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ROLE OF 5' ADENOSINE MONOPHOSPHATE-ACTIVATED PROTEIN KINASE (AMPK) IN CARDIOVASCULAR HEALTH AND DISEASE-A REVIEW

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Abstract: AMP-activated protein kinase (AMPK) is a heterotrimeric enzyme that is expressed in most mammalian tissues including cardiac muscle. Among the multiple biological processes influenced by AMPK, regulation of fuel supply and energy-generating pathways in response to the metabolic needs of the organism is fundamental and likely accounts for the remarkable evolutionary conservation of this enzyme complex. By regulating the activity of acetyl coenzyme A carboxylase, AMPK affects levels of malonyl coenzyme A, a key energy regulator in the cell. AMPK is generally quiescent under normal conditions but is activated in response to hormonal signals and stresses sufficient to produce an increase in AMP/ATP ratio, such as hypoglycemia, strenuous exercise, anoxia, and ischemia. Once active, muscle AMPK enhances uptake and oxidative metabolism of fatty acids as well as increases glucose transport and glycolysis. Data from AMPK deficiency models suggest that AMPK activity might influence the pathophysiology and therapy of diabetes and increase heart tolerance to ischemia. Effects that are not as well understood include AMPK regulation of transcription. Different AMPK isoforms are found in distinct locations within the cell and have distinct functions in different tissues. A principal mode of AMPK activation is phosphorylation by upstream kinases (eg, LKB1). These kinases have a fundamental role in cell-cycle regulation and protein synthesis, suggesting involvement in a number of human disorders including cardiac hypertrophy, apoptosis, cancer, and atherosclerosis. The physiological role played by AMPK during health and disease is far from being clearly defined. Naturally occurring mutations affecting the nucleotide-sensing modules in the regulatory subunit of AMPK lead to enzyme dysregulation and in appropriate activation under resting conditions. Glycogen accumulation ensues, leading to human disease manifesting as cardiac hypertrophy, accessory atrioventricular connections, and degeneration of the physiological conduction system. Whether AMPK is a key participant or bystander in other disease states and whether its selective manipulation may significantly benefit these conditions remain important questions.

Keywords: AMPK, Acetyl coenzyme A carboxylase, Malonyl coenzyme A, Hypoglycemia, Cardiac hypertrophy, Apoptosis

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INTRODUCTION

Adenosine monophosphate (AMP)–activated protein kinase (AMPK) is a heterotrimeric protein consisting of one catalytic subunit (a) and two noncatalytic subunits (b and g) two isoforms of the a-subunit have been identified and both are expressed in the heart[1-3]. Recently, there has been emerging evidence suggesting that AMPK regulates fatty acid oxidation and glucose uptake in the heart and skeletal muscle in response to altered energy supply and/or demand[4–7]. These studies suggest that an increased ratio of AMP to ATP (AMP/ATP), caused by depletion of high-energy phosphates, activates AMPK and increases energy production by stimulating substrate utilization. Regulation of AMPK activity by AMP/ATP is mediated by both allosteric and covalent mechanisms. Besides a direct allosteric activation, binding of AMP to AMPK also facilitates its phosphorylation by an upstream kinase, AMPK kinase (AMPKK), and inhibits its dephosphorylation by protein phosphatases. Moreover, increased [AMP] allosterically activates AMPKK[8]. In striated muscle, AMPK activity is also regulated by the ratio of phosphocreatine (PCr) to creatine (PCr/Cr). Decreased PCr/Cr can activate AMPK by two mechanisms: either direct activation or altering the AMP/ATP ratio by increasing AMP concentration through the creatine kinase reaction and the adenylate kinase reaction[9]. Hearts with hypertrophy caused by pressure overload are characterized by chronic depletion of near normal ATP. It is not known whether these changes result in altered AMPK activity. Furthermore, in hypertrophied hearts of animal models and patients, glucose uptake is increased, but the mechanism responsible for this increase remains largely unclear[10-13]. Interestingly, in rat hearts with pressure overload–induced left ventricular hypertrophy (LVH), we have observed a close relation between increased glucose uptake and decreased [PCr], a key regulator of AMPK[12]. Taken together, these studies raise the possibility that glucose utilization in cardiac hypertrophy is regulated by myocardial energetics, with AMPK as a key signaling intermediary. The function and survival of all organisms is dependent on the dynamic control of energy metabolism, when energy demand is matched to energy supply. The AMP-activated protein kinase (AMPK) αβγ heterotrimer has emerged as an important integrator of signals that control energy balance through the regulation of multiple biochemical pathways in all eukaryotes. In this review, we begin with the discovery of the AMPK family and discuss the recent structural studies that have revealed the molecular basis for AMP binding to the enzyme's γ subunit. AMPK's regulation involves autoinhibitory features and phosphorylation of both the catalytic α subunit and the β-targeting subunit. We review the role of AMPK at the cellular level through examination of its many substrates and discuss how it controls cellular energy balance. We look at how AMPK integrates stress responses such as exercise as well as nutrient and hormonal signals to control food intake, energy expenditure, and substrate utilization at the whole body level. Lastly, we review the possible role of AMPK in multiple common diseases and the role of the new age of drugs targeting AMPK signaling.

ROLE AND FUNCTION OF AMPK IN CARDIO VASCULAR DISEASE

Adenosine monophosphate (AMP), activated protein kinase (AMPK) is a heterotrimeric protein consisting of one catalytic subunit (a) and two non catalytic subunits (b and g)[1,2]. Two isoforms of the a-subunit have been identified (a1 and a2), and both are expressed in the heart[3]. Recently, there has been emerging evidence suggesting that AMPK regulates fatty acid oxidation and glucose uptake in the heart and skeletal muscle in response to altered energy supply and/or demand[4–7]. These studies suggest that an increased ratio of AMP to ATP (AMP/ATP), caused by depletion of high-energy phosphates, activates AMPK and increases energy production by stimulating substrate utilization. Regulation of AMPK activity by AMP/ATP is mediated by both allosteric and covalent mechanisms. Besides a direct allosteric activation, binding of AMP to AMPK also facilitates its phosphorylation by an upstream kinase, AMPK kinase (AMPKK), and inhibits its dephosphorylation by protein phosphatases. Moreover, increased AMP allosterically activates AMPKK[1,8]. In striated muscle, AMPK activity is also regulated by the ratio of phosphocreatine (PCr) to creatine (PCr/Cr)[1,2,4]. Decreased PCr/Cr can activate AMPK by two mechanisms: either direct activation or altering the AMP/ATP ratio by increasing AMP concentration through the creatine kinase reaction and the adenylate kinase reaction[2,4,9]. Hearts with hypertrophy caused by pressure overload are characterized by chronic depletion of PCr but near normal ATP. It is not known whether these changes result in altered AMPK activity. Furthermore, in hypertrophied hearts of animal models and patients, glucose uptake is increased, but the mechanism responsible for this increase remains largely unclear. Interestingly, in rat hearts with pressure overload induced left ventricular hypertrophy (LVH), we have observed a close relation between increased glucose uptake and decreased PCr, a key regulator of AMPK[12]. Taken together, these studies raise the possibility that glucose utilization in cardiac hypertrophy is regulated by myocardial energetics, with AMPK as a key signal intermediary.

AMPK regulates energy metabolism by phosphorylation of key enzymes in metabolic pathways, such as acetyl coenzyme A (acetyl-CoA) carboxylase (ACC) or mTOR (mammalian target of rapamycin), by modulating their activities, and by regulating the activity of transcription factors and transcriptional cofactor. Experiments conducted by Hardie, Carling, and colleagues defined the specific activity of AMPK, substrate specificity, and the role of the enzyme in hepatic lipid metabolism[10–13]. AMPK regulates carbohydrate and lipid metabolism in muscle tissue, by controlling uptake and catabolism for energy generation[14–16]. These studies were complemented by extensive analyses of the yeast AMPK homolog, the sucrose non fermenting protein 1 (SNF1)/SNF4 complex, which plays a critical role in cell adaptation to glucose-free environment. In yeast, SNF1/SNF4 mediates its activity through modulation of gene transcription, whereas, in mammalian cells, AMPK regulates metabolism both by regulating

gene transcription and direct control of metabolic enzymes. Although generally recognized as a cellular energy gauge. The precise role that AMPK plays in cardiac metabolism remain incompletely understood. Important questions include whether the various AMPK isoforms have unique roles that are relevant to particular metabolic state, identification of the full repertoire of cardiac protein targets of AMPK activity, and definition of the stimuli (such as increased AMP/ATP ratios) and kinases that activate AMPK. Studies of skeletal muscle AMPK have addressed some of these questions. Exercise, hypoxia, ischemia, and neurohumoral factors leptin, adiponectin, and adrenoreceptor agonist stimulate AMPK. Whether these factors mediate AMPK activity in the heart is under active investigation. Two AMPK kinases have been identified in the heart, LKB1 and calmodulin-dependent protein kinase. The serine/threonine kinase LKB1 appears to function in a myriad of cell processes including AMPK activation. LKB1 responds to anoxic stress by phosphorylating the AMPK α subunit at the Thr172 residue. Notably, this response is independent of AMP concentration[17]. LKB1 activity as an AMPK kinase is also dependent on a number of associated proteins[18]. During ischemia, LKB1 selectively phosphorylates the βα2 but not the α1 subunit, suggesting differential regulation and distinct physiological roles of various catalytic complexes and their upstream kinases[19]. Calmodulin dependent kinase appears to be part of an independent regulatory pathway. Nucleotides released from damaged tissue activate AMPK in endothelial cells through Ca^{2+} calmodulin-dependent kinase pathway but independently of AMP/ATP ratio. Activation of AMPK can influence cardiac metabolism by regulating uptake and oxidative phosphorylation of fatty acids (reviewed by Dyck and Lopaschuk), the primary source of ATP in normal myocardium. AMPK phosphorylates acetyl-CoA carboxylase (ACC), which adds a carboxyl moiety to acetyl-CoA to yield the 3-carbon molecule malonyl-CoA. AMPK-mediated phosphorylation of ACC at serine residues (Ser79 in the hepatic isoform and Ser221 in muscle isoform) inactivates ACC. In the liver, malonyl-CoA is required for fatty acid synthesis. In the heart, malonyl-C as an inhibitor of carnitine palmitoyl transferase (CPT1), which is located at the outer mitochondrial membrane and is the rate-limiting step for internalizing and rendering fatty acids as substrates for oxidative phosphorylation. Hence activation of AMPK inhibits ACC and triggers a cascade of regulatory events that ultimately activate CPT1 and provide cardiac mitochondria with a preferred substrate for energy. This pathway is also regulated by malonyl-CoA decarboxylase (MCD), which degrades malonyl-CoA. There is controversy over whether or not MCD is activated during muscle contraction by AMPK[20,21]. In vitro transfection studies by Sambandam *et* al[41] suggest that this regulation occurs within mitochondria, rather than in the cytosol. In addition to preventing malonyl-CoA formation, AMPK appears to inhibit triglyceride synthesis in liver and muscle by interacting with glycerol-3-phosphate acyl transferase[22]. Further evidence for the role of AMPK in stimulating cardiac fatty acid metabolism comes from analyses of individual cardiomyocytes. Addition of fatty acids to culture medium increases AMPK activity by an unknown mechanism. Thus, concomitant with delivery of acyl carnitine to

mitochondria, AMPK increases the uptake of free fatty acids to cardiomyocytes. In addition, AMPK stimulation increases protein expression and plasmalemma content of fatty acid transporter (FAT/CD36) and membrane-associated fatty acid binding protein (FABPpm)[23]. The availability of free fatty acids in the vicinity of cardiomyocytes is further enhanced by increased synthesis and recruitment of lipoprotein lipase to capillary lumen. Stimulation of glucose is another established activity of AMPK, although the mechanism and physiological relevance of this activity are not well understood[24]. AMPK stimulation of glucose uptake occurs via an insulin-independent mechanism. Early studies demonstrating that activated AMPK increased expression of glucose transporters, GLUT1 and GLUT4, suggested that GLUT4 is translocated from intracellular reservoirs to the sarcolemma to mediate AMPK effect on glucose uptake[25,26] Yang and Holman suggested that AMPK increases the sarcolemmal GLUT4 transporter number by decreasing endocytosis[27]. The molecular mediators of these events and the specific phosphorylation targets of AMPK may include protein kinase C-28 endothelial nitric oxide synthase, 29 and p38 mitogen-activated protein kinase transforming growth factor β activated protein kinase 1– binding protein 1 (TAB1) complex[30]. This AMPK dependent pathway does not cross-communicate with the insulin– glycogen synthase kinase pathway and in fact may be mutually antagonistic. Insulin inactivates AMPK through Akt, and AMPK may inhibit insulin signaling[31]. Alternatively, under pharmacological stimuli, AMPK can potentiate insulin signaling downstream protein kinase B, thereby helping to overcome insulin resistance[32]. The physiological importance of AMPK-mediated glucose uptake at rest and aerobic activity is not well defined. AMPK appears to control a different intracellular pool of transporters from that mobilized by insulin. The effect of insulin predominates at rest or during exercise, whereas the contribution of AMPK to glucose uptake and metabolism becomes central during oxidative stress,[27] ischemia,and pathological hypertrophy. AMPK stimulates glycolysis directly by activating phosphofructokinases, which provide the primary regulatory step in the glycolytic pathway. AMPK indirectly activates phosphofructokinase (PFK1) through its phosphorylation of phosphofructokinase 2 (PFK2), which converts fructose 6 phosphate to fructose-2,6-diphosphate, an allosteric stimulator of PFK1. The capacity of AMPK to activate PFK and increase glycolysis makes it an essential component in mounting the cellular response to energetic stress like anaerobic exercise, anoxia, and low-flow ischemia. Finally, AMPK plays an important role in regulating glycogen metabolism. AMPK mutations affect glycogen metabolism,[33] whereas primary defects in glycogen metabolism (eg, muscle phosphorylase [GP] deficiency, glycogen storage disease type II, or McArdle disease) activate AMPK while decreasing glycogen synthase (GS) activity. Carling and Hardie suggested that GS and phosphorylase kinase might be phosphorylated by AMPK. AMPK phosphorylates GS at Ser7 and inhibits it in skeletal myoblasts, rendering it glucose-6-phosphate (G-6-P) dependent. Paradoxically, repeat AICAR administration resulted in increased glycogen. The issue could be settled if AICAR increased the level of G-6-P, thereby overcoming GS inhibition[34]. In another

study, AICAR inhibited GS and activated GP in white but not red gastrocnemius in vivo[35]. These effects could not be reproduced in vitro, which led these authors to conclude that the observed increase in glycogen was attributable to increased glucose uptake but not altered GS or GP. In isolated rat hearts, AMPK-activator AICAR increased glycogen breakdown but did not affect GS or GP activity, leaving the mechanism unknown[36]. Hence, the pathways by which AMPK controls glycogen synthesis and breakdown and the precise phosphorylation targets remain to be elucidated. Whether AMPK mediates this action by controlling transcription or by controlling glycogen metabolism directly is also not known. Although many studies have focused on the role of AMPK as a direct mediator of intermediary metabolism, AMPK also mediates transcription of genes involved in lipid and glucose metabolism. Recurrent muscle AMPK stimulation reproduces the effect of exercise training by increasing the expression of GLUT-4 transporter, hexokinase II, mitochondrial biogenesis, and other mitochondrial enzymes. An important mediator of many of these effects is peroxisome proliferator–activated receptor-γ(PPARγ) coactivator 1α (PGC-1α), which increases expression of transcription factors: nuclear respiratory factors 1 and2, mitochondrial transcription factor A, and PPARα. Importantly, each of these has its own downstream targets. For instance, the PPARα controls the genes involved in lipid metabolism. The target genes regulated by AMPK in cardiac muscle are undefined at present. Recent evidence indicates that AMPK mediated modification of gene expression leads to cardiac protection from anoxia and ischemia. Decrease in protein synthesis through eEF2 phosphorylation affords better tolerance to hypoxia-induced stress[37,38]. The late phase of ischemic preconditioning involves increased expression and translocation of GLUT-4, which appears to be related to PKC dependent AMPK activation. Presumably α2 associated AMPK mediates this activity because the predilection of the α2 subunit for nuclear localization[39]. Therefore, the role of AMPK as transcriptional mediator may be particularly important in the human heart, where α2 is much more abundant than α1 subunit. Future studies will be required to distinguish the roles of AMPK as a direct regulator of cardiac metabolism and as a mediator of cardiac transcription.

THE ROLE OF AMPK IN PHYSIOLOGICAL AND OTHER FORMS OF CARDIAC MYOCYTE HYPERTROPHY

Physiological hypertrophy, or pressure-overload hypertrophy, is the cardiac response to hypertension. This hypertrophy response has a significant effect on cardiac energetics. In the mouse heart, AMPK is activated during pressure overload– induced hypertrophy,[40] and reduced activity in adiponectin deficient mice has been associated with worse hypertrophy and outcome after aortic banding[41-43]. In addition, AMPK appears to have a role in regulating Akt1-induced hypertrophy in neonatal cardiomyocytes[44]. Hypertrophic hearts undergo relative oxygen deficit and switch from using fatty acids as the primary energy source to glucose[45]. The

role of AMPK may be to compensate for an increased AMP/ATP ratio secondary to pressure overload rather than being the cause of the pathogenic response. Alternatively, AMPK has been implicated as a regulator of the Akt-mTOR pathway, which leads to decreased protein synthesis through inactivation of eukaryote elongation factor 2, eEF-2 and inhibition of mTOR complex[46]. Because of the wide range of AMPK targets, defining its role in the hypertrophic process has proven difficult. For example, AMPK can be either pro or antiapoptotic, depending on experimental conditions. The ability of AMPK to upregulate p53[47] and mediate Bax translocation to mitochondria suggests an antiproliferative function[48]. By contrast, AMPK activity also protects cardiomyocytes from palmitate load or anoxic damage associated with antiapoptotic activity[26,49]. Transgenic mice associated with constitutive AMPK activity had a net increase in heart muscle mass and increased expression of hypertrophy associated genes[53]. These are most probably secondary phenomenon resulting from the evolving pathological process and not directly from changes in AMPK activity. Because various models associated with decreased AMPK activity did not show any hypertrophy, AMPK may be more of a bystander than a key player in the hypertrophic process. In conclusion, the role of AMPK in cardiac hypertrophy remains unknown. AMPK may play other roles in altering cardiac myocyte morphology and proliferation. The main AMPK activator, LKB1, is in fact a tumor suppressor gene. AMPK activates the tuberous sclerosis tumor suppressor protein complex TSC1-TSC2 to inhibit mTOR signaling and cell growth and proliferation (recently reviewed Motoshima *et al*). Interestingly, inactivating mutations in TSC1-TSC2 causes tuberous sclerosis, a syndrome associated with benign tumors including multiple hamartomas and cardiac rhabdomyomas[50]. Whether these regulatory functions are related to cardiac disorders should be answered in the near future. One recent exciting study addressed the potential of AMPK stimulation to inhibit coronary atherosclerosis. AMPK activation suppressed vascular smooth muscle proliferation through a p53- dependent mechanism[47]. This action is expected to work in synergism with lipid lowering effect and improved endothelial functionto attenuate the leading cause of morbidity and mortality in the western hemisphere of AMPK[52]. If this rule applies to the heart, one could expect inhibition of ion currents to contribute to brady arrhythmia and conduction system disease in PRKAG2 cardiomyopathy. Although PRKAG2 mutations may affect ion channels, studies of murine models strongly suggest that the primary mechanism leading to conduction disease is an abnormal anatomical substrate for electrical coupling between atria and ventricles. Either electrical connections, which normally exist during embryonic life, do not involute or they develop postnatally by muscle tissue penetrating through annulus fibrosis[54]. Transgenic mice develop glycogen storage, multiple atrioventricular communications, and preexcitation several weeks after birth[53,57,55]. Other glycogen storage disorders, such as Danon disease, also cause preexcitation, probably by a similar mechanism[42]. Most likely, there are two mechanisms leading to cardiac preexcitation: some forms are caused by glycogen accumulation, whereas others are caused by the formation of accessory bypass tracts formed

from abnormal cell proliferation or growth[54,57]. This may explain relatively low prevalence of typical WPW arrhythmia (atrioventricular reentrant tachycardia) in PRKAG2 cardiomyopathy despite multiple accessory pathways in the affected individuals humans and animal models have demonstrated.

AMPK IN CARDIOVASCULAR PHYSIOLOGY

Homeostatic mechanisms in the heart and the vascular endothelium are critical in maintaining cardiovascular health. These mechanisms are involved in diverse cardiovascular functions like regulation of vascular tone, maintenance of tissue perfusion, vascular permeability, myocardial function, anticoagulant activity and inflammatory responses[58-60].

AMPK IN THE HEART

While AMPK signaling has a specific physiological role in the heart, its importance is accentuated under conditions that place a stress on this organ. Such stressors include excess hemodynamic load, myocardial ischemia and hypoxia. Specifically, AMPK activates the glycolytic pathway by phosphorylating phosphofructokinase-2, enhances fatty acid β-oxidation and enhances ATP availability[61-62]. It also promotes translocation of glucose transporter-4 (GLUT-4) to the plasma membrane in the cardiomyocyte thus increasing the uptake of glucose to serve as the primary energy substrate.

AMPK IN ENDOTHELIAL CELLS

Endothelial cells in the CVS can sense changes in haemodynamic forces, ambient pO2 and local blood borne signals. They can then respond with appropriate control and regulatory processes to maintain homeostasis. Such responses can include release of paracrine mediators such as nitric oxide (NO), prostacyclin and endothelin-1 (ET-1) In addition, activity of cell surface enzymes can be modulated such as angiotensin converting enzyme (ACE) which regulates the bioactivity of vasculoactive molecules like angiotensin II and bradykinin. Furthermore cell surface adhesion molecules are also modulated in addition to promotion of immune cell recruitment and migration in case of vascular injury along with increased vascular permeability and intravascular thrombosis. The central signaling molecule involved in endothelial function is nitric oxide (NO) which is synthesized from L-arginine by NO synthase (NOS) in endothelial cells. NOS exists as three isoforms: neuronal (nNOS), inducible (iNOS) and endothelial (eNOS). All three isoforms share a carboxyl terminal domain homologous to cytochrome P-450 reductase and has binding sites for nicotinamide adenine dinucleotide phosphate-oxidase (NADPH), Flavin mononucleotide (FMN) and tetrahydrobiopterin (BH4)[82]. eNOS is typically activated by an increase in [Ca^{2+}]. A strong physiological stimulus for NO synthesis via eNOS activation is the shear stress caused by increases in blood flow[82]. It has also been demonstrated that other

physiological stimuli such as insulin, estrogen and vascular endothelial growth factor (VEGF) can activate the phosphatidyl inositol-3 kinase (PI3K)/Akt system and in turn this phosphorylates NOS. AMPK is the only kinase recognized to date that can also potentially phosphorylate eNOS on more than one site. These sites have been reported as (activating sites on the reductase domain of eNOS), and at Thr495 (inhibitory at the CaM binding domain of the enzyme). Many reports have identified that AMPK-dependent eNOS activation can occur following endothelial cell stimulation by diverse agents and these include VEGF peroxisome proliferator-activated receptors γ (PPAR-γ) agonists, and metformin[64,65]. In 2004, it was reported that the biguanide drug, metformin activated AMPK mediated by mitochondrial RNS (reactive nitrogen species) and the PI3K pathway[66]. Specifically, this report demonstrated that metformin activated AMPK and increased the phosphorylation of ACC (its downstream effector) at Ser79 in cultured BAEC (bovine aortic endothelial cells), that this was mediated through c-Src and was PI3Kdependent, and NO dependent that the peroxynitrile oxidant was sourced to the mitochondrion and that inhibition of mitochondrial complex I activated AMPK. Further, the paper also validated these findings *in vivo*. Further studies have also established that metformin-induced AMPK activation is beneficial to endothelial function via Heat Shock Protein[80] (hsp90) mediated activation of eNOS[65]. These investigations have revealed that metformin can increase the conversion of arginine into citrulline in BAECs in a dose-dependent fashion, suggesting that NO synthesis was occurring via activation of eNOS. This study also revealed that eNOS activation by metformin was PI3K-dependent, was clearly mediated through the activation of AMPK and that it enhanced the association of hsp80 (an important stress response marker) with eNOS. Recently, evidence has also emerged that NO itself might act as an endogenous activator of AMPK[67]. In this report, we have shown that NO activates AMPK in endothelial cells through a Ca^{2+} dependent mechanism involving CaMKKβ and that AMPK activation can itself increase NO release through AMPK-dependent phosphorylation of eNOS. These data imply that a positive feedback relationship might exist between eNOS and AMPK activation. However, further investigation of this possibility is warranted. Once NO is synthesized via the activation of eNOS it diffuses to the surrounding vascular smooth tissue and exerts its multiple physiological effects which include muscle relaxation and proliferation, and inhibiting leukocyte adhesion and migration, platelet adhesion and aggregation and expression of adhesion molecules. In disease conditions, the endothelium undergoes complex changes and loses its protective and pro-homeostatic function and acquires pro-atherosclerotic properties. Collectively these abnormalities are referred to as “endothelial dysfunction” and are typified by reduced bioavailability of NO[68]. In these alterations, AMPK also appears to play an important role.

AMPK AND VASCULAR SMOOTH MUSCLE

AMPK activation in the endothelium is also linked to endothelial control of vascular smooth muscle function, particularly vasorelaxation by NO which appears to be regulated, by AMPK activity. Vasorelaxation is a critical arm of vascular tone and tone is a central determinant of blood pressure regulation. Sustained high blood pressure is responsible for a variety of serious disease states such as hypertrophic cardiomyopathy, coronary artery insufficiency, myocardial infarction, hypertensive encephalopathy, cerebrovascular disease, hypertensive retinopathy and hypertensive nephropathy being important examples. It has been established that metformin therapy is beneficial for the cardiovascular system by virtue of its ability to improve vasodilatory function[69,70]. It is possible that these effects of metformin are dependent on eNOS activation as demonstrated by various investigators. Another possibility that merits consideration is the fact that endothelium dependent vasorelaxation is not exclusively regulated by NO. It is provocative to speculate that AMPK might be linked to vasorelaxation via the generation of epoxyeicosatrienoic acids by the cytochrome P450 epoxygenases. In fact LKB1 and AMPK can be activated by stimulation of the constitutive androstane receptor and pregnane X receptor (both of the nuclear receptor super family that are thought to be involved in the detoxification of xenobiotics), using Phenobarbital (a classic P450 inducer)[71]. In mice that lack hepatic α1 and α2 isoforms of AMPK, this response is not detectable[72]. In keeping with the vasorelaxative role of AMPK, it is now fairly well established that AICAR and metformin can both relax arterial preparations *ex vivo*. Interestingly, there seems to be a species and vessel dependent difference in published literature in terms of chemical sensitivity of AMPK activation. For example, unlike rat and mouse aortae, porcine carotid artery smooth muscle is reportedly insensitive to AICAR and metformin, even though this tissue can have AMPK activation by other means, such as by hypoxia and 2-deoxyglucose (which causes a metabolic block in the glycolytic pathway)[73].

AMPK AS A REDOX SENSOR AND MODULATOR

Oxidative stress and a shift in the cellular redox balance are critical underpinnings of endothelial dysfunction. In turn, endothelial disturbances underlie cardiovascular pathology. The decreased bioavailability of NO is also associated with the generation of reactive oxygen species (ROS) in the vessel wall such as peroxynitrite[74]. Many studies have now established that there is an intricate balance between AMPK signaling and the redox balance in the vascular milieu. For example, AMPK has been shown to inhibit the formation of reactive oxygen species (ROS) by NADPH oxidase and stimulate NO production be eNOS[81]. Furthermore, AMPK has also been implicated in JNK activation, NF-κB-mediated transcription, E-selectin expression and vascular cell adhesion molecule-1 (VCAM-1) expression, in endothelial cells that have been exposed to H_2O_2, TNF-α or fatty acids. As a consequence, these signaling events lead to

attenuated monocyte adhesion to the endothelial surface[75-78]. Silencing AMPKα1 has also been reported to decrease the expression of Manganese Superoxide Dismutase (MnSOD), catalase, γ glutamyl cysteine synthase and thioredoxin, in endothelial cells[83]. AMPK also appears to have direct links to NADPH oxidase, a membrane bound enzyme complex which is normally latent in neutrophils and is activated to assemble in the membranes during the respiratory burst.

CONCLUSION

In summary, AMPK plays a critical role in cardiac metabolism, as it does in a variety of cell types. Seven different genes encode the various α, β, and γ subunit isoforms, which appear to be able to assort in all combinations producing as many as 12 different AMPK proteins. The expression of these different subunits varies considerably from tissue to tissue. One approach to defining the role of these different AMPKs is to identify naturally occurring mutations that alter physiology. Two different types of naturally occurring mutations in AMPK subunits have been described. In pigs, missense mutations in the γ3 subunit cause muscle glycogen accumulation. In humans, γ2 (or PRKAG2) missense mutations cause glycogen-storage cardiomyopathy. Studies in both humans and animal models have demonstrated that these mutations in the subunit lead to constitutive activation of PRKAG2. In mouse heart, the α2/γ2 AMPK isoform appears to be responsible for most of the disease manifestations. The mutationally altered PRKAG2 leads to altered glycogen metabolism and to dramatic accumulation of cardiac glycogen, which is responsible for both cardiac hypertrophy and preexcitation. Whether activated AMPK also triggers other pathways that may contribute to the increase in cardiac mass or electrophysiological abnormalities is unknown. Similarly, accumulated glycogen in the cardiac myocytes leads to disruption of the annulus fibrosis and bypass tracts, which contribute to preexcitation.

In this review, we examine the role of cardiac AMPK using the concepts derived from assessing phenotypes of human patients bearing AMPK mutations. Investigators have speculated on the multiple pros and cons of pharmacological AMPK manipulation for treatment of cardiovascular disease. To date, we have no sufficient understanding of the role of AMPK in cardiac health and disease to guide pharmacological manipulations for patients. AMPK may eventually emerge as a major regulator of muscle/heart glycogen stores. However, glycogen storage that occurs in response to constitutive PRKAG2 mutations may not reflect normal cardiac physiology, and more research is required to understand the contribution of AMPK to normal glycogen metabolism. Over the next several years, we expect that the precise molecular mechanisms, both direct regulation of metabolism and indirectly by mediating transcription, by which AMPK mutations lead to glycogen accumulation will be dissected. Identification of these mechanisms will lead to better understanding of the role of AMPK in regulating cardiac energy metabolism

as well as energy metabolism in other cells. In addition, we anticipate that definition of these mechanisms will lead to therapies directed toward reducing glycogen accumulation in PRKAG2 cardiomyopathy.

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