Berberine attenuates cAMP-induced lipolysis via reducing the inhibition of phosphodiesterase in 3T3-L1 adipocytes

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Abstract

Berberine, a hypoglycemic agent, has been shown to decrease plasma free fatty acids (FFAs) level in insulin-resistant rats. In the present study, we explored the mechanism responsible for the antilipolytic effect of berberine in 3T3-L1 adipocytes. It was shown that berberine attenuated lipolysis induced by catecholamines, cAMP-raising agents, and a hydrolyzable cAMP analog, but not by tumor necrosis factor α and a nonhydrolyzable cAMP analog. Unlike insulin, the inhibitory effect of berberine on lipolysis in response to isoproterenol was not abrogated by wortmannin, an inhibitor of phosphatidylinositol 3-kinase (PI3K). Stimulation of adipocytes with berberine increased phosphodiesterase (PDE) 3B activity, measured by hydrolysis of [3H]cAMP. These results suggest that berberine exerts an antilipolytic effect mainly by reducing the inhibition of PDE, leading to a decrease in cAMP and hormone-sensitive lipase (HSL) phosphorylation independent of AMPK pathway.

1. Introduction

Type 2 diabetes is characterized by insulin resistance and impaired insulin secretion [1]. Elevated plasma levels of free fatty acids (FFAs) are thought to play a major role in the pathogenesis of insulin resistance and type 2 diabetes by increasing glucose uptake and utilization by muscle and causing increased glucose output by the liver [2–5]. In the pancreas, lipid accumulation in β cells may impair insulin secretion [6]. The increases in plasma FFA levels are the result of increased mobilization from adipose tissue. Hormone-sensitive lipase (HSL), thought to be the rate-limiting enzyme in adipose tissue lipolysis, hydrolyzes the stored triglycerides into monoglycerides and FFAs [7].

Cyclic AMP is an important second messenger in the signaling pathways that mobilize fat stores [8]. Catecholamines stimulate adipocyte lipolysis by binding to β-adrenoceptors, which activate adenyl cyclase via the stimulatory guanine nucleotide binding protein (Gs), leading to an increase in intracellular cAMP. cAMP activates the protein kinase A (PKA). PKA then phosphorylates both perilipins and HSL. The phosphorylation of HSL is associated with an increase in hydrolytic activity of the enzyme and the translocation of HSL from the cytosol to the lipid droplet [9]. In contrast, insulin acutely inhibits lipolysis via inhibition of the above cAMP-dependent pathway by PKB-dependent phosphorylation and activation of phosphodiesterase 3B (PDE3B), which in turn lowers cAMP levels [10].

Berberine, one of the major constituents of Chinese herb Rhizoma coptidis, is an isoquinoline alkaloid. Clinical trials and animal experiments reported that berberine improved insulin resistance and lowered...
2. Materials and methods

2.1. Materials

Dulbecco’s modified Eagle’s 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). Human insulin (Humulin R) was from Eli Lilly S.A. S. (Fegersheim, France). 8-Bromo-cAMP, dibutyryl cAMP, cAMP, forskolin, isoproterenol, noradrenaline, 3-isobutyl-1-methylxanthine (IBMX), 5-aminooimidazole-4-carboxamide riboside (AICAR), PD98059, wortmannin, H89, TNF-α, cilostamide, rolipram, fatty acid-free bovine serum albumin (BSA), anti-perilipin A, GPO-Trinder glycerol assay reagent, N-Ter, serum albumin (BSA), anti-perilipin A, GPO-Trinder glycerol assay reagent, N-Ter™ Nanoparticle siRNA transfection reagent, and direct cAMP enzyme immunoassay kit were purchased from Sigma (St Louis, MO, USA). anti-extracellular signal-regulated kinase 1/2 (ERK 1/2), anti-phospho-ERK1/2, anti-AKB, anti-phospho-AKB (Thr172), anti-ace-
yl-coenzyme A carboxylase (ACC), anti-phospho-Ser-79 ACC, anti-ACC, anti-phospho-HSL (Ser-563), anti-phospho-HSL (Ser-565), anti-phospho-HSL (Ser-660), anti-rabbit IgG conjugated with horseradish peroxidase were from Cell Signaling Technology (Beverly, MA, USA). Compound C was purchased from Calbiochem (San Diego, CA). [3H] cAMP was from PerkinElmer Life Sciences (Boston, MA, USA). Murine-derived 3T3-L1 fibroblasts were purchased from American Type Culture Collection (Rockville, MD). 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 mM glucose plus 10% fetal bovine serum (FBS). For adipocyte differentiation, 2-day postconfluence cells were placed in 10% FBS-DMEM with 250 nM dexamethasone, 0.5 mM IBMX, and 1 µg/ml insulin. After 2 days, the medium was changed to 10% FBS-DMEM containing 1 µg/ml insulin alone for 2 additional days and was replaced with 10% FBS-DMEM. Thereafter, the medium was changed every 2 days [18]. Cells were used between days 8 and 10 postdifferentiation and between passages 6 and 12.

2.3. Measurement of glycerol release

The differentiated 3T3-L1 adipocytes in 96-well plates were preincubated with serum-free DMEM containing 0.2% BSA for 12 h before lipolysis experiments. Cells were subjected to the treatments described in each figure legend in phenol red-free DMEM containing 2% fatty acid-free BSA. Glycerol content in the incubation medium was used as an index for lipolysis and was measured using a colorimetric assay (GPO-Trinder, Sigma, St. Louis, MO, USA). Briefly, supernatant was mixed with GPO-Trinder reagent A, and optical density was measured at 540 nm [19].

2.4. cAMP measurement

To measure cAMP levels, the differentiated 3T3-L1 adipocytes in 24-cell plates were incubated in serum-free DMEM containing 0.2% BSA for 12 h and subjected to 10 µM berberine for 1 h followed by 1 µM isoproterenol, 10 µM forskolin, or 0.5 mM IBMX stimulation for another 1 h. The cells were immediately lysed, and the cellular cAMP levels were measured using an enzyme immunoassay kit (Sigma, St Louis, MO, USA).

2.5. Assay of PDE activity

PDE activity was measured as described previously [20]: 100 µl assay volume contained 50 mmol/l Tris-HCl buffer, pH 7.8, 10 mmol/l MgCl₂, 2 µmol/l cAMP, and [³H]cAMP (40,000 cpm/assay). Reactions were started by addition of 25 µl cell lysate and incubated at 24 °C for 30 min and stopped by addition of 0.2 ml of 0.2 mol/l ZnSO₄ and 0.2 ml of 0.2 mol/l Ba(OH)₂. The samples were vortexed and centrifuged at 10,000 × g for 3 min. The labeled product of the reaction [³H]-cAMP was precipitated with BaSO₄, and the unreacted [³H]-cAMP remained in the supernatant. Radioactivity in the supernatant was determined by liquid scintillation counter. The PDE activity was determined as the amount of cAMP hydrolyzed during the reaction time.

2.6. Western blotting

Cells in six-well plates were washed twice with ice-cold PBS and placed immediately in lysis buffer containing 25 mM HEPES (pH 7.4), 1% Nonidet P-40, 100 mM NaCl, 2% glycercol, 5 mM NaF, 1 mM EDTA, 1 mM Na₃VO₄, 1 mM NaPPI, 1 mM phenylmethylsulfonyl fluoride (PMSF), 10 µg/ml aprotinin, 5 µg/ml leupeptin, and 5 µg/ml pepstatin. Lysates were gently mixed for 10 min at 4 °C and then centrifuged at 13,000 × g for 15 min at 4 °C. The protein concentration of the extracts was determined according to the method of Bradford, using BSA as the standard. Protein samples were separated by 10% SDS-PAGE 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).

2.7. Knockdown of AMPKα by RNA interference

To reduce levels of endogenous AMPKα, 3T3-L1 adipocytes were transfected with a pool of three siRNAs for AMPKα (sc-45313; Santa Cruz Biotechnology) using N-Ter™ Nanoparticle siRNA transfection reagent and incubated for 72 h. The specific interference of AMPK protein expression was confirmed by Western blot.

2.8. Statistics

Data are presented as means ± SEM. Significance between groups was determined using an unpaired two-tailed Student’s t-test or one-way ANOVA when appropriate. Significance was established at P < 0.05.

3. Results

3.1. Berberine attenuated lipolysis stimulated by catecholamines, but not by TNF-α

Fully differentiated 3T3-L1 adipocytes were preincubated with various concentrations of berberine for 1 h followed by the incubation.
with 1 μM isoproterenol for further 1 h, and glycerol release was
determined as a measure of the rate of lipolysis. As shown in
Fig. 1A, berberine decreased lipolysis induced by isoproterenol in 3T3-L1
adipocytes with the maximal effect at the concentration of 10 μM. A
time course study depicted in Fig. 1B showed that berberine exerted a
similar magnitude of antilipolytic effect with 31–39% decrease in
glycerol release after 1-, 6-, and 24-h preincubation. 3T3-L1 adipocytes
incubated with 1 μM noradrenaline for 1 h showed a 616% increase in
glycerol release, which was suppressed 48% by 10 μM berberine
(Fig. 1C). In the basal state, treatment with 1–100 μM berberine for
24 h did not affect glycerol release significantly (data not shown). To
determine whether berberine also exerts an antilipolytic effect on TNF-
α-stimulated lipolysis, 3T3-L1 adipocytes were incubated in the
presence of 10 ng/ml TNF-α in combination with 10 μM berberine.
After treatment with TNF-α for 6, 12, and 24 h, glycerol release
increased by 88%, 121%, and 70%, respectively. However, berberine had
no effect on lipolysis in response to TNF-α (data not shown).

3.2. Berberine attenuates lipolysis stimulated by forskolin, IBMX, and
cAMP

Catecholamines stimulate lipolysis through increasing intracellular
cAMP and activating HSL [9]. To determine whether berberine exerts the
same effect on other cAMP-raising agents–stimulated lipolysis, 3T3-L1
adipocytes were preincubated with 10 μM berberine for 1 h and then
stimulated by 10 μM forskolin, an activator of adenyl cyclase, and
0.5 mM IBMX, a nonselective PDE inhibitor, for another 1 h. The results
showed that forskolin– and IBMX-stimulated lipolysis were decreased by
46% and 29%, respectively (P < 0.01). Furthermore, pretreatment with
berberine also resulted in a 56% decrease in lipolysis induced by S-
bromo-cAMP, a hydrolyzable cAMP analog (P < 0.01, Fig. 2A). However,
berberine did not decrease dibutyryl cAMP, a nonhydrolyzable cAMP
analog–stimulated lipolysis, which was inhibited by H89, a PKA inhibitor
(P < 0.01, Fig. 2B).

3.3. Effect of various kinases inhibitors on berberine–inhibited lipolysis

It has been shown that components regulating adipocyte lipolysis
are cytoplasmic targets of mitogen-activated protein kinase (MAPK)
and that a portion of β-adrenergic-stimulated lipolysis in 3T3-L1
adipocytes is mediated via ERK [21]. As expected, PD98059, an ERK
kinase inhibitor, inhibited ERK phosphorylation (Fig. 3A). Fig. 3B
showed that PD98059 and berberine decreased lipolysis by 54% and
30%, respectively. Combined treatment with PD98059 and berberine
completely abolished isoproterenol–stimulated lipolysis, suggesting
that both agents act synergistically via different mechanisms. The
lipolysis in response to isoproterenol combined with IBMX was
markedly suppressed by berberine plus PD98059, not by berberine
alone (Fig. 3B). Many studies indicate that insulin activates phosphoi-
nositide 3-kinase (PI3K). Wortmannin, a selective and potent inhibitor
of this enzyme, antagonizes all of the metabolic effects of the hormone,
including inhibition of lipolysis in rat adipocytes [22]. Our previous
studies showed that berberine stimulated glucose uptake independent
of PI3K signaling pathway [23]. To further investigate whether berberine
inhibits lipolysis via a mechanism distinct from insulin, we examined
the effect of wortmannin on berberine–inhibited lipolysis. As reported
previously [24], 1 μM wortmannin significantly reversed the antilipo-
ytic effect of insulin in the presence of isoproterenol but had no effect on
berberine–inhibited lipolysis (Fig. 3C).

Recent study showed that AMPK activation inhibited lipolysis [25]. It
has been shown that berberine stimulated AMPK activity in 3T3-L1
adipocytes, as well as in other cells [14,16,26]. To clarify whether AMPK
activation is involved in berberine–inhibited lipolysis, 10, 20, and 50 μM
compound C, an AMPK inhibitor, was preincubated 30 min before 10 μM
berberine was added. As shown in Fig. 3D and E, compound C did not
reverse berberine–inhibited lipolysis in response to isoproterenol.

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reverse berberine–inhibited lipolysis in response to isoproterenol.
3.4. Berberine decreased intracellular cAMP production

To test whether berberine mediates its antilipolytic effect via lowering cAMP production, we detected intracellular cAMP levels. After preincubation with 10 μM berberine for 1 h, 3T3-L1 adipocytes were stimulated with 10 μM isoproterenol, 10 μM forskolin, and 0.5 mM IBMX for another 1 h. The results showed that no significant change in intracellular cAMP was detected after stimulation of 3T3-L1 adipocytes with berberine in the basal state. Isoproterenol, forskolin, and IBMX treatment strongly elevated cAMP levels. Berberine markedly decreased isoproterenol-, forskolin-, and IBMX-potentiated cAMP production by 39%, 43%, and 23%, respectively (Fig. 4A).

3.5. Effect of berberine on PDE activity

As the aforementioned results suggested that berberine decreased lipolysis via hydrolyzing cAMP, we further detected whether berberine increased PDE activity. In basal state, PDE activity was not changed by berberine; however, berberine increased IBMX, cilostamide, and rolipram-inhibited PDE activity by 48%, 26%, and 41%, respectively (Fig. 4B).

3.6. Effect of berberine on HSL and AMPK phosphorylation

The activity of HSL is regulated by both phosphorylation and translocation to the lipid droplet. PKA phosphorylates HSL at Ser-563, Ser-659, and Ser-660 [27]. AMPK activation stimulates the phosphorylation of HSL at Ser-563 [28]. We detected the HSL phosphorylation at Ser-563, Ser-659, and Ser-660. Berberine at the concentrations of 0.1–100 μM completely inhibited HSL Ser-563 phosphorylation mediated by isoproterenol. However, HSL Ser-659 phosphorylation was completely inhibited by berberine only at the concentration of 10 μM or higher (Fig. 5A). Consistent with this result, 10 μM berberine also dramatically inhibited HSL Ser-563 and Ser-660 phosphorylation induced by noradrenaline, forskolin, and IBMX, as well as by 8-bromo-cAMP (Fig. 5B and C). Unlike Ser-563 and Ser-660, HSL Ser-565 was phosphorylated to a high degree already in non-stimulated adipocytes. Berberine further enhanced HSL Ser-565 phosphorylation (Fig. 5B).

To evaluate the role of AMPK in the regulation of berberine on lipolysis, we used compound C to detect the effect of berberine on HSL phosphorylation. Compound C did not reverse the regulation of berberine on HSL Ser-563, Ser-659, and Ser-660 phosphorylation in the presence of isoproterenol (Fig. 5D). We then detected the phosphorylation state and activity of the enzyme after exposure of 3T3-L1 adipocytes to berberine for various time points. The increased phosphorylation of AMPK and ACC was detectable 15 min after the addition of berberine and reached the maximum at 30 min (Fig. 6A). Compound C inhibited AICAR-stimulated the phosphorylations of ACC and AMPK, not berberine-stimulated ACC and AMPK phosphorylations (Fig. 6B and C). We further transfected AMPKα siRNA into 3T3-L1 adipocytes using N-TER™ Nanoparticle siRNA transfection reagent. AMPKα protein expression was knocked down at least 50% (Fig. 6D). However, berberine-suppressed lipolysis was not reversed (Fig. 6E). After 3T3-L1 adipocytes were incubated with forskolin and IBMX for 1 h, AMPK was also activated, but two agents and berberine had no additive effect on AMPK phosphorylation (Fig. 6F).

3.7. Effect of berberine on perilipin and ERK1/2 phosphorylation

Perilipin, like HSL, is described to be phosphorylated in response to PKA activation and is involved in the lipolytic reaction of fat cells. To examine whether the antilipolytic effect of berberine is related to phosphorylation of perilipin in differentiated 3T3-L1 adipocytes, we detected perilipin electrophoretic shift after adipocytes were incubated with isoproterenol, forskolin, and berberine. As depicted in Fig. 7, isoproterenol and forskolin induced the characteristic shift in perilipin, whereas such electrophoretic shift was not changed by berberine. However, PD98059 inhibited isoproterenol-stimulated perilipin and ERK1/2 phosphorylation. Interestingly, berberine also suppressed ERK1/2 phosphorylation induced by isoproterenol and forskolin.

4. Discussion

Obesity and type 2 diabetes mellitus are associated with increased levels of circulating FFA, which are thought to induce insulin resistance [29]. We found that berberine treatment significantly decreased plasma FFA in insulin-resistant rats induced by high-fat diet feeding for 20 weeks [16], which, at least in part, contributes to the mechanism underlying berberine increasing insulin sensitivity. The present study showed that berberine directly decreased catecholamines-stimulated lipolysis in 3T3-L1 adipocytes, not basal and TNF-α-stimulated lipolysis. As shown in Fig. 1A, berberine exerted the maximal effect on lipolysis at the concentration of 10 μM, in consistent with its effect on glucose uptake in 3T3-L1 adipocytes [23]. However, the time course study showed that the antilipolytic effect of berberine did not increase with
further extension of incubation, which is different from its effect on glucose uptake [23], suggesting that there exist different mechanisms for the effects of berberine on lipolysis and glucose uptake.

There is much to suggest that catecholamines activate not only PKA but also ERK 1/2. PKA and ERK1/2 work in concert to stimulate lipolysis [20]. As shown in Fig. 4A, isoproterenol-stimulated lipolysis
was partially reversed by PD98059 or berberine alone, but completely abrogated by combined treatment with berberine and PD98059, suggesting that cAMP-PKA signaling pathway is involved in the inhibitory effect of berberine on lipolysis induced by catecholamines. The ability of insulin to antagonize hormone-induced lipolysis is to a large extent accounted for by its ability to lower cAMP levels and therefore PKA activity. The decrease in cAMP is mainly the result of an insulin activation of PDE3B via PKB signaling [30]. As shown in Fig. 3B, wortmannin, a PI3K inhibitor, abrogated insulin-mediated antilipolytic effect. However, wortmannin had no effect on berberine-inhibited lipolysis, in agreement with the results of our previous study that berberine stimulated glucose uptake independent of PI3K pathway [23].

Catecholamines stimulated lipolysis primarily via cAMP-mediated activation of PKA. In addition to catecholamines, lipolysis was also stimulated by a variety of agonists such as forskolin (an activator of adenylyl cyclase), IBMX (a nonselective PDE inhibitor), and 8-bromo-cAMP (a hydrolyzable cAMP analog), which function at different levels of the lipolytic cascade [31,32]. The present study showed that berberine attenuated the three agonists-stimulated lipolysis but had no effect on dibutylryl cAMP (a nonhydrolyzable cAMP analog)-stimulated lipolysis. Furthermore, it was confirmed that berberine decreased the intracellular cAMP production induced by isoproterenol, forskolin, and IBMX. These results suggest that berberine may exert its antilipolytic effect via a step before PKA, activation of PDE.
expected, berberine increased PDE activity in the presence of IBMX, cilostamide (a PDE3B inhibitor), or rolipram (a PDE4 inhibitor).

Acute activation of HSL has been shown to be controlled by phosphorylation/dephosphorylation events, which are regulated by the respective activities of cAMP-dependent PKA and protein phosphatase [33]. The enzyme is subject to complex activity regulation involving multisite phosphorylation. For a long time, Ser-563 was believed to be the only PKA site of HSL, which confers activity control. Using site-directed mutagenesis, it was possible to elucidate that sites Ser-659 and Ser-660 are critical activity-controlling sites in the activation of HSL upon phosphorylation with PKA, whereas Ser-563 plays a minor role in direct activation of HSL. It also showed that the phosphorylation state of Ser-660 is presumably more important in adipocytes at maximal lipolysis [34]. All three PKA sites of HSL are phosphorylated both in vivo in response to stimulation of rat adipocytes by isopreterenol and in vitro upon incubation with PKA. In the present study, 0.1 μM berberine completely abolished isopreterenol-stimulated HSL phosphorylation at Ser-563, not at Ser-660. At this concentration, berberine did not reduce

Fig. 6. Knockdown of AMPKα did not reverse berberine-suppressed lipolysis. (A) 3T3-L1 adipocytes were incubated with 10 μM berberine (BBR) for the indicated time. The phosphorylation of ACC (Ser-79) and AMPK (Thr172) was detected by Western blot. (B, C) 3T3-L1 adipocytes were preincubated with 10, 20, or 50 μM compound C for 30 min followed by incubation with 10 μM BBR and 1 mM AICAR for 1 h. The phosphorylation of ACC (Ser-79) and AMPK (Thr172) was detected by Western blot. (D) 3T3-L1 adipocytes were transfected with AMPKα or control scrambled siRNAs (60 nM) for 72 h. Total AMPKα protein expression was determined by Western blot. (E) 72 h post-transfection, 3T3-L1 adipocytes were preincubated with 10 μM berberine for 1 h, and stimulated with 1 μM isoproterenol (ISO) for another 1 h. The medium was removed for glycerol measurement. (F) 3T3-L1 adipocytes were incubated with 10 μM BBR, 10 μM forskolin (FKL), and 0.5 mM 3-isobutyl-1-methylxanthine (IBMX) for 1 h. The cell lysates were analyzed by Western blot for the phosphorylation of ACC and AMPK. The figure shown is 1 of 3 independent experiments. All 3 experiments showed similar results.
favoring the lipid deposition. When HSL translocates to lipidic drop

The function of perilipins is to prevent lipolysis in basal conditions, which may be due to the antilipolytic effect of berberine. In the present study, forskolin and IBMX activated AMPK, but the two cAMP-phosphorylation sites can account for the antilipolytic effect of berberine to a large extent.

In addition to the three PKA sites, one additional phosphorylation site in HSL, Ser-565, has been shown to be phosphorylated in vitro as well as in isolated primary adipocytes [8]. It has been shown in vitro that prior phosphorylation of HSL by AMPK, which is the kinase proposed to be responsible for phosphorylation of this site, prevents subsequent phosphorylation by PKA and vice versa. When AMPK activity is increased by AICAR, phenformin, or by the expression of a constitutively active form, isoproterenol-induced phosphorylation is strongly reduced [24]. Based on the mutual exclusion between phosphorylation of Ser-565 and the PKA sites, phosphorylation of the AMPK site has been proposed to play an antilipolytic role [8]. As shown in Fig. 6A, AMPK activity was already increased after 3T3-L1 adipocytes were incubated with berberine for 15 min and stayed at high level until 24 h (data not shown). However, compound C, an AMPK inhibitor, did not reverse the antilipolytic effect of berberine, nor the phosphorylations of ACC and AMPK stimulated by berberine. As expected, berberine stimulated HSL Ser-565 phosphorylation. Whereas, compound C did not reverse berberine-suppressed HSL phosphorylation at Ser-660, Ser-563, and Ser-565. Previously, we found that compound C completely reversed troglitazone-suppressed glucose-stimulated insulin secretion (GSIS), which may be responsible for the antilipolytic effect of berberine to a large extent.

It has recently been demonstrated that AMPK is activated by CAMP-raising agents as a consequence of lipolysis in the adipocyte [35,36]. In our previous study, fatty acids themselves can lead to an increased activation of AMPK in isolated rat islets and MIN 6 beta cells [37]. In the present study, forskolin and IBMX activated AMPK, but the two CAMP-raising agents and berberine had no synergic effect on AMPK activity, which may be due to the antilipolytic effect of berberine.

Perilipins belong to a family of hydrophobic lipid droplet-associated phosphoproteins that are phosphorylated by PKA in multiple residues. The function of perilipins is to prevent lipolysis in basal conditions, favoring the lipid deposition. When HSL translocates to lipidic drop surface, perilipins, which act as a barrier to enzyme action, are phosphorylated by PKA and lose their blocking capability [38]. Berberine completely reversed HSL Ser-563 and Ser-660 phosphorylation induced by isoproterenol, but without effect on perilipin phosphorylation. This may be why berberine partially decreased isoproterenol-stimulated lipolysis, not completely. Additionally, we found that berberine inhibited isoproterenol and forskolin-stimulated ERK1/2 phosphorylation. Previous studies have shown that CAMP can activate mitogen-activated protein kinase (MAPK) pathways [39,40]. Furthermore, we found that 8-bromo-cAMP and IBMX-induced ERK1/2 phosphorylation was also decreased by berberine (data not shown). Therefore, berberine-suppressed ERK1/2 phosphorylation stimulated by these agents may be attributed to the decrease of the intracellular CAMP.

In conclusion, berberine attenuates catecholamines-stimulated lipolysis in vitro, which is attributable to the decrease of PDE inhibition, reduction of cAMP production, and inhibition of HSL activation. In our previous study, berberine decreased plasma FFA in insulin-resistant rats [16]. Whether berberine decreased lipolysis in vivo by these mechanisms need to be further investigated.

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References


