New targets of AMP-activated protein kinase

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Abstract

The discovery of the AMP-activated protein kinase (AMPK) more than a decade ago has shed much light on the cellular response to stresses characterized by a fall in the concentration of ATP and an increase in the AMP/ATP ratio. All conditions known to increase this ratio activate AMPK, whose major role is to act as an emergency signal to conserve ATP. It does so by inhibiting anabolic processes and by activating pathways producing ATP. In recent years, our laboratory has discovered new targets of AMPK. The purpose of this short review is to summarize our contribution to this field.

Control of AMP-activated protein kinase (AMPK) activity

AMPK is a well-conserved eukaryotic heterotrimeric protein kinase that senses nutritional and environmental stresses and acts as a metabolic master switch (for a review, see [1]). The control of AMPK activity is complex and involves allosteric stimulation by AMP as well as phosphorylation by AMPK kinase. AMPK activation requires phosphorylation of Thr-172 in the activation loop of its catalytic α subunit, by an upstream AMPK kinase sensitive to AMP [2]. Moreover, AMPK is allosterically activated by a decreased phosphocreatine/creatine ratio, thus reinforcing the activity-dependence of AMPK on the energy state of the cell [3]. In normoxic cells, the very low AMP/ATP ratio (about 0.05) keeps AMPK mainly in the inactive form. In contrast, any energy inbalance increases the AMP/ATP ratio and activates AMPK. This is the case when oxygen supply is limited or when energy demand exceeds supply. An adenosine analogue, 5-amino-4-imidazolecarboxamide riboside (AICAR), is commonly used to activate AMPK in certain cells after its conversion into ZMP (AICAR monophosphate), which is an AMP analogue. This does not occur in all cell types, probably because of a limited transport or phosphorylation of AICAR [4–7]. In addition, caution should be exerted because AMP, and conceivably ZMP, interact with enzymes such as glycogen phosphorylase, 6-phosphofructo-1-kinase (PFK-1) and fructose-1,6-bisphosphatase.

In normoxic heart, insulin decreases the basal activity of AMPK [8,9]. We found that AMPK activation by ischaemia was also inhibited in hearts treated with insulin [10]. This effect of insulin was wortmannin-sensititive, presumably involving the phosphoinositide 3-kinase pathway, and decreased the phosphorylation state of Thr-172 in AMPK. More importantly, the anti-AMPK effect of insulin occurred without a change in the AMP/ATP ratio. Indeed, evidence is growing that more than one upstream AMPK kinase could be involved in the control of AMPK activity independently of changes in energy charge.

Known targets of AMPK

In various tissues (liver, adipose tissue, muscle), AMPK is known to phosphorylate key enzymes, such as acetyl-CoA carboxylase, 3-hydroxy-3-methylglutaryl-CoA reductase, glycogen synthase and creatine kinase [1,3,11], which control the synthesis of fatty acids, cholesterol, glycogen and phosphocreatine, respectively. It also phosphorylates adipose tissue hormone-sensitive lipase [12]. In muscle, AMPK mediates the recruitment of glucose transporters (GLUT4) by a mechanism that differs from that of insulin [13–15]. The endothelial isoform of nitric oxide synthase is also phosphorylated and activated by AMPK [16]. The phosphorylation of malonyl-CoA decarboxylase has been reported [17] but not confirmed [18]. The long-term action of AMPK prolongs its short-term effect by inhibiting the transcription of fatty acid synthase, L-type pyruvate kinase and Spot 14 in hepatocytes, a down-regulation expected during a low-carbohydrate diet. It also increases the expression of proteins involved in glucose transport, hexokinase and several mitochondrial enzymes [19–24].

The Pasteur effect revisited

Oxygen deprivation induces a rapid stimulation of glycolysis, a phenomenon known as the Pasteur effect. The control of glycolysis is exerted at several steps: (i) the supply of glucose 6-phosphate (glucose transport and phosphorylation, glycogenolysis) and (ii) the reaction catalysed by PFK-1 [25]. PFK-1 activity is controlled allosterically by negative effectors, such as citrate, which act synergistically with ATP, and by positive effectors, such as AMP, acting in synergy with fructose 6-phosphate. Fructose 2,6-bisphosphate (Fru-2,6-P_2) is the most potent positive effector of mammalian PFK-1 and is regarded as a ubiquitous glycolytic signal [26]. The concentration of Fru-2,6-P_2 depends on the activity

Key words: apoptosis, glycolysis, Pasteur Effect, protein synthesis.

Abbreviations used: AMPK, AMP-activated protein kinase; AICAR, 5-amino-4-imidazolecarboxamide riboside; PFK-1, 6-phosphofructo-1-kinase; iPFK-2, inducible PFK-2; Fru-2,6-P_2, fructose 2,6-bisphosphate; FBPase-2, fructose-2,6-bisphosphatase; JNK, c-Jun N-terminal kinase.

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of the bifunctional enzyme PFK-2/fructose-2,6-bisphosphatase (FBPase-2), which controls its synthesis and degradation. Distinct isoenzymes of PFK-2/FBPase-2 exist in mammalian tissues and some are regulated by phosphorylation/dephosphorylation (for reviews see [27,28]). The C-terminal end of the heart PFK-2 contains two serine residues, Ser-466 and Ser-483, which are located within recognition motifs for a large number of protein kinases, including several insulin-stimulated protein kinases [29]. Phosphorylation of Ser-466 increases the V_{max} of PFK-2 about 2.5-fold without changing FBPase-2 activity [30].

The classical explanation for the Pasteur effect is an allosteric stimulation of PFK-1 by an increase in the AMP/ATP ratio. We studied whether AMPK could participate in this phenomenon by activating PFK-2 [31]. It was found that heart PFK-2 is a substrate of AMPK *in vitro*. Phosphorylation occurs at Ser-466 and, as expected, activates PFK-2 without changing FBPase-2. In perfused ischaemic rat hearts, AMPK activation correlated with PFK-2 activation and with the increase in Fru-2,6-P_2 content. In cultured cells expressing heart PFK-2, treatment with oligomycin, an inhibitor of oxidative phosphorylation, resulted in a parallel activation of AMPK and PFK-2, whose Ser-466 was phosphorylated. A dominant-negative construct of AMPK prevented both the phosphorylation and activation of heart PFK-2 by oligomycin. Therefore the stimulation of glycolysis in ischaemic hearts is due to the concomitant increase in glucose 6-phosphate supply, and the stimulation of PFK-1, directly by AMP and indirectly through the AMPK-mediated activation of PFK-2 and the resulting increase in Fru-2,6-P_2.

AMPK and PFK-2 are also partners in activated monocytes [32]. The activation of monocytes by pro-inflammatory agents involves a stimulation of glycolysis, release of potent inflammatory mediators and alterations in gene expression. These processes are further increased under hypoxic conditions. Activated monocytes express an isoform of PFK-2 resembling heart PFK-2 and called inducible PFK-2 (iPFK-2) [33]. We studied whether iPFK-2, like heart PFK-2, could be a substrate of AMPK, thereby participating in the stimulation of glycolysis by hypoxic conditions [32]. iPFK-2 was phosphorylated on the homologous serine (Ser-461) and activated by AMPK *in vitro*. Activation of human monocytes by lipopolysaccharide induced iPFK-2 expression and increased Fru-2,6-P_2 content and glycolysis. Incubation of these activated monocytes under hypoxic conditions activated AMPK and further increased iPFK-2 activity, Fru-2,6-P_2 content and glycolysis. Remarkably, this hypoxia-induced stimulation did not occur in non-activated monocytes, suggesting that expression of iPFK-2 was required.

In skeletal muscle, AMPK activation mediates the stimulation of glycolysis that results from either oxygen deprivation or muscle contraction, both of which increase the AMP/ATP ratio [34]. Whether AMPK was involved in the stimulation of heart glycolysis by contraction was tested in isolated working hearts in which contraction can be readily modulated by changing the 'afterload'. We showed that, in contrast to the situation in skeletal muscle, AMPK was not activated by increasing heart workload and that the activation of PFK-2 occurring under these conditions was wortmannin-sensitive [35].

AMPK switches off protein synthesis

Protein synthesis is an energy-consuming process, which is regulated by phosphorylation/dephosphorylation of translation factors and ribosomal proteins. We studied whether any of the factors known to control translation could be affected by AMPK. In hepatocytes under anoxia or treated with AICAR, AMPK was activated and protein synthesis was inhibited in parallel [36]. The inhibition of protein synthesis could not be explained by changes in the phosphorylation state of the known translation-initiation factors, 4E-BP1 or eIF2α. However, the phosphorylation state of the elongation factor eEF2 was increased. Although eEF2 was not a direct substrate of AMPK, the upstream eEF2 kinase was activated under these conditions. A dominant negative AMPK abolished the oligomycin-induced inhibition of protein synthesis and eEF2 phosphorylation. Therefore, the activation of eEF2 kinase by AMPK, resulting in the phosphorylation and inactivation of eEF2, provides a novel mechanism for the inhibition of protein synthesis.

Certain amino acids, like glutamine and leucine, induce an anabolic response in the liver. Among other effects, they activate p70 ribosomal protein S6 kinase (p70S6K), which is involved in the control of protein synthesis [37]. Activation of p70S6K involves multiple serine/threonine phosphorylations by several protein kinases. One such kinase is the mammalian target of rapamycin, which activates p70S6K by phosphorylating Thr-389 and is inhibited by the immunosuppressant rapamycin. We found that the activation of p70S6K by amino acids in liver was blocked or reversed when AMPK was activated [38]. AMPK did not act directly on p70S6K but was found to decrease Thr-389 phosphorylation. Similar results have been reported elsewhere [39,40].

Sustained activation of AMPK induces c-Jun kinase activation and apoptosis in liver cells

The consequences of prolonged stress and sustained AMPK activation on survival were studied in liver cells [41]. Sustained AMPK activation was obtained by AICAR or constitutively active AMPK. These treatments triggered apoptosis and activated c-Jun N-terminal kinase (JNK) and caspase-3. Experiments with iodotubercidin, dicoumarol and benzyloxycarbonyl-Val-Ala-DL-Asp-fluoromethylketone (z-VAD-fmk), which inhibited activation of AMPK, JNK and caspase-3, respectively, supported the notion that prolonged AMPK activation in liver cells induces apoptosis through an activation pathway involving JNK and caspase-3 [41]. Our results are at variance with earlier reports showing that AICAR inhibits apoptosis in several cell types [42–44] in which AMPK antagonized the pro-apoptotic effect of various compounds. Although we have no simple explanation for

the different results, it is likely that the effect of AMPK on cell survival depends on the cell type, the environmental conditions and the duration of kinase activation.

Role of AMPK

AMPK becomes activated in response to ATP depletion. As such, it behaves as an emergency signal to restore cell energy homoeostasis. We have shown that AMPK participates in the Pasteur effect, i.e. that it contributes to furnishing ATP when oxygen supply is restricted. It does so by stimulating glycolysis, at least in cells that express the PFK-2 isoform containing the C-terminal AMPK-recognition motif. Certain cancer cells express iPFK-2, which therefore might participate in the Warburg effect, namely the high glycolytic rate characteristic of proliferating cells. Besides its effect on protein synthesis and apoptosis, AMPK could also be implicated in pathological situations. The regulation of lipid and carbohydrate metabolism by AMPK makes it a good candidate to reverse some metabolic abnormalities in Type 2 diabetes [45]. Indeed, AICAR administration to diabetic rats improved their metabolic condition [46]. Interestingly, the therapeutic effect of metformin, a drug used to treat Type 2 diabetes, appears to be mediated by AMPK [47]. In addition, links between mutations in AMPK and several cardiomyopathies have been reported [48,49], suggesting that AMPK action extends beyond cell energy homoeostasis and might have other physiological roles, such as the mediation of leptin action [50].

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