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Effect of acute activation of 5'-AMP-activated protein kinase on glycogen regulation in isolated rat skeletal muscle

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1Department of Medicine and Clinical Science, Graduate School of Medicine, 2Laboratory of Nutrition Chemistry, Division of Food Science and Biotechnology, Graduate School of Agriculture, and 3Laboratory of Sports and Exercise Medicine, Graduate School of Human and Environmental Studies, Kyoto University, Kyoto, Japan

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Miyamoto L, Toyoda T, Hayashi T, Yonemitsu S, Nakano M, Tanaka S, Ebihara K, Masuzaki H, Hosoda K, Ogawa Y, Inoue G, Fushiki T, Nakao K. Effect of acute activation of 5'-AMP-activated protein kinase on glycogen regulation in isolated rat skeletal muscle. J Appl Physiol 102: 1007–1013, 2007. First published November 22, 2006; doi:10.1152/japplphysiol.01034.2006.—5'-AMP-activated protein kinase (AMPK) has been implicated in glycogen metabolism in skeletal muscle. However, the physiological relevance of increased AMPK activity during exercise has not been fully clarified. This study was performed to determine the direct effects of acute AMPK activation on muscle glycogen regulation. For this purpose, we used an isolated rat muscle preparation and pharmacologically activated AMPK with 5-aminoimidazole-4-carboxamide-1β-D-ribofuranoside (AICAR). Tetanic contraction in vitro markedly activated the α1- and α2-isofoms of AMPK, with a corresponding increase in the rate of 3-O-methylglycerol uptake. Incubation with AICAR elicited similar enhancement of AMPK activity and 3-O-methylglycerol uptake in rat epitrochlearis muscle. In contrast, whereas contraction stimulated glycogen synthase (GS), AICAR treatment decreased GS activity. Insulin-stimulated GS activity also decreased after AICAR treatment. Whereas contraction activated glycogen phosphorylase (GP), AICAR did not alter GP activity. The muscle glycogen content decreased in response to contraction but was unchanged by AICAR. Lactate release was markedly increased when muscles were stimulated with AICAR in buffer containing glucose, indicating that the glucose taken up into the muscle was catabolized via glycolysis. Our results suggest that AMPK does not mediate contraction-stimulated glycogen synthesis or glycogenolysis in skeletal muscle and that acute AMPK activation leads to an increased glycolytic flux by antagonizing contraction-stimulated glycogen synthesis.

contraction; glycogen synthase; glycogen phosphorylase; epitrochlearis muscle; glycolysis

EXERCISE PROFOUNDLY AFFECTS glycogen metabolism by stimulating glycogenolysis during exercise, which is followed by the resynthesis of glycogen after exercise. Intracellular glycogen plays a major role as a fuel for acute muscle contraction, and its concentration is dramatically reduced in response to a single bout of exercise (6, 14, 25, 38). The facilitation of glycogen resynthesis after exercise causes a marked accumulation of glycogen, i.e., the well-known phenomenon of glycogen supercompensation. Similar to exercise, insulin causes strong glucose uptake and glycogen synthesis. Therefore, the increase in insulin sensitivity in skeletal muscle after exercise also plays an important role in glucose metabolism (11, 25, 36). In contrast to the physiological relevance of muscle glycogen, the signaling mechanisms leading to the regulation of glycogen synthesis and glycogenolysis in contracting muscle have not been fully clarified.

Several aspects of the contraction-evoked metabolic events in skeletal muscle have been shown to be related to 5'-AMP-activated protein kinase (AMPK), including GLUT4 translocation and glucose transport (1, 4, 18, 19, 27, 31, 32), fatty acid oxidation via the inactivation of acetyl-CoA carboxylase (23, 32, 41, 43), GLUT4 expression (7, 21, 22, 32, 35, 44, 48), and insulin sensitivity (12, 24, 32). AMPK, a heterotrimeric serine/threonine protein kinase, consists of a catalytic α-subunit and regulatory β- and γ-subunits. Two distinct α-isofoms, α1 and α2, are expressed in skeletal muscle (39), and both isoforms can be activated in response to muscle contraction (40). AMPK is allosterically activated in response to an elevation in AMP concentration or in the AMP-to-ATP ratio. It is also activated when phosphorylated by upstream kinases (42, 46). Therefore, it has been hypothesized that AMPK acts as an intracellular energy sensor that plays a key role in regulating cellular metabolism in skeletal muscle (9, 13, 18, 19, 23, 37, 41, 43).

Recent studies of natural and manipulated mutations in AMPK suggest that chronic changes in AMPK action have substantial effects on glycogen metabolism in skeletal muscle. The glycogen concentration in the gastrocnemius muscles of mice that muscle-specifically express a dominant-negative kinase-dead form of the α-subunit is about half that observed in nontransgenic mice (31), although glycogen synthase (GS) activity and glycogen phosphorylase (GP) activity are not significantly different between the transgenic and nontransgenic animals under basal conditions or after contraction (30). The AMPK R225Q mutation in the PRKAG3 gene, which encodes the muscle-specific isoform of the γ2-subunit of AMPK, leads to increased glycogen accumulation in Hampshire pig skeletal muscle (29). The amount of glycogen in the gastrocnemius muscle is twofold higher in the R225Q PRKAG3 transgenic mouse than in the wild-type mouse but is unaltered in the PRKAG3-null mouse (5). In contrast, another mutation has been identified in the pig PRKAG3 gene (V224I) that is associated with low muscle glycogen (10). It has also been suggested that mutations in PRKAG2, the gene for the γ2-subunit of AMPK, cause glycogen-associated vacuoles to

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form in myocytes, leading to a glycogen storage disease of the cardiac muscle (2).

The physiological relevance of the acute elevation of AMPK activity during exercise to muscle glycogen regulation has not been fully elucidated. Halse et al. (16) reported GS inactivation in accordance with AMPK activation after 2 h of glucose starvation in the medium in human myoblast cells in culture. They also showed that stimulation by 5-aminoimidazole-4-carboxamide-1-B-d-ribofuranoside (AICAR) or hydrogen peroxide caused AMPK activation and concurrent GS inactivation. Wojtaszewski et al. (45) demonstrated that the addition of AICAR to the circulation medium resulted in increased AMPK-α2 activity and decreased GS activity in perfused rat skeletal muscles. AICAR is taken up into muscle cells and metabolized to form ZMP, a monophosphorylated derivative that mimics the stimulatory effects of AMP on AMPK without changing the concentration of AMP or ATP (17). In fact, GS Ser57 can be phosphorylated and inactivated by AMPK in vitro (8) and in vivo (26). Wojtaszewski et al. also found significant activation of α2-AMPK and inactivation of GS when muscle glycogen was depleted by prolonged exercise and restricted diet. In contrast, repeated activation of AMPK by the administration of AICAR for 5–28 days increased glycogen concentration in rat skeletal muscles (7, 21, 44). Aschenbach et al. (3) demonstrated acute activation of AMPK-α2 in red and white gastrocnemius muscles in normal rats after a single intraperitoneal dose of AICAR. However, GS activity was reduced only in white gastrocnemius muscle, whereas GS activity conversely increased in red gastrocnemius muscle (3). They also made the contradictory observation that in vitro incubation with AICAR had no effect on GS activity in isolated rat muscles (epitrochlearis or flexor digitorum brevis) (3).

Therefore, we undertook the present study to elucidate the direct effects of acute AMPK activation, which is comparable to activation in contracting muscle on glycogenolysis and glycogen synthesis in mature skeletal muscle. For this purpose, we used an isolated rat muscle preparation and pharmacologically manipulated muscle AMPK with AICAR stimulation, which allowed us to activate AMPK in red and white skeletal muscle and facilitates the regeneration of ATP via glycogenolysis.

### MATERIALS AND METHODS

**Materials.** All radioactive materials ([γ-32P]ATP, 3-O-methyl-[3H]glucose, [14C]mannitol, [14C]UDP-glucose, and [14C]glucose 1-phosphate) were obtained from Perkin Elmer Japan (Yokohama, Japan), human insulin (Humulin R) from Eli Lilly (Indianapolis, IN), P81 filter paper from Whatman (Brentford, UK), and protein A-Sepharose from GE Healthcare Bioscience (Little Chalfont, UK). All other reagents were of analytic grade and were obtained from Sigma-Aldrich (St. Louis, MO), unless otherwise stated.

**Animals.** Male Sprague-Dawley rats (120–130 g body wt; Japan SLC, Hamamatsu, Japan) were housed in an animal facility maintained at 20°C with a 12:12-h light-dark cycle and allowed free access to water and standard rodent chow. After an overnight fast, the rats were randomly assigned to experimental groups. The Kyoto University Graduate School of Medicine Committee on Animal Research approved all experimental procedures.

**Muscle sample preparation.** Animals were killed by cervical dislocation. The epitrochlearis muscles were rapidly isolated and incubated as previously described (19), with some modifications. Briefly, the muscles were preincubated for 40 min in 6 ml of Krebs-Ringer bicarbonate (KRB) buffer, pH 7.4, containing 2 mM sodium pyruvate (KRB-P). For the dose-response and time-course changes in AICAR-stimulated AMPK activity, muscles were incubated in the absence or presence of AICAR (0.03, 0.125, 0.5, 2, or 8 mM) for 40 min or in the presence of 2 mM AICAR for 10, 30, 40, or 60 min, respectively. On the basis of these experiments, we decided to stimulate muscles with 2 mM AICAR for 40 min in other experiments (see RESULTS).

For insulin treatment, muscles were preincubated and then incubated in the presence of 1 μM insulin for 40 min. For epinephrine treatment, muscles were preincubated and then incubated in the presence of 3 μg/ml epinephrine for the last 15 min of the incubation period (total 40 min). Muscle contractions were induced by preincubation of muscles and then incubation in KRB-P for 40 min, followed by electrical stimulation during the last 10 min, as described previously (19) (1/min train rate, 10-s train duration, 100-Hz pulse rate, 0.1-ms pulse duration, 100 V). The buffers were continuously gassed with 95% O2-5% CO2 and maintained at 37°C. The muscles were then used for glucose uptake measurements (see Glucose uptake) or trimmed and immediately frozen in liquid nitrogen for other assays. For the measurement of lactate release, we used KRB containing 8 mM glucose during preincubation and stimulation. AICAR treatment and muscle contractions were performed as described above (see RESULTS).

**Isoform-specific AMPK activity.** AMPK activity was determined as described previously, with slight modifications (40). Frozen muscles were homogenized in ice-cold lysis buffer (1:60, wt/vol) containing 20 mM HEPES (pH 7.4), 1% Triton X-100, 50 mM sodium chloride, 50 mM sodium fluoride, 5 mM sodium pyrophosphate, 2 mM dithiothreitol, 4 mg/ml leupeptin, 50 μg/ml trypsin inhibitor, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonfluoride and then centrifuged at 14,000 g for 20 min at 4°C. The supernatants (200 μg of protein) were immunoprecipitated with isofrom-specific antibodies directed against the α1- or α2-subunit of AMPK (40) and protein A-Sepharose beads. The immune complex was washed extensively with 240 mM HEPES (pH 7.0) and 480 mM sodium chloride. Kinase activity was determined by the phosphorylation of the SAMS peptide (40). The kinase reaction was carried out in 40 mM HEPES (pH 7.0), 0.1 mM SAMS, 0.2 mM AMP, 80 mM sodium chloride, 0.8 mM dithiothreitol, 5 mM magnesium chloride, and 0.2 mM ATP (2 μCi of [γ-32P]ATP) for 20 min at 37°C. The reaction products were then spotted on P81 filter papers, which were extensively washed in 1% phosphoric acid, and the radioactivity on the dried papers was quantitated with a liquid scintillation counter (Aloka, Tokyo, Japan).

**Glucose uptake.** We evaluated 3-O-methylglucose (3-MG) uptake as an index of glucose uptake activity. 3-MG uptake was determined as described previously (19), with modifications. After the incubation period, muscles were incubated in KRB containing 1 mM 3-MG, 1.5 μCi/ml [3H]-3-MG, 7 mM mannitol, and 0.3 μCi/ml [14C]mannitol at 30°C for 10 min. The muscles were then trimmed and frozen in liquid nitrogen. The muscles were processed by incubation in 1 M NaOH at 85°C for 10 min, and the digestates were neutralized with HCl. The radioactivity in aliquots of the digestates was determined by liquid scintillation counting of dual labels, and the intracellular and intracellular spaces were calculated. The rate of 3-MG uptake was expressed as micromoles of 3-MG per milliliter of intracellular space per hour.

**GS activity.** GS activity was determined as described previously (19), with modifications. Frozen muscles were homogenized in lysis buffer (buffer A) containing 20 mM HEPES (pH 7.4), 1% Triton X-100, 50 mM sodium chloride, 50 mM sodium fluoride, 5 mM sodium pyrophosphate, 2 mM EGTA, 50 mM β-glycerophosphate, 4 mg/ml leupeptin, 10 μg/ml aprotinin, 3 mM benzamidine, and 0.5 mM

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glyphosate by 30 min. The stimulatory effect of AICAR was not observed 10 min after the start of incubation but became

**extensively** in 66% (vol/vol) ice-cold ethanol and then evaluated in a liquid scintillation counter for $^{14}$C incorporation into glycogen. GS activity in the muscle was expressed as the active form ratio: G-6-P-AMP-independent activity $/\div$ total GS activity. The effect of ZMP on basal GS activity was evaluated by measurement of absolute GS activity in the presence of 6 mM ZMP, instead of 10 mM G-6-P, in the assay mixture. Absolulate GS activity was expressed as $^{14}$C incorporation activity (nmol/min $\div$ mg protein $^{-1}$).

**GP activity.** Muscle GP activity was measured as described previously (15). Frozen muscles were homogenized in buffer A, and the supernatants were prepared as described for GS activity. Supernatants (40 $\mu$g of protein) were added to 80 $\mu$l of assay mixture containing 50 mM MES (pH 6.1), 100 mM glucose 1-phosphate, 200 mM potassium fluoride, 10 mg/ml glycogen, and 2.5 mM $[^{14}$C]UDP-glucose 1-phosphate, in the absence and presence of 6 mM ZMP, instead of 6 mM 5'-AMP, to measure AMP-independent and total GP activities, respectively. After 15 min the reaction solution was spotted onto a filter paper to terminate the reaction. The filter papers were washed extensively in 66% (vol/vol) ice-cold ethanol and then evaluated in a liquid scintillation counter for $^{14}$C incorporation into glycogen. GP activity in the muscle was expressed as the active form ratio: 5'-AMP-independent activity $/\div$ total GP activity. The effect of ZMP on basal GP activity was evaluated by measurement of absolute GP activity in the presence of 6 mM ZMP, instead of 10 mM G-6-P, in the assay mixture. Absolute GP activity was expressed as $^{14}$C incorporation activity (nmol/min $\div$ mg protein $^{-1}$).

**Muscle glycogen.** Frozen muscles were processed by incubation in 1 M NaOH at 85°C for 10 min. The digestates were neutralized with HCl. The glycogen in the digestates was hydrolyzed by incubation in 2 N HCl for 2 h at 85°C. The digestates were neutralized with NaOH, and the glucose released from the glycogen was measured enzymatically using a hexokinase glucose assay reagent (Sigma-Aldrich). Glycogen content was expressed as micromoles of glucose unit per gram wet muscle weight.

**Lactate.** Muscles were incubated and stimulated in KRB containing 8 mM glucose as described above. After muscle contraction for 10 min or AICAR stimulation for 40 min, aliquots of the incubation buffer were collected, and the lactate in the buffer was determined using the Determiner-LA kit (Kyowa Medex, Tokyo, Japan). The lactate released into the buffer was calculated and normalized to the wet muscle weight.

**Statistical analysis.** Values are means $\div$ SE. Multiple means were compared by ANOVA. Two means were compared by Student’s t-test. P < 0.05 was considered statistically significant.

**RESULTS**

**AICAR acutely stimulates muscle AMPK in a dose- and time-dependent manner to a level comparable to that achieved by contraction.** To evaluate the effects of AICAR stimulation on rat epitrochlearis muscle, we determined the dose and time dependency of its effects on isoform-specific AMPK activities. Pharmacological stimulation with AICAR caused a two- to threefold activation of both isoforms of AMPK (Fig. 1, A and B). The effects of AICAR stimulation on AMPK activity were not observed 10 min after the start of incubation but became prominent by 30 min. The stimulatory effect of AICAR was maximal at 40 min (Fig. 1B) in a dose-dependent manner (Fig. 1A). Therefore, we judged that stimulation with 2 mM AICAR for 40 min causes maximal AMPK activation of both $\alpha$-isoforms. We also compared AICAR- and contraction-stimulated AMPK activities. Both treatments caused almost equal increases in AMPK-$\alpha_1$ and $\alpha_2$ activities (Fig. 1C).

**Contraction and AICAR activate glucose uptake to similar levels, comparable to the level achieved by a maximally effective dose of insulin.** We determined whether muscle contraction and AICAR stimulation, both of which activate AMPK to similar levels (Fig. 1), increase glucose uptake. The almost identical four- to fivefold increases in 3-MG uptake stimulated by muscle contraction and AICAR (Fig. 2) are similar to that achieved by stimulation with a maximally effective dose (1 $\mu$M) of insulin.

**Fig. 1.** Changes in $\alpha_1$- and $\alpha_2$-isoform-specific 5'-AMP-activated protein kinase (AMPK) activities in rat epitrochlearis muscle. Isolated muscles were incubated and stimulated by 0.03–8 mM 5-aminimidazole-4-carboxamide-1-$\beta$-d-ribonucleoside (AICAR) for 40 min (A), 2 mM AICAR for 10–60 min (B), and in vitro contraction (10 min) or 2 mM AICAR for 40 min (C). Values are means $\pm$ SE (n = 4–6/group). * P < 0.05; ** P < 0.01 vs. basal.
Contraction activates, but AICAR inhibits GS activity. To determine whether the activation of AMPK affects the activity of GS, the rate-limiting enzyme of glycogen synthesis, we measured GS activity. Whereas muscle contraction caused a marked increase in GS activity, AICAR stimulation conversely decreased GS activity (Fig. 3A). Insulin also increased GS activity, but the effect of insulin was antagonized by the presence of AICAR (active form ratio (%) = 28.0 ± 1.0 (basal), 52.7 ± 1.5 (contraction), 24.4 ± 1.1 (AICAR), 36.1 ± 2.0 (AICAR + insulin), and 45.4 ± 1.5 (insulin); Fig. 3A). AICAR-induced GS inactivation was dose dependent (Fig. 3B), in parallel with AMPK activity (Fig. 1A).

Contraction activates, but AICAR does not alter, GP activity. To determine the effect of AMPK activation on glycogenolysis, we examined the effect of AICAR stimulation on the activity of GP, which is the rate-limiting enzyme of glycogenolysis. Whereas muscle contraction and epinephrine stimulation markedly increased GP activity, AICAR did not alter GP activity (active form ratio (%) = 22.4 ± 1.0 (basal), 78.8 ± 4.4 (contraction), 20.5 ± 1.4 (AICAR), and 64.5 ± 1.7 (epinephrine); Fig. 4).

Contraction decreases, but AICAR does not alter, muscle glycogen content. We examined the effect of AICAR-stimulated AMPK activation on the concentration of glycogen. Glycogen content was reduced in contracting and epinephrine-stimulated muscles. In contrast, glycogen was unchanged by AICAR (23.3 ± 1.3, 12.4 ± 0.9, 25.6 ± 1.3, 23.7 ± 1.2, and 13.1 ± 2.4 μmol glucosyl unit/g wet muscle wt for the basal state, contraction, AICAR, insulin, and epinephrine, respectively; Fig. 5). These results are consistent with the increase in GP activity induced by contraction or epinephrine, which was unchanged by AICAR (Fig. 4).

AICAR increases lactate release from muscle. Because AICAR stimulation resulted in the inactivation of GS, despite a marked increase in glucose uptake, we investigated the amount of lactate released into the incubation buffer to clarify
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whether the glucose taken up into the muscle is degraded via the glycolysis pathway. The amount of lactate was measured in buffer containing glucose, instead of pyruvate. Similar to muscle contraction, AICAR caused a significant increase in lactate release (12.7 ± 0.85, 38.6 ± 2.11, and 20.1 ± 1.14 µg lactate/mg muscle for the basal state, contraction, and AICAR, respectively; Fig. 6).

**ZMP stimulates GP activity, but does not affect GS activity, in vitro.** When skeletal muscle is incubated with AICAR, AICAR is taken up into the muscle. It is then phosphorylated to form the AMP-like compound ZMP (17). Consequently, it is possible that increased intracellular ZMP directly modulates GS or GP activity as an AMPK-independent effect. Therefore, we measured GS and GP activities in the presence of ZMP in vitro. GS activity was unchanged in the presence of ZMP, whereas it was markedly elevated in the presence of G-6-P, a strong allosteric activator of GS (6,669 ± 446, 36,907 ± 1,085, and 6,409 ± 267 pmol/min/mg protein without G-6-P and without ZMP, with G-6-P, and with ZMP, respectively; Fig. 7A). GP activity was elevated in the presence of ZMP to an extent similar to that observed with AMP, a potent allosteric activator of GP (664.2 ± 36.8, 1,335 ± 61.2, and 1,416 ± 92.4 pmol/min/mg protein without AMP and without ZMP, with AMP, and with ZMP, respectively; Fig. 7B).

**DISCUSSION**

Studies of AICAR have provided important information about the function of acute AMPK activation in muscle glucose metabolism. The specificity of AICAR as an AMPK stimulator has been established by Mu et al. (31), who blocked AMPK activity in mouse skeletal muscle with the muscle-specific expression of a dominant-negative kinase-dead form of AMPK. In that mouse, the stimulatory effects of AICAR on glucose transport (31) and GLUT4 expression (22) were abolished completely. On the basis of findings suggesting that acute AMPK stimulation in vitro activates AMPK and glucose transport in fast-twitch muscles but has no effect on the slow-twitch soleus muscle of the rat (1, 4) and that AICAR administration also has the greatest effect on the GLUT4 content of fast-twitch muscles (7, 21, 44), we analyzed the rat epitycholeal muscle. Differential ATPase staining of rat epitrochlearis demonstrated >80% fast-twitch and only 15% slow-twitch fibers (33, 34). In the present study, AICAR treatment activated AMPK to the extent observed in skeletal muscle after contraction. AICAR (2 mM, 40 min) and tetanic contraction (10 s, 10 times) activated AMPK-α1 and -α2 (Fig. 1C), with a corresponding increase in the rate of 3-MG uptake to the level achieved by a maximally effective dose (1 µM) of insulin (Fig. 2).

We used isolated muscle incubated in vitro to eliminate the effects of systemic confounders, such as humoral factors and blood flow, because exercise in vivo evokes a number of dynamic changes, many of which can potentially alter fuel metabolism in contracting skeletal muscles (20). For example, exercise increases the blood concentration of epinephrine, a potent activator of glycogen breakdown (Fig. 4). Our method, using isolated muscle, made it possible to examine the direct effects of pharmacological manipulation and contraction on skeletal muscle metabolism.

In the present study, AICAR stimulation caused a decrease in GS activity, in contrast to muscle contraction (Fig. 3A). GS inactivation was dose dependent, in parallel with AMPK activation (Figs. 1A and 3B). Furthermore, insulin-stimulated GS activation was partially antagonized in the presence of AICAR (Fig. 3A). We eliminated the possibility of a direct inactivation of GS by ZMP, an intracellular metabolite of AICAR (Fig. 7A). Our observation is consistent with a previous report by Wojtaszewski et al. (45), who showed an inhibition of GS activity in rat hindlimb muscle after perfusion with AICAR. They also showed that the inactivation of GS was accompanied by a decrease in gel mobility and was abolished by protein
phosphatase treatment, indicating that AICAR stimulation causes GS phosphorylation (45). More recently, Jørgensen et al. (26) showed that AICAR treatment in vitro increases GS phosphorylation at site 2 (Ser1012) and decreases GS activity in mouse extensor digitorum longus muscle. Interestingly, however, Aschenbach et al. (3) showed that intraperitoneal administration of AICAR to a living rat inhibited GS activity in white gastrocnemius muscle, whereas AICAR administration also activated GS activity in red gastrocnemius muscle. They reported that in vivo incubation of the epitrochlearis and flexor digitorum brevis muscles with AICAR stimulated AMPK-α2 but had no effect on GS activity (3). The reasons for these discrepancies in the responses of the red and white gastrocnemius muscles and between the in vivo and in vitro experiments are unclear. However, Aschenbach et al. speculate that these AICAR-stimulated changes in GS activity may be due to the secondary effects of glucose transport and glycogen accumulation, rather than the direct effects of AMPK on GS.

The idea that active AMPK prevents glycogen synthesis in skeletal muscle may appear to be inconsistent with the chronic accumulation of glycogen in rat skeletal muscle induced by repeated administrations of AICAR. Several reports have shown that once-a-day administration of AICAR for 5–28 days in vivo causes a marked increase in the glycogen concentration of rat muscles (7, 21, 44). The levels of GLUT4 and hexokinase proteins are also upregulated by AICAR (7, 21, 44). Furthermore, each AICAR treatment induces GLUT4 translocation and increases the rate of glucose transport into muscle cells (Fig. 2) (19, 27). Increased glucose transport and hexokinase expression may result in increased concentrations of G-6-P, a potent allosteric activator of GS (Fig. 7A). They may also override the effects of the inhibitory phosphorylation of GS by AMPK. Thus these combined effects of AICAR on protein expression and glucose transport may predominate and facilitate glycogen synthesis, despite the deactivation of GS by AICAR.

In the present study, epinephrine stimulation and contraction caused increases in GP activity (Fig. 4), with corresponding decreases in glycogen content (Fig. 5), whereas AICAR stimulation neither increased GP activity nor altered the glycogen content (Fig. 4). Therefore, acute AMPK activation is considered to have no significant effect on GP activity in skeletal muscle. Our findings are consistent with the report by Aschenbach et al. (3), who found that in vitro AICAR treatment had no effect on GP activity in isolated epitrochlearis or flexor digitorum brevis muscles, despite significant activation of AMPK-α2. Interestingly, however, they also reported activation of GP in gastrocnemius muscle after intraperitoneal administration of AICAR in vivo, with a corresponding increase in AMPK-α2 activity (3). The cause of this contradictory change in GP activity is unknown. However, Aschenbach et al. speculate that it may be due to secondary effects in response to in vivo AICAR treatment. Our findings also appear to be inconsistent with another report by Young et al. (47), who showed that GP is activated in rat soleus muscle incubated with AICAR in vitro. However, as mentioned above, AICAR stimulation has no effect on AMPK activity in rat soleus muscle (1–4). Therefore, the GP activation observed in rat soleus muscle might not be related to AMPK activity. In support of this idea, AICAR did not alter the rate of glycogen synthesis in rat soleus in the basal state or a maximally stimulatory concentration of insulin (47). However, we found that AICAR decreased the basal and insulin-stimulated GS activity in rat epitrochlearis muscle (Fig. 3A), with a significant increase in AMPK activity (Fig. 1). Young et al. also demonstrated that the AICAR metabolite ZMP mimics the stimulatory effect of AMP, a known allosteric activator of GP, in extracts of rat soleus muscle. In the present study, we also found a marked elevation in GP activity when ZMP was added directly to muscle lysate to a level similar to that observed when AMP was added to the lysate (Fig. 7B). Longnus et al. (28) showed that AICAR activates GP in isolated rat myocardium in a dose-dependent manner, with no accompanying activation of AMPK. On the basis of these data, it seems reasonable that AICAR-induced GP activation is due to allosteric activation by ZMP, as in the rat myocardium.

On the basis of these observations, we propose that acute AMPK activation during muscle contraction antagonizes contraction-stimulated GS activity and that this effect consequently facilitates a glycolytic flux. Our proposal is consistent with the idea that AMPK acts as an energy sensor, switching off ATP-consuming pathways and switching on alternative pathways for ATP regeneration when cells sense low energy (17). The muscle glycogen accumulation induced by repeated AICAR stimulation may be due to the stimulatory effect of AMPK on glucose transport and on the expression of proteins such as GLUT4 and hexokinase. These effects may override the inhibitory action of AMPK on GS activity, resulting in enhanced glycogen synthesis in skeletal muscle. In conclusion, AMPK does not directly mediate contraction-stimulated GS or GP activation. However, AMPK may act as a metabolic regulator that leads to an increased glycolytic flux in contracting skeletal muscle.

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