Effect of AMPK activation on muscle glucose metabolism in conscious rats

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Am. J. Physiol. 276 (Endocrinol. Metab. 39): E938–E944, 1999.—The effect of AMP-activated protein kinase (AMPK) activation on skeletal muscle glucose metabolism was examined in awake rats by infusing them with 5-aminoimidazole-4-carboxamide 1-β-D-ribofuranoside (AICAR; 40 mg/kg bolus and 7.5 mg·kg⁻¹·min⁻¹ constant infusion) along with a variable infusion of glucose (49.1 ± 2.4 μmol·kg⁻¹·min⁻¹) to maintain euglycemia. Activation of AMPK by AICAR caused 2-deoxy-o-[1,2-³H]glucose uptake to increase more than twofold in the soleus and the lateral and medial gastrocnemius compared with saline infusion and occurred without phosphatidylinositol 3-kinase activation. Glucose uptake was also assessed in vitro by use of the epitrochlearis muscle incubated either with AICAR (0.5 mM) or insulin (20 mU/ml) or both in the presence or absence of wortmannin (1.0 μM). AICAR and insulin increased muscle 2-DG uptake rates by ~2- and 2.7-fold, respectively, compared with basal rates. Combining AICAR and insulin led to a fully additive effect on muscle glucose transport activity. Wortmannin inhibited insulin-stimulated glucose uptake. However, neither wortmannin nor 8-(p-sulfophenyl)-theophylline (10 μM), an adenosine receptor antagonist, inhibited the AICAR-induced activation of glucose uptake. Electrical stimulation led to an about threefold increase in glucose uptake over basal rates, whereas no additive effect was found when AICAR and contractions were combined. In conclusion, the activation of AMPK by AICAR increases skeletal muscle glucose transport activity both in vivo and in vitro. This cellular pathway may play an important role in exercise-induced increase in glucose transport activity.

5'-aminoimidazole-4-carboxamide 1-β-D-ribofuranoside; phosphatidylinositol 3-kinase; glucose transport; adenosine receptor; exercise

INSULIN INCREASES GLUCOSE TRANSPORT ACTIVITY in skeletal muscle through the translocation of glucose transporter proteins (specifically GLUT-4) from an intracellular location to the plasma membrane. This process involves the phosphorylation of the insulin receptor, insulin receptor substrates 1 and 2 (42), and activation of phosphatidylinositol (PI) 3-kinase (8). In the absence of insulin, muscle contractile activity is known also to increase skeletal muscle glucose transport (33) and GLUT-4 translocation (7).

There are three lines of evidence suggesting that insulin and contractile activity stimulate glucose transport via two separate pathways in skeletal muscle. One is that the effects of contraction and maximal insulin stimulation on glucose transport activity are additive (30, 50). The second is that contraction-stimulated glucose transport is not impaired in muscle from insulin-resistant rats (3, 19) in which insulin-mediated glucose transport is severely impaired. Third, it is possible to inhibit insulin-mediated glucose transport selectively with the PI 3-kinase inhibitor wortmannin without inhibiting contraction-mediated glucose transport (25, 26).

Muscle contraction causes ATP degradation and AMP accumulation (5), leading to the activation of AMP-activated protein kinase (AMPK) (17). The effect of AMPK activation on the regulation of free fatty acid oxidation in the skeletal muscle is well described (11). However, the role of AMPK in the regulation of skeletal muscle glucose transport remains unclear. 5-Aminimidazole-4-carboxamide 1-β-D-ribofuranoside (AICAR) was recently found to activate AMPK and increase glucose uptake in the perfused rat hindlimb (28) and in the isolated muscle preparation (13). On the other hand, the only in vivo study did not find increased muscle glucose utilization with AICAR (39). Furthermore, there is the possibility that AICAR increases skeletal muscle glucose transport through the release of adenosine from the tissue, as reported previously (9), and thereby activates the adenosine receptor, which has been proposed to modulate muscle glucose transport (10, 38).

The objectives of the present study were to examine the in vivo effects of AICAR infusion on muscle glucose transport and PI 3-kinase activities in the awake rat and to determine whether AICAR's effects on glucose transport activity in the isolated muscle strips are additive with 1) insulin or 2) contractile activity or are 3) inhibited in the presence of an adenosine receptor antagonist.

METHODS

In vivo measurement of 2-deoxy-o-[1,2-³H]glucose muscle glucose uptake. To establish the effect of AICAR on skeletal muscle glucose transport activity in vivo, male Sprague-Dawley rats (Charles River, Raleigh, NC) were maintained on standard rat chow (Ralston Purina, St. Louis, MO) and housed in an environmentally controlled room with a 12:12-h light-dark cycle. Rats were chronically catheterized in the
right jugular vein and carotid artery, as described previously (35). The catheters were externalized through a skin incision at the back of the head. The rats were allowed to recover until they were at least of preoperative weight (5–8 days).

Overnight-fasted animals (100–130 g) were randomly infused either with isotonic saline (n = 9) or AICAR (n = 12; primed: 40 mg/kg; constant infusion: 7.5 mg·kg⁻¹·min⁻¹; Sigma, St. Louis, MO) for 75 min. Simultaneous infusions of somatostatin (1.0 µg·kg⁻¹·min⁻¹) and basal replacement of insulin (0.1 mU·kg⁻¹·min⁻¹) were performed in both groups to avoid variations of plasma insulin concentrations. The plasma glucose concentration was maintained at basal fasting levels by use of a variable-rate glucose infusion in the AICAR group to prevent hypoglycemia (39). The 20% glucose solution was enriched with [6,6-²H]glucose (30) to determine rates of hepatic glucose production (HGP) calculated from the following formula: 

\[ \text{HGP} = \text{GIR} \times \left( \frac{E_{\text{inf}}}{E_{\text{plasma}}} - 1 \right) \]

where GIR is the mean glucose infusion rate over the last 15 min of the clamp, Einf is the exogenous [6,6-²H]glucose infusion enrichment (%), and Eplasma is the steady-state damped plasma [6,6-²H]glucose enrichment (%). Blood was sampled for assessment of basal and posttreatment plasma glucose, insulin, and free fatty acid (FFA) concentrations. Skeletal muscle glucose uptake was measured according to a method previously described (21). Briefly, 30 min after the beginning of the infusions, [35]C of 2-deoxy-1,2-³H]glucose (2-DOG) were injected as a bolus. Plasma samples were obtained at 2, 5, 10, 15, 20, 30, and 45 min after the bolus infusion to estimate the plasma tracer activity. At the end of the experiment, animals were anesthetized with pentobarbital sodium (10 mg·kg⁻¹·min⁻¹). Calf muscles from the left hindlimb were quickly freeze-clamped in situ for biochemical analysis, and the soleus and the lateral and medial gastrocnemius muscles of the right hindlimb were individually freeze-clamped for determination of glucose uptake rates on the basis of 2-DOG 6-phosphate counts present in the tissue. Glucose uptake rate calculations were based on mean plasma glucose and 2-DOG tissue concentrations and the area under the plasma 2-DOG curve, as described by Kraegen et al. (21).

Measurement of glucose transport activity in isolated muscle. Epitrochlearis muscles from overnight-fasted rats weighing 90–120 g were isolated as previously described (29), placed in 2 ml of oxygenated Krebs-Henseleit bicarbonate (KHB) buffer containing 2 mM pyruvate, 36 mM mannitol, and 0.1% BSA, and were preincubated in a shaking bath at 29°C. Muscles were preincubated for 35 min in KHB alone or in KHB with AICAR (0.5 mM), insulin (20 µU·kg⁻¹·min⁻¹), or both simultaneously in the presence or absence of wortmannin (1.0 µM; Sigma), which is known to inhibit insulin-stimulated muscle glucose transport (25). A second series of experiments was performed in which epitrochlearis muscles were also preincubated for 35 min with an adenosine receptor antagonist, 8-(p-sulfophenyl)-theophylline (8-SPT; 10 µM), in the presence or absence of AICAR (2.0 mM). To induce contractile activity, epitrochlearis muscles were electrically stimulated in situ using subminiature electrodes (Harvard Apparatus, Boston, MA) attached directly to the proximal-medial aspect of the epitrochlearis muscle, which was stimulated for 10 s every min over a 10-min interval, as described previously (16). Tetanic contractions were induced with supramaximal (10 V) square-wave pulses of 0.5 ms at a frequency of 50 Hz. At the end of the electrical stimulation protocol, epitrochlearis muscles were rapidly excised and preincubated either with or without AICAR (0.5 mM) for 35 min. The gas phase in the flasks was 95%O₂–5%CO₂. After the initial preincubation, muscles were transferred to flasks containing 1 mM 2-DOG (190 µCi/ml; NEN, Boston, MA) and 39 mM [¹³C]mannitol (3.9 µCi/ml; NEN) and were incubated at 29°C for 20 min. At the end of the incubation, muscles were rinsed in ice-cold saline, blotted on filter paper, and kept in liquid nitrogen until analysis. The rate of 2-DOG uptake was measured according to the procedure described by Young et al. (46) and expressed as micromoles per milliliter for every 20-min period.

Analytical procedures. Plasma glucose and lactate concentrations were determined using an automated analyzer (YSI Instruments, Yellow Spring, OH). Enrichment of deuterated glucose in plasma was determined by gas chromatography-mass spectrometry by using the pentaacetate derivative of glucose (44). Immunoreactive insulin was assayed using a double-antibody immunoassay kit (Linco Research, St. Louis, MO); plasma FFA were assayed with a colorimetric kit (Wako Pure Chemical Industries, Osaka, J apan). AICAR plasma levels were determined spectrophotometrically (6). Phosphorylated tissue 2-DOG was separated from a perchloric acid muscle extract with ion chromatography (27), and counts in tissue and plasma were assessed with liquid scintillation counting. Muscle glycogen was assayed using the amyloglucosidase method (41). Tissue lactate was assayed spectrophotometrically (22), pyruvate by 2,4-dinitrophenylhydrazine (23), and creatine phosphate (15) concentrations were determined as previously described. Muscle nucleoside and nucleotide were separated and quantified by anion exchange chromatography according to a modified version of a method described earlier (37). Briefly, samples were injected onto a Supelcosil SAX1 ion exchange column (25 cm × 4.6 mm ID, 5 µm particle size) from Supelco (Park Bellefonte, PA) coupled to a Rainin (Woburn, MA) HPLC system. Nucleotide peaks were analyzed by comparison of retention time with external standards and relative absorbance at 254 nm, and results are expressed as micromoles per gram of wet weight. Skeletal muscle AMPK activity and PI 3-kinase activity were determined according to methods previously described (8, 43). Hindlimb muscles from additional rats treated with insulin (20 µU·kg⁻¹·min⁻¹; n = 4) or AICAR + insulin (n = 3) served as a positive control for the effect of insulin on PI 3-kinase activity.

Statistical analyses. All data are reported as means ± SE. Data from the incubated epitrochlearis muscles and the awake animal model were analyzed by two-way and one-way ANOVAs, respectively. Both analyses were followed by the Newman-Keuls test for post hoc comparisons. Differences were considered significant at P < 0.05.

RESULTS

Metabolic effects of AICAR in vivo. The infusion of AICAR resulted in plasma AICAR concentrations of 0.58 ± 0.07 mmol/l. Plasma glucose concentrations during the euglycemic clamp remained stable throughout the experiment and did not differ significantly between the two groups (AICAR: 5.3 ± 0.1 vs. control: 5.8 ± 0.1 mmol/l; not significant). The exogenous glucose infusion rate required to maintain euglycemia in the AICAR-infused animals was 49.1 ± 2.4 µmol·kg body weight⁻¹·min⁻¹, whereas no glucose was infused in the control group (Table 1). HGP was almost completely suppressed in the AICAR-infused rats. Baseline fasting plasma insulin, FFA, and lactate concentrations were similar in AICAR and control groups (insulin: 18 ± 2 vs. 25 ± 5 µU/ml, FFA: 1.5 ± 0.2 vs. 1.5 ± 0.2 mmol/l, and lactate: 0.6 ± 0.1 vs. 0.6 ± 0.1 mmol/l, respectively) and did not change during the experiment in the saline-infused animals. However, AICAR infu-
Table 1. In vivo glucose metabolism and kinase activities after AICAR infusion

<table>
<thead>
<tr>
<th>Glucose infusion rate, μmol·kg body weight⁻¹·min⁻¹</th>
<th>Control</th>
<th>AICAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatic glucose production, μmol·kg body weight⁻¹·min⁻¹</td>
<td>ND</td>
<td>0.05±0.01</td>
</tr>
<tr>
<td>AMPK activity, mmol·g muscle⁻¹·min⁻¹</td>
<td>ND</td>
<td>0.55±0.004</td>
</tr>
<tr>
<td>PL 3-kinase activity, % of control</td>
<td>ND</td>
<td>0.27±0.006</td>
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Values are means ± SE after 5-aminoimidazole-4-carboxamide 1-β-o-ribofuranoside (AICAR; n = 9) or saline infusion (Control; n = 12). Muscle mass is expressed as wet weight. AMPK, AMP-activated protein kinase; PI, phosphatidylinositol; ND, not determined. *P < 0.05 vs. Control.

AICAR infusion resulted in the activation of AMPK (Table 1) activity ~80% above controls (P < 0.05). Under these circumstances, glucose uptake rates in the soleus and the medial and lateral gastrocnemius increased significantly by 215, 80, and 115%, respectively, with AICAR compared with saline infusion (Fig. 1). Muscle PI 3-kinase activity (Table 1) was not affected by AICAR (99±18% of control; n = 7) compared with control rats (100±13%; n = 8). In contrast, PI 3-kinase activity was increased by ~3.5-fold (P < 0.0001 vs. control; n = 4) and ~3.9-fold (P < 0.0001 vs. control; n = 3; data not shown) in the insulin- and AICAR+ insulin-infused rats, respectively. The muscle glycogen content (Table 2) was not significantly altered by AICAR, whereas muscle glucose 6-phosphate concentrations were increased by more than 10-fold (P < 0.001) in muscles of AICAR-treated animals compared with those of saline-infused rats (P < 0.05). Muscle nucleotide and creatine phosphate concentrations (Table 2) were unchanged by the treatment with AICAR, whereas AICAR-monophosphate (ZMP) levels that were undetectable under saline infusion reached levels of 0.35 ± 0.04 μmol/g wet wt. Finally, lactate and pyruvate concentrations, as well as the lactate-to-pyruvate ratio, increased significantly in the AICAR-treated rats (Table 2).

Glucose uptake in epitrochlearis muscle in vitro. AICAR and insulin increased glucose uptake (Fig. 2A) in incubated epitrochlearis muscles by 100 and 170%, respectively, compared with basal values (P < 0.001). Combining AICAR with a maximal dose of insulin further increased muscle 2-DG uptake to rates that were 280% above basal (P < 0.005 vs. either insulin or AICAR alone). The addition of wortmannin to insulin inhibited the insulin-stimulated glucose uptake (P < 0.01 vs. insulin), whereas it did not inhibit AICAR-induced stimulation of glucose transport. Adding wortmannin to AICAR+insulin caused a significant decrease in 2-DG uptake (P < 0.001 vs. AICAR+insulin), although resulting rates were still higher than basal. Importantly, glucose uptake in the AICAR+insulin+ wortmannin and AICAR+wortmannin groups was similar (1.05 ± 0.10 and 1.24 ± 0.28 μmol·ml⁻¹·20 min⁻¹, respectively). The AICAR-induced increase in muscle glucose uptake was not inhibited by 8-SPT, an adenosine receptor antagonist (Fig. 2B). Electrical stimulation significantly increased muscle glucose transport activity (Fig. 3) compared with AICAR and basal groups (1.55 ± 0.16 vs. 1.04 ± 0.09 and 0.53 ± 0.14 μmol·ml⁻¹·20 min⁻¹, respectively; P < 0.01). Combining AICAR and contractile activity did not result in any significant increase in muscle glucose uptake compared with the effect of contractile activity alone (1.61 ± 0.22 μmol·ml⁻¹·20 min⁻¹).

DISCUSSION

The present study demonstrates that AICAR stimulates rat skeletal muscle glucose transport activity through a PI 3-kinase-independent pathway that may play an important role in the exercise-induced increase in skeletal muscle glucose transport activity. This conclusion is supported by 1) in vitro data from incubated epitrochlearis muscle showing an additive effect of both AICAR and insulin on glucose uptake, 2) the
absence of inhibition of AICAR-stimulated glucose transport with wortmannin, and 3) the nonadditive effect of AICAR and contractile activity on glucose uptake. Furthermore, this stimulatory effect of AICAR on glucose uptake and transport could also be demonstrated in vivo without PI 3-kinase activation.

There is substantial evidence to suggest that exercise and insulin act to promote glucose transport in the skeletal muscle through different mechanisms (14). The most convincing lines of evidence come from the demonstration that 1) insulin-induced glucose transport can be selectively inhibited without affecting contraction-mediated glucose uptake (25, 26) and that 2) exercise and maximal insulin stimulation have an additive effect on glucose transport (30, 50) and GLUT-4 translocation to the cell membrane (26).

Several cellular pathways proposed to play a potential role in the exercise-mediated increase in glucose transport have been studied. Intracellular calcium release from the sarcoplasmic reticulum, which occurs transiently during muscle fiber contraction, may facilitate glucose transport (24, 45). Protein kinase C activation, which is triggered by increases in intracellular calcium concentration, has also been proposed to modulate exercise-stimulated glucose transport, since inhibition of this protein reduces glucose transport during contraction (47). Nitric oxide, which is released in the skeletal muscle during contraction (1), has also recently been proposed to mediate the contraction-induced increase in muscle glucose transport (34, 49), although blockade of nitric oxide synthesis failed to inhibit the contraction-induced increase in muscle glucose uptake (18), suggesting that this pathway is nitric oxide independent. Finally, bradykinin has also recently been suggested to be involved in the exercise-induced muscle glucose transport and translocation of GLUT-4 to the cell membrane (20).

Contractile activity is also known to increase the concentration of the purine nucleotide AMP in the skeletal muscle (5, 17). Increases in the cellular concentration of AMP activate AMPK both allosterically and covalently through the phosphorylation of AMPK by AMPK kinase (12). The nucleoside AICAR diffuses into the cells, where it is phosphorylated by adenosine kinase to ZMP (2), which can activate AMPK (4). A recent study that examined the effect of AMPK activation by AICAR on the regulation of fat oxidation in a perfused rat hindlimb preparation also found that this compound increased skeletal muscle glucose uptake by using the arteriovenous balance technique (28). More recently, AICAR was found to increase muscle glucose transport in an isolated muscle preparation in a PI 3-kinase-independent way (13). However, AICAR has been shown to increase adenosine release from ischemic heart tissue (9), and recent data suggest that the adenosine receptor modulates the stimulation of muscle glucose transport by insulin and contraction (10, 38).

In the present experiment, we measured 2-DG muscle uptake to specifically evaluate glucose transport and phosphorylation induced by AICAR. When used at a
concentration of 0.5 mM, AICAR caused an ~100% increase in glucose uptake in the incubated muscle preparation, whereas insulin alone increased glucose uptake by ~170%. Using the same preparation, Hayashi et al. (13) reported that 2.0 mM AICAR increased glucose transport by ~3.5-fold compared with basal and slightly more with a maximally stimulating dose of insulin. Although the amplitude of the increases in glucose transport caused by AICAR or insulin alone in the present study was smaller, the ratio between the AICAR and the insulin-induced stimulations of glucose transport activity is similar in both studies. When AICAR and insulin were used in combination, glucose uptake increased by ~280% compared with basal values. Furthermore, the addition of 1.0 µM wortmannin, which is known to inhibit insulin-induced stimulation of PI 3-kinase activity, successfully blocked the effect of insulin on glucose transport activity. On the other hand, adding wortmannin to the muscles treated with AICAR did not affect glucose uptake. The addition of wortmannin to AICAR+insulin-treated muscles decreased glucose uptake comparably to those observed in the AICAR group. The lack of additivity of contractile activity and AICAR on glucose transport activity observed in the present study suggests that exercise-induced increase in skeletal muscle glucose transport and AICAR act through the same cellular pathway. The present data obtained from the isolated muscle preparation support those reported by Hayashi et al., showing that AICAR stimulates glucose transport in a PI 3-kinase-independent fashion and suggesting that this pathway, together with nitric oxide and bradykinin as well as other mechanisms not yet fully understood, may be involved in the exercise-induced stimulation of muscle glucose transport. Interestingly, Han et al. (10) recently showed that blocking the adenosine receptor diminishes the contraction-induced increase in glucose uptake, therefore supporting a role for the adenosine receptor in the exercised-induced stimulation of skeletal muscle glucose transport. However, the present study demonstrates that blocking the adenosine receptor with 8-SPT did not inhibit AICAR’s effect on muscle glucose transport. These data suggest that the AICAR-induced increase in muscle glucose transport activity is mainly due to AMPK activation and not to adenosine receptor activation.

The cellular mechanism by which AICAR-stimulated skeletal glucose transport activity is likely to be mediated is through the increase in GLUT-4 translocation to the cell membrane. Although not measured in the present experiment, this assumption is supported by immunofluorescence and cell-fractionation studies conducted in our laboratories on heart muscle showing that there was translocation of GLUT-4 to the cell membrane in response to stimulation with AICAR (36).

The infusion of AICAR in vivo led to an ~10-fold increase in plasma lactate concentrations. This could be due to AICAR inhibition of hepatic gluconeogenesis and/or activation of anaerobic glycolysis in muscle and other tissues. In regard to the former possibility, we found that HGP was almost completely inhibited by the infusion of AICAR in 18-h-fasted rats, when most of the endogenous glucose production can be attributed to hepatic gluconeogenesis. These data are consistent with the findings of Vincent et al. (40) obtained from isolated rat hepatocytes. In regard to the latter possibility, we found that AICAR infusion resulted in an ~10-fold increase in intracellular glucose 6-phosphate concentration, which was associated with an increase in intracellular concentrations of pyruvate and lactate without any changes in ATP/ADP/AMP/IMP, creatine phosphate, or muscle glycogen content. These data suggest that the AICAR-induced increase in glucose transport activity resulted in an increase in anaerobic glycolysis, as previously suggested (48), which also contributed to the increase in plasma lactate concentrations.

AICAR infusion resulted in an increase in AMPK activity of ~80% over saline-infused controls. This activation occurred without significant changes in skeletal muscle nucleotides, as previously reported (28, 37), and without a decrease in creatine phosphate concentrations. ZMP reached tissue concentrations that, according to studies conducted on hepatocytes (4), are known to activate AMPK, suggesting that the increase in muscle glucose transport was likely to be caused by the activation of AMPK rather than modification of the skeletal muscle energetic state.

In summary, this study was the first to examine AICAR’s effect on glucose metabolism in vivo. AICAR simultaneously increased skeletal muscle glucose transport activity independent of PI 3-kinase activation and inhibited HGP. Taken together with our in vitro studies, these data support the hypothesis that the AMPK pathway may play an important role in exercised-induced increase in glucose transport.

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