Inhibition of the TNF-α–Induced Serine Phosphorylation of IRS-1 at 636/639 by AICAR

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Abstract. AMP-activated protein kinase (AMPK) contributes to the acceleration of insulin signaling. However, the mechanism by which AMPK regulates insulin signaling remains unclear. Serine phosphorylation of insulin receptor substrate (IRS)-1 negatively regulates insulin signaling. Here we investigated the role of AMPK in serine phosphorylation of IRS-1 at 636/639 and 307, which is induced by tumor necrosis factor (TNF)-α in 3T3L1 adipocytes. We demonstrated that the AMPK activator 5-aminoimidazole-4-carboxamide-1-d-ribofuranoside (AICAR) significantly inhibited the TNF-α–induced serine phosphorylation of IRS-1 at 636/639 and 307 by suppression of extracellular signal–regulated kinase (ERK) phosphorylation but not c-Jun-NH2-terminal kinase (JNK) phosphorylation. In addition, AICAR stimulation resulted in enhanced interaction between ERK and MAP kinase phosphatase-4 (DUSP9/MKP-4) without affecting DUSP9/MPK4 mRNA synthesis. Moreover, intraperitoneal administration (0.25 g/kg) of AICAR to db/db mice improved blood glucose levels and inhibited the phosphorylation of ERK in adipose tissue. In conclusion, we propose a new mechanism in which AICAR suppresses TNF-α–induced serine phosphorylation of IRS-1 at 636/639 and 307 by enhancing the interaction between ERK and DUSP9/MKP-4. Taken together, these findings provide evidence that AMPK plays a crucial role in improving type 2 diabetes.

Keywords: 3T3L1 adipocyte, AICAR, insulin receptor substrate (IRS)-1, DUSP9/MKP-4, insulin resistance

Introduction

Insulin resistance has been implicated in the pathophysiology of type 2 diabetes mellitus (1). One mechanism mediating insulin resistance involves phosphorylation of serine residues in insulin receptor substrate-1 (IRS-1) leading to the suppression of tyrosine residues phosphorylation, which is needed to activate signal molecules related to insulin signaling acceleration, including phosphatidylinositol 3-kinase and Akt (2). There are several serine phosphorylation sites on IRS-1 and its phosphorylation is stimulated by cytokines, including tumor necrosis factor (TNF)-α, interleukin (IL)-1, and IL-6 (3–5).

TNF-α, an inflammatory cytokine, is a major risk factor for insulin resistance in obesity and chronic inflammation (6). TNF-α can induce IRS-1 serine phosphorylation at multiple sites through activation of several serine kinases, including c-Jun-NH2-terminal kinase (JNK) and extracellular signal–regulated kinase (ERK) (7). IRS-1 serine phosphorylation sites 307 and 636/639 are located close to the PTB domain of IRS-1 and the major PI3-kinase binding site, respectively. Phosphorylation of these two IRS-1 residues resulted in reduced interaction between IRS-1 and the insulin receptor or PI3-kinase. Therefore, these two phosphorylation sites of IRS-1 play critical roles in inhibiting insulin signaling (8, 9). Although the serine phosphorylation of IRS-1 at 307, which is induced by TNF-α, is well studied in 3T3L1 adipocytes and has been reported to play a critical role in inhibiting insulin signaling, it has not been reported that TNF-α can induce serine phosphorylation
of IRS-1 at 636/639 in 3T3L1 adipocytes.

ERK and JNK, which are members of mitogen-activated protein kinases (MAPKs) are activated by a variety of cytokines (10, 11). ERK and JNK regulate many signal transduction pathways related to cell growth and apoptosis (12, 13). These two MAPKs are negatively regulated by protein phosphatases such as dual-specificity protein phosphatase (DUSP) and protein phosphatase 2A (PP2A) (14 – 16). Therefore, both of these have important roles in modulating many signal transductions.

Adenosine monophosphate–activated protein kinase (AMPK) plays a key role in the regulation of energy homeostasis and monitors cellular energy charge, acting as a “metabolic master switch” for regulating adenosine triphosphate concentrations in the face of stresses that reduce cellular energy levels (17). 5-Aminoimidazole-4-carboxamide 1-d-ribofuranoside (AICAR) is a well-known activator of AMPK (18). It is transported inside the cells through the adenosine transporter and phosphorylated by adenosine kinase to form zeatin riboside-5-monophosphate (ZMP), which mimics the stimulatory actions of AMP on AMPK. Although the activation of AMPK stimulated by AICAR is known to translocate glucose transporter (GLUT) 4 to the plasma membrane through the phosphorylation of Akt substrate of 160 kD (AS160) (19), it is not known whether AMPK regulates IRS-1.

Given our hypothesis that AICAR can act upstream of AS160 to influence insulin signaling, we focused on the role of IRS-1. Therefore, in the present study we investigated whether the AMPK agonist AICAR inhibits the TNF-α–induced serine phosphorylation of IRS-1 at 636/639 and 307 in vitro and in vivo.

Materials and Methods

Drugs and reagents

Mouse recombinant TNF-α, AICAR, aprotinin, leupeptin, phenylmethylsulfonyl fluoride (PMSF), and bovine serum albumin (BSA) were purchased from Sigma (St. Louis, MO, USA). Dulbecco’s modified Eagle’s medium (DMEM), isobutylmethylxanthine (IBMX), Glucose CII-Test Wako, ISOGEN II, and dexamethasone were purchased from Wako Pure Chemical Industries (Osaka). The DC protein assay kit and sodium azide, and 5% nonfat dry milk). The membrane was blocked with blocking buffer (PBS, 0.1% Tween 20, 0.5 mM sodium vanadate, 0.02% sodium azide, and 5% nonfat dry milk). The membranes

MA, USA). Phospho-IRS-1 (Ser 636/639) antibody, IRS-1 antibody, phospho-p42/44 MAPK (Erk1/2) (Thr202/Tyr204) antibody, p42/44 MAPK (Erk1/2) (137F5) rabbit mAb, Phospho-AMPKα (Thr172) (40H9) rabbit mAb, AMPKα antibody, and anti-mouse and anti-rabbit immunoglobulin G secondary antibodies were purchased from Cell Signaling Technology (Beverly, MA, USA). p-JNK, JNK, MKP-4, and normal mouse IgG were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The PrimeScript® II 1st Strand cDNA Synthesis Kit and SYBR® Premix Ex Taq™ II (Tli RNaseH Plus) were purchased from Takara Bio, Inc. (Shiga). The mouse insulin assay kit was purchased from Morinaga Institute of Biological Science, Inc. (Yokohama).

Cell culture

Mouse 3T3L1 fibroblasts were obtained from Health Science Research Resources Bank (Osaka) and were maintained in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% FBS in an atmosphere of 10% CO₂ at 37°C (20). Three days after the fibroblasts had reached confluence, differentiation was induced by treating the cells with DMEM containing 100 μU/mL insulin, 0.5 mM IBMX, and 250 nM dexamethasone and 10% FBS for 3 days. The cells were then maintained in DMEM containing 10% FBS and 100 μU/mL insulin (medium changed every 3 days) until the cells were used for the experimentation (i.e., 12 – 16 days after the induction of differentiation), when > 95% of the cells had the morphological and biochemical properties of adipocytes.

Western blot analyses

3T3-L1 adipocytes grown on 6-well plates were stimulated as described in the results. The cells were then washed 3 times with ice-cold phosphate-buffered saline (PBS) and lysed in a lysis buffer (25 mM Tris, pH 7.5, 150 mM NaCl, 10 mM sodium fluoride, 1 mM sodium vanadate, 1 mM PMSF, 10 μg/mL aprotinin, 10 μg/mL leupeptin, and 1% Nonidet 40) for 30 min at 4°C. The cell lysates were centrifuged to remove insoluble materials. The protein concentrations of the supernatants were measured using the DC protein assay kit. For western blot analyses, whole cell lysates (50 μg proteins per lane) were denatured by boiling in SDS sample buffer (50 mM Tris, 2% SDS, 10% glycerol, 50 mM dithiothreitol, and 0.1% bromophenol blue). Samples were electrophoresed on 7.5% SDS-polyacrylamide gels and transferred to a polyvinylidene difluoride membrane. The membrane was blocked with blocking buffer (PBS, 0.1% Tween 20, 0.5 mM sodium vanadate, 0.02% sodium azide, and 5% nonfat dry milk). The membranes
were then exposed to primary antibodies overnight at 4°C. After incubation with the alkaline phosphatase-linked secondary antibody for 1 h at room temperature, immunoreactive proteins were visualized by the CDP-Star Reagent. The results were quantified by densitometry in the linear range of film exposure with Scion Image software (NIH).

**Immunoprecipitation**

3T3L1 adipocytes grown on 60-mm plates were stimulated as described in the results. The cells were then washed, lysed, and centrifuged, and the protein concentrations were determined as described above. The supernatant (250 μg of protein) was mixed with 1 μg of primary antibody overnight at 4°C and then with protein A-Sepharose for 3.5 h at 4°C. The protein A-Sepharose was collected by centrifugation at 10,000 rpm for 2 min, washed with lysis buffer 3 times, and resuspended in 1 × SDS sample buffer for western blot analyses.

**Real-time reverse transcription–polymerase chain reaction (real-time RT-PCR)**

Total RNA was extracted with ISOGEN II according to the manufacturer’s instructions. Total mRNA was reverse-transcribed to cDNA using the PrimeScript® II 1st Strand cDNA Synthesis Kit according to the manufacturer’s instructions. Real-time PCR was performed using the Fast 7500 Real-Time PCR system (Applied Biosystems, Foster City, CA, USA) with SYBR® Premix Ex Taq™ II (Tli RNaseH Plus). The thermal profiles consisted of 30 s at 95°C for denaturing followed by 40 cycles at 95°C for 3 s; annealing at 60°C for 30 s; and 95°C for 15 s, 60°C for 1 min, 95°C for 15 s, and 60°C for 15 s for melting curve analysis. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was used as the housekeeping gene, and all data are represented as the fold change relative to its expression (using the standard curve methods). The following primers were used: DUSP9/MKP-4 forward primer, 5′-AAGGCT ACCCAGCATACTACCTACA-3′ and reverse primer, 5′-CTGGTTTCACACAGGTGAGGAC-3′; TNF-α forward primer, 5′-CCTCCCTCTCATCAGTTCTA-3′ and reverse primer, 5′-ACTTGGTGTTTTGCTACGAC-3′; GAPDH forward primer, 5′-TGAAGCAGGCATCTGAGTGGGAG-3′ and reverse primer, 5′-CGAAGGTGGAAGAGTGGGAG-3′.

**Animals**

The animal experimental plan was approved by the president of Hokkaido Pharmaceutical University School of Pharmacy (No. 10-006), and conformed to the Guiding Principles for the Care and Use of Experimental Animals in Hokkaido Pharmaceutical University School of Pharmacy. C57BL/6 db/db and db/+ (C57BLKS/J lar+Leprdb/+Leprdb and m+/+Leprdb) mice were obtained from Sankyo Labo Service Corporation, Inc. (Tokyo). The mice were randomly divided into control and AICAR-treated groups at 6 – 7 weeks of age. They were housed in a temperature-controlled room with a 12-h light–dark cycle and given free access to water and normal chow. After starvation for 16 h, mice were intraperitoneally injected with saline or AICAR (250 mg/kg) (21). After 1 h, epididymal adipose tissues were removed from the mice under pentobarbital (100 mg/kg) anesthesia and immediately stored at −80°C until use. For western blot analyses, adipose tissue was homogenized in ice-cold lysis buffer (25 mM Tris pH 7.5, 150 mM NaCl, 10 mM sodium fluoride, 1 mM sodium vanadate, 1 mM PMSF, 10 μg/mL aprotinin, 10 μg/mL leupeptin, and 1% Nonidet 40).

**Measurement of plasma glucose and insulin concentrations**

Blood samples were obtained from the postcaval vein under pentobarbital (100 mg/kg) anesthesia and immediately stored at −20°C for later analysis. Plasma glucose levels were measured using Glucose C II-Test Wako, and plasma insulin levels were measured using a mouse insulin assay kit from Morinaga according to manufacturer’s instructions.

**Statistical analyses**

Data are presented as the mean ± S.E.M. Statistical significance between the 2 groups was evaluated using one-way ANOVA followed by Student’s t-test with StatView (SAS Institute Inc., Cary, NC, USA). P-values less than 0.05 were considered as statistically significant.

**Results**

**TNF-α stimulates the serine phosphorylation of IRS-1 at 636/639 through JNK and ERK**

We first examined whether TNF-α induces the serine phosphorylation of IRS-1 at 636/639 in 3T3L1 adipocytes. In 3T3L1 adipocytes, we observed that TNF-α stimulation led to a transient increase in the serine phosphorylation of IRS-1 at 636/639, which reached a peak at 15 min (Fig. 1). On the other hand, the serine phosphorylation of IRS-1 at 307 was gradually increased after stimulation with TNF-α (Fig. 1). In addition, JNK and ERK phosphorylation induced by TNF-α exhibited a transient increase, reaching a maximum at 15 min (Fig. 2A). Next, we examined whether the TNF-α–induced serine phosphorylation of IRS-1 at 636/639 was mediated by JNK and ERK in 3T3L1 adipocytes by using the JNK inhibitor SP600125 and the MEK
inhibitor U0126. As shown in Fig. 2, B and C, the TNF-α–
induced serine phosphorylation of IRS-1 at 636/639
was significantly inhibited by SP600125 and U0126
treatment in 3T3L1 adipocytes. SP600125 and U0126
alone had no effect on the serine phosphorylation of
IRS-1 at 636/639. We have also observed that the
serine phosphorylation of IRS-1 at 307 was significantly
inhibited by SP600125 and U0126 treatment in 3T3L1
adipocytes (data not shown).

AICAR stimulation inhibits the TNF-α–induced serine
phosphorylation of IRS-1 at 636/639 through inhibition
of ERK phosphorylation but not JNK phosphorylation

We investigated the potential roles of AICAR in the
signal transduction related to TNF-α–induced serine
phosphorylation of IRS-1 at 636/639 and 307 in 3T3L1
adipocytes with the AMPK activator AICAR. For stimu-
lation, 1mM AICAR was used because this concentration
has been shown to significantly increase the phosphory-
lation of AMPK in 3T3L1 adipocytes (Fig. 3). The TNF-
α–induced serine phosphorylation of IRS-1 at 636/639
and 307 was significantly inhibited in the presence of
AICAR (Fig. 4: A and B). Interestingly, treatment with
AICAR significantly inhibited the TNF-α–induced phos-
phorylation of ERK, but did not affect the phosphoryla-
tion of JNK (Fig. 4: C and D). Treatment with AICAR
alone had no effects on the serine phosphorylation of
IRS-1 at 636/639 and 307 and JNK, but the phosphoryla-
tion of ERK was significantly inhibited.

AICAR inhibits ERK phosphorylation through increased
DUSP9/MKP-4 and ERK interaction without affecting
PP2A activity

We further investigated the inhibitory effects of AICAR
on TNF-α–induced phosphorylation of ERK. We focused
on PP2A and DUSP9/MKP-4, both of which are critical
ERK threonine/tyrosine phosphatases. First, we exam-
AICAR Inhibits pIRS-1 Ser636/639

pp38 eliminates the effects of PP2A on the ERK phosphorylation inhibited by AICAR using the PP2A inhibitor okadaic acid. We showed that both basal and TNF-α stimulation resulted in enhanced phosphorylation of ERK in okadaic acid–treated cells compared with that in controls (Fig. 5). In addition, AICAR stimulation markedly inhibited the TNF-α–induced ERK phosphorylation in the absence or presence of okadaic acid (Fig. 5). Next, we tested

AICAR stimulation inhibits TNF-α–induced ERK phosphorylation without affecting PP2A in 3T3L1 adipocytes. Cells were treated with or without AICAR (1 mM) for 24 h and okadaic acid (100 nM) for 45 min before 20 ng/mL TNF-α stimulation for 15 min. The samples were then subjected to SDS-PAGE and subsequently immunoblotted with the indicated antibodies. Data shown are representative of 3 independent experiments.

Fig. 5. AICAR stimulation inhibits TNF-α–induced ERK phosphorylation without affecting PP2A in 3T3L1 adipocytes. Cells were treated with or without AICAR (1 mM) for 24 h and okadaic acid (100 nM) for 45 min before 20 ng/mL TNF-α stimulation for 15 min. The samples were then subjected to SDS-PAGE and subsequently immunoblotted with the indicated antibodies. Data shown are representative of 3 independent experiments.
and found that AICAR has no effect on the levels of DUSP9/MKP-4 mRNA expression (Fig. 6A). We next examined whether ERK interacts with DUSP9/MKP-4 with or without AICAR stimulation in 3T3L1 adipocytes. AICAR stimulation resulted in increased association between DUSP9/MKP-4 and ERK and this interaction was further increased in response to TNF-α plus AICAR (Fig. 6B).

**AICAR injection decreased blood glucose level, ERK phosphorylation, and serine phosphorylation of IRS-1 at 636/639 in db/db mice**

To investigate this signaling pathway and the effects of AICAR on the phosphorylation of ERK in a diabetic situation in vivo, we used db/+ and db/db mice. First, we examined the levels of TNF-α mRNA level in both mice and found that TNF-α mRNA levels in epididymal adipose tissue of the db/db mice were higher than those in epididymal adipose tissue db/+ mice (Fig. 7A). In addition, ERK and JNK phosphorylation and the serine phosphorylation of IRS-1 at 636/639 were increased in adipose tissue of the db/db mice compared to that in adipose tissue of the db/+ mice (Fig. 7B). We further tested the influence of AICAR injection on the insulin and glucose levels in these mice. We observed that both blood glucose and insulin levels were increased in the db/db mice compared to those in the db/+ mice (Fig. 8: A and B). To determine whether AICAR injection could affect blood glucose and insulin levels, we measured blood glucose and insulin levels in saline or AICAR-injected db/db and db/+ mice. Blood glucose and insulin concentrations in the db/db and db/+ mice injected with AICAR significantly decreased compared with those in
Fig. 8. AICAR decreases plasma glucose and insulin concentrations in vivo. Db/db and db/+ mice were injected with saline or AICAR (250 mg/kg) after 16 h of starvation. One hour after the AICAR injection, blood samples were collected from the postcaval vein. A) Plasma glucose concentrations were measured by the glucose oxidase method. B) Plasma insulin concentrations were determined using an enzyme immunoassay. Data are represented as the mean ± S.E.M. *P < 0.05, compared with db/+ vs. db/db injected with saline. #P < 0.05, compared with saline vs. AICAR in db/+ and db/db.

Fig. 9. AICAR suppresses ERK and JNK phosphorylation and the serine phosphorylation of IRS-1 at 636/639 but not the phosphorylation at 307 in vivo. Db/db mice were injected with saline or AICAR (250 mg/kg) after 16 h of starvation. One hour after the AICAR injection, epididymal adipose tissue was removed from each mouse and homogenized. The samples were then subjected to SDS-PAGE and immunoblotted with the indicated antibodies. Data shown are representative of 3 – 4 independent experiments. *P < 0.05, compared with saline vs. AICAR in db/db.
mice injected with saline. Examination of effects of AICAR injections on JNK and ERK phosphorylation and the serine phosphorylation of IRS-1 at 636/639 in the db/db mice revealed that their phosphorylations decreased in the adipose tissue of the db/db mice that were intraperitoneally administered with AICAR. However, AICAR did not affect the serine phosphorylation of IRS-1 at 307 in this condition (Fig. 9).

Discussion

The present study demonstrated that the AMPK activator AICAR inhibits the TNF-α–induced serine phosphorylation of IRS-1 at 636/639 and 307 by inducing increased association between ERK and DUSP9/MKP-4 in 3T3L1 adipocytes. We also showed that intraperitoneal administration of AICAR to db/db mice inhibits ERK phosphorylation, inhibits serine phosphorylation of IRS-1 at 636/639, and improves fasting glucose and insulin levels.

Serine phosphorylation of IRS-1 induced by cytokines such as TNF-α and interleukins interferes with insulin signal transduction in many ways and resulted in the development of insulin resistance (4–6). Furthermore, it was reported that the basal serine phosphorylation of IRS-1 at 636/639 and 307 was increased in skeletal muscle cells of patients with type 2 diabetes compared with those in skeletal muscle cells of lean subjects (22, 23), suggesting that these two serine phosphorylation sites are critical physiological and pathophysiological mediators of insulin resistance. Therefore, the suppression of the serine phosphorylation of IRS-1 results in improvement in insulin resistance. It has been proposed that AMPK directly phosphorylates AS160, which leads to the translocation of GLUT4 vesicles to the plasma membrane (19). However, it is still unclear how AMPK activation induced by AICAR affects the serine phosphorylation of IRS-1 in 3T3L1 adipocytes. In this study, we demonstrated that TNF-α induced serine phosphorylation of IRS-1 at 636/639 and that this response was almost completely inhibited by pretreatment of either with SP600125 (JNK inhibitor) or U0126 (MEK inhibitor). In agreement with previous reports, we confirmed that the serine phosphorylation of IRS-1 at 307 induced by TNF-α was mediated through the ERK and JNK pathways (data not shown). These data suggest that both JNK and ERK are important molecules for modulating the serine phosphorylation of IRS-1 at 636/639 and 307 in 3T3L1 adipocytes. Interestingly, we demonstrated that AICAR pretreatment completely inhibited TNF-α–induced ERK phosphorylation and subsequently serine phosphorylation of IRS-1 at 636/639 and 307 but not JNK phosphorylation, suggesting that AMPK may interact specifically with ERK. In contrast, it has been reported that AMPK activated by AICAR phosphorylates ERK and JNK in PC12 cells (24), indicating that AMPK may negatively regulate insulin signaling through ERK and JNK phosphorylation in some situations. In particular, the differences between our results and those of the previous study may be explained by AMPK acting as a suppressor protein for the phosphorylation of ERK when it is extracellularly stimulated by cytokines. It has been reported that the serine phosphorylation of IRS-1 at 636/639 is also regulated by the Ser/Thr kinase mammalian target of rapamycin (mTOR) (25), which is activated by TNF-α. This cascade seems to exist both dependent and independent of the MEK/ERK or JNK pathways (26, 27). Therefore, AICAR may inhibit the serine phosphorylation of IRS-1 at 636/639 and 307 through the inhibition of mTOR signaling. Indeed, a previous study has demonstrated that AMPK activation results in reduced mTOR activation in glioma cell lines (28).

Next we investigated how AICAR suppresses the TNF-α–induced phosphorylation of ERK in 3T3L1 adipocytes. The dephosphorylation of ERK is regulated by a variety of protein phosphatases, including protein PP2A and DUSP9/MKP-4 (14–16). Therefore, we examined whether PP2A and DUSP9/MKP-4 were involved in the dephosphorylation of ERK induced by AICAR. We clearly showed that DUSP9/MKP-4 interacted with ERK in response to AICAR stimulation and that AICAR stimulation had no influence on DUSP9/MKP-4 mRNA. In support of our proposed mechanism in which AICAR inhibits the phosphorylation of ERK and subsequently the serine phosphorylation of IRS-1 at 636/639 and 307, it has been proposed that DUSP6/MKP-3, which has a high sequence homology to DUSP9/MKP-4, specifically within its catalytic domain, can form a complex with ERK, leading to enhanced DUSP6/MKP-3 activity (29). Moreover, it has been reported that overexpression of an adenovirus encoding DUSP9/MKP-4 protects against stress-induced insulin resistance in 3T3L1 adipocytes (30). On the other hand, we demonstrated that the PP2A inhibitor okadaic acid did not restore ERK phosphorylation levels suppressed by AICAR stimulation, indicating that AICAR does not increase PP2A activity. Taken together, while these publications support our proposed mechanism, we cannot exclude the possibility that other DUSP family proteins suppress the serine phosphorylation of IRS-1. Indeed, DUSP1/MKP-1 also exists in adipocytes and can inhibit ERK activity (31). Further study will be necessary to understand the mechanisms by which ERK binds to DUSP9/MKP-4 in response to AMPK activation induced by AICAR.
Next, we investigated the effects of AICAR on insulin resistance in \( db/db \) mice. We confirmed that TNF-\( \alpha \) mRNA levels are significantly increased in adipose tissue of \( db/db \) mice compared with that of \( db/+ \) mice, suggesting that TNF-\( \alpha \) may result in impaired insulin signaling in adipose tissue. We also showed that the intraperitoneal administration of AICAR to \( db/db \) mice inhibits the phosphorylation of ERK and JNK and the serine phosphorylation of IRS-1 at 636/639 but not that of IRS-1 at 307. Moreover, we here showed that AICAR administration significantly reduced fasting blood glucose and insulin levels. Several lines of evidence have suggested that type 2 diabetes is improved by AICAR treatment in vivo (32). In agreement with these past reports, our results suggest that in adipose tissue, the inhibition of ERK phosphorylation and the serine phosphorylation of IRS-1 at 636/639 by AICAR injections greatly contributed to improvement in the diabetic state. However, despite the results that AICAR did not inhibit TNF-\( \alpha \)–induced JNK phosphorylation in 3T3L1 adipocytes, AICAR administration inhibited JNK phosphorylation in vivo. Moreover, despite the result that AICAR significantly inhibited the TNF-\( \alpha \)–induced serine phosphorylation of IRS-1 at 307 in 3T3L1 adipocytes, AICAR administration did not affect serine phosphorylation of IRS-1 at 307 in vivo. Although we cannot explain these phenomenon precisely, this difference could be explained by the fact that adipose tissue is exposed to various extracellular stimuli in an in vivo system, which are absent in an in vitro system.

In conclusion, we propose a new mechanism in which the AMPK activated by AICAR suppresses the cytokine-induced serine phosphorylation of IRS-1 at 636/639 and 307 by enhancing interactions between ERK and DUSP9/MKP-4 in 3T3L1 adipocytes. Taken together, these findings provide evidence that AMPK plays a crucial role in the improvement in type 2 diabetes.

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References


