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AMPK in myocardial infarction and diabetes: the yin/yang effect

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Abstract

There has been considerable progress in our understanding of cardiac cell metabolism in health and disease, yet important gaps remain in basic knowledge and its translation to clinical care. AMP-activated protein kinase (AMPK) functions either to conserve ATP or to promote alternative methods of ATP generation. Since the discovery of AMPK more than three decades ago and demonstration of its expression in the heart, interest has grown exponentially in this major fuel gauge as a modulator of the cellular response to ischemia. Such pathway may potentially explain the strong association between metabolic syndrome and ischemic heart disease. Still missing from our most recent cardiology textbooks, this article aims to summarize our understanding so far of the role of AMPK in coordinating the cellular response to ischemic stress and reperfusion injury in the heart. We aim to provide a focused update on the pharmacological agents activating AMPK for treatment of diabetes that show potential cardioprotective effects. Our hope is to stimulate future researchers to the potential benefits of harnessing the AMPK signaling pathway, or better one of its novel downstream targets for the treatment of myocardial ischemia.

Keywords

Ischemia-reperfusion injury; Myocardial infarction; AMPK; Myocardial ischemia; Diabetes; Agonists; Glucose homeostasis

1. Introduction

Heart disease remains the leading cause of death worldwide in spite of the dramatic improvements in the management of acute myocardial infarction. While the underlying pathophysiology of the atherosclerotic process is not significantly different in diabetic subjects, the prothrombotic and procoa-gulant state with which diabetes is associated is thought to contribute to the higher incidence of and worse prognosis after myocardial infarction[1]. Following reperfusion with percutaneous coronary intervention or thrombolytics, infarct size propagation seemed inevitable[2]. That was when attention has turned to cellular adaptive mechanisms in the hope to improve mortality and morbidity of myocardial infarction. AMPK has stood out over the past decade as a master conductor

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orchestrating the intracellular metabolic process, both at the cellular level and in experimental animal models[3–6]. It functions as a sensor during low energy states, such as ischemia, to change substrate utilization and thereby increase ATP synthesis. AMPK also seemed to put the cell in 'survival mode' conserving energy that would otherwise be consumed in non-urgent anabolic processes[7–9]. Since then AMPK has emerged as a very promising target for cytoprotective therapy[10–15]. Our perception of the AMPK pathway continues to evolve as we better understand the intricate relationships between AMPK and its upstream and downstream kinases. The aim of the current review is to discuss the benefits and potential harms of AMPK activation during myocardial infarction, especially in the diabetic population and shed light on the pros and cons of currently available AMPK activators.

2. Structure of AMPK

AMPK is a widely distributed, highly conserved heterotrimeric enzyme composed of a catalytic α (63 kDa) subunit and the non-catalytic β and γ subunits[5] (Fig. 1). There are two genes encoding isoforms of both the α and β subunits (α1, α2, β1 and β2). The α2 isoform is the subunit of AMPK found predominantly within cardiac muscles[16]. AMPK is activated by AMPK kinase (AMPKK) by phosphorylation of threonine-172 at the N-terminal end of the α subunits[17,18]. The close proximity of AMPK to cellular glycogen stores allows it to rapidly affect changes in glycogen metabolism in response to changes in the metabolic demands[19]. Binding sites for AMP are found on the regulatory γ subunits which also bind ATP[16,20]. Of clinical significance is the observation that mutations in the γ2 subunit, the dominant isoform in the heart (gene symbol PRKAG2), are associated with abnormal glycogen accumulation in cardiomyoctes manifesting as Wolff–Parkinson–White syndrome and familial hypertrophic cardiomyopathy[21]. The binding of AMP and ATP to AMPK occurs in a mutually exclusive manner[17]. An increased AMP to ATP ratio leads to a conformational change in AMPK that makes it a poorer substrate for degrading protein phosphatases and a better substrate for AMPKK[5,17]. Because of its dual effect, small rises in AMP levels can induce a dramatic rise in the activity of AMPK[22]. Adenylate kinase maintains its reaction (2ADP↔ATP+AMP) close to equilibrium, therefore the AMP:ATP ratio varies as the square of the ADP:ATP ratio, and this makes the AMP:ATP ratio a very sensitive indicator of cellular energy charge[23]. Similar positive allosterism is also observed with an increased creatinine:phosphocreatine ratio[20]. Activation of AMPK leads to phosphorylation of many target proteins important for ATP synthesis and utilization as well as the inhibition of ATP-consuming pathways such as fatty acid synthesis[11,24]. Once the energy stores are replenished, the binding of ATP maintains the activity of AMPK low[25].

3. Upstream regulators of AMPK

There are at least three different upstream AMPK kinases (AMPKKs), the most widely expressed being LKB1 (with two accessory subunits, STRADα/β and MO25α/β) which is critical for the gluconeogenic flux and consequently glucose homeostasis[26,27]. AMPK phosphorylation during myocardial ischemia however seems to be related, in addition, to AMPKKs other than LKB1 as observed by Altarejos et al.[28] AMPK activity was noted to be increased in mild ischemia before the classic shift in the AMP:ATP and

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creatine:phosphocreatinine ratios[17,29]. One other activator of AMPK is Ca^{2+}-calmodulin–protein kinase kinase β (CaMKK-β) which phosphorylates and activates AMPK in response to increased Ca^{2+} fluxes providing a mechanism for cells to anticipate the increased demand for ATP[30,31]. An interesting phenomenon described by Anderson et al.[32,33] is the autophosphorylation of CaMKK-β after sustained exposure to Ca^{2+}. This might contribute to sustained myocardial stunning and ischemia reperfusion injury (RI) following myocardial infarction. The third AMPK phosphorylating kinase is transforming growth factor-β-activated kinase 1 (TAK1)[34]. TAK1, a member of the mitogen-activated protein kinase kinase kinase (MAPKKK) family, is a key mediator of proinflammatory and stress signals[29,35]. TAK1 was initially found to activate the AMPK analogue in yeast, Snf1 before being linked to mammalian AMPK[34]. A large body of promising research is being invested in each upstream kinase to determine which one is predominantly expressed in the heart and is the first domino in the cardiomyocyte response to ischemic stress cascade and post-MI remodeling. Detailed discussion of each of these kinases is beyond the scope of this article and is readily available for the avid reader. Most likely the cellular response to ischemia initiating AMPK activation is interplay of all three kinases and others. Adiponectin, a plasma protein with cardioprotective, antidiabetic and anti-inflammatory properties also exerts its action through AMPK activation[36,37]. Of note is that adiponectin levels were found to correlate inversely with insulin resistance, obesity and coronary artery disease possibly explaining why patients with diabetes and ischemic heart disease have worse prognoses[38]. Furthermore sestrins, a family of conserved proteins that accumulate in cells exposed to stress, and lack kinase activity have been recently shown to potentiate the activation of AMPK independent of AMP:ATP[39]. Little is known about which phosphatase regulates AMPK. Both type 2A and type 2C protein phosphatases (PP2A and PP2C, respectively) are good candidates since they can dephosphorylate and deactivate AMPK *in vitro*[40].

4. Downstream targets of AMPK in the heart

Activation of AMPK in the heart leads to a myriad of changes affecting glucose and lipid metabolism, protein synthesis and gene expression[3,41]. AMPK activation induces the translocation of glucose transporter 4 (GLUT4) to the plasma membrane by a mechanism distinct to that of insulin and not clearly understood[42,43]. It augments the binding of transcription factor myocyte enhancing factor-2 (MEF-2) to promoters in the GLUT4 gene increasing its expression[44]. Under hypoxic conditions in the heart, AMPK activates phosphofructokinase-2 (PFK-2), the product of which is fructose 2, 6-biphosphate (Fru2, 6-P2)[45,46]. This molecule allosterically regulates phosphofructokinase-1 and in the liver fructose 1, 6-biphosphatase, favoring glycolysis over gluconeogenesis. This phenomenon, recognized as the Pasteur Effect is responsible for increased ATP production through anaerobic glycolysis during ischemia[45]. AMPK also inhibits both isoforms of Acetyl-CoA carboxylase (ACC1 and ACC2) through phosphorylation[47]. This eventually leads to decreased inhibition of carnitine palmitoyltransferase I (CPTI) in the mitochondria and increased β-oxidation of fatty acids[4,6]. Increased fatty acid oxidation, like increased glycolysis will lead to valuable increases in ATP production in stress conditions. One important concept in energy production is the Randle cycle where the utilization of one nutrient inhibits the use of the other directly and without hormonal mediation[48]. The

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glucose-fatty acid cycle is thus a biochemical mechanism that controls fuel selection and adapts substrate supply and demand in normal tissues in coordination with hormones controlling substrate concentrations in the circulation[49].

In addition AMPK phosphorylates and inactivates HMG-CoA reductase, hormone-sensitive lipase, as well as transcription factors hepatic nuclear factor 4α (HNF4α) and sterol regulatory element binding proteins 1c and −2 (SREBP-1c and −2)[50]. This reduces energy consumption at the cellular level and in the long term atherogenic dyslipidemia. AMPK further conserves ATP expenditure by inhibiting liver glycogen synthase and creatine kinase[51].

With regards to protein metabolism, activation of AMPK phosphorylates and activates endothelial nitric oxide synthase (eNOS) in cardiac endothelium causing NO-mediated vasodilatation and improved perfusion[4,42]. NO activation in platelets leads to a decrease in thrombin-induced aggregation, thereby, limiting the pro-coagulant effects of platelet activation[52]. AMPK activation regulates protein synthesis through inhibition of elongation factor 2 (eEF2) and indirectly through inhibition of mammalian target of rapamycin (mTOR)[53]. Of clinical significance is the use of mTOR inhibitors in drug-eluting stents to inhibit protein synthesis and restenosis following reestablishing blood flow. Another promising transcription factor and downstream target of AMPK recently linked to improved cardiac oxidative stress is Forkhead box protein (FoxO)[54]. Mice with deletion of FoxO1 and FoxO3 were found to have increased scar formation, induction of stress-responsive signaling, and apoptotic cell death in response to ischemia[55]. Peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1α) is a multi-functional transcriptional coactivator involved in the regulation of cardiac mitochondrial biogenesis and oxidative phosphorylation[56]. It is activated by AMPK and sirtuin 1 (SIRT1) through phosphorylation and deacetylation, respectively[57]. Phosphorylated AMPK also indirectly activates PGC-1α through SIRT1 by promoting an intracellular increase in NAD^+ levels, the rate-limiting substrate for the deacetylase activity of SIRT1[58]. Once turned on, PGC-1α boosts mitochondrial activity to adapt to the increased energy requirements.

5. AMPK regulates autophagy

AMPK is also involved in triggering autophagy in the heart during ischemia and reperfusion[59]. Autophagy is a lysosomal pathway involved in the turnover of cell's own macromolecules and organelles[9]. This has been identified as an adaptive cell response allowing the cell to survive otherwise lethal challenges by removing damaged organelles and misfolded proteins. The principle is to conserve energy that would otherwise be used in non-essential pathways. In addition free fatty acids and amino acids produced by the degradation of these organelles are then recycled to maintain mitochondrial ATP production and ribosomal protein synthesis[60]. Recent studies have shown that AMPK is involved in triggering autophagy in the heart in response to ischemia[59]. Activated AMPK inhibits mammalian target of rapamycin complex 1 (mTORC1/mTOR) activity *via* a pathway involving tuberous sclerosis complex 1 and 2 (TSC1/2) thus stimulating autophagy[53]. Recent studies unveiled that ULK1 is a mediator for AMPK modulating autophagy[9,61,62].

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6. AMPK and myocardial ischemia

Utilizing the AMPK pathway seems to have a beneficial effect during myocardial ischemia[11,12,14,31,63,64]. There is a fairly stereotypical sequence of physiologic changes that develop during an episode of spontaneous transmural ischemia[3,20]. Under physiological conditions, energy necessary to maintain myocardial contraction/relaxation cycle is derived from mitochondrial oxidation of long chain fatty acids (60–70%) and carbohydrates (20–30%)[3,65,66]. Coronary occlusion results in an immediate fall in coronary venous oxygen saturation, with a reduction in ATP production[64]. This causes a decline in regional contraction within several beats, reaching dyskinesis within 1 min[67]. As regional contraction ceases, there is a reduction in global LV contractility (dP/dt), a progressive rise in LV end-diastolic pressure and a fall in systolic pressure. AMPK therefore is activated in response to the decreased concentration of ATP in order to boost energy production and sustain the cardiomyocyte during this time of metabolic stress[3,4]. The magnitude of systemic hemodynamic changes varies with the severity of ischemia and the amount of the left ventricle subjected to ischemia. ST-segment changes on EKG develop within 2 min as efflux of potassium into the extracellular space reaches a critical level[68]. Irreversible injury begins after 20 min following a total occlusion but is delayed for up to 5 h following a partial occlusion[69]. The timing of administering a drug that activates AMPK is thus crucial, ideally as soon as possible following presentation.

7. AMPK in reperfusion injury

Following restoration of blood flow before irreversible injury, several types of RI have been observed in experimental animals that exacerbate myocardial infarction[70]. These consist of the following: (1) lethal reperfusion injury – reperfusion-induced death of cells that were still viable at the time of restoration of coronary blood flow; (2) vascular reperfusion injury – progressive damage to the microvasculature so that there is an expanding area of no reflow and loss of coronary vasodilatory reserve; (3) stunned myocardium – salvaged myocytes display a prolonged period of contractile dysfunction following restoration of blood flow because of abnormalities of intracellular metabolism leading to reduced energy production; and (4) reperfusion arrhythmias – bursts of ventricular tachycardia and, on occasion, ventricular fibrillation – that occur within seconds of reperfusion. The exact mechanism of RI remains incompletely understood, but is thought to include (i) cytosolic and mitochondrial Ca^{2+}-overload, (ii) release of reactive oxygen species (ROS), (iii) changes in pH and intramitochondrial H^{+}[71], (iv) shift in substrate use, and (v) recruitment of inflammatory cells attacking viable myocytes. In concert, these derangements result in the formation of mitochondrial permeability transition pore (mPTP) upon reperfusion (Fig. 2). It spans both the inner and outer membranes and, when open, causes collapse of the transmembrane voltage gradient C and uncoupling of the mitochondria. Pore formation would prevent mitochondrial ATP production at reperfusion, a time when the ischemic but still salvageable myocytes would need it the most[72]. We now know that AMPK is stimulated by all of these changes. AMPK is activated by CaMKK-β in response to (i)[73] and is redox-sensitive to (ii) and (iii)[74]. Decreased ATP:AMP (iv) and TAK1 in response to inflammatory cytokines (v) also activate AMPK. In addition, AMPK seems to inhibit the opening of mPTP and reduce infarct size in animal models[75]. Transgenic mice heart expressing a kinase dead

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form of AMPK demonstrated impaired recovery of left ventricular contractility and increased apoptosis on reperfusion[11]. A group of protective kinases, referred to as reperfusion injury salvage kinases (RISK), were found to somehow prevent transition pore formation at reperfusion. This comprises phosphatidylinositol 3-kinase (PI3K), Protein kinase B (Akt) and p42/p44 extra-cellular signal-regulated kinases (Erk 1/2). Even though AMPK has not been identified as a member of the RISKs, many of the agents that activate AMPK were also found to activate the RISK pathway including metformin, pioglitazone, statins and adenosine[76]. While autophagy in myocardial ischemia is cardioprotective as a means to conserve energy, continued autophagy upon reperfusion seems to be harmful with a shift more towards programmed cell death[59]. This however was shown to be due to beclin 1, independent of AMPK[77].

Needless to say that the graveness of reperfusion injury is proportional to the duration of ischemia. The effectiveness of agents directed against reperfusion injury rapidly declines the later they are administered after reperfusion; eventually, no beneficial effect is detectable in animal models after 45 to 60 min of reperfusion has elapsed[75].

8. Pharmacological agents

A very interesting combination of drugs has emerged that utilize the AMPK pathway. There are two groups of antihyperglycemics: metformin and glitazones (TZDs), an adenosine analogue tested in bypass surgery, a drug formerly used in sepsis, a thienopyridone and statins, which are the only drugs currently approved for acute coronary syndrome. Cannabinoids, present in marijuana have also been found, in a small study, to stimulate AMPK in the heart and hypothalamus of rats[78].

8.1. Metformin

First used in 1957, it is the most commonly used drug for treatment of type 2 diabetes. The activation of AMPK by metformin is mediated indirectly through increased AMP:ATP ratio. This is hypothesized to occur through weak inhibition of complex I of the respiratory chain and a relative fall in cellular ATP concentration[79]. This inhibits AMPK dephosphorylation thereby potentiating its phosphorylation by the upstream kinase LKB1[80]. Adenine nucleotide-independent mechanisms have also been postulated but poorly understood[81,82]. The cardioprotective actions of metformin through AMPK are not solely limited to its ability to reduce myocardial infarct size, as demonstrated by Calvert et al.[83] with the acute administration before ischemia or at reperfusion. The dose used for AMPK activation of 125 μg/kg[79] was much less than the maximum therapeutic dose of 2550 mg[84]. A very recent study has shown the cardioprotective benefits of metformin extend beyond acute coronary syndrome (ACS) to cardiac transplantation. Murine hearts treated with metformin perioperatively were found to have significantly better graft beating scores and less luminal narrowing, an effect that lasted more than 50 day post surgery[79]. This is believed to be mainly due to decreased RI, apoptosis and cardiac allograft vasculopathy secondary to AMPK activation. Chronic metformin therapy in diabetic patients is also known to improve lipoprotein profiles, reduce oxidative stress, and improve vascular stability[85]. Dogs with tachycardia-induced heart failure were found to have improved left ventricular function with

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chronic AMPK activation with metformin or AICAR[86]. The metformin dose used there was 100 mg/kg/day, similar to the therapeutic dose for diabetes. Advantages of metformin use are its good bioavailability and relatively safe profile. The dose for optimal AMPK activation is yet to be determined. An interesting question arises when considering metformin in ameliorating ischemia/reperfusion injury in the setting of ACS. Would diabetic patients already on chronic metformin therapy (assuming AMPK activation) benefit from a 'booster' dose of metformin as much as naive patients?

8.2. Glitazones (TZDs)

Rosiglitazone and pioglitazone are insulin sensitizers. Pioglitazone is the only drug remaining in this class that may be prescribed without restriction. TZDs bind to the nuclear membrane receptors PPARγ (peroxisome proliferator-activated receptors gamma) and affect DNA expression. Like metformin, TZDs have been found to increase the AMP:ATP ratio leading to AMPK activation[87]. PPARγ agonists further activate AMPK through increased expression of adiponectin[88,89]. Most experiments have been done using rosiglitazone as it is the most selective and potent PPARγ agonist in its class[90]. Rosiglitazone treatment also has been shown to reduce myocardial apoptosis and infarction size post RI by restoring the balance between the pro-apoptotic and antiapoptotic mitogen-activated protein kinases (MAPKs), increasing phosphatidylinositol-3-kinase (PI3K)-Akt phosphorylation, and inhibiting p42/44 MAPK[91].

The association between TZDs and heart failure is well recognized as a class effect[3]. Fluid retention is mediated through increased sodium reabsorption of the renal PPARγ-dependent pathway in the collecting tubules. In 2007, a meta-analysis by Nissen et al.[92] suggested that use of rosiglitazone in type 2 diabetes was associated with a significant increase in the risk of myocardial infarction and with an increase in the risk of death from cardiovascular causes that had borderline significance. Although the method and statistical analysis used in this study have been criticized, subsequent meta-analyses showed similar concerns[93]. In spite of these concerns, one can argue that acute rosiglitazone administration may prove beneficial in ACS through AMPK activation regardless of the implicating long-term increased mortality[3,64]. Unpublished results from our lab suggest that the benefit of acute rosiglitazone therapy during myocardial ischemia is even more pronounced in diabetic mice.

8.3. Activated protein C (APC)

APC is a vitamin K-dependent plasma serine protease that down-regulates clotting and inflammatory pathways[94]. Because of such properties, APC was approved in septic shock in 2001 before a Cochrane review a decade later showed no significant mortality benefit[95]. In the interim it was found that APC exerts cardioprotective effects by decreasing apoptosis in cardiomyocytes and inhibiting expression of inflammatory mediators after myocardial ischemia through AMPK. The activation of cardiac AMPK by APC was mediated through the APC receptors: endothelial protein C receptor (EPCR) and protease activated receptor-1 (PAR-1), and was dependent on CaMKK-β. Moreover, APC decreased inflammatory signaling pathways JNK and NF-κB, and down-regulated the expression of inflammatory cytokines in ischemic hearts. These cardioprotective effects of APC were abrogated in AMPKα2 knockout mice[31].

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8.4. 5-Aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR)

AICAR is a drug that is metabolized inside cells into ZMP (5-aminoimidazole-4-carboxamide-riboside), an analogue of AMP[96]. ZMP activates AMPK by direct and allosteric activation although less effective than AMP[17]. Balschi et al.[97] demonstrated the AICAR-dependant phosphorylation of AMPK in isolated mouse heart. Interestingly AICAR made it to phase III trials, as early as 1994 in (Coronary Artery Bypass Graft) CABG surgery, long before our current understanding of the AMPK pathway. This was simply because of the rationale that increased levels of adenosine potentiates vasodilatation, preconditioning and inhibits inflammation[98]. RED-CABG in 2009, the last phase III trial involving AICAR, was terminated early when an interim analysis showed low probability of success. Limitations to its use include the requirement for intravenous infusion and variable potency. It also causes bradycardia and can lead to hypoglycemia when administered intravenously. One interesting conflict is that AICAR has been shown to induce protein kinase B (Akt), a potent activator of mTORC1, in an AMPK-independent manner[99]. Activation of mTORC1 inhibits autophagy which has been shown to play a role in ameliorating cardiac ischemic injury.

8.5. A-769662

A cell-permeable small organic compound was identified as an effective AMPK activator by screening a chemical library of over 70,000 compounds followed by lead optimization[100]. First found to have beneficial effects on metabolism in *db/db* mice, A-769662 was shown to activate AMPK both allosterically and by inhibiting dephosphorylation of AMPK on Thr[172], analogous to the effects of AMP[101]. Similarly macrophage migration inhibitory factor (MIF), a pleiotropic cytokine that is released by ischemic cardiomyocytes was found to exert cardioprotective effects by activating AMPK[10,12]. While developing small molecule antagonists of the MIF receptor for immunomodulatory applications[102], the compound MIF20 was uncovered that significantly augmented AMPK phosphorylation in the presence of MIF (unpublished data). The clinical benefit of those two compounds however remains largely investigational.

8.6. Statins

HMG-CoA reductase inhibitors have been shown to have cardiovascular benefits in patients with and without diabetes beyond their lipid-lowering effects. Actually the pleiotropic benefits of statins extend to patients without cardiovascular disease and with normal cholesterol levels[103,104]. Statins activate AMPK in the myocardium and endothelial cells by mechanisms that are not fully understood. Up-regulation of eNOS by atorvastatin in endothelial cells of mice without altering AMP:ATP ratio suggests another indirect mechanism of AMPK activation[105]. Rossoni et al.[106] have shown that acute simvastatin-mediated relaxation of healthy endothelial cells of rats was mediated *via* AMPK and eNOS phosphorylation. This effect was totally blunted after adding the AMPK inhibitor, Compound C[106].

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9. AMPK and diabetes

Type 2 diabetes mellitus is a fasting hyperglycemic state of insulin resistance leading to impaired peripheral glucose utilization. A key contributing factor to these abnormalities is the failure of insulin to suppress gluconeogenesis and hepatic glucose production. AMPK activity is thought to be impaired in diabetes as AMPK stimulation in animal models has been shown to improve glycemia. This was also established using AMPK activators other than the antidiabetic drugs: metformin and glitazones[107,108]. Increasing glucose uptake into the cells and suppressing endogenous glucose production and lipolysis suggest AMPK activators as a possible new class of antidiabetic agents.

10. Insulin/AMPK interaction

The use of insulin for ACS seemed logical in 1963 by Sodi-Pallares, with the intention of facilitating potassium flux in the ischemic myocardium, the so-called polarizing therapy. Glucose and potassium were later added to counteract the effects of large dose insulin to become known as GIK therapy[109]. With the identification of the insulin signaling pathway, insulin was found to have comprehensive anti-inflammatory, antioxidant and antiapoptotic properties through PI3K and Akt (RISK pathway) independent of AMPK[110]. Insulin also suppressed the high blood fatty acid levels commonly observed in patients with ACS. Insulin-mediated activation of PI3K-Akt led to activation of endothelial nitric oxide synthase (eNOS) which inhibited mPTP opening in cardiomyocyte mitochondria. Yet the promising non-metabolic effects of insulin failed to translate to significant mortality benefit in a trial comprising 20,000 patients with myocardial infarction (MI)[111]. One possible explanation is the dynamic interaction between insulin and AMPK during ischemia then reperfusion in the heart. Insulin activates Akt which directly phosphorylates AMPKα1/α2 on Ser$^{485/491}$ preventing AMPKK from phosphorylating AMPKα at its primary activation site, Thr^{172}.[112] This is believed to be beneficial as decreased AMPK-mediated oxidation of fatty acids relieved the inhibition of glucose oxidation mainly at the level of pyruvate dehydrogenase. This rechanneled the end products of glycolysis into aerobic respiration with subsequent decrease in the detrimental protons and lactate accumulation. However this cardioprotective effect demonstrated in isolated hearts was abolished in the presence of palmitate, simulating the high free circulating fatty acid levels present during reperfusion[113]; an effect that was observed at an insulin concentration of 100 nM but not, the near-physiological, 0.6 nM. Interestingly palmitate seems to also activate PP2A which dephosphorylates and inactivates AMPK[114]. This appears to be in response to negative feedback by ceramide, a second messenger in the sphingomyelin signaling pathway. Added to that, heparin that is conventionally used in ACS, stimulates lipoprotein lipase increasing further FFA levels and favoring their oxidation. More studies are needed with different levels of insulin to better understand the relationship between anabolic insulin and catabolic AMPK.

11. Non-pharmacological AMPK activators

11.1. Caloric restriction

One independent factor increasing AMPK activity is caloric restriction (CR)[115]. CR being defined as moderate restriction (20–40%) of caloric intake compared to *ad libitum* feeding.

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CR has shown promising results in increasing longevity and reducing the risk factors for cardiovascular disease, diabetes and cancer[116]. The exact mechanism of AMPK activation remains elusive, but the general belief is that CR simulates an energy-deficient state that is sensed by AMPK as an increase in the AMP:ATP ratio. Resveratrol, a small polyphenol present in red grapes, was found to have CR-like metabolic effects primarily through activation of AMPK[117]. While utilizing CR as an inducer of AMPK seems impractical in the setting of acute myocardial infarction, it is nonetheless a weak one. On a clinical note, current medical practices entail that most patients admitted for ACS and planned intervention be in a fasting state to reduce the risk of aspiration. Further research in CR is needed to be able to answer when to resume feeds in those patients and how aggressive we should be controlling glucose levels in the diabetic subgroup.

11.2. Preconditioning and human in vivo model for reperfusion injury

Charles Murry, a fellow then in Duke University laboratory, first demonstrated the principle of preconditioning (PC) in canine hearts in 1986. He reported that subjecting the heart to four cycles of 5-min coronary occlusion/5-min reperfusion would make the heart very resistant to infarction from a subsequent lethal ischemic insult[119]. This seems to be the strongest endogenous protective mechanism of the heart. Later PC was divided into early and late phases, the latter mediated by upregulation of cardioprotective genes by mechanisms that are still unclear. This involved the upregulation of inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX2), aldose reductase, 12-Lipoxygenase (12-LO) and Mn-superoxide dismutase (Mn-SOD) leading to enhanced production of NO and PGI2/PGE2 and removal of oxygen free radicals. One established link is that PC activates AMPK and upregulates GLUT4 expression in a PKC-dependent manner[120]. Others include activation of the RISK pathway and eventual prevention of mPTP formation. The clinical applicability of ischemic PC remains limited because of the obligate need to initiate it before the onset of ischemia which is seldom possible to predict. However, several strategies using AMPK activators have been developed that can be applied before or at the time of reperfusion which mimic ischemic PC.

Worth mentioning is the *in vivo* model of skeletal muscle preconditioning in humans by Rongen et al.[121] from the Netherlands. The value of such experiment is that it was the first one to demonstrate preconditioning in humans with fairly reproducible results. There, ischemia was induced in non-dominant hands by inflating a blood pressure cuff to 200 mmHg followed by intermittent hand gripping till exhaustion for 10 min followed by reperfusion. Annexin A5 scintigraphy was used as a marker for apoptosis. Ischemia–reperfusion injury was then quantified as the percentage difference in uptake between experimental and control hands. Preconditioning was simulated by 10 min of forearm ischemia and a 10-min period of reperfusion without simultaneous exercise. Pharmacological preconditioning was done with intra-arterial adenosine infusion with similar results. Using the same model in subjects with metabolic syndrome, Rongen et al.[122] have demonstrated improved reperfusion injury in a crossover study of 4 mg bid of AMPK inducer rosiglitazone *vs*. placebo for 8 weeks.

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Numerous papers have been published worldwide showing promising results regarding utilizing AMPK phosphorylation in ameliorating ischemia/reperfusion injury on various animal models[10,14,31,64]. Only a handful of these have translated to human research. This in part is due to variations in the protocols used and reproducibility of the published results. Some even argue that using animal models is misleading with the conviction that human myocardium is biochemically more tolerant to ischemia than animal models. If 20 min of total occlusion is how long it takes for irreversible myocardial injury to occur in humans, how much time of acute occlusion should be simulated in disease-free coronaries with no collaterals of mice, rabbits or pigs corrected for their relative metabolisms? This has lead to the Consortium for preclinicAl assESsment of cARdioprotective therapies (CAESAR), an initiative from the NHLBI aimed at the preclinical research[118]. The plan of which is to perform systematic preclinical testing of cardioprotective therapies using standardized protocols performed by blinded investigators and analyzed by a single statistical core as done for randomized, multicenter clinical trials. Pharmacological agents showing consistent results and statistical power will be promoted to clinical trials.

12. Conclusions

The actions of activated AMPK previously described have generated many questions regarding its potential role in cardiovascular disease. AMPK activation during myocardial ischemia might indeed protect the heart against injury. Additional tremendous applications can include adjuvant therapy to cardiac bypass surgery, protecting donor hearts before transplant and treatment of acute ischemic strokes. We now know that activated AMPK effectively preconditions the heart, improves glucose uptake, glycoysis and fatty acid oxidation to provide ATP. It also stimulates autophagy and inhibits cholesterol and glycogen synthesis to conserve energy during ischemic stress. Other benefits to AMPK activation during ischemia extend beyond cardiomyocytes to improve endothelial metabolism, function and angiogenesis. How much of this response is beneficial and how much is harmful is not clear. How is the cellular response to physiological stress different from infarction? To what degree can we extrapolate the cellular responses in physiological stress situations as exercise and caloric restriction to pathological situations as myocardial infarction without making it harmful? Since AMPK is rapidly inactivated during reperfusion, sustained iatrogenic AMPK activation may prove detrimental. Continued ‘switched on’ autophagy would eventually lead to cell death. Favoring fatty acid over glucose oxidation would exaggerate the uncoupled ATP production from glycolysis leading to increased proton and lactate production, cardiac inefficiency and contractile dysfunction.

If one may formulate a drug for the treatment of myocardial infarction with our current understanding of cellular metabolism, it would be a single-use drug that can be given within the first hour of suspected ACS. It would have the same potency to directly activate AMPK as Compound A-769662, with a similar safety and bioavailability as metformin. It would be a drug selective to AMPK unlike AICAR which seems to inhibit autophagy through other pathways, but has a similar half-life as to not persist long after reperfusion. It would be one that does not cross the blood brain barrier as to not affect hypothalamic AMP-activated protein kinase. And finally it would be a drug that allows fatty acid and glucose oxidation to go hand in hand like perhaps an AMPK activator and just the right amount of insulin.

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Ultimately, manufacturing such a drug for clinical use must involve our pharmaceutical friends which, understandably are not excited about this as clinicians are. With the survival rate for U.S. patients hospitalized with MI being approximately 95% using conventional therapy, pharmaceutical companies are less interested in investing into a new single-use drug that must be administered in a timely fashion prior to reperfusion. The market for chronic treatments to survivors of MI is much broader and much more lucrative. Furthermore, enthusiasm for development of such a drug with so many downstream effects would be dampened by the required expense of the clinical trials that would be necessary to obtain approval. Hopefully with the amount of research being invested in cardiac cell metabolism and the CAESAR initiative the pharmaceutical companies will soon pick up the scent.

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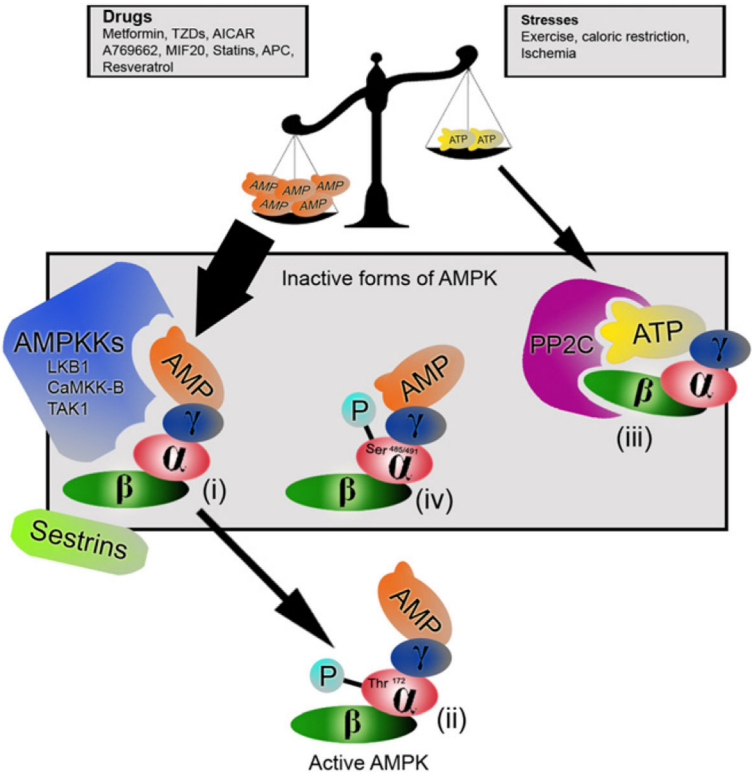

Figure 1.

Schematic representation of the different forms of AMPK. Increases in AMP relative to ATP, sensed as a low energy state, allows AMPKK to phosphorylate Thr^{172} and activate AMPK at the N-terminal of the α subunit (i). This occurs in situations of stress and may be simulated by different pharmacological agents. The active form of AMPK (ii) is now available to lead the myriad of changes in cellular metabolism. Abundance of ATP, as in high energy states, allows ATP to bind to AMPK causing a conformational change that renders it vulnerable to degrading phosphatases (iii). Phosphorylation of AMPK by Akt at $Ser^{485/491}$ in response to insulin causes another conformational change that prevents AMPKKs from binding and activating AMPK (iv).

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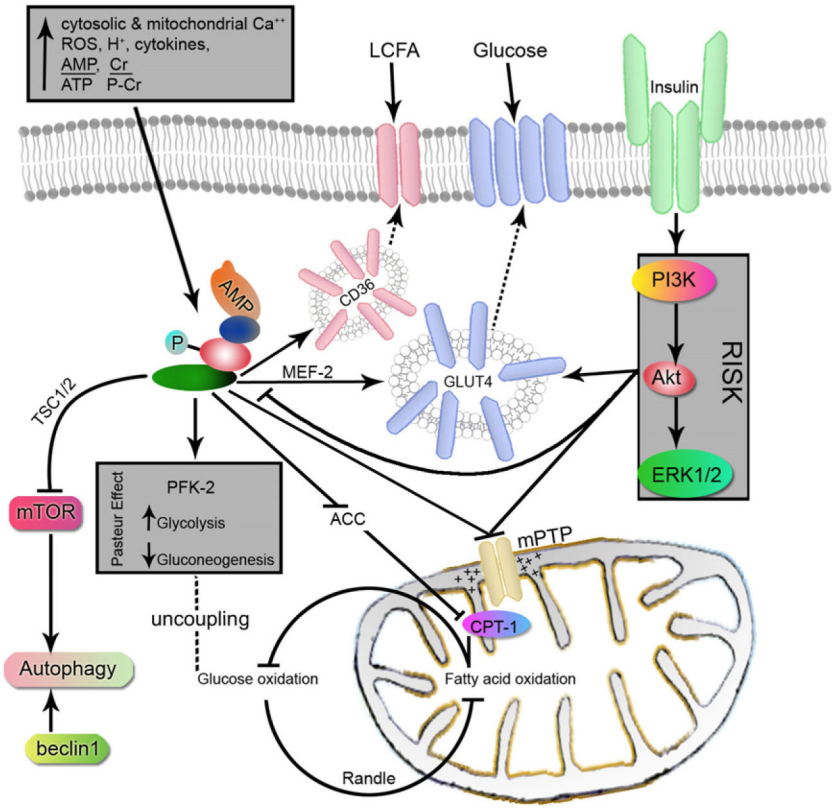

Figure 2.

Illustration of the role of AMPK during reperfusion. AMPK activation in early reperfusion increases the uptake of glucose and long chain fatty acids (LCFA) into the cell. This coincides with increased anaerobic glycolysis and fatty acid oxidation for ATP production. In the absence of insulin, fatty acids are preferred over glucose for oxidation resulting in the uncoupling of end products of glycolysis. Both AMPK and RISK function to prevent the opening of mPTP and loss of the electrochemical gradient within the mitochondria. Autophagy is turned on initially by AMPK inhibition of mTOR and later by beclin 1.