AMP kinase is not required for the GLUT4 response to exercise and denervation in skeletal muscle

Burton F. Holmes,1 David B. Lang,1 Morris J. Birnbaum,2 James Mu,2 and G. Lynis Dohm1

1East Carolina University, Greenville, North Carolina 27858; and 2Howard Hughes Medical Institute, Philadelphia, Pennsylvania 19104

Submitted 20 February 2004; accepted in final form 26 May 2004

Holmes, Burton F., David B. Lang, Morris J. Birnbaum, James Mu, and G. Lynis Dohm. AMP kinase is not required for the GLUT4 response to exercise and denervation in skeletal muscle. Am J Physiol Endocrinol Metab 287: E739–E743, 2004. First published June 15, 2004; 10.1152/ajpendo.00080.2004.—An acute bout of exercise increases muscle GLUT4 mRNA in mice, and denervation decreases GLUT4 mRNA. AMP-activated protein kinase (AMPK) activity in skeletal muscle is also increased by exercise, and GLUT4 mRNA is increased in mouse skeletal muscle after treatment with AMPK activator 5′-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR). These findings suggest that AMPK activation might be responsible for the increase in GLUT4 mRNA expression in response to exercise. To investigate the role of AMPK in GLUT4 regulation in response to exercise and denervation, transgenic mice with a mutated AMPK α-subunit (dominant negative; AMPK-DN) were studied. GLUT4 did not increase in AMPK-DN mice that were treated with AICAR, demonstrating that muscle AMPK is inactive. Exercise (two 3-h bouts of treadmill running separated by 1 h of rest) increased GLUT4 mRNA in both wild-type and AMPK-DN mice. Likewise, denervation decreased GLUT4 mRNA in both wild-type and AMPK-DN mice. GLUT4 mRNA was also increased by AICAR treatment in both the innervated and denervated muscles. These data demonstrate that AMPK is not required for the response of GLUT4 mRNA to exercise and denervation.

Adenine 5′-monophosphate; glucose transporter isoform 4; denervation; exercise

EXERCISE TRAINING has been shown to increase total GLUT4 protein in skeletal muscle in both rats and humans (2, 5, 10, 13, 30, 31). One exercise session increased GLUT4 protein content 50% in epitrochlearis. After a second session, rats exhibited a twofold increase in GLUT4 protein (29). In studies with trained and sedentary men, the GLUT4 protein content of the trained group was roughly twofold greater than that of the sedentary group (11). After 1 wk of cycle ergometer training there was a 2.8-fold increase in muscle GLUT4 protein content in sedentary males (12).

One week of training significantly increased GLUT4 protein content (1.7-fold increase), although there was no significant increase in message 24 h after the last training bout. Transcription of the GLUT4 gene was significantly increased at 3 h after exercise in both the trained and untrained groups; however, the increase in GLUT4 gene transcription in the untrained rats was lower (1.4-fold) compared with the trained rats (1.8-fold). No increase in transcription was seen at 30 min or 24 h after exercise (24). This suggests that exercise transiently increased GLUT4 gene transcription, whereas the level of GLUT4 mRNA is increased each day after exercise and returns to baseline. However, GLUT4 protein increases each day during endurance exercise training because of the transient increase in GLUT4 mRNA. A more recent study using transgenic mice confirmed the increases in GLUT4 gene transcription (20). An acute exercise bout resulted in a significant increase in endogenous mRNA (20). Work with transgenic mice demonstrated that 895 bp of the human GLUT4 promoter were sufficient for normal GLUT4 response to exercise (20). Numerous other studies have shown an increase in GLUT4 gene expression in skeletal muscle due to physical activity (3, 7, 17, 18, 29, 33).

In contrast to exercise, denervation results in a decrease in muscle GLUT4 protein. Three to seven days postdenervation, GLUT4 protein and mRNA were decreased 50% in skeletal muscle (1). Denervation of the rat hindlimb leads to a reduction in both GLUT4 content and 3-O-methylglucose uptake in most hindlimb muscles, and the effects are greatest in oxidative tissue (21). A study from our laboratory showed that denervated mice have an 85% decrease in skeletal muscle GLUT4 mRNA (16). Work in denervated rat hindlimb showed that 5′-AMP-activated protein kinase activator 5′-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) treatment prevented the effects of denervation on GLUT4 protein levels in gastrocnemius (28). This suggests that AMPK is downstream of denervation and that artificial activation with AICAR can bypass the neuronal control to reestablish normal GLUT4 protein levels, if we assume that neuronal regulation acts through AMPK.

Although it is well established that exercise increases and denervation decreases GLUT4 expression, the mechanisms are unknown. One possible mediator that might be involved is AMPK. Exercise increases AMPK activity in skeletal muscle, and GLUT4 mRNA is increased in mouse skeletal muscle after treatment with AICAR. Previous work from our laboratory showed that the time course for GLUT4 mRNA upregulation is the same for both AICAR and treadmill exercise (20, 34). Likewise, it appears that the same DNA promoter sequences are required for the GLUT4 response to AICAR and exercise. When the mice carrying only −730 bp of the human GLUT4 promoter are exercised, there is no GLUT4 mRNA increase (20). In vivo treatment of transgenic mice with AICAR also resulted in a significant increase in relative GLUT4 mRNA levels in the −895-bp, but not the −730-bp, promoter (34).

In this study we tested the hypothesis that activated AMPK is required for the changes in muscle GLUT4 with exercise and/or denervation. Transgenic mice with inactive AMPK...
(dominant negative: AMPK-DN) were exercised or denervated, and GLUT4 mRNA was measured.

METHODS

Animal care and housing. All procedures were approved by the East Carolina University Animal Care and Use Committee. Eight-week-old mice were housed with controlled room temperature and lighting (20–22°C and 12:12-h light-dark cycle) and free access to food and water (one group of mice were fasted 24 h before treatment; however, there was no difference in results, so the remainder of the groups were fed ad libitum). Transgenic mice expressing a mutated α-subunit of the AMPK enzyme (AMPK-DN), as previously described (22), were used. AMPK-DN transgenic mice were identified by PCR analysis of isolated tail DNA by use of a DNA isolation kit (Epicentre). Another transgenic mouse, previously described (32) with the 895-bp human GLUT4 (895-bp hG4) promoter region driving the chloramphenicol acetyltransferase (CAT) gene, was kindly provided by Dr. Ann Louise Olson.

Exercise schedule. Wild-type (WT; n = 21) and AMPK-DN (n = 17) mice were exercised for two 3-h bouts of treadmill running (0% incline, 20–28 m/min) separated by 1 h of rest to study the acute effects of exercise on GLUT4 mRNA expression. Rested WT (n = 23) and transgenic (n = 13) mice were used as controls. Muscles were excised 12 h after the second bout of exercise.

Denervation. The right hindlimbs of WT and Tg mice (AMPK-DN and mouse expressing the CAT gene, driven by 895-bp hG4), were denervated as described previously (16). Briefly, the right hindlimb was denervated by severing the sciatic nerve, and the contralateral hindlimb was sham operated.

AICAR treatment. WT, AMPK-DN, and 895-bp hG4 Tg mice were injected with AICAR (1 mg/g body wt) or saline, and muscle was removed 12 h later. The 895-bp hG4 mice were injected 60 h postdenervation, and tissue was taken 72 h postdenervation.

RNA isolation and Northern analysis. RNA was isolated from quick frozen muscle samples with the TRIzol (Life Technologies, Rockville, MD) reagent, according to the manufacturer’s instructions, as our laboratory has previously described (15). For Northern analyses, 10 μg of total RNA per sample were fractionated on a 1.25% agarose-2 M formaldehyde gel and then electrotransferred to a Hybond N+ membrane (Amersham, Piscataway, NJ). The membrane blots were cross-linked under ultraviolet light for 90 s and prehybridized with ULTRAhyb buffer (Ambion, Austin, TX) for 1 h at 45°C. Blots were then hybridized at 45°C.

RNase protection assays. Radiolabeled (α-32P]UTP, 800 Ci/mmol) antisense RNA probes were transcribed from the pTRI-GAPDH-mouse antisense control template (Ambion) and the Bsu 361 linearized mouse GLUT4-CAT plasmid, p469GLUT4-CAT (27) by use of Ambion’s T3/T7 MAXiScrip in vitro transcription kit. Briefly, RNase protection assays (RPA) were performed with the streamlined procedure of Ambion’s RPA II kit, as previously described (15, 16). Ten micrograms of total RNA per sample and 32P-labeled antisense probes were hybridized overnight at 45°C. Nonhybridized RNA was digested for 1 h at 37°C with 1:1,000 dilution of RNase T1/A from the RPA II kit. The protected RNA fragments were separated by 6% acrylamide 7.5 M urea gel electrophoresis and were then exposed to PhosphorImager and quantitated with Imagequant software (Molecular Dynamics). The size of the protected fragments was estimated by RNA transcripts labeled with [α-32P]UTP from Century Marker Templates (Ambion) by use of T7 RNA polymerase.

Statistical analysis. Mean differences from each experiment were analyzed by two-way ANOVA, and a Tukey post hoc test was used when significance was found. Statistical significance was set at P ≤ 0.05.

RESULTS

Effect of AICAR on GLUT4 mRNA levels in AMPK-DN mice. AICAR treatment of the AMPK-inactive, or AMPK-DN, mice and WT littermate controls was conducted 12 h before muscle samples were taken. In agreement with our previous report (34), GLUT4 mRNA was significantly increased by AICAR treatment in WT mice [WT control = 0.97 ± 0.07 (n = 10), WT AICAR treated = 1.22 ± 0.05 (n = 11, P < 0.05)]. There was no effect of AICAR treatment on muscle GLUT4 mRNA in the AMPK-DN mice [AMPK-DN control = 0.96 ± 0.06 (n = 14), AMPK-DN AICAR treated = 0.94 ± 0.07 (n = 18) arbitrary units; Fig. 1].

Effect of exercise on GLUT4 mRNA levels in AMPK-DN mice. Groups of both fasted and fed Tg (AMPK-DN) and WT littermates were exercised for two 3-h bouts separated by 1 h of rest. No difference was seen between the fasted and fed groups, so the data were combined. Results from Northern analysis showed no differences in muscle GLUT4 mRNA between WT and AMPK-DN mice in either the rested or exercised state [WT rested = 0.86 ± 0.06, WT exercised = 1.12 ± 0.07; AMPK-DN rested = 0.88 ± 0.09, AMPK-DN = 1.13 ± 0.1 arbitrary units (AU); Fig. 2]. The increase in GLUT4 mRNA in response to exercise was statistically significant (P < 0.01) for both groups.

Effect of denervation on GLUT4 mRNA levels in AMPK-DN mice. AMPK-DN mice and WT littermates were unilaterally denervated by severing the sciatic nerve in the right hindlimb, and the contralateral limb was sham operated to serve as control. Seventy-two hours postdenervation, the gastrocnemius/plantar is muscle group was removed for RNA isolation and Northern blots to determine GLUT4 mRNA levels. The decrease in GLUT4 mRNA between the denervated and control muscle was highly significant (P < 0.001), although there was no difference between the AMPK-DN and WT mice (WT denervated = 0.62 ± 0.09, WT control = 1.36 ± 0.32, AMPK-DN denervated = 0.60 ± 0.08, AMPK-DN control = 1.40 ± 0.28 AU; Fig. 3).

Fig. 1. GLUT4 mRNA response to AICAR treatment in wild-type (WT, n = 10 and 11 for control and AICAR, respectively) and AMPK dominant negative (AMPK-DN) mice (n = 14 and 18 for control and AICAR, respectively). All values are reported as means ± SE. *Statistically significant difference (P < 0.01) between AICAR-treated and control animals, by Student’s t-test.
Effect of AICAR on GLUT4 mRNA levels in denervated muscle. Denervated 895-bp hG4 Tg mice were injected with AICAR or saline 60 h postdenervation and 12 h before collection of tissue. The AICAR- and denervation-induced changes in GLUT4 mRNA (Fig. 4A) and CAT (Fig. 4B) mRNA were qualitatively the same, but the changes in GLUT4 were of less magnitude. Denervation resulted in a 73% decrease in CAT mRNA and a 39% decrease in GLUT4 mRNA. AICAR increased CAT expression 5.4-fold in denervated muscle and 3.9-fold in control muscle. CAT mRNA in denervated and AICAR-treated muscle was 1.4-fold higher than control values but did not reach the control AICAR-treated muscle values. The same relationship between groups was found for GLUT4 mRNA.

**DISCUSSION**

The importance of GLUT4 in maintaining euglycemia has been shown in a study by Gibbs et al. (6) with a mouse model for type 2 diabetes. Introducing a GLUT4 transgene to cause overexpression of GLUT4, Gibbs et al. showed that upregulation of the GLUT4 protein restored glycemic control in diabetic mice (6). Thus, although depressed GLUT4 protein may not be responsible for insulin resistance (4), increased synthesis of GLUT4 protein could be a therapeutic target for the treatment of diabetes. Understanding the factors that affect GLUT4 expression is paramount in understanding GLUT4 regulation and may provide clues for manipulation of GLUT4 protein concentration in muscle.

Although there are numerous factors that appear to regulate GLUT4 expression, the scope of this discussion is to focus on
the necessity for AMPK activation in GLUT4 regulation during the exercised or denervated state and during AICAR treatment in rats and mice. The results from our laboratory and others indicate that AMPK activation is necessary for increased GLUT4 mRNA and protein content with exercise (8, 18–20, 23) and/or AICAR treatment (9, 14, 26, 34).

To determine whether AMPK may be regulating GLUT4 during exercise and denervation, we have utilized Tg mice with inactive muscle AMPK. In characterizing these mice, it was shown that there is no AMPK-α1 or -α2 activity. The inactive subunit is expressed in 50-fold excess over the α1- and α2-subunits and therefore is bound in such great abundance to the β complex that the endogenous α1- and α2-subunits do not bind (22). AMPK-DN and WT mice EDL muscles were exposed to electrical stimulation, and AMPK activity was measured by the SAMS (HMRSAMGLHLVKRR) peptide kinase activity method. AMPK activity was elevated nearly fourfold in the WT mice, whereas activity was almost non-existant in the AMPK-DN mice (22). As an indication of AMPK activity, in this study we treated AMPK-DN mice with AICAR and measured GLUT4 mRNA. Previously our laboratory had shown that AICAR significantly increased GLUT4 mRNA 12 h postinjection (34). When the AMPK-DN mice were treated with AICAR, there was no GLUT4 mRNA increase, whereas the WT littermates had a significant increase in GLUT4 mRNA.

Although denervation is not an exact model for inactivity, it does provide insight into muscle response when neuronal control is removed. Our data demonstrate that AICAR partially restores the decrease in GLUT4 mRNA with denervation in Tg mice. Additionally, denervation in the AMPK-DN mice results in no change in GLUT4 mRNA levels compared with control denervated mice. The combined data from the denervated and AICAR-treated plus denervated AMPK-DN mice suggest that denervation does not have a dominant-negative effect over AICAR treatment and that neuronal stimulation does not act through the AMPK-signaling pathway.

The results from the exercise and denervation experiments on the AMPK-inactive mice indicate that AMPK activity is not required to increase GLUT4 mRNA expression in mouse skeletal muscle during treadmill exercise, and that AMPK is not a regulatory enzyme in the neuronal pathway for GLUT4 expression. This therefore suggests that other pathways are involved in regulation of GLUT4 transcription in response to these stimuli. That AMPK is not required for an increase in GLUT4 mRNA with exercise does not mean that AMPK is not important during exercise. During different intensities of exercise, different pathways may be recruited or employed to increase GLUT4 mRNA and therefore improve glucose regulation. For example, at higher intensities AMPK may be essential, but at lower intensities, such as those in this study, Ca2+ may be the primary regulator, as has been suggested by recent research in L6 cells (25). The exercise protocol we followed for this study, although sufficient to increase GLUT4 expression, may not have been at an intensity that absolutely requires AMPK activation. This study and a previous report (22) both indicate that AMPK-DN mice are poor exercise performers and have a decreased ability to exercise at the intensity of their WT littermates. Therefore, the inactive AMPK in these AMPK-DN mice may play a role in their decreased ability to exercise at higher intensities.

Grants

This work was supported by a grant from the National Institutes of Health (RO1-DK-38416).

References


