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Berberine stimulates glucose transport through a mechanism distinct from insulin

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Abstract

Berberine exerts a hypoglycemic effect, but the mechanism remains unknown. In the present study, the effect of berberine on glucose uptake was characterized in 3T3-L1 adipocytes. It was revealed that berberine stimulated glucose uptake in 3T3-L1 adipocytes in a dose- and time-dependent manner with the maximal effect at 12 hours. Glucose uptake was increased by berberine in 3T3-L1 preadipocytes as well. Berberine-stimulated glucose uptake was additive to that of insulin in 3T3-L1 adipocytes, even at the maximal effective concentrations of both components. Unlike insulin, the effect of berberine on glucose uptake was insensitive to wortmannin, an inhibitor of phosphatidylinositol 3-kinase, and SB203580, an inhibitor of p38 mitogen-activated protein kinase. Berberine activated extracellular signal-regulated kinase (ERK) 1/2, but PD98059, an ERK kinase inhibitor, only decreased berberine-stimulated glucose uptake by 32%. Berberine did not induce Ser473 phosphorylation of Akt nor enhance insulin-induced phosphorylation of Akt. Meanwhile, the expression and cellular localization of glucose transporter 4 (GLUT4) were not altered by berberine. Berberine did not increase GLUT1 gene expression. However, genistein, a tyrosine kinase inhibitor, completely blocked berberine-stimulated glucose uptake in 3T3-L1 adipocytes and preadipocytes, suggesting that berberine may induce glucose transport via increasing GLUT1 activity. In addition, berberine increased adenosine monophosphate–activated protein kinase and acetyl-coenzyme A carboxylase phosphorylation. These findings suggest that berberine increases glucose uptake through a mechanism distinct from insulin, and activated adenosine monophosphate–activated protein kinase seems to be involved in the metabolic effect of berberine.

1. Introduction

Berberine ($[C_{20}H_{18}NO_4]^+$), one of the major constituents of Chinese herb *Rhizoma coptidis*, is a kind of isoquinoline alkaloid. *Rhizoma coptidis* has been used to treat diabetes for more than 1000 years in the long history of Chinese medicine [1]. Berberine has a wide range of pharmacologic actions such as antidiarrheic, antimicrobial, anticancer, antiinflammatory, and antiarrhythmic activities [2]. Berberine began to be used in the treatment of type 2 diabetes mellitus in China in the 1980s when its hypoglycemic effect was accidentally found in treating patients with diarrhea and diabetes. Since then, a large number of clinical trials and animal experiments about berberine improving insulin resistance and lowering hyperglycemia were reported [3,4]. However, the underlying mechanisms remain uncertain.

Type 2 diabetes mellitus is a heterogeneous metabolic disorder characterized by the impairment of insulin secretion from pancreatic beta cells and insulin resistance in peripheral tissues such as liver, skeletal muscle, and adipose tissue [5]. Therefore, several studies have focused on the effect of berberine on insulin secretion and insulin resistance. It is widely accepted that berberine can improve insulin resistance. However, the results of berberine-stimulated insulin secretion were controversial [6]. Adipose tissue plays an important role in the pathogenesis of insulin resistance and type 2 diabetes mellitus. Glucose transport is the rate-limiting step in glucose utilization in adipocytes. There is considerable evidence that a defect in glucose transport is

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responsible for the acquired insulin resistance of glucose uptake observed in diabetes. This metabolic impairment could conceivably be explained by a variety of defects in glucose transporter 4 (GLUT4) regulation, including alterations in GLUT4 expression and translocation, defects in the insulin signaling pathway, and alterations in the temporal and spatial pattern of signaling molecules [7]. Insulin-responsive tissues also express GLUT1, a ubiquitous glucose transporter largely responsible for basal uptake. It is now apparent that increased plasma membrane glucose transporter content is insufficient to fully account for the insulin-stimulated elevation in glucose uptake. Thus, it has been proposed that the intrinsic activity of cell surface glucose transporters may be regulated by insulin and other agents that stimulate glucose uptake [8].

3T3-L1 cells were originally selected from Swiss mouse embryo fibroblasts for their ability to differentiate into adipoctyes, accompanied by the acquisition of the insulin-dependent glucose transport and the expression of GLUT4. The present study aimed to characterize the effect of berberine on glucose uptake in 3T3-L1 adipocytes and to investigate whether the action of berberine is involved in insulin signaling pathway.

2. Materials and methods

2.1. Materials

Dulbecco modified Eagle medium (DMEM) and other culture reagents were obtained from Gibco Life Technologies (Grand Island, NY). The cell culture plates were purchased from Nalge Nunc International (Roskilde, Denmark). Methylthiotetrazole (MTT), dexamethasone, 3-isobutyl-1-methylxanthine, 5-aminoimidazole-4-carboxamide riboside (AICAR), wortmannin, SB203580, PD98059, and genistein were purchased from Sigma (St Louis, MO). Bovine serum albumin (BSA) was from Shanghai Shisheng Cell Biological Technologies (Shanghai, China), glucose oxidase was from Shanghai Shensuo Reagents (Shanghai, China), and human insulin (Humulin R) was from Eli Lilly Canada (Toronto, Ontario). Anti-Akt, anti–phospho-Akt (Ser473), anti-AMPK (adenosine monophosphate–activated protein kinase), anti–phospho-AMPK (Thr172), anti–phospho-Ser79 ACC (acetyl-coenzyme A carboxylase), anti–ERK1/2 (extracellular signal-regulated kinase 1/2), and anti–phospho-ERK1/2 were from Cell Signaling Technology (Beverly, MA), and anti-GLUT1 (N-20) and anti-GLUT4 (C-20) from Santa Cruz Biotechnology (Santa Cruz, CA). Murine-derived 3T3-L1 fibroblasts and CHO cells were purchased from American Type Culture Collection (Rockville, MD). 2-Deoxy-[^{3}H]-D-deoxyglucose was from Amersham Pharmacia Biotech (Piscataway, NJ). Anti-rabbit immunoglobulin G conjugated with horseradish peroxidase was obtained from Sigma (St Louis, MO). Berberine was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China).

2.2. Cell culture and differentiation

3T3-L1 preadipocytes were grown and passaged in DMEM containing 25 mmol/L glucose plus 10% fetal bovine serum (FBS). For adipocyte differentiation, 2-day postconfluent cells were placed in 10% FBS-DMEM with 250 nmol/L dexamethasone, 0.5 mmol/L 1-methyl-3-isobutylxanthine, and 1 μg/mL insulin. After 2 days, the medium was changed to 10% FBS-DMEM containing 1 μg/mL insulin alone for 2 more days and was then replaced with 10% FBS-DMEM. Thereafter, the medium was changed every 2 days [9]. Cells were used between days 8 and 12 postdifferentiation and between passages 6 and 12.

2.3. Glucose consumption

The differentiated 3T3-L1 adipocytes that were plated into 96-well plates were preincubated with DMEM containing 0.2% BSA for 12 hours and were then incubated with various concentrations of berberine for 24 hours. The medium was removed and its glucose concentrations were determined by the glucose oxidase method. The amount of glucose consumption was calculated by the glucose concentrations of blank wells subtracting the remaining glucose in the cell-plated wells.

2.4. Methylthiotetrazole method

Methylthiotetrazole was dissolved at a concentration of 5 mg/mL in sterile phosphate-buffered saline (PBS). One volume of 5 mg/mL stock solution of MTT was mixed with 9 vol DMEM. It was added to the 96-well plates when the test of glucose consumption was finished. After 4 hours of incubation at 37°C, the MTT medium was replaced with dimethyl sulfoxide. After it was shaken, the optical densities at 570 nm were measured using a Multiskan MS (Labsystems, Vantaa, Finland) [10].

2.5. Glucose transport assay

3T3-L1 adipocytes and preadipocytes in 24-well plates were preincubated with 0.2% BSA-DMEM for 12 hours and were then incubated with drugs for the indicated time. The cells were rapidly washed 3 times at 37°C with 1.0 mL of Krebs-Ringer phosphate buffer at pH 7.4 (NaCl, 131.2 mmol/L; KCl, 4.7 mmol/L; $CaCl_2$, 2.47 mmol/L; $MgSO_4$, 1.24 mmol/L; $NaPO_4$, 2.48 mmol/L; HEPES, 10 mmol/L). The cells were incubated in the presence or absence of insulin at 37°C for 30 minutes. Glucose transport was initiated by the addition of 2-deoxy-[1-^{3}H]glucose (18.5 kBq/mL) for 10 minutes. Uptake was terminated with 3 rapid washes in 1.0 mL of ice-cold PBS, after which the cells were dissolved in 0.1 N NaOH and were counted by liquid scintillation [11].

2.6. Western blotting

Cells in 6-well plates were washed twice with ice-cold PBS and placed immediately in lysis buffer containing 25 mmol/L HEPES (pH 7.4), 1% Nonidet P-40, 100 mmol/L

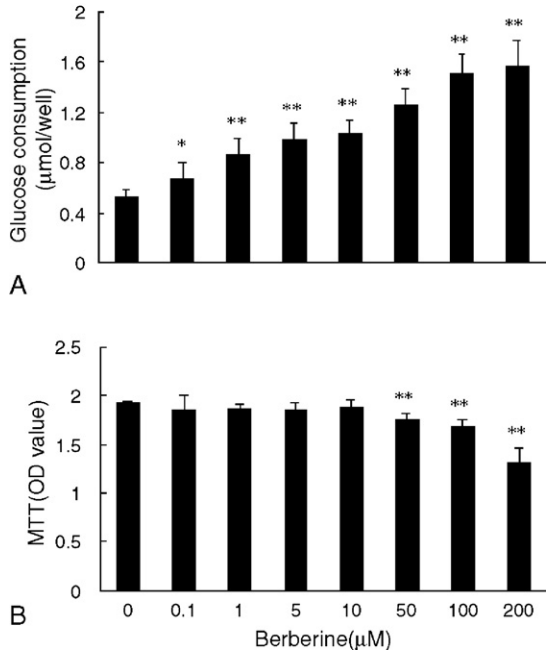

Fig. 1. Dose-dependent effect of berberine on glucose consumption in 3T3-L1 adipocytes. The differentiated 3T3-L1 adipocytes in 96-well plates were preincubated with DMEM containing 0.2% BSA for 12 hours and then incubated with various concentrations of berberine for 24 hours. The amount of glucose that disappeared in the medium was determined as the amount of glucose consumption. The remaining cells were incubated with 0.5 mg/mL MTT for 4 hours to detect the cells' viability. A, Glucose consumption. B, MTT value. Values were mean ± SD (n = 8). *$P < .05$, **$P < .01$ compared with basal.

NaCl, 2% glycerol, 5 mmol/L NaF, 1 mmol/L EDTA, 1 mmol/L Na_3VO_4, 1 mmol/L NaPPi, 1 mmol/L phenylmethylsulfonyl fluoride, 10 μg/mL aprotinin, 5 μg/mL leupeptin, and 5 μg/mL pepstatin. Lysates were gently mixed for 10 minutes at 4°C and then centrifuged at 13 000*g* for 15 minutes at 4°C. The protein concentration of the extracts was determined according to the Bradford method, using BSA as the standard. Protein samples were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. The transferred membranes were blocked, washed, and incubated with various primary antibodies, followed by horseradish peroxidase-conjugated secondary antibody. Visualization was detected with chemiluminescence reagent, using the ECL Western blotting analysis system (Amersham Biosciences, Piscataway, NJ).

2.7. Glucose transporter 4 translocation

On 24-well culture plates, 1×10^5 CHO cells were seeded. On the next day, the cells were transfected with pCDNA3-GLUT4eGFP (a gift from Alan R. Saltiel) using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) at a 3:1 ratio according to the manufacturer's instructions. Twenty-four hours posttransfection, the cells were serum starved for 4 hours and treated with insulin or berberine at various times. Glucose transporter 4 translocation was observed under fluorescence microscopy (Leica, Wetzler, Germany).

2.8. Statistical analysis

Results are expressed as means ± SD. Statistical analyses were performed by unpaired Student *t* test. $P < .05$ was considered statistically significant.

3. Results

3.1. Effect of berberine on glucose consumption in 3T3-L1 adipocytes

Berberine significantly increased basal glucose consumption of 3T3-L1 adipocytes in a dose-dependent manner. In the presence of 0.1 and 200 μmol/L berberine, glucose consumption increased 1.3-fold ($P < .05$) and 3-fold ($P < .01$) compared with the basal level, respectively (Fig. 1A).

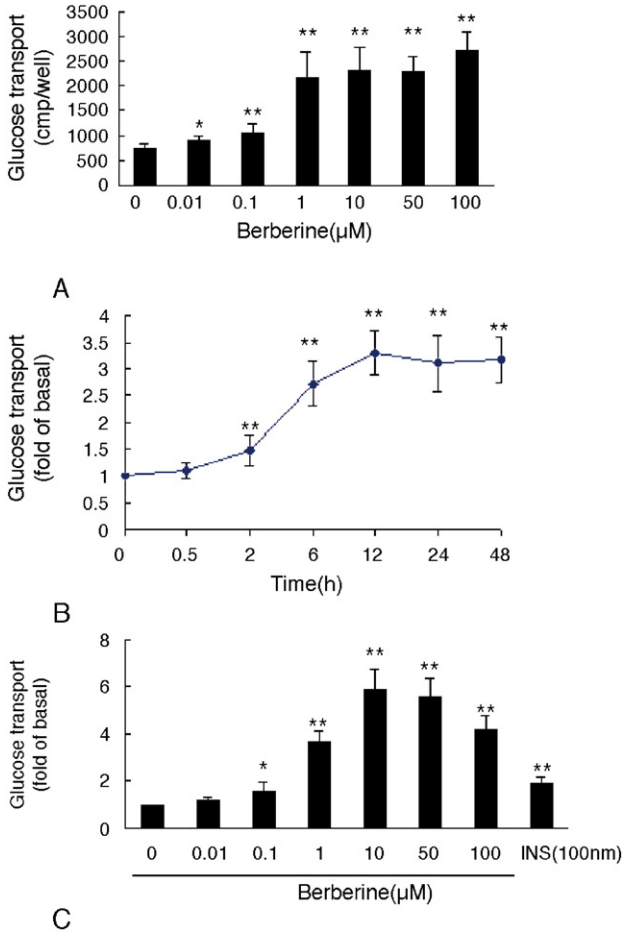

Fig. 2. Berberine-stimulated glucose transport in 3T3-L1 adipocytes and preadipocytes. A, 3T3-L1 adipocytes were treated with the indicated concentrations of berberine for 24 hours and assayed for 2-DOG transport. B, 3T3-L1 adipocytes were treated with 10 μmol/L berberine for the indicated time and assayed for 2-DOG transport. C, Ninety percent confluent preadipocytes were incubated with various concentrations of berberine for 24 hours. Glucose transport was assayed. Values were mean ± SD of 3 separate experiments, *$P < .05$, **$P < .01$ compared with basal.

Cell viability was assayed by the MTT method. The results showed that the optical density significantly decreased at 50 μmol/L berberine (Fig. 1B), suggesting that berberine exerts a toxic effect on 3T3-L1 adipocytes at this concentration or higher.

3.2. Berberine stimulated glucose uptake in 3T3-L1 adipocytes and preadipocytes

Incubation of 3T3-L1 adipocytes with berberine stimulated glucose uptake in a dose- and time-dependent manner. Uptake of 2-deoxy-D-[^{3}H]glucose (2-DOG) increased significantly at 0.01 μmol/L berberine ($P < .05$) and reached the plateau at 1 μmol/L berberine (Fig. 2A). The 2-DOG uptake increased markedly at 2 hours, reached the peak at 12 hours, and remained at that high level until 48 hours after incubation with 10 μmol/L berberine (Fig. 2B), which is different from the characteristics of insulin action. Berberine increased glucose uptake in a dose-dependent manner as in 3T3-L1 adipocyte with the maximal effect at 10 μmol/L (Fig. 2C), whereas 100 nmol/L insulin only stimulated glucose uptake 1.9-fold in 3T3-L1 preadipocytes. As shown in Fig. 3, 1 and 100 nmol/L insulin stimulated glucose uptake 2.4- and 3.7-fold, respectively. The effect of insulin on glucose uptake reached the peak level at 100 nmol/L (data not shown), as previously reported [12]. The effect of 1 nmol/L insulin is half of the maximum. At 10 μmol/L, berberine-stimulated glucose transport approached the maximum as well. However, 2-DOG uptake increased 6-fold in the presence of 100 nmol/L insulin in combination with 10 μmol/L berberine, which was markedly higher than that in the presence of 10 μmol/L berberine alone (3.6-fold). Glucose uptake increased 3.7-fold in the presence of 1 nmol/L insulin in combination with 0.1 μmol/L berberine.

3.3. Effect of various inhibitors of insulin signaling pathway on berberine-stimulated glucose uptake

The activation of phosphatidylinositol 3-kinase (PI3K) is necessary for insulin-stimulated glucose transport. To clarify whether berberine-stimulated glucose uptake is mediated through PI3K activation, we examined the effects of wortmannin, a selective inhibitor of PI3K, on berberine-stimulated glucose uptake. We confirmed that insulin-stimulated glucose uptake was completely abrogated to the basal level by 100 nmol/L wortmannin in 3T3-L1 adipocytes. However, berberine-stimulated glucose uptake was not decreased by wortmannin (Fig. 4A). These results suggest that the signal transduction leading to glucose uptake by berberine is possibly mediated via a PI3K-independent pathway.

PD98059 is an inhibitor of ERK kinase, and its effect is uncertain on insulin-stimulated glucose uptake [13,14]. In

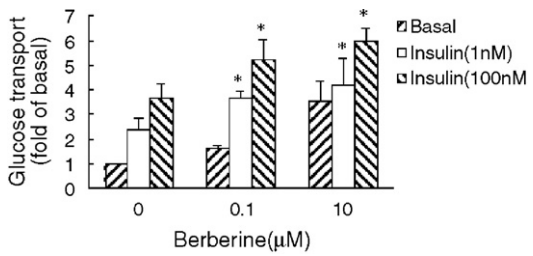

Fig. 3. Berberine enhanced insulin-stimulated glucose transport. The cells were incubated in the presence or absence of insulin for 30 minutes after 3T3-L1 adipocytes were treated with 0.1 or 10 μmol/L berberine for 24 hours. Glucose transport was assayed. Values were mean ± SD of at least 3 separate experiments. *$P < .01$ compared with the same concentration of insulin alone.

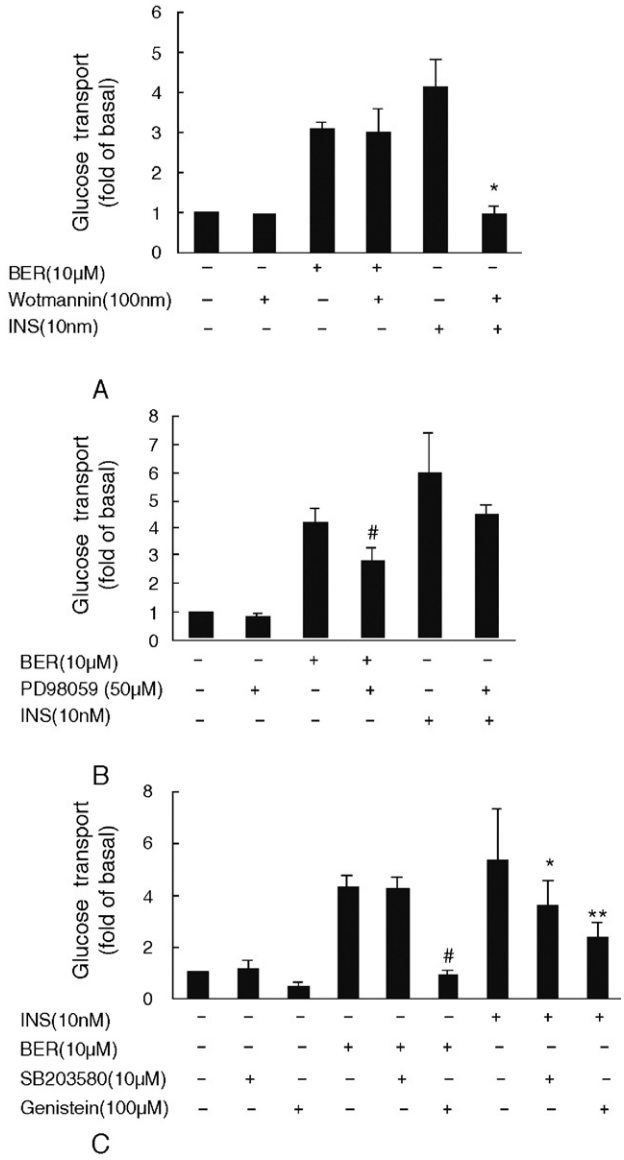

Fig. 4. Effect of pharmacological inhibitors on berberine-induced glucose transport. After 3T3-L1 adipocytes were incubated in the presence of 100 nmol/L wortmannin, 50 μmol/L PD98059, 10 μmol/L SB203580, or 100 μmol/L genistein for 30 minutes, 10 μmol/L berberine was added and incubation was continued for another 12 hours or with 10 nmol/L insulin for another 30 minutes. Glucose transport was measured. Values were mean ± SD of at least 3 separate experiments. A, Wortmannin. B, PD98059. C, SB203580 and genistein. *$P < .05$, **$P < .01$ compared with insulin alone. #$P < .05$ compared with berberine alone.

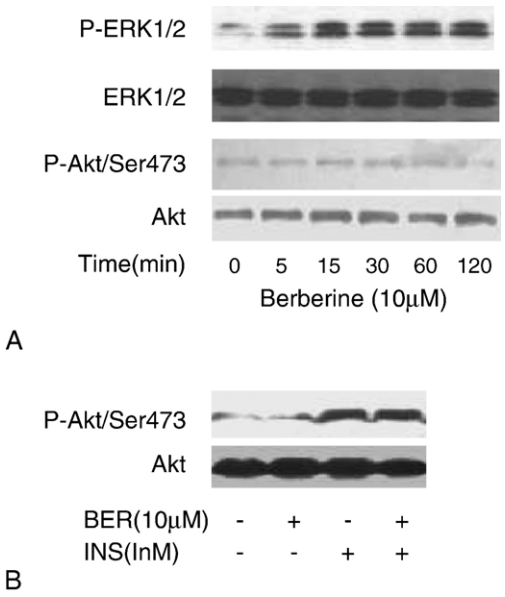

Fig. 5. Berberine activated ERK1/2 but not Akt in 3T3-L1 adipocytes. A, 3T3-L1 adipocytes were treated with 10 μmol/L berberine for the indicated time. The cell lysates were analyzed by Western blot using the primary antibodies as described above. B, 3T3-L1 adipocytes were treated with 10 μmol/L berberine for 24 hours, and then incubated with 1 nmol/L insulin for 10 minutes. The cell lysates were analyzed by Western blot for the phosphorylation of Akt on Ser473. The figure shown is 1of 3 independent experiments. All 3 experiments showed similar results.

the present study, PD98059 decreased insulin- and berberine-stimulated glucose uptake by 25% and 32%, respectively (Fig. 4B). SB203580 is a pharmacological inhibitor of the mitogen-activated protein kinase (MAPK) family member p38 and has been shown to inhibit insulin-induced glucose uptake in 3T3-L1 adipocytes [15,16]. As illustrated in Fig. 4C, SB203580 decreased insulin-stimulated glucose uptake by 32%, but did not reduce berberine-stimulated glucose uptake. Genistein inhibits glucose transport through GLUT1 and GLUT4 [17,18]. We treated 3T3-L1 adipocytes with 100 μmol/L genistein for 30 minutes and then with insulin for 30 minutes or with berberine for 12 hours in the continued presence of genistein. As shown in Fig. 4C, genistein reduced insulin-stimulated glucose uptake by 55%. Berberine-stimulated glucose uptake was completely blocked by genistein. The same result was obtained when genistein was added in 3T3-L1 adipocytes as well as in 3T3-L1 preadipocytes at the last hour of the 12-hour incubation with berberine (data not shown).

3.4. Berberine induced phosphorylation of ERK1/2 but not of Akt

Berberine was recently reported to increase low-density lipoprotein receptor via activating p44/p42 ERK1/2 in HepG2 cells [19]. We investigated whether berberine also activated ERK1/2 in 3T3-L1 adipocytes. As shown in Fig. 5A, the phosphorylated ERK1/2 significantly increased after 3T3-L1 adipocytes were incubated with 10 μmol/L berberine at different intervals. The activation of Akt is necessary for insulin to stimulate glucose uptake. In accord with the results of glucose transport, the activity of Akt did not increase after treatment with berberine at different intervals (Fig. 5A). Exposure of 3T3-L1 adipoctyes to berberine for 24 hours did not enhance insulin-stimulated phosphorylation of Akt on Ser473 (Fig. 5B).

3.5. Berberine had no effect on the expression and GLUT1 and GLUT4 and the cellular localization of GLUT4

Berberine had no effect on total cellular levels of GLUT1 and GLUT4, as assessed by Western blotting (Fig. 6A). Insulin promotes glucose uptake in muscle and fat tissue through the translocation of GLUT4 to the plasma membrane. CHO cells were transfected with pCDNA3-GLUT4eGFP to assay whether berberine stimulated GLUT4 translocation. After the encoded proteins were allowed to express for 24 hours, the cells were starved in serum-free medium for 4 hours and were stimulated with insulin or berberine. After 20 minutes of insulin stimulation, the eGFP-tagged GLUT4 was seen at the plasma membrane as a clear ring with a concomitant decrease in the cytoplasm under fluorescence microscopy. After the CHO cells transfected with GLUT4eGFP were incubated with 10 μmol/L berberine for 2, 6, 12 and 24 hours, the eGFP-tagged GLUT4 was predominantly sequestered intracellularly in punctuate structures throughout the cell (Fig. 6B only showed 12-hour incubation), suggesting that berberine did not significantly stimulate GLUT4 translocation.

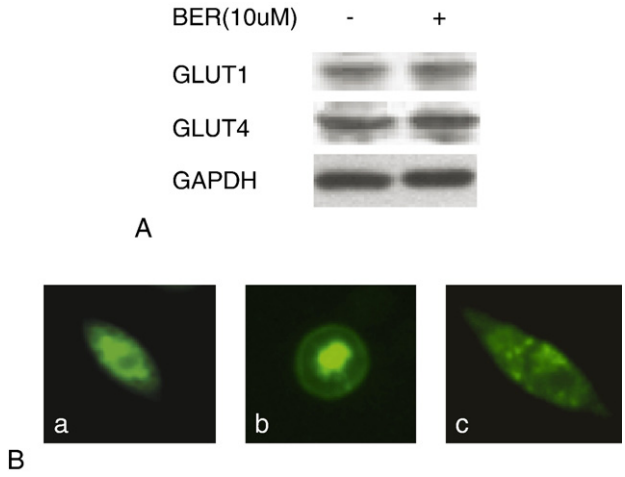

Fig. 6. Berberine did not stimulate GLUT1 and GLUT4 expression and GLUT4 translocation. A, 3T3-L1 adipocytes were incubated with 10 μmol/L berberine for 24 hours. Total cellular level of GLUT1 and GLUT4 was detected by Western blot. B, CHO cells were transfected with pCDNA30-GLUT4eGFP using Lipofectamine 2000. Twenty-four hours posttransfection, the cells were serum starved for 4 hours and treated with insulin for 20 minutes or with berberine for 12 hours. The cellular localization of GLUT4eGFP was observed under fluorescence microscopy. a, Without treatment. b, Insulin treatment. c, Berberine treatment. The figure shown is 1 of 3 independent experiments. All 3 experiments showed similar results.

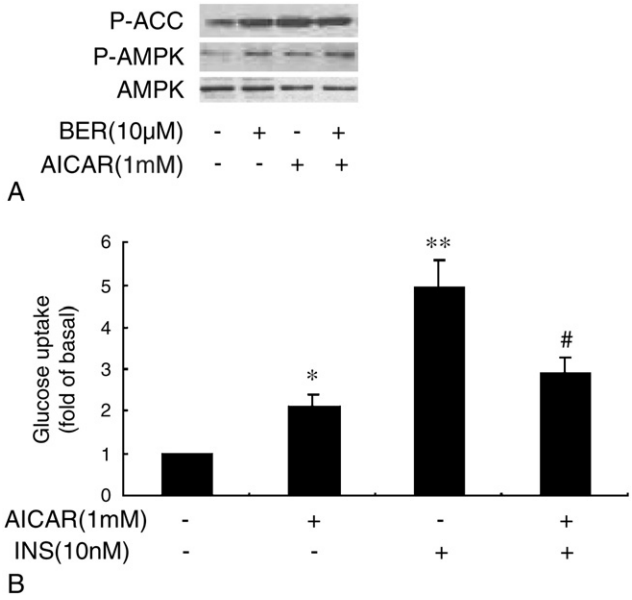

Fig. 7. Berberine stimulated AMPK activity. A, 3T3-L1 adipocytes were incubated in the presence of 10 μmol/L berberine or 1 mmol/L AICAR for 2 hours. The phosphorylation of ACC and AMPK was detected by Western blot. B, The cells were incubated in the presence or absence of 10 nmol/L insulin for 30 minutes after 3T3-L1 adipocytes were treated with 1 mmol/L AICAR for 12 hours. Glucose transport was assayed. *$P < .05$, **$P < .01$ compared with basal. $^{\#}P < .05$ compared with insulin alone.

3.6. Berberine activated AMPK

Adenosine monophosphate–activated protein kinase is emerging as a metabolic master switch. Changes in AMPK activity have been shown to regulate glucose transport in muscle and adipocytes. Acetyl-coenzyme A carboxylase is phosphorylated by AMPK and is inactivated [20]. Therefore, we detected the phosphorylation of AMPK and ACC to determine the activity of AMPK in 3T3-L1 adipocytes. The phosphorylation of AMPK and ACC significantly increased after incubation with 10 μmol/L berberine for 2 hours as AICAR, an AMPK activator. However, berberine did not enhance AICAR-stimulated AMPK and ACC phosphorylation (Fig. 7A). In 3T3-L1 adipocytes, we also found that AICAR increased basal glucose uptake, but inhibited insulin-stimulated glucose uptake (Fig. 7B), as previously reported [21].

4. Discussion

Our previous study showed that berberine did not stimulate insulin secretion in βTC3 cell lines, but it increased glucose consumption in HepG2 cells [22]. Therefore, in the present study, we characterized the effect of berberine on glucose metabolism in 3T3-L1 adipocyte, an ideal cell model for the study of hypoglycemic agents. It was shown that berberine increased glucose consumption of 3T3-L1 adipocytes in a dose-dependent manner, suggesting that glucose uptake was stimulated. As expected, berberine induced a dose- and time-dependent increase of glucose uptake in the absence of insulin. However, our result is different from that of Ko et al [6], in which berberine did not stimulate basal glucose uptake and only enhanced insulin-stimulated glucose uptake. In our study, however, 10 μmol/L berberine increased glucose uptake to a similar extent as 100 nmol/L insulin did (Fig. 3). Previously it was reported that insulin rapidly stimulated glucose uptake in 3T3-L1 cells [23], whereas with berberine, the onset of the increase in glucose transport was slow at the start with at least a 30-minute lag (Fig. 3). Glucose uptake significantly increased within 2 hours and reached the plateau 12 hours after berberine incubation. Therefore, this feature of action between insulin and berberine is obvious. Furthermore, we compared the effect of insulin and berberine on glucose uptake in 3T3-L1 preadipocytes, which are relatively insensitive to insulin compared with 3T3-L1 adipocytes. Berberine increased glucose uptake in 3T3-L1 preadipocytes as in 3T3-L1 adipocytes, suggesting that berberine stimulates glucose uptake through a mechanism distinct from insulin. This was further supported by the fact that berberine- and insulin-stimulated glucose uptake was additive in 3T3-L1 adipocytes. Although 100 nmol/L insulin-stimulated glucose uptake has already reached the peak level, berberine could exert an additive effect.

PI3K/Akt is required for insulin-induced glucose uptake into muscle and fat [24]. Akt was not activated by berberine as berberine-stimulated glucose was insensitive to wortmannin, suggesting that berberine induces glucose uptake via a PI3K-independent pathway. Recently, it was shown that MAPK pathway also played a role in insulin-stimulated glucose uptake. In our study, PD98059, an ERK kinase inhibitor, and SB203580, a p38 MAPK inhibitor, partially inhibited insulin-stimulated glucose uptake, as previous studies showed [14,15]. Berberine was reported to alleviate cardiovascular disease by decreasing levels of low-density lipoprotein receptor via activating ERK1/2 [19]. In the present study, berberine also activated ERK1/2 in 3T3-L1 adipocytes, but PD98059 only decreased berberine-stimulated glucose uptake by 32%, and SB203580 had no effect.

3T3-L1 fibroblasts predominantly express insulin-insensitive GLUT1, whereas 3T3-L1 adipocytes express more of the insulin-responsive GLUT4. Basal glucose transport is mainly mediated through GLUT1. The effector of the insulin-induced glucose transport in adipocytes is GLUT4. The phosphorylation of Akt on Ser473 and Thr308 sites by insulin led to an increase in GLUT4 translocation [25]. As Akt was not activated by berberine in the absence of insulin, berberine did not stimulate GLUT4 translocation into the plasma membrane. Berberine-stimulated glucose uptake began to increase after 2 hours of incubation, suggesting that some gene expressions were up-regulated for the action. However, total cellular GLUT1 and GLUT4 protein expression was not increased in the treatment of berberine for 24 hours.

The naturally occurring isoflavone derivative genistein is commonly applied as a tyrosine kinase inhibitor. However, recent study showed that genistein acted as a direct inhibitor of insulin-induced glucose uptake in 3T3-L1 adipocyte without effect on insulin-induced tyrosine kinase activity of the insulin receptor or activation of Akt [18]. Previous studies showed that genistein acted at the GLUT1 adenosine triphosphate–binding domain and inhibited glucose uptake [17]. In our study, berberine-stimulated glucose uptake was completely blocked by genistein in both 3T3-L1 adipocytes and 3T3-L1 preadipocytes, suggesting that berberine induces glucose transport through increasing GLUT1 activity, which needs further verification.

It has been shown that stress-inducing stimuli such as shock, H_2O_2, and arsenite increase GLUT4-mediated glucose uptake in adipocytes via PI3K-independent pathway, whereas reducing insulin-stimulated glucose transport correlated with the inhibition of insulin signaling [26-28]. Berberine augmented basal glucose uptake independent of GLUT4 and enhanced insulin-stimulated glucose uptake without altering insulin signaling, suggesting that the underlying mechanism of berberine-stimulated glucose uptake seems completely different from that of the stress-inducing stimuli.

Adenosine monophosphate–activated protein kinase is a phylogenetically conserved intracellular energy sensor that has been implicated in the regulation of glucose and lipid homeostasis [29]. Hence, AMPK is emerging as a potentially interesting drug target for the treatment of diabetes [20]. It has been shown that metformin and rosiglitazone increased insulin sensitivity by activating AMPK [30]. Activated AMPK stimulated glucose uptake in muscle cells as well as in adipocytes, which are independent from the insulin signaling pathway [21]. Therefore, we studied the possibility that berberine could activate AMPK. It is currently accepted that the ACC phosphorylation levels in the cells represent a marker of AMPK activity [31]. In our study, berberine induced AMPK and ACC phosphorylation in 3T3-L1 adipocytes, as recently reported [32]. Berberine did not further enhance AICAR-stimulated AMPK activity. We also compared the effect of berberine and AICAR on glucose uptake in 3T3-L1 adipocytes. 5-Aminoimidazole-4-carboxamide riboside increased basal glucose uptake and inhibited insulin-stimulated glucose uptake, which is a feature not similar to that of berberine. However, AICAR is not a specific activator of AMPK, and it promotes insulin-stimulated glucose uptake in muscle. Overexpression of the dominant negative AMPKα2 mutant had no effect on AICAR-induced glucose transport in 3T3-L1 adipocytes, although AMPK activation was almost completely abolished. By contrast, overexpression of the dominant negative AMPKα2 mutant in muscle markedly suppressed both AICAR-induced glucose uptake and AMPK activation [33]. Therefore, more studies are needed to confirm whether berberine regulates glucose and lipid metabolism via activating AMPK as metformin [30].

Obesity is a major risk factor for metabolic syndrome and type 2 diabetes mellitus. However, most antidiabetic drugs that are hypoglycemic also promote weight gain, alleviating one symptom of type 2 diabetes mellitus while aggravating a major risk factor that leads to type 2 diabetes mellitus. Adipogenesis, the differentiation and proliferation of adipocytes, is a major mechanism leading to weight gain and obesity [34]. It is highly desirable to develop pharmaceuticals and treatments for type 2 diabetes mellitus that reduce blood glucose levels without inducing adipogenesis in patients. Previously, we reported that berberine inhibited the differentiation of 3T3-L1 preadipocytes [35], which was confirmed by Ko et al [6] and Huang et al [36]. In addition, berberine has been shown to have a hypolipidemic action by a mechanism different from that of statins [19]. The present study demonstrates that berberine stimulates glucose uptake through a mechanism distinct from insulin and enhances insulin-stimulated glucose uptake. Therefore, if proved clinically, berberine may be considered as a promising drug for the prevention and treatment of metabolic syndrome and type 2 diabetes mellitus.

Acknowledgments

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