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AMPK – A NOVEL THERAPEUTIC TARGET IN CARDIOVASCULAR DISEASES

F. Heidrich[1], J. Schuenemann[2], S.A. Mokashi[3] and J.D. Schmitto[1,3]

[1]Department of Thoracic, Cardiac and Vascular Surgery, University of Goettingen, Goettingen, Germany; [2]Department of Neurology, University of Goettingen, Goettingen, Germany; [3]Division of Cardiac Surgery, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

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SUMMARY

AMP-activated protein kinase (AMPK) is a heterotrimeric protein functioning as a sensitive molecular switch that is activated by physical and chemical stress. In the myocardium, its triggers include hypoxia, ischemia, myocardial hypertrophy and ventricular dysfunction. AMPK subsequently activates several potent cardioprotective signaling cascades, which lead to reversible phosphorylation of key metabolic enzymes. The major effect is to harness myocardial energy metabolism to store or unlock energy in terms of ATP. For this reason, AMPK is a rational target for pharmacological intervention. For instance, application of metformin is associated with an improvement of ventricular function and survival in heart failure and confers cardioprotection against myocardial infarction by activating the AMPK cascade.

INTRODUCTION

AMP-activated protein kinase (AMPK) was first identified by Carlson and colleagues in 1973 during an investigation of the acetyl-CoA carboxylase (ACC) enzyme. ACC functions as a key enzyme involved in fatty acid synthesis. In their experiments, Carlson et al. proved that ACC was regulated through phosphorylation and dephosphorylation (1). Specifically, Carlson described AMPK as responsible for the phosphorylation of ACC in response to energetic stress. AMPK activity itself is dependent on the intracellular ATP/AMP quotient. Therefore, AMPK plays an important role in the glucose and fatty acid metabolism of the heart and has a tremendous influence on the intracellular ATP/AMP ratio.

The AMPK cascade is activated by cellular stresses, e.g., hypoxia, pressure overload, hypertrophy or ATP deficiency (2, 3). Hence, the activity of AMPK also increases during myocardial ischemia induced by coronary microembolization or iatrogenically by percutaneous coronary intervention, both of which diminish the concentration of intracellular ATP. ATP depletion is the major signal activating AMPK by phosphorylation of the α-subunit (2). AMPK itself acts by reversible phosphorylation (stimulation) and dephosphorylation (inhibition) of key metabolic enzymes. This causes, on the one hand, a reduction of ischemic myocardial damage, and on the other hand, economization of cardiac metabolism (3-5). Economization implies an increase of catabolic pathways to generate energy and a decrease of anabolic pathways to decelerate the waste of ATP for nonessential biosynthesis. Modulation of the energy metabolism by phosphorylation and dephosphorylation is vital in terms of storing and unlocking energy as ATP. This allows the maintenance of vital transmembrane ion transporters, such as the sodium/potassium antiport, to preserve membrane potential.

AMPK is an attractive therapeutic target for minimizing the consequences of metabolic dysregulation after myocardial infarction or in the context of type 2 diabetes. In vitro analyses in isolated rat adipocytes have revealed that there is 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR)-induced phosphorylation of AMPK, which in turn inhibits basal and insulin-stimulated glucose uptake, lipid synthesis and fatty acid oxidation (6). Furthermore, investigations at the level of the skeletal muscle indicate AICAR-induced glucose uptake (7).

In vivo, AMPK is an important target of metformin. This pharmaceutical is used in patients with type 2 diabetes to reduce glucose and lipid production (8). Interestingly, metformin therapy confers protection against myocardial infarction (9). Furthermore, metformin therapy improves left ventricular function and survival in heart failure (10). This indicates that AMPK could be a pivotal target in the therapy of heart failure and type 2 diabetes.

In summary, AMPK is an important regulatory enzyme during periods of myocardial stress and plays an important role in regulating

Correspondence: Jan D. Schmitto, M.D., Ph.D., Division of Cardiac Surgery, Brigham and Women's Hospital, Harvard Medical School, 75 Francis St., Boston, MA 02115, USA. E-mail: jschmitto@partners.org.

myocardial energy metabolism, with major cardioprotective effects (11, 12). Therefore, AMPK may represent a novel therapeutic target in cardiovascular diseases.

STRUCTURE AND REGULATION

Activated AMPK is a heterotrimeric protein, which is composed of a catalytic α-subunit (63 kDa), a regulatory β-subunit (38 kDa) and a regulatory γ-subunit (38 kDa), which is allosterically adjusted by ATP and AMP (13). The binding of AMP on the γ-subunit is the step which activates AMPK. This occurs by three different mechanisms: allosteric activation, conformational change and inhibition of dephosphorylation. As the concentration of ATP increases, ATP competitively inhibits further phosphorylation of AMPK. In the second pathway, a conformational change in the α-subunit favors the phosphorylation of the α-subunit by upstream AMPK kinases such as serine/threonine-protein kinase LKB1 and calcium/calmodulin-dependent protein kinase kinase (CaMKK) (11, 14). This is supported by experiments with LKB1-deficient hearts, which leads to hypertrophy and dysfunction due to alterations in AMPK and mTOR/p70s6k/eEF-2 signaling (15). The calcium concentration plays a pivotal role in the contraction and relaxation of the myocardium. As the calcium concentration rises, CaMKK activates AMPK by phosphorylation (16). Calcium-dependent ATPases are localized in the endoplasmic reticulum and orchestrate the intracellular calcium concentrations. However, without highly regulated transporters, unregulated calcium management leads to a mismatch between myocardial contractility and relaxation. The third pathway, inhibition of dephosphorylation, can occur by several protein phosphatases (11).

AMPK IN CARDIAC METABOLISM

AMPK plays an important role in the cardiac metabolism of glucose. Second to fatty acid oxidation, it produces the most ATP in the myocardium (17). After AMPK is activated, the levels of glucose uptake increase via upregulation of either glucose transporter type 4 (GLUT-4) or type 1 (GLUT-1). Glucose transporters are necessary for glucose absorption and become translocated into the cell membranes of cardiomyocytes (18, 19). AMPK phosphorylates 6-phosphofructo-2-kinase, a key enzyme for glucose depletion, activating glycolysis during states of myocardial ischemia, exercise and anoxia. This activation is competitively regulated. Fructose-2,6-bisphosphate stimulates 6-phosphofructo-1-kinase, which is an irreversible and essential regulatory step in the glycolytic pathway (5). The direct phosphorylation of glycogen synthase and phosphorylase kinase, key enzymes of glycogen metabolism, influences glycogen storage. Thus, AMPK induces efficient storage and utilization of glucose (13). Importantly, the AMPK-mediated modulation of the above-mentioned enzymes leads to cardiac protection from anoxia and ischemia (20, 21).

Furthermore, AMPK acts in a different way in fatty acid metabolism. Here, AMPK phosphorylates ACC at the amino acids Ser79, Ser1200 and Ser1215 (22). This phosphorylation inhibits ACC and stimulates the transport of fatty acids into mitochondria, where β-oxidation is localized. Consequently, fatty acids are shunted into the catabolic pathway. Processing and consumption of fatty acids by cardiomyocytes requires the carnitine carrier system (CCS). The CCS transports acylcarnitine into the mitochondria and carnitine into the cytosol. Carnitine palmitoyltransferase (CPT I), which synthesizes acylcarnitine, is regulated by malonyl-CoA, the first product of fatty acid biosynthesis. Allosteric regulation of CPT I by malonyl-CoA enables the biosynthesis of fatty acids (1).

Increasing the translocation of lipoprotein lipase into the membrane subsequently increases the supply of fatty acids. This enzyme segregates blood lipids such as chylomicrons, triacylglycerides, very-low-density lipoproteins (VLDLs) and low-density lipoproteins (LDLs) into fatty acids. These fatty acids are channeled into the β-oxidation system to generate reducing equivalents to produce energy via the citric acid cycle (3, 4).

Other regulators of energy metabolism are the upstream fatty acid transporter (FAT/CD36) and the plasma membrane-associated fatty acid binding protein (FABP), which are responsible for fatty acid uptake into the heart (3, 4). Here, AMPK upregulates the expression of the relevant proteins to transport free fatty acids into cardiomyocytes. In the absence of AMPK, a decrease in fatty acid oxidation is seen in cardiomyocytes, which thus rely on other substrates such as lipids or pyruvate (23).

Each of these pathways increases the concentration of $NADH/H^+$ and FADH2 to extract ATP. $NADH/H^+$ and FADH2 function as reducing equivalents which are necessary to sustain the respiratory chain, which is located on the inner mitochondrial membrane, with electrons. Sufficient generation of ATP is essential to maintain cardiomyocyte electrolyte balance and cellular protein volume.

AMPK IN HEART FAILURE

As noted above, AMPK is an important regulatory enzyme during periods of myocardial stress, e.g., physiological or mechanical stress, and plays an important role in regulating energy metabolism and protein synthesis. Therefore, one principal task of AMPK is cardiovascular protection under several conditions of stress (3, 5).

In vitro and in vivo studies

In vivo studies show attenuation of experimentally induced heart failure in dogs with metformin therapy. Consequently, AMPK appears to be a rational target for pharmaceutical intervention to prevent progression of heart failure and reduce cardiomyocyte apoptosis (24). Furthermore, Ko et al. show that inhibition of AMPK reduces glucose metabolism and activates inflammation in mice (25). AMPK could be regulated by LKB1, but deletion of LKB1 leads to hypertrophy and dysfunction. The main steps are alterations in AMPK or mTOR/p70s6k/eEF-2 signaling (15). Russell et al. described comparable results. They found an increase in glucose and oleate oxidation, as well as glucose uptake, during ischemia in "kinase-dead" mice compared to wild-type animals. These findings suggest that AMPK mediates ischemic glucose uptake and prevents postischemic cardiac dysfunction, apoptosis and injury (20). Solskov et al. demonstrated cardioprotection in rats with ischemia/reperfusion (I/R) injury 24 h after administration of metformin, which was based on activation of AMPK (26). In contrast, in the absence of regulation by AMPK, cell death and ultimately necrosis, as seen in multiple cases of heart disease, occur.

AMPK could be an attractive therapeutic target to minimize the consequences of myocardial infarction. The mechanism is mentioned

above, e.g., activation of glucose absorption by translocating GLUT-4 and -1 into the cardiac cellular membrane or stimulation of fatty acid oxidation via inhibition of ACC (3, 4). Acute metformin therapy conferred cardioprotection against myocardial infarction in a murine model of myocardial I/R injury during transient myocardial ischemia for a period of 30 min (9). Furthermore, metformin therapy improved left ventricular function and survival in a murine model of heart failure. In this model, heart failure was induced either by permanent left coronary artery occlusion or by 60-min left coronary artery occlusion followed by reperfusion for 4 weeks (10). In another study, the role of AMPK was investigated in a model of chronic heart failure induced by multiple sequential intracoronary microembolizations (27-29). Quantitative analysis in this study showed significantly higher phosphorylation of AMPK in animals with chronic heart failure compared to control myocardium (30).

These animal models clarify the important interaction between AMPK and metformin, which leads to rational therapy of cardiovascular diseases and type 2 diabetes (31). Consequently, AMPK represents an important target in the therapy of heart failure. Similarly, AMPK is an important target of metformin in type 2 diabetes therapy, reducing glucose and lipid production (8). AMPK plays a key role as a protein kinase in several metabolic pathways of the heart, including cellular energy sensing and cardiovascular protection (Fig. 1).

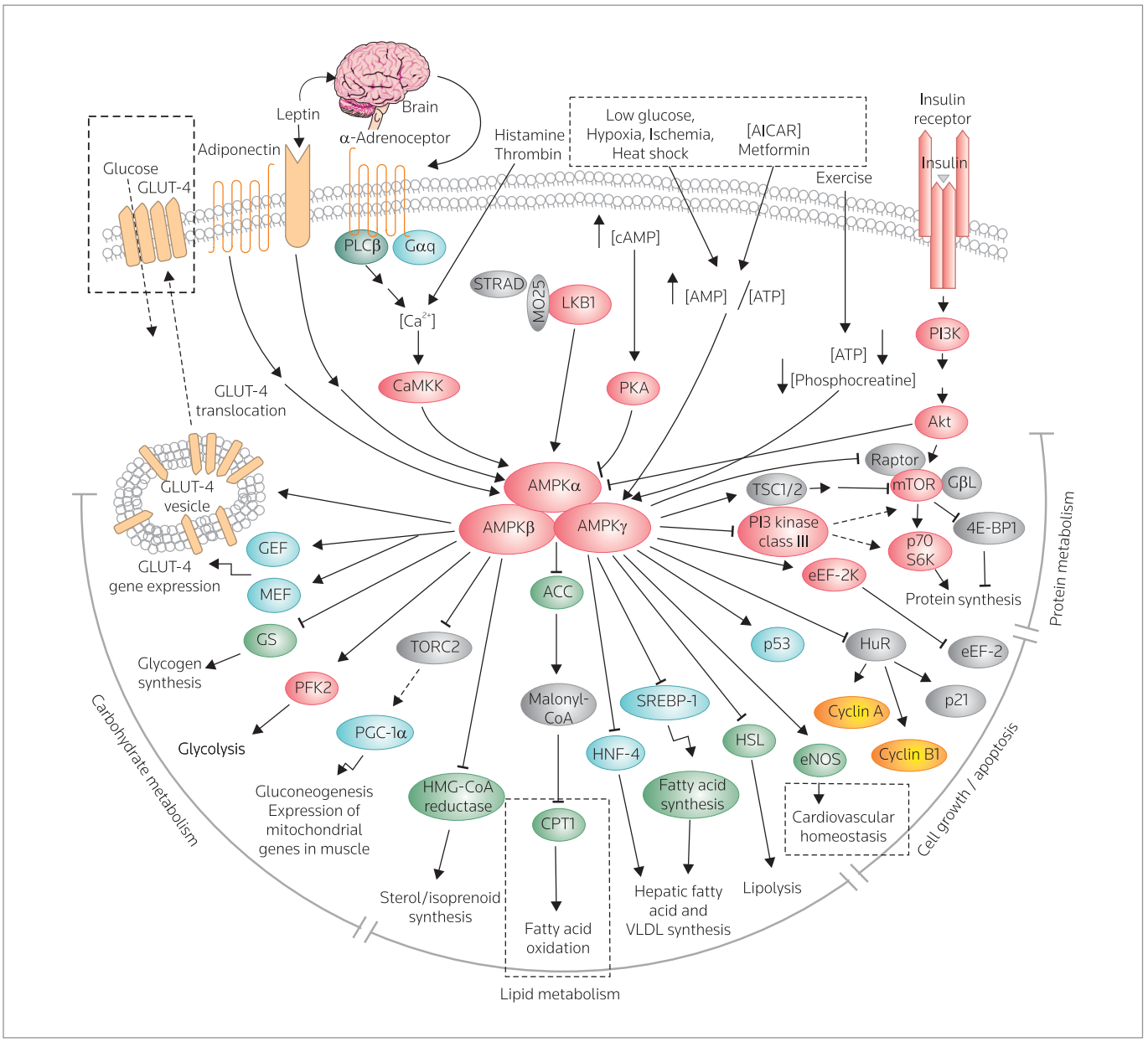

Figure 1. Signaling pathways involving AMPK. Important cardiovascular pathways indicated by boxes. Pathway diagram reproduced courtesy of Cell Signaling Technology, Inc. (49).

These results illustrate the important ways in which AMPK regulates cardiac energy metabolism. Wong et al. demonstrated that AMPK has an important role in the mechanism of action of metformin, thiazolidinediones and statins, which could harness the beneficial effects of AMPK (32). This is also supported by evidence from Calvert et al. and Gundewar et al. (9, 10). Research by Kim et al. indicates that AMPK functions as a core signaling pathway in the heart and emphasizes the therapeutic potential of targeting AMPK in the treatment of myocardial ischemia or cardiac hypertrophy (11). Subsequent studies showed that mutations in the AMPK gamma2 subunit are responsible for heart failure or arrhythmias. Furthermore, the absence of myocardial AMPK in transgenic mice is associated with ventricular dysfunction, apoptosis and necrosis (33). Similarly, AMPK alpha2 deficiency causes pressure overload-induced left ventricular hypertrophy and dysfunction (5).

Thus, AMPK deficiency enhances myocardial I/R injury (34). Once activated, AMPK reversibly phosphorylates and dephosphorylates key enzymes in cardiac energy metabolism. Thus, AMPK plays a key role in the coordination of cardiac anabolic and catabolic pathways. The role of AMPK during periods of myocardial stress or cardiac hypertrophy, e.g., aortic constriction or hypertension, is controversial (3, 19, 34). The exact steps necessary for AMPK-regulated physiological hypertrophy are debatable, as almost every situation of increased physical requirements is accompanied by morphological hypertrophy (14, 20) (Figs. 2 and 3).

AMPK functions during inflammation and cardiac remodeling

Hemodynamic disorders, such as oxidative stress or heart failure, facilitate an inflammatory reaction. The migration of macrophages,

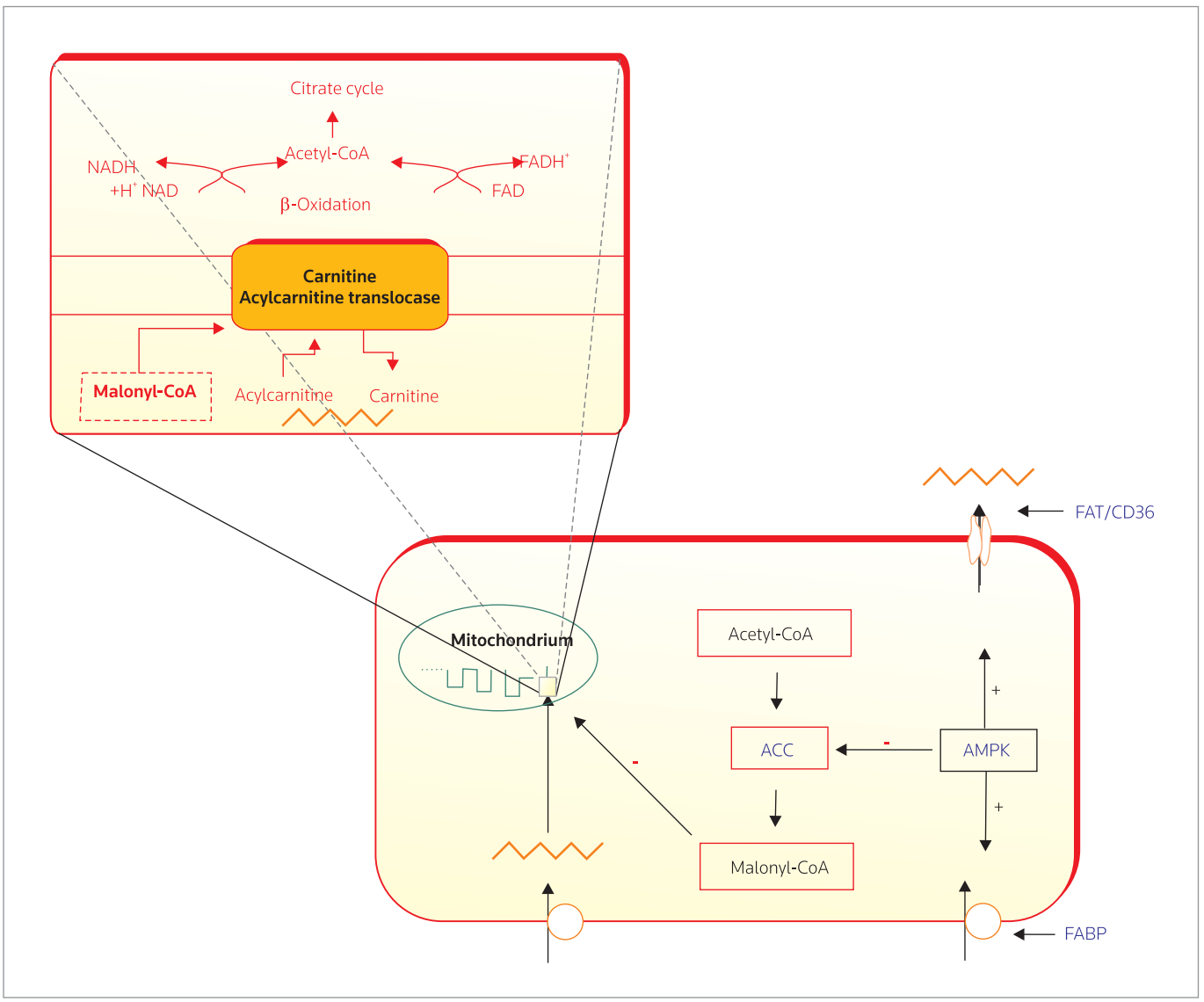

Figure 2. Function of AMPK in fatty acid metabolism. ACC, acetyl-CoA carboxylase; FABP, plasma membrane-associated fatty acid binding protein; FAT/CD36, fatty acid transporter.

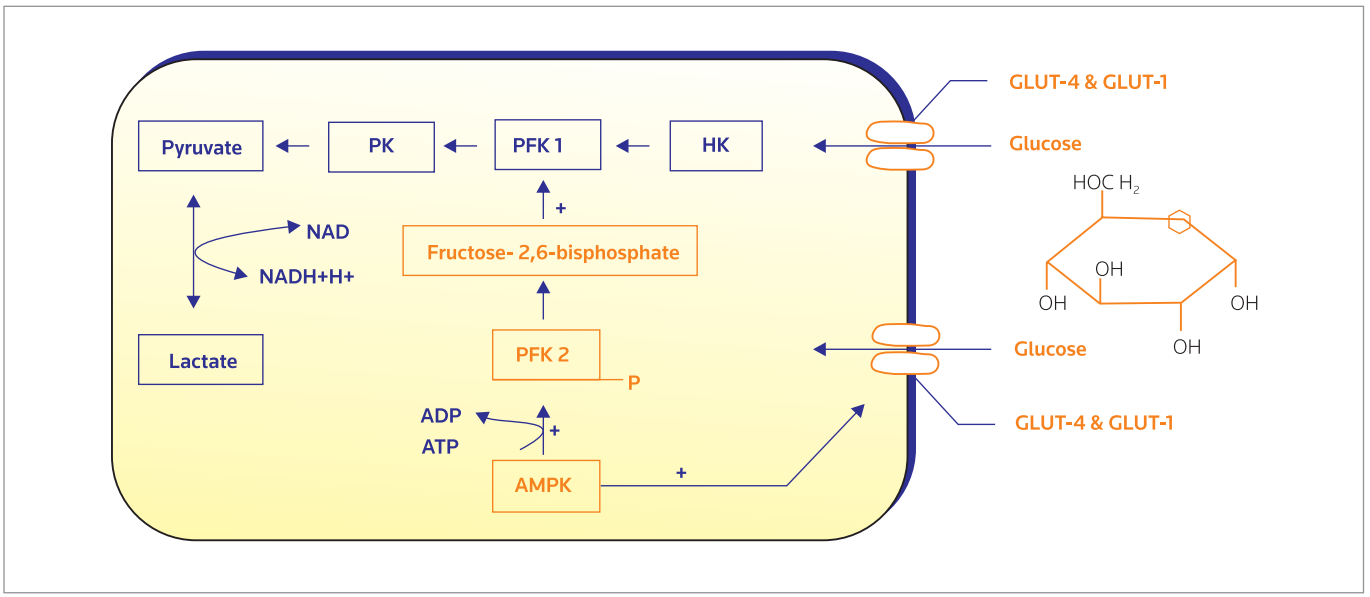

Figure 3. Scheme of the glucose metabolic pathway including the stimulation of translocation of GLUT-1 and GLUT-4 into the cell membrane. HK, hexokinase; PFK 1, 6-phosphofructo-1-kinase; PFK 2, 6-phosphofructo-2-kinase; PK, pyruvate kinase; GLUT, glucose transporter.

monocytes and leukocytes, and the expression of different proinflammatory cytokines, including TNF-α, IL-1α and IL-6, are responsible for the systemic reactions (35, 36).

During an inflammatory reaction, the activity of AMPK increases secondary to the release of MIF (macrophage migration inhibitory factor). In this context, MIF, a cytokine influencing multiple aspects of systemic reactions during inflammation, stimulates AMPK through CD74 (22). In addition, MIF has the ability to modulate glucose uptake in hypoxic conditions. Consequently, AMPK can regulate several pathways of cardiac energy metabolism that are active during inflammation (37).

Inflammatory reactions are associated with high protein degradation. This implies an enhanced performance of intracellular transport. The Golgi apparatus and endoplasmic reticulum undergo continuous rebuilding. Alleviating endoplasmic reticulum stress and endoplasmic reticulum-specific apoptotic pathways by AMPK activation protects cardiomyocytes from hypoxic injury (21).

AMPK is an important regulatory enzyme during periods of myocardial stress, such as physiological or mechanical stress, and plays an important role in regulating energy metabolism and protein synthesis. Consequently, a principal task of AMPK is cardiovascular protection under different stress conditions. Various cell types, e.g., cardiomyocytes and extracellular matrix cells (ECMs), actively proliferate and differentiate as an adaptation to changes in physiological conditions. Specifically, AMPK plays a central role in myocardial remodeling by attenuating the growth and proliferation of cardiac fibroblasts. The extracellular signal-regulated kinase (ERK), which influences the growth and proliferation of cardiac fibroblasts, exhibits an interaction with AMPK (38, 39).

In addition, adiponectin mediates antiatherosclerotic activity via the adventitia–AMPK–inducible nitric oxide synthase (iNOS) pathway combined with proliferation, mobilization and translation of adventitial fibroblasts via activity of iNOS (40).

INNOVATIVE FUTURE PROSPECTS

New compounds under active development emphasize that AMPK could be a potential pharmacological target. Currently, numerous studies (preclinical, clinical, etc.) are under way to verify the positive influence of AMPK activity in pharmacological interventions.

The treatment of type 2 diabetes with metformin, which acts as an AMPK activator, showed very positive results, and combination with pioglitazone reduced all-cause mortality compared to metformin alone (41). Preclinical studies with BG-8702, an AMPK activator candidate, demonstrated a significant decrease in glucose and triglycerides and improvement in insulin sensitivity in a mouse model (42). Comparable results were seen for antidiabetic therapy with DRL-16536 (phase I for type 2 diabetes) in a mouse model (43).

Also, studies with AMPK have indicated a potential approach to the treatment of myocardial infarction. For example, acadesine stimulates AMPK and is currently in phase III trials (44). Initial results indicated that acadesine reduces mortality and improves cardiac outcome after coronary artery bypass grafting (CABG) (45, 46). Acadesine is postulated as a new drug that may improve myocardial protection during CABG (46). In addition, AMPK showed versatile functions to support cardiac surgery, such as heart valve interventions (47, 48).

CONCLUSIONS

AMPK is a multifaceted enzyme in cardiac metabolism and works as a key signaling pathway to reduce postischemic injury, alleviate mortality and protect the myocardium. Likewise, in the treatment of type 2 diabetes or in cardiac surgery, AMPK also appears to be a promising pharmacological target.

DISCLOSURES

The authors state no conflicts of interest.

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