

The AMP-activated protein kinase activator AICAR does not induce GLUT4 translocation to transverse tubules but stimulates glucose uptake and p38 mitogen-activated protein kinases α and β in skeletal muscle

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ABSTRACT The AMP-activated protein kinase (AMPK) pathway participates in the metabolic effects of contraction on muscle glucose uptake. We have shown that contraction increases both GLUT4 translocation to the cell surface and p38 mitogen-activated protein kinase (p38 MAPK) activity. The latter pathway may be involved in the activation of GLUT4. Here we investigated whether the AMPK activator AICAR increases glucose uptake by inducing translocation of GLUT4 and/or by activating the p38 MAPK pathway. AICAR infusion into glucose-clamped rats increased muscle glucose uptake and GLUT4 translocation from an intracellular fraction to the plasma membrane but not to T-tubules. AICAR also caused recruitment of the transferrin receptor to the plasma membrane and increased [¹²⁵I]-transferrin uptake in isolated muscle. AICAR treatment in vivo or in vitro activated both p38 MAPK α and β (1.6- to 2.8-fold) in EDL muscles with a time course identical to that of stimulation of AMPK and glucose transport. The p38 MAPK inhibitor SB203580 abrogated the stimulatory effect of AICAR on glucose uptake. These results suggest that AICAR increases muscle glucose uptake by two mechanisms: 1) inducing selective recruitment of GLUT4 to the plasma membrane, and 2) activating p38 MAPK α and β , which may be involved in the activation of GLUT4—Lemieux, K., Konrad, D., Klip, A., Marette, A. The AMP-activated protein kinase activator AICAR does not induce GLUT4 translocation to transverse tubules but stimulates glucose uptake and p38 mitogen-activated protein kinases α and β in skeletal muscle.—Lemieux, K., Konrad, D., Klip, A., Marette, A. The AMP-activated protein kinase activator AICAR does not induce GLUT4 translocation to transverse tubules but stimulates glucose uptake via p38 mitogen-activated protein kinases α and β in skeletal muscle. *FASEB J.* 17, 1658–1665 (2003)

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MUSCLE CONTRACTION increases glucose uptake and utilization by triggering translocation of GLUT4 glu-

cose transporters from intracellular vesicles to both the plasma membrane and T-tubule sarcolemmal domains of muscle fibers (1, 2). Recent studies have suggested a role for AMP-activated protein kinase (AMPK) in the stimulatory effects of contraction on glucose uptake. Thus, exercise (3, 4) or electrically stimulated contraction (5–7) increase the activity of AMPK, a heterotrimeric protein consisting of one catalytic subunit (α) and two noncatalytic subunits (β , γ) (8, 9). Moreover, several studies have shown that the cell-permeable AMPK activator 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) stimulates glucose uptake in fast-twitch muscles and that its effect is not additive to that of contraction (5, 10–12). Further evidence for a role of AMPK in contraction-induced glucose uptake came from the work of Mu et al. (13), who showed that expression of a dominant inhibitory mutant of AMPK in transgenic mouse muscle reduced contraction-induced glucose uptake by 30–40%. AICAR has been reported to increase GLUT4 translocation to the plasma membrane in hindlimb muscles (14). However, it is still unknown whether AICAR also triggers the recruitment of GLUT4 to the T-tubules, which represent 60 to 80% of the total cell surface area in skeletal muscle fibers (15, 16).

Exercise and contraction also activate p38 MAPK in rat skeletal muscle (17–20). We recently showed that incubation of isolated EDL muscles with the p38 MAPK inhibitor SB203580 reduced contraction-induced glucose uptake by ~ 50% (20). SB203580 inhibits insulin-induced glucose transport in both L6 muscle cells and 3T3-L1 adipocytes without affecting GLUT4 exposure at the cell surface (21). It was thus proposed that activation of p38 MAPK by contraction may be involved in the activation of GLUT4 in rat skeletal muscle. It was

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recently reported that AICAR increases the activity of GLUT1 transporters in clone 9 cells (22) and that this effect is mediated by activation of p38 MAPK (23).

The goal of the present study was to test whether the AMPK activator AICAR increases muscle glucose uptake by inducing GLUT4 translocation to the plasma membrane and the T-tubule surface domains and/or by activating the p38 MAPK pathway. The results show that AICAR induces GLUT4 translocation but that the transporter is recruited only to the plasma membrane and not to the T-tubules. On the other hand, AICAR activates both p38 MAPK α and p38 MAPK β , and SB203580 fully inhibits the ability of AICAR to stimulate glucose uptake in isolated muscle.

MATERIALS AND METHODS

Whole-body and muscle glucose uptake measurements

This study was approved by the Animal Care and Handling Committee of Laval University. Male Sprague-Dawley rats (175–200 g) purchased from Charles River (Montreal, QC, Canada) were randomly assigned to AICAR or control groups and housed in individual cages, maintained on a 12 h dark and light schedule, and fed ad libitum with Purina rat chow. Whole-body AICAR action was determined by the clamp procedure as detailed previously (24) with modifications based on Bergeron et al. (10). Measurement of steady-state AICAR-stimulated glucose uptake in muscle tissues was performed by measuring the incorporation of radiolabeled 2-deoxy-D-glucose [2-[1,2-³H(N)]-deoxyglucose (DG)] as previously described (24). Immediately after the clamp, rats were killed by decapitation and hindlimb muscles (EDL, tibialis, gastrocnemius, and quadriceps) were rapidly excised, cleaned of extraneous tissues, and frozen in liquid nitrogen. The muscles were kept at -80°C until use for 2-[³H]-DG incorporation or subcellular fractionation.

For enzyme activity determinations after *in vivo* AICAR treatment, male Sprague-Dawley rats (200–250 g) were injected with AICAR (0.7 g/kg *i.p.*) for 30 min and EDL muscles were rapidly removed, clamp frozen, and stored at -80°C until processed as described below.

Subcellular membrane fractionation and Western blot

Plasma membranes, T-tubules, and intracellular membranes were isolated from 7–8 g of muscles (tibialis, gastrocnemius, and quadriceps) using a procedure developed in our laboratory (25). This subcellular fractionation protocol has been extensively characterized with immunologic and enzymatic markers (1, 2, 25). Membrane fractions (10 μg) were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblot was performed as previously described (25). Immunoreactive bands were detected by the enhanced chemiluminescence method (Renaissance ECL kit, NEN Life Science, Boston, MA, USA).

Incubation protocols of isolated rat skeletal muscles and 2-deoxyglucose uptake

Isolation of extensor digitorum longus (EDL) muscles and determination of glucose uptake rates were performed as recently described (20). Muscles were incubated with AICAR (2 mM) and 2-deoxyglucose uptake was determined. In some

experiments, muscles were preincubated with 10 μM SB203580 (or DMSO) for 20 min, then incubated for 30 min with AICAR in the absence or continued presence of SB203580. AICAR and SB203580 treatments were continued during the glucose uptake experiments if they were present during the previous incubations. For *in vitro* stimulation of AMPK and p38 MAPK activity by AICAR or insulin, EDL muscles were incubated with or without AICAR (2 mM) or insulin (0.2 mU/mL) for the times indicated in the figures, immediately blotted at 4°C , clamp frozen, and stored at -80°C until processed as described below.

AMPK activity and phosphorylation

AMPK activity was measured as described earlier (7). AMPK was immunopurified from muscles lysates with protein A-G Sepharose beads coupled to antibody directed against the α -2 subunit of AMPK heterotrimer (kind gift of Dr. N. B. Ruderman). The immunocomplexes were collected and washed extensively. The AMPK was assayed against the SAMS peptide with the sequence HMRSAMSGHLHLVKRR (26). Phosphorylation of AMPK was assessed by immunoblot using an anti-phospho-AMPK (Thr172) (1:1000 dilution; Cell Signaling, Beverly, MA, USA), as previously described (27).

p38 MAPK phosphorylation and kinase assay

p38 MAPK phosphorylation and activity were measured as described (20, 28) with the following modifications: p38 MAPK was immunoprecipitated from 300 μg of total proteins for 2–3 h with polyclonal anti-p38 MAPK α or anti-p38 MAPK β antibodies (Santa Cruz, Santa Cruz, CA, USA) or with p38 MAPK γ antibody (kind gift from K. Sedorf, Novo Nordisk, Denmark) preadsorbed to protein G-Sepharose beads. Immunocomplexes were isolated and washed four times as previously described (20), and then used for Western blot with anti-phospho p38 (T180, Tyr182) MAPK antibody (Cell Signaling). In some experiments, muscle lysates (150 μg proteins) were directly subjected to SDS-PAGE and analyzed by Western blot with anti-phospho p38 MAPK antibody. When used for kinase assays, washed immunocomplexes were incubated for 30 min at 30°C with 50 μL reaction mixture (kinase buffer containing 200 μM ATP, 2 μg ATF-2 fusion protein per condition) in the absence or presence of 10 μM SB203580 on a platform shaker. The reaction was stopped by adding 25 μL 2 \times Laemmli sample buffer. Samples were heated for 30 min at 65°C , then vortexed and centrifuged for 5 min (1000 rpm). Fifty microliters of the supernatant were resolved by 10% SDS-PAGE, and immunoblotted for phospho-ATF-2 (1:500 dilution, cell signaling). Immunoblots were scanned within the linear range and quantitated using the computer software NIH image version 1.61 (NIH, Bethesda, MD, USA).

RESULTS

Effect of AICAR on skeletal muscle glucose uptake and GLUT4 translocation.

The effect of AICAR on glucose disposal and GLUT4 translocation in hindlimb muscles was first investigated *in vivo* using the glucose clamp technique coupled with tracer ³H-2-DG injection and subcellular fractionation. The exogenous glucose infusion rate required to maintain euglycemia in the AICAR-infused rats was $16.5 \pm 3.9 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; no glucose was infused in the

TABLE 1. AICAR increases whole-body glucose disposal and skeletal muscle glucose uptake in rats^a

	CONTROL	AICAR
Body weight (g)	184.6 ± 7.9	198.0 ± 9.3
GDR (mg · kg ⁻¹ · min ⁻¹)	—	16.5 ± 3.9
2 DG-uptake (nmol · min ⁻¹ · g ⁻¹)	51.7 ± 10.8	220.9 ± 65.7*

^aData are means ± SE of 3–5 rats. GDR, glucose disposal rate. 2-deoxy-D-glucose uptake was measured in tibialis, gastrocnemius, and quadriceps muscles. **P* < 0.01 vs. control.

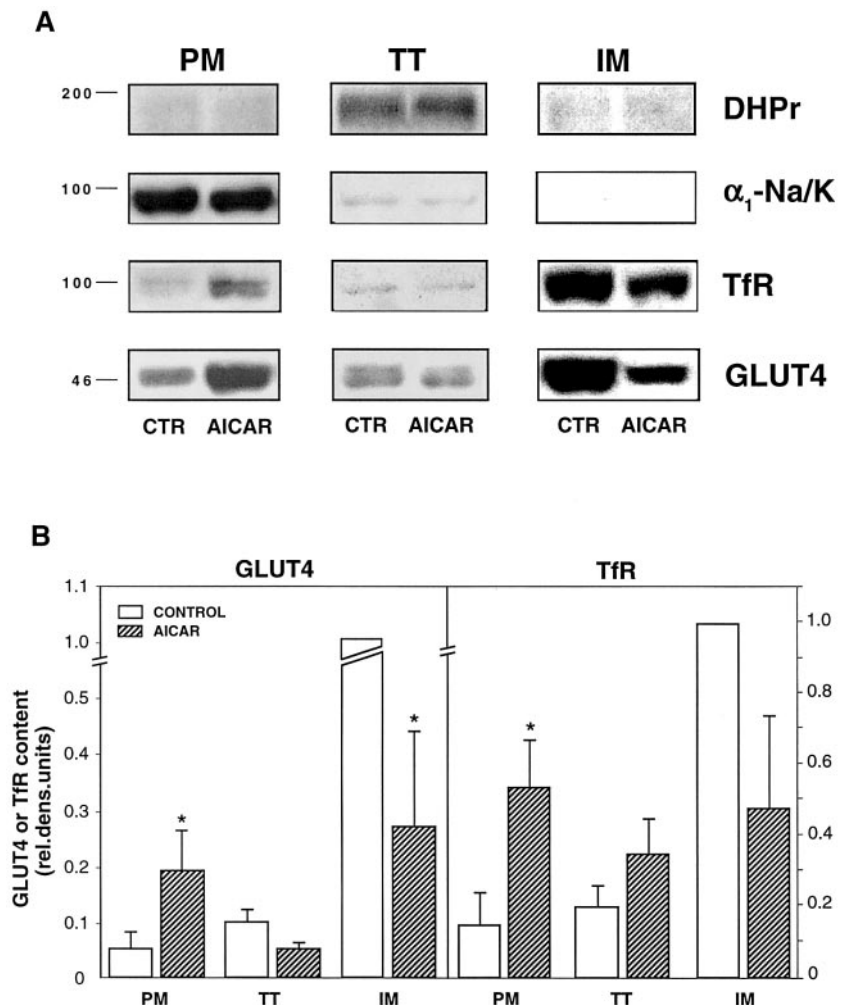
control group (Table 1). This was associated with a 4.25-fold stimulation of 2-deoxyglucose uptake in hindlimb muscles of AICAR-treated rats relative to control animals.

The effect of in vivo AICAR treatment on GLUT4 translocation is shown in Fig. 1. GLUT4-enriched intracellular membrane (IM), plasma membrane (PM), and T-tubule (TT) fractions were isolated by subcellular fractionation of saline (vehicle control) and AICAR-stimulated muscles. Protein recoveries of PM, TT, and IM fractions were similar between control and AICAR-stimulated muscles (see legend to Fig. 1). Figure 1A shows immunoblots of one representative experiment

whereas the mean effects of three or four independent membrane preparations are represented in Fig. 1B. In accordance with previous studies (1, 2, 25), the plasma membrane marker α 1-Na/K-ATPase was mainly enriched in the PM fraction whereas the α 1-subunit of the dihydropyridine receptor (DHPr), a T-tubule-specific marker, was recovered mostly in the TT fraction. AICAR treatment induced GLUT4 recruitment from the IM fraction to the plasma membrane cell surface domain. Surprisingly, however, AICAR failed to increase GLUT4 translocation to the T-tubules.

Electrically induced contraction also induces the translocation of the transferrin receptor (TfR) from the intracellular membrane fraction to the plasma membrane (29). We therefore tested whether AICAR stimulated the translocation of TfR to the plasma membrane. As shown in Fig. 1A, B, AICAR induced recruitment of the TfR from the GLUT4-enriched intracellular fraction to the plasma membrane but not to the T-tubule domain of the sarcolemma. To further confirm the effect of AICAR on TfR translocation to the plasma membrane, EDL muscles were isolated from normal rats, incubated with [¹²⁵I]-transferrin (0.02 μ Ci/mL), and AICAR or saline were added for 30 min. AICAR doubled (2.0 ± 0.4-fold, *P* < 0.05) the uptake of

Figure 1. Effects of AICAR on GLUT4 translocation in rat skeletal muscle. *A*) Representative immunoblots showing the subcellular distribution of the dihydropyridine receptor (DHPr), α ₁-Na/K ATPase, transferrin receptor (TfR) and GLUT4 content in plasma membranes (PM), transverse tubules (TT), and LiBr-released intracellular membrane (IM) fractions isolated from control (CTR) and AICAR-stimulated rat muscles (tibialis, gastrocnemius, and quadriceps). 10 μ g of membrane proteins was used for Western blot analysis, as described in Materials and Methods. Molecular weight standards are shown on the left. *B*) Effects of AICAR on GLUT4 and TfR translocation in hindlimb muscles. Densitometric values are means ± SE of data obtained from 3 or 4 individual membrane preparations. **P* < 0.05 vs. controls in each membrane fraction. Protein recoveries of isolated PM (CTR: 67.7 ± 8.1, AICAR: 65.2 ± 11.9), TT (CTR: 332.5 ± 69.6, AICAR: 348.6 ± 97.9), and IM (CTR: 93.5 ± 32.2, AICAR: 97.0 ± 12.8) membrane fractions were not different among groups.



[¹²⁵I]-transferrin, confirming that it increases TfR recycling in muscle.

Role of p38 MAPK in AICAR-induced glucose uptake in muscle

In the next experiments, we tested the hypothesis that AICAR may also increase glucose transport in muscle by activation of the p38 MAPK pathway. We first tested the effects of in vivo AICAR treatment on p38 MAPK phosphorylation and activation in the EDL muscle. As shown in Fig. 2A, B, AICAR treatment increased the

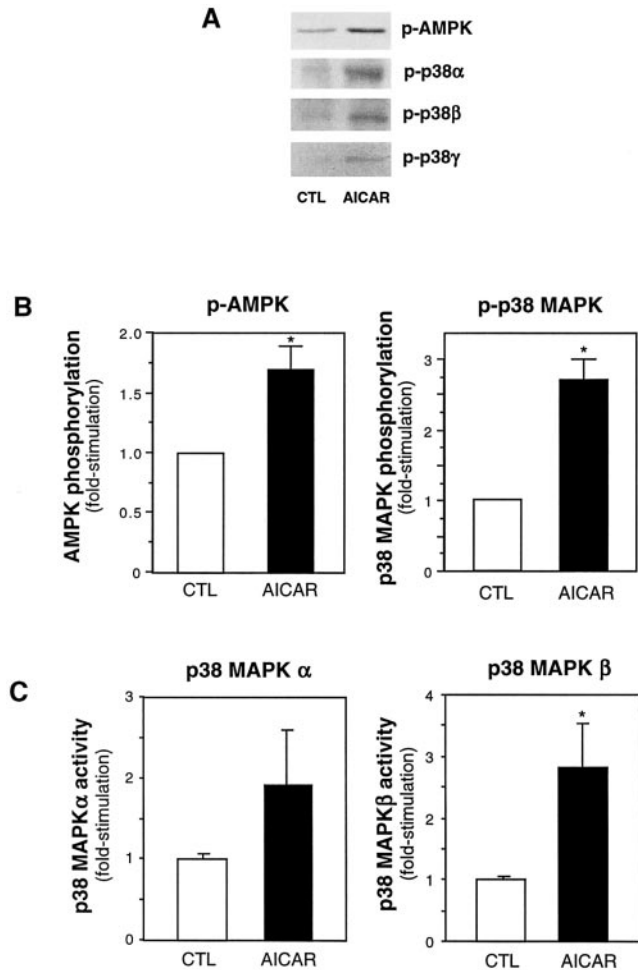


Figure 2. Effects of in vivo AICAR treatment on AMPK and p38 MAPK in EDL muscles. Male Sprague-Dawley rats (200–250 g) were injected with AICAR (0.7 g/kg i.p.) for 30 min and EDL muscles were rapidly removed, clamp frozen, and stored at -80°C until processed as described in Materials and Methods. *A, B*) Total muscle lysates (150 μg proteins) or p38 MAPK α , β , or γ immunoprecipitated from 300 μg of proteins were analyzed for Western blot with anti-phospho AMPK or anti-phospho p38 MAPK antibody. *C*) p38 MAPK α and p38 MAPK β kinase activities were assayed as described in Materials and Methods. Immunoblots in panel *A* are representative of 4 different experiments; results in panels *B* and *C* are the mean \pm SE of 4 individual experiments with different rats. Basal (CTL) level of phosphorylation or kinase activity were assigned a value of 1.0 and AICAR values are expressed relative to this value.

phosphorylation of AMPK on Thr172, a site known to activate the enzyme (30) and a good correlate of its activation (27). In vivo AICAR stimulation also enhanced phosphorylation of p38 MAPK in the EDL muscle (Fig. 2B). Immunoprecipitation of individual isoforms further indicated that AICAR increased the phosphorylation of p38 MAPK α and p38 MAPK β (Fig. 2A). Phosphorylation of p38 MAPK γ was slightly but not significantly increased by AICAR (Fig. 2A). Kinase activity measurements confirmed activation of both p38 MAPK α and β isoforms (Fig. 2C). However, only p38 MAPK β activation by AICAR reached the level of statistical significance in these in vivo studies.

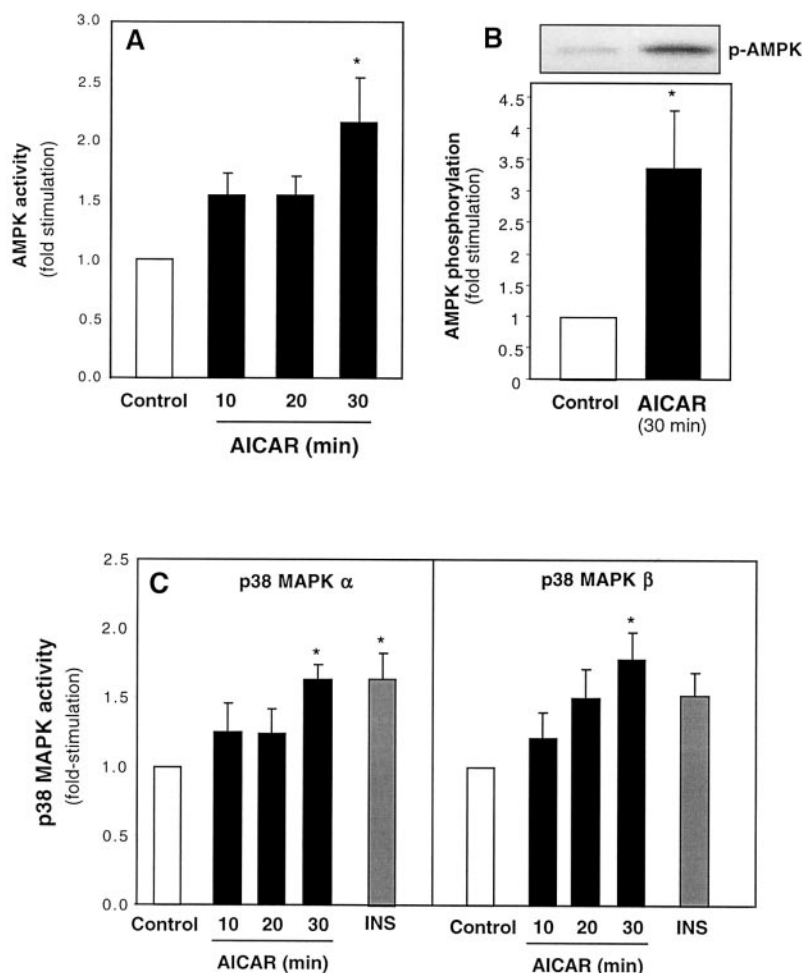
It has been suggested that systemic factors may contribute to activation of p38 MAPK by exercise (19). To avoid the possible contribution of systemic or humoral factors in the AICAR effect on p38 MAPK, we conducted ex vivo studies in isolated EDL muscles. As depicted in Fig. 3, AICAR induced a time-dependent increase in AMPK activity in EDL muscle, reaching an \sim twofold stimulation after 30 min (Fig. 3A) and an \sim threefold increase in AMPK phosphorylation (Fig. 3B). Figure 3C further shows that AICAR increased p38 MAPK activity with a similar time course. AICAR enhanced the kinase activity of p38 MAPK α and p38 MAPK β isoforms by 1.6 ± 0.1 and 1.8 ± 0.2 -fold after 30 min, respectively. The stimulatory effect of AICAR on p38 MAPK was similar to that observed for insulin in the same conditions (Fig. 3C). p38 MAPK activity was also inhibited when the p38 MAPK inhibitor SB203580 (10 μM) was added directly to the immunoprecipitates during the kinase assay, confirming the specificity of the immunoprecipitation (data not shown).

Finally, we investigated whether AICAR-induced p38 MAPK activity is implicated in the stimulation of muscle glucose uptake. Addition of 2 mM AICAR to isolated EDL muscles induced a maximal twofold increase in glucose uptake after 40–45 min but a significant effect was already observed after 30 min of treatment (Fig. 4A). Longer exposure to AICAR did not further increase glucose uptake (data not shown). Addition of SB203580 (10 μM) abrogated the effect of AICAR on muscle glucose uptake but failed to affect basal glucose uptake (Fig. 4B). The p38 MAPK inhibitor did not affect AMPK activation by AICAR as assessed by its phosphorylation on Thr172 (Fig. 4C).

DISCUSSION

The present study was designed to delineate the molecular mechanisms coupling the AMPK pathway to increased glucose uptake in skeletal muscle. The stimulatory effect of contraction/exercise on glucose uptake is linked to the translocation of GLUT4 glucose transporters to the muscle cell surface. We (1, 2, 29) and others (31) have shown that contraction increases the amount of cell surface GLUT4 transporter proteins by inducing the translocation of the transporter to the plasma membrane and the T-tubules, the two subdo-

Figure 3. Effects of AICAR on AMPK and p38 MAPK activity in isolated EDL muscles. Isolated EDL muscles were incubated with 2 mM AICAR and *A*) AMPK activity, *B*) AMPK phosphorylation on Thr172, or *C*) p38 MAPK α and p38 MAPK β activities were assayed as described in Materials and Methods. For comparison, the effect of insulin (0.2 mU/mL, 4 min) on p38 MAPK α and p38 MAPK β is shown in panel *C*. Results are the mean \pm SE of 4–6 individual experiments. Basal kinase activity was assigned a value of 1.0, and activity values of AICAR, or insulin samples are expressed relative to this value.



mains of the muscle cell surface. AICAR infusion caused GLUT4 translocation to the plasma membrane in skeletal muscle (14); however, translocation to the T-tubules, the principal component of the surface area in muscle cells (15, 16), was not assessed. The present work provides evidence that AMPK activation selectively increases GLUT4 translocation to the plasma membrane but not to the T-tubules. AICAR is the first stimulus known to increase muscle glucose uptake without detectable GLUT4 recruitment to the T-tubules. These results suggest that contraction stimulates GLUT4 translocation to the T-tubules through an AMPK-independent mechanism, which is consistent with the finding that contraction-induced glucose uptake is only partially inhibited in skeletal muscle expressing a dominant-negative AMPK (13).

The biochemical nature of the AICAR-sensitive intracellular GLUT4 compartment remains to be defined. Recent studies (29, 31) suggest that GLUT4 traffic into the recycling endosomes is implicated in contraction-stimulated GLUT4 recruitment in muscle. Indeed, contraction triggers GLUT4 translocation from a TfR-enriched vesicular compartment in skeletal muscle. Furthermore, translocation of GLUT4 from the TfR-enriched compartment was selective to the plasma membrane domain of the sarcolemma whereas GLUT4 from a TfR-depleted pool was also recruited to the

T-tubules after contraction stimulation (29). The fact that AICAR induced GLUT4 translocation only to the plasma membrane suggests that the transporters were mobilized from the TfR-enriched compartment. Two additional observations are in accordance with a selective GLUT4 recruitment from the TfR-enriched intracellular compartment. First, AICAR induced TfR protein translocation to the plasma membrane but not to the T-tubules. Second, the AMPK activator increased recycling of the TfR, as revealed by measurement of [125 I]-transferrin uptake in isolated muscles. Whether this AICAR-responsive TfR-enriched GLUT4 pool represents recycling endosomes, as previously suggested (29, 31), remains to be firmly established by immunocytochemical studies complemented by detailed kinetics of [125 I]-transferrin internalization in isolated muscles.

Given that the plasma membrane represents only 20–40% of the sarcolemmal surface area of muscle cells (15, 16), it is unlikely that the stimulatory effect of AICAR on glucose uptake could be solely explained by its ability to induce GLUT4 translocation. Another potential mechanism by which AICAR may increase glucose uptake into skeletal muscle is through activation of cell surface GLUT4 transporters. It has been shown that p38 MAPK functions in a signaling pathway which modulates the activity of GLUT4 transporters in

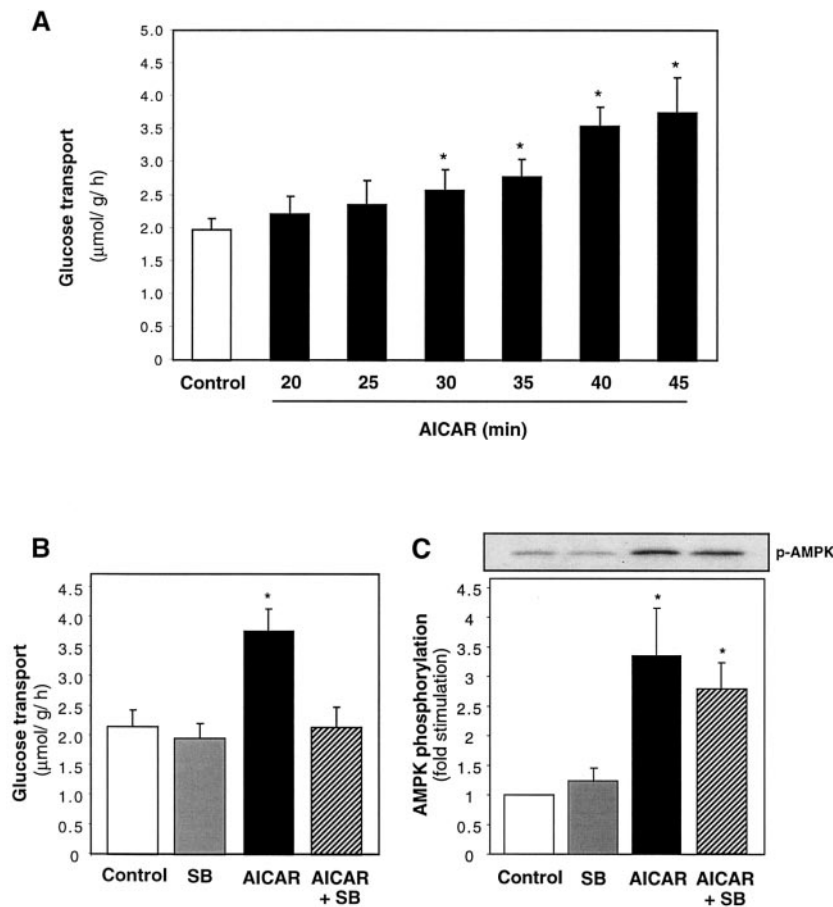


Figure 4. AICAR-stimulated 2-deoxyglucose uptake is reduced by SB203580. *A*) Isolated EDL muscles were incubated with or without 2 mM AICAR for the indicated times and 2-deoxyglucose uptake was then determined. *B*, *C*) Muscles were preincubated with 10 μ M SB203580 (or DMSO) for 20 min, then incubated for 30 min with AICAR or saline in the absence or continued presence of SB203580 (SB). 2-Deoxyglucose uptake (*B*) or AMPK phosphorylation on Thr172 (*C*) were then determined as described in Materials and Methods. Results represent the mean \pm SE of 3–6 individual experiments. * $P < 0.05$ compared with samples not treated with AICAR.

muscle and fat cells (21). In skeletal muscle, p38 MAPK is activated by insulin (20, 32, 33) and by contraction (17, 19, 20) and the p38 MAPK inhibitor SB203580 reduces insulin- and contraction-stimulated glucose uptake in isolated EDL muscles *in vitro* (20). AICAR increases the activity of GLUT1 transporters in clone 9 cells (22), and this effect is mediated by activation of p38 MAPK (23). It was therefore of interest to test whether AMPK signals through p38 MAPK for increasing glucose transport in skeletal muscle. Our data show that AICAR rapidly increases the phosphorylation and kinase activity of both p38 MAPK α and p38 MAPK β in the EDL muscle. The finding that AICAR also induced p38 MAPK activation *in vitro* in isolated EDL muscles indicates that the *in vivo* effect cannot be solely attributed to humoral/systemic factors. Although p38 MAPK γ is also expressed in skeletal muscle and previously reported to be activated by long-term exercise (34), it was not found to be responsive to acute AICAR treatment. Thus AICAR, unlike contraction, may not activate p38 MAPK γ . However, it should be noted that p38 MAPK γ activity was found to be increased after prolonged (180–210 min marathon running) exercise, which is certainly not comparable with the acute (30 min) AICAR treatment used in our study. Furthermore, the responsiveness of p38 MAPK γ to exercise and AICAR may differ between rats and humans.

The activation of p38 MAPK α and β isoforms was temporally related to the stimulation of AMPK and

muscle glucose uptake by AICAR. The effect of AICAR on glucose uptake was abrogated by 10 μ M SB203580, suggesting that p38 MAPK α and β act downstream of AMPK to stimulate glucose uptake. The same concentration of SB203580 reduced the effect of contraction on glucose uptake by only 40% (20), possibly because contraction also activates p38 MAPK γ , which is not inhibited by SB203580 (35). This raises the interesting hypothesis that p38 MAPK γ activation by contraction may be part of a signaling cascade that stimulates GLUT4 translocation to the T-tubules, which, as suggested in the present study, is independent of AMPK activation.

Even with the most specific protein kinase inhibitors, caution must be expressed when used in cell-based assays (35). Although SB203580 is widely used as a potent and selective inhibitor of p38 MAPK α and β , it was recently shown to inhibit nucleoside transport in K562 cells and AMPK activation by AICAR in H-2K cells (36, 37). However, we found that SB203580 does not affect AMPK activation by AICAR in EDL muscle, in line with previous studies in clone 9 cells (23). Thus, SB203580 abolishes AICAR-induced glucose transport most likely by inhibiting p38 MAPK and not by inhibiting transport of AICAR and activation of AMPK in muscle.

It is difficult to directly assess whether activation of p38 MAPK by AICAR increases GLUT4 activity. Current methods to assess GLUT4 translocation cannot distin-

guish between transporters that are fully inserted into the plasma membrane and T-tubules and those that are docked but not fused. The exofacial label ATB-³H]BMBA cannot be used because it reacts with the active site of glucose transporters and, hence, does not distinguish between changes in number vs. activity of cell surface GLUT4 transporters. Determination of cell surface GLUT4 appearance using a different exofacial marker will be needed to resolve this important question.

In summary, the present results show that AICAR increases glucose uptake in skeletal muscle by two distinct mechanisms: 1) inducing selective recruitment of GLUT4 to the plasma membrane, and 2) activating both p38 MAPK α and β , which may be involved in the activation of GLUT4 at the cell surface. **FJ**

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