Energy-sensing and signaling by AMP-activated protein kinase in skeletal muscle

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Winder, W. W. Energy-sensing and signaling by AMP-activated protein kinase in skeletal muscle. J Appl Physiol 91: 1017–1028, 2001.—AMP-activated protein kinase (AMPK) is emerging as an important energy-sensing/signaling system in skeletal muscle. This kinase is activated allosterically by 5'-AMP and inhibited allosterically by creatine phosphate. Phosphorylation of AMPK by an upstream kinase, AMPK kinase (also activated allosterically by 5'-AMP), results in activation. It is activated in both rat and human muscle in response to muscle contraction, the extent of activation depending on work rate and muscle glycogen concentration. AMPK can also be activated chemically in resting muscle with 5-aminoimidazole-4-carboxamide-riboside, which enters the muscle and is phosphorylated to form ZMP, a nucleotide that mimics the effect of 5'-AMP. Once activated, AMPK is hypothesized to phosphorylate proteins involved in triggering fatty acid oxidation and glucose uptake. Evidence is also accumulating for a role of AMPK in inducing some of the adaptations to endurance training, including the increase in muscle GLUT-4, hexokinase, uncoupling protein 3, and some of the mitochondrial oxidative enzymes. It thus appears that AMPK has the capability of monitoring intramuscular energy charge and then acutely stimulating fat oxidation and glucose uptake to counteract the increased rates of ATP utilization during muscle contraction. In addition, this system may have the capability of enhancing capacity for ATP production when the muscle is exposed to endurance training.

fatty acid oxidation; GLUT-4; glucose transport; malonyl-CoA; muscle mitochondria

ONE OF THE FASCINATING PURSUITS in the field of exercise physiology involves elucidation of the intramuscular signals that allow matching of the rate of ATP production with the increased energy requirements during exercise. What is the intramuscular change that triggers an enhancement of glucose uptake and fatty acid oxidation during a single bout of exercise? How is the availability of energy stores monitored? When a muscle is exposed repeatedly to endurance exercise, an adaptation occurs that allows enhancement of the rate of ATP production, thus allowing increased endurance at higher intensities. During the course of an endurance training program, what are the intramuscular signals that trigger accumulation of increased quantities of mitochondrial oxidative enzymes responsible for oxidation of carbohydrate and fat? Recent studies have provided initial evidence that at least one of the energy-sensing/signaling proteins of the muscle is AMP-activated protein kinase (AMPK) (16, 34–36, 99). This protein appears to be designed to monitor the energy charge of the muscle fiber and to initiate responses that prevent high-energy phosphate depletion. This signaling system is currently being characterized with respect to muscle phosphorylation targets. These protein targets of AMPK appear to be involved in both regulation of short-term metabolic responses and chronic adaptation to exercise. In this review, the AMPK system will be described in terms of its occurrence in skeletal muscle, the mechanisms of activation, processes influenced by AMPK, recently described targets of the kinase, possible roles in Type 2 diabetes and
obesity, and feasibility of manipulation of the kinase in treatment of obesity and diabetes.

STRUCTURE, DISTRIBUTION, AND QUANTITATION OF AMPK

AMPK is a protein consisting of three subunits designated α, β, and γ (34, 35) (Fig. 1). The approximate molecular masses are 63 and 38 kDa for the α- and β-subunits, respectively (34, 35, 99). The γ-subunit molecular mass varies considerably among isoforms (15). The α-subunit is the catalytic subunit containing the kinase domain, which transfers a phosphate from ATP to the target protein (34, 35). The β- and γ-subunits are considered regulatory components (15, 34, 35). All three subunits are required for expression of full activity (Fig. 1) (15, 24, 104). Each subunit has two or three isoforms, designated α1, α2, β1, β2, γ1, γ2, and γ3 (9, 15, 35). Information is available on tissue distribution based on immunoprecipitation studies (15,000 g supernatant) using antibodies to all three subunits and in combination with AMPK activity measurements (15). Both α1- and α2-isoforms are found in skeletal muscle, but the α3-isoform is more abundant, representing 80% of the total AMPK activity (15, 82, 83, 88, 91). The α2-isoform is more sensitive to AMP (81). Both β1 and β2 are found in extensor digitorum longus (EDL; predominantly type II fibers), but only β1 appears in the soleus (type I fibers) (13). Data on distribution of all subunits in the different muscle types are not currently available. On the basis of immunoprecipitation studies, the γ1-isoform predominates in skeletal muscle (muscle type unspecified), but these data are not consistent with Northern blot analyses, which indicate high relative abundance of the γ3-isoform mRNA in muscle (15). Because Western blots were performed only on postnuclear supernatants, it is possible that AMPK containing the γs-isoform sedimented with the cellular particulate fraction. The γ-subunits appear to play a role in determining sensitivity to AMP (15).

Activity of AMPK can be measured on ammonium sulfate precipitates, polyethylene glycol precipitates, or immunoprecipitates of muscle homogenates (34, 35, 91, 98). The assay measures incorporation of [32P]-labeled phosphate from [32P]ATP into an artificial peptide substrate resembling an AMPK phosphorylation target site on acetyl-CoA carboxylase (ACC). Most investigators have used a 15-amino acid peptide called “SAMS” peptide as substrate. It should be remembered that an increase in AMPK activity measured in these assays represents increases due to phosphorylation of the enzyme. As will be discussed in detail later, 5′-AMP (AMP) can allosterically activate AMPK and creatine phosphate (CP) can allosterically inhibit AMPK. The phosphorylated fraction of the enzyme could be activated allosterically in the intact muscle, and this increased AMPK activity would not show up in the assay, since AMP and CP would for the most part be discarded in the supernatant after collection of the precipitated fraction. In the assay mix, AMP is added in maximally effective activating concentrations.

MECHANISMS OF MUSCLE AMPK ACTIVATION

In addition to the glycolytic pathway and oxidative phosphorylation, two enzymes are responsible for maintaining relatively stable ATP concentrations in the muscle as muscle begins to contract and utilize ATP at increased rates (Fig. 2). Myokinase (adenylate kinase) transfers a phosphate from one ADP to another, resulting in the products AMP and ATP. An increase in free ADP concentration resulting from in-
AMPK could be considered a protein that monitors the energy state of the muscle cell and triggers metabolic processes, which when activated are designed to restore high-energy phosphate concentrations. This enzyme is regulated by both allosteric and covalent mechanisms (Figs. 1 and 3). AMPK is activated allosterically by an increase in AMP concentration (12, 16, 37). In addition, it is inhibited by CP and ATP (16, 19, 37, 73). The increase in AMP and the decrease in CP as muscle contracts result in allosteric activation of AMPK. AMP binds to AMPK, making it a better substrate for the upstream kinase, AMPK kinase (AMPKK) (37). AMP also directly activates AMPKK, which phosphorylates the α-subunit of AMPK at threonine 172, resulting in activation (37). AMP binding also makes AMPK a poor substrate for protein phosphatase 2C, the phosphatase that appears to be responsible for removal of the phosphate at threonine 172 and inactivation of the enzyme (19). CP apparently has no effect on the phosphorylation of AMPK; its inhibitory effects are entirely allosteric (see Ref. 99).

AMPKK and AMPK can also be activated artificially by use of the chemical 5-aminoimidazole-4-carboxamide-riboside (AICAR) (17, 41, 42, 60, 79, 87). AICAR has since been determined to be effective in activating AMPK in perfused hindlimb muscle, incubated muscle of the rat, cultured mouse muscle cells, and muscle of injected rats in vivo (29, 38, 50, 60, 101).

**CONTRACTION-INDUCED ACTIVATION OF AMPK IN MUSCLE**

The first indication that AMPK could be involved in regulation of muscle metabolism during exercise came from a study reported in 1996 (98). Rats were run on a treadmill at 21 m/min up a 15% grade for periods up to 30 min. The deep red region of the quadriceps muscle was quickly removed and frozen for AMPK isolation and analyses. AMPK activity was found to increase two- to threefold within 5 min of the beginning of exercise and remained elevated for as long as the rat continued to run. In rats running on the treadmill, the increase in AMPK activity was found to be dependent on work rate (75), and the activity remained elevated for several minutes following 5- and 30-min exercise bouts (74). When rat gastrocnemius muscle was stimulated in situ at a frequency of once per second, 10-ms duration, the estimated free AMP concentration increased, CP decreased, and AMPK activity increased (53). Interestingly, the decline in ACC activity (phosphorylation target for AMPK, a reporter for AMPK activity) correlated more closely with the decline in CP than with the rise in free AMP or with the increase in measurable AMPK activity. This illustrates the importance of the allosteric mechanisms. AMPK activity also increased in incubated epitrochlearis muscles in response to contraction and AICAR treatment (38).

Vavvas et al. (91) published the first information on the activation of specific isoforms of AMPK in response to muscle contraction. The gastrocnemius muscle was stimulated via the sciatic nerve (5 pulses/s, 100-ms trains, 50 Hz, 10-ms duration) to contract in situ for several minutes following 5- and 30-min exercise bouts (74). When rat gastrocnemius muscle was stimulated in situ at a frequency of once per second, 10-ms duration, the estimated free AMP concentration increased, CP decreased, and AMPK activity increased (53). Interestingly, the decline in ACC activity (phosphorylation target for AMPK, a reporter for AMPK activity) correlated more closely with the decline in CP than with the rise in free AMP or with the increase in measurable AMPK activity. This illustrates the importance of the allosteric mechanisms. AMPK activity also increased in incubated epitrochlearis muscles in response to contraction and AICAR treatment (38).

Studies published during the last year indicate that the glycogen content of muscle may modulate the AMPK response to contraction (21, 55). Derave et al. (21) subjected rats to 2 h of swimming, followed by feeding with a 100% fat diet overnight or with normal chow + 20% glucose in drinking water. The two protocols produced rats with low and high muscle glycogen, respectively. Hindlimbs of these rats were perfused with cell-free medium. After a 5-min washout period, one sciatic nerve was stimulated for 10 min with 100-ms trains (100 Hz) at 2-s intervals. In fast-twitch
white fibers from the gastrocnemius, AMPK activity measured in postnuclear supernatants of stimulated muscles was increased 1.8-fold over resting muscles in the high-glycogen group compared with 4.6-fold over resting muscles in the low-glycogen group. In slow-twitch soleus muscle, a contraction-stimulated activation of AMPK occurred in the muscles with low glycogen but not in those with high glycogen. The activity of ACC declined during contraction, however, indicating that the AMPK may have been activated allosterically. This would not have been detected in the final AMPK assay procedure. Studies using the incubated epitrochlearis muscle (fast twitch) have also demonstrated an attenuation of the AMPK response in muscles with high glycogen (55). It is unclear whether there is a direct effect of glycogen on AMPK activity or whether the attenuation is due to blunting of changes in ATP, AMP, and CP. ATP and CP responses to stimulation did not appear to be significantly altered in the soleus regardless of glycogen content, although the time course of changes were not studied (21).

Recent studies have clearly demonstrated AMPK to be activated in human muscle during exercise. Wojtaszewski et al. (103) exercised (cycle ergometer) human subjects at 50% of maximal oxygen consumption for 90 min or at 55 min at 75% followed by 5 min at 90% of maximal oxygen consumption. They reported no change in AMPK activity in needle biopsies from the vastus lateralis in response to the lower intensity work bout but a three- to fourfold increase in activity of the \( \alpha_2 \)-isoform in response to the higher intensity work bout. The activity of the \( \alpha_1 \)-isoform was not changed in response to either work rate. The activity of the \( \alpha_2 \)-isoform had returned to baseline by 3 h postexercise. Fugii et al. (30) earlier the same year reported no change in either isoform of AMPK in the vastus lateralis of human subjects in response to 20 min of work at 50% of maximal oxygen consumption, whereas activity of the \( \alpha_2 \)-isoform increased after 20 and 60 min of work at 70% of maximal oxygen consumption on the cycle ergometer. Muscle CP and glycogen were significantly decreased in response to subjects working at 70% but not at 50% of maximal oxygen consumption. One recent report indicates that the activity of both \( \alpha_1 \)- and \( \alpha_2 \)-isoforms may increase with very high-intensity work. Chen et al. (14) had subjects do a 30-s sprint (peak power = 834 W) on a cycle ergometer and found the activity of both isoforms to be increased. Richter et al. (76a) recently obtained data from human subjects indicating that elevated glycogen prevents the increase in AMPK activity in response to working at 70% of maximal oxygen consumption for 60 min, confirming the relationship between glycogen content and AMPK activity first demonstrated in animal studies (personal communication).

In summary, from the currently available data, it appears that the muscle AMPK increases in response to muscle contraction. The increase is dependent on work rate. In prolonged exercise at work rates exceeding 70% of maximal oxygen consumption, only activity of the \( \alpha_2 \)-isoform increases. In 30-s maximal sprints, activities of both isoforms increase. The presence of high glycogen in muscle appears to attenuate the increase in AMPK during prolonged exercise.

**REGULATION OF MUSCLE FATTY ACID OXIDATION BY AMPK**

The rate of fatty acid oxidation in muscle is controlled in part at the level of carnitine palmitoyltransferase-1 (CPT-1) (Fig. 4). This enzyme is subject to inhibition by malonyl-CoA (59, 78). Malonyl-CoA declines rapidly in the red region of the quadriceps muscle of rats during exercise, presumably relieving inhibition and allowing fatty acid oxidation to increase as long-chain acyl-CoA becomes available (74, 75, 97). Malonyl-CoA is synthesized by the muscle isoform of ACC, which has a molecular mass of \( \sim 272 \text{kDa} \) (89). The ACC isoform isolated from rat hindlimb muscle can be phosphorylated in vitro by both cAMP-dependent protein kinase (PKA) and by AMPK, but only phosphorylation by AMPK inactivates the enzyme, thus differing from principal isoforms in both liver and heart, which respond to phosphorylation by PKA with a decline in activity (98, 102).

The muscle isoform of ACC is activated by citrate over a range of 0–20 mM (89, 91, 98). The shape of this activation curve is dependent on the phosphorylation state of the enzyme (91, 98). Phosphorylation by AMPK causes the curve to shift to the right, and the maximal activation at high-citrate concentrations \( V_{\text{max}} \) is reduced. The activation constant \( K_a \) for citrate increases as a consequence of phosphorylation. Although the determination of the citrate activation curve (activity of ACC with citrate varied between 0 and 20 mM) is important in characterizing ACC and its phosphorylation state, muscle levels of citrate are in the range of 0.2 mM. The phosphorylated ACC is essentially inactive at physiological citrate concentrations. Nevertheless, measurement of the citrate activation curve for ACC isolated from muscle is a convenient way to assess
whether AMPK is activated (either allosterically or by phosphorylation). ACC activity thus serves as a reporter for in vivo activation of AMPK.

ACC is inactivated concurrently with AMPK activation in muscle of rats running on the treadmill (75, 98) and in muscle stimulated electrically (53, 91). It is also clear that ACC can be inactivated early during the time course of prolonged submaximal muscle contraction with no detectable increase in AMPK activity (53), implying that the AMPK may be activated allosterically or that other mechanisms exist for inactivation of ACC. When AMPK is activated using AICAR in resting perfused muscle of rats, ACC activity declines, malonyl-CoA declines, and the rate of radiolabeled palmitate oxidation increases (60, 61). These effects are also observed in incubated soleus (3) and in cultured C2C12 myocytes and myotubes (63). The rate of palmitate oxidation is dependent on palmitate concentration, but inclusion of AICAR in the medium increased the rate of oxidation regardless of palmitate concentration (61).

The processes regulating the substrate oxidation mix (carbohydrate vs. fat) in muscle are poorly understood. Although a number of factors may be important in determining this mix (including availability of glucose, concentration of insulin, muscle glycogen, availability of fatty acids, availability of carnitine and CoA, and so forth; see Ref. 94), it is likely that AMPK plays a role. Addition of insulin to medium during rat hindlimb perfusion increases glucose uptake and tends to reduce the rate of palmitate oxidation in perfused muscles (100). If AICAR is also included in the medium to activate AMPK, inactivate ACC, and reduce malonyl-CoA, the presence of insulin, even at very high concentrations, does not prevent an acceleration of the rate of palmitate oxidation. With the use of this method, a wide range of malonyl-CoA concentrations were generated in perfused rat hindlimbs. A curvilinear relationship between malonyl-CoA and palmitate oxidation was observed, with a half-maximal effect inhibition seen at ~0.6 nmol malonyl-CoA/g of muscle. This is much higher than would have been predicted from data generated from malonyl-CoA inhibition curves in isolated mitochondria (59, 78).

Although a reduction of ACC activity would be expected to reduce the rate of malonyl-CoA synthesis during muscle contraction, there remained the question of what actually caused the reduction in malonyl-CoA concentration. This question was approached by Saha et al. (80), who studied muscle responses of malonyl-CoA decarboxylase (MCD) activity to electrical stimulation and AICAR treatment. MCD is thought to catalyze the major pathway for degradation of malonyl-CoA in muscle, removing one carbon and producing acetyl-CoA. MCD activity increased approximately twofold in the gastrocnemius muscle in response to electrical stimulation. Treatment of muscle extracts with protein phosphatase 2A reversed this stimulation of activity, providing evidence that phosphorylation is involved. In addition, MCD could be activated in the

EDL by incubation with AICAR. Phosphorylation of purified MCD with purified AMPK in vitro has not yet been reported. The AICAR data provide initial evidence that AMPK regulates not only synthesis but also degradation of malonyl-CoA (Fig. 5).

The role of the AMPK system in control of fatty acid oxidation in human muscle is less well defined. All components of the signaling system are present in human muscle, including AMPK, ACC, and malonyl-CoA (14, 20). Evidence has been presented above for activation of AMPK in muscle of human subjects during exercise. Data indicate that ACC is phosphorylated (14) and inactivated (14, 20) in muscle during exercise. A small decline in malonyl-CoA has been reported by one group (20) but not so in the majority of studies (68–70). The concentration of malonyl-CoA is much lower (~0.1) in human muscle than in rat muscle, making it more difficult to measure, particularly in small muscle biopsy samples (7, 20, 68–70, 77). It is also more difficult to obtain muscle samples that are homogeneous with respect to fiber type, as can be done in rat studies. In one of the first rat studies, malonyl-CoA was found to decline rapidly in type IIA fibers but not in IIB fibers (97). If a mixture of contracting and noncontracting fibers was sampled in the biopsy of human muscle, any change in contracting fibers would be diluted. One possibility is that ACC in human muscle is arranged in close proximity to CPT-1 so that local concentration of malonyl-CoA is responsible for regulation. In this case, total tissue malonyl-CoA may not change significantly, yet the concentration near CPT-1 could vary markedly depending on the ACC phosphorylation state. This is entirely speculative, and it is possible that malonyl-CoA-independent mechanisms (see Refs. 68–70, 84, 94) are more important in control of fatty acid oxidation in human muscle. The fact remains, however, that ACC is present in the muscle. It is phosphorylated at the AMPK target site, and its activity decreases during exercise.
ROLE IN GLUCOSE UPTAKE

During the first hindlimb perfusion studies on the role of AMPK in regulation of fatty acid oxidation in muscle, it was noted that AICAR not only activated AMPK and increased palmitate oxidation but also it stimulated an increase in glucose uptake (60). It was hypothesized at the time that AMPK activated by muscle contraction stimulated both an increase in fatty acid oxidation and an increase in glucose uptake to meet the energy demands of working muscle (60).

The insulin-like effect of muscle contraction has been extensively studied (31, 32, 40, 47). The insulin-sensitive glucose transporter, GLUT-4, resides in two locations in the muscle (31, 32, 40, 47). In the basal state (low insulin), the GLUT-4 transporters reside in microvesicles beneath the sarcolemma and T-tubule membranes. In response to insulin or contraction, these transporters are translocated and inserted into membranes of the T tubules and sarcolemma, thus increasing the capacity for moving glucose into the muscle fiber (Fig. 6). The signaling pathways for the two systems differ. The insulin pathway involves occupied receptor-catalyzed phosphorylation of insulin receptor substrate 1 followed by activation of phosphatidylinositol 3-kinase, whereas the contraction pathway does not involve either of these signaling proteins (31, 32, 40, 47). The maximal effect of contraction on translocation of GLUT-4 is additive with the effects of high insulin. Although careful and extensive efforts have been expended by many laboratories in attempts to elucidate initial steps in the contraction-mediated GLUT-4 translocation, the signaling steps have been elusive. The possibility that AMPK activation may be involved has therefore generated considerable interest.

Using the incubated epitrochlearis, it was determined that AICAR would increase 3-methylglucose (3-MG) transport with characteristics similar to the contractions. It was hypothesized that AMPK activation may mediate the effect of AICAR on stimulation of glucose transport. Two studies (29, 39) have now demonstrated that both the contraction-induced increase in AMPK activity and the contraction stimulation of glucose uptake are additive with those of insulin but not with those of contraction. In perfused hindlimbs, AICAR was found to stimulate glucose uptake in the absence of insulin (47). Infusion of AICAR into live rats resulted in increased labeled hexose uptake into all three muscle fiber types (8). More recent studies have demonstrated that several factors that decrease CP and glycogen in incubated epitrochlearis (contraction, hypoxia, dinitrophenol, rotenone, and sorbitol) also activate AMPK and stimulate glucose transport (29, 39). These data provide evidence that AMPK activation mediates the effect of contraction on stimulation of glucose transport.

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Role of AMPK in Regulation of Fat Oxidation

AMPK is a key regulator of energy metabolism in muscle. It is activated by phosphorylation of Thr172 by upstream kinases, such as AMPK α2 regulatory subunit and α1 catalytic subunit. Upon activation, AMPK phosphorylates and inhibits key enzymes involved in glucose and lipid metabolism, such as pyruvate dehydrogenase and acetyl-CoA carboxylase, and accelerates fatty acid oxidation.

AMPK activation by contraction or AICAR. Stimulatory effects of AICAR were additive with those of insulin but not with those of contraction. In perfused hindlimbs, AICAR was found to stimulate glucose uptake in the absence of insulin and to cause GLUT-4 translocation to the sarcolemmal fraction from the microvesicle fraction (56). Infusion of AICAR into live rats resulted in increased labeled hexose uptake into all three muscle fiber types (8). More recent studies have demonstrated that several factors that decrease CP and glycogen in incubated epitrochlearis (contraction, hypoxia, dinitrophenol, rotenone, and sorbitol) also activate AMPK and stimulate glucose transport (29, 39). These data provide evidence that AMPK activation mediates the effect of contraction on stimulation of glucose transport.

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AMPK resulted in a depression of endogenous AMPK activity in both the resting and contracting muscle. Stimulation of hexose transport was completely blocked in AICAR-treated and hypoxic muscles but was only partially blocked in electrically stimulated contracting muscles. The authors concluded that a redundant AMPK-independent pathway must in part mediate the effect of contraction on glucose uptake in muscle. It is unclear whether residual endogenous AMPK could have been activated allosterically to mediate contraction-stimulated glucose transport in these transgenic mice. No mention was made of measurement of ACC activity as a reporter of possible allosteric activation.

Not all effects of AMPK on glucose transport may be due to effects on translocation of GLUT-4. Abbud et al. (1) studied effects of AICAR on glucose transport in C2C12 myoblasts, which rely on GLUT-1 to transport glucose. In these cells, a two- to threefold increase in glucose transport was observed in response to incubation with AICAR. Thus enhancement of the activity of other isoforms of glucose transporters may also help explain the effect of AICAR on glucose transport in muscle.

Some attention has been given to identifying the specific target protein(s) for mediating the effect of AMPK on GLUT-4 translocation and glucose transport. Previous studies have demonstrated a possible role of nitric oxide (NO) in contraction stimulation of glucose transport (5, 6). Fryer et al. (29) recently reported nitric oxide synthase (NOS) activity to be increased along with glucose transport in H-2K myotubes in response to activation of AMPK with AICAR. NOS inhibitors blocked the increase in glucose transport after AMPK activation. Both the neuronal NOS and endothelial NOS isoforms can be phosphorylated by AMPK in vitro. NO activates guanylate cyclase, which then catalyzes synthesis of cGMP. Treatment of these cells with an inhibitor of guanylate cyclase prevents the increase in glucose transport wrought by AICAR. In soleus and EDL muscle strips, the stimulatory effect of AICAR on glucose transport was also blocked by the NOS inhibitor, N\textsuperscript{6}-nitro-L-arginine methyl ester. These results provide evidence that an obligatory target of AMPK in inducing an increase in glucose transport is NOS.

A more recent study using isolated EDL and soleus muscles provides evidence against NO being a mediator of the contraction-induced activation of glucose uptake (43). Incubation of muscles with sodium nitroprusside (SNP = NO donor) resulted in stimulation of 2-deoxy-D-glucose uptake. SNP was also reported to activate \( \alpha_1 \) but not the \( \alpha_2 \)-isoform of AMPK. Inclusion of a NOS inhibitor in the incubation medium failed to block the effect of contraction on 3-MG transport. The authors proposed that NO activates glucose uptake by a pathway separate from the insulin- and contraction-induced signaling pathways. The reason for the apparently contradicting results in these two studies is unclear at this time.

ROLE IN CONTROL OF GLUT-4 AND HEXOKINASE

One of the benefits of performing regular endurance exercise is that muscles tend to become more sensitive to the action of insulin (4, 22, 26, 47–49, 54, 67, 76, 90, 93). This is thought to be due in part to the increased amounts of GLUT-4 that accumulate in muscle in response to repetitive bouts of exercise over a several-day period (26, 52, 54, 76). With daily exposure of the muscle to exercise, there is an increase in the amount of GLUT-4 mRNA that accumulates in muscle after each bout (66, 76). The GLUT-4 gene has a regulatory region that appears to have what is termed an “exercise response element” that mediates the effect of regular bouts of contraction on GLUT-4 transcription (27, 66). Although the presence of this regulatory element has been demonstrated, it is unclear what transcription factor(s) binds to this region and how this factor (or factors) is influenced by the contractile process. AMPK appears to be a candidate for controlling the putative transcription factors, thereby mediating adaptive responses to exercise. AMPK would be expected to be activated with each bout of exercise. Could it then phosphorylate a target transcription factor, which would then be more effective in turning on the GLUT-4 gene? Another possibility is that activated AMPK could trigger synthesis of relevant transcription factors? AMPK has previously been reported to be responsible for negative regulation of several genes involved in gluconeogenesis and fatty acid metabolism in liver and in pancreatic islet \( \beta \)-cell metabolism (18, 28, 57, 58).

If AMPK activation is involved in inducing an increase in GLUT-4 transcription, it should be possible to mimic the effect of training with chronic chemical activation of AMPK in resting muscle. Injection of a high dose (1 mg/g body wt) of AICAR into resting rats was found to produce an increase in muscle ZMP, induce activation of AMPK, and induce a decrease in ACC activity and in malonyl-CoA content (50). The AMPK was activated in muscle for at least 2 h in response to a single injection. When muscle AMPK was activated daily with AICAR in resting rats for a 5-day period, the total amount of GLUT-4 in epitrochlearis and gastrocnemius muscle was increased by 100% and 60%, respectively. Hexokinase activity was also significantly increased (~2.5-fold) in both muscles. The AICAR-treated rats exhibited glycogen supercompensation the morning after injection, similarly to trained rats after an overnight resting period. This increase in GLUT-4 was maintained for at least 4 wk with daily treatment of rats with AICAR (101). The increase in muscle GLUT-4 induced by AICAR injection into rats has recently been confirmed and extended (11, 107). The increase in GLUT-4 protein is accompanied by increases in mRNA (11, 107) and increases in glucose uptake of isolated epitrochlearis in response to insulin (11). Ojuka et al. (71) reported that incubation of epitrochlearis muscles with 0.5 mM AICAR for 18 h induced an increase in both GLUT-4 protein and hexokinase activity, implying that the in-
increase in these proteins in muscle was due to direct effects of AMPK activation and not to some indirect or humoral change (92, 107).

Transgenic mice have been produced with regions of the 5′-flanking region of the human GLUT-4 gene controlling expression of the bacterial chloramphenicol acetyltransferase (CAT) reporter gene. These transgenes have been utilized to determine which region of the promoter is responsible for the AICAR induction of mRNA synthesis (107). The transgenic mice with either 1,154 or 895 bp of the promoter responded to AICAR with an increase in CAT mRNA expression, but the transgenic with 730 bp was not responsive. This implies that the region between −730 and −895 bp from the GLUT-4 transcription start site contains an element essential for induction of GLUT-4 mRNA synthesis by AMPK activation. Evidence was also obtained from gel-shift assays for possible roles of MEF-2A and/or MEF-2D transcription factors in this enhancement of transcription (107). A recent preliminary report indicates hexokinase II transcription (using nuclear run-on analysis) can be enhanced 6.5- and 10-fold, respectively, in red and white gastrocnemius locally infused with AICAR (86).

ROLE IN CONTROL OF MITOCHONDRIAL PROTEINS

When muscle is exposed to repeated bouts of exercise over several days and weeks, adaptations occur that result in an increase in the maximal capacity to generate ATP. These adaptations include increases in mitochondrial enzymes of the citric acid cycle and fatty acid oxidation, proteins of the electron transport chain, and myoglobin (10, 25, 44–46, 51). Although considerable work has been done in characterizing these adaptations, the intramuscular signaling mechanisms responsible for the accumulation of these proteins are not clearly defined (see Ref. 51). Recently reported data provide evidence of AMPK involvement in inducing these adaptations (101). When muscle AMPK is activated by AICAR injection into nonexercising rats for periods up to 4 wk, citrate synthase, succinate dehydrogenase, cytochrome c, δ-aminolevulinate synthetase, and hexokinase increase in a fiber-type-specific manner. Significant increases (in the range of 40–50%) were seen in the white quadriceps (type IIb fibers) and soleus (type I fibers) but not in the red region of the quadriceps (type IIa fibers). However, the muscle AMPK response to AICAR injection was less at the end of 4 wk than at the beginning. When rats were given intermittent injections of AICAR rather than daily injections (to minimize the downregulation), a significant increase in citrate synthase in the red quadriceps was observed after 2-wk treatment (101). Enzymes of fatty acid oxidation were not significantly influenced by AICAR treatment. A more recent study, however, provides evidence of a twofold increase in the rate of CPT-1 gene transcription in response to local infusion of AICAR into muscle of conscious rats in vivo (86). An increase in transcription of CPT-1 along with other metabolic genes has also been reported for human muscle in the postexercise period (72).

It should be clear that AICAR injection does not mimic all changes in high-energy phosphate concentrations that occur during muscle contraction. ATP and CP are not markedly influenced. ZMP increases to mimic the effects of AMP. ZTP was also found to increase, and this change may interfere allosterically with full AMPK activation (101). These differences may explain why changes in mitochondrial enzymes were not of the magnitude seen with daily 2-h bouts of exercise (44, 101). The decline in CP may be essential for full manifestation of the effect of contraction on these adaptations. Nevertheless, these data suggest that the regular activation of AMPK during daily bouts of exercise may be responsible for inducing increases in some, but not all, mitochondrial oxidative enzymes.

The mitochondrial uncoupling protein 3 (UCP3) has also recently been discovered to be under the influence of AMPK (108). Treatment of incubated muscles with AICAR resulted in an increased UCP3 mRNA expression within 30 min. These findings are supported by recent work indicating a marked stimulation of skeletal muscle UCP3 gene transcription after in vivo infusion of AICAR into muscle of conscious rats (86). Although the function of UCP3 in muscle is not entirely clear, if it is indeed functioning as an uncoupler of oxidative phosphorylation, more energy substrate would be consumed for producing a given amount of ATP when mitochondrial UCP3 is increased. Is it possible that transient increases in UCP3 after exercise bouts during training could contribute to an increase in oxygen consumption and caloric expenditure during each postexercise period? Teleologically speaking, it is advantageous for those involved in regular endurance exercise to have a lower body mass. Could a transient increase in UCP3 after daily bouts of exercise contribute to the weight loss and low-fat content of those who train by running, swimming, or cycling many hours per week?

CLINICAL CONSIDERATIONS

Two very exciting reports this year emphasize the importance of continued research into this exciting field and possible application of the basic research findings to treatment or prevention of metabolic diseases. Musi et al. (64) reported that the α2-isoform of AMPK is activated in muscle of patients with Type 2 diabetes in response to 20 and 45 min of exercise. They also demonstrated with Western blots that both α-isoforms are expressed normally in muscles of these patients. It has been previously suggested that some forms of Type 2 diabetes could possibly be due to deficiencies in the AMPK signaling pathway (98). This may still prove to be the case, since subjects used in the above-mentioned study were not obese and were capable of exercising at work rates required to activate AMPK. A recent preliminary report indicates that transgenic mice deficient in muscle AMPK activity have reduced exercise tolerance (62). When the numer-
uous actions of AMPK are considered (activation of glucose transport in muscle, control of GLUT-4 expression in muscle, activation of muscle fatty acid oxidation, inhibition of hepatic cholesterol synthesis and lipogenesis, stimulation of hepatic fatty acid oxidation, and ketogenesis), it is not difficult to imagine that a deficiency in AMPK could lead to insulin resistance and obesity. Nevertheless, in those individuals with intact AMPK signaling, exercise of sufficient duration and intensity will most likely be beneficial in prevention and possibly treatment of Type 2 diabetes.

Another major finding this year was from a study in mice lacking the skeletal muscle isoform of ACC (2). The consequent low malonyl-CoA in skeletal muscle was postulated to result in chronically increased fatty acid oxidation in muscle. Labeled palmitate oxidation was 30% higher in incubated soleus muscle from these mice compared with that of wild-type mice. In addition, insulin suppression of fatty acid oxidation was not observed in soleus muscles from the ACC-deficient mice. These mice accumulated less fat in epididymal fat pads while eating normally. This confirms the studies in rats that demonstrated that daily AICAR injections (which results in activation of AMPK, inactivation of ACC, and decreased malonyl-CoA in muscle) reduced fat pad size compared with pair-fed controls (101).

It has been suggested previously that development of pharmaceutical activators of AMPK may be a feasible approach for treatment of obesity and Type 2 diabetes (50, 96, 99–101). AICAR probably will not be the drug of choice, since large quantities are required for induction of AMPK activation in vivo. Liver hypertrophy develops when large doses of AICAR are given for a prolonged period (101). Much more potent and specific AMPK activators must be developed.

SUMMARY AND FUTURE DIRECTIONS

Evidence has been presented to support the hypothesis that AMPK, activated in response to muscle contraction, is involved in regulation of fatty acid oxidation and stimulation of glucose uptake during exercise (Fig. 7). The phosphorylation targets for AMPK include ACC and possibly MCD, which then induce a decline in malonyl-CoA in rat muscle (but not in human muscle) and allow an increased rate of entrance of long-chain fatty acyl-CoA into the mitochondria for oxidation. In AMPK stimulation of glucose uptake, NOS has been hypothesized to be a phosphorylation target, but data have been presented that are inconsistent with the hypothesis that NOS activation mediates the effect of muscle contraction. Certainly, it will be important to focus research efforts on this action of AMPK to discern the downstream signaling mechanisms responsible for GLUT-4 translocation and stimulation of glucose uptake. Development of specific inhibitors of AMPK and the use of transgenic models will be important. Both of these rapid actions in muscle would have the effects of enhancing the rate of ATP production during acute bouts of exercise.

On a broader scale, evidence is accumulating for a role of chronic AMPK activation in increasing the maximal capacity for ATP production. This includes enhancement of the rate of transcription of the GLUT-4 gene, allowing increased amounts of GLUT-4 to accumulate in the muscle in response to training. This would allow increases in maximal glucose uptake during very heavy exercise. The increase in mitochondrial oxidative enzymes, hypothesized to be due in part to the chronic activation of AMPK, would allow increased maximal rates of pyruvate oxidation and ATP production as the glucose is metabolized. Thus the AMPK energy-sensing and signaling system appears to be involved in countering high-energy phosphate depletion during single bouts of exercise and, in the long term, in inducing adaptations that counteract high-energy phosphate depletion during prolonged heavy exercise. The phosphorylation targets of AMPK responsible for mediating increases in GLUT-4 and mitochondrial proteins are yet to be identified.

The basic research on the AMPK signaling pathway provides additional strong rationale for the use of exercise in prevention and treatment of Type 2 diabetes and obesity (96). Regular activation of this system appears to increase fatty acid oxidation, glucose uptake, and responsiveness of muscle to insulin (by increasing GLUT-4). It may be also feasible to develop suitable pharmaceutical AMPK activators, which would mimic many of the effects of exercise. Obviously, exercise will always be the most economical and most effective way to activate this kinase and produce the beneficial effects, but, for those who are unable to exercise, the development of AMPK activators may have clinical application (96).
REFERENCES


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