AMP-activated protein kinase activity and glucose uptake in rat skeletal muscle

NICOLAS MUSI,*1 TATSUYA HAYASHI,1* NOBUHARU FUJII,1 MICHAEL F. HIRSHMAN,1 LEE A. WITTERS,2 AND LAURIE J. GOODYEAR1
1Research Division, Joslin Diabetes Center and Department of Medicine, Brigham and Women’s Hospital and Harvard Medical School, Boston, Massachusetts 02215; and 2Endocrine-Metabolism Division, Department of Medicine and Biochemistry, Dartmouth Medical School, Hanover, New Hampshire 03755

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Musi, Nicolas, Tatsuya Hayashi, Nobuharu Fujii, Michael F. Hirshman, Lee A. Witters, and Laurie J. Good- year. AMP-activated protein kinase activity and glucose uptake in rat skeletal muscle. Am J Physiol Endocrinol Metab 280: E677–E684, 2001.—The AMP-activated protein kinase (AMPK) has been hypothesized to mediate contraction and 5-adenosynimidazole-4-carboxamide 1-β-D-ribonucleoside (AICAR)-induced increases in glucose uptake in skeletal muscle. The purpose of the current study was to determine whether treadmill exercise and isolated muscle contractions in rat skeletal muscle increase the activity of the AMPKa1 and AMPKa2 catalytic subunits in a dose-dependent manner and to evaluate the effects of the putative AMPK inhibitors adenosine 9-β-D-arabinofuranoside (ara-A), 8-bromo-AMP, and iodotubercidin on AMPK activity and 3-O-methyl-D-glucose (3-MG) uptake. There were dose-dependent increases in AMPKa2 activity and 3-MG uptake in rat epitrochlearis muscles with treadmill running exercise but no effect of exercise on AMPKa1 activity. Tetanic contractions of isolated epitrochlearis muscles in vitro significantly increased the activity of both AMPK isoforms in a dose-dependent manner and at a similar rate compared with increases in 3-MG uptake. In isolated muscles, the putative AMPK inhibitors ara-A, 8-bromo-AMP, and iodotubercidin fully inhibited AICAR-stimulated AMPKa2 activity and 3-MG uptake but had little effect on AMPKa1 activity. In contrast, these compounds had absent or minimal effects on contraction-stimulated AMPKa1 and AMPKa2 activity and 3-MG uptake. Although the AMPKa1 and AMPKa2 isoforms are activated during tetanic muscle contractions in vitro, in fast-glycolytic fibers, the activation of AMPKα2-containing complexes may be more important in regulating exercise-mediated skeletal muscle metabolism in vivo. Development of new compounds will be required to study contraction regulation of AMPK by pharmacological inhibition.

adepthosine 5′-monophosphate-activated protein kinase; contraction

Physical exercise decreases blood glucose concentrations in people with diabetes, and this is due in part to an increase in the rate of glucose uptake into the contracting muscles (4, 20, 33). It is well established that the mechanism by which insulin and contraction stimulate glucose uptake into skeletal muscle involves the translocation of the GLUT-4 glucose transporter to the cell surface (17). However, there is considerable evidence that the underlying mechanisms responsible for insulin and contraction-stimulated GLUT-4 translocation and glucose uptake are different. For example, the combination of contraction and insulin has additive or partially additive effects on glucose uptake in skeletal muscle (17, 21). In the insulin-resistant (fa/fa) rat (24, 25) and people with type 2 diabetes mellitus (23, 44), insulin-stimulated GLUT-4 translocation is impaired (25, 44) but exercise-stimulated GLUT-4 translocation is normal (23, 24). Studies investigating the signaling mechanisms regulating glucose uptake in muscle have shown that phosphatidylinositol 3-kinase (PI 3-kinase) is necessary for insulin-stimulated, but not contraction-stimulated, glucose uptake (10, 27, 28, 43).

There is increasing evidence that the AMP-activated protein kinase (AMPK) is a key mediator of contraction-stimulated glucose uptake in skeletal muscle (3, 15, 16, 26, 30). AMPK is active as a heterotrimer consisting of one catalytic subunit (α) and two noncatalytic subunits (β, γ) (14, 22). AMPK is activated allosterically in muscle by increases in the creatine-to-phosphocreatine and AMP-to-ATP ratios and is also activated by phosphorylation by an upstream kinase (AMPKK) and is inhibited by the activity of protein phosphatases (13, 14, 32). Treadmill-running exercise (35, 40) and muscle contractions induced by electrical stimulation (16, 34, 39) in rats significantly increase AMPK activity. Most studies suggesting a role for AMPK in the regulation of muscle glucose uptake are based on experiments using 5-adenosynimidazole-4-carboxamide 1-β-D-ribonucleoside (AICAR), a compound that is converted to 5-adenosynimidazole-4-carboxamide ribonucleotide (ZMP) within muscle (30). ZMP can then mimic the effect of AMP to increase AMPK activity (19). Acute AICAR treatment increases glucose up-

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take in skeletal muscle (3, 16, 30) and promotes GLUT-4 translocation to the plasma membrane (26). The combination of insulin and AICAR treatments have additive effects on glucose uptake, whereas there is no additivity with the combination of AICAR plus contraction (3, 16). Furthermore, similar to contraction-simulated uptake, AICAR-stimulated uptake is not inhibited by wortmannin, a pharmacological inhibitor of PI 3-kinase (3, 16). This is evidence that both muscle contraction and AICAR stimulate glucose uptake through a common insulin-independent mechanism. A recent study from our group (15) also showed a close correlation between increases in AMPK activity and glucose uptake in rat skeletal muscle under numerous conditions of metabolic stress, suggesting that activation of AMPK may be a common mechanism mediating insulin-independent glucose uptake aimed at restoring cellular energy stores.

The AMP analog adenine 9-b-D-arabinofuranoside (ara-A) and the adenosine kinase inhibitor iodotubercidin decrease the activity of AMPK in isolated hepatocytes in vitro (19). Subsequently, it was demonstrated that these two compounds decrease AICAR- and cyanide-stimulated glucose uptake in isolated rat papillary muscles (36). Although this proved that ara-A and iodotubercidin inhibit AICAR- and cyanide-stimulated glucose uptake in an isolated papillary muscle preparation, AMPK activity was not measured in this study. The effects of these compounds on contraction-stimulated AMPK activity and glucose uptake in skeletal muscle have not been evaluated.

In the present study, we investigated the relationship between isoform-specific AMPK activation and glucose uptake. We examined whether increasing the number of contractions in an isolated muscle preparation in vitro and different exercise intensities in vivo activate AMPK in a dose-dependent manner, and we compared changes in AMPK activity to changes in glucose uptake. We also evaluated the effects of ara-A, iodotubercidin, and 8-bromo-AMP (another AMP analog) on AICAR- and contraction-stimulated glucose uptake and isoform-specific AMPK activity.

**METHODS**

**Experimental animals.** Male Sprague-Dawley rats weighing 120–140 g were purchased from Taconic Farms (Germantown, NY). Animals were housed in an animal room maintained at 23°C with a 12:12-h light-dark cycle and fed standard laboratory chow and water ad libitum. Food was removed from rats at 2300, allowing for 4 h of feeding during the dark cycle, and experiments were performed between 0900 and 1100. Protocols for animal use and euthanasia were reviewed and approved by the Institutional Animal Care and Use Committee of the Joslin Diabetes Center and were in accordance with National Institutes of Health guidelines.

**Materials.** [γ-32P]ATP, 3-O-methyl-D-[3H]glucose (3-MG) and D-[14C]mannitol were obtained from New England Nuclear (Boston, MA), and protein A/G agarose beads were from Santa Cruz Biotechnology (Santa Cruz, CA). AICAR, ara-A, 8-bromoadenosine 5′-monophosphate (8-bromo-AMP), 4-amino-5-iodo-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine (iodotubercidin), and all other standard reagents were purchased from Sigma Chemical (St. Louis, MO).

**Treadmill exercise studies.** Rats were accustomed to a rodent treadmill (Quinton Instruments, Seattle, WA) for 5 min/day for 2 days before the experiment. Rats ran on the treadmill for 1 h at 18 or 32 m/min up a 10% incline. Animals were decapitated immediately after exercise, and epitrochlearis muscles were rapidly dissected, frozen in liquid nitrogen, and used for AMPK activity determination (see Assays for muscle enzymes and metabolites). The time elapsed between cessation of exercise and the freezing of muscles was 1 min, and there was no significant muscle activity of the forelimbs before dissection of the muscles. To measure 3-MG uptake, muscles were mounted on an incubation apparatus as previously described (16), and the elapsed time between cessation of exercise and mounting of the muscles on the apparatus was 2 min. Muscles were then preincubated for 20 min in Krebs-Ringer bicarbonate (KRB) buffer containing 2 mM pyruvate at 37°C and transferred to transport buffer for assessment of glucose uptake (see 3-MG uptake).

**Contraction and AICAR treatment in isolated muscles.** Animals were killed by decapitation, and the epitrochlearis muscles were rapidly dissected. Both ends of each muscle were tied with suture (silk 4–0) and mounted on the incubation apparatus. The buffers were continuously gassed with 95% O2-5% CO2. Muscles were preincubated in KRB containing 2 mM pyruvate for 30 min at 37°C. For the isolated muscle contraction dose-response studies, muscles were stimulated for 1, 3, 10, or 15 min (one 10-s contraction/min, train rate = 1/min, train duration = 10 s, pulse rate = 100 pulse/s, duration = 0.1 ms, volts = 100 V). The postcontraction experiments had the same preincubation and 10-min contraction protocol as described above; muscles were then incubated at rest for 10, 30, or 60 min in KRB containing 2 mM pyruvate at 37°C. muscles were then frozen in liquid nitrogen and were used for AMPK activity measurements or to measure glucose uptake (see 3-MG uptake).

For the inhibitor studies, isolated rat epitrochlearis muscles were preincubated in KRB containing 2 mM pyruvate at 37°C for 30 min in the presence or absence of ara-A (2.5 mM), 8-bromo-AMP (1 mM), or iodotubercidin (10 μM). These concentrations were determined on the basis of previous reports (36) and our own preliminary dose-response studies. The muscles were then incubated for 20 min in buffer containing 2 mM AICAR or contracted for 10 min as described above. When added, the inhibitors ara-A, 8-bromo-AMP, and iodotubercidin were present throughout the entire incubation. Muscles were then immediately frozen in liquid nitrogen and subsequently analyzed for AMPK activity or used to measure glucose uptake (see 3-MG uptake).

**3-MG uptake.** 3-MG uptake was measured in 2 ml KRB containing 1 mM 3-O-methyl-D-[3H]glucose (1.5 μCi/ml) and 1 mM D-[14C]mannitol (0.45 μCi/ml) at 30°C for 10 min. AICAR (2 mM), ara-A (2.5 mM), 8-bromo-AMP (1 mM), and iodotubercidin (10 μM) were added to the buffer if they had been present during the previous incubation period. Muscles were processed, radioactivity was determined by liquid scintillation counting for dual labels, and the transport rate was determined as previously described (16).

**Assays for muscle enzymes and metabolites.** For the measurement of isoform-specific AMPK activity, muscles were homogenized in ice-cold lysate buffer (1:100, wt/vol) containing 20 mM Tris·HCl (pH 7.4), 1% Triton X-100, 50 mM NaCl, 250 mM sucrose, 50 mM NaF, 5 mM sodium pyrophosphate, 2 mM dithiothreitol, 4 mg/l leupeptin, 50 mg/l trypsin inhibitor, 0.1 mM benzamidine, and 0.5 mM phenylmethylsulfonyl fluoride and then centrifuged at 14,000 g for 20 min at 4°C.
The supernatants (200 μg protein) were immunoprecipitated with isofrom-specific antibodies to the α1 or α2 catalytic subunits of AMPK and protein AG/G beads. These are antipeptide antibodies made to the amino acid sequences DFY-LATSPPDSFLDDHILTR (339–358) of α1 and MDDSAM-HIPPGKLKPH (352–366) of α2 (38). Immunoprecipitates were washed twice in lysis buffer and twice in wash buffer (240 mM HEPES and 480 mM NaCl). Kinase reactions were performed in 40 mM HEPES (pH 7.0), 0.1 mM SAMS peptide (6), 0.2 mM AMP, 80 mM NaCl, 0.8 mM dithiothreitol, 5 mM MgCl₂, and 0.2 mM ATP (2 μCi [γ-32P]ATP) in a final volume of 40 μl for 20 min at 30°C. At the end of the reaction, a 20-μl aliquot was removed and spotted on Whatman P81 paper. The papers were washed for 20 min six times in 1% phosphoric acid and once with acetone. Radioactivity was quantitated with a scintillation counter.

Statistical analysis. Data are expressed as means ± SE. Comparison of means was by one-way ANOVA followed by post hoc comparison using the Fisher’s protected least significant difference method. For comparison of two means, an unpaired Student’s t-test was performed. P < 0.05 was considered statistically significant.

RESULTS

Effects of exercise intensity on AMPK activation and 3-MG uptake. We measured isofrom-specific AMPK activity and 3-MG uptake in rat epitrochlearis muscles in response to different intensities of treadmill exercise. Rats performed treadmill running at 18 or 32 m/min for 1 h, up a 10% grade. Figure 1A shows that, with lower-intensity exercise, there was no increase in α1 activity and only a tendency for α2 activity with higher-intensity exercise. On the other hand, there was a tendency for an increase in α2 activity with low-intensity exercise and a significant increase in α2 activity with higher-intensity exercise (2.1-fold above sedentary, P < 0.05). 3-MG uptake increased twofold above sedentary levels with low-intensity exercise (P < 0.05) and 4.6-fold with high-intensity exercise (P < 0.01, Fig. 1B). Muscle glycogen concentrations decreased 24% below baseline with low-intensity exercise (P < 0.05) and 52% with high-intensity exercise (P < 0.001), confirming that both exercise intensities of treadmill exercise caused significant recruitment of epitrochlearis muscles. These findings show that, under these conditions, AMPKα2 is preferentially activated during treadmill exercise and that the magnitude of the increases in AMPKα2 activity and glucose uptake depends on exercise intensity. Western blotting of immunoprecipitates and supernatants showed that the efficiency of immunoprecipitation was 75% for AMPKα1 and 85% for AMPKα2 and that there was no difference between basal and stimulated samples.

Effect of contraction number on AMPK activation and 3-MG uptake. In vitro muscle contractions increase total AMPK activity as measured in ammonium sulfate precipitates (16). To determine whether there is a dose-dependent increase in isoform-specific AMPK activity with contraction, isolated epitrochlearis muscles were contracted tetanically, with one 10-s contraction/min for various periods of time. Figure 2 shows that a single contraction was enough to begin to increase the activity of both the α1 and α2 isoforms. Maximal activity of both AMPK isoforms was observed with 10 contractions, with no further increase with 15 contractions. Importantly, there was a striking similarity between the increases in AMPKα1 and AMPKα2 activities and the increases in 3-MG uptake (Fig. 2). Muscle glycogen concentrations tended to decrease with a single contraction (17% below baseline) and decreased significantly with higher numbers of contractions (28% decrease with 3 and 32% decrease with 10 contractions, P < 0.05). No further decreases in glycogen concentrations were observed with 15 contractions.

AMPK activity and glucose uptake in the period after contraction. The period after contraction is characterized by enhanced glucose uptake into muscle (12). To determine whether AMPK activity remains elevated after contractions, rat epitrochlearis muscles were contracted in vitro for 10 min and then incubated at rest for an additional 10, 30, or 60 min. The activity of both AMPK isoforms decreased rapidly after the cessation of contraction (t½ = 8 min). In contrast, the rate of decrease in 3-MG uptake was much slower, with only a 48% decrease by 60 min after the contractions (Fig. 3).
Effect of putative AMPK inhibitors on isoform-specific AMPK activity and glucose uptake.

We evaluated the effect of preincubating isolated skeletal muscle with the reported AMPK inhibitors ara-A (2.5 mM) and iodo-tubercidin (10 μM) and the AMP analog 8-bromo-AMP (1 mM). After preincubation, muscles were treated for 20 min with AICAR (2 mM) or contracted for 10 min in the presence or absence of the different compounds. Contraction force was measured by a force transducer, and tracings were obtained using a chart recorder. The compounds did not have any effect on contraction force. Ara-A did not alter basal levels of AMPKα1 activity (Fig. 4A), α2 activity (Fig. 4B), or 3-MG uptake (Fig. 4C). Ara-A fully inhibited AICAR-stimulated AMPKα2 activity and 3-MG uptake (P < 0.05) but had no effect on AMPKα1 activity. In contrast, ara-A had no inhibitory effect on contraction-stimulated AMPKα1 or α2 activity and had a mild effect on 3-MG uptake (30% decrease, P < 0.05). Similar to ara-A, 8-bromo-AMP inhibited AICAR-stimulated AMPKα2 activity and 3-MG uptake but had minimal inhibitory effects on contraction-stimulated AMPK activity and 3-MG uptake (data not shown). During the preliminary dose-response studies, higher concentrations of the compounds did not result in further decreases in AICAR- or contraction-stimulated AMPK activity.

Figure 5 shows that iodo-tubercidin decreased basal AMPKα2 activity by 30% (P < 0.05) but had no effect on basal α1 activity or 3-MG uptake. Similar to the effects of ara-A and 8-bromo-AMP, iodo-tubercidin fully inhibited AICAR-stimulated AMPKα2 activity (P < 0.01) and 3-MG uptake (P < 0.01) but did not significantly alter AMPKα1 activity. Iodo-tubercidin did not decrease contraction-induced activation of either AMPK isoform and did not affect 3-MG uptake in the skeletal muscle.

DISCUSSION

In skeletal muscle, both the α1 and α2 isoforms of the AMPK catalytic subunit are expressed (38). In
INS-1 cells, there are differences in the subcellular localization of AMPKα1 and -α2, with α1 localized predominantly in the cytosol whereas α2 is found in both the cytosol and the nucleus (37). A study done in rat liver also showed that AMPKα1 and AMPKα2 have different substrate specificity (42). These differences in the subcellular localization (37) and substrate specificity (42) between AMPKα1 and AMPKα2 suggest that there are distinct functions of the isoforms in the regulation of metabolic processes (22). Our current data, showing that in epitrochlearis muscles the α2 but not the α1 isoform of the catalytic subunit of AMPK was activated during treadmill exercise along with recent work showing that moderate-intensity exercise preferentially activates the α2 isoform in human skeletal muscle (9, 41), also support the concept of differential regulation of the α1 and α2 isoforms.

In contrast to the differential regulation of AMPKα1 and AMPKα2 during in vivo exercise, we found that both isoforms are activated in isolated epitrochlearis muscles contracted in vitro. These muscles are composed mostly of fast-glycolytic (FG) fibers and there is evidence that treadmill running in rats causes more depletion of glycogen in fast-oxidative glycolytic (FOG) and slow-oxidative (SO) fibers than in FG fibers (2), suggesting that FOG and SO fibers might be recruited more significantly during treadmill running. In the present study, we observed significant depletion of muscle glycogen during treadmill exercise, especially at the higher intensity (52%), confirming the recruitment of epitrochlearis muscles during the protocol. This fall in glycogen was associated with significant stimulation of AMPKα2 but not -α1 activity; nevertheless, in future studies it will be necessary to evaluate the effect of exercise on isoform-specific AMPK activity in different fiber types.

A previous study using gastrocnemius and soleus muscles found that, during in situ contractions induced by electrical stimulation of the sciatic nerve, the α2 but not the α1 AMPK isoform was activated (39). This is in accordance with the effect of exercise on the activity of the AMPK isoforms found in the present study but opposite to the response observed with isolated muscle contractions. The cause of the discrepancy in AMPKα1 activation induced by these models is yet to be determined; however, these findings suggest that the changes in energy status induced by exercise are more similar to the effects of sciatic nerve-stimulated contractions in situ than to the changes induced by contraction of isolated muscles in vitro. A recent study showed that super-maximal sprint cycle exercise for 30 s leads to activation of both AMPK isoforms in human skeletal muscle (5). Therefore, it is possible that the α1 isoform is more resistant and is activated in

**Fig. 4.** Effects of 9-β-D-arabinofuranoside (ara-A) on 5-aminoimidazole-4-carboxamide 1-β-D-ribonucleoside (AICAR)- and contraction-stimulated AMPKα1 activity (A), AMPKα2 activity (B), and 3-MG uptake (C). Isolated rat epitrochlearis muscles were preincubated and then incubated in the presence or absence of 2.5 mM ara-A for 30 min and then treated with 2 mM AICAR for 20 min or contracted (contr.) for 10 min. Data are means ± SE; n = 3–9 per group. *P < 0.05 vs. group not treated with ara-A.

**Fig. 5.** Effects of iodotubercidin on AICAR- and contraction-stimulated AMPKα1 activity (A), AMPKα2 activity (B), and 3-MG uptake (C). Isolated rat epitrochlearis muscles were preincubated and then incubated in the presence or absence of 10 μM iodotubercidin for 30 min and then treated with 2 mM AICAR for 20 min or contracted for 10 min. Data are means ± SE; n = 3–9 per group. *P < 0.05 vs. group not treated with iodotubercidin, †P < 0.01 vs. group not treated with iodotubercidin.
situations of extreme contraction intensity, such as in vitro tetanic contraction of isolated muscles and sprint exercise.

It has been postulated that AMPK is a key mediator of exercise-induced glucose uptake in muscle (3, 15, 16, 26, 30). If the activity of this enzyme increases in a dose-dependent manner in parallel with increases in glucose uptake, this would further strengthen the possibility of an important role for AMPK in mediating rates of glucose uptake. As shown in Fig. 1, the degree of activation of AMPKα2 showed a similar trend compared with the increases in 3-MG uptake in response to running exercise. We also found a strong relationship between the activation of the AMPKα1 and α2 isoforms and the rate of contraction-stimulated 3-MG uptake (Fig. 2). The AMPK activity assay used in the current study is performed under saturating concentrations of AMP measuring only AMPKK-induced changes in AMPK activity. Therefore, because potential allosteric activation of the AMPK isoforms during exercise is not determined by the assay, it is possible that there is some degree of AMPKα1 activation in vivo that is not detected using currently available assay systems.

There is evidence that the soleus muscle (SO fiber) is particularly resistant to the effects of AICAR in increasing glucose uptake and AMPK activity (Ref. 7 and T. Hayashi, M. F. Hirshman, and L. J. Goodyear, unpublished data). Recently, in a model using muscles saturated with glycogen, contraction led to an increase in glucose uptake but no change in total AMPK activity in soleus muscle, whereas in white gastrocnemius muscle, both glucose uptake and AMPK activity increased in response to contraction. This finding suggests that, in soleus under these specific conditions, contraction-mediated glucose uptake is AMPK independent (8). In isolated soleus muscles from fasted rats (not glycogen supercompensated), we found that contraction increases the activity of both AMPK isoforms as well as glucose uptake (T. Hayashi, M. F. Hirshman, and L. J. Goodyear, unpublished data). Thus contraction-induced changes in AMPK activity may vary depending on the preexisting glycogen content. It will be important to measure isoform-specific AMPK activity in the glycogen-supercompensated model used by Derave et al. (8), because the lack of an increase in total AMPK activity in the supercompensated red muscle with contraction was still associated with a significant alteration in acetyl-CoA carboxylase activity, a sensitive intracellular reporter of AMPK activity.

The period after contraction is characterized by increased glucose disposal into skeletal muscle (12). In the present study, we found that the activity of both isoforms decreased rapidly in the postcontraction period, whereas 3-MG uptake remained elevated (Fig. 3). This suggests that, although AMPK may be involved in initiating increases in glucose uptake during contraction, sustained enzyme activity is not necessary to maintain uptake after contraction. Studies of GLUT-4 vesicle kinetics suggest that plasma membrane GLUT-4 is elevated immediately and 30 min after treadmill exercise in rats, and that by 2 h after exercise, plasma membrane GLUT-4 returns to baseline values (11). Therefore, AMPK may be involved in triggering GLUT-4 to translocate to the plasma membrane, but another mechanism may be responsible for maintaining transporters at the membrane in the period after exercise. This mechanism could be AMPK independent, or it could be due to the prolonged activation of one or more putative “downstream” substrates of AMPK.

Although most data now show that AMPK is activated during contraction concurrently with an increase in glucose uptake (3, 15, 16, 26, 30), studies evaluating the effects of AMPK inhibitors on stimulated enzyme activity could provide more direct evidence of the enzyme’s involvement in exercise-mediated glucose uptake. In isolated rat hepatocytes, the AMP analog ara-A, a precursor of ara-ATP, significantly inhibited AMPK activity in the absence of AMP (19). The competitive inhibitor of adenosine kinase iodotubercidin (18, 31), which is also a potent serine-threonine kinase inhibitor (29), had similar inhibitory effects on AMPK (19). Subsequently, in isolated heart papillary muscles, these two compounds were shown to inhibit AICAR- and cyanide-stimulated glucose uptake, but the effect of the compound on AMPK activity was not determined (36). We found that iodotubercidin, ara-A, and 8-bromo-AMP are not effective inhibitors of the AMPKα1 isoform, suggesting that the α1 isoform is resistant to the effects of these compounds. These compounds did, however, significantly inhibit AICAR-stimulated AMPKα2 activity and glucose uptake. Ara-A had a mild AMPK-independent inhibitory effect on contraction-stimulated glucose uptake, suggesting that, similar to the glycogen-supercompensated model, in certain situations AMPK may not be indispensable for contraction-regulated uptake. The ability of the inhibitors to blunt AICAR-stimulated AMPKα2 activity and not contraction-stimulated activity suggests that AICAR and contraction do not necessarily share a common pathway to increase AMPK activity and glucose uptake. However, it is also possible that these compounds are acting upstream of AMPK and not directly inhibiting the kinase. In fact, iodotubercidin has recently been shown to alter AICAR-stimulated AMPK activity by decreasing the concentrations of ZMP (1). Ara-A and 8-bromo-AMP could also be competing with AICAR at the level of the nucleoside transporter and/or adenosine kinase, reducing intracellular availability of ZMP. Overall, the lack of effect of the compounds on contraction-stimulated AMPK activity makes them unsatisfactory agents for the study of AMPK as a possible mediator of contraction-induced glucose uptake. The use of more specific and potent AMPK inhibitors or the development of AMPK knockout animal models will be necessary to fully clarify this issue and to definitively determine the role of AMPK in regulating skeletal muscle metabolism.

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