AMP-activated protein kinase: implications on ischemic diseases

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Ischemia is a blockade of blood supply due to an embolism or a hemorrhage in a blood vessel. When an organ cannot receive oxygenated blood and can therefore no longer replenish its blood supply due to ischemia, stresses, such as the disruption of blood glucose homeostasis, hypoglycemia and hypoxia, activate the AMPK complex. LKB1 and CaMKKβ are essential activators of the AMPK signaling pathway. AMPK triggers proangiogenic effects through the eNOS protein in tissues with ischemic conditions, where cells are vulnerable to apoptosis, autophagy and necrosis. The AMPK complex acts to restore blood glucose levels and ATP levels back to homeostasis. This review will discuss AMPK, as well as its key activators (LKB1 and CaMKKβ), as a central energy regulator and evaluate the upstream and downstream regulating pathways of AMPK. We will also discuss how we can control this important enzyme in ischemic conditions to prevent harmful effects in patients with vascular damage. [BMB Reports 2012; 45(9): 489-495]

INTRODUCTION

Ischemia is a condition in which blood flow to a certain organ, such as the brain or the heart, cannot be maintained or is stopped entirely (1). People who have atherosclerosis, hypertension and cerebral vascular diseases are at a higher risk of developing an ischemic disease (2). Ischemic disease is characterized by inflammatory responses, metabolic disorganization and eventual cell death (3, 4). Mammalian 5-adenosine monophosphate [AMP]-activated protein kinase (AMPK) is an enzyme involved in cellular energy homeostasis. AMPK is the master metabolic regulator, and its activation leads to the down-regulation of several energy-demanding, anabolic processes, such as fatty acid oxidation, glucose uptake, and glycogen synthesis (5, 6). It is the intracellular low fuel warning system in the body, which is active during exercise and high AMP/ATP state (6). This pathway can be activated by many factors including LKB1 and CaMKKβ (7, 8). AMPK propagates a cascade of events within cells in response to stress signals in the body that can be caused by exercise, calorie restriction, low carbohydrate diet and ischemic damage to the body (9). When blood gas and blood glucose homeostasis is disrupted and the AMP/ATP level is high, the body activates the AMPK pathway to generate ATP and restore blood gas and blood glucose back to a state of homeostatic balance (6). AMPK is activated by two complexes. The LKB1 complex activates AMPK during the normal resting state, and CaMKKβ activates AMPK during exercise and physical stress, when calcium levels are high in the body (5, 7). LKB1 is a tumor suppressor serine/threonine kinase that is encoded by the STK11 gene and is the key upstream regulator of the AMPK pathway (10). LKB1 requires two binding partners, STRAD and MO25, to activate AMPK (8). CaMKKβ, calcium dependent protein kinase kinase beta, is an upstream activator of the AMPK pathway (7). Under stress or following injury, our bodies naturally activate the AMPK pathway to turn on key metabolic pathways, such as ribosome and protein biogenesis, cell growth and proliferation, cellular autophagy, cell apoptosis, lipolysis, fatty acid synthesis, steroid synthesis, gluconeogenesis, glycogen synthesis and ultimately, cardiovascular homeostasis via the eNOS complex which is a major regulator of vascular tone. In this review, we summarize the current understanding of the associations between the AMPK signaling pathway and ischemic disease and discuss therapeutic strategies. We also speculate on the importance of the AMPK signaling pathway and its many activators in ischemic conditions to help uncover potentially effective therapeutic methods and reverse the damaging effects made by ischemic injury.

AMPK COMPLEX AND ITS MOLECULAR COMPOSITION

AMPK exists as a heterotrimeric protein complex and is expressed in virtually all eukaryotic cells, including plants, fungi...
AMPK ACTIVATION AND INHIBITION

A wide variety of agents activate AMPK (Table 1), and AMP activation occurs mainly via the 5-amino-4-imidazolecarboxamide riboside-1-β-D-ribofuranoside (AICAR) analogue, 5-amino-4-imidazolecarboxamide ribotide (ZMP) (9). ZMP phosphorylates and activates a complex consisting of STK11 (LKB1), mouse protein 25 (MO25) and the pseudokinase STE-related adaptor protein (STRAD) (8). This LKB1/MO25/STRAD complex phosphorylates AMPK on its active site, Thr172; this phosphorylation is required for AMPK to become active (8, 17). LKB1 is a tumor suppressor and is mutated in the Peutz-Jeghers syndrome (8). LKB1 is the primary AMPK activator in skeletal muscle, and ablation of LKB1 eliminates the activation of AMPK (12). It requires two binding partners STRAD and MO25 (17). LKB1 phosphorylates serine-threonine kinase (Thr172) in AMPK. AMPK can also be phosphorylated by the calcium-dependent protein kinase kinase beta (CaMKKβ) protein. Inhibition of CaMKK also leads to an inhibition of AMPK phosphorylation in muscles after a few minutes of contraction. Decrease in inhibition resulted in a low contraction because exercise is activating (18). CaMKK inhibitors had no effect on AMPK activation via AICAR phosphorylation (9). Muscle contraction activates the AMPK complex via an adenylate kinase reaction that results in high AMP/ATP levels (1, 9, 19). Adenylate kinase converts two ADP molecules into one ATP molecule and one AMP molecule (Fig. 3) (19, 20). The newly synthesized AMP molecules bind to the active site Thr172 and phosphorylate and activate the AMPK complex. C75, fatty acid synthase inhibitor, inhibits AMPK in hypothalamus (53). Compound C and adenosine 9-β-D-arabinofuranosyl-
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Table 1. Activators and Inhibitors of AMPK

<table>
<thead>
<tr>
<th>Anabolic processes</th>
<th>Hepatic Fatty Acid Oxidation (17)</th>
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<tbody>
<tr>
<td></td>
<td>Ketogenesis (17)</td>
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<tr>
<td></td>
<td>Lipogenesis (35)</td>
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<tr>
<td></td>
<td>Mitochondrial Biogenesis (36)</td>
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<td></td>
<td>Triglyceride Synthesis (36)</td>
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<td></td>
<td>Inhibition of Adipocyte (37)</td>
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<td>Lipolysis (37)</td>
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<td>Inhibition of Adipocyte (35)</td>
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<td></td>
<td>Lipogenesis (35)</td>
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<tr>
<td></td>
<td>Decrease Malonyl-CoA (36)</td>
</tr>
<tr>
<td></td>
<td>Increase Co-activator-1 (PGC-1) (36) and associated proteins (36)</td>
</tr>
</tbody>
</table>

AMPK activators

(supplemental and drug)

- AICAR (9)
- CaMKKβ (7)
- Metformin (38)
- Phenformin (39)
- Resveratrol (40)
- Ghrelin (41)
- Lipoic Acid (35)
- Leptin (42)
- ADIPONECTIN (42)
- Galegine (39)
- Phenobarbital (39)
- Captopril (2)
- Berberine (39)
- Exercise (9, 40)
- Ischemia (43)
- Decreased Glucose Levels (44)
- Increased CA2+ Levels (9)
- Low Calorie Consumption (9, 21)
- Low Carbohydrate Consumption (9, 21)
- Insulin (9)

AMPK activators (natural)

- A769662
- ZMP
- AMP

AMPK inhibitors

- Compound C (2)
- CS7 53
- 9-ß-D-arabinofuranoside (araA) 54

AMPK activators

- A769662
- AICAR
- 2-deoxy glucose
- Metformin
- Phenformin
- Galegine
- Phenobarbital
- Troglitazone
- Oxidative stress
- Berberine
- Oligomycin
- Respiratory chain
- ATP synthase
- CA2+

AMPK inhibitors

- Compound C (2)
- CS7 53
- 9-ß-D-arabinofuranoside (araA) 54

Side (araA) inhibit AMPK (54).

UPSTREAM AND DOWNSTREAM DELETED OF AMPK SIGNALING PATHWAYS

In mammals, there are several alternative upstream kinases, including the tumor suppressor LKB1, CaMKKα and CaMKKβ (10, 21-24). The AMPK signaling pathway responds to various energy stresses that cause fluctuation in the intricate balance of AMP/ATP ratio. As ATP levels fall, there is an increase in the AMP levels, which triggers the activation of the AMPK pathway (Fig. 3) (3, 6). The upstream regulator of AMPK, LKB1, allows for AMP phosphorylation at a specific site (Thr172) on the α subunit of AMPK (25). Two other subunits, STRAD and MO25, form a complex with LKB1 to enhance this process (16, 19). CaMKK is also able to activate AMPK (10, 21) Of the CaMKK proteins, CaMKKβ has the strongest regulation of AMPK (10). CaMKKβ regulates AMPK in response to changes in intracellular calcium concentrations but not to changes in the AMP:ATP ratio (22, 23). Downstream kinases of AMPK include eukaryotic elongation factor-2 kinase (eEF-2), mammalian target of rapamycin (mTOR) and ULK1. AMPK directly phosphorylates eEF-2 at Ser398 and inhibits protein synthesis by inhibiting protein elongation (26). AMPKα2 activation by AICAR regulates cell growth by decreased activation of mTOR on Ser2448 (27) and evidence suggests that mTOR inhibition by AMPK activation is mediated by Tsc2 (28). In Tsc2−/− MEFs cells, cell growth is controlled by AMPK activation and results in the direct phosphorylation of Raptor of mTORC1 (29, 30). AMPK directly phosphorylates the ULK1 complex (31-34).

AMPK AND ISCHEMIC DISEASES

Ischemic disease results in a perturbation of blood glucose homoeostasis that leads to activation of AMPK. There are two major pathways to activate AMPK. CaMKKβ can be activated through changes in calcium concentrations, or LKB1 can become active through a currently unknown mechanism (43). The activation of AMPK leads to the phosphorylation of eNOS at residue Ser1177, resulting in the production of nitric oxide. Therefore, it is reasonable to state that the activation of AMPK has a proangiogenic effect because it stimulates the eNOS pathway and allows new blood vessels to form in endothelial cells during ischemic condition (45, 46). Additionally, the Akt pathway is activated by AMPK through a mechanism that remains elusive (21). The activation of Akt is crucial for cell survival. Akt can be activated through PI3K - this is activated by insulin (41). It has been shown that PI3K also plays a crit-
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Fig. 4. Representative signaling in the AMPK pathway. Ischemic conditions increase AMP concentrations, leading to AMP binding to the CBS regions of the γ subunits of the AMPK complex and subsequent AMPK complex activation. Two AMP molecules bind to the γ subunit at each Bateman region, which is made up of two CBS regions (5). Additionally, there are three common ways in which the AMPK complex may be activated: 1) direct activation via AICAR (9, 16), 2) phosphorylation at the active site Thr172 by the LKB1/MO25/STRAD complex or the Ca2+/calmodulin complex (16, 18), and 3) phosphorylation induced by the mis-regulation of the ADP/ATP (4, 6). Metformin activates the AMPK complex by decreasing ATP levels, as well phosphorylating the active site Thr172 (38).

CONTROLLING AMPK SIGNALING PATHWAYS AND THERAPEUTIC IMPLICATIONS IN ISCHEMIC DISEASES

Once activated, AMPK induces a signaling cascade that works to boost ATP levels back to homeostatic levels. In this way, the AMPK pathway is the most important energy-regulating pathway in the body (4). AMPK activation also exerts its effect on glucose homeostasis, lipid metabolism, and protein synthesis in the liver, pancreas, skeletal muscle, heart, and brain (44). AMPK is the master regulator of energy, glucose, and fat storage. In diseased conditions, AMPK is less active, and therefore, activators are essential for the proper upkeep and regulation of these anabolic processes and proper body functions (4). While many activators exist for this important pathway, LKB1 and CaMKKβ are the major primary activators of AMPK (8, 16). The actions of LKB1 and CaMKKβ are well understood, and, as previously mentioned, extensive research has been focused on these activators to prevent the inhibition or breakdown of the AMPK pathway in disease conditions, such as ischemic injury. Many AMPK studies have focused on other human diseases, particularly breast cancer, diabetes mellitus, obesity, cardiovascular disease, and neurological disease, such as ischemia and stroke (9, 52). Further study is necessary to understand the potential of targeting AMPK activators for treatment in ischemic disease. Currently, there are two drugs that are agonists of AMPK: AICAR and metformin. AICAR enhances insulin-stimulated glucose uptake; metformin is an oral drug used to treat type II diabetes (9, 52). Metformin suppresses the activation of Complex I, thereby shifting the AMP/ATP ratio which results in the activation of AMPK (38). Compound C has been used to inhibit AMPK (2). Although this compound is commercially available, most studies focus on activating AMPK to trigger its beneficial effects (35). In terms of ischemic diseases, activation of AMPK would be far more beneficial than inhibition of AMPK.

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REFERENCES


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