ORIGINAL PAPER

Effect of AMPK activation on monocarboxylate transporter (MCT)1 and MCT4 in denervated muscle

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Abstract It is now evident that exercise training leads to increases in monocarboxylate transporter (MCT)1 and MCT4, but little is known about the mechanisms of coupling muscle contraction with these changes. The aim of this study was to investigate the effect of 5-aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside (AICAR) induced activation of AMP-activated protein kinase (AMPK) on MCT1, MCT4, and GLUT4 in denervated muscle. Protein levels of MCT4 and GLUT4 after 10 days of denervation were significantly decreased in mice gastrocnemius muscle, while MCT1 protein levels were not altered. AICAR treatment for 10 days significantly increased MCT4, and GLUT4 protein levels in innervated muscle as shown in previous studies. We found that the MCT1 protein level was also increased in AICAR treated innervated muscle. AICAR treatment prevented the decline in MCT4 and GLUT4 protein levels in denervated muscle. Thus, the current study suggests that MCT1 and MCT4 protein expression in muscles, as well as GLUT4, may be regulated by AMPK-mediated signal pathways, and AMPK activation can prevent denervation-induced decline in MCT4 protein.

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Introduction

Lactate transport across the plasma membrane is facilitated by a family of monocarboxylate transporters (MCTs) [1]. Among the 14 identified MCT isoforms, MCT1 and MCT4 are considered to be the key transporters in skeletal muscle; MCT1 facilitates lactate uptake, while MCT4 facilitates lactate extrusion [2, 3]. Studies have shown that muscle contractile activity appears to be important for the regulation of MCTs in skeletal muscle [4]. Protein contents of MCT1 and MCT4 can be increased by exercise training, and returned to the baseline levels after training cessation [5]. However, the signal pathways involved in changes in protein expression of MCTs remain largely unknown.

AMP-activated protein kinase (AMPK) is known as a master sensor of changes in cellular energy metabolism, and AMPK activation by 5-aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside (AICAR) administration stimulates the expression of proteins involved with carbohydrate metabolism, mimicking effects of exercise training [6, 7]. Skeletal muscle AMPK activity is increased during exercise [8, 9]. Moreover, GLUT4 protein levels, glycogen stores, and the activity of hexokinase and mitochondria oxidative enzymes are all increased in response to both exercise training and chronic AICAR administration [10]. Recently, it has been reported that MCT4 protein content was increased by AICAR treatment in mice [11]. Hence, it is possible that muscle contractile activity dependent changes in lactate transporters may also be dependent on AMPK.

To examine this issue, muscle denervation provides a suitable model. Neural regulation of muscle contraction is eliminated in denervated muscle, while the contralateral muscle from the same animal serves as control; all other conditions can be kept the same. MCT1 and MCT4 proteins have been shown to decrease in denervated muscle [12]. In the light of these previous findings, the aim of this study was to investigate the effect of activation of AMPK on MCT1 and MCT4 with or without immobilization due to denervation. We hypothesized that AMPK activation could rescue the decreases in MCT proteins with muscle inactivity.

Materials and methods

Animals

Eight-week-old male ICR mice (CLEA Japan, Japan) were used. Food (Oriental Yeast, Japan) and water were provided ad libitum. The room temperature was maintained at 23 ± 2 °C with a 12 h light and 12 h dark cycle. All protocols were approved by the Animal Experimental Committee of the University of Tsukuba and were in accordance with the Regulations for Animal Experiments at the University and Fundamental Guidelines for Proper Conduct of Animal Experiments and Related Activities in Academic Research Institutions under the jurisdiction of the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

Experimental design

Mice were anesthetized with ethyl ether, and the sciatic nerve of the right hindlimb was severed as we previously described [13]. Briefly, a small incision was made in the posterior aspect of the hindlimb to expose the sciatic nerve at the level of the femoral trochanter, and then the exposed nerve portion was cut off using small operating scissors at least 5.0 mm. The skin was closed with surgical glue. The contralateral hindlimb served as control. After the surgery, mice were subcutaneously injected with AICAR (0.5 mg/g body wt) (Toronto Research Chemicals, Toronto, Canada) or saline for 10 days. We selected 10 days because we found that MCT1 and MCT4 proteins were increased more robustly after 10 days of AICAR treatment than after 5 days in preliminary experiments (data not shown). After 24 h from the last injection, mice were sacrificed by cervical dislocation. The gastrocnemius muscle was dissected out quickly from each mouse, and frozen in liquid nitrogen and stored at −80 °C until further analysis. Although it is known that the gastrocnemius muscle can be visually divided into red and white portions as in our previous

reports [14, 15], we used the whole gastrocnemius muscle in this study to avoid the possibility of some artificial effects given that the muscle size would be dramatically changed after denervation.

Western blotting

Muscle samples were homogenized and prepared for electrophoresis, followed by Western blotting as we have previously described [16, 17]. Protein concentrations of homogenate samples were measured using a BCA protein assay kit (Pierce, Pittsburgh, PA, USA). Antibodies against MCT1 and MCT4 were raised in rabbits against the oligopeptide corresponding to the C-terminal region of the respective MCT (Qiagen, Japan) and have been used in previous studies [16, 18]. GLUT4 and peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) antibodies were obtained from Millipore (Billerica, MA, USA). Total AMPK-α, phospho-AMPK-α (Thr172) antibodies were obtained from Cell Signaling (Danvers, MA, USA). Blots were developed via enhanced chemiluminescence (GE Healthcare, Amersham, UK) and subsequently quantified with the ChemiDoc system (Bio-Rad Laboratories, Hercules, CA, USA). Ponceau S staining was used to control for equal loading and transfer between lanes [19].

Statistical analysis

All data were expressed as mean ± SE. The significance of differences between groups was evaluated by one-way analysis of variance (ANOVA), followed by Bonferroni post hoc tests (GraphPad Prism 5.0, La Jolla, CA, USA). Statistical significance was defined as $p < 0.05$.

Results

Table 1 summarizes the results of body weight and gastrocnemius muscle weight. The gastrocnemius muscles

Table 1 Body and gastrocnemius muscle weight in 10-day-denervated and contralateral control muscles of mice treated with AICAR or saline

Treatment	Body weight (g)	Gastrocnemius muscle weight (mg)
Saline-treated control	40.56 ± 0.68	181.42 ± 3.57
Saline-treated denervated		109.34 ± 2.38**
AICAR-treated control	39.79 ± 0.55	179.68 ± 8.01
AICAR-treated denervated		117.72 ± 8.62**

Values are mean ± SE ($n = 6$). Values are mean ± SE ($n = 6$).

** Significantly different from saline-treated control and AICAR-treated control groups ($p < 0.01$)

from saline-treated denervated (SD) and AICAR-treated denervated (AD) groups were significantly atrophied after 10 days compared with muscles of saline-treated control (SC) and AICAR-treated control (AC) groups (Table 1). There was no difference in either body weight or gastrocnemius muscle weight between saline-treated (SC and SD) and AICAR-treated (AC and AD) groups (Table 1). MCT1 (+32 %), MCT4 (+30 %), GLUT4 (+76 %), and PGC-1α (+22 %) protein levels were significantly higher in the AC group compared with the SC group (Fig. 1). Protein levels of MCT4 (−59 %), GLUT4 (−45 %), and PGC-1α (−37 %) were significantly lower in the SD group compared with the SC group, while MCT1 protein level was not altered by 10 days of denervation (Fig. 1). Total

AMPK protein level was significantly lower in the SD group compared with the SC group (Fig. 2). There was no difference in phospho-AMPK protein levels between the SC group and all other groups (Fig. 2).

Discussion

Although it is now clear that exercise training leads to increases in MCT1 and MCT4, little is known about the mechanisms of coupling muscle contraction with these changes. AMPK is a possible candidate for regulating exercise-induced changes in MCT expression, because it is known to regulate various proteins related energy

Fig. 1 MCT1, MCT4, GLUT4, and PGC-1α protein expression in gastrocnemius muscle in the saline-treated control group (SC) and saline-treated denervated group (SD), AICAR-treated control group (AC), and AICAR-treated denervated group (AD). All values are reported as mean ± SE (n = 6). *Significantly different from SC group (p < 0.05)
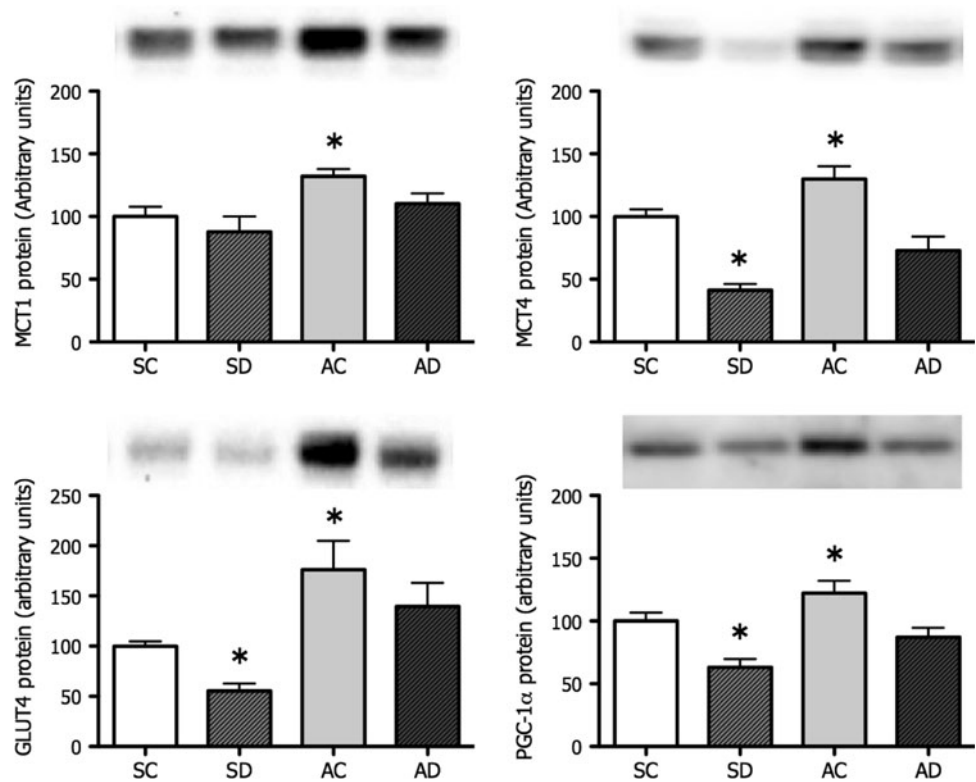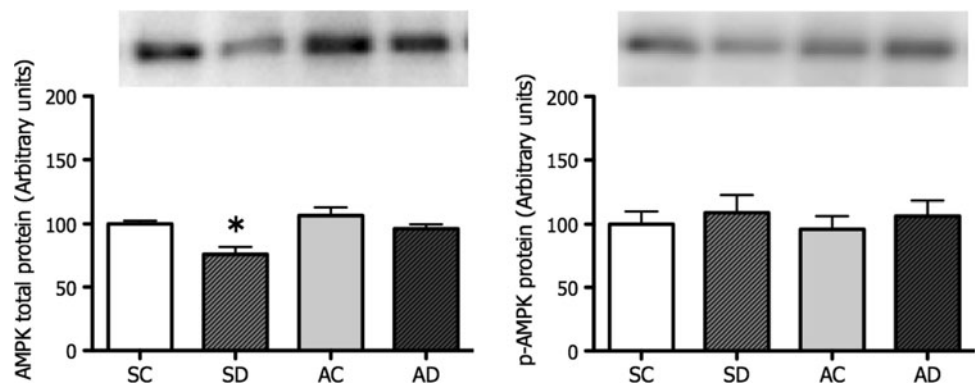

Fig. 2 Total AMPK and Phosphorylated AMPK (at Thr172) protein expression in gastrocnemius muscle in the saline-treated control group (SC) and saline-treated denervated group (SD), AICAR-treated control group (AC), and AICAR-treated denervated group (AD). All values are reported as mean ± SE (n = 6). *Significantly different from SC group (p < 0.05)

metabolism. In the current study, we found that AICAR treatment for 10 days significantly increased MCT1, MCT4, and GLUT4 protein levels in innervated muscle. To our knowledge, this is the first study to report the increase in MCT1 protein by AICAR treatment, while increases in GLUT4 and MCT4 matched previous reports [11, 20]. These results suggested that AMPK activation is important for up-regulating glucose and lactate transporters in skeletal muscle. Previously, Furugen et al. [11] reported that AMPK activation increased MCT4 protein both in white and red gastrocnemius muscles. Therefore, we speculate that there is little effect of fiber types at least in gastrocnemius muscle.

In this study, the levels of GLUT4 and MCT4 proteins were decreased when muscle activity was eliminated by denervation. Moreover, we demonstrated that these declines in GLUT4 and MCT4 proteins could be prevented by AMPK activation. It is reported that AICAR treatment prevented the decrease in GLUT4 and mitochondrial proteins that is typically seen after denervation [20, 21]. Together, these results strongly support the notion that AMPK is important for muscle contractile activity dependent changes in proteins related to energy metabolism, including MCT4. Supporting the importance of AMPK for MCT expression, AICAR-induced MCT4 up-regulation can be blocked by Compound C, an AMPK inhibitor [11]. In the current study, we found that the total AMPK protein level was decreased in denervated muscle, while the phospho-AMPK protein level was not altered. AMPK activity is known to return to basal a few hours after a single AICAR injection [22] or acute exercise [23]. Moreover, exercise training does not alter AMPK activity at rest [24]. Thus, the repeated transient activation of AMPK might be needed to increase or maintain the downstream targets.

At present, the downstream pathways of AMPK for regulating MCT are mostly unknown. As a downstream target of AMPK, PGC-1α is known as a master regulator of genes involved oxidative metabolism [25, 26]. Benton et al. [19] reported a strong relationship between PGC-1α and MCT1 proteins both in chronically stimulated and PGC-1α transfected muscles. We found that AICAR treatment increased PGC-1α protein content both in innervated and denervated muscles. Importantly, it is reported that PGC-1α is required for AICAR-induced up-regulation of GLUT4 [27]. Therefore, AICAR-induced changes in MCT1 may be dependent on PGC-1α. In contrast to PGC-1α, hypoxia inducible factor-1α (HIF-1α) regulates genes involved in glycolytic metabolism, including MCT4 [28, 29]. However, because AICAR treatment does not activate HIF-1α under normoxic conditions [30, 31], AICAR-induced up-regulation of MCT4 may be through a HIF-1α independent mechanism. Another possible mechanism regulating MCT is its substrate concentration, as a previous

study has demonstrated that MCT1 expression was increased in L6 muscle cells with high lactate concentrations [32]. Moreover, mitochondrial myopathy patients with lactic acidosis have higher MCT1 and MCT4 protein content, in spite of the apparent decrease in physical activity in daily life [33]. Importantly, it is well known that ACIAR induces an increase in glycolytic flux [34] and the subsequent increase in blood lactate concentration [35, 36]. Further study is needed to investigate molecular mechanisms of regulating MCT expression.

Previous studies reported that MCT1 and MCT4 proteins in rat muscles were decreased after denervation for 3 weeks with concomitant decreases in lactate transport [37, 38], but not after 3 days [12]. In the current study, while we found the decline in MCT4 protein after 10 days of denervation, the MCT1 protein level was not altered. This could imply that the duration of denervation was not enough to down-regulate MCT1. Furthermore, we previously reported that the protein level of MCT1 but not MCT4 could be maintained by moderate-intensity training in well-trained horses [39]. Thus, we speculate that MCT4 might be more sensitive to muscle contractile activity than MCT1. MCT1 is a high-affinity lactate transporter (Km: ∼3.5–8.3 mM), and MCT4 is a low-affinity lactate transporter (Km: 25–31 mM) [40]. Our results might suggest that MCT1 is more important in most physiological conditions than MCT4, except when lactate concentration rises to very high levels, such as during intense exercise.

In summary, we have shown that chronic activation of AMPK by AICAR injection increased MCT1, MCT4, and GLUT4 protein levels in innervated muscle, and prevented the decline in MCT4 and GLUT4 protein levels in denervated muscle. These results suggest that MCT1, MCT, and GLUT4 proteins, as well as PGC-1α, may be regulated by AMPK-mediated signal pathways in skeletal muscles, and activation of the AMPK-mediated signal pathways can prevent denervation-induced decline in MCT4 protein. Therefore, it is possible that AMPK activation is one of the key factors in the exercise-induced adaptations of lactate transporters in skeletal muscle. Our data could be beneficial for future clinical application.

Conflict of interest There is no conflict of interest.

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