Bypassing the glucose/fatty acid cycle: AMP-activated protein kinase

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Abstract

The AMPK (AMP-activated protein kinase) cascade plays a key role in regulating energy metabolism. Conditions which cause a decrease in the ATP/AMP ratio lead to activation of AMPK. Once activated, AMPK initiates a series of responses that act to restore the energy balance of the cell. In skeletal muscle, activation of AMPK increases both glucose uptake and fatty acid oxidation, raising the possibility that AMPK can bypass the glucose/fatty acid cycle. This review focuses on the role of AMPK in the regulation of glucose and fatty acid metabolism in muscle. Recently, naturally occurring mutations within the γ isoforms have been identified which lead to altered metabolic regulation in cardiac and skeletal muscle and suggest an important role for the kinase in regulating glycogen metabolism.

Introduction

AMPK (AMP-activated protein kinase) is the central component of a protein kinase cascade that plays an important role in the regulation of energy metabolism [1,2]. AMPK is a heterotrimeric enzyme that has been highly conserved throughout evolution and homologues of all three subunits have been identified in plants, yeast, nematode worms, flies and mammals [1]. Studies in mammalian cells have shown that AMPK is activated in response to conditions that deplete ATP. Once activated, AMPK initiates a series of responses aimed at restoring ATP levels. Two major consequences of AMPK activation are switching-off of ATP-consuming pathways and switching-on of ATP-generating pathways. This review will concentrate on the role of AMPK in skeletal and cardiac muscle, highlighting the important role AMPK plays in regulating energy metabolism and discussing the recent findings that mutations in the γ subunit isoforms lead to marked alterations in metabolism in these tissues.

Regulation of muscle metabolism by AMPK

Skeletal muscle plays a major part in determining whole-body energy metabolism. In humans, skeletal muscle accounts for over 70% of total body glucose disposal [3]. Moreover, muscle is a versatile tissue in terms of its substrate utilization and can use a number of fuels, including fatty acids. The regulation of energy utilization in muscle, which is subject to large fluctuations, e.g. during the change from rest to contraction and back to rest again, requires tight control. The first major clue to indicate that AMPK plays an important role in regulating skeletal muscle energy metabolism came from studies in which rat muscle was perfused with AICA (5-amino-4-imidazole carboxamide) riboside [4]. This compound is taken up by cells and phosphorylated to the monophosphate form, ZMP (AICA riboside monophosphate), which can accumulate to relatively high levels within certain cell types. ZMP mimics the activity of AMP on the AMPK cascade [5,6] and has been used in numerous studies to investigate the physiological consequences of AMPK activation. Perfusion of rat hindlimb muscle with AICA riboside led to an increase in the rate of fatty acid oxidation and an increase in glucose uptake [4]. The increased rate of fatty acid oxidation correlated with inactivation of ACC (acetyl-CoA carboxylase) and a subsequent fall in the concentration of malonyl-CoA, the end product of the reaction catalysed by ACC. There are two isoforms of ACC, encoded by separate genes [7,8]. ACC1 (ACCα; molecular mass 265 kDa) is expressed primarily in lipogenic tissues, such as liver, adipose and lactating mammary gland, and produces malonyl-CoA for fatty acid synthesis. ACC2 (ACCβ; 280 kDa) is predominantly expressed in cardiac and skeletal muscle and malonyl-CoA generated by this isoform influences the transport of fatty acids into the mitochondria through inhibition of carnitine-palmitoyl-CoA transferase I, regulating the rate of fatty acid oxidation [9]. A number of studies from several groups have demonstrated that AMPK phosphorylates and inactivates both of these isoforms [1]. Phospho-specific antibodies have been developed to the major AMPK phosphorylation site within ACC1 (Ser-79) and these cross-react with the equivalent site within ACC2 (Ser-227 in human ACC2), allowing a simple method for determining phosphorylation of ACC by AMPK in tissues.

Recently, leptin was found to activate AMPK in skeletal muscle, providing a molecular basis for the stimulatory effect of this hormone on fatty acid oxidation [10]. The activation of AMPK by leptin showed a marked bi-phasic response. A rapid (15-min) response is likely to be a direct effect of leptin binding to receptors on the muscle cell. This...
is supported by the finding that leptin activates AMPK in H-2Kb muscle cells in culture. In contrast, a slower (6-h) response appears to be mediated via the hypothalamus and stimulation of α-adrenergic receptors. The precise molecular mechanism(s) by which leptin activates AMPK are not fully understood, although there is evidence that at least part of the action of leptin on AMPK may involve a nucleotide-independent pathway (see below). Whereas leptin appears to increase fatty acid oxidation in skeletal muscle through activation of AMPK, this does not appear to be the case in cardiac muscle [11]. This finding suggests that AMPK has tissue-specific effects and this is likely to be an important consideration when examining the physiological role of the kinase.

In addition to activating fatty acid oxidation, AICA riboside perfusion of rat muscle led to an increase in glucose uptake [4]. The increase in glucose transport induced by AICA riboside was accompanied by increased translocation of GLUT4 glucose transporters to the plasma membrane [12]. A number of studies have shown that AMPK is activated in muscle in response to contraction, raising the possibility that AMPK could mediate the effect of contraction on glucose uptake [13]. In order to address this question directly, Birnbaum’s group [14] generated transgenic mice expressing a dominant-negative form of AMPK in muscle. The activation of glucose uptake in these mice by either AICA riboside or hypoxia was completely abolished, whereas the activation in response to contraction was partially inhibited. These findings suggest that AMPK mediates the effect of AICA riboside and hypoxia on glucose uptake, but there must be at least two mechanisms involved in the contraction-mediated increase in glucose uptake: an AMPK-dependent and an AMPK-independent pathway. In H-2Kb muscle cells activation of glucose uptake in response to AICA riboside or hyperosmotic stress was completely blocked by adenoviral expression of a dominant-negative form of AMPK [15]. In the same study, expression of a constitutively active form of AMPK stimulated glucose uptake concomitant with increased translocation of both GLUT1 and GLUT4 to the plasma membrane [15]. Precisely how AMPK regulates glucose transport remains enigmatic, although it is clear that it is distinct from the insulin-mediated pathway which involves stimulation of phosphoinositide 3-kinase [13].

Activation of AMPK

A key finding early on in the study of AMPK was the observation that the kinase cascade was activated in response to ATP depletion [16]. Since then many studies have demonstrated the activation of AMPK following a rise in the AMP/ATP ratio within the cell. The AMPK cascade is activated by AMP via a number of separate mechanisms and all of these are antagonized by high concentrations of ATP, resulting in an ultrasensitive system responding to changes in the AMP/ATP ratio [17]. More recently, however, evidence has emerged that the AMPK cascade can also be activated in response to stimuli that do not cause a detectable change in the AMP/ATP ratio. Several studies have shown that metformin, a widely used oral anti-hyperglycaemic agent [18], activates AMPK without altering cellular nucleotide levels [19–21]. In addition, hyperosmotic stress activates AMPK in H-2Kb muscle cells without an increase in the AMP/ATP ratio [20]. The mechanism for the nucleotide-independent activation of AMPK is not known, but it does involve phosphorylation of threonine-172 within the catalytic α-subunit of AMPK, the major site phosphorylated by the upstream kinase, AMPK kinase [20,22]. Whether there are multiple upstream kinases acting on AMPK in response to different stimuli, or whether different signalling pathways converge on the same upstream kinase, is unclear. Identification of the upstream kinase(s) in the AMPK cascade will play a significant role in helping elucidate this issue.

Mutations in the γ-subunit isoforms lead to muscle abnormalities

Three γ-subunit isoforms of AMPK have been identified [23] and all have a highly conserved C-terminal domain that contains four copies of a motif known as a CBS (cystathionine β-synthase) domain [24]. The function of this domain, which is found in a wide variety of proteins, including CBS itself, is not known, although there is evidence to suggest that in AMPK these domains may be involved in nucleotide binding [23]. A dominant mutation present in Hampshire pigs that causes high glycogen content in skeletal muscle was found to map to the γ3 gene [25]. A single amino acid substitution changing Arg-200 to a Gln (R200Q) was found to be uniquely associated with the affected allele [25]. Two potential initiating sites have been identified within pig γ3, and the numbering used here refers to residues downstream of the second initiating methionine, which was the form that was originally reported for the pig γ3 sequence [25]. The equivalent residue in human γ3, taken from the first initiating methionine, is Arg-226 [23]. In humans, mutations have been identified within γ2 that cause cardiac hypertrophy associated with Wolff–Parkinson–White syndrome [26–29]. Intriguingly, some of the mutations identified in γ2 lie in equivalent positions to the γ3 mutation within individual CBS domains (Figure 1). The effects of four different mutations within γ2 were studied by co-expressing γ2 with α1β1 or α2β1 in mammalian cells [30]. Three of these mutations occur within the CBS domains and had a significant effect on regulation of the kinase by AMP. No direct effect on activity was observed with a fourth mutation, corresponding to the insertion of a leucine residue between two adjacent CBS domains. These findings suggest that the CBS domains play a role in AMP binding and that mutations which affect AMP binding may have significant consequences for the normal development and function of the heart. In addition, the results also raise the possibility that distinct mutations leading to the same disease phenotype have different effects on AMPK activity, indicating that alternative mechanisms may contribute to similar pathogenesis. In a separate study the effect of introducing a mutation into γ1 equivalent to the R302Q mutation in γ2 or the R200Q mutation in γ3 was...
Figure 1  | Locations of naturally occurring mutations within the CBS domains of AMPK γ2 and γ3

The amino acid sequences of the CBS domains of human γ2, in which the R302Q (CBS1), H383R (CBS2) and R531G (CBS4) mutations are located, are shown aligned. The CBS domain of pig γ3 (CBS1), which contains the R200Q mutation, is also shown aligned. Amino acid identities in the CBS domains of the γ isoforms are shaded in black and conservative substitutions in grey. The residues at which the mutations occur are denoted by arrows above the sequences.

determined [31]. In that study, the mutation was also found to reduce the AMP dependence of the kinase, but in contrast to γ2 [30] the mutation in γ1 was reported to cause constitutive activation. At present it is unclear whether these differences reflect differences in the γ isoforms, or whether there are other factors that complicate the findings.

Unusual vacuoles, possibly containing glycogen, were found to be present in cardiomyocytes from patients harbouring two different mutations in γ2 (N488I and T400N) [29]. This is particularly interesting, since the γ3 mutation in pigs leads to high skeletal muscle glycogen content. Taken together these results suggest that a common mechanism may exist by which mutations in γ2 and γ3 lead to excess glycogen accumulation in heart and skeletal muscle, respectively. However, as mentioned above, it is possible that the different mutations within the γ2-subunit do not have the same effect on AMPK activity. Furthermore, the effects of the γ2 and γ3 mutations appear to be restricted either to heart (γ2) or skeletal muscle (γ3). In the case of γ3 this may simply reflect the expression pattern of the protein which is confined almost exclusively to skeletal muscle [23]. However, γ2 is expressed in a number of tissues, raising the possibility that γ2 has a specific function in heart and that changes in this function lead to the disease phenotype. This seems an attractive hypothesis given the finding that γ1 accounts for the majority of AMPK activity in all tissues, including heart and skeletal muscle [23].

AMPK and glycogen

A recent observation which seems likely to feature strongly in the regulation of AMPK is the discovery of a motif within the β-subunit that appears to play a role in glycogen binding [32,33]. This motif, originally known as an N-isoamylase domain, is found in a number of enzymes, most of which are involved in the metabolism of α1-6 branches in α1-4 glucans. In the AMPK β-subunits there is evidence that this domain binds glycogen and so it has been renamed a glycogen-binding domain [32,33]. At present the role of the glycogen-binding domain in the β-subunit isoforms is not fully understood although it is tempting to speculate that it will be involved in the regulation of glycogen metabolism by AMPK. Previously, AMPK has been shown to phosphorylate and inactivate glycogen synthase in vitro [34] and high muscle glycogen has been shown to suppress the activation of AMPK in response to AICA riboside [35]. These combined findings clearly implicate a complex link between AMPK activity, glycogen synthesis and the level of glycogen within the cell (Figure 2). How these different aspects linking AMPK to glycogen metabolism are coordinated remains to be established.
glycogen metabolism fit with the effect of γ-subunit mutations on glycogen accumulation in vivo remains to be elucidated. The efforts of a number of groups working in this area are likely to ensure that some of the pieces of the jigsaw will fall into place in the near future.

References

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