Stress Signaling in the Heart by AMP-activated Protein Kinase

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The stress-signaling protein, adenosine monophosphate–activated protein kinase (AMPK), regulates a variety of pathways in cells that 1) increase the provision and utilization of energy-providing substrates such as glucose and fatty acids, 2) inhibit energy-requiring pathways such as cholesterol biosynthesis and protein synthesis, and 3) increase the transcription of genes involved in energy metabolism and mitochondrial biogenesis. In the heart, AMPK therefore becomes very important in protecting against ischemia-reperfusion injury and regulating substrate metabolism in the face of changes in workload. This review summarizes the regulation of AMPK activity in the heart and discusses the effects of AMPK activation.

Introduction

Adenosine monophosphate–activated protein kinase (AMPK) is increasingly recognized as a vital signaling molecule in the cellular response to stress. Originally two proteins were described: 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase kinase, inhibiting cholesterol synthesis, and acetyl-CoA carboxylase (ACC) kinase, stimulating fatty acid metabolism by decreasing the conversion of acetyl-CoA to malonyl-CoA. The structure of the two proteins was found to be identical and it was revealed that this protein, which is sensitive to changes in the energy state of the cell, plays a central role in regulating metabolism [1].

Metabolic and Molecular Regulation of AMPK

AMPK is a serine/threonine kinase that plays a major role in the response of mammalian cells to metabolic stress (Fig. 1). As the name indicates, AMPK activity increases in response to increases in the intracellular content of AMP, which generally occur as a result of hydrolysis of adenosine triphosphate (ATP). As noted above, AMPK inhibits both cholesterol synthesis and protein synthesis [2,3], which are energy-requiring cellular functions. AMPK also activates energy-producing metabolic pathways, including glycolysis [4,5••,6,7] and fatty acid oxidation [8,9]. Therefore, the overall effect of AMPK activation is to shift the balance from ATP consumption to ATP production to compensate for cellular metabolic stress. As a result, AMPK has been referred to as a "metabolic fuel gauge" [10] or "metabolic master switch" [11].

AMPK consists of three subunits, the catalytic α subunit and the noncatalytic β and γ subunits, which regulate the activity of AMPK and its sensitivity to activation by AMP [12]. There are two isoforms of the α subunit, α1 and α2, which might be responsible for the regulation of different downstream targets [13]. In addition, the α2 subunit is present in the nucleus as well as in the cytosol, suggesting a role for AMPK in gene expression [14]. It has been demonstrated that chronic chemical activation of AMPK with the nucleoside 5-aminoimidazole-4-carboxyamide-1-ribofuranoside (AICAR) increases the expression of key metabolic genes in skeletal muscle [15–19]. In addition, stimulation of AMPK with the AMP analog precursor AICAR in isolated cardiac myocytes and perfused hearts increases the expression of two fatty acid transporters, FABPpm and FAT/CD36 [20]. Interestingly, chronic stimulation of AMPK improves insulin sensitivity in animal models of diabetes and obesity [21,22]. Although little work has been done to demonstrate the effect of chronic activation of AMPK in the heart, mutations in the γ2 subunit of AMPK in humans result in increased AMPK activity and a cardiomyopathy characterized by intracellular glycogen accumulation and pre-excitation syndrome [23,24].

Regulation of AMPK occurs through both covalent and allosteric mechanisms. With respect to the covalent regulation of AMPK, phosphorylation of the catalytic α subunit at threonine 172 (Thr172) results in increased activity. This phosphorylation occurs through the activity of upstream kinases, known collectively as AMPK kinases (AMPKKs). The family of AMPKKs includes the tumor suppressor LKB1 [25••,26] and calmodulin-dependent

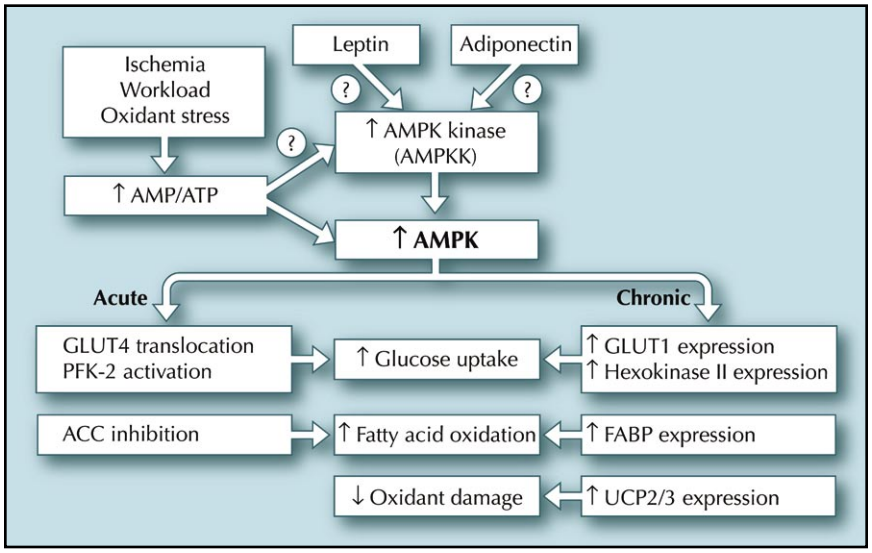

Figure 1. The adenosine monophosphate–activated protein kinase (AMPK) signaling pathway and the downstream metabolic effects of AMPK activation. ACC—acetyl-CoA carboxylase; ATP—adenosine triphosphate; FABP—fatty acid binding protein; GLUT—facilitative glucose transporter; PFK—phosphofructokinase; UCP—uncoupling protein.

protein kinase kinase-β [27,28], although there may be other members of this family of kinases that are responsible for Thr172 phosphorylation and ultimately AMPK activation. This is supported by recent findings that loss of LKB1 function in the heart prevents the Thr172 phosphorylation of the α2 subunit but does not affect phosphorylation and activation of the α1 subunit [29].

5′-Adenosine monophosphate regulates AMPK through noncovalent binding with the γ subunit. This noncovalent interaction has several effects that increase AMPK activity. First, the AMP-γ subunit complex interacts with an autoinhibitory region in the catalytic α subunit, increasing the interaction of the α subunit with target proteins [12]. Second, the binding of AMP to the γ subunit makes AMPK less susceptible to the actions of protein phosphatases, which can desphosphorylate Thr172 of the α subunit [30].

As expected, stimuli that cause changes in the energy charge of the cell will increase the activity of AMPK. These include ischemia [5••,8], increased contractile work [6,31,32], oxidant stress [33,34], and osmotic stress [35]. In addition, the chemical agent AICAR stimulates AMPK activity by being metabolized to an AMP analog, ZMP, albeit at concentrations in the millimolar range, in which there may be nonspecific effects [36,37]. However, AMPK can be activated by other stimuli that do not affect the AMP/ATP ratio. Specifically, the hypoglycemic agents rosiglitazone and metformin can cause Thr172 phosphorylation and AMPK activation [38,39], although at concentrations well above the serum concentrations that are observed with clinical use of the drugs. Nitric oxide (NO) also can stimulate AMPK activation [40], which is interesting given the fact that AMPK can phosphorylate and activate endothelial NO synthase (eNOS) [41], and NO production has been implicated in the stimulation of glucose uptake by AMPK [42•]. Furthermore, the adipokines leptin [43,44] and adiponectin [45,46] can stimulate AMPK activity, although some of these effects may be tissue-specific [47].

Role of AMPK in the Cardiovascular System

The first (and still the most studied) stimulus for AMPK activation in the heart is myocardial ischemia. Myocardial ischemia results in a switch in the main source of energy production from fatty acid oxidation to glycolysis. During reperfusion, with restored provision of oxygen to the heart, the rate of fatty acid oxidation increases over the rate observed under basal conditions. These metabolic switches during ischemia and reperfusion can be explained by activation of AMPK. AMPK is activated very rapidly with the onset of ischemia [48••]. This increase in AMPK activity is maintained during ischemia and for at least 30 minutes during reperfusion [5••]. Early studies using AICAR demonstrated that AMPK activation causes translocation of the facilitative glucose transporter GLUT4 from an intracellular storage pool to the cell surface, where it is biologically active, increasing glucose uptake [4]. Subsequent studies using transgenic mice in which AMPK was rendered inactive demonstrated that there is an absolute requirement for AMPK activation for increased glucose uptake in the setting of myocardial ischemia [5••,49]. Furthermore, AMPK activation enhances glycolysis by activating phosphofructokinase (PFK)-2, which generates fructose 2,6-bisphosphate, an activator of the glycolytic enzyme PFK-1 [7].

As noted above, AMPK phosphorylates and thereby inactivates ACC, which results in increased fatty acid oxidation. The phosphorylation of ACC decreases its ability to convert acetyl-CoA to malonyl-CoA, which is an allosteric inhibitor of carnitine palmitoyltransferase-1 (CPT-1). CPT-1 regulates the transport of fatty acids into the mitochondria and is therefore the rate-limiting enzyme of fatty acid β-oxidation by the mitochondria. Although AMPK activation during ischemia will result in the phosphorylation of ACC, the oxidation of fatty acids does not increase; instead, it **decreases** because of the lack of sufficient oxygen to maintain β-oxidation. During reperfusion, however, when adequate blood flow and oxy-

genation are reestablished, the inhibition of ACC results in enhanced fatty acid oxidation [5••,8,9].

The vital role that AMPK plays in the ischemic heart is underscored by studies assessing the effect of loss of AMPK function on the response of the heart to ischemia. In the setting of no-flow ischemia, loss of AMPK function results in a more rapid onset of ischemic contracture [49]. With low-flow ischemia followed by reperfusion, loss of AMPK function results in poorer postischemic recovery of function [5••]. Furthermore, this contractile dysfunction is associated with greater myocyte damage and increased apoptosis. It remains to be determined if this antiapoptotic effect of AMPK is related to the metabolic effects of this stress protein or if there is some direct effect on the apoptotic pathways that is mediated by AMPK.

Because of the rapid hydrolysis of ATP to adenosine diphosphate (ADP) and AMP in the setting of myocardial ischemia [50], rapid activation of AMPK is to be expected, and detectable changes in AMPK activity have been demonstrated within 1 minute of the onset of ischemia [48••]. This activation is mirrored by an increase in AMPKK activity, as determined by Thr172 phosphorylation of AMPK. Interestingly, in contrast to AMPK, which demonstrates clear allosteric activation by AMP, AMPKK activity is not affected by AMP concentrations [48••]. Furthermore, in vitro activity of one of the putative AMPKKs, LKB1, is not increased by myocardial ischemia, suggesting either that LKB1 does not play a significant role in the regulation of AMPK in the heart [51] or that other mechanisms, such as the association of LKB1 with the accessory subunits MO25 and STRAD, are responsible for increased AMPKK activity [25••].

The effects of AMPK on fatty acid metabolism are rather direct, with phosphorylation and inactivation of ACC being responsible for increased fatty acid oxidation, but the downstream mechanisms responsible for GLUT4 translocation are only beginning to be characterized. Recent studies have demonstrated that inhibition of p38 mitogen-activated protein kinase (MAPK) results in partial inhibition of the increase in glucose uptake caused by either hypoxia or AICAR stimulation and decreased translocation of GLUT4 to the cell surface [52•]. As mentioned above, AMPK activation results in phosphorylation of eNOS. However, treatment of heart muscle with NO inhibitors incompletely attenuates the increase in glucose uptake in response to AICAR stimulation [42•]. It is not clear whether the incomplete nature of the attenuation of the effects of AMPK stimulation on glucose metabolism by NO inhibitors is due to incomplete inactivation of p38 MAPK or NO production or whether p38 MAPK and eNOS are only some of the parallel mediators of the AMPK signaling cascade.

The role of AMPK in the heart's response to ischemia discussed above represents an acute response with little effect on gene transcription and protein expression. However, several studies have investigated the effects of chronic stimuli on AMPK activity in the heart. Chronic pressure overload, induced by ascending aortic banding, increases both α1- and α2-isoform–specific AMPK activity, although the expression of the α1 isoform increases while expression of the α2 isoform decreases, suggesting that there may be greater Thr172 phosphorylation of the α2 isoform to explain the increased α2 activity [53]. Interestingly, volume overload inhibits the developmental increases in AMPK expression [54]. Metabolic signals, such as prolonged exposure of isolated cardiac myocytes to the saturated fatty acid palmitate, cause a decrease in AMPK expression and increase apoptosis [55]. Furthermore, perfusion of isolated hearts with high concentrations of free fatty acids has been shown to increase Thr172 phosphorylation of the α subunits of AMPK, as does 24 hours of fasting [56]. Chronic caloric restriction did not change myocardial AMPK activity in another study, however [57]. Furthermore, in insulin-resistant rats with increased circulating free fatty acid concentrations, no change in myocardial AMPK activity was noted [58]. The conflicting results of the above studies demonstrate the need for further research in the area of chronic regulation of AMPK.

Although most of this review has focused on the role of AMPK in cardiac myocytes, it is important to point out that AMPK also plays a critical role in endothelial cells. Endothelial cells derive the bulk of their ATP from glycolysis, although glucose and fatty acid oxidation can contribute variable amounts to ATP production [59]. As mentioned above, AMPK activation leads to eNOS phosphorylation, which may be responsible, in part, for the increase in glucose utilization by endothelial cells [40,41]. Endothelial-cell AMPK activation by AICAR can increase the rates of oxidation of both glucose and fatty acids [59,60]. The significance of endothelial-cell AMPK activation by metabolic stress in vivo remains to be determined, although AMPK activation can decrease reactive oxygen species generation and apoptosis induced by hyperglycemia in endothelial cells [61,62]. Furthermore, loss of AMPK activity inhibits the expression of vascular endothelial growth factor and angiogenesis in response to hypoxic stress [63,64].

Conclusions

Recognition of the importance of AMPK in the response to metabolic and hemodynamic stressors in the cardiovascular system continues to increase, with AMPK found to be responsible for metabolic adaptations to ischemia, changes in workload, and alterations in circulating substrate concentrations, as well as for genomic-level adaptations with changes in expression of key proteins and protection against cell injury and death. Based on the growing body of information concerning the role of AMPK in the heart and vasculature, increased interest will be focused on pharmacologic manipulation of the AMPK signaling pathway. There may also be interest in possible methods of noninvasively assessing the activity of this pathway in disease states.

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