Protective effects of AMP-activated protein kinase in the cardiovascular system

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Abstract

Cardiovascular diseases remain the leading cause of mortality worldwide. Recent studies of AMP-activated protein kinase (AMPK), a highly conserved sensor of cellular energy status, suggest that there might be therapeutic value in targeting the AMPK signaling pathway. AMPK is found in most mammalian tissues, including those of the cardiovascular system. As cardiovascular diseases are typically associated with blood flow occlusion and blood occlusion may induce rapid energy deficit, AMPK activation may occur during the early phase upon nutrient deprivation in cardiovascular organs. Therefore, investigation of AMPK in cardiovascular organs may help us to understand the pathophysiology of defence mechanisms in these organs. Recent studies have provided proof of concept for the idea that AMPK is protective in heart as well as in vascular endothelial and smooth muscle cells. Moreover, dysfunction of the AMPK signalling pathway is involved in the genesis and development of various cardiovascular diseases, including atherosclerosis, hypertension and stroke. The roles of AMPK in the cardiovascular system, as they are currently understood, will be presented in this review. The interaction between AMPK and other cardiovascular signalling pathways such as nitric oxide signalling is also discussed.

Keywords: AMPK • cardiovascular system • therapeutic target • energy stress • protection

Introduction

AMP-activated protein kinase (AMPK) is a phylogenetically conserved serine/threonine protein kinase [1]; AMPK is a member of the SNF1/AMPK protein kinase family and is found in all eucaryotes [2, 3]. It is a critical metabolic controller and stress sensor that is activated by nutrient deprivation, hypoxia/ischaemia, starvation, exercise and some therapeutic agents [1]. The name AMPK was first suggested in 1988 when Munday *et al.* showed that the enzyme formerly known as acetyl-CoA carboxylase (ACC) kinase-3 is the primary kinase responsible for regulation of the ACC V_{max} [4]. Later, the name AMPK was used in 1989 by Carling *et al.* [5], who demonstrated that AMPK is the major 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase kinase in rat liver. Activated AMPK not only stimulates a number of energy-producing metabolic pathways, but also inhibits energy-consuming pathways to reserve energy for cell survival. Thus, AMPK is a signal switch that monitors and regulates systemic and cellular energy status [6].

As the name suggests, AMPK activity increases in response to increases in the intracellular level of adenosine monophosphate (AMP), which generally occurs as a product of hydrolysis of adenosine triphosphate (ATP) in metabolic processes. In response to a reduction in energy charge (decrease in the ATP/AMP ratio), AMPK shuts down anabolic pathways such as fatty acid, triglyceride and cholesterol synthesis, as well as transcriptional processes that consume ATP, and switches on catabolic pathways that generate ATP, including fatty acid oxidation, glycolysis and protein decomposition [7]. Increases in the AMP/ATP ratio activate AMPK by a number of mechanisms, including direct allosteric activation, and render it more easily phosphorylated by an AMP-dependent

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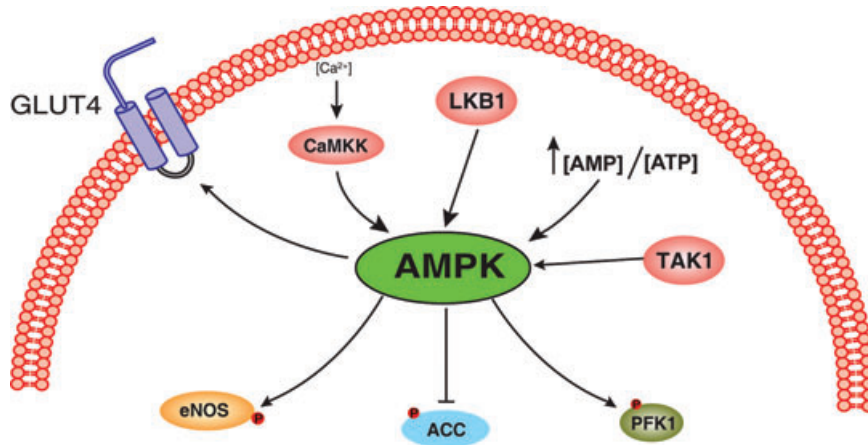E-mail: siliangyi@yahoo.com

Fig. 1 Proposed model illustrating protein factors upstream and downstream of AMPK (refer to text for additional detail). Upper arrows show kinase-mediated activation of AMPK via Ca^{2+}/calmodulin-dependent protein kinase β (CaMKKβ), LKB1 and TGFβ-activated kinase (TAK1). Lower arrows indicate targets of AMPK, including endothelial nitric oxide synthase (eNOS), acetyl-CoA carboxylase (ACC), glucose transporter type 4 (GLUT4) and 6-phosphofructo-1-kinase (PFK1).

AMPK kinase (AMPKK) on several sites of the α-subunit, including at Thr^{172} [8, 9]. Thus, the ubiquitous expression of AMPK and its involvement in the uptake, mobilization and utilization of glucose, fatty acids and protein, stress the general importance of this pathway. Apart from these diverse functions in metabolism, AMPK is, in fact, a key regulator involved in many biological functions of the cardiovascular system. In this review, we specifically focus on the roles of AMPK in cardiovascular tissues and data suggesting that dysfunction in AMPK signalling contributes to the genesis and progression of cardiovascular diseases.

AMPK structure and function

AMPK is a heterotrimeric complex consisting of a catalytic α subunit and regulatory β and γ subunits [1]. The α and β subunits each exist as two isoforms: α1 and α2 and β1 and β2. The γ subunit has three known isoforms: γ1, γ2 and γ3 [10, 11]. Although the C-terminal region of the α subunit is required for association with the β/γ subunits in both α1 and α2 isoforms, the subunits have distinct expression patterns. For example, in peripheral metabolic tissues, AMPK complexes containing the α1 catalytic subunit are mainly expressed in adipose tissue [12–14], whereas those containing the α2 catalytic subunit pre-dominate in skeletal and heart muscle [15–17]. In central nervous system tissues, the α1 catalytic subunit is almost absent [18]. The serine/threonine kinase activity of AMPK is located at the α subunit and is characterized by the presence of a threonine residue (Thr172) in a loop that must be phosphorylated for activation [19–21]. The AMPK α catalytic subunit can also be phosphorylated at other sites: Thr258 and Ser485 for α1 and Ser491 for α2. However, the upstream kinase and the biological significance of these phosphorylation sites are currently unclear [22]. The β subunits are modified by both myristoylation and multi-site phosphorylation, including Ser24/25, Ser96, Ser101, Ser108 and Ser182. These modifications appear to be required for the activation of AMPK and its subcellular localization [22, 23]. The γ subunits are responsible for the binding of AMP. Mutations at the AMP/ATP binding sites (CBS or Bateman domains) lead to reduction of AMPK activity and cause glycogen accumulation [24, 25].

The phosphorylation of the sites referred to above is regulated by three upstream AMPK kinases (AMPKKs): LKB1, Ca^{2+}/calmodulin-dependent protein kinase β (CaMKKβ) and transforming growth factor β activated kinase 1 (TAK1) shown schematically in Fig. 1. LKB1 is an upstream AMPKK that regulates AMPK and AMP-related kinases [20] and is believed to be the most potent positive regulator of AMPK [26, 27]. A cytosolic protein complex containing LKB1, putative kinase STRAD and the MO25 scaffold protein can interact with, and directly phosphorylate, AMPK [20]. When the AMP/ATP ratio increases, the phosphorylation of AMPK by LKB1 is enhanced; the LKB1 pathway is thought to be the major route by which AMPK is activated in response to energy demand and to maintain metabolic homeostasis. In addition, an increasing number of studies indicate that LKB1 is the principal provider of AMPKK activity, especially in peripheral tissues such as muscle, adipose tissue and liver. The second AMPKK, CaMKKβ, is particularly relevant to situations where Ca^{2+} signalling pathways are activated. Several studies have shown that Ca^{2+} signalling activates AMPK by CaMKKβ-dependent phosphorylation [28–30]. Some investigators have suggested that the CaMKKβ–AMPK axis plays an important role in cerebral Ca^{2+} signal transduction under physiological conditions as well as in ischaemic states (*e.g.*, ischaemia-induced calcium influx through NMDA receptor activation) [31, 32]. Recently, TAK1, the third putative upstream AMPKK, (also referred to as MAPKK kinase-7 or MAP3K7) was identified by Momcilovic *et al.* [33, 34]. Subsequently, TAK1 has been shown to activate AMPK-dependent cytoprotective autophagy in tumour necrosis factor (TNF)-related apoptosis-inducing ligand-treated epithelial cells [35]. However, the precise role of TAK1 as an AMPKK requires further investigation.

Role of AMPK in the heart

The role of AMPK in the heart is currently under intensive study. The heart can be viewed simplistically as a circulating pump. In

performing its function, the heart consumes considerable energy as a result of oxidation of various substrates, including fatty acids and glucose [36, 37]. Therefore, reduction of oxygen availability, as would occur during an ischaemic episode, has been postulated to trigger AMPK activation, which then acts as an emergency signal to restore cell energy homeostasis in the cardiomyocytes [38, 39]. Consistent with this, numerous reports have indicated that AMPK is very important in regulating substrate metabolism and in protecting against ischaemic reperfusion injury in the heart [24, 40, 41]. Further, two of the AMPKKs described earlier are found in the heart, LKB1 and CaMKKβ [28, 42]. Importantly, it has been shown that LKB1 responds to ischaemic stress in heart tissue by phosphorylating the AMPK α subunit at Thr172 [43]. In a recent study, Sakamoto *et al.* demonstrated that under the ischaemic status LKB1 selectively phosphorylates the α2 but not the α1 subunit, suggesting differential regulation and distinct physiological roles of various catalytic complexes and their upstream kinases [16].

AMPK protects ischaemic cardiomyocytes, although several mechanisms. First, it increases glucose uptake by stimulating the translocation of glucose transporter type 4 (GLUT4, Fig. 1) to the sarcolemmal membrane. Nishino *et al.* found that ischaemic pre-conditioning activates AMPK and up-regulates GLUT4 expression in a manner dependent on protein kinase C (PKC), suggesting that these upregulation events may contribute to attenuation of myocardial stunning [44]. The nitric oxide pathway also contributes to AMPK stimulation of glucose uptake and GLUT4 translocation in heart muscle [45]. Recently, Horie *et al.* demonstrated that oxidative stress induces GLUT4 translocation by activation of phospho-inositide 3-kinase (PI3K) and Akt and by dual AMPKK (CaMKKβ and LKB1) activation in cardiac myocytes [46]. Secondly, AMPK indirectly stimulates 6-phosphofructo-1-kinase (PFK1, Fig. 1) activity by phosphorylating and activating 6-phosphofructo-2-kinase (PFK2), the enzyme that synthesizes fructose 2,6-bisphosphate, to generate more energy. Third, AMPK protects cardiocytes by inhibiting apoptosis through several mechanisms, thus promoting cell survival. In 2005, Shibata *et al.* showed that the AMPK pathway is essential for the apoptosis-inhibiting effects of adiponectin in heart [47]. The translocation of Bax, a pro-apoptotic protein, to mitochondria is thought to be an early step in apoptosis induced by ischaemia and this process appears to occur in response to activation of p38 MAPK downstream of AMPK in heart [48]. Recently, AMPK was further defined as a powerful cardiac protector against cardiomyocyte apoptosis triggered by TNF-α through phosphorylation of Bad; subsequent suppression of the interaction between Bad and Bcl-xL limits cytochrome *c* release and caspase-3 activation [49]. Because ischaemic injury concurrently elevates TNF-α production, cytochrome *c* release, the caspase cascade and AMPK activation, these newly identified anti-apoptosis functions of AMPK may represent important pathogenic activities of AMPK pathway. Finally, AMPK is a critical controller in the autophagy process in ischaemic heart. Autophagy has been identified as a survival mechanism in ischaemic myocardium that reserves energy and substances necessary for cell survival [50–53]. In 2007, Liang *et al.* first demonstrated that the LKB1–AMPK pathway dictates entry into autophagy or apoptosis

under ischaemic conditions by regulating p27(kip1) phosphorylation in heart [54]. Of relevance, it was found that ischaemia stimulates autophagy through an AMPK-dependent mechanism, and this autophagy is cardioprotective [55]. Collectively, these studies provide compelling evidence for the beneficial effects of AMPK on cardiac function.

Animal models have been applied to address the question whether AMPK plays a positive or negative role during processes associated with ischaemia/reperfusion [56]. The α2 isoform is the pre-dominant catalytic isoform in the heart. The phosphorylation of the main substrate of AMPK, acetyl-CoA carboxylase (ACC, Fig. 1), is clearly decreased in both normoxic and ischaemic conditions in AMPKα2 knockout mice [57, 58]. These studies also indicated that AMPK α2 is necessary for maintaining myocardial energy homeostasis during ischaemia. This phenotype was further confirmed in AMPK α2-dominant negative (DN) mouse hearts [59, 60]. Moreover, genetic manipulation of the AMPK pathway indicated additional roles for this enzyme in heart. In AMPKα2-DN mouse hearts, AMPK critically contributes to p38 MAPK activation during ischaemia by promoting auto-phosphorylation of p38 MAPK through interaction with the scaffold protein transforming growth factor β activated protein kinase 1 binding protein 1 [61]. In a similar fashion, it was found that AMPK mediates pre-conditioning in cardiac cells by regulating the activity and recruitment of sarcolemmal K (ATP) channels without being a part of the signalling pathway that regulates mitochondrial membrane potential [62]. Furthermore, using a knock-down approach, Ahmad *et al.* demonstrated that the AMPK α2, rather than the α1 subunit, is the primary mediator of the effects of the 5'-AMP-activated protein kinase subunit γ2 gene mutation, such as cardiomyopathy with cardiac hypertrophy, pre-excitation and glycogen deposition [63].

Role of AMPK in endothelial cells

The endothelium is a single layer of cells covering the inner surface of all blood vessels, including conduit arteries, resistance and capacitance vessels and capillaries. Endothelial cells are involved in many aspects of vascular biology, including control of blood pressure, thrombosis, fibrinolysis, atherosclerosis and inflammation. The pre-dominant catalytic isoform of AMPK expressed in vascular endothelial cells is α1 rather than α2 [64–66]. Endothelium-dependent vasodilatation is a vital mechanism of systemic blood flow regulation that occurs during periods of increased metabolic demand such as glucose deprivation and hypoxia. As a metabolic sensor, vascular AMPK is believed to be involved in the metabolic regulation of blood flow. It is now well established that a major function of endothelial cells is nitric oxide synthesis, which is regulated by the activity of endothelial nitric oxide synthase (eNOS, Fig. 1) [67–68]. Chen *et al.* (1999) first suggested that eNOS might represent a target for AMPK in endothelium [69]. In 2008, Hu *et al.* demonstrated that AMPK could phosphorylate eNOS at Ser1179, enhancing activity of this enzyme [70]. Recently, a second phosphorylation site on eNOS,

Ser633, was identified; the authors confirmed that AMPK phosphorylation of eNOS Ser633 is a functional signalling event contributing to nitric oxide bioavailability in endothelial cells [71]. Thus, AMPK directly affects endothelial cells function, although regulation of the phosphorylation of eNOS.

Consistent with these reports, we demonstrated in cultured human umbilical vein endothelial cells (HUVECs) that resveratrol, a natural extract, enhances phosphorylation of eNOS at Ser1177 and increases nitric oxide production *via* the AMPK pathway. Further, the enhanced eNOS activity and nitric oxide production is totally blocked by the specific AMPK inhibitor, Compound C [72]. Moreover, the AMPK pathway is required for the resveratrol-induced relaxation of aorta isolated from mice fed a high glucose diet [72]. In addition to these effects on eNOS, AMPK also appears to play an important role in the level of oxidative stress in endothelial cells. Zou *et al.* suggested that the increased generation of reactive oxygen species (ROS) promotes AMPK activation by the LKB1 complex in cultured endothelial cells [73]. Further, experiments by Ido *et al.* [74] showed that the AMPK activator, 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR), increases AMPK activity and completely prevents the marked increase in HUVEC apoptosis that results from oxidative stress.

The AMPK pathway may represent a 'common pathway' involved in the protective effects of many exogenous drugs or endogenous factors on endothelial cells. For example, it has been reported that a class of drugs that target peroxisome proliferator-activated receptor α, used to modify blood lipids levels, stimulate eNOS phosphorylation and nitric oxide production through AMPK activation [75, 76]. In addition, metformin, a commonly used anti-diabetes drug, reportedly exerts its therapeutic effects by activating the AMPK signalling pathway in endothelial cells [77–79]. The mechanisms underlying this protective effect include prevention of inflammatory cytokine production [77] and increased angiogenesis [79]. Rosiglitazone, an anti-diabetic drug in the thiazolidinedione class of drugs, not only protects endothelial cells against glucose-induced oxidative stress in an AMPK-dependent manner [80] but also stimulates nitric oxide synthesis in human aortic endothelial cells *via* AMPK [81]. In addition, some important hormones, such as adiponectin [82–84], ghrelin [85] and leptin [86], exert their biological functions though the AMPK signalling pathway. Collectively, these studies support the notion that the AMPK pathway is a physiologically relevant and potent protection mechanism in endothelial cells.

Role of AMPK in vascular smooth muscle cells

The most important function of vascular smooth muscle cells (VSMC) is vasoconstriction in response to many physiological and pathological stimuli. Moreover, proliferation and migration of VSMCs play critical roles in vascular inflammation and development of atherosclerosis. Vascular smooth muscle AMPK differs in its expression profile compared to striated and cardiac muscle cells, with both α1 and α2 catalytic subunits being evident [64, 65, 87]. Further, the relative proportion of the α1 and α2 subunits varies between vessels [64, 65, 87]. Studies from Rubin *et al.* demonstrated that endothelium-denuded porcine carotid artery segments exhibit an increase in AMPK activity within 1 minute of being metabolically challenged with 2-deoxyglucose (2DG) and 2-deoxyglucose plus N_2 (N_2-2DG) [65]. Further, AMPK activation by N2-2DG was associated with a rapid and pronounced reduction in phosphorylation of Akt and ERK 1/2. Moreover, it has been reported that AMPK can phosphorylate and desensitize smooth muscle myosin light chain kinase to attenuate vasoconstriction, implying that AMPK contributes to the reduced ATP turnover in the tonic phase of smooth muscle contraction [88]. Using AMPK α1 and α2 knockout mice, Goirand *et al.* demonstrated that activation of AMPK α1, but not α2, leads to relaxation of aorta in mice in an endothelium- and eNOS-independent manner [87]. Consistent with these reports, AMPK was also found to be a key regulator of the contractile response of pulmonary arteries to acute hypoxia [89, 90]. Therefore, AMPK is a player in the complex signalling pathways that regulate vascular smooth muscle tone.

Apart from the acute regulation of vascular tone, AMPK is also involved in the proliferation of VSMC. AMPK expression blocks the growth of vascular smooth muscle induced by angiotensin-II [91]. In a rat femoral artery wire injury model, continuous activation of AMPK by AICAR injection significantly inhibits neointima formation [91]. Igata *et al.* showed that AMPK increased the expression of the cyclin-dependent kinase inhibitor p21cip via inhibition of phosphorylation of the retinoblastoma gene product, leading to inhibition of the proliferation of vascular smooth muscle [92]. Moreover, Liang *et al.* demonstrated that berberine, a herbal extract, inhibits platelet-derived growth factor-induced growth and migration in VSMCs, partly through the AMPK pathway [93]. Interestingly, AMPK also contributes to the activation of NAD(P)H:quinone oxidoreductase 1, which can prevent arterial restenosis by suppressing VSMC proliferation [94].

Role of AMPK in vascular angiogenesis

Angiogenesis is a physiological process involving the growth of new blood vessels from pre-existing vessels. It represents a normal and pivotal biological process in growth and development and is also important in wound healing and tumour growth. A critical role for AMPK in angiogenesis was first identified in cancer cells under nutrient deprivation. The introduction of AMPK antisense RNA expression vectors into pancreatic cancer cell lines, PANC-1 and AsPC-1, diminished their tolerance to glucose deprivation; in addition, the stable transfection of AMPK antisense RNA into PANC-1 cells inhibited tumour growth in nude mice [95]. Results from the studies of Nagata *et al.* demonstrated that AMPK signalling was a potent regulator of vascular angiogenesis and was specifically required for endothelial cell migration and differentiation

under conditions of hypoxia [96]. These observations suggest that endothelial AMPK signalling may be a critical determinant of blood vessel recruitment to tissues exposed to ischaemic stress. Subsequently, the involvement of AMPK signalling in the pro-angiogenesis actions of adiponectin was observed [97]. Moreover, Yun *et al.* found that glucose deprivation increased mRNA stability of vascular endothelial growth factor (VEGF), a key hormone regulating angiogenesis, through activation of AMPK [98]. In another report, Orchi *et al.* provided the first direct evidence that AMPK activation by AICAR treatment increased VEGF expression and accelerated angiogenic repair of ischaemic hindlimbs in mice [99]. The mechanisms by which AMPK promotes vascular angiogenesis are being gradually elucidated. Levine *et al.* suggested that an AMPK-Rac1-Akt-eNOS axis in endothelial cells plays a central role in the vascular angiogenesis process [100]. Although several other studies support this hypothesis [101, 102], data from the studies of Zwetsloot *et al.* [103] suggest that AMPK is not necessary for the angiogenic response to exercise, indicating that the role of AMPK may be more complex than it currently appears.

Role of AMPK in cardiovascular diseases

Atherosclerosis

Atherosclerosis is a chronic disease affecting medium and large arteries. In the past several decades, there has been a marked increase in our understanding of the pathogenesis of this vascular disorder [104]. Previously, atherosclerosis was presumed to be primarily a plumbing problem. Now it is deemed a chronic inflammatory disease in the walls of arteries, which originates from the interaction between cells of the arterial wall, lipoproteins and inflammatory cells, leading to the development of complex lesions or plaques. AMPK not only regulates proliferation, migration, apoptosis and autophagy of vascular endothelial and smooth muscle cells but also affects biological functions of macrophages, which are critical activators of atherosclerosis. Also relevant to the development of atherosclerosis is the fact that AMPK participates in whole body glucose, lipid and protein homeostasis, thus affecting serum lipid and glucose levels and insulin resistance. Therefore, it is an attractive hypothesis that AMPK-mediated signalling is intimately involved in the pathophysiological process of atherosclerosis.

The entry of vascular cells, especially VSMCs, into the cell cycle plays an important role in the development and progression of atherosclerosis. Studies have reported that treatment of human aortic smooth muscle cells (HASMC), with the AMPK activator AICAR results in phosphorylation of AMPK and ACC while significantly inhibiting HASMC proliferation induced by either platelet-derived growth factor-BB or fetal calf serum [92]. Cell cycle analysis showed that AMPK activation increases the number of cells in G0/G1-phase and reduces numbers in S- and G2/M-phase, sug-

gesting that AMPK activation can cause cell cycle arrest [105]. Consistent with this report, AICAR inhibits angiotensin II-stimulated VSMC thymidine incorporation, and administration of AICAR prevents neointimal formation in the rat balloon injury model. This suggests that AMPK might serve as a therapeutic target in treatment of atherosclerosis. However, there is currently no direct evidence conclusively showing AMPK to be protective in atherosclerosis. In 2006, Zang *et al.* showed that polyphenols increase AMPK and ACC phosphorylation and inhibit the hepatic lipid accumulation, hyperlipidaemia and accelerated aortic lesion formation seen in type 1 diabetic LDL receptor-deficient mice. These data imply that activation of AMPK may represent a potential protective mechanism against atherosclerosis [106]. Studies from Devaraj *et al.* revealed that adiponectin decreased C-reactive protein synthesis and secretion by endothelial cells, which could be mimicked by the AMPK activator AICAR and reversed by inhibition of AMPK [107]. Moreover, AMPK activation was found to contribute to the activity of adiponectin, which inhibits insulin-like growth factor-1 induced cell migration in VSMCs [108]. AMPK regulates the antioxidant status of vascular endothelial cells [109], and reduction of AMPK increases endoplasmic reticulum stress and atherosclerosis *in vivo* [110]. In the latter report, Dong *et al.* also demonstrated that AMPK α2 is the main physiological suppressor of endoplasmic reticulum stress in endothelial cells [110]. Notably, AMPK suppresses oxidized low-density lipoprotein-induced macrophage proliferation, which is a key event underlying the development of atherosclerosis [111]. Collectively, these studies suggest that AMPK protects the vascular wall from atherosclerosis and may represent a new therapeutic target in the treatment of atherosclerosis.

Hypertension

Vessel remodelling and/or vessel hypertrophy are responsible for the alterations in vascular structure observed in hypertension. Vessel remodelling occurs through reorganization of existing intracellular and extracellular components around the vascular lumen. In contrast, hyperplasia involves VSMC proliferation, migration, hypertrophy and polyploidy. Thus, AMPK may be involved in the genesis and development of hypertension. Studies from Kurdi *et al.* (2004) comparing left ventricle gene expression between severely hypertensive rats (transgenic rats that express the renin gene in the salivary gland) and normotensive control rats by cDNA macroarray revealed that AMPK is overexpressed in hypertensive rats [112]. Hattori *et al.* reported that metformin attenuates cytokine-induced expression of proinflammatory and adhesion molecule genes by inhibiting NF-κB activation via AMPK activation [77], which is pivotal in development of hypertension [113]. Moreover, impaired adiponectin-AMPK signalling has been reported in insulin-sensitive tissues of hypertensive rats by Rodrìguez *et al.* [114]. Recently, it was further reported that deletion of AMPK α2 enhances the increase of phosphate-4E binding protein-1 seen in transverse aortic constriction and augments the T transverse aortic constriction-induced increase of phosphate-Akt, indicating that the AMPK signalling pathway exerts a cardiac

protective effect against pressure-overload-induced ventricular hypertrophy and dysfunction [115]. Collectively, the results from these studies support the notion that AMPK is protective against hypertension. However, it should be noted that activation of AMPK was found to enhance angiotensin II-induced proliferation in cardiac fibroblasts [116], implying AMPK might exert different effects in different cells in hypertension. Therefore, further investigation is needed to reveal the precise role of AMPK in hypertension, including the relevance of AMPK activation to the end-organ damage induced by hypertension.

Ischaemic stroke

Whether AMPK is beneficial or detrimental to the ischaemic brain is in dispute. Evidence from several independent groups indicates that AMPK is neuroprotective in ischaemic stroke [117–119], whereas there are also reports showing activation of AMPK is harmful in ischaemic stroke [120–121]. The brain, a high energy-consuming organ with very low energy reserves, relies heavily on a continuous flow of blood. Thus, AMPK in brain is likely to be activated when the ischaemic stroke occurs; a suggestion which is supported by a number of studies [117–121]. During severe energy depletion, neurons attempt to reserve energy. Apoptosis and autophagy both occur in neuronal cell outcomes after ischaemic stroke [122]. Moreover, different from other organs, AMPK $\alpha 1$ is expressed at low levels in brain; the AMPK $\alpha 2$ catalytic subunit accounts for more than 90% of AMPK in cerebral tissue [18]. In 2001, Culmsee *et al.* reported that AMPK activator AICAR protected hippocampal neurons against death induced by glucose deprivation, chemical hypoxia and exposure to glutamate and amyloid β peptide [119]. Dasgupta *et al.* found that resvera-trol protects neurons through stimulation of AMPK activity [118]. Also, AMPK is associated with GABA(B) receptors, serving to enhance neuronal survival after ischaemia [32, 117]. It is worth noting that the detrimental effects of AMPK in stroke have been reported by only one group [120, 121]. Future studies using more selective AMPK activators or inhibitors, selective genetic manipulation in animal models and isolated genetic knockout neuronal cells will provide more information on this role of AMPK in stroke.

Conclusion and perspectives

Although the AMPK pathway is traditionally thought of as an intracellular fuel switch and controller of metabolism, the significance of AMPK in the cardiovascular system has recently been recognized [123]. A large body of evidence indicates that approaches aimed at increasing AMPK activity in the cardiovascular system may be therapeutic. A-769662, a thienopyridone, has been identified as a potent AMPK activator which directly stimulates partially purified rat liver AMPK [124]. Treatment of *ob/ob* mice with this agent lowered plasma glucose by 40%, reduced body-weight gain and significantly decreased both plasma and liver triglyceride levels. Evaluation of small molecular weight AMPK activators will shed new light on the treatment of cardiovascular disease.

Conflicts of interest

The authors confirm that there are no conflicts of interest.

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