Published in final edited form as:

J Neurosci Res. 2013 August ; 91(8): 1018–1029. doi:10.1002/jnr.23207.

Function of the Master Energy Regulator Adenosine Monophosphate-Activated Protein Kinase in Stroke

Bharti Manwani¹ and Louise D. McCullough^{1,2,★}

¹Department of Neuroscience, University of Connecticut Health Center, Farmington, Connecticut

²Department of Neurology, University of Connecticut Health Center, Farmington, Connecticut

Abstract

Adenosine monophosphate-activated protein kinase (AMPK) is an evolutionarily conserved signaling molecule that is emerging as one of the most important energy sensors in the body. AMPK monitors cellular energy status and is activated via phosphorylation when energy stores are low. This allows for maintenance of energy homeostasis by promoting catabolic pathways for ATP production and limiting processes that consume ATP. Growing number of stimuli have been shown to activate AMPK, and AMPK has been implicated in many diverse biological processes, including cell polarity, autophagy, and senescence. The effect of AMPK activation and its biological functions are extremely diverse and depend on both the overall energy “milieu” and the location and duration of activation. AMPK has tissue- and isoform-specific functions in the brain vs. periphery. These functions and the pathways activated also appear to differ by cell location (hypothalamus vs. cortex), cell type (astrocyte vs. neuron), and duration of exposure. Short bursts of AMPK activation have been found to be involved in ischemic preconditioning and neuronal survival; however, prolonged AMPK activity during ischemia leads to neuronal cell death. AMPK may also underlie some of the beneficial effects of hypothermia, a potential therapy for ischemic brain injury. This review discusses the role of AMPK in ischemic stroke, a condition of severe energy depletion.

Keywords

AMPK; adenosine monophosphate-activated protein kinase; energy regulation; ischemic stroke; hypothermia; metformin; compound C; preconditioning; astrocyte; neuron

The dynamics of metabolic processes in any living organism revolve around adenosine-5'-triphosphate (ATP), the energy currency molecule of the cell (Knowles, 1980). Anabolic processes utilize ATP (endergonic) to build cellular machinery from small molecules, and catabolic processes produce ATP (exergonic). Energy utilization leads to the hydrolysis of one or two high-energy phosphate bonds in ATP, leading to the formation of adenosine diphosphate (ADP) or adenosine monophosphate (AMP), respectively, and these reaction products are interconvertible. Therefore, a high ADP/ATP or AMP/ATP ratio implies falling

energy stores, leading to signals that activate pathways to restore energy homeostasis. Functioning of an organism depends on the maintenance of this delicate energy homeostasis.

Molecules that can sense the disruption of energy balance are critical to survival (Hardie et al., 2012). AMP-activated protein kinase (AMPK), a serine threonine kinase, is emerging as a critical energy monitor for many organisms, from yeast to mammals (Carling et al., 2012). It is the master energy sensor that not only senses but also controls and responds to altered energy status in most eukaryotic cells (Stein et al., 2000). AMPK is activated through phosphorylation at threonine172 (Thr¹⁷²; Hawley et al., 1996) under conditions of metabolic stress, whether physiological (exercise) or pathological (hypoxia, starvation). Activation of AMPK via phosphorylation by upstream kinases (see below) leads to the restoration of energy balance by activation of catabolic pathways that produce ATP (fatty acid oxidation, glycolysis, protein breakdown) and inhibition of anabolic pathways that use energy (fatty acid synthesis, cholesterol, triglyceride synthesis; Steinberg and Kemp, 2009).

AMPK stands at the crossroads of various metabolic processes and is involved in the metabolic changes that occur with aging, obesity, cell death, and cell survival. It is increasingly recognized that AMPK activation can lead to different outcomes depending on the physiological status of the organism, the severity of the stressor, and the tissue examined. For example, AMPK activation can protect the heart, endothelial cells, and smooth muscle cells (Xu and Si, 2010) under certain stress conditions. However, AMPK activation enhances neuronal death in several models of acute and chronic neurodegeneration, including stroke and Huntington's disease (Ju et al., 2011). This review focuses on the role of AMPK in experimental ischemic stroke.

AMPK SUBUNITS, EXPRESSION, AND FUNCTION IN THE BRAIN

AMPK is a heterotrimeric complex comprised of three subunits, $\alpha\beta\gamma$; α is the catalytic subunit, β and γ are regulatory subunits (Kahn et al., 2005). The α subunit has two isoforms $\alpha1$, $\alpha2$; the β subunit has two isoforms, $\beta1$, $\beta2$; and the γ subunit has three isoforms, $\gamma1$, $\gamma2$, and $\gamma3$ (Stapleton et al., 1996). Each subunit has specific expression patterns in CNS, and different combinations of subunits may be important for the heterogeneity of AMPK function in different brain areas or cell types. The $\alpha2$ catalytic subunit is highly expressed in neurons and activated astrocytes, whereas the $\alpha1$ subunit is expressed at low levels throughout the neuropil (Turnley et al., 1999). The γ subunits are found primarily in neurons, except for the $\gamma2$ isoform, which is expressed in Bergmann glia. Several AMPK subunits are expressed in astrocytes, including the $\alpha2$, $\beta2$, and to a lesser extent $\beta1$. The $\alpha1$ subunit is not expressed in astrocytes, a key cell type involved in the response to ischemia (see below), which implies that some specialization of AMPK function can occur based on subunit expression. Interestingly, in neurons, subunits $\alpha2$, $\beta1$, and $\gamma1$ are found primarily in the nucleus, whereas $\alpha1$, $\beta2$, $\gamma2$ have a cytoplasmic distribution, suggesting that the former may be more involved in gene transcription and regulation (Turnley et al., 1999; Li and McCullough, 2010). However, there is evidence that, in Huntington's disease (HD), the $\alpha1$ subunit can translocate to the nucleus and contribute to mutant huntingtin (mHtt)-induced cell death (Ju et al., 2011). This study by Ju et al. 2011 provides insight into a novel

mechanism by which specific isoforms of AMPK mediate the pathogenesis of brain diseases. We review the function of each of these isoforms specifically in the brain.

The AMPK α -subunit contains the catalytic domain and the residue Thr¹⁷² on the N-terminus. The C-terminal domain is important for interacting with the regulatory β and γ subunits (Suter et al., 2006; Zaha and Young, 2012). A complete knockout of the catalytic subunit α leads to embryonic lethality in mice (Viollet et al., 2009). AMPK α 1/ α 2 double conditional knockout mice show normal cortical neuronal development, suggesting that AMPK catalytic activity is not required constitutively at least for neurogenesis in early life (Williams et al., 2011). However, this phenotype changes under conditions of metabolic stress (discussed below). The AMPK α 2 isoform of the catalytic subunit is induced by hypoxia and promotes VEGF expression in human glioblastoma cell lines (Neurath et al., 2006). AMPK α 2 also modulates the activity of the autonomic nervous system, insofar as AMPK α 2 knockout mice (but not AMPK α 1 knockout mice) have increased catecholamine urinary excretion and increased adrenergic tone (Viollet et al., 2003). Genetic deletion of the α 2 isoform leads to neuroprotection in acute experimental stroke models (see below), demonstrating that AMPK α 2 is critical in the response to an ischemic challenge (Li et al., 2007).

Activation of AMPK α 1, the other catalytic isoform, has been seen in striatal neurons of both humans and mice with HD (Ju et al., 2011). Overactivation of AMPK in the striatum led to brain atrophy and increased neuronal loss in a transgenic mouse model of HD. Prevention of nuclear translocation or inactivation of AMPK α 1 reduces cell death (Ju et al., 2011) and has recently been shown to protect against diet-induced obesity and insulin resistance (Zhang et al., 2012). In contrast, experimental models of ischemic stroke have shown no protection in AMPK α 1 knock out mice. (Li et al., 2007). Thus, the α 1 isoform may be important in more chronic neurodegenerative disease models but not in models of acute ischemic stress. However, studies focused on more chronic assessment of poststroke angiogenesis and functional recovery are lacking in both α 1 and α 2 knockout mice. Further research is required to determine if α 1 subunit plays a role in other models of ischemic stroke (i.e., permanent occlusion), has a nuclear translocation phase or is involved in poststroke recovery and repair.

The β regulatory subunits possess a glycogen-binding region (GBR) on the N-terminus and also modulate AMPK activity (Hudson et al., 2003; Polekhina et al., 2003; McBride et al., 2009). The C-terminus of β subunits bears a binding domain that stabilizes the interaction between the α and the γ subunits (Woods et al., 1996; Iseli et al., 2005). Deficiency of the AMPK β subunit in the *Drosophila* eye causes increased autophagy and neurodegeneration in the photoreceptor neurons of retina (Poels et al., 2012). In murine models, it was demonstrated that AMPK β 1 is critical for normal neurogenesis and neuronal differentiation (Dasgupta and Milbrandt, 2009). However, another study (Dzamko et al., 2010) failed to find any brain developmental defects in AMPK β 1 knockout mice. Thus, at this time, the functional significance of the β subunit in neurogenesis and neuronal survival is controversial, and its role in other neurological diseases is unknown.

The γ subunits contain four repeating cystathionine- β -synthase (CBS) units; two CBS units constitute one Bateman domain, which serves as a regulatory AMP- and ATP-binding site within the AMPK complex (Sanders et al., 2007; Xiao et al., 2007). The role and function of this subunit in brain pathologies is yet to be explored.

REGULATION OF AMPK

AMPK activation requires phosphorylation at Thr¹⁷², which lies in the activation segment of the N-terminal domain in the α subunit (Stein et al., 2000). Phosphorylation at the Thr¹⁷² site is regulated by relative activity of upstream kinases, such as liver kinase B1 (LKB1), Ca²⁺/calmodulin-dependent protein kinase kinase β (CaMKK β), and transforming growth factor- β -activated kinase 1 (TAK1), and by protein phosphatases. LKB1 (also known as serine-threonine kinase 11) is the Peutz-Jeghers syndrome tumor suppressor kinase and is required for activation of AMPK in response to stress (Hemminki et al., 1998; Xie et al., 2009). LKB1 appears to be the predominant regulator of AMPK activation insofar as its deletion abolishes AMPK activity in different cell types and tissues (Carling, 2004; Weisova et al., 2012b). In *C. elegans* and in ischemic mouse heart, AMPK α 2 activation has been shown to be dependent on LKB1 (Lee et al., 2008). It may also be of primary importance in the ischemic brain, as whole-brain homogenates from middle cerebral artery occlusion (MCAO) mice show increased phosphorylation of LKB1 (Li et al., 2007).

The activation of AMPK by CaMKK β occurs by increases in intracellular stores of Ca²⁺ and not by changes in the AMP/ATP ratio (Hawley et al., 2005; Hurley et al., 2005). AMPK α 2 activation by CaMKK β has been known to function in the hypothalamus in controlling food intake (Anderson et al., 2008). TAK1, a member of the mitogen-activated protein kinase family, has also been shown to activate AMPK in yeast (Momcilovic et al., 2006). In stroke models, it has been demonstrated that short-term inhibition of TAK1 is protective both in vitro (oxygen glucose deprivation; Neubert et al., 2011) and in vivo (MCAO), but this effect may be independent of AMPK activation (White et al., 2012).

Optimal activity of AMPK is induced when the AMP/ATP level is high and when two AMP molecules bind in the Bateman domains in the γ subunit. This AMP binding induces an allosteric change in the AMPK complex, exposing the active site (Thr¹⁷²) on the α subunit (Cheung et al., 2000). This site can then be phosphorylated by an upstream kinase to induce AMPK activity (Oakhill et al., 2010). The binding of AMP to the γ subunit of AMPK also inhibits the dephosphorylation of Thr¹⁷² by PP2C α (protein phosphatase; Sanders et al., 2007) or PP2A (Wu et al., 2007), prolonging AMPK in its active state. The level of this AMP-mediated allosteric AMPK activation is dependent on the presence of specific isoforms of both α and γ subunits in the AMPK complex; the greatest activation occurs in complexes having α 2 and γ 2 isoforms (Cheung et al., 2000). Recent studies examining the crystal structure of active AMPK complex, have shown that ADP protects AMPK from dephosphorylation after binding to one of the two exchangeable AXP (AMP/ADP/ATP) sites in the γ subunit regulatory domain (Xiao et al., 2011). Thus, ADP, like AMP binds to γ 1 and γ 3 sites but does not cause direct activation of the AMPK Thr¹⁷² site (Oakhill et al., 2011). Tables I and II review the role of AMPK in different pathologies affecting the human brain. The remainder of this review focuses on the role of AMPK in cerebral ischemia.

AMPK IN STROKE

Stroke is the third leading cause of death in the United States and the most common cause of disability (Rosamond et al., 2008). Ischemic stroke leads to a cascade of oxidative and cell death pathways, leading to cellular energy failure. This energy imbalance leads to an increase in phosphorylated AMPK (pAMPK; the active form of the kinase) in an attempt to restore ATP levels in the ischemic brain. pAMPK enhances glycolysis, inhibits glycogen synthesis, increases glucose transport, and enhances fatty acid oxidation to produce energy for the cell (Almeida et al., 2004; Ronnett et al., 2009). However, whether AMPK activation is beneficial or detrimental depends on many factors, and conflicting results have led to a considerable debate in literature. Some of this confusion comes from the models utilized, many of which have focused on the effects of injury in culture systems in which only one cell type is examined and conditions are far from physiological. It has been observed that maintaining the physiological glucose (Kleman et al., 2008) and oxygen levels (Zhu et al., 2011) in culture medium makes AMPK more responsive to metabolic changes. AMPK activation with 5 aminoimidazole-4-carboxamide ribonucleoside (AICAR) increases survival in cultured embryonic rat hippocampal neurons subjected to glucose deprivation, chemical hypoxia, or glutamate exposure (Culmsee et al., 2001). Inhibition of AMPK by compound C decreases viability of primary cortical neuronal cultures under physiological oxygen and glucose conditions (Zhu et al., 2011). Similarly, a recent study shows that AMPK inhibition by compound C increases oxygen glucose deprivation (OGD) induced neuronal injury in pure neuronal cultures, whereas AMPK activation by AICAR decreases it (Wang et al., 2011). On the other hand, several other studies have demonstrated that AMPK activation is detrimental. It has been shown that AMPK activation leads to neuronal apoptosis, as in human undifferentiated neuroblastoma cells SH-SY5Y exposed to AICAR (Garcia-Gil et al., 2003) or cortical neurons exposed to the neurotoxin tributyltin (Nakatsu et al., 2008).

Increasing recognition of the concept that the effects of AMPK are dependent on the duration and extent of its activation, may also explain some of these divergent results. A positive correlation has been found in pAMPK levels induced by AICAR and cell death in hypoxic neurons, suggesting that cell death depends on the extent of AMPK activation (Zhang et al., 2010). AMPK overactivation not only enhances cell death but also affects axonal polarization and repair. Studies in cortical neuronal cultures show that AMPK overactivation inhibits axogenesis under conditions of metabolic stress (simulated by AICAR or metformin treatments; Williams et al., 2011). The mechanism of this has been recently explored. AMPK activation leads to phosphorylation of the kinesin light chain of the Kif5 neurite motor protein, causing a disruption of its association with phosphatidylinositol 3-kinase (PI3K; Amato et al., 2011). Thus, PI3K cannot target the axonal tip, and axonal polarization and growth are inhibited (Amato et al., 2011). It is possible that AMPK activation also inhibits axogenesis and repair after an ischemic event. Future studies aimed at identifying the more chronic effects of AMPK activation on axogenesis in animal models of ischemic stroke are required.

However, the effect of AMPK manipulation in vivo, when astrocytes and neurons are in close proximity and work cooperatively to maintain metabolism in an “in vivo” setting, may

differ. In vivo studies using a transient MCAO model in mice have shown that AMPK activation is detrimental, at least when damage is assessed acutely (days after stroke). AICAR administration in mice increases injury in an MCAO reperfusion model, and pharmacological inhibition of AMPK using C75 (which modulates neuronal ATP levels) or compound C (an AMPK inhibitor) reduces injury (McCullough et al., 2005), demonstrating that acute AMPK inhibition is protective in stroke. These studies examined AMPK signaling after severe, prolonged ischemia and reperfusion. It is increasingly clear that this initial AMPK activation is an attempt to restore homeostasis in the ischemic brain. However, after cell death begins, further attempts to produce ATP from cells that are metabolically “damaged” leads only to an exacerbation of injury and further metabolic dysfunction. It should be noted that pharmacological activation of AMPK with drugs such as AICAR may lead to off-target effects (Weisova et al., 2011). AICAR has both low specificity and low potency (Arad et al., 2007), so more conclusive studies using genetic models have also been pursued. These studies have further clarified the role of AMPK in ischemic stroke.

AMPK Gene Deletion Models in Stroke

Examination of mouse models with deletion of either the $\alpha 1$ or $\alpha 2$ catalytic subunit of AMPK has further elucidated the role of AMPK in cerebral ischemia, although these models also have limitations. As mentioned above, a complete knockout of α subunit is embryonically lethal (Viollet et al., 2009). AMPK $\alpha 2$ knockout mice have less injury compared with wildtype littermates in an MCAO reperfusion model (Li et al., 2007), whereas no difference in injury was seen in $\alpha 1$ knockout mice (Li et al., 2007). This ischemic protection seen in AMPK $\alpha 2$ knockout mice has been also found in other studies (Venna et al., 2011; White et al., 2012). Administration of compound C (an AMPK inhibitor) decreased injury in wild-type mice but not in $\alpha 2$ knockout mice, suggesting that the detrimental effects of AMPK in stroke are mediated by the $\alpha 2$ subunit (Li et al., 2007). However, another group has demonstrated increased infarct volumes in AMPK $\alpha 2$ knockout mice compared with wild type, possibly resulting from the use of a more prolonged ischemic insult (120 min of MCAO; Wang et al., 2011).

Studies utilizing the AMPK activator metformin in AMPK knockout mice have further highlighted the complexity of AMPK signaling. Metformin, a biguanide used in type 2 diabetes mellitus treatment (Smiley and Umpierrez, 2007), has been shown to decrease the risk of cardiovascular disease by 36% in clinical populations (UKPDS, 1998), suggesting that chronic AMPK activation can be beneficial for disease prevention. We investigated whether treatment with metformin prior to an ischemic event led to improved stroke outcomes. Surprisingly, metformin treatment for 3 days (acute treatment) prior to an induced stroke (MCAO model) led to an enhancement of injury, increased AMPK activation, and exacerbated stroke-induced lactic acidosis (Li et al., 2010). The deleterious effects of acute metformin treatment were abolished in AMPK $\alpha 2$ knockout mice, confirming that metformin acts through AMPK pathways. These results are consistent with the hypothesis that AMPK activation is detrimental in stroke but question the mechanism leading to its benefits for stroke prevention in clinical populations. When the duration of treatment was changed from an acute to a chronic regimen (3 weeks prior to an induced stroke), a significant decrease in ischemic injury was seen. Interestingly, it was found that chronic

treatment with metformin led to significant downregulation of stroke-induced pAMPK levels, reduced lactate formation, and improved infarct damage (Li et al., 2010). This work suggests that the duration and chronicity of AMPK activation (acute vs. chronic) are critical determinants of stroke outcome.

Hypothermia is neuroprotective after experimental ischemia (Zhao et al., 2007), likely by reducing metabolic demand. In the MCAO model in mice, 6 hr of hypothermia led to a significant reduction in infarct volume (Li et al., 2011) and also a reduction in pAMPK in the brain (Li et al., 2011). The neuroprotective effect of hypothermia was mediated by the reduction in stroke-induced pAMPK levels, because both pharmacological inhibition (with compound C) and AMPK α 2 gene deletion provided no reduction in injury from hypothermia (beyond that of loss of AMPK alone). If hypothermia-induced protection was mediated by a non-AMPK mechanism, an additive effect (enhanced protection) would be expected (Li et al., 2011). However, these results must be interpreted cautiously, because a “floor” effect may occur when there is less injury, reducing the chance of observing meaningful differences in infarct volume. Interestingly, mice given compound C spontaneously developed hypothermia, suggesting that a reduction in the stimulus that signals metabolic demand (low pAMPK levels) leads to hypothermia. This may have clinical relevance, in that both hypothermia and AMPK inhibition decrease neuronal metabolism, potentially prolonging the time window for reperfusion therapy.

On the other hand, other studies have suggested that AMPK activation (Kuramoto et al., 2007) may be beneficial during ischemic injury. Stroke-induced AMPK activation led to the phosphorylation of serine783 in the R2 subunit of the GABA_B receptor, enhancing slow synaptic inhibition by inwardly rectifying K⁺ channels. It has been proposed that this caused a reduction in excitotoxic damage leading to enhanced neuronal survival. It is worth noting that injury-induced phosphorylation of GABA was seen mainly in the hippocampal region, which is not usually injured with occlusion of the MCA. Ongoing studies should clarify these disparate results.

Cell-Type-Specific Effects in Stroke

In the classical view, under normal conditions (normoxia and glucose availability), neurons utilize predominantly glucose as their main energy substrate via oxidative metabolism (Chih and Roberts, 2003; Pellerin and Magistretti, 2012) rather than obtaining energy through glycolysis (for a discussion of energy substrates utilized by neurons see Pellerin et al., 2007; Genc et al., 2011; Pellerin and Magistretti, 2012). According to the “astrocyte–neuron lactate shuttle (ANLS)” model, astrocytes utilize glucose predominantly via anaerobic glycolysis to produce pyruvate and lactate (Chih and Roberts, 2003; Pellerin and Magistretti, 2012). This lactate is taken up by the neuron for further oxidative degradation and production of ATP. However, under conditions of hypoxia and hypoglycemia, oxidative metabolism ceases, and differential metabolic activity is induced in neurons and astrocytes (Genc et al., 2011; Pellerin and Magistretti, 2012). AMPK is activated in an attempt to restore energy balance, induced by rising AMP levels. Phosphorylation of AMPK leads to an increase in glycolysis, inhibition of glycogen synthesis, enhancement of glucose transport (increasing glucose availability), and increased fatty acid oxidation (Almeida et al., 2004;

Ronnett et al., 2009). Under such stress conditions, neurons (unlike astrocytes) are unable to upregulate glycolysis, because they virtually lack the enzyme 6-phosphofructo-2-kinase (PFK2) that synthesizes fructose-2,6-bisphosphate (F2,6P2; Almeida et al., 2004; Bolanos et al., 2010). F2,6P2 is a powerful allosteric activator of PFK1, the rate-limiting enzyme of glycolysis (Almeida et al., 2004). Although both neurons and astrocytes have PFK1, constant proteasomal degradation of PFK2 by anaphase-promoting complex/cyclosome (APC/C)-Cdh1 in neurons leads to a lower glycolytic rate in neurons than in astrocytes (Herrero-Mendez et al., 2009; Bolanos et al., 2010). Moreover, neurons have minimal glycogen stores and do not oxidize fatty acids efficiently, making them more sensitive to ischemic damage (Vilchez et al., 2007; Li and McCullough, 2010).

Astrocytes have low (APC/C)-Cdh1 activity, leading to higher PFK2 enzyme activity and an enhanced glycolysis rate under stress conditions compared with neurons (Herrero-Mendez et al., 2009; Bolanos et al., 2010). Astrocytes store and utilize glycogen and efficiently oxidize fatty acids to produce ATP (Fig. 1). It has been shown that enhanced astrocyte energy production promotes neuronal survival (Pellerin, 2005). Thus, it appears that astrocytes (unlike neurons) are equipped to enhance energy production via AMPK signaling mechanisms, even under conditions of energy failure and ischemic stress. AMPK activation would then be expected to enhance astrocytic metabolism and survival, and thus provide a better supportive environment for neurons via enhancement of lactate. It has also been suggested that the lower glycolytic rate in neurons can divert glucose-6-phosphate (G6P) through the pentose phosphate pathway (PPP) and can generate antioxidants, promoting survival (Bolanos et al., 2010). Thus, the importance of examining the effects of loss of AMPK selectively in astrocytes or neurons is clear. Separating the unique features and effects of AMPK signaling in neurons or astrocytes was previously impossible in “in vivo” models. To address this, cell-type-specific knockouts utilizing a floxed AMPK α 2 parental line (Zou and Wu, 2008) have been generated. Preliminary data show that cell-specific deletion of astrocytic AMPK is deleterious after injury, leading to enhanced edema, increased infarct volumes, poorer behavioral outcomes, and enhanced hemorrhagic transformation, suggesting that astrocytic AMPK may protect the brain from ischemic injury (unpublished results). The effect of neuronal specific deletion of AMPK after stroke is under investigation.

In endothelial cells, hypoxia also leads to AMPK activation (Nagata et al., 2003), but, in contrast to what is seen in neurons, AMPK activation is protective in endothelial cells (Zou and Wu, 2008). The protective effects of AMPK in endothelial cells may be directly due to an enhancement in endothelial NO synthase (eNOS) and acute vasodilatation (Osuka et al., 2009). AMPK activation is also specifically required for endothelial cell migration and differentiation under conditions of hypoxia (Nagata et al., 2003; Stahmann et al., 2010). Post-stroke angiogenesis is required for repair and recovery. This specific effect on endothelial cells suggests that the timing of administration of AMPK inhibitors (which are neuroprotective in animal models), will be critical, because poststroke repair must be maintained (Stahmann et al., 2010; Su et al., 2012).

Ischemic stroke is also a powerful stimulus for influx of inflammatory cells in the brain (Kamel and Iadecola, 2012), and this inflammatory response is a critical determinant of

stroke outcome. Poststroke inflammation is an attractive therapeutic target as the peak of inflammation occurs many hours to days after injury (Dirnagl et al., 1999). AMPK activation inhibits inflammation in acute and chronic colitis (Bai et al., 2010), experimental allergic encephalomyelitis (EAE; an animal model of multiple sclerosis; Nath et al., 2005) and cystic fibrosis (Myerburg et al., 2010). AMPK can also influence T-cell development, homeostasis, and effector function (Blagih et al., 2012), suggesting that AMPK signaling also has unique effects on inflammatory cells. The role of AMPK in stroke-induced inflammation has not yet been explored. Future studies utilizing myeloid-specific knockout models or bone marrow chimeras will be required to understand more fully the role of AMPK in the immune system after stroke.

AMPK AND ISCHEMIC PRECONDITIONING

Ischemic preconditioning is a phenomenon by which exposure to brief, sublethal ischemia stimulus leads to protection of the organism from subsequent severe ischemic insults (Dirnagl et al., 2009). At this injury threshold, activation of a neuroprotective “program” (Stenzel-Poore et al., 2007) leads to the development of tolerance and resistance to future injurious stimuli. The protective effects of preconditioning have been linked to AMPK activity. Brief glutamate exposure (excitotoxic injury) led to AMPK activation and translocation of glucose transporter 3 (GLUT3) to the plasma membrane (Weisova et al., 2009) in cerebellar granule cells. A hyperpolarization of mitochondrial membrane potential (Ψ_m) was also seen (Ward et al., 2007). Ψ_m is critical to the maintenance of mitochondrial Ca^{2+} homeostasis. Excess calcium leads to loss of both mitochondrial energetics and neuronal integrity (Luetjens et al., 2000; Ward et al., 2000, 2006, 2007). Silencing the GLUT3 gene reduced the Ψ_m and sensitized the neurons to excitotoxic injury (Weisova et al., 2009), demonstrating that regulation of GLUT3 expression by AMPK mediates this tolerance to glutamate excitation.

A mild mitochondrial uncoupling stimulus can also lead to neuronal preconditioning (Atlante et al., 1996) as long as the stress remains “sublethal.” A recent report suggests that this mild mitochondrial uncoupling also leads to mitochondrial AMPK activation, GLUT3 translocation, and subsequent hyperpolarization of Ψ_m (Weisova et al., 2012a). An important weakness in these in vitro studies is that GLUT3 activation and the subsequent rapid increase in glucose during reperfusion can lead to significant neuronal hyperglycemia, an increase in injury or enhanced hemorrhagic transformation rates in vivo (Berger and Hakim, 1986; Tomlinson and Gardiner, 2008).

Recent work in vivo demonstrated that ischemic preconditioning (induced by a brief, 3-min ischemia 72 hr prior to stroke; IPC) leads to neuroprotection (Venna et al., 2011). These beneficial effects occurred in parallel with a significant inhibition of pAMPK protein expression. Although both pharmacological inhibition of AMPK or IPC led to neuroprotection, IPC offered no additional protective effects when coadministered with an AMPK inhibitor. Pharmacological activation of AMPK with metformin abolished the neuroprotective effects of IPC, and AMPK- $\alpha 2$ null mice failed to demonstrate a preconditioning response.

It is becoming evident that brief periods of AMPK activation enhance neuronal survival, whereas sustained activation of AMPK induces cell death. Prolonged AMPK activation activates the proapoptotic protein Bcl-2 homology domain 3 (BH3)-only protein (Bim; Concannon et al., 2010). Interestingly, activation of Bim during excitotoxic injury not only requires dephosphorylation and nuclear translocation of the Forkhead transcription factor FOXO3, but also a second activation step in which FOXO3 in the nucleus has to be directly phosphorylated by AMPK (Davila et al., 2012). FOXO3 has been shown to promote resistance to oxidative stress in *C. elegans* (Greer et al., 2007a,b). Thus, short-term AMPK exposure causes only nuclear translocation of FOXO3, preconditioning, and cell survival. However, prolonged pAMPK activity stimulates the second activation step of FOXO3, leading to Bim expression and cell death. It is being proposed that this second FOXO3 activation step can be the molecular switch from cell survival to cell death.

AMPK AND AUTOPHAGY

Energy failure after cerebral ischemia leads to cell death by necrosis and a delayed cell death by apoptosis. Another cell death mechanism called autophagy, is also induced in the ischemic brain (Puyal et al., 2009). Autophagy allows cells to produce energy during periods of nutrient starvation via engulfment of cytoplasmic material and organelles and allows recycling of amino acid and other nutrients. AMPK induces autophagy in response to energy need (Hardie, 2011; Kim et al., 2011) by inactivation of target of rapamycin complex-1 (TORC1) and direct phosphorylation of the protein kinase mammalian autophagy initiating kinase (ULK1; Hardie, 2010; Egan et al., 2011; Kim et al., 2011). Energy depletion in *Drosophila* AMPK mutants (β -AMPK, *alc* mutants) has been shown to result in an increase in autophagy and promote neurodegeneration (Poels et al., 2012). Whether AMPK stimulated autophagy occurs in stroke is not known. Future studies are needed to investigate the contribution of autophagy to infarct damage after cerebral ischemia.

CONCLUSIONS AND FUTURE DIRECTIONS

AMPK is a major energy sensor in the brain. Emerging work suggests that AMPK signaling plays an important role in stroke. AMPK activation may have differential effects in neurons vs. astrocytes, and the effects of AMPK may be cell type specific in the setting of cerebral ischemia. Future studies utilizing cell-type-specific (astrocyte/ neuron) knockout mice should address these gaps in our knowledge. Over the past 5 years, the early conflicting results from in vivo and in vitro work have been clarified. It is increasingly evident that AMPK activation can be both helpful and harmful in the ischemic brain, and the duration of exposure is key to outcome. Data from preconditioning studies suggests that transient AMPK activation may act as a sublethal stimulus, similar to caloric restriction or mild mitochondrial uncoupling, protecting the cell from subsequent stressors. In this regard, the adage “what doesn’t kill you makes you stronger” is especially relevant. Our understanding of AMPK in stroke is still in its infancy. After years of research, the only FDA-approved treatment for stroke is the thrombolytic tissue plasminogen activator. Novel approaches for stroke therapy are urgently required. Because ischemic stroke is a condition of severe energy depletion and AMPK is the master energy regulator, it may be a promising target for future development of stroke therapies.

Acknowledgments

Contract grant sponsor: NIH; Contract grant number: 11PRE7440068 (to B.M.); Contract grant number: R01-NS055215-06A1 (to L.D.M.).

References

- Almeida A, Moncada S, Bolanos JP. Nitric oxide switches on glycolysis through the AMP protein kinase and 6-phosphofructo-2-kinase pathway. *Nat Cell Biol.* 2004; 6:45–51. [PubMed: 14688792]
- Amato S, Liu X, Zheng B, Cantley L, Rakic P, Man HY. AMP-activated protein kinase regulates neuronal polarization by interfering with PI 3-kinase localization. *Science.* 2011; 332:247–251. [PubMed: 21436401]
- Anderson KA, Ribar TJ, Lin F, Noeldner PK, Green MF, Muehlbauer MJ, Witters LA, Kemp BE, Means AR. Hypothalamic CaMKK2 contributes to the regulation of energy balance. *Cell Metab.* 2008; 7:377–388. [PubMed: 18460329]
- Arad M, Seidman CE, Seidman JG. AMP-activated protein kinase in the heart: role during health and disease. *Circ Res.* 2007; 100:474–488. [PubMed: 17332438]
- Atlante A, Gagliardi S, Minervini GM, Marra E, Passarella S, Calissano P. Rapid uncoupling of oxidative phosphorylation accompanies glutamate toxicity in rat cerebellar granule cells. *Neuroreport.* 1996; 7:2519–2523. [PubMed: 8981415]
- Bai A, Ma AG, Yong M, Weiss CR, Ma Y, Guan Q, Bernstein CN, Peng Z. AMPK agonist downregulates innate and adaptive immune responses in TNBS-induced murine acute and relapsing colitis. *Biochem Pharmacol.* 2010; 80:1708–1717. [PubMed: 20797389]
- Berger L, Hakim AM. The association of hyperglycemia with cerebral edema in stroke. *Stroke.* 1986; 17:865–871. [PubMed: 3764956]
- Blagih J, Krawczyk CM, Jones RG. LKB1 and AMPK: central regulators of lymphocyte metabolism and function. *Immunol Rev.* 2012; 249:59–71. [PubMed: 22889215]
- Bolanos JP, Almeida A, Moncada S. Glycolysis: a bioenergetic or a survival pathway? *Trends Biochem Sci.* 2010; 35:145–149. [PubMed: 20006513]
- Carling D. The AMP-activated protein kinase cascade—a unifying system for energy control. *Trends Biochem Sci.* 2004; 29:18–24. [PubMed: 14729328]
- Carling D, Thornton C, Woods A, Sanders MJ. AMP-activated protein kinase: new regulation, new roles? *Biochem J.* 2012; 445:11–27. [PubMed: 22702974]
- Cheung PC, Salt IP, Davies SP, Hardie DG, Carling D. Characterization of AMP-activated protein kinase gamma-subunit isoforms and their role in AMP binding. *Biochem J.* 2000; 346:659–669. [PubMed: 10698692]
- Chih CP, Roberts EL Jr. Energy substrates for neurons during neural activity: a critical review of the astrocyte-neuron lactate shuttle hypothesis. *J Cereb Blood Flow Metab.* 2003; 23:1263–1281. [PubMed: 14600433]
- Choi JS, Park C, Jeong JW. AMP-activated protein kinase is activated in Parkinson's disease models mediated by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Biochem Biophys Res Commun.* 2010; 391:147–151. [PubMed: 19903456]
- Concannon CG, Tuffy LP, Weisova P, Bonner HP, Davila D, Bonner C, Devocelle MC, Strasser A, Ward MW, Prehn JH. AMP kinase-mediated activation of the BH3-only protein Bim couples energy depletion to stress-induced apoptosis. *J Cell Biol.* 2010; 189:83–94. [PubMed: 20351066]
- Culmsee C, Monnig J, Kemp BE, Mattson MP. AMP-activated protein kinase is highly expressed in neurons in the developing rat brain and promotes neuronal survival following glucose deprivation. *J Mol Neurosci.* 2001; 17:45–58. [PubMed: 11665862]
- Dasgupta B, Milbrandt J. AMP-activated protein kinase phosphorylates retinoblastoma protein to control mammalian brain development. *Dev Cell.* 2009; 16:256–270. [PubMed: 19217427]
- Davila D, Connolly NM, Bonner H, Weisova P, Dussmann H, Concannon CG, Huber HJ, Prehn JH. Two-step activation of FOXO3 by AMPK generates a coherent feed-forward loop determining excitotoxic cell fate. *Cell Death Differ.* 2012 (in press).

- Dirnagl U, Iadecola C, Moskowitz MA. Pathobiology of ischaemic stroke: an integrated view. *Trends Neurosci.* 1999; 22:391–397. [PubMed: 10441299]
- Dirnagl U, Becker K, Meisel A. Preconditioning and tolerance against cerebral ischaemia: from experimental strategies to clinical use. *Lancet Neurol.* 2009; 8:398–412. [PubMed: 19296922]
- Dzamko N, van Denderen BJ, Hevener AL, Jorgensen SB, Honeyman J, Galic S, Chen ZP, Watt MJ, Campbell DJ, Steinberg GR, Kemp BE. AMPK beta1 deletion reduces appetite, preventing obesity and hepatic insulin resistance. *J Biol Chem.* 2010; 285:115–122. [PubMed: 19892703]
- Egan DF, Shackelford DB, Mihaylova MM, Gelino S, Kohnz RA, Mair W, Vasquez DS, Joshi A, Gwinn DM, Taylor R, Asara JM, Fitzpatrick J, Dillin A, Viollet B, Kundu M, Hansen M, Shaw RJ. Phosphorylation of ULK1 (hATG1) by AMP-activated protein kinase connects energy sensing to mitophagy. *Science.* 2011; 331:456–461. [PubMed: 21205641]
- Garcia-Gil M, Pesi R, Perna S, Allegrini S, Giannecchini M, Camici M, Tozzi MG. 5'-Aminoimidazole-4-carboxamide riboside induces apoptosis in human neuroblastoma cells. *Neuroscience.* 2003; 117:811–820. [PubMed: 12654334]
- Genc S, Kurnaz IA, Ozilgen M. Astrocyte–neuron lactate shuttle may boost more ATP supply to the neuron under hypoxic conditions—in silico study supported by in vitro expression data. *BMC Syst Biol.* 2011; 5:162. [PubMed: 21995951]
- Greco SJ, Sarkar S, Johnston JM, Tezapsidis N. Leptin regulates tau phosphorylation and amyloid through AMPK in neuronal cells. *Biochem Biophys Res Commun.* 2009; 380:98–104. [PubMed: 19166821]
- Greer EL, Dowlatshahi D, Banko MR, Villen J, Hoang K, Blanchard D, Gygi SP, Brunet A. An AMPK-FOXO pathway mediates longevity induced by a novel method of dietary restriction in *C. elegans*. *Curr Biol.* 2007a; 17:1646–1656. [PubMed: 17900900]
- Greer EL, Oskoui PR, Banko MR, Maniar JM, Gygi MP, Gygi SP, Brunet A. The energy sensor AMP-activated protein kinase directly regulates the mammalian FOXO3 transcription factor. *J Biol Chem.* 2007b; 282:30107–30119. [PubMed: 17711846]
- Han YX, Lin YT, Xu JJ, Cao LL, Liu XW, Jiang H, Chi ZF. Status epilepticus stimulates peroxisome proliferator-activated receptor gamma coactivator 1-alpha/mitochondrial antioxidant system pathway by a nitric oxide-dependent mechanism. *Neuroscience.* 2011; 186:128–134. [PubMed: 21536107]
- Harada S, Kishimoto M, Kobayashi M, Nakamoto K, Fujita-Hamabe W, Chen HH, Chan MH, Tokuyama S. Honokiol suppresses the development of post-ischemic glucose intolerance and neuronal damage in mice. *J Nat Med.* 2012; 66:591–599. [PubMed: 22261858]
- Hardie DG. AMPK and autophagy get connected. *EMBO J.* 2010; 30:634–635. [PubMed: 21326174]
- Hardie DG. AMP-activated protein kinase: an energy sensor that regulates all aspects of cell function. *Genes Dev.* 2011; 25:1895–1908. [PubMed: 21937710]
- Hardie DG, Ross FA, Hawley SA. AMPK: a nutrient and energy sensor that maintains energy homeostasis. *Nat Rev Mol Cell Biol.* 2012; 13:251–262. [PubMed: 22436748]
- Hawley SA, Davison M, Woods A, Davies SP, Beri RK, Carling D, Hardie DG. Characterization of the AMP-activated protein kinase kinase from rat liver and identification of threonine 172 as the major site at which it phosphorylates AMP-activated protein kinase. *J Biol Chem.* 1996; 271:27879–27887. [PubMed: 8910387]
- Hawley SA, Pan DA, Mustard KJ, Ross L, Bain J, Edelman AM, Frenguelli BG, Hardie DG. Calmodulin-dependent protein kinase kinase-beta is an alternative upstream kinase for AMP-activated protein kinase. *Cell Metab.* 2005; 2:9–19. [PubMed: 16054095]
- Hemminki A, Markie D, Tomlinson I, Avizienyte E, Roth S, Loukola A, Bignell G, Warren W, Aminoff M, Hoglund P, Jarvinen H, Kristo P, Pelin K, Ridanpaa M, Salovaara R, Toro T, Bodmer W, Olschwang S, Olsen AS, Stratton MR, de la Chapelle A, Aaltonen LA. A serine/threonine kinase gene defective in Peutz-Jeghers syndrome. *Nature.* 1998; 391:184–187. [PubMed: 9428765]
- Herrero-Mendez A, Almeida A, Fernandez E, Maestre C, Moncada S, Bolanos JP. The bioenergetic and antioxidant status of neurons is controlled by continuous degradation of a key glycolytic enzyme by APC/C-Cdh1. *Nat Cell Biol.* 2009; 11:747–752. [PubMed: 19448625]

- Horvath TL, Erion DM, Elsworth JD, Roth RH, Shulman GI, Andrews ZB. GPA protects the nigrostriatal dopamine system by enhancing mitochondrial function. *Neurobiol Dis.* 2011; 43:152–162. [PubMed: 21406233]
- Hudson ER, Pan DA, James J, Lucocq JM, Hawley SA, Green KA, Baba O, Terashima T, Hardie DG. A novel domain in AMP-activated protein kinase causes glycogen storage bodies similar to those seen in hereditary cardiac arrhythmias. *Curr Biol.* 2003; 13:861–866. [PubMed: 12747836]
- Hurley RL, Anderson KA, Franzone JM, Kemp BE, Means AR, Witters LA. The Ca^{2+} /calmodulin-dependent protein kinase kinases are AMP-activated protein kinase kinases. *J Biol Chem.* 2005; 280:29060–29066. [PubMed: 15980064]
- Iseli TJ, Walter M, van Denderen BJ, Katsis F, Witters LA, Kemp BE, Michell BJ, Stapleton D. AMP-activated protein kinase beta subunit tethers alpha and gamma subunits via its C-terminal sequence (186–270). *J Biol Chem.* 2005; 280:13395–13400. [PubMed: 15695819]
- Ju TC, Chen HM, Lin JT, Chang CP, Chang WC, Kang JJ, Sun CP, Tao MH, Tu PH, Chang C, Dickson DW, Chern Y. Nuclear translocation of AMPK- α 1 potentiates striatal neurodegeneration in Huntington's disease. *J Cell Biol.* 2011; 194:209–227. [PubMed: 21768291]
- Kahn BB, Alquier T, Carling D, Hardie DG. AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism. *Cell Metab.* 2005; 1:15–25. [PubMed: 16054041]
- Kamel H, Iadecola C. Brain-immune interactions and ischemic stroke: clinical implications. *Arch Neurol.* 2012; 69:576–581. [PubMed: 22782509]
- Kim J, Kundu M, Viollet B, Guan KL. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat Cell Biol.* 2011; 13:132–141. [PubMed: 21258367]
- Kleman AM, Yuan JY, Aja S, Ronnett GV, Landree LE. Physiological glucose is critical for optimized neuronal viability and AMPK responsiveness in vitro. *J Neurosci Methods.* 2008; 167:292–301. [PubMed: 17936912]
- Knowles JR. Enzyme-catalyzed phosphoryl transfer reactions. *Annu Rev Biochem.* 1980; 49:877–919. [PubMed: 6250450]
- Kuramoto N, Wilkins ME, Fairfax BP, Revilla-Sanchez R, Terunuma M, Tamaki K, Iemata M, Warren N, Couve A, Calver A, Horvath Z, Freeman K, Carling D, Huang L, Gonzales C, Cooper E, Smart TG, Pangalos MN, Moss SJ. Phospho-dependent functional modulation of GABA_B receptors by the metabolic sensor AMP-dependent protein kinase. *Neuron.* 2007; 53:233–247. [PubMed: 17224405]
- Lee H, Cho JS, Lambacher N, Lee J, Lee SJ, Lee TH, Gartner A, Koo HS. The *Caenorhabditis elegans* AMP-activated protein kinase AAK-2 is phosphorylated by LKB1 and is required for resistance to oxidative stress and for normal motility and foraging behavior. *J Biol Chem.* 2008; 283:14988–14993. [PubMed: 18408008]
- Li J, McCullough LD. Effects of AMP-activated protein kinase in cerebral ischemia. *J Cereb Blood Flow Metab.* 2010; 30:480–492. [PubMed: 20010958]
- Li J, Zeng Z, Viollet B, Ronnett GV, McCullough LD. Neuroprotective effects of adenosine monophosphate-activated protein kinase inhibition and gene deletion in stroke. *Stroke.* 2007; 38:2992–2999. [PubMed: 17901380]
- Li J, Benashski SE, Venna VR, McCullough LD. Effects of metformin in experimental stroke. *Stroke.* 2010; 41:2645–2652. [PubMed: 20847317]
- Li J, Benashski S, McCullough LD. Post-stroke hypothermia provides neuroprotection through inhibition of AMP-activated protein kinase. *J Neurotrauma.* 2011; 28:1281–1288. [PubMed: 21446786]
- Liu F, Benashski SE, Persky R, Xu Y, Li J, McCullough LD. Age-related changes in AMP-activated protein kinase after stroke. *Age (Dordr).* 2012; 34:157–168. [PubMed: 21360073]
- Luetjens CM, Bui NT, Sengpiel B, Munstermann G, Poppe M, Krohn AJ, Bauerbach E, Kriegelstein J, Prehn JH. Delayed mitochondrial dysfunction in excitotoxic neuron death: cytochrome c release and a secondary increase in superoxide production. *J Neurosci.* 2000; 20:5715–5723. [PubMed: 10908611]

- McBride A, Ghilagaber S, Nikolaev A, Hardie DG. The glycogen-binding domain on the AMPK beta subunit allows the kinase to act as a glycogen sensor. *Cell Metab.* 2009; 9:23–34. [PubMed: 19117544]
- McCullough LD, Zeng Z, Li H, Landree LE, McFadden J, Ronnett GV. Pharmacological inhibition of AMP-activated protein kinase provides neuroprotection in stroke. *J Biol Chem.* 2005; 280:20493–20502. [PubMed: 15772080]
- Momcilovic M, Hong SP, Carlson M. Mammalian TAK1 activates Snf1 protein kinase in yeast and phosphorylates AMP-activated protein kinase in vitro. *J Biol Chem.* 2006; 281:25336–25343. [PubMed: 16835226]
- Myerburg MM, King JD Jr, Oyster NM, Fitch AC, Magill A, Baty CJ, Watkins SC, Kolls JK, Pilewski JM, Hallows KR. AMPK agonists ameliorate sodium and fluid transport and inflammation in cystic fibrosis airway epithelial cells. *Am J Respir Cell Mol Biol.* 2010; 42:676–684. [PubMed: 19617399]
- Nagata D, Mogi M, Walsh K. AMP-activated protein kinase (AMPK) signaling in endothelial cells is essential for angiogenesis in response to hypoxic stress. *J Biol Chem.* 2003; 278:31000–31006. [PubMed: 12788940]
- Nakatsu Y, Kotake Y, Hino A, Ohta S. Activation of AMP-activated protein kinase by tributyltin induces neuronal cell death. *Toxicol Appl Pharmacol.* 2008; 230:358–363. [PubMed: 18511093]
- Nath N, Giri S, Prasad R, Salem ML, Singh AK, Singh I. 5-Aminoimidazole-4-carboxamide ribonucleoside: a novel immunomodulator with therapeutic efficacy in experimental autoimmune encephalomyelitis. *J Immunol.* 2005; 175:566–574. [PubMed: 15972693]
- Nath N, Khan M, Paintlia MK, Singh I, Hoda MN, Giri S. Metformin attenuated the autoimmune disease of the central nervous system in animal models of multiple sclerosis. *J Immunol.* 2009a; 182:8005–8014. [PubMed: 19494326]
- Nath N, Khan M, Rattan R, Mangalam A, Makkar RS, de Meester C, Bertrand L, Singh I, Chen Y, Viollet B, Giri S. Loss of AMPK exacerbates experimental autoimmune encephalomyelitis disease severity. *Biochem Biophys Res Commun.* 2009b; 386:16–20. [PubMed: 19486896]
- Neubert M, Ridder DA, Bargiotas P, Akira S, Schwaninger M. Acute inhibition of TAK1 protects against neuronal death in cerebral ischemia. *Cell Death Differ.* 2011; 18:1521–1530. [PubMed: 21475303]
- Neurath KM, Keough MP, Mikkelsen T, Claffey KP. AMP-dependent protein kinase alpha 2 isoform promotes hypoxia-induced VEGF expression in human glioblastoma. *Glia.* 2006; 53:733–743. [PubMed: 16518831]
- Ng CH, Guan MS, Koh C, Ouyang X, Yu F, Tan EK, O'Neill SP, Zhang X, Chung J, Lim KL. APM Kinase Activation Mitigates Dopaminergic Dysfunction and Mitochondrial Abnormalities in Drosophila Models of Parkinson's Disease. *J Neurosci.* 2012; 32:14311–14317. [PubMed: 23055502]
- Oakhill JS, Chen ZP, Scott JW, Steel R, Castelli LA, Ling N, Macaulay SL, Kemp BE. Beta-subunit myristoylation is the gatekeeper for initiating metabolic stress sensing by AMP-activated protein kinase (AMPK). *Proc Natl Acad Sci U S A.* 2010; 107:19237–19241. [PubMed: 20974912]
- Oakhill JS, Steel R, Chen ZP, Scott JW, Ling N, Tam S, Kemp BE. AMPK is a direct adenylate charge-regulated protein kinase. *Science.* 2011; 332:1433–1435. [PubMed: 21680840]
- Osuka K, Watanabe Y, Usuda N, Atsuzawa K, Yoshida J, Takayasu M. Modification of endothelial nitric oxide synthase through AMPK after experimental subarachnoid hemorrhage. *J Neurotrauma.* 2009; 26:1157–1165. [PubMed: 19226196]
- Pellerin L. How astrocytes feed hungry neurons. *Mol Neurobiol.* 2005; 32:59–72. [PubMed: 16077184]
- Pellerin L, Magistretti PJ. Sweet sixteen for ANLS. *J Cereb Blood Flow Metab.* 2012; 32:1152–1166. [PubMed: 22027938]
- Pellerin L, Bouzier-Sore AK, Aubert A, Serres S, Merle M, Costalat R, Magistretti PJ. Activity-dependent regulation of energy metabolism by astrocytes: an update. *Glia.* 2007; 55:1251–1262. [PubMed: 17659524]
- Poels J, Spasic MR, Gistelinck M, Mutert J, Schellens A, Callaerts P, Norga KK. Autophagy and phagocytosis-like cell cannibalism exert opposing effects on cellular survival during metabolic stress. *Cell Death Differ.* 2012 (in press).

- Polekhina G, Gupta A, Michell BJ, van Denderen B, Murthy S, Feil SC, Jennings IG, Campbell DJ, Witters LA, Parker MW, Kemp BE, Stapleton D. AMPK beta subunit targets metabolic stress sensing to glycogen. *Curr Biol*. 2003; 13:867–871. [PubMed: 12747837]
- Puyal J, Vaslin A, Mottier V, Clarke PG. Postischemic treatment of neonatal cerebral ischemia should target autophagy. *Ann Neurol*. 2009; 66:378–389. [PubMed: 19551849]
- Ronnett GV, Ramamurthy S, Kleman AM, Landree LE, Aja S. AMPK in the brain: its roles in energy balance and neuroprotection. *J Neurochem*. 2009; 109(Suppl 1):17–23. [PubMed: 19393004]
- Rosamond W, Flegal K, Furie K, Go A, Greenlund K, Haase N, Hailpern SM, Ho M, Howard V, Kissela B, Kittner S, Lloyd-Jones D, McDermott M, Meigs J, Moy C, Nichol G, O'Donnell C, Roger V, Sorlie P, Steinberger J, Thom T, Wilson M, Hong Y. Heart disease and stroke statistics—2008 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation*. 2008; 117:e25–e146. [PubMed: 18086926]
- Sanders MJ, Grondin PO, Hegarty BD, Snowden MA, Carling D. Investigating the mechanism for AMP activation of the AMP-activated protein kinase cascade. *Biochem J*. 2007; 403:139–148. [PubMed: 17147517]
- Sheng B, Liu J, Li GH. Metformin preconditioning protects *Daphnia pulex* from lethal hypoxic insult involving AMPK, HIF and mTOR signaling. *Comp Biochem Physiol B Biochem Mol Biol*. 2012; 163:51–58. [PubMed: 22564403]
- Smiley D, Umpierrez G. Metformin/rosiglitazone combination pill (Avandamet) for the treatment of patients with Type 2 diabetes. *Expert Opin Pharmacother*. 2007; 8:1353–1364. [PubMed: 17563269]
- Stahmann N, Woods A, Spengler K, Heslegrave A, Bauer R, Krause S, Viollet B, Carling D, Heller R. Activation of AMP-activated protein kinase by vascular endothelial growth factor mediates endothelial angiogenesis independently of nitric-oxide synthase. *J Biol Chem*. 2010; 285:10638–10652. [PubMed: 20129920]
- Stapleton D, Mitchelhill KI, Gao G, Widmer J, Michell BJ, Teh T, House CM, Fernandez CS, Cox T, Witters LA, Kemp BE. Mammalian AMP-activated protein kinase subfamily. *J Biol Chem*. 1996; 271:611–614. [PubMed: 8557660]
- Stein SC, Woods A, Jones NA, Davison MD, Carling D. The regulation of AMP-activated protein kinase by phosphorylation. *Biochem J*. 2000; 345:437–443. [PubMed: 10642499]
- Steinberg GR, Kemp BE. AMPK in health and disease. *Physiol Rev*. 2009; 89:1025–1078. [PubMed: 19584320]
- Stenzel-Poore MP, Stevens SL, King JS, Simon RP. Preconditioning reprograms the response to ischemic injury and primes the emergence of unique endogenous neuroprotective phenotypes: a speculative synthesis. *Stroke*. 2007; 38(Suppl 2):680–685. [PubMed: 17261715]
- Su KH, Yu YB, Hou HH, Zhao JF, Kou YR, Cheng LC, Shyue SK, Lee TS. AMP-activated protein kinase mediates erythropoietin-induced activation of endothelial nitric oxide synthase. *J Cell Physiol*. 2012; 227:3053–3062. [PubMed: 22021095]
- Suter M, Riek U, Tuerk R, Schlattner U, Wallimann T, Neumann D. Dissecting the role of 5'-AMP for allosteric stimulation, activation, and deactivation of AMP-activated protein kinase. *J Biol Chem*. 2006; 281:32207–32216. [PubMed: 16943194]
- Thornton C, Bright NJ, Sastre M, Muckett PJ, Carling D. AMP-activated protein kinase (AMPK) is a tau kinase, activated in response to amyloid beta-peptide exposure. *Biochem J*. 2011; 434:503–512. [PubMed: 21204788]
- Tomlinson DR, Gardiner NJ. Glucose neurotoxicity. *Nat Rev Neurosci*. 2008; 9:36–45. [PubMed: 18094705]
- Turnley AM, Stapleton D, Mann RJ, Witters LA, Kemp BE, Bartlett PF. Cellular distribution and developmental expression of AMP-activated protein kinase isoforms in mouse central nervous system. *J Neurochem*. 1999; 72:1707–1716. [PubMed: 10098881]
- UKPDS. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). UK Prospective Diabetes Study (UKPDS) Group. *Lancet*. 1998; 352:854–865. [PubMed: 9742977]

- Venna VR, Li J, Benashski SE, Tarabishy S, McCullough LD. Preconditioning induces sustained neuroprotection by downregulation of adenosine 5'-monophosphate-activated protein kinase. *Neuroscience*. 2011; 201:280–287. [PubMed: 22120436]
- Vingtdeux V, Davies P, Dickson DW, Marambaud P. AMPK is abnormally activated in tangle- and pre-tangle-bearing neurons in Alzheimer's disease and other tauopathies. *Acta Neuropathol*. 2011; 121:337–349. [PubMed: 20957377]
- Vilchez D, Ros S, Cifuentes D, Pujadas L, Valles J, Garcia-Fojeda B, Criado-Garcia O, Fernandez-Sanchez E, Medrano-Fernandez I, Dominguez J, Garcia-Rocha M, Soriano E, Rodriguez de Cordoba S, Guinovart JJ. Mechanism suppressing glycogen synthesis in neurons and its demise in progressive myoclonus epilepsy. *Nat Neurosci*. 2007; 10:1407–1413. [PubMed: 17952067]
- Viollet B, Andreelli F, Jorgensen SB, Perrin C, Flamez D, Mu J, Wojtaszewski JF, Schuit FC, Birnbaum M, Richter E, Burcelin R, Vaulont S. Physiological role of AMP-activated protein kinase (AMPK): insights from knockout mouse models. *Biochem Soc Trans*. 2003; 31:216–219. [PubMed: 12546688]
- Viollet B, Athea Y, Mounier R, Guigas B, Zarrinpashneh E, Horman S, Lantier L, Hebrard S, Devin-Leclerc J, Beauloye C, Foretz M, Andreelli F, Ventura-Clapier R, Bertrand L. AMPK: lessons from transgenic and knockout animals. *Front Biosci*. 2009; 14:19–44.
- Wallace BK, Foroutan S, O'Donnell ME. Ischemia-induced stimulation of Na-K-Cl cotransport in cerebral microvascular endothelial cells involves AMP kinase. *Am J Physiol Cell Physiol*. 2011; 301:C316–326. [PubMed: 21562306]
- Wang P, Xu TY, Guan YF, Tian WW, Viollet B, Rui YC, Zhai QW, Su DF, Miao CY. Nicotinamide phosphoribosyltransferase protects against ischemic stroke through SIRT1-dependent adenosine monophosphate-activated kinase pathway. *Ann Neurol*. 2011; 69:360–374. [PubMed: 21246601]
- Ward MW, Rego AC, Frenguelli BG, Nicholls DG. Mitochondrial membrane potential and glutamate excitotoxicity in cultured cerebellar granule cells. *J Neurosci*. 2000; 20:7208–7219. [PubMed: 11007877]
- Ward MW, Rehm M, Duessmann H, Kacmar S, Concannon CG, Prehn JH. Real time single cell analysis of Bid cleavage and Bid translocation during caspase-dependent and neuronal caspase-independent apoptosis. *J Biol Chem*. 2006; 281:5837–5844. [PubMed: 16407197]
- Ward MW, Huber HJ, Weisova P, Dussmann H, Nicholls DG, Prehn JH. Mitochondrial and plasma membrane potential of cultured cerebellar neurons during glutamate-induced necrosis, apoptosis, and tolerance. *J Neurosci*. 2007; 27:8238–8249. [PubMed: 17670970]
- Weisova P, Concannon CG, Devocelle M, Prehn JH, Ward MW. Regulation of glucose transporter 3 surface expression by the AMP-activated protein kinase mediates tolerance to glutamate excitation in neurons. *J Neurosci*. 2009; 29:2997–3008. [PubMed: 19261894]
- Weisova P, Davila D, Tuffy LP, Ward MW, Concannon CG, Prehn JH. Role of 5'-adenosine monophosphate-activated protein kinase in cell survival and death responses in neurons. *Antiox Redox Signal*. 2011; 14:1863–1876.
- Weisova P, Anilkumar U, Ryan C, Concannon CG, Prehn JH, Ward MW. "Mild mitochondrial uncoupling" induced protection against neuronal excitotoxicity requires AMPK activity. *Biochim Biophys Acta*. 2012a; 1817:744–753. [PubMed: 22336583]
- Weisova P, Davila D, Tuffy LP, Ward MW, Concannon CG, Prehn JH. Role of 5'-adenosine monophosphate-activated protein kinase in cell survival and death responses in neurons. *Antiox Redox Signal*. 2012b; 14:1863–1876.
- White BJ, Tarabishy S, Venna VR, Manwani B, Benashski S, McCullough LD, Li J. Protection from cerebral ischemia by inhibition of TGFbeta-activated kinase. *Exp Neurol*. 2012 (in press).
- Williams T, Courchet J, Viollet B, Brenman JE, Polleux F. AMP-activated protein kinase (AMPK) activity is not required for neuronal development but regulates axogenesis during metabolic stress. *Proc Natl Acad Sci U S A*. 2011; 108:5849–5854. [PubMed: 21436046]
- Won JS, Im YB, Kim J, Singh AK, Singh I. Involvement of AMP-activated-protein-kinase (AMPK) in neuronal amyloidogenesis. *Biochem Biophys Res Commun*. 2010; 399:487–491. [PubMed: 20659426]

- Woods A, Cheung PC, Smith FC, Davison MD, Scott J, Beri RK, Carling D. Characterization of AMP-activated protein kinase beta and gamma subunits. Assembly of the heterotrimeric complex in vitro. *J Biol Chem*. 1996; 271:10282–10290. [PubMed: 8626596]
- Wu Y, Song P, Xu J, Zhang M, Zou MH. Activation of protein phosphatase 2A by palmitate inhibits AMP-activated protein kinase. *J Biol Chem*. 2007; 282:9777–9788. [PubMed: 17255104]
- Xiao B, Heath R, Saiu P, Leiper FC, Leone P, Jing C, Walker PA, Haire L, Eccleston JF, Davis CT, Martin SR, Carling D, Gamblin SJ. Structural basis for AMP binding to mammalian AMP-activated protein kinase. *Nature*. 2007; 449:496–500. [PubMed: 17851531]
- Xiao B, Sanders MJ, Underwood E, Heath R, Mayer FV, Carmena D, Jing C, Walker PA, Eccleston JF, Haire LF, Saiu P, Howell SA, Aasland R, Martin SR, Carling D, Gamblin SJ. Structure of mammalian AMPK and its regulation by ADP. *Nature*. 2011; 472:230–233. [PubMed: 21399626]
- Xie Z, Dong Y, Zhang J, Scholz R, Neumann D, Zou MH. Identification of the serine 307 of LKB1 as a novel phosphorylation site essential for its nucleocytoplasmic transport and endothelial cell angiogenesis. *Mol Cell Biol*. 2009; 29:3582–3596. [PubMed: 19414597]
- Xu Q, Si LY. Protective effects of AMP-activated protein kinase in the cardiovascular system. *J Cell Mol Med*. 2010; 14:2604–2613. [PubMed: 20874722]
- Zaha VG, Young LH. AMP-activated protein kinase regulation and biological actions in the heart. *Circ Res*. 2012; 111:800–814. [PubMed: 22935535]
- Zhang W, Zhang X, Wang H, Guo X, Li H, Wang Y, Xu X, Tan L, Mashek MT, Zhang C, Chen Y, Mashek DG, Foretz M, Zhu C, Zhou H, Liu X, Viollet B, Wu C, Huo Y. AMP-activated protein kinase alpha1 protects against diet-induced insulin resistance and obesity. *Diabetes*. 2012 (in press).
- Zhang X, Gao R, Li J, Qi Y, Song X, Zhao L, Wang H, Pu Y, Xu K. A pharmacological activator of AMP-activated protein kinase protects hypoxic neurons in a concentration-dependent manner. *Neurochem Res*. 2010; 35:1281–1289. [PubMed: 20490918]
- Zhao H, Steinberg GK, Sapolsky RM. General vs. specific actions of mild-moderate hypothermia in attenuating cerebral ischemic damage. *J Cereb Blood Flow Metab*. 2007; 27:1879–1894. [PubMed: 17684517]
- Zhu J, Aja S, Kim EK, Park MJ, Ramamurthy S, Jia J, Hu X, Geng P, Ronnett GV. Physiological oxygen level is critical for modeling neuronal metabolism in vitro. *J Neurosci Res*. 2011; 90:422–434. [PubMed: 22002503]
- Zou MH, Wu Y. AMP-activated protein kinase activation as a strategy for protecting vascular endothelial function. *Clin Exp Pharmacol Physiol*. 2008; 35:535–545. [PubMed: 18177481]

Fig. 1. Schematic demonstrating the effects of AMPK signaling in stroke. +, Activation; -, inhibition; F6P, fructose-6-phosphate; F2-6P, fructose-2,6-bisphosphate; PFK2, phosphofructokinase 2; PPP, pentose phosphate pathway; G6P, glucose-6-phosphate. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

TABLE I

Role of AMPK in Brain Pathologies

	Disease	AMPK activation (good/bad)	Role of AMPK	Reference
1	Huntington's disease (HD)	Bad	AICAR (AMPK activation) ↑neuronal death, ↓life span of HD mice, promoted death of neuronal cell lines; this effect reversed by an AMPK inhibitor	Ju et al., 2011
2	Alzheimer's disease	Good/bad?	Leptin causes inhibition of amyloid β production and tau phosphorylation through activation of AMPK	Greco et al., 2009
			AMPK phosphorylates tau on a number of sites altering the binding of microtubules to tau	Thornton et al., 2011
			AMPK can directly phosphorylate tau	Vingtdeux et al., 2011
			AICAR (AMPK activation) ↓A β production	Won et al., 2010
3	Multiple sclerosis (MS)	Good	Metformin (AMPK activator) attenuates EAE (models MS) by restricting mononuclear cell infiltration, downregulation of proinflammatory cytokines (IFN γ , TNF- α , IL-6, IL-17, and iNOS), cell adhesion molecules, matrix metalloproteinase 9, and RANTES	Nath et al., 2009a
			AMPK is downregulated in all immune cells of EAE; AMPK α 1(-/-) mice have increased disease severity	Nath et al., 2009b
4	Parkinson's disease (PD)	Good	AMPK activation may prevent neuronal cell death and can be a survival factor in MPTP model of PD	Choi et al., 2010
			Guanidinopropionic acid (GPA)-fed mice exhibited no loss of TH neurons in the substantia nigra; these effects were mediated partially by increases in AMPK activity	Horvath et al., 2011
			AMPK activation suppresses dopaminergic and mitochondrial dysfunction in mutant parkin and LRRK2 (mutations in familial PD) flies	Ng et al., 2012
5	Status epilepticus (SE)	Good	Compound C (AMPK inhibitor) exacerbates neuronal loss and oxidative stress in pilocarpine-induced SE	Han et al., 2011

TABLE II

Studies of AMPK in Stroke/Hypoxia

Study design	Results	Reference
AMPK-activating agent AICAR in cultured embryonic rat hippocampal neurons subjected to glucose deprivation, chemical hypoxia, and exposure to glutamate and A β peptide	AICAR increased neuronal survival	Culmsee et al., 2001
Cortical and hippocampal neurons (in vitro) subjected to oxygen glucose deprivation (OGD) and C57BL/6 mice subjected to 120 min of MCAO	Compound C and C75 (AMPK inhibitors) are neuroprotective; AICAR (AMPK activator) exacerbates injury	McCullough et al., 2005
Wild- type and AMPK α 2 and - α 1 gene knockout mice subjected to 90 min of MCAO in C57BL/6 mice	Compound C treatment neuroprotective; infarct volumes decreased in AMPK α 2 gene deletion male mice; AMPK α 1 gene deletion mice had no effect	Li et al., 2007
90 Min of MCAO in C57BL/6 wild-type and AMPK α 2 gene knockout ovariectomized female mice	Infarct volumes decreased with compound C treatment and in AMPK α 2 gene deletion ovariectomized female mice; effect of AMPK is sex independent	Li and McCullough, 2010
Male C57BL6 mice subjected to acute (3 days) or chronic (3 weeks) Metformin prior to 90 min of MCAO	Acute metformin treatment increased infarct volumes (enhanced AMPK activation); chronic metformin treatment decrease infarct volumes (ameliorated stroke-induced activation of AMPK)	Li et al., 2010
Bovine cerebral microvascular endothelial cells (CMEC) subjected to hypoxia, aglycemia, OGD	pAMPK levels increased; administration of compound C decreased pAMPK and CMEC Na-K-Cl cotransporter activity	Wallace et al., 2011
Brief, 3-min MCAO, 3 days before (IPC) 90 min of MCAO in C57BL6 mice	IPC decreases infarct volumes through inhibition of AMPK activation	Venna et al., 2011
Hypothermia after 90 min of MCAO in C57BL6 mice	Hypothermia decreases infarct volumes, mediated through inhibition of AMPK activation	Li et al., 2011
Nampt overexpression in wild-type and AMPK α 2 gene knockout mice subjected to 120 min of MCAO in C57BL/6 mice	Nampt overexpression decreased injury but had no effect in AMPK α 2 gene knockout mice; NAMPT acts by AMPK activation	Wang et al., 2011
<i>Daphnia pulex</i> received AMPK-activating agent metformin for 3 hr and then were subjected to 30 min hypoxic insult using 4 mM sodium hyposulfite	Metformin treatment increased survival; effect partially through AMPK activation	Sheng et al., 2012
Inhibition of AMPK by compound C in young and aged C57BL6 male mice subjected to 90 min of MCAO	Young mice had AMPK activation, response muted in the aging; compound C neuroprotective in young but no effect in aging	Liu et al., 2012
Inhibitor of TAK1 by 5Z-7-oxozeanol, then the male C57BL/6 mice were subjected 90 min of MCAO	5Z-7-oxozeanol decreases infarct volumes; effect is independent of AMPK	White et al., 2012
Male ddY mice treated with Honokiol, subjected to 120 min of MCAO	Honokiol increases hepatic pAMPK levels (day 1 post-MCAO) and decreases infarct volumes (peripheral effects?)	Harada et al., 2012