Transgenic models – a scientific tool to understand exercise-induced metabolism: the regulatory role of AMPK (5′-AMP-activated protein kinase) in glucose transport and glycogen synthase activity in skeletal muscle


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Abstract

The AMPK (5′-AMP-activated protein kinase) is becoming recognized as a critical regulator of energy metabolism. However, many of these effects in muscle metabolism have been ascribed to AMPK based on the use of the unspecific activator AICAR (5-aminoimidazole-4-carboxamide-1-β-d-ribofuranoside). Using mouse models in which AMPK activity has been specifically blocked (kinase dead) or knocked out we and others have been able to conduct studies gaining more conclusive data on the role of AMPK in muscle metabolism. In this mini-review focus is on AMPK and its regulatory role for glucose transport and GS (glycogen synthase) activity in skeletal muscle, indicating that AMPK is a GS kinase in vivo which might influence GS activity during exercise and that AMPK is involved in AICAR/hypoxia-induced glucose transport but not or only partially in contraction-stimulated glucose transport.

Introduction

Physical activity has profound effects on whole-body metabolism but in particular in the working skeletal muscle. Energy consumption may increase radically during exercise and the muscle fibres need to adjust the substrate oxidation adequately and in accordance with fuel availability. Besides endogenous fuels (glycogen and triacylglycerols) the working muscle is also dependent on the delivery of exogenous fuels, mainly glucose and fatty acids. During exercise carbohydrate oxidation contributes significantly to energy production, the source being either glucose or muscle glycogen [1].

Transgenic models have increasingly been used to study the importance of several key elements involved in glucose and glycogen metabolism during exercise, including proteins such as glucose transporter GLUT1 and GLUT4, GS (glycogen synthase), glycogen-targeting subunits (Gm) and hexokinase. In addition, the role of signalling molecules such as the insulin receptor, insulin receptor substrates (IRS1/IRS2) and very recently AMPK (5′-AMP-activated protein kinase) has been studied. In this mini-review AMPK and its regulatory role for glucose transport and as a GS kinase in skeletal muscle is discussed.

Key words: 5′-AMP-activated protein kinase (AMPK), exercise, 5-aminoimidazole-4-carboxamide-1-β-d-ribofuranoside (AICAR), glucose transport, glycogen synthase, skeletal muscle.

Abbreviations used: AMPK, 5′-AMP-activated protein kinase; AICAR, 5-aminoimidazole-4-carboxamide-1-β-d-ribofuranoside; ZMP, AICAR monophosphate; GS, glycogen synthase; GLUT, glucose transporter.

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affected by the intracellular ratio of phosphocreatine/creatinine [9,10].

The role of AMPK has been widely explored using the adenosine analogue AICAR (5-aminimidazole-4-carboxamide-1-β-D-ribofuranoside). AICAR is taken up by the cells and via phosphorylation converted to ZMP (AICAR monophosphate) [11]. ZMP mimics AMP and thereby activates AMPK, among other AMP-dependent enzymes, e.g. glycogen phosphorylase. Thus, AICAR is not a specific AMPK activator and data obtained solely using AICAR should be interpreted in this view.

**AMPK and GS**

**GS**

Besides allosteric regulation (e.g. by glucose 6-phosphate) GS activity is regulated by reversible phosphorylation and dephosphorylation leading to deactivation and activation, respectively. GS activation may therefore take place through inactivation of GS kinases and by activation of GS phosphatases. A number of these upstream enzymes are identified, but their relative importance in regulation of GS activity in vivo is unclear. GS is subjected to phosphorylation on several sites of which sites 2 and 2a are located near the N-terminus and at least seven sites (sites 3a–3c, 4, 5, 1a, 1b) are located in proximity to the C-terminus of GS. Phosphorylation of sites 2, 2a, 3a and 3b generally decreases the activity more than phosphorylation of the remaining sites that have minor or no effect on GS activity in vitro (recently reviewed by [12]).

Based on the view that AMPK is a sensor of cellular energy status, turning off ATP-consuming anabolic pathways [13], it could be hypothesized that AMPK activation may work to decrease the activity of GS, slowing down the energy-consuming incorporation of UDP-glucose into glycogen. But is AMPK a GS kinase in vivo?

**Regulation of GS by AICAR: role of AMPK**

The first study indicating AMPK as a GS kinase was performed in vitro showing that partially purified rat liver AMPK phosphorylates GS purified from rabbit skeletal muscle, leading to a partial inhibition of GS activity [14]. The phosphorylation site was shown to be in the N-terminus, most likely site 2. Later it was shown that GS co-immunoprecipitates with AMPK in skeletal muscle [15]. Recently, several observations from studies of intact muscle add further to this view. Thus in incubated slow- and fast-twitch muscles from mouse and rat, in perfused rat muscles and in fast-twitch muscles in vivo, treatment with AICAR leads to deactivation of GS ([16–18], and S.B. Jørgensen, J.N. Nielsen, S. Vaulont, D.G. Hardie, E.A. Richter, B.F. Hansen and J.F.P. Wojtaszewski, unpublished work). This deactivation is associated with a decreased gel mobility of GS, an indication of increased covalent modification, presumably phosphorylation [16]. In fact, we recently observed that GS from AICAR-treated rat skeletal muscles is heavily phosphorylated on site 2 (confirming the original study in vitro) and on site 2a (likely induced by the constitutive active casein kinase 1) whereas the phosphorylation on sites 3a and 3b is unaffected (S.B. Jørgensen, J.N. Nielsen, S. Vaulont, D.G. Hardie, E.A. Richter, B.F. Hansen and J.F.P. Wojtaszewski, unpublished work). In AICAR-perfused rat skeletal muscle this deactivation of GS is tightly inversely correlated to the activation associated with the α2 catalytic subunit of AMPK [16]. Since the accumulation of ZMP in muscle is not a specific activator of AMPK, these findings do not prove that AMPK mediates the phosphorylation and deactivation of GS. To firmly provide this link, we have investigated the effects of AICAR in isolated muscles (extensor digitorum longus and soleus) from both the α1 and α2 AMPK knockout mouse [19]. In the absence of the α1 catalytic subunit of AMPK, basal GS activity is unaffected and AICAR treatment leads to a deactivation of GS comparable with the effect seen in wild-type muscle. In contrast, in the absence of the α2 catalytic subunit basal GS activity is increased, and the deactivation of GS mediated by AICAR treatment observed in wild-type muscle is no longer present (S.B. Jørgensen, J.N. Nielsen, S. Vaulont, D.G. Hardie, E.A. Richter, B.F. Hansen and J.F.P. Wojtaszewski, unpublished work). These data provide evidence that AMPK is reducing GS activity at rest, and is mediating the deactivation of GS in response to AICAR treatment. These effects are solely mediated by the α2 catalytic subunits as these do not occur in the absence of α1-AMPK. It might seem puzzling that muscle from animals undergoing chronic AICAR treatment has enhanced, rather than normal or decreased, glycogen levels [20]. One likely explanation for this is that although GS is rate limiting for glycogen synthesis from endogenous glucose, glucose needs to be taken up into the cell, and as discussed later, this process is greatly enhanced by AICAR (by both acute processes and by long-term regulation of protein expression). This enhanced glucose availability then pushes glucose into glycogen, via mechanisms which involve glucose 6-phosphate-induced GS activation (allosterically) counteracting the AMPK-induced GS phosphorylation/deactivation [21].

**GS regulation by exercise: role of AMPK**

Breakdown of glycogen can be a major contributor to carbohydrate metabolism during exercise. When the glycogen utilization decreases either as a result of low levels of glycogen or as a result of failure to break down the glycogen, e.g. as seen in patients with McArdle’s disease, exercise performance is markedly reduced [22]. In muscle, glycogen phosphorylase activity is in excess of GS activity ensuring that the capacity for glycogenolysis is always present. It is a rather common observation that GS activity is increased in muscle after exercise during which glycogen has been utilized. This indicates that a substrate cycle is being activated under these conditions. In vivo, this ongoing incorporation of glucose into glycogen during exercise has been observed using NMR techniques (reviewed in [23]). For each substrate cycle one ATP is utilized and it is not clear what the physiological
relevance of this is under conditions where energy is needed for contractile activity. Adding to the picture however is that during very intense exercise, where glycolysis can reach very high rates, the increase in GS activity is blunted or even reduced below resting levels [24]. Thus, dependent on exercise duration and intensity, GS activity is regulated in opposite directions suggesting a complex mode of regulation.

AMPK is activated during exercise in an intensity-dependent manner and might play a pivotal role in the down-regulation of GS activity during more intense exercise. Studies performed in muscles from mouse expressing a dominant-negative AMPK construct (AMPK-dead mouse), and in muscles from either α1- or α2-AMPK-knockout mice, have revealed that GS activity is normally increased in response to 10 min of in vitro contractions [25], and S.B. Jørgensen, J.N. Nielsen, S. Vaulont, D.G. Hardie, E.A. Richter, B.F. Hansen and J.F.P. Wojtaszewski, unpublished work). This suggests that AMPK does not play a major role in the activation of GS at this point during contractions. However, GS from in vitro-contracted rat muscle is markedly phosphorylated on site 2 as long as contraction is continued. This indicates an active role of AMPK (or another endogenous activated site 2 kinase). In fact, this phosphorylation of site 2 seems to maintain GS activity at basal or sub-basal levels until GS phosphorylation decreases on sites 3a and 3b which leads to activation of GS (S.B. Jørgensen, J.N. Nielsen, S. Vaulont, D.G. Hardie, E.A. Richter, B.F. Hansen and J.F.P. Wojtaszewski, unpublished work). At present, the available data from transgenic and knockout mice (obtained after 10 min of contraction) are not sufficient to fully understand the role of AMPK in GS regulation during contraction, and further studies are needed in order to obtain more conclusive data. As mentioned before, the capacity for glycogen breakdown far exceeds that of synthesis, and breakdown under most conditions is independent of the activity of GS. Thus it is not surprising that muscle glycogen breakdown during in vivo exercise is similar in wild-type mouse and AMPK-dead mouse as well as the α2-AMPK-knockout mouse. However, in recovery from exercise glycogen repletion is slower in muscle from both of these genetically modified mice despite an apparent unchanged GS activation after exercise [25], and S.B. Jørgensen, J.N. Nielsen, S. Vaulont, D.G. Hardie, E.A. Richter, B.F. Hansen and J.F.P. Wojtaszewski, unpublished work). Again, the most likely explanation is related to the post-exercise cellular glucose availability which might be compromised to some extent because, at least in the AMPK-dead mouse, exercise-stimulated glucose transport is decreased somewhat as discussed below [26].

AMPK and glucose transport

Glucose transport

Multiple processes need to be accelerated for muscle glucose uptake to increase in vivo, including increased glucose supply (increased capillary perfusion), increased membrane transport capacity (recruitment of the GLUT4 glucose transporter protein to the outer membranes and probably through further activation of the recruited GLUT4 molecules) and increased intracellular glucose metabolism [1].

Role of AMPK in AICAR-stimulated muscle glucose transport

Since the first observation that AICAR treatment of perfused muscle increased muscle glucose uptake many studies have aimed to elucidate whether this was an effect of AMPK activation per se [27]. As discussed below, many, if not all, of these studies have focused on the transmembrane transport of glucose, but the observation that AICAR increases muscle glucose uptake in the intact animal suggests that conditions are present in vivo for this to take place [28]. At the membrane level AICAR treatment of human and rodent muscles leads to GLUT4 recruitment [29,30] and the increase in transport capacity is tightly and positively related to the degree of AMPK activation during AICAR treatment (and other stimuli activating AMPK, e.g. hypoxia) [31]. The molecular signalling utilized for this is distinct from that utilized by insulin but might be somewhat similar to that of contraction, because the effects on glucose transport of maximal effective stimuli of insulin and AICAR are additive whereas the effects of contraction and AICAR are found to be either not [28,32] or partially additive [33]. In line with these observations, AICAR-stimulated glucose transport is independent of the phosphoinositide 3-kinase whereas insulin is not [32]. Thus numerous studies suggest that AMPK mediates the increase in glucose transport in response to AICAR, but the most conclusive data so far have been obtained in studies of transgenic and knockout animals. Thus, in fast-twitch muscle from the AMPK-dead mouse, AICAR did not stimulate glucose transport [26]. Studies from our laboratory using knockout mice indicate that only the α2, and not the α1, isoform of the catalytic subunit of AMPK is responsible for the AICAR-induced muscle glucose transport in both slow- and fast-twitch muscles (S.B. Jørgensen, J.N. Nielsen, S. Vaulont, D.G. Hardie, E.A. Richter, B.F. Hansen and J.F.P. Wojtaszewski, unpublished work). Taken together, these studies show that the α2 subunit of AMPK is necessary for AICAR to stimulate glucose transport. Interestingly, in the period after AICAR treatment (acute and chronic), insulin’s ability to activate glucose transport is enhanced similar to the effect of prior exercise [34–36]. Whether these effects are due to actions through AMPK is not clear but it is an intriguing observation and has led to the hypothesis that exercise-induced improved muscle insulin sensitivity is mediated through AMPK. However, this needs to be proven.

Role of AMPK in exercise-stimulated muscle glucose transport

Winder and Hardie [36a] first observed that AMPK was activated by exercise. Taken together with the before-mentioned observation that AICAR increases glucose uptake in rat skeletal muscle, a long line of studies have added further
support for the possible involvement of AMPK in contraction-induced glucose transport (recently reviewed in [37,38]), although other studies have questioned this view as discussed later [39,40]. In favour of such a relationship is (i) that both AICAR and contraction recruit GLUT4 to the plasma membrane eliciting glucose transport which is additive to the effect of insulin, but not (or only partially) to each other [28,32,33], (ii) that both AICAR and contraction stimulate glucose transport in a phosphoinositide 3-kinase-independent manner [28,32] and (iii) that a positive relationship between the activation of glucose transport and AMPK is observed in isolated rat skeletal muscle when subjected to a range of metabolic stressors (e.g. hypoxia and dinitrophenol), electrical stimulation and AICAR [31]. Thus, numerous observations have been supportive of AMPK playing a role in exercise-induced glucose transport, but none of these studies has provided conclusive evidence. The most definite evidence again comes from experiments in transgenic mice that in fact brought serious doubt to an essential role for AMPK in contraction-induced glucose transport. Thus in the AMPK-dead mouse it was demonstrated that AICAR- and hypoxia-stimulated glucose transport was totally abolished in skeletal muscle, whereas glucose transport was reduced by only ≈40% after in vitro contraction and by ≈30% after in situ electrical stimulation [26]. This indicates that AMPK regulates glucose transport during contraction but is not necessary for contraction to stimulate glucose transport. Our own studies of the isoform-specific α1- and α2-knockout mice unfortunately do not bring more definitive answers to this topic at the moment. This is so because in muscles from these mice contraction in vitro elicits an increase in glucose transport similar to that observed in wild-type muscles (S.B. Jørgensen, J.N. Nielsen, S. Vaulont, D.G. Hardie, E.A. Richter, B.F. Hansen and J.F.P. Wojtaszewski, unpublished work). This indicates that AMPK is either not involved in contraction-stimulated glucose transport at all or that the lack of one catalytic isoform is compensated for by the remaining isoform. If AMPK is not involved in contraction-stimulated glucose transport then one needs to argue that the ‘component’ inhibited during contraction in the study of the AMPK-dead mouse is related to an AMPK-dependent contributing factor, e.g. hypoxia, present during the contraction conditions applied. This is however not necessarily so, and from the available data, it seems more fair to conclude that AMPK is partially responsible for regulation of glucose transport during exercise.

Final remarks

AMPK has marked effects on muscle glucose metabolism in skeletal muscle, and thus regulation of AMPK might have a great impact on whole-body glucose metabolism, making AMPK activation by exercise or drugs a potential target for treatment of diseases associated with insulin resistance. However, the full potential for AMPK as target is not yet clear and future studies are needed to confirm and extend our present knowledge. In this process transgenic and knockout mouse models will prove their merit.

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