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Review Article

AMP-Activated Protein Kinase (AMPK) and Energy-Sensing in the Brain

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5'-adenosine monophosphate-activated protein kinase (AMPK) is an evolutionarily conserved cellular and organismal energy integrator that responds to numerous stimuli with the overall intention to facilitate energy conservation and enhance energy balance while also affecting cellular survival and behaviors. AMPK has been appreciated for many years to function in peripheral organs that contribute to the generation or disposition of cellular energy, while its role in the brain has been only recently elucidated. While acknowledged to respond to organismal energy balance, we now recognize that energy balance within neurons also affects the brain's response to these peripheral signals. In this review, we discuss AMPK's regulation and its ever-expanding role as a neuronal energy integrator at both the cellular and systems levels.

Key words: 5'-adenosine monophosphate-activated kinase, AMPK, energy balance, neuronal metabolism, stroke, feeding behavior

INTRODUCTION

AMPK, or 5'-adenosine monophosphate-activated protein kinase, is an evolutionarily conserved serine/threonine kinase that plays a crucial role in regulating energy homeostasis. In higher order eukaryotes, it is expressed in multiple systems and serves as an integrator of cellular and organismal energy balance and energy-dependent responses [1]. As its name implies, AMPK is activated by physiological or pathophysiological stimuli that cause elevations in cellular AMP concentration, as well as hormones, drugs, and xenobiotics. Upon activation, AMPK phosphorylates its targets with the goal of acutely stimulating catabolic processes while inhibiting anabolic processes to restore cellular energy

homeostasis, and chronically altering gene transcription and controlling cellular fate. In this review, we focus on recent advances in AMPK and energy-sensing in the brain. As the AMPK field is rapidly growing, for further discussion of the regulation of AMPK and its role in coordinating various aspects of cell and whole organism function, readers are directed towards several recent reviews [1-4].

STRUCTURE AND REGULATION

AMPK is a heterotrimeric enzyme complex, comprised of a catalytic α subunit and regulatory β and γ subunits. There are two alpha subunit genes (α1 and α2), two beta subunit genes (β1 and β2), and three gamma subunit genes (γ1, γ2, and γ3) which are capable of assembling in different combinations in a tissue-specific manner. In the rodent brain, all the isoforms have been detected except the γ3, which is primarily thought to be a muscle-specific subunit [5,6]. Activation of the AMPK complex requires
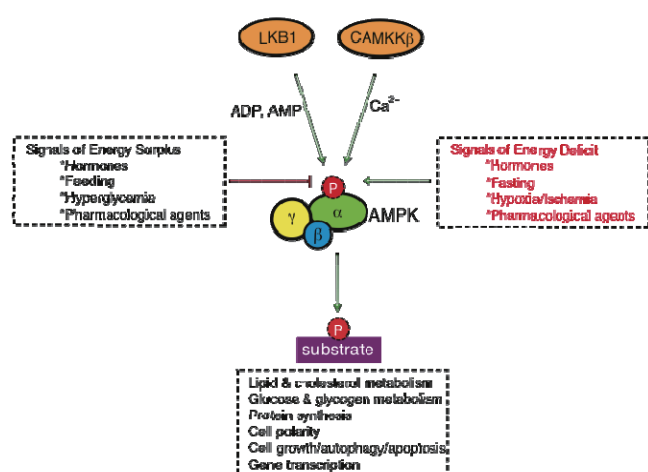
Fig. 1. Regulation of AMPK activation. AMPK is activated under conditions of energy-deficit by either physiological or pathophysiological signals. Signals that increase cellular AMP or ADP levels activate AMPK through LKB1, whereas those that raise intracellular Ca^{2+} activate AMPK through CaMKKβ. In contrast signals of energy-surplus inhibit AMPK activation and promote deactivation. Activated AMPK phosphorylates numerous targets to promote catabolic processes and inhibit anabolic cellular processes.

phosphorylation of the α subunit at threonine-172 (T172) in the kinase loop by one of two identified upstream kinases, LKB1 (Liver kinase B1/serine threonine kinase 11) or CaMKKβ (Ca^{2+}/ calmodulin-dependent kinase kinase β) (Fig. 1). The β subunits contain a carbohydrate-binding domain that acts as a cellular glycogen sensor. In addition, a myristoylation site in the β subunit has been identified that is required for α subunit phosphorylation and allosteric activation by AMP [7, 8]. The γ subunits contain four cystathionine β-synthase domains arranged as tandem pairs (also known as CBS or Bateman domains), which serve as AMP, ADP, and ATP binding sites. More recently, through biochemical and structural studies, evidence has been provided that AMPK also responds to elevations in both cellular AMP and ADP [9]. The current model for complete activation of AMPK appears to have several steps. Initially, phosphorylation of T172 by the upstream kinase increases AMPK activity by several hundred-fold. Additionally, binding of AMP or ADP to site 1 and site 3 of the CBS domains induce further allosteric activation (induced by AMP alone) and protect the kinase from dephosphorylation (induced by both AMP and ADP) [10]. In this manner, several thousand-fold increases in AMPK activity can be achieved in the presence of elevated levels of AMP after α subunit phosphorylation. In contrast, high concentrations of ATP bind to the γ subunit and promote α subunit dephosphorylation and kinase inactivation. Therefore due to the fact that AMPK can directly monitor AMP and ADP levels and indirectly sense

ATP levels, it can appropriately be considered an "adenosine nucleotide" sensor. It has been proposed that AMPK acts at the cellular level through changes in adenine nucleotides via LKB1 phosphorylation, and at the whole organism level through elevations in intracellular Ca^{2+} and CaMKKβ activation [11]. In addition to LKB1 and CaMKKβ, it has also been suggested that TAK1 (transforming growth factor β-activated kinase 1) may serve as an upstream AMPK kinase [12, 13]. However, the exact mechanism of this regulation and its *in vivo* significance in the brain is less well understood.

The list of AMPK targets that are phosphorylated upon activation is ever expanding [4]. These targets can be broadly categorized into enzymes involved in glucose and lipid metabolism, protein synthesis, cell growth, autophagy, cell polarity, and gene transcription. Based on numerous studies, it appears that the overall consequence of AMPK activation is context and stimulus-specific. Under normal physiological situations in the brain, AMPK serves as a master energy sensor and integrator of signals in response to long-term energy deficits. In pathophysiological situations such as ischemia, over activation of AMPK is deleterious.

AMPK AND REGULATION OF FOOD INTAKE AND ENERGY BALANCE

Early histological studies revealed that AMPK subunits are highly expressed in the developing and adult rodent brain [5, 14]. Although neurons represent a minority of cells in the brain, it appears that AMPK subunits are enriched in these cells irrespective of brain area. In particular, hypothalamic AMPK is proposed to be an "energy integrator" and is poised to sense not only cellular energy status, but also to monitor whole-organism energy balance.

The role of AMPK in the regulation of food intake and energy balance was elucidated by several groups. It was shown that signals such as fasting and the orexigenic hormone ghrelin, increased hypothalamic AMPK activity, whereas refeeding and the anorexigenic hormone leptin, decreased AMPK activity [15, 16]. As further evidence that it is indeed alterations in hypothalamic AMPK that mediate changes in food intake, it was demonstrated that direct pharmacological activation of AMPK by i.c.v. injection of AICAR (5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside) or expression of a constitutively active AMPK adenovirus increase food intake and body weight. Similarly, injection of a dominant negative AMPK adenovirus produced a reduction in food intake and body weight. Previously, we developed the compound C75 (3-carboxy-4-octyl-2-methylenebutyrolactone) as a fatty-acid synthase inhibitor/

carnitne palmitoyltransferase-1 stimulator and demonstrated its ability to reduce food intake and body weight in mice [17-19]. It was observed that C75 caused rapid profound anorexia, reversible weight loss, and increased peripheral energy expenditure. Subsequently, when examining the mechanism of action of C75, we determined that C75, either given i.p or i.c.v, mediates its effects through AMPK inhibition in the hypothalamus [20, 21]. We demonstrated that direct stimulation of AMPK with AICAR reverses the effects of C75 on AMPK inhibition and feeding reduction. In contrast, i.c.v. injection of the AMPK inhibitor compound C (6-[4-(2-Piperidin-1-yl-ethoxy)-phenyl)]-3-pyridin-4-yl-pyrrazolo[1,5-a]-pyrimidine), reduced hypothalamic AMPK activation and food intake. Furthermore, it was demonstrated the altering hypothalamic neuronal energy balance and inhibiting AMPK activity with C75 treatment produced long-term effects on neuropeptide Y (NPY) gene expression and feeding behavior, suggesting a mechanism for C75 action in the CNS.

In the subsequent years, it has been demonstrated by many laboratories that hypothalamic AMPK can be regulated by numerous orexigenic signals which indicate "energy deficit" and anorexigenic signals which indicate "energy surplus." Fasting, hypoglycemia, ghrelin, adiponectin, agouti-related peptide (AGRP), endocannabinoids, thyroid hormone, glucocorticoids, are among the orexigenic cues that increase hypothalamic AMPK and feeding [22-30]. In contrast, refeeding, hyperglycemia, leptin, insulin, resistin, α-melanocyte stimulating hormone (α-MSH), ciliary neurotrophic factor (CNTF), glucagon-like peptide 1 (GLP-1), and angiopoeitin-like protein 4 (Angpt14/Fiaf) are among the anorexigenic signals that inhibit hypothalamic AMPK and reduce food intake [15, 16, 26, 31-34].

Several transgenic and knockout mouse model systems provided further evidence supporting the importance of hypothalamic AMPK in energy balance. Initially, global deletion of either α1 or α2 subunits did not reveal any differences in food intake, body weight, or energy expenditure [35-37]. This lack of central effect could be attributed to compensatory up-regulation or redundancy in function of the α subunits. To overcome this limitation, a conditional deletion approach was employed. In this regard, mice with a targeted deletion of α2 (on an global α1-null background) in pro-opiomelanocortin (POMC) neurons of the arcuate (ARC) nucleus developed obesity and exhibited a reduced metabolic rate [38]. By contrast in the same study, mice with a deletion of α2 in agouti-relate peptide (AGRP) neurons were leaner than wild type mice. Unexpectedly, neurons from both of these knockout mouse models retained acute sensitivity to leptin and insulin signaling, but were blunted in their response to glucose alterations. These findings suggest that AMPK is important for glucose-sensing in POMC and AGRP neurons but is not required for hormonal responses. As an alternative strategy, AMPK β subunit knockout mouse models have also provided interesting insight into enzyme regulation in feeding centers. It was recently observed that mice with a global β1 deletion have reduced food intake, adiposity, and total body mass when fed either a standard or high fat diet [39]. In contrast, mice with a global deletion of β2 subunits that were fed a high fat diet gained more weight and developed hyperinsulinemia and glucose intolerance [40]. The finding that each of the β subunit deletion mice exhibits defined phenotypes is interesting and may be explained by differential subunit expression in the various tissues involved in metabolic regulation (e.g, hypothalamus, muscle, liver, adipose tissue). Furthermore, while these knockout models have yielded significant and interesting findings that are sometimes surprising, they underscore the complexity of regulation of AMPK in different hypothalamic nuclei and neuronal subpopulations. To further address the role of AMPK in the hypothalamus, it would be useful to have conditional and inducible control of gene deletion in specific neuronal populations with appropriate Cre-recombinase mouse lines. It is likely that such tools are already in development and studies will yield valuable information soon.

AMPK AND CNS ISCHEMIC STROKE

As ischemic brain injury is essentially an acute disorder of energy deficit, it is not surprising that AMPK would be implicated in this process. Stroke injury involves multiple cellular and tissue stresses including hypoxia, hypoglycemia, lactic acidosis, calcium overload, and oxidative and nitrosative stresses, apoptosis, and necrosis [41-43]. Accumulating evidence suggests that AMPK is activated by various metabolic stresses in the brain and elicits downstream compensatory responses [44]. However, the overall consequence of AMPK activation appears to be dependent on the degree of activation, the cell-type in which it is activated and the specific metabolic status of the cell.

In vitro, it was demonstrated that AMPK activation with a low dose of AICAR protects primary hippocampal neurons from hypoglycemic stress and glutamate excitotoxicity and protects primary astrocytes from ceramide-mediated apoptosis [14, 45]. In contrast, studies using cell lines determined that AICAR-stimulated AMPK activation was detrimental to overall cell survival in SH-SY5Y neuroblastoma cells and mouse neuroblastoma cells [46, 47]. Similarly, it was found that AMPK activation mediated by tributlyltin induced primary cortical neuronal cell death [48]. Furthermore, it was shown that the

AMPK inhibitor compound C was able protect neurons from tributyltin toxicity. Studies from another group showed that AICAR could protect rat primary cortical neurons from hypoxic death in a concentration-dependent manner [49]. Interestingly, whereas low doses were neuroprotective, higher doses lost their efficacy. Most recently, another study provided further mechanistic insight into the role of AMPK in excitotoxic injury [50]. It was found that NMDA or glutamate excitotoxic stress produced activation of AMPK in primary cortical or cerebellar granule cells. Moreover, overactivation of AMPK was found to be involved in increased gene transcription and activation of the pro-apoptotic protein Bim, a Bcl-2 family member. In this regard, constitutively active AMPK or AICAR were found to activate an ATP-dependent cell death process known as "excitotoxic apoptosis."

Based on *in vitro* studies, it is evident that discrepancies exist regarding the role of AMPK in the neuronal response to stress. One important consideration is that many studies typically use neuronal culture medium containing 25 mM glucose whereas the physiological concentrations of glucose *in vivo* have been estimated to be 0.82~2.4 mM [51-53]. To facilitate the study of AMPK, neuronal metabolism, and their responses to physiological and pathophysiological stress conditions we established a new neuronal culture system that would more closely mimic the in vivo situation [54]. We observed that when neurons are cultured in 3 mM glucose as opposed to 25 mM, they respond to metabolic alterations more similar to that seen *in vivo*. More recently, we have also optimized this culture paradigm by culturing cells at 5% O_2 tension (as opposed to 21% or ambient O_2 tension), which is more reflective of that seen in the brain [55]. Interestingly, when neurons were grown in both 3 mM glucose and 5% O_2 they exhibit increased survival, reduced reactive oxygen species, improved mitochondrial function. Moreover, cellular ATP levels were increased through increased glucose uptake and flux through the glycolytic pathway. While activated AMPK levels were elevated, oxidation of both glucose and fatty acids were decreased. These data suggest that under physiological glucose and oxygen culture conditions, activated AMPK is reset at a new setpoint where it is better poised to respond to and integrate energy flux signals as it may *in vivo*.

In addition to accumulating data from *in vitro* experiments, several studies have examined the role of AMPK in ischemia reperfusion injury *in vivo*. Work from our group demonstrated that AMPK is activated by oxygen-glucose deprivation (OGD) stress in rat hippocampal slices [56]. Similar findings were observed in the mouse cortex using a middle cerebral artery occlusion (MCAO) model of transient focal ischemia. Furthermore, it was found that administration of C75, which

inhibits AMPK indirectly, or compound C, which inhibits AMPK directly, produced significant reductions in infarct size and behavioral defects, suggesting that AMPK over-activation is detrimental to overall stroke outcome. Additional data to support the involvement of AMPK in ischemic damage was provided in studies using α1- or α2-deletion mice [57]. It was found that mice with global α2 deletion had significantly smaller cortical, striatal, and total infarct volumes compared to wild-type mice. In contrast α1-deletion mice were not significantly neuroprotected compared to wild-type controls. Furthermore, compound C, which was shown to be neuroprotective in WT mice, lost its effects in α2 knockout mice. It was also found that the neuroprotective effect of AMPK α2 deletion was equally evident in male and female mice [58]. In another series of experiments, it was demonstrated that acute treatment with the commonly used anti-diabetic drug and AMPK activator, metformin, exacerbated stroke outcome after MCAO [59]. This detrimental effect was lost in both nNOS and α2 knockout mice, demonstrating that over-activation of this pathway is harmful in ischemic injury. Most intriguingly, it was also shown that chronic metformin actually improved stroke outcome by *reducing* AMPK activation. It was suggested that the duration and degree of AMPK activation are the critical factors that determine cellular responses in ischemia. Based on these data, it was proposed that over-activation of AMPK especially in astrocytes enhances glycolysis and produces extreme lactic acidosis which exacerbates ischemic injury. However, it cannot be ruled out that over-activation of AMPK in neurons induces a cell-autonomous death pathway such as autophagy [60]. Indeed it has recently been demonstrated that AMPK activation is a key signal in the initiation of this cascade [61, 62]. Although the precise mechanism remains unclear, accumulating *in vivo* data using a combination of genetic and pharmacological tools has shown that over activation of AMPK is deleterious in stroke and that AMPK inhibition is neuroprotective.

OTHER ROLES OF AMPK IN THE BRAIN

While the role of AMPK in hypothalamic energy-sensing and ischemic/metabolic injury is well studied, recent work implicates AMPK in other aspects of brain physiology. As the complete deletion of both AMPK α subunits results in embryonic lethality, several authors have investigated the role of AMPK in neuronal development using transgenic and alternate methods. Dasgupta and Milbrandt generated a transgenic mouse expressing an inactive β1-β-galactosidase fusion protein. These mice exhibited a striking neurodegeneration phenotype and early post-natal lethality suggesting that β1 subunit is required for normal brain

development [63]. However using an alternative β1 deletion strategy, another group was unable to confirm these severe neurological deficits [39]. Given the lack of a striking neurological phenotype in the α1, α2, or the β2 deletion animals, it is likely that the phenotype observed by Dasgupta and Milbrandt reflects the expression of a β1-fusion protein rather than a β1 deletion [37, 40]. Clearly additional studies are required to test the role of the β1 subunit in neuronal development.

More recently, using a conditional neuronal deletion of α2 on a whole body α1-deletion background, it was shown that AMPK activity is not required for cortical neuron development but regulates axon formation during metabolic stress [64]. It was also shown that overactivation of AMPK during a critical phase of neuronal development inhibits polarization through phosphorylation of KIf5 and disruption of PI3-kinase transport in growing axons [65]. These data also lend support to the notion that over-activation of AMPK in neurons is detrimental and suggest a mechanism of damage to developing neurons in conditions such as perinatal hypoxic-ischemic injury.

AMPK has also been implicated in neurodegenerative diseases such as Alzheimer's (AD) and Huntington's disease (HD) [66, 67]. In this regard, it has been shown that there is overactivation of AMPK in striatal neurons in a transgenic mouse model of HD. More recently, a mechanism was identified by which the α1 subunit abnormally translocates to the nucleus to mediate neurotoxicity via suppression of expression of the anti-apoptotic protein Bcl2. It was also found that a pharmacological agent that ameliorates HD symptoms acts in part by preventing α1 translocation to the nucleus.

The role of AMPK in the pathogenesis of AD is less clear. Initially, it was demonstrated that AMPK activation, either directly or indirectly, inhibits tau phosphorylation in rat cortical neurons [68]. In contrast, others demonstrated that AMPK could phosphorylate tau on multiple sites and disrupt the binding of tau to microtubules [69,70]. Interestingly, it was demonstrated that exposure of mouse cortical neurons to amyloid β peptide (a.a. 1-42) induced Ca^{2+}-CaMKKβ-dependent activation of AMPK and hyperphosphorylation of tau. In human patients it was observed that AMPK is abnormally activated and translocalized from the nucleus to the cytoplasm in AD tangles as well as other tauopathies [70]. Thus it is evident that although AMPK is activated in the Alzheimer's tangle formation process, it is not yet established if this is a primary cause or a cellular response in an attempt to repair disordered metabolic balance and signaling.

Other studies have examined the role of AMPK on amyloid plaques, the other major component of AD pathology. Several studies in this field have shown that AMPK activation represses amyloidogenesis in neurons [68, 71, 72]. Furthermore, it was observed that amyloid-β production is increased in cortical neurons from AMPK α2 knockout mice, whereas activators of AMPK such as AICAR and resveratrol are able to reduce Aβ production and facilitate its clearance. As Alzheimer's disease is a complex neurodegenerative process encompassing numerous molecular players, a complete understanding of its details is lacking. However, based on accumulating data over the past few years, it is likely that AMPK plays an important role in several aspects of AD pathogenesis. Therefore, selective modulation of AMPK signaling may be a potential consideration for AD therapy.

CONCLUSION

AMPK is a multi-functional energy sensor in many organ systems. In the hypothalamus, AMPK serves as an energy integrator which responds to signaling by hormonal and nutrient cues. In pathological conditions such as stroke and neurodegenerative diseases, its overactivation is deleterious. Information from various transgenic and knockout AMPK mouse model systems will help to elucidate precise cellular mechanisms of AMPK activation in both normal and pathophysiology. Due to the fact the AMPK is implicated in a variety of processes, it is a valuable pharmacological target by either or indirect manipulation.

ACKNOWLEDGEMENTS

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