Glycogen-Dependent Effects of 5-Aminomidazole-4-Carboxamide (AICA)-Riboside on AMP-Activated Protein Kinase and Glycogen Synthase Activities in Rat Skeletal Muscle

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5′-AMP-activated protein kinase (AMPK) functions as a metabolic switch in mammalian cells and can be artificially activated by 5-aminimidazole-4-carboxamide (AICA)-riboside. AMPK activation during muscle contraction is dependent on muscle glycogen concentrations, but whether glycogen also modifies the activation of AMPK and its possible downstream effectors (glycogen synthase and glucose transport) by AICA-riboside in resting muscle is not known. Thus, we have altered muscle glycogen levels in rats by a combination of swimming exercise and diet and investigated the effects of AICA-riboside in the perfused rat hindlimb muscle. Two groups of rats, one with super-compensated muscle glycogen, (white gastrocnemius, white soleus, red gastrocnemius, soleus) and one with moderately lowered muscle glycogen (white gastrocnemius, low glycogen [LG]), were generated. In both groups, the degree of activation of the α2 isoform of AMPK by AICA-riboside depended on muscle type (white gastrocnemius > red gastrocnemius > soleus). Basal and AICA-riboside-induced α2-AMPK activity were markedly lowered in the HG group (~50%) compared with the LG group. Muscle 2-deoxyglucose uptake was also increased and glycogen synthase activity decreased by AICA-riboside. Especially in white gastrocnemius, these effects, as well as the absolute activity levels of AMPK-α2, were markedly reduced in the HG group compared with the LG group. The inactivation of glycogen synthase by AICA-riboside was accompanied by decreased gel mobility and was eliminated by protein phosphatase treatment. We conclude that acute AICA-riboside treatment leads to phosphorylation and deactivation of glycogen synthase in skeletal muscle. Although the data do not exclude a role of other kinases/phosphatases, they suggest that glycogen synthase may be a target for AMPK in vivo. Basal and AICA-riboside–induced AMPK-α2 and glycogen synthase activities, as well as glucose transport, are depressed when the glycogen stores are plentiful. Because the glycogen level did not affect adenine nucleotide concentrations, our data suggest that glycogen may directly affect the activation state of AMPK in skeletal muscle. Diabetes 51:284–292, 2002

5′-AMP-activated protein kinase (AMPK) functions as a metabolic master switch in mammalian cells (1). Upon stimulation, AMPK switches off ATP-consuming anabolic pathways and activates ATP-producing catabolic processes. In skeletal muscle, AMPK is activated in response to metabolic stresses such as hypoxia, hyperosmolarity, mitochondrial uncoupling, contractile activity, and exercise in vivo (2–8). The levels of AMP, ATP, and creatin phosphate (CrP) seem to be the major determinants for the activation of AMPK, by direct allosteric regulation and by phosphorylation induced by upstream kinases (1,9). Muscle AMPK is also activated by the adenosine analog 5-aminimidazole-4-carboxamide (AICA)-riboside when given to the intact animal in vivo or to isolated muscle in vitro (4,10,11). AICA-riboside is taken up by muscle cells and phosphorylated to form 5-aminimidazole-4-carboxamide ribonucleotide (ZMP), a 5′-AMP mimicking compound (12). Thus, ZMP accumulates in skeletal muscle and leads to activation of AMPK (both directly by allosteric mechanisms and by phosphorylation [1,9]), although with somewhat lower affinity than AMP (13,14).

Carling and Hardie (15) have shown that AMPK from rat liver phosphorylates glycogen synthase (GS) at Ser-7 in vitro. Phosphorylation at Ser-7 is known to promote phosphorylation at Ser-10 by casein kinase-1, and these two phosphorylations cause inactivation of GS (16). GS also coprecipitated with AMPK when the α2 catalytic subunit of AMPK was immunoprecipitated from rat hindlimb muscles (17). Recently, the dominant RN− mutation, found in Hampshire pigs, was described as a substitution in the PRKAG3 gene, encoding a muscle specific isoform (γ3) of the regulatory γ subunit of AMPK (18). Muscle AMPK activity appeared to be decreased in this strain by two-thirds, and interestingly, glycogen content was 70% higher in muscles from the RN− compared with the RN+.
pig. These data suggest that AMPK, by association with and phosphorylation of GS, regulates GS activity upon stimulation in vivo. Alternatively, the finding of a lower AMPK activity in the m\(^{-1}/RN\^-1\) muscle could be a secondary event caused by the high glycogen level in RN muscle.

AICA-riboside increases glucose uptake in muscles of the intact rat (19) as well as in perfused (10) and isolated (4) muscles. This effect involves the recruitment of GLUT4 protein to the plasma membrane (11) and the activation of AMPK (20). Thus, in this regard AICA-riboside–induced glucose transport resembles the effect of acute muscle contraction. Recently, studies performed in our laboratory, as well as in others, have shown that both contraction- and insulin-stimulated muscle glucose transport (21–24) as well as GS activity (25) are inversely correlated to muscle glycogen content. Furthermore, AMPK activity during contractions was also markedly decreased in muscle with high compared with low glycogen concentrations (26). The molecular mechanism(s) for these relationships, and whether they apply to other modulators of muscle glucose transport and synthase activity, are currently unknown. If contraction-stimulated glucose transport involves activation of AMPK (20), a glycogen dependency of AICA-riboside–induced transport similar to that observed for contraction-induced glucose transport is to be expected.

Using AICA-riboside, we investigated the relationship between muscle glycogen content and the activities of AMPK and GS. Specifically, we measured the AICA-riboside–induced activation of specific \(\alpha\) isoforms of AMPK in muscles pretreated to have different glycogen content, and we examined the ability of AICA-riboside to inactivate GS and activate glucose transport. The muscle glycogen levels in rats were altered by a combination of swimming exercise and diet, and the effects of AICA-riboside in the perfused rat hindlimb were investigated. This model allows a simultaneous study of different calf muscles, which have varying compositions of muscle fiber type.

**RESEARCH DESIGN AND METHODS**

**Animals.** All experiments were approved by the Danish Animal Experimental Inspectorate and complied with the European Convention for the Protection of Vertebrate Animals used for Experiments and other Scientific Purposes (council of Europe no. 123, Strasbourg, France, 1985). Male Wistar rats weighing 110–120 g were used in the study. The rats were maintained on a 12 h:12 h light-dark cycle and received standard rat chow and water ad libitum.

Animals were subjected to 2 h of swimming in water maintained at 32–35°C with weights attached to their tails (5.5% of body weight). In the 24 h preceding the swim exercise, their food availability was restricted to 4 g (~45% of normal intake). After swimming, they were fed ad libitum with land and tap water (low glycogen [LG]) or with normal rat food, tap water, and a 20% glucose drinking solution (high glycogen [HG]), until 3–6 h before perfusion. Rats were perfused 18–24 h after swimming exercise.

**Surgical procedure.** On the day of the experiment, rats were anesthetized by an intraperitoneal injection of pentobarbital sodium (4–5 mg/100 g body wt). Surgery for two-legged perfusion was performed as described by Ruderman et al. (27).

**Perfusion medium.** All perfusions were carried out with cell-free medium consisting of Krebs-Ringer bicarbonate buffer solution, 4% BSA (fraction V; Sigma-Aldrich, Copenhagen, Denmark) diazoyed twice for 24 h against 25 vol of Krebs-Ringer bicarbonate buffer solution (pore size 10–15 kDa), 0.15 mmol/l pyruvate, and 4.2 U/ml heparin. Media having this simple composition were used initially during all perfusions. The perfusate subsequently applied for the measurement of glucose transport contained 8 mmol/l 2-deoxy-D-glucose and 1 mmol/l mannitol, in addition to radiolabeled tracers (2-deoxy-D-[2,6-\(^3^H\)]glucose, specific activity 51 mCi/mmol, and [U-\(^1^C\)]mannitol, specific activity 57 mCi/mmol, yielding an activity of 0.128 and 0.087 uCi/ml; Amer sham Pharmacia Biotech, Uppsala, Sweden). When AICA-riboside was included, it was present in the perfusate at a concentration of 2 mmol/l.

**Perfusion procedure.** The perfusion apparatus included an artificial lung by means of which the arterial perfusate was continuously gassed with a mixture of 95% O\(_2\)/5% CO\(_2\). The O\(_2\) pressure of the arterial perfusate was on average 565 ± 5 mmHg, and pH 7.4 ± 0.1 (n = 32). The arterial perfusate was heated to a temperature of 35°C, resulting in a muscle temperature (calf muscles) of ~32°C. With respect to the viability of the presently used muscle preparation, we have previously shown that muscle ATP and CrP values during 45 min of basal perfusion with a cell-free medium are not different from the values obtained from rested anesthetized rat muscle (28). Preliminary experiments were carried out to investigate the time course and the dose-dependent activation of hindlimb glucose uptake by AICA-riboside. These studies showed a maximal effect by AICA-riboside at 2 mmol/l, reaching steady-state level after 25 min of perfusion (data not shown). Therefore, all perfusions consisted of a 15 min preperfusion without addition of AICA-riboside followed by perfusion with or without 2 mmol/l AICA-riboside for an additional 35 min. All perfusions were carried out with a nonrecycle perfusate flow using a flow rate of 0.5 ml · min \(^{-1}\) · g \(^{-1}\) muscle. The hindlimb was perfused with media containing radioactive tracers as given above during the last 10 (AICA-riboside) or 20 (basal) min of perfusion. Muscle biopsies were taken from the quadriceps muscles at the end of the experiment of the calf musculature while the muscles were still being perfused: the most superficial part of m. gastrocnemius medialis (consisting mainly of type IIA fibers) (white gastrocnemius [WG]); the profound medial portion of m. gastrocnemius (consisting mainly of type Ila fibers) (red gastrocnemius [RG]); and m. soleus (consisting mainly of type I fibers) (29). The biopsies were quickly dissected, trimmed of connective tissue, blotted, and freeze-clamped with aluminum clamps cooled in liquid nitrogen. Biopsies were stored at ~80°C until analyzed. During perfusion, a number of perfusate samples were obtained at various time points. O\(_2\) pressure, CO\(_2\) pressure, pH, and lactate concentrations were immediately measured (model ABL 510 acid-base analyzer; Radiometer, Copenhagen) (YSI-3700; Yellow Springs Instruments, Yellow Springs, OH). Furthermore, samples of arterial perfusate needed for assessment of glucose transport were taken before muscle biopsy sampling and stored at ~8°C until analysis.

**Glucose transport.** Muscle 2-deoxyglucose uptake was assessed by measuring radioactivity in perchloric acid extracts of homogenates of the various muscles and in perchloric acid precipitates of perfusate using a liquid scintillation counter (model 2000 Tri-Carb; Packard Instruments, Gennevilliers, France, as previously described (30).

**Glycogen.** Muscle glycogen content was determined as glycosyl units after acid hydrolysis (31).

**Nucleotides.** Muscle specimens were extracted with perchloric acid, neutralized, and analyzed for nucleotides. Contents of ATP, ADP, AMP, and ZMP were determined by reverse-phase high-performance liquid chromatography according to a previously described method (32).

**Glycogen synthase activity.** Muscle GS activity was measured in a homogenate by a modification of the method of Thomas et al. (33). GS activity was determined in the presence of 0.02, 0.17, and 8 mmol/l glucose-6-phosphate (G6P) and presented either as the percent G6P-dependent glycogen synthase activity (referred to as the 0.02/8.0 activity ratio) (100 × activity in the presence of 0.02 mmol/l G6P divided by the activity at 8 mmol/l G6P [saturated]) or as the fractional velocity (100 × activity in the presence of 0.17 mmol/l G6P divided by the activity at 8 mmol/l G6P). Measurements of GS in the presence of ZMP (3 mmol/l) or AICA-riboside (2 mmol/l) were done to ensure that any muscle content of ZMP and/or AICA-riboside did not influence the in vitro GS assay.

**Dephosphorylation assay.** GS dephosphorylation was achieved by preparing muscle homogenates using a buffer without the phosphate inhibitor sodium fluoride. In some cases, the catalytic subunit of protein phosphatase-1 (10 U/ml; PP1; Sigma-Aldrich) was also added to the buffer together with 5 mmol/l MnCl\(_2\). The homogenates were stored on ice for 1 h before freezing. The influence of dephosphorylation on GS was evaluated by activity assay.

**Gel-shift analysis.** Proteins in homogenates (5 \(\mu\)g) were resolved by 7.5% SDS-PAGE (Criterion-system; Bio-Rad, Herlev, Denmark) and transferred to polyvinylidene difluoride (PVDF) membranes. After blocking in 5% BSA (in Tris-buffered saline with Tween [TBST; 10 mmol/l Tris-base, 0.09% NaCl, 0.05% Tween-20, pH 7.4]), membranes were incubated at room temperature for 2 h with a rabbit anti-GS antibody (34) (provided by Prof. Oluf Pedersen, Denmark), followed by incubation with a secondary anti-rabbit antibody conjugated with alkaline phosphatase (PVDF). Membranes were exposed to x-ray film for 1–2 days.
incubation with a chemifluorescence substrate (ECF, Amersham Pharmacia Biotech).

AMP-activated protein kinase activity. Isoform-specific AMPK activity was measured in immunoprecipitates from 200 μg of muscle lysate protein using anti-α1 or anti-α2 antibodies, as previously described (6).

Western blotting of the α2 isoform of AMPK. Proteins in homogenates (25 μg) were resolved by 10% SDS-PAGE and transferred to PVDF membranes. After blocking in 5% BSA in TBST, membranes were incubated at room temperature overnight with an anti-α2 antibody, followed by incubation with a secondary anti-sheep antibody conjugated with alkaline phosphatase (Pierce). A single 63-kDa band, corresponding to the α2 isoform of AMPK, was visible using a Kodak Image Station after incubation with ECF-substrate.

Calculations and statistics. Control samples were added to all activity assays, and assay-to-assay variation was accounted for by expressing the data relative to these samples. Data are expressed as means ± SE. Statistical evaluation was performed by two-way ANOVA. When ANOVA revealed significant differences, a post hoc test was used to correct for multiple comparisons (Student-Newman-Keuls test). Differences between groups were considered statistically significant if P was <0.05.

RESULTS

Swimming exercise and diet pretreatment modulates muscle glycogen content. The animals in the present study underwent a pretreatment the day before the experiment. This consisted of swimming for 2 h followed by either a carbohydrate-rich or -poor diet. The next day, the animals were investigated using the perfused rat hindlimb model. Muscle glycogen content was measured after perfusions and was not affected by the presence of 2 mM AICA-riboside for 40 min (Table 1). Independent of intervention, glycogen content was dependent on muscle type (WG > RG > soleus), and the pretreatment of the rats resulted in levels of muscle glycogen that were 2-fold (soleus) to 3.5-fold (RG and WG) higher in HG compared with LG (P < 0.001).

AICA-riboside induces a muscle type-specific increase in muscle ZMP content. Including AICA-riboside in the perfusate induced an increase in ZMP content in all muscle types (P < 0.001) (Table 2). The accumulation of ZMP was highest in RG (~1.5 mmol/kg w.w.) and similar in soleus and WG (~1 mmol/kg w.w.). Neither AICA-riboside nor glycogen content affected the levels of ATP, ADP, or AMP in any of the muscle types.

Glycogen affects the activity of the α2 isoform of AMPK to a greater extent than AICA-riboside in rat skeletal muscle. The basal activity of the α2 isoform of AMPK was muscle type specific (WG > RG > soleus). AICA-riboside increased α2 activity only minimally in soleus (51%, P = 0.03) and in RG (31%, P = 0.1) (Fig. 1). In contrast, a robust increase was observed in WG (240%, P < 0.001). In muscles with high glycogen levels, there were decreased basal (average ~52%) and AICA-riboside–induced (average ~46%) α2 activity (all muscle types, P < 0.001). It was confirmed by Western blotting of immunoprecipitates that the efficiency of the α2 immunoprecipitation was independent of both glycogen content and AICA-riboside stimulation (data not shown). Similarly, direct blotting of the α2 subunit revealed no differences in α2 protein expression due to glycogen manipulation or AICA-riboside stimulation or among the three muscle types, although the soleus muscle tended to have a lower expression compared with RG and WG (data not shown).

AICA-riboside and glycogen affect the activity of the α1 isoform of AMPK only modestly. In general, the level of α1 activity was only modestly (25%, P < 0.05) (soleus) or not at all (RG and WG) lowered by glycogen supercompensation (Fig. 2). Likewise, AICA-riboside had a small but significant effect on α1 activity only in WG (10–30%, P < 0.05). Basal α1 activity was dependent on muscle type, with soleus (2 pmol · min⁻¹ · mg⁻¹) having

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<td>Muscle glycogen content (mmol/kg w.w.)</td>
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<td><strong>Soleus</strong></td>
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Data are means ± SE, with (numbers of replicates). *Significant difference from LG (P < 0.001).

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<td>Muscle nucleotide content (mmol/kg w.w.)</td>
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<td><strong>Soleus</strong></td>
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Data are means ± SE, with (numbers of replicates). †Significant difference from zero (P < 0.001); ‡significant differences from WG and soleus (P < 0.001). ND, not detectable.
the highest and WG (1 pmol · min⁻¹ · mg⁻¹) having the lowest level. In absolute values, basal α1 activity was higher than basal α2 activity in all muscle types (9.4-, 3.3-, and 2.5-fold for soleus, RG, and WG, respectively).

**Glycogen and AICA-riboside lower glycogen synthase activity in perfused muscle.** GS activity was measured both as the 0.02:8.0 activity ratio and as fractional velocity. These measures of synthase activity gave nearly identical results. GS activity was lowered by glycogen super-compensation in a muscle type–dependent manner (WG > RG > soleus) both in the basal state and in the presence of AICA-riboside (Figs. 3 and 4). In addition, stimulation with AICA-riboside decreased GS activity significantly in all muscle types (LG ~42%, HG ~34%). The lowering effect of AICA-riboside was significantly reduced by glycogen super-compensation in all three muscle types (all three, P < 0.05) such that in HG the effect of AICA-riboside was in most cases not significant. AICA-riboside did not affect the total activity of GS (data not shown).

**FIG. 1.** Activity of the α2 isoform of AMPK in different rat hindlimb muscles after perfusion with or without (basal) 2 mmol/l AICA-riboside. Animals were pretreated to have either a high (HG) or a low (LG) muscle glycogen content. General differences between HG and LG are indicated on each graph. A significant interaction between AICA-riboside treatment and level of glycogen was present in WG only (P < 0.02). Means ± SE, n = 7–8. *Significant difference from basal in the same group (soleus P = 0.05, WG P < 0.001).

**FIG. 2.** Activity of the α1 isoform of AMPK in different rat hindlimb muscles after perfusion with or without (basal) 2 mmol/l AICA-riboside. Animals were pretreated to have either a high (HG) or a low (LG) muscle glycogen content. General differences between HG and LG are indicated on each graph. Means ± SE, n = 7–8. *Significant difference from basal in the same group (P = 0.04).
the homogenate from the muscles exposed to AICA-riboside during perfusion. The decreased GS activity in AICA-riboside–treated muscles was restored after dephosphorylation by endogenous phosphatase activity (i.e., by incubation of extracts on ice in the absence of sodium fluoride) (Fig. 6). GS mobility was decreased in HG versus LG. In addition, GS from AICA-riboside–treated muscle had a lower mobility compared with control, especially during LG (Fig. 7). Collectively, these data support the idea that AICA-riboside treatment leads to phosphorylation and subsequent inactivation of GS in vivo. The markedly decreased activity of GS from glycogen supercompensated muscles was only partially reversed by phosphatase treatment (Fig. 6). Addition of the catalytic subunit of PP1-α to the homogenate did not increase the activity of GS further, compared with the activity seen after endogenous phosphatase treatment (data not shown). This indicates that glycogen supercompensation suppresses GS activity possibly by phosphorylation-independent mechanisms.

**AICA-riboside increases 2 deoxyglucose uptake in a glycogen-dependent manner.** 2 deoxyglucose uptake was increased by AICA-riboside in all muscle types in both LG and HG (Fig. 8). The response to AICA-riboside was greatest in WG (three- and sixfold in HG and LG, respectively). The effect of AICA-riboside was significantly lower
in the HG group compared with the LG group in both RG and WG (both, $P < 0.001$), but not in soleus. The marked effect of glycogen super-compensation in WG is illustrated by the fact that the AICA-riboside–induced 2 deoxyglucose uptake in HG was similar to the basal level in the LG condition.

**DISCUSSION**

The combined exercise-diet pretreatment resulted in two groups of animals: one with a minor reduction (−20% below normal) and one with a marked increase (−100–250% above normal) in glycogen level compared with non-pretreated rat muscle. Thus, in the present study differences induced by this pretreatment should be viewed as a result of glycogen super-compensation rather than glycogen depletion.

FIG. 5. Linear regression analysis of the AICA-riboside–induced α2 and GS activities. Data are from the LG condition only. ¶, WG; ◦, RG. Both correlations are significant at $P < 0.05$ ($n = 7$).

FIG. 6. GS activity measured in muscle lysate prepared in the presence (+) or absence (−) of the phosphatase inhibitor sodium fluoride. Muscles were pretreated to have either a high (HG) or a low (LG) glycogen content and perfused in the absence (−) or presence (+) or 2 mmol/l AICA-riboside. Samples of muscles were processed in buffer with or without sodium fluoride and were left on ice for 1 h before freezing. Addition of the α catalytic subunit of PP1 and 5 mmol/l MnCl$_2$ to the homogenates did not change GS activity compared to homogenates without sodium fluoride (not shown). All data presented are from WG and are means ± SE, $n = 3$.

Glycogen super-compensation markedly lowered basal activities of glycogen synthase and glucose transport as well as the activity of the α2 isoform of AMPK in all three muscles. In addition, the effect of AICA-riboside on these three processes was also significantly reduced by glycogen super-compensation. These findings are in agreement with observations made by us and others that exercise-induced glucose transport and AMPK activity, as well as insulin-induced glucose transport and Akt activity, are markedly lowered by glycogen super-compensation in rodent skeletal muscles (21,22,24,26,35). Recently, we reported that the glycogen levels also influenced basal GS activity and the ability of muscle contraction and insulin to activate GS (25). Although the effect of AICA-riboside opposes that of contraction and insulin, its ability to decrease GS activity was also dependent on glycogen. Thus, glycogen super-compensation not only affects several physiological responses by a variety of stimuli, but also seems to affect signaling molecules involved in the regulation.

One important finding in the present study is the marked AICA-riboside–induced decrease in the 0.02/8.0 activity ratio or fractional velocity activities of GS without changes in total activity. GS in AICA-riboside–treated muscles also had a lower gel mobility compared with control. The difference in activity was not present when muscles were homogenized without inhibition of endogenous phosphatase activity. Collectively, these data indicate that AICA-riboside induces phosphorylation of GS in vivo. It has been shown that AMPK from rat liver phosphorylates GS at Ser-7 in vitro (15). Phosphorylation by other protein kinases at Ser-7 is known to promote phosphorylation at Ser-10 by casein kinase I, and together these phosphorylation events cause inactivation of GS (16). In fact, during the LG condition in the present study, a negative correlation existed between the level of α2 activity and GS activity during AICA-riboside stimulation in both RG and WG. Thus, AMPK activation by AICA-riboside might mediate the phosphorylation of GS in vivo. AICA-riboside is not a completely specific stimulus for AMPK, and the possibility exists that one or more other protein kinases may be activated by ZMP, leading to phosphorylation of GS. In favor of such a view would be the observation that NH$_2$-terminal phosphorylation in vitro—by, for example, cyclic AMP–dependent protein kinase—does not cause a significant mobility shift of GS (30). The identity of such other putative GS kinases activated by AICA-riboside is
not known to us, although in vitro data have shown that three of the major protein kinases acting on GS—phosphorylase kinase, cyclic AMP—dependent protein kinase, and glycogen synthase kinase-3—are not activated by 1 mmol/l ZMP in vitro (Bo Falk Hansen, Novo Nordisk, personal communication). Similarly, control experiments showed that AICA-riboside and ZMP do not affect GS activity in vitro. Comparing the effects of AICA-riboside in the three muscles, it is clear (Figs. 1, 3, and 4) that very different levels of stable α2 activation mediate an almost comparable inactivation of GS. For example, in soleus we observed a smaller and in WG a larger stable activation of α2, whereas the inactivating effect of AICA-riboside on GS activity was nearly the same in the two muscle types. In addition to many fiber type–related differences that might explain this discrepancy, at least two methodological factors should be considered before any conclusions can be drawn from these comparisons. First, any allosteric regulation of AMPK occurring in vivo is not reflected in the in vitro assay. Second, because Ser-7 is only one of multiple phosphorylation sites on GS, changes in activity ratio in the present study may not relate solely to phosphorylation at this site.

A role for AMPK in inactivation of GS is consistent with the view that AMPK is a sensor of cellular energy charge, turning off ATP-consuming anabolic pathways, including the incorporation of UDP-glucose into glycogen. In addition, the observed AMPK activation during exercise of higher intensities in human skeletal muscle (6–8) may therefore indirectly lead to a higher rate of net glycogenolysis via inactivation of GS. In many studies, GS activity has been reported to increase with exercise (37–40), making the above view appear to be inconsistent with existing results. However, the degree of glycogen depletion and the mode of exercise seem to be important for this observation. For instance, during exercise, decreasing glycogen concentrations may act to increase GS activity whereas, at the same time, increased AMPK activity may act to decrease activity of GS. Thus, the resultant activity of GS in exercising muscle may depend on the relative magnitude of these two opposing signals. In accordance with such an interpretation is our recent finding that GS activity is decreased during exercise in patients with myophosphorylase deficiency who consequently are unable to break down muscle glycogen during exercise (J.N. Nielsen, J.Vissing, E.A.R., unpublished observations). In addition, immediately after strenuous isometric exercise, during which glycogen depletion is modest and AMPK activity presumably is very high, GS activity is decreased (41). A few minutes after contractions, however, GS activity reverted to an increase, presumably because AMPK activity was diminished, allowing the effect of glycogen depletion to be expressed (41).

Once-a-day administration of AICA-riboside for 5 days causes a marked increase in the glycogen content of rat muscle (42,43). These findings are not at first sight consistent with our present observation of decreased GS activity as an acute response to AICA-riboside. In the above-mentioned studies, however, the protein levels of GLUT4 and hexokinase were markedly upregulated. Similar effects were seen in isolated epitrochlearis muscles treated for 18 h with AICA-riboside in vitro (44). Thus, changes in protein expression in response to the chronic AICA-riboside treatment may lead to an increased rate of glycogen synthesis despite a transiently decreased GS activity following each acute treatment. In addition, increased GLUT4 and hexokinase expression may lead to increased muscle concentrations of G6P, activating GS allosterically and overriding the direct effect of AMPK on GS phosphorylation.

In Hampshire pigs, a mutation in the PRKAG3 (γ3) gene is associated with elevated glycogen content and apparently lowered AMPK activity in skeletal muscle (18). This is consistent with our observations, since depressed AMPK activity could lead to decreased phosphorylation.
and thus increased GS activity. However, caution is needed in this interpretation, because simply increasing the level of glycogen in muscles lowers basal AMPK activity and its ability to be activated by both AICA-riboside and contraction in rodents and by bicycling in humans (23,24,45). In COS cells and fibroblasts, AMPK activity was increased when the Hampshire pig mutation was introduced in the γ1 subunit (46). If this finding is transferable to muscles of the pig, then an inhibitory role of glycogen loading seems even more likely; the depressed AMPK activity in the muscle of Hampshire pigs could be the result of a feedback inhibition due to the high glycogen concentrations in the muscle cells.

The lower absolute level of α2 activity in soleus compared with RG and WG could in part be explained by a lower level of expression of this isoform in soleus compared with RG and WG. However, this would not explain the lower degree of activation in soleus upon AICA-riboside stimulation. Interestingly, this lower degree of activation occurs despite the fact that ZMP accumulates to the same extent in soleus as in WG. Similarly, ZMP activation occurs despite the fact that ZMP accumulates to a level ~50% higher in RG compared with WG and soleus. Nevertheless, the stable activation by AICA-riboside was also modest in this muscle. This suggests that the upstream AMPK kinases in soleus and RG might be expressed at lower levels or might be less ZMP-dependent or that the AMPK-phosphatase activity might be expressed at lower levels or might be less regulated in response to glycogen and AICA-riboside. This may reflect that α1 complexes are activated to a lower extent by AMP than α2 complexes (47).

As has been observed by others, AICA-riboside increased muscle glucose transport (4,10,19). The effect was dependent on muscle type and followed the AICA-riboside-induced α2 activation pattern. This suggests a causal relationship between α2 activation and glucose transport during stimulation with AICA-riboside. Recently, experiments using isolated muscles from mice expressing a kinase-inactive dominant-negative α-AMPK construct provided strong evidence that AICA-riboside–induced glucose transport is mediated through AMPK (20). Muscle type–specific effects of AICA-riboside are, interestingly, also found in studies with chronic AICA-riboside treatment. Thus, in contrast to WG, GLUT4 expression was not affected and glycogen content only minimally increased in RG after 5 days of treatment (43). Longer treatment (4 weeks) with AICA-riboside may provoke an increased expression for some proteins (GLUT4 and hexokinase) but not for others (mitochondrial enzymes) in RG (48). The reason for this fiber type dependency is not known.

AICA-riboside has been reported to activate glycogen phosphorylase and glycogenolysis in skeletal muscle (10,11,49). This is presumably due to the ZMP accumulation and ZMP activation of phosphorylase b rather than activation of phosphorylase kinase (50). In the present study, we were unable to detect any indication of enhanced glycogenolysis during 40 min of AICA-riboside exposure. Thus, muscle glycogen levels (Table 1) and lactate release (LG 6.1 ± 0.4 vs. 5.6 ± 0.5, HG 11.2 ± 0.6 vs. 11.0 ± 0.8 μmol · g⁻¹ · h⁻¹, n = 8, for basal and AICA-riboside, respectively) during perfusion, were unaffected by AICA-riboside. The reason for the unchanged lactate release with AICA-riboside treatment in the present study might be linked to the use of perfusate without D-glucose. When glucose is present during studies of isolated or perfused muscles or during in vivo conditions, measurable lactate release or muscle lactate accumulation is observed with AICA-riboside treatment (10,11,19,42,49).

In summary, the present study shows that AICA-riboside enhances glucose transport, inactivates GS, and activates the α2 isoform of AMPK in a muscle fiber type–dependent manner. Both basal and AICA-riboside–induced activities of these processes are depressed when the cellular glycogen content is high. Since concentrations of the adenine nucleotides were unaffected by glycogen levels, our data suggest that glycogen may directly affect the activation state of AMPK in skeletal muscle. Finally, our data also suggest that AMPK might phosphorylate GS in skeletal muscle in response to AICA-riboside in vivo.

ACKNOWLEDGMENTS

J.F.P.W. was supported by a postdoctoral fellowship from the Danish Medical Research Council. D.G.H. was supported by a Program Grant from the Wellcome Trust. The study was supported by Grant #504-14 from the Danish National Research Foundation.

Bo Falk Hansen, Novo Nordisk, is acknowledged for sharing unpublished data and for helpful comments during the preparation of this manuscript, as is Oluf Pedersen, Steno Diabetes Center, for sharing the GS antibody. We thank Betina Bølgren and Karina Olsen for skilled technical assistance.

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