AMP-activated protein kinase activates transcription of the UCP3 and HKII genes in rat skeletal muscle

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AMP-activated protein kinase (AMPK) has recently emerged as a key signaling protein in skeletal muscle, coordinating the activation of both glucose and fatty acid metabolism in response to increased cellular energy demand. To determine whether AMPK signaling may also regulate transcription in muscle, rats were given a single subcutaneous injection (1 mg/g) of the AMP analog 5-aminoimidazole-4-carboxamide-1-beta-D-ribonucleoside (AICAR). AICAR injection also elicited (P < 0.05) an acute drop (60%) in blood glucose and a sustained (2-h) increase in blood lactate, prompting concern regarding the specificity of AICAR on transcription. To maximize AMPK activation in muscle while minimizing potential systemic counterregulatory responses, a single-leg arterial infusion technique was employed in fully conscious rats. Relative to saline-infused controls, single-leg arterial infusion of AICAR (0.125, 0.5, and 2.5 μg·g−1·min−1 for 60 min) induced a dose-dependent increase (2- to 4-fold, P < 0.05) in UCP3 and HKII transcription in both red and white skeletal muscle. Importantly, AICAR infusion activated transcription only in muscle from the infused leg and had no effect on blood glucose or lactate levels. These data provide evidence that AMPK signaling is linked to the transcriptional regulation of select metabolic genes in skeletal muscle.

5-aminoimidazole-4-carboxamide ribonucleoside; single-leg arterial infusion; rat; AMP kinase phosphorylation

AMP-activated protein kinase (AMPK) is a highly conserved metabolite-sensing protein kinase found in all eukaryotic cells (12). In mammals, the AMPK enzyme is composed of a catalytic α-subunit and two regulatory subunits, β and γ, each of which is encoded for by either two (α1, α2, β1, β2) (40, 42) or three (γ1, γ2, γ3) (6) genes. Although not completely defined, it is thought that AMP activates AMPK by binding to the interface between the α- and γ-subunits, disrupting an autoinhibitory domain within the α-subunit (13). AMPK is also stimulated by phosphorylation of a regulatory site (Thr172) in the catalytic domain of the α-subunit, a reaction catalyzed by an upstream kinase, AMPK kinase (AMPKK), which is also activated by AMP (14, 15, 41). Thus full activation of AMPK is achieved by a combination of AMP-mediated allosteric activation of AMPK and its upstream kinase AMPKK, and by covalent phosphorylation of the AMPK α-subunit by AMPKK. Although the resting concentration of AMP is typically 100-fold lower than ATP (a competitive inhibitor of AMPK), AMP concentration increases dramatically under conditions of accelerated ATP utilization, due in part to the adenylate kinase reaction (ADP + AMP = ATP + AMP). Therefore, AMPK is thought to represent an extremely sensitive intracellular energy charge sensor (13).

In skeletal muscle, AMPK is activated by contraction and hypoxia in vitro and by exercise, creatine, and leptin in vivo (10, 16, 24, 26, 34, 51). Activation of AMPK in muscle stimulates a rapid phosphorylation and inactivation of acetyl-CoA carboxylase (ACC), a fall in malonyl-CoA concentration, and a release of malonyl-CoA-mediated allosteric inhibition of a carnitine palmitoyltransferase I (CPT I), all of which lead to an increase in fatty acid oxidation (12, 49). In addition, recent evidence suggests that AMPK is also responsible, at least in part, for the contraction/exercise-stimulated activation of glucose transport, the rate-limiting step for glucose utilization in muscle (3, 16, 23, 26). Similar to both insulin and exercise, activation of AMPK stimulates translocation of the GLUT4 transporter protein to the cell surface (21, 37). Thus AMPK has emerged in skeletal muscle as a key signaling protein believed to coordinate the overall metabolic response to increased energy demand (13, 36, 47, 49).

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The importance of AMPK signaling may extend well beyond control of acute substrate utilization. In *Saccharomyces cerevisiae*, for example, the yeast homolog of AMPK known as sucrose nonfermenting 1 kinase (SNF1) is activated when yeast are switched from the preferred carbon source of glucose to an alternative sugar (5). Importantly, the downstream effects of SNF1 are mediated by the direct induction of genes necessary to metabolize nonglucose carbon sources (5). Several lines of evidence suggest that AMPK may also be linked to the regulation of gene expression in mammals. AMPK complexes containing the α2 isoform, the major isoform expressed in skeletal muscle, preferentially localize to the nucleus in both INS-1 and CCL13 cells (rat pancreatic β-cell and hepatoma cell lines, respectively) (38). AMPK has been shown to directly phosphorylate the nuclear protein p300, a transcriptional coactivator that interacts with a variety of nuclear receptors, including peroxisome proliferator-activated receptor-γ, AMP response element-binding proteins, thyroid receptor, and retinoic acid receptor, as well as other coactivator proteins (52). Evidence for AMPK regulation of gene expression in vivo has come exclusively from experiments in which rodents have been administered 5-aminoimidazole-4-carboxamide-1-β-D-ribonucleoside (AICAR), a compound that forms a nonmetabolized analog of AMP, ZMP, that activates both AMPK and AMPKK (7, 23). In rats, the expression and/or activity of GLUT4, hexokinase II (HKII), and several mitochondrial enzymes is increased by daily subcutaneous injections of AICAR (19, 50). Zheng et al. (54) recently extended these findings, demonstrating that a single injection of AICAR in transgenic mice carrying ∼1,000 bp of the GLUT4 promoter elicits a significant increase in reporter gene mRNA content in gastrocnemius muscle. Collectively, these findings provide evidence that the AMPK-signaling pathway may regulate mammalian gene expression.

The purpose of the present study was to determine whether the acute administration of AICAR directly influences the transcriptional regulation of metabolic genes in skeletal muscle of rats. The genes analyzed in the present study have previously been shown to increase in expression in response to exercise or chronic AICAR administration. They included two glucose (hexokinase II (HKII) and GLUT4) and two lipid (lipoprotein lipase (LPL) and CPT I) metabolism genes as well as uncoupling protein 3 (UCP3), a muscle-specific gene with a putative role in regulating free radical production and metabolic thermogenesis (9, 19, 33, 50). On the basis of previous reports (50), initial experiments were conducted by delivering AICAR as a single subcutaneous injection. To minimize the potential influence of systemic metabolic stress induced by whole body AICAR administration, a second study was also conducted in which AICAR was infused into the femoral artery (via the saphenous artery) of one leg in fully conscious, free-living rats.

**Materials and Methods**

**Materials.** Male Sprague-Dawley rats were bred in-house or were purchased from the Charles River Laboratory (Wilmington, MA). All rats were housed individually in a temperature (22°C) and reverse light-controlled (dark 9:00 AM-9:00 PM) room and were given free access to food (Purina Rodent Diet) and water. AICAR was purchased from Sigma Chemical, [γ-32P]ATP was obtained from New England Nuclear (Boston, MA), and protein A/G agarose beads were from Santa Cruz Biotechnology (Santa Cruz, CA). All other chemicals were of molecular biology grade and were purchased from either Boehringer Mannheim, GIBCO-BRL, or Promega.

**AICAR injection.** To determine the effects of acute activation of AMPK, rats weighing 314 ± 1.9 g were given a single subcutaneous injection of AICAR (1 mg/g body wt) in vehicle (sterile saline) or vehicle alone. Injections were given between 10:00 AM and 12:00 noon. Within 15 min, 1 h, or 2 h (n = 6–8/group) after injection, rats were anesthetized (5.0 mg pentobarbital sodium/100 g body wt ip) and placed on a heating pad to maintain body temperature. Gastrocnemius muscles were removed from the animal, and red and white portions were quickly dissected free. One portion (∼350 mg) was immediately placed in ice-cold nuclei isolation buffer while the other portion (∼50 mg) was quickly frozen in liquid nitrogen. Blood (∼4 ml) was collected via cardiac puncture, placed in heparinized tubes, and spun, and the plasma was removed and frozen for later analysis.

**Single-leg AICAR infusion.** In an attempt to minimize the metabolic disturbances (e.g., drop in blood glucose, elevated lactate; see RESULTS) induced by systemic AICAR administration, a single-leg arterial infusion technique was employed. The technique allows for delivery of agent at a relatively high concentration to the lower limb musculature of a single leg in fully conscious rats while systemic exposure is minimized. Rats (348 ± 13.6 g) were surgically instrumented with polyethylene catheters placed in the greater saphenous artery and were advanced retrograde to the femoral artery (at the popliteal branch of the right hindlimb, threaded under the skin, and externalized through an incision on the back of the animal. Catheters were also placed in the right atrium (via the jugular vein) for blood sampling. Both catheters were secured/protected using a spring harness system and kept patent with a heparin lock and daily flushing. Rats were given analgesic (topical and in drinking water) for 12 h postsurgery and were monitored (food and water intake, activity) for 48 h for any adverse signs. After recovery (72 h), fully conscious animals were infused for 60 min with saline or AICAR at rates of 0.125, 0.5, or 2.5 μg·g⁻¹·min⁻¹. The highest infusion dose was selected to generate a concentration of AICAR in the infused leg of ∼2 mM on the basis of an estimated blood flow of ∼1.4 ml/min (single leg) in a 350-g rat. Food was removed 4 h before infusion, and infusion was performed between 9:00 and 11:00 AM (early in the dark cycle). During the infusion protocol, a 0.5-ml blood sample was taken at time 0 and at 20, 40, and 60 min for determination of serum glucose and lactate (YSI Glucose/Lactate Analyzer, YSI, Yellow Springs, OH). At the end of the infusion period, rats were anesthetized, and red and white portions of the gastrocnemius muscle (from both the infused and contralateral legs) were removed for nuclei isolation, as described above. Separate infusion experiments were conducted to determine the effects of AICAR infusion on AMPK activity, AMPK phosphorylation, and ACC phosphorylation.

**Determination of muscle AMPK activity, AMPK phosphorylation, and ACC phosphorylation.** Measurements of isoform-specific AMPK activity were performed as described (26) by use of the SAMS peptide as substrate and were expressed as picomoles of ATP incorporated per milligram of muscle lysate protein per minute. AMPK and ACC phosphorylation was determined by Western blotting with antibodies that recognize AMPK phosphorylated on Thr172.
Nuclei isolation and nuclear run-on analysis. Nuclei were isolated from red and white portions of the rat gastrocnemius muscle and subjected to RT-PCR-based nuclear run-on analysis, as previously described (17). To account for differences in initial nuclei content among samples before the run-on reaction, RT products were diluted with nuclease-free H2O based on the relative genomic DNA content of each nuclei preparation. PCR primer pairs (described previously in Ref. 17) were designed from rat-specific sequence data (Entrez; National Institutes of Health) with DNA analysis computer software (Lazergene; DNASTAR). Annealing temperature, MgCl2 concentration, and PCR cycle number were determined for each primer pair by pretesting to ensure that conditions were optimized and within the linear range for PCR amplification. Control and experimental samples were run in parallel to permit direct relative comparisons. Amplification products were separated by gel (2.5% agarose) electrophoresis, stained with ethidium bromide, visualized, and quantified by ultraviolet exposure with a charge-coupled device integrating camera (Gel Doc; Bio-Rad) and analysis software (Molecular Analyst; Bio-Rad) under nonsaturating conditions.

Statistical analysis. Transcription data for all metabolic genes were expressed relative to the transcription of the β-actin gene. All data across experimental treatments were expressed relative to data from control rats, with the control mean set to 1.0. Statistical analyses were performed using either one-way (injection data) or two-way (infusion data) ANOVA, with all pairwise multiple comparisons among groups performed with the Student-Newman-Keuls method.

The level of significance was set at P < 0.05.

RESULTS

Effects of subcutaneous AICAR injection. AMPK-α2 activity increased (P < 0.05) by 2- to 2.5-fold in both red and white gastrocnemius muscle within 60 min after AICAR injection and remained elevated through 120 min. AICAR injection did not affect AMPK-α1 activity (Fig. 1).

As shown in Fig. 2, AICAR injection induced a significant increase in transcription of the UCP3 and HKII genes (P < 0.001). Transcription of UCP3 increased ~3.0-fold in both red and white gastrocnemius muscle 60 min after AICAR injection and continued to rise 4.4-fold (red) and 3.5-fold (white) 120 min after AICAR injection. Transcription of the HKII gene responded even more dramatically, increasing ~6- to 8-fold 60 min after AICAR injection in both red and white gastrocnemius muscle. In red gastrocnemius muscle, HKII transcription remained significantly elevated (6.5-fold) through 120 min; in white gastrocnemius muscle, HKII transcription continued to increase to ~10-fold above that of saline-injected controls 120 min after AICAR injection. Similar to previous reports (3, 19, 44), subcutaneous injection of AICAR (1 mg/g body wt) triggered an acute drop in blood glucose and a marked increase in circulating lactate concentration (Table 1).

Effect of single-leg arterial infusion of AICAR. The dramatic effects of AICAR injection on blood glucose and lactate prompted concern that the observed changes in UCP3 and HKII transcription may have arisen as a consequence of the counterregulatory measures to the systemic effects of AICAR rather than as a direct effect of AMPK signaling in skeletal muscle. In an effort to minimize whole body AICAR exposure, a single-leg arterial infusion technique was employed to deliver AICAR at much lower concentrations directly to the lower hindlimb muscle of one leg in free living rats. In contrast to subcutaneous injection, infusion of AICAR via the saphenous artery at doses of 0.125, 0.5, and 2.5 μg·g−1·min−1 body wt had no effect on serum glucose or lactate concentrations (Table 2). Overall, AMPK-α2 activity was significantly higher (main effect, P < 0.05) in the infused vs. the contralateral leg in both red and white gastrocnemius muscle (Fig. 3A). Although no specific differences were found in red gastrocnemius muscle, in white gastrocnemius muscle AICAR infusion at 2.5 μg·g−1·min−1 induced a significant increase in AMPK-α2 activity relative to all other infusion conditions in the infused leg, as well as rela-
AMPK activates transcription in skeletal muscle

The major finding of the present study is that administration of AICAR, a chemical activator of the intracellular signaling protein AMPK, activates transcription of the UCP3 and HKII genes in both red and white skeletal muscle of rats. The acute effects of AMPK activation were initially studied in response to a single subcutaneous injection of AICAR (1 mg/g body wt). AMPK-α2 activity increased within 60 min after AICAR injection and coincided with 2- to 11-fold increases in transcription of the UCP3 and HKII genes, respectively, in both red and white skeletal muscle. However, AICAR injection also resulted in an acute drop in blood glucose (~60% 15 min after injection) and prolonged increase in blood lactate (~3-fold through 120 min after injection), raising concern that the effects of AICAR on these genes may not be physiological in the context of whole-body metabolism.

Table 1. Blood glucose and lactate concentrations in response to AICAR injection

<table>
<thead>
<tr>
<th>Time After AICAR Injection, min</th>
<th>Control</th>
<th>15</th>
<th>60</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood glucose, mM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>9.89 ± 0.5</td>
<td>2.94 ± 0.15</td>
<td>8.33 ± 1.16</td>
<td>8.38 ± 1.11</td>
</tr>
<tr>
<td>60</td>
<td>2.72 ± 0.11</td>
<td>6.65 ± 0.76</td>
<td>8.92 ± 0.65</td>
<td>6.85 ± 0.21</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 6-8/time point). *Significantly (P < 0.05) different from Control.

(P < 0.05) in both UCP3 and HKII transcription relative to saline-infused control animals (Fig. 4 and Fig. 5). The effect of AICAR was specific for the infused leg, as UCP3 and HKII transcription were not changed in either red or white gastrocnemius muscle from the contralateral leg. AICAR infused at the lowest dose (0.125 μg·g⁻¹·min⁻¹) did not elicit any significant increase in transcription. Although GLUT4 transcription tended to increase in the infused leg (<2-fold, P = 0.1), AICAR infusion was not associated with any significant change in transcription of the GLUT4, LPL (Figs. 4 and 5), or CPT I (not shown) genes.

DISCUSSION

The major finding of the present study is that administration of AICAR, a chemical activator of the intracellular signaling protein AMPK, activates transcription of the UCP3 and HKII genes in both red and white skeletal muscle of rats. The acute effects of AMPK activation were initially studied in response to a single subcutaneous injection of AICAR (1 mg/g body wt). AMPK-α2 activity increased within 60 min after AICAR injection and coincided with 2- to 11-fold increases in transcription of the UCP3 and HKII genes, respectively, in both red and white skeletal muscle. However, AICAR injection also resulted in an acute drop in blood glucose (~60% 15 min after injection) and prolonged increase in blood lactate (~3-fold through 120 min after injection), raising concern that the effects of AICAR on these genes may not be physiological in the context of whole-body metabolism.

Table 2. Blood glucose and lactate concentrations during AICAR infusion

<table>
<thead>
<tr>
<th>Infusion Rate, μg·g body wt⁻¹·min⁻¹</th>
<th>0 min</th>
<th>20 min</th>
<th>40 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood glucose, mM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>5.85 ± 0.16</td>
<td>6.40 ± 0.26</td>
<td>6.47 ± 0.20</td>
<td>6.64 ± 0.20</td>
</tr>
<tr>
<td>AICAR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.125</td>
<td>5.76 ± 0.37</td>
<td>5.98 ± 0.38</td>
<td>6.52 ± 0.45</td>
<td>6.64 ± 0.44</td>
</tr>
<tr>
<td>0.5</td>
<td>5.17 ± 0.22</td>
<td>5.61 ± 0.14</td>
<td>5.73 ± 0.12</td>
<td>6.11 ± 0.17</td>
</tr>
<tr>
<td>2.5</td>
<td>5.6 ± 0.25</td>
<td>5.69 ± 0.11</td>
<td>6.02 ± 0.12</td>
<td>6.08 ± 0.21</td>
</tr>
</tbody>
</table>

Blood lactate, mM

| Saline                              | 2.84 ± 0.23 | 2.34 ± 0.16 | 2.10 ± 0.18 | 1.77 ± 0.18 |
| AICAR                               |       |        |        |        |
| 0.125                               | 4.62 ± 0.71 | 3.61 ± 0.79 | 3.43 ± 0.77 | 2.95 ± 0.81 |
| 0.5                                 | 3.86 ± 0.53 | 3.21 ± 0.38 | 3.25 ± 0.45 | 2.89 ± 0.45 |
| 2.5                                 | 3.53 ± 0.23 | 3.29 ± 0.35 | 3.19 ± 0.35 | 3.11 ± 0.38 |

Values are means ± SE (n = 6-8/time point).
The effects of AICAR on UCP3 and HKII expression may have been related to systemic metabolic disturbances rather than a direct consequence of AMPK signaling in skeletal muscle. However, AICAR infusion via the saphenous artery of one leg in fully conscious rats elicited a dose-dependent activation of UCP3 and HKII transcription in both red and white gastrocnemius muscle. Importantly, AICAR infusion activated transcription only in muscle from the infused leg (no effect in the contralateral leg) and had no effect on blood glucose or lactate levels. Thus these data provide evidence in vivo that activation of the AMPK-signaling pathway in skeletal muscle regulates the transcription of select metabolic genes.

The effect of AICAR administration on the AMPK signaling was assessed by measurements of AMPK activity, AMPK phosphorylation (indication of AMPKK activity), and ACC phosphorylation (a primary target for AMPK). Subcutaneous injection of AICAR increased AMPK-α2 activity in both red and white gastrocnemius muscle but had no effect on AMPK-α1 activity (Fig. 1). Although infusion of AICAR also induced an increase in AMPK-α2 activity (and AMPK phosphorylation) in both red and white gastrocnemius muscle (main effect), specific differences were noted only at the highest infusion rate in white gastrocnemius muscle (Fig. 3, A and B). Differences in the effective dose of AICAR between the two studies may have at least
partially accounted for the findings; i.e., subcutaneous injection of AICAR delivered ~350 mg in one acute dose, whereas infusion of AICAR over 60 min delivered a total of ~2, 13, and 65 mg for the three infusion rates, respectively. It is also important to note that the AMPK activity assay currently available is only a measure of the activation induced by phosphorylation, and it therefore provides no information on the degree of direct allosteric activation of AMPK (47). ACC activity/phosphorylation is thought to represent a more accurate measure of in vivo AMPK activity in skeletal muscle, and, indeed, ACC phosphorylation increased in response to AICAR infusion in both red and white gastrocnemius muscle (Fig. 3C). Surprisingly, however, infusion of AICAR also increased phosphorylation of ACC in muscle from the contralateral leg, a finding that appears to be at odds with the lack of a significant effect of AICAR infusion on transcription in the contralateral leg. One possibility is that phosphorylation of ACC may be more sensitive to changes in AMPK activity relative to the control of transcription. Given that AICAR is not a specific activator of AMPK, it is also possible that ACC phosphorylation may be regulated by other factors sensitive to AICAR. For example, Winder et al. (50) recently reported that 4 wk of AICAR injection elicited a persistent and significant depression in muscle ACC activity despite no detectable increase in AMPK activity. A clear dissociation between muscle AMPK-α2 activity and ACC phosphorylation has also recently been found in humans during prolonged (3-h) low-intensity exercise, in which AMPK-α2 activity and phosphorylation were found to progressively increase 2- to 3-fold, whereas ACC phosphorylation peaked (~4-fold) after 1 h and then declined to basal levels by the end of exercise (J. F. P. Wojtaszewski, M. Mourtzakis, T. Hilling, B. Saltin, and H. ...

Fig. 4. Effect of single-leg arterial infusion of AICAR on transcription of UCP3, HKII, GLUT4, and lipoprotein lipase (LPL) genes in red gastrocnemius muscle. Experiments were conducted as described in Fig. 3. All data were normalized to β-actin transcription and expressed relative to saline-infused data (set to 1.0) from corresponding leg. Data are means ± SE. *Significantly \( P < 0.05 \) different from saline control within infused leg. †Significantly \( P < 0.05 \) different from corresponding contralateral leg.
Collectively, these findings indicate the need for a more definitive means of quantifying total in vivo AMPK activity. The idea that disruptions in energy charge may be a critical factor driving the adaptive response of skeletal muscle to increased contractile activity first arose from work of Pette and Vrbova (32) with chronic motor nerve stimulation. In this model, the tibialis anterior muscle of the rabbit is stimulated to contract continuously (24 h/day), eliciting over several weeks a profound adaptive, metabolic, and functional transformation from a glycolytically based fast-twitch muscle to a nearly complete oxidatively based slow-twitch muscle (31, 32). In contrast to most metabolites, phosphorylation potential (ATP/ADP × P_i) was found to be the only metabolic disturbance persistently depressed during the course of 50 days of continuous motor nerve stimulation, suggesting that cellular energy charge may be a key signaling factor to regulate activity-induced changes in gene expression (11, 30). In rats, administration of the nonmetabolized creatine analog β-guanadinopropionic acid (β-GPA) also reduces phosphorylation potential in skeletal muscle and, when administered over several weeks, results in marked increases in the expression of several metabolic genes (22, 25, 35, 53). Similarly, skeletal muscle from mice in which the muscle-specific creatine kinase gene has been knocked out (KO) is characterized by lower ATP/AMP ratios and increased mitochondrial content (43). Thus each of these conditions (chronic motor nerve stimulation, β-GPA feeding, creatine kinase KO) in which metabolic homeostasis is disrupted in skeletal muscle on a chronic basis elicits similar adaptive increases in metabolic gene expression, changes that are also qualitatively similar to those evoked by endurance exercise training (46).
There is now considerable evidence to suggest that a mechanism by which myofibers sense and respond to disruptions in energy charge involves the activation of AMPK. Exercise (10, 16, 48, 51), isolated muscle contractions (20, 26), and leptin (24) activate AMPK activity in skeletal muscle. Although only a few direct substrates for AMPK have been identified thus far, AMPK is thought to activate a number of cellular processes, including both glucose uptake and fatty acid oxidation, thus serving to coordinate the metabolic response to an increase in energy demand. Recently, evidence has also accumulated that AMPK signaling may be involved in regulating muscle gene expression. Chronic activation of AMPK in rats induced by daily injections of AICAR leads to an increase in GLUT4 and HKII protein content as well as in the activity of several mitochondrial enzymes, changes similar to the adaptive responses seen in muscle with endurance exercise training (47).

Acute activation of muscle AMPK in vivo by AICAR injection or exposure of incubating muscle to AICAR in vitro has been shown to increase GLUT4 and UCP3 mRNA (28, 54, 55), implying pretranslational regulation. Regulation at the level of transcription has been found in response to AICAR injection in transgenic mice carrying a human GLUT4 promoter/chloramphenicol acetyltransferase reporter gene construct (54). However, muscle incubated in vitro is not stable at the point whether a more prolonged period of AMPK activation is needed to determine the factors targeted by AMPK to increase both muscle AMPK activity and nuclear extracts isolated from AICAR-treated mice (54).

In contrast to UCP3 and HKII, GLUT4 and LPL failed to show any significant response to either acute injection or single-leg infusion of AICAR. This is somewhat surprising, as GLUT4 protein content has been reported to increase after both 5 and 28 successive days of AICAR administration (19, 50). It is unclear at this point whether a more prolonged period of AMPK activation is required for GLUT4 induction or whether the activation of GLUT4 transcription was simply below the detection limits of the nuclear run-on assay in the present study. Interestingly, genes involved in fatty acid oxidation do not appear to be targets for AMPK signaling (47, 50). In the present study, acute administration of AICAR did not affect transcription of the LPL or CPT I genes (Figs. 4 and 5 and unpublished observations). Winder et al. (50) also found that chronic administration of AICAR (4 wk) does not induce any significant change in CPT I or hydroxyacyl-CoA dehydrogenase activity. LPL, CPT I, and hydroxyacyl-CoA dehydrogenase are all genes whose expression increases with exercise/contractile activity (4, 18, 46), providing evidence that the exercise-induced activation of transcription likely involves multiple signaling mechanisms.

The mechanism by which AMPK regulates transcription is not known. The idea that AMPK may be directly involved in gene regulation actually stems from studies conducted in S. cerevisiae in which SNF1 kinase, the yeast homolog for AMPK, exerts its effects almost exclusively by regulating gene expression (5). In response to glucose deprivation, SNF1 kinase is activated by AMP and phosphorylates Mig1, a nuclear protein that normally represses the expression of genes required for metabolism of nonglucose carbon sources. When phosphorylated, Mig1 is inactivated and exported from the nucleus, thereby allowing the appropriate metabolic genes to be expressed (8, 39). In mammalian cells, there is strong evidence suggesting that AMPK complexes containing the α2-isoform predominately localize to the nucleus (38), raising the distinct possibility that AMPK-α2 may function in a manner analogous to SNF1 to regulate mammalian gene expression (13). The transcriptional coactivator p300 has been shown to be a direct target of AMPK (52), although the functional significance of p300 phosphorylation has not been established. Increased myocyte enhancer factor-2 (MEF-2) binding activity, a response element found within the GLUT4 and myoglobin genes, has recently been reported using muscle nuclear extracts isolated from AICAR-treated mice (54).

Chronic β-GPA feeding has also recently been reported to increase both muscle AMPK activity and nuclear respiratory factor-1 (NRF-1) DNA-binding activity. Both the MEF-2 and NRF-1 transcription factors are thought to be involved in the regulation of metabolic gene expression in skeletal muscle (29, 45). Whether these or other transcription factors are targets for AMPK phosphorylation remains to be established.

In summary, the results from the present study demonstrate that acute AICAR administration activates transcription of the UCP3 and HKII genes in both red and white skeletal muscle of rats, suggesting that the AMPK-signaling pathway may be directly linked to the transcriptional regulation of gene expression in skeletal muscle. These findings also support the hypothesis that intracellular energy charge may be a key factor leading to the transcriptional regulation of exercise-responsive genes. Further investigation is needed to determine the factors targeted by AMPK to regulate gene expression in response to cellular energy status.

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