myotubes expressing GLUT4myc were treated with insulin (1 μM, 30 min) or scriptide (1 μM, 6 days). Binding of antibodies to myc at the cell surface was measured as described in Materials and Methods. Results are expressed as a percentage of the cell surface binding measured under basal conditions for the myotubes and are presented as mean ± SEM of three experiments. *: P<0.5; compared with those under respective basal conditions.

Fig. 4. Effects of Scriptide on PPARγ Target- and Glucose/Lipid Metabolism-genes in 3T3-L1 Adipocytes.
Scriptide was added to the differentiated adipocytes at day 10, and cells were collected for RT-PCR analysis 4-days later.

Stably expressing GLUT4myc. The results showed that scriptide increased cell-surface GLUT4myc by 251% of its basal level. This effect of scriptide significantly exceeded that of insulin (137%). These findings suggested that scriptide promoted GLUT4 translocation from the cytoplasm to the plasma membrane in addition to increasing the cellular content of GLUT4.

Scriptide does not alter transcription of the PPARγ target genes
Next, we examined the effects of scriptide on the transcription of genes known to be the targets of PPARγ and genes involved in glucose or lipid metabolism. As shown in Fig. 4, Neither PPARγ itself nor the genes encoding aP2, lipoprotein lipase, HMG CoA reductase, acetyl-CoA carboxylase, and MCAD were activated by scriptide in 3T3-L1 cells. These data suggested that the observed effects of scriptide were not brought about via PPARγ. In addition, UCP-2 and insulin receptor (IR) genes were not stimulated by scriptide in the same cells.

Discussion
Scriptide works its effect after a long period following administration to target cells (>24 hr), unlike the instantaneous effect of insulin, implying that prolonged exposure to scriptide may cause changes in
the expression levels of certain proteins. Our results showed that treatment of myotubes with scriptide increased the cellular content of GLUT4 and its translocation. These findings suggest that the hypoglycemic effect of scriptide, and probably TSA, result mainly from an increase of translocated GLUT4. The GLUT4 mRNA level did not change while GLUT4 protein was increased by scriptide treatment, and scriptide increased exogenous GLUT4myc at the cell surface, suggesting that scriptide is involved in a posttranscriptional regulation such as translation or stabilization of the GLUT4 in addition to its translocation.

An increase of UCP2 might also contribute to the stimulation of GLUT4 translocation and subsequent glucose uptake by scriptide. Indeed scriptide (at 1 μM for 3–6 days) caused a 240% increase of UCP2 transcription in myotubes (Fig. 2A). UCP2 is postulated to be important in the regulation of thermogenesis and the resting metabolic rate.14 An increase of UCP2 by scriptide could explain its metabolic action to increase energy expenditure and the requirement of glucose as energy source. Conceivably, the increased UCP2 level and subsequent over-activated metabolism may influence GLUT4 translocation.

Scriptide does not stimulate glucose uptake in cells other than myotubes, suggesting that scriptide affects muscle-specific molecules. We demonstrated that in L6 myotubes, MyoD transcription decreased in response to long-term treatment with scriptide (Fig. 2A). This finding is consistent with a report that TSA selectively blocked the transcriptional induction of the mouse UCP2 gene, a putative regulatory motif specific gene expressions.16 In the promoter region of the mouse UCP2 gene, a putative regulatory motif responsible for MyoD has been found.17 Though it is not clear whether MyoD binds to this motif in the UCP2 gene, increased UCP2 transcription might also be due to repression of MyoD caused by scriptide.

Previous studies reported the reappearance of actin stress fibers in T24 and HeLa cells when HDAC activity was inhibited by TSA.12 On the other hand, it was recently suggested that actin remodeling after insulin stimulation expedites the delivery of GLUT4-containing vesicles to the plasma membrane.18 The morphological changes of scriptide-treated L6 myotubes suggested promotion of actin stress fiber regeneration. These results imply that scriptide is involved in vesicle transportation via actin remodeling. Further studies are needed to clarify the effects of scriptide on actin remodeling, UCP2 action on muscle energy metabolism, and regulation of UCP2 expression by MyoD, in order to understand the mechanism of scriptide effects on GLUT4. We also speculate that scriptide interacts with another muscle-specific protein yet to be identified which is involved in translocation and posttranscriptional regulation of GLUT4. It would be of interest to investigate whether proteins known to be involved in insulin-mediated translocation of GLUT4, such as SNARE and its associated proteins19 play roles in the mechanism underlying scriptide-mediated GLUT4 translocation.

Crystallographic studies showed direct interaction of TSA and HDAC. The hydroxamic acid group on TSA is attached to a five-carbon aliphatic chain (including double bonds) that fits into the catalytic site of histone deacetylase, which has a tubular pocket structure with a zinc atom at its base. The bulky end-group on the opposite end of the aliphatic chain is positioned outside the entrance to the pocket. Our structure-activity-relation analysis suggests that the effect of scriptide on glucose uptake in the myotubes is strictly dependent on the structural moiety necessary for HDAC inhibition, as reported in the case for TSA, therefore, we postulate that the effect of scriptide on glucose uptake stimulation is mediated via its HDAC inhibitory activity. However, as the effects are detectable only after prolonged treatment, we assume there are multiple molecules involved in this signaling process, and the immediate molecule(s) promoting glucose uptake may not be the direct target of HDAC inhibition. To further elucidate the molecular mechanism, the effects of other types of HDAC inhibitors on GLUT4 and glucose uptake still require investigation.

It has been reported that some types of PPAR ligands, fibrates or thiazolidinediones, stimulate glucose uptake and UCP2 expression in L6 myotubes.20,21 We observed by RT-PCR that scriptide did not modify mRNA levels of PPAR target genes such as carnitine palmitoyl transferase (CPT) 1, acyl CoA thiolase, and malic enzyme in the HepG2 hepatoma cell line, and medium-chain acyl CoA dehydrogenase in HepG2 and 3T3-L1 adipocytes, aP2, and lipoprotein lipase in 3T3-L1 adipocytes (Fig. 4 and unpublished results). These observations imply that the effect of scriptide is mediated through a pathway that is essentially distinct from that of PPAR ligands of antidiabetic drugs.

Moreover, our results showed that scriptide did not stimulate transcription of genes participating in lipid or glucose metabolism, such as HMG-CoA reductase, acetyl-CoA carboxylase, paraoxonase-2, fatty acid synthase, ATP citrate lyase, aromatase, PECK, long-chain acyl CoA dehydrogenase, CPT2 and LDL receptor in HepG2, HMG-CoA reductase, and acetyl-CoA carboxylase and the insulin receptor in 3T3-L1 adipocytes (Fig. 4 and unpublished results). Therefore, it is reasonable to speculate that scriptide does not influence the metabolism in adipose tissue and liver, but only stimulates muscle metabolism. Understanding of the molecular
mechanism underlying muscle-specific stimulation of glucose uptake will need further dissection of the action of scriptide which may have a broad influence on gene transcription through HDAC inhibition.\(^9\)

In summary, we have demonstrated that scriptide, a HDAC inhibitor, stimulates glucose transport in muscle cells, but not in adipocytes, independent of insulin action. The stimulation of glucose uptake and GLUT4 translocation by scriptide is significantly greater than that of insulin, implying a novel potential for blood glucose control. Further examination of the effects of scriptide on GLUT4 translocation and posttranscriptional regulation may provide a clue to our understanding of the role of hydroxamic acid-based HDAC inhibitors in the regulation of glucose uptake in muscle cells.

Acknowledgment

We thank Dr. Amira Klip (The Hospital for Sick Children, Toronto, Canada) for providing us with the L6 muscle cells and L6/GLUT4myc myoblast cell line. We also thank Mr. Shinichi Nishimoto for his assistance in manuscript preparation.

References


Stimulation of Glucose Uptake in Myotubes by Scriptide 1505

